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medical**

USMLE **Step 1**

Lecture Notes

Biochemistry

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SECTION I

**Molecular Biology
and Biochemistry**

Nucleic Acid Structure and Organization

1

OVERVIEW: THE CENTRAL DOGMA OF MOLECULAR BIOLOGY

An organism must be able to store and preserve its genetic information, pass that information along to future generations, and express that information as it carries out all the processes of life. The major steps involved in handling genetic information are illustrated by the central dogma of molecular biology (Figure I-1-1). Genetic information is stored in the base sequence of DNA molecules. Ultimately, during the process of gene expression, this information is used to synthesize all the proteins made by an organism. Classically, a gene is a unit of the DNA that encodes a particular protein or RNA molecule. Although this definition is now complicated by our increased appreciation of the ways in which genes may be expressed, it is still useful as a general, working definition.

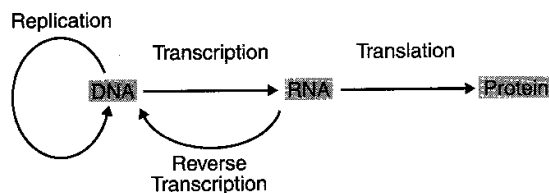


Figure I-1-1. The Central Dogma of Molecular Biology

Gene Expression and DNA Replication

Gene expression and DNA replication are compared in Table I-1-1. Transcription, the first stage in gene expression, involves transfer of information found in a double-stranded DNA molecule to the base sequence of a single-stranded RNA molecule. If the RNA molecule is a messenger RNA, then the process known as translation converts the information in the RNA base sequence to the amino acid sequence of a protein.

When cells divide, each daughter cell must receive an accurate copy of the genetic information. DNA replication is the process in which each chromosome is duplicated before cell division.

Table I-1-1. Comparison of Gene Expression and DNA Replication

Gene Expression	DNA Replication
Produces all the proteins an organism requires	Duplicates the chromosomes before cell division
Transcription of DNA: RNA copy of a small section of a chromosome (average size of human gene, 10^4 – 10^5 nucleotide pairs)	DNA copy of entire chromosome (average size of human chromosome, 10^8 nucleotide pairs)
Translation of RNA: protein synthesis	
Occurs throughout interphase	Occurs during S phase
Transcription in nucleus	Replication in nucleus
Translation in cytoplasm	

The concept of the cell cycle (Figure I-1-2) can be used to describe the timing of some of these events in a eukaryotic cell. The M phase (mitosis) is the time in which the cell divides to form two daughter cells. Interphase is the term used to describe the time between two cell divisions or mitoses. Gene expression occurs throughout all stages of interphase. Interphase is subdivided as follows:

- G_1 phase (gap 1) is a period of cellular growth preceding DNA synthesis. Cells that have stopped cycling, such as muscle and nerve cells, are said to be in a special state called G_0 .
- S phase (DNA synthesis) is the period of time during which DNA replication occurs. At the end of S phase, each chromosome has doubled its DNA content and is composed of two identical sister chromatids linked at the centromere.
- G_2 phase (gap 2) is a period of cellular growth after DNA synthesis but preceding mitosis. Replicated DNA is checked for any errors before cell division.

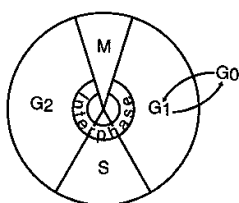


Figure I-1-2.
The Eukaryotic Cell Cycle

Reverse transcription, which produces DNA copies of an RNA, is more commonly associated with life cycles of retroviruses, which replicate and express their genome through a DNA intermediate (an integrated provirus). Reverse transcription also occurs to a limited extent in human cells, where it plays a role in amplifying certain highly repetitive sequences in the DNA (Chapter 7).

NUCLEOTIDE STRUCTURE AND NOMENCLATURE

Nucleic acids (DNA and RNA) are assembled from nucleotides, which consist of three components: a nitrogenous base, a five-carbon sugar (pentose), and phosphate.

Five-Carbon Sugars

Nucleic acids (as well as nucleosides and nucleotides) are classified according to the pentose they contain. If the pentose is ribose, the nucleic acid is RNA (ribonucleic acid); if the pentose is deoxyribose, the nucleic acid is DNA (deoxyribonucleic acid) (Figure I-1-3).

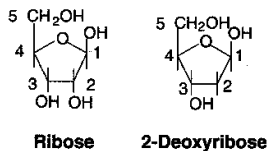


Figure I-1-3.
Five-Carbon Sugars
Commonly Found
in Nucleic Acids

Bases

There are two types of nitrogen-containing bases commonly found in nucleotides: purines and pyrimidines (Figure I-1-4):

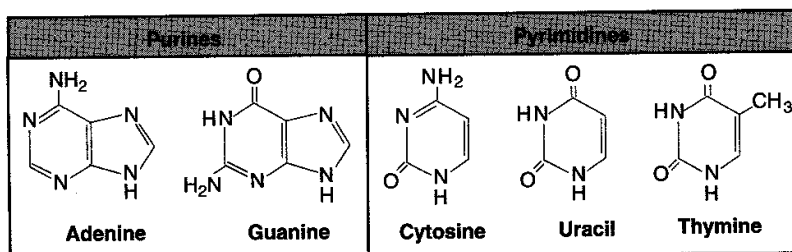


Figure I-1-4. Bases Commonly Found in Nucleic Acids

- Purines contain two rings in their structure. The two purines commonly found in nucleic acids are adenine (A) and guanine (G); both are found in DNA and RNA. Other purine metabolites, not usually found in nucleic acids, include xanthine, hypoxanthine, and uric acid.
- Pyrimidines have only one ring. Cytosine (C) is present in both DNA and RNA. Thymine (T) is usually found only in DNA, whereas uracil (U) is found only in RNA.

Nucleosides and Nucleotides

Nucleosides are formed by covalently linking a base to the number 1 carbon of a sugar (Figure I-1-5). The numbers identifying the carbons of the sugar are labeled with "primes" in nucleosides and nucleotides.

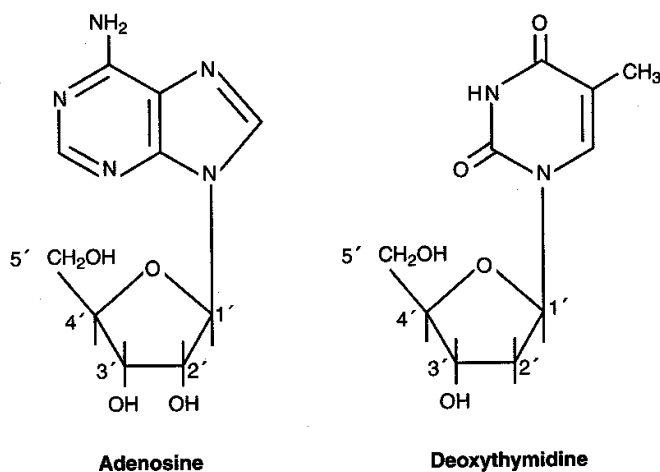


Figure I-1-5. Examples of Nucleosides

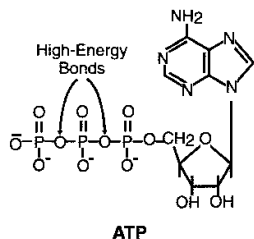


Figure I-1-7.
High-Energy Bonds in
a Nucleoside Triphosphate

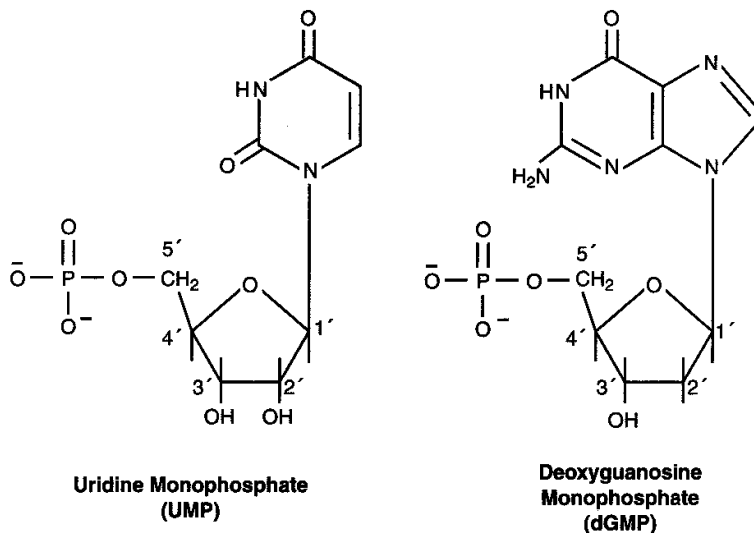


Figure I-1-6. Examples of Nucleotides

The nomenclature for the commonly found bases, nucleosides, and nucleotides is shown in Tables I-1-2a and -2b. Note that the “deoxy” part of the names deoxythymidine, dTMP, etc., is sometimes understood, and not expressly stated, because thymine is almost always found attached to deoxyribose.

Table I-1-2a. Nomenclature of the Ribonucleotide Series of Compounds

Base	Nucleoside	Nucleotides		
Adenine	Adenosine	Adenylic acid	Adenosine diphosphate (ADP)	Adenosine triphosphate (ATP)
		Adenosine monophosphate (AMP)		
Guanine	Guanosine	Guanylic acid	Guanosine diphosphate (GDP)	Guanosine triphosphate (GTP)
		Guanosine monophosphate (GMP)		
Cytosine	Cytidine	Cytidylic acid	Cytidine diphosphate (CDP)	Cytidine triphosphate (CTP)
		Cytidine monophosphate (CMP)		
Uracil	Uridine	Uridylic acid	Uridine diphosphate (UDP)	Uridine triphosphate (UTP)
		Uridine monophosphate (UMP)		

Table I-1-2b. Nomenclature of the Deoxyribonucleotide Series of Compounds

Base	Deoxynucleoside	Deoxynucleotides		
Adenine	Deoxyadenosine	Deoxyadenylic acid	Deoxyadenosine diphosphate (dADP)	Deoxyadenosine triphosphate (dATP)
		Deoxyadenosine monophosphate (dAMP)		
Guanine	Deoxyguanosine	Deoxyguanylic acid	Deoxyguanosine diphosphate (dGDP)	Deoxyguanosine triphosphate (dGTP)
		Deoxyguanosine monophosphate (dGMP)		
Cytosine	Deoxycytidine	Deoxycytidylic acid	Deoxycytidine diphosphate (dCDP)	Deoxycytidine triphosphate (dCTP)
		Deoxycytidine monophosphate (dCMP)		
Thymine	Deoxythymidine	Deoxythymidylic acid	Deoxythymidine diphosphate (dTDP)	Deoxythymidine triphosphate (dTTP)
		Deoxythymidine monophosphate (dTMP)		

In a Nutshell**Nucleic Acids**

- Nucleotides linked by 3', 5' phosphodiester bonds
- Have distinct 3' and 5' ends, thus polarity
- Sequence is always specified as 5'→3'

NUCLEIC ACIDS

Nucleic acids are polymers of nucleotides joined by 3', 5'-phosphodiester bonds; that is, a phosphate group links the 3' carbon of a sugar to the 5' carbon of the next sugar in the chain. Each strand has a distinct 5' end and 3' end, and thus has polarity. A phosphate group is often found at the 5' end, and a hydroxyl group is often found at the 3' end.

The base sequence of a nucleic acid strand is written by convention, in the 5'→3' direction (left to right). According to this convention, the sequence of the strand on the left in Figure I-1-8 must be written

5'-TCAG-3' or TCAG:

- If written backward, the ends must be labeled: 3'-GACT-5'
- The positions of phosphates may be shown: pTpCpApG
- In DNA, a "d" (deoxy) may be included: dTdTdAdG

In eukaryotes, DNA is generally double-stranded (dsDNA) and RNA is generally single-stranded (ssRNA). Exceptions occur in certain viruses, some of which have ssDNA genomes and some of which have dsRNA genomes.

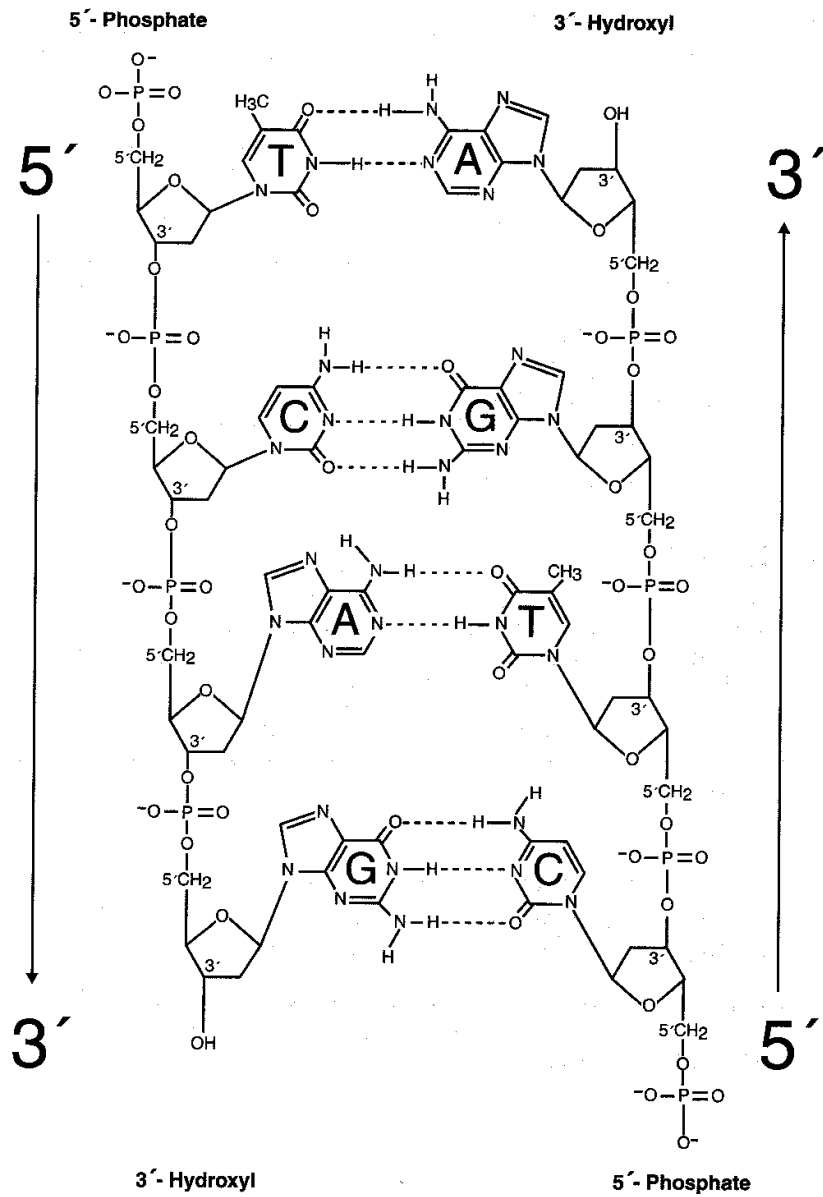


Figure I-1-8. Hydrogen-Bonded Base Pairs in DNA

DNA Structure

Figure I-1-8 shows an example of a double-stranded DNA molecule. Some of the features of double-stranded DNA include:

- The two strands are antiparallel (opposite in direction).
- The two strands are complementary. A always pairs with T (two hydrogen bonds), and G always pairs with C (three hydrogen bonds). Thus, the base sequence on one strand defines the base sequence on the other strand.
- Because of the specific base pairing, the amount of A equals the amount of T, and the amount of G equals the amount of C. Thus, total purines equals total pyrimidines. These properties are known as Chargaff's rules.

With minor modification (substitution of U for T) these rules also apply to dsRNA.

Most DNA occurs in nature as a right-handed double-helical molecule known as Watson-Crick DNA or B-DNA (Fig I-1-9). The hydrophilic sugar-phosphate backbone of each strand is on the outside of the double helix. The hydrogen-bonded base pairs are stacked in the center of the molecule. There are about 10 base pairs per complete turn of the helix. A rare left-handed double-helical form of DNA that occurs in G-C-rich sequences is known as Z-DNA. The biologic function of Z-DNA is unknown, but may be related to gene regulation.

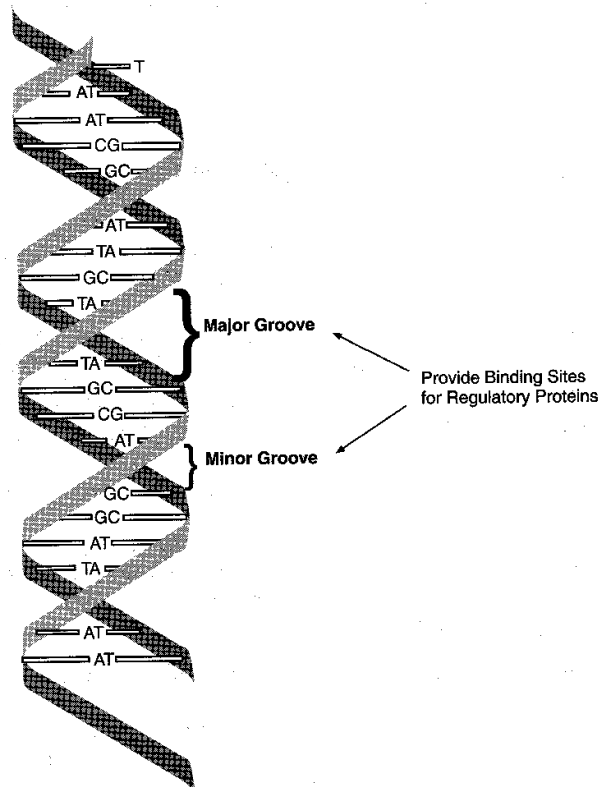


Figure I-1-9. The B-DNA Double Helix

Note

Using Chargaff's Rules

In dsDNA (or dsRNA) (ds = double-stranded)

$$\% A = \% T \quad (\% U)$$

$$\% G = \% C$$

$$\% \text{ purines} = \% \text{ pyrimidines}$$

A sample of DNA has 10% G; what is the % T?

$$10\% G + 10\% C = 20\%$$

therefore, % A + % T must total 80%

$$40\% A \text{ and } 40\% T$$

Ans: 40% T

Denaturation and Renaturation of DNA

Double-helical DNA can be denatured by conditions that disrupt hydrogen bonding and base stacking, resulting in the “melting” of the double helix into two single strands that separate from each other. No covalent bonds are broken in this process. Heat, alkaline pH, and chemicals such as formamide and urea are commonly used to denature DNA.

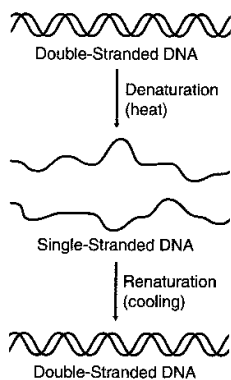


Figure I-1-11. Denaturation and Renaturation of DNA

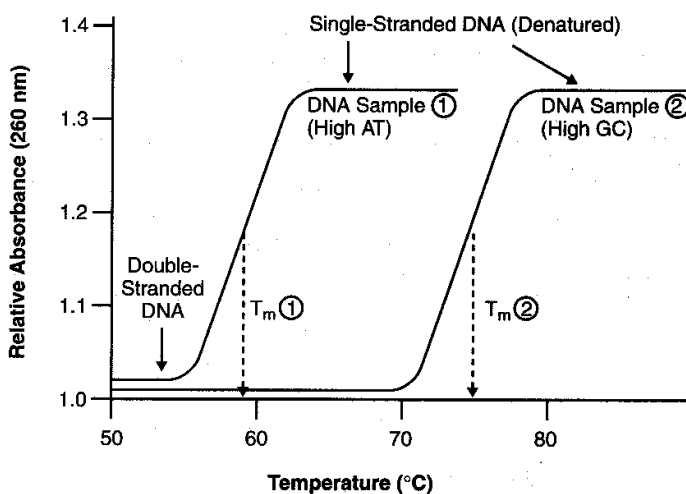


Figure I-1-10. DNA Melting Point

A melting curve for the denaturation of DNA by heat is shown in Figure I-1-10. In such a melting curve:

- Denaturation can be detected by the increase in absorbance of a DNA solution at a wavelength of 260 nm. This increase is known as the hyperchromic effect.
- The melting temperature (T_m) is the temperature at which the molecule is half denatured. This represents the point at which enough heat energy is present to break half the hydrogen bonds holding the two strands together.
- A DNA sample with a high G-C content will have a high T_m because G-C base pairs have three hydrogen bonds, whereas A-T base pairs have only two.

Denatured single-stranded DNA can be renatured (annealed) if the denaturing condition is slowly removed. For example, if a solution containing heat-denatured DNA is slowly cooled, the two complementary strands can become base-paired again (Figure I-1-11).

Such renaturation or annealing of complementary DNA strands is an important step in probing a Southern blot and in performing the polymerase chain reaction (reviewed in Chapter 7). In these techniques, a well-characterized probe DNA is added to a mixture of target DNA molecules. The mixed sample is denatured and then renatured. When probe DNA binds to target DNA sequences of sufficient complementarity, the process is called hybridization.

ORGANIZATION OF DNA

Large DNA molecules must be packaged in such a way that they can fit inside the cell and still be functional.

Supercoiling

Mitochondrial DNA and the DNA of most prokaryotes are closed circular structures. These molecules may exist as relaxed circles or as supercoiled structures in which the helix is twisted around itself in three-dimensional space. Supercoiling results from strain on the molecule caused by under- or overwinding the double helix:

- Negatively supercoiled DNA is formed if the DNA is wound more loosely than in Watson-Crick DNA. This form is required for most biologic reactions.
- Positively supercoiled DNA is formed if the DNA is wound more tightly than in Watson-Crick DNA.
- Topoisomerases are enzymes that can change the amount of supercoiling in DNA molecules. They make transient breaks in DNA strands by alternately breaking and resealing the sugar-phosphate backbone. For example, in *Escherichia coli*, DNA gyrase (DNA topoisomerase II) can introduce negative supercoiling into DNA, whereas DNA topoisomerase I can relax the supercoils (Figure I-1-12).

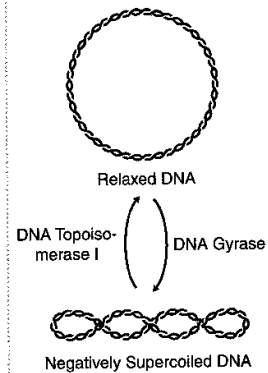


Figure I-1-12. Supercoiling of Circular DNA

Nucleosomes and Chromatin

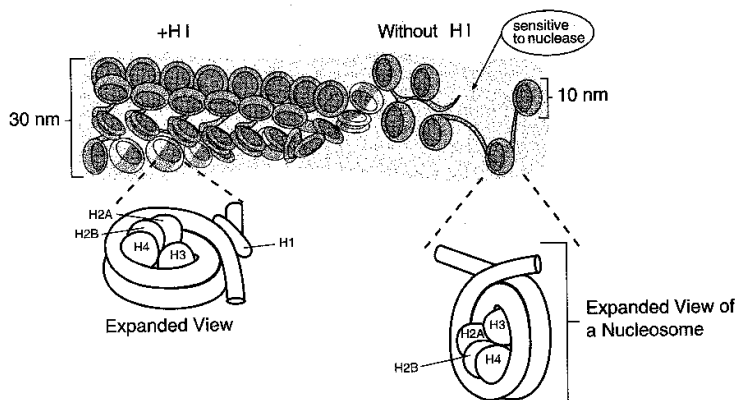


Figure I-1-13. Nucleosome and Nucleofilament Structure in Eukaryotic DNA

Nuclear DNA in eukaryotes is found in chromatin associated with histones and nonhistone proteins. The basic packaging unit of chromatin is the nucleosome (Figure I-1-13):

- Histones are rich in lysine and arginine, which confer a positive charge on the proteins.
- Two copies each of histones H2A, H2B, H3, and H4 aggregate to form the histone octamer.
- DNA is wound around the outside of this octamer to form a nucleosome (a series of nucleosomes is sometimes called “beads on a string”).

- Histone H1 is associated with the linker DNA found between nucleosomes to help package them into a solenoid-like structure, which is a thick 30-nm fiber.
- Further condensation occurs to eventually form the chromosome. Each eukaryotic chromosome contains one linear molecule of DNA.

Cells in interphase contain two types of chromatin:

- Euchromatin is loosely packaged and transcriptionally active.
- Heterochromatin is tightly packaged and inactive.

Euchromatin generally corresponds to looped 30-nm fibers. Heterochromatin is more highly condensed. Figure I-1-14 shows an electron micrograph of an interphase nucleus containing euchromatin, heterochromatin, and a nucleolus. The nucleolus is a nuclear region specialized for ribosome assembly (discussed in Chapter 3).

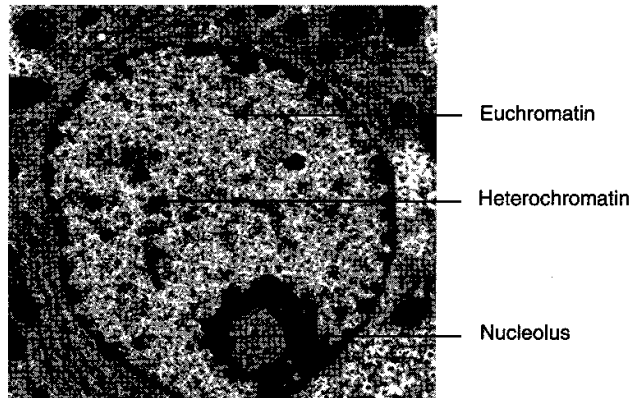


Figure I-1-14. An Interphase Nucleus

Gene expression requires that chromatin be opened for access by transcription complexes (RNA polymerase and transcription factors, Chapter 5). Chromatin-modifying activities include:

- Histone acetylation
- Histone phosphorylation

During mitosis, all the DNA is highly condensed to allow separation of the sister chromatids. This is the only time in the cell cycle when the chromosome structure is visible. Chromosome abnormalities may be assessed on mitotic chromosomes by karyotype analysis (metaphase chromosomes) and by banding techniques (prophase or prometaphase), which identify aneuploidy, translocations, deletions, inversions, and duplications.

Chapter Summary

Nucleic acids:

- RNA and DNA
- Nucleotides (nucleoside monophosphates) linked by phosphodiester bonds
- Have polarity (3' end versus 5' end)
- Sequence always specified 5'-to-3' (left to right on page)

Double-stranded nucleic acids:

- Two strands associate by hydrogen bonding
- Sequences are complementary and antiparallel

Eukaryotic DNA in the nucleus:

- Packaged with histones (H2a, H2b, H3, H4)₂ to form nucleosomes (10-nm fiber)
- 10-nm fiber associates with H1 (30-nm fiber).
- 10-nm fiber and 30-nm fiber comprise euchromatin (active gene expression).
- Higher-order packaging forms heterochromatin (no gene expression).
- Mitotic DNA most condensed (no gene expression)

Review Questions

Select the ONE best answer.

1. Cytosine arabinoside (araC) is used as an effective chemotherapeutic agent for cancer, although resistance to this drug may eventually develop. In certain cases, resistance is related to an increase in the enzyme cytidine deaminase in the tumor cells. This enzyme would inactivate araC to form
 - A. cytosine
 - B. cytidylic acid
 - C. thymidine arabinoside
 - D. uracil arabinoside
 - E. cytidine
2. A double-stranded RNA genome isolated from a virus in the stool of a child with gastroenteritis was found to contain 15% uracil. What is the percentage of guanine in this genome?
 - A. 15
 - B. 25
 - C. 35
 - D. 75
 - E. 85

3. Endonuclease activation and chromatin fragmentation are characteristic features of eukaryotic cell death by apoptosis. Which of the following chromosome structures would most likely be degraded first in an apoptotic cell?
- A. Barr body
 - B. 10 nm fiber
 - C. 30 nm fiber
 - D. Centromere
 - E. Heterochromatin

Answers

1. **Answer: D.** Deamination of cytosine would produce uracil.
2. **Answer: C.** $U = A = 15\%$.
Since $A + G = 50\%$, $G = 35\%$.
Alternatively, $U = A = 15\%$, then $U + A = 30\%$
 $C + G = 70\%$, and
 $G = 35\%$.
3. **Answer: B.** The more "opened" the DNA, the more sensitive it is to enzyme attack. The 10 nm fiber, without the H1 is the most open structure listed. The endonuclease would attack the region of unprotected DNA between the nucleosomes.

DNA Replication and Repair

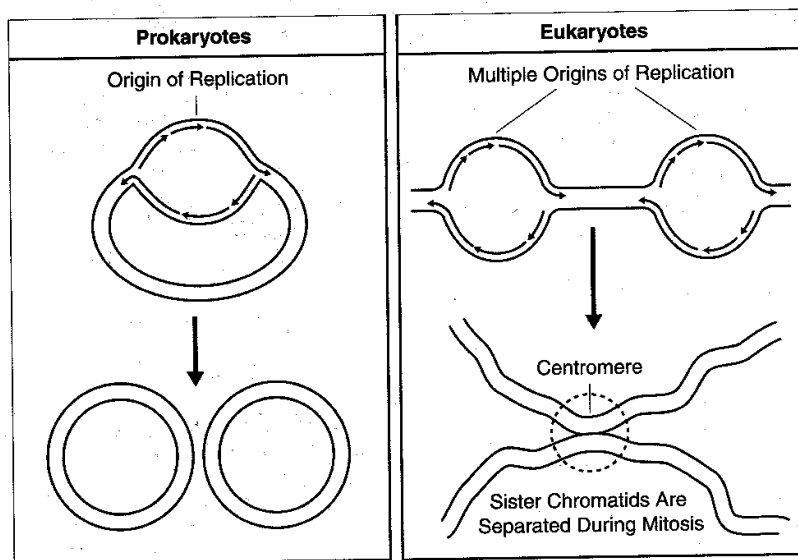
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OVERVIEW OF DNA REPLICATION

Genetic information is transmitted from parent to progeny by replication of parental DNA, a process in which two daughter DNA molecules are produced that are each identical to the parental DNA molecule. During DNA replication, the two complementary strands of parental DNA are pulled apart. Each of these parental strands is then used as a template for the synthesis of a new complementary strand (semiconservative replication). During cell division, each daughter cell receives one of the two identical DNA molecules.

Replication of Prokaryotic and Eukaryotic Chromosomes

The overall process of DNA replication in prokaryotes and eukaryotes is compared in Figure I-2-1.



DNA Replication by a Semi-Conservative, Bidirectional Mechanism

Figure I-2-1

The bacterial chromosome is a closed, double-stranded circular DNA molecule having a single origin of replication. Separation of the two parental strands of DNA creates two replication forks that move away from each other in opposite directions around the circle. Replication is,

In a Nutshell**Polymerases and Nucleases**

Polymerases are enzymes that synthesize nucleic acids by forming phosphodiester (PDE) bonds. Nucleases are enzymes that hydrolyze PDE bonds.

- Exonucleases remove nucleotides from either the 5' or the 3' end of a nucleic acid.
- Endonucleases cut within the nucleic acid and release nucleic acid fragments.

thus, a bidirectional process. The two replication forks eventually meet, resulting in the production of two identical circular molecules of DNA.

Each eukaryotic chromosome contains one linear molecule of DNA having multiple origins of replication. Bidirectional replication occurs by means of a pair of replication forks produced at each origin. Completion of the process results in the production of two identical linear molecules of DNA. DNA replication occurs in the nucleus during the S phase of the eukaryotic cell cycle. The two identical sister chromatids are separated from each other when the cell divides during mitosis.

COMPARISON OF DNA AND RNA SYNTHESIS

The overall process of DNA replication requires the synthesis of both DNA and RNA. These two types of nucleic acids are synthesized by DNA polymerases and RNA polymerases, respectively. DNA synthesis and RNA synthesis are compared in Figure I-2-2 and Table I-2-1.

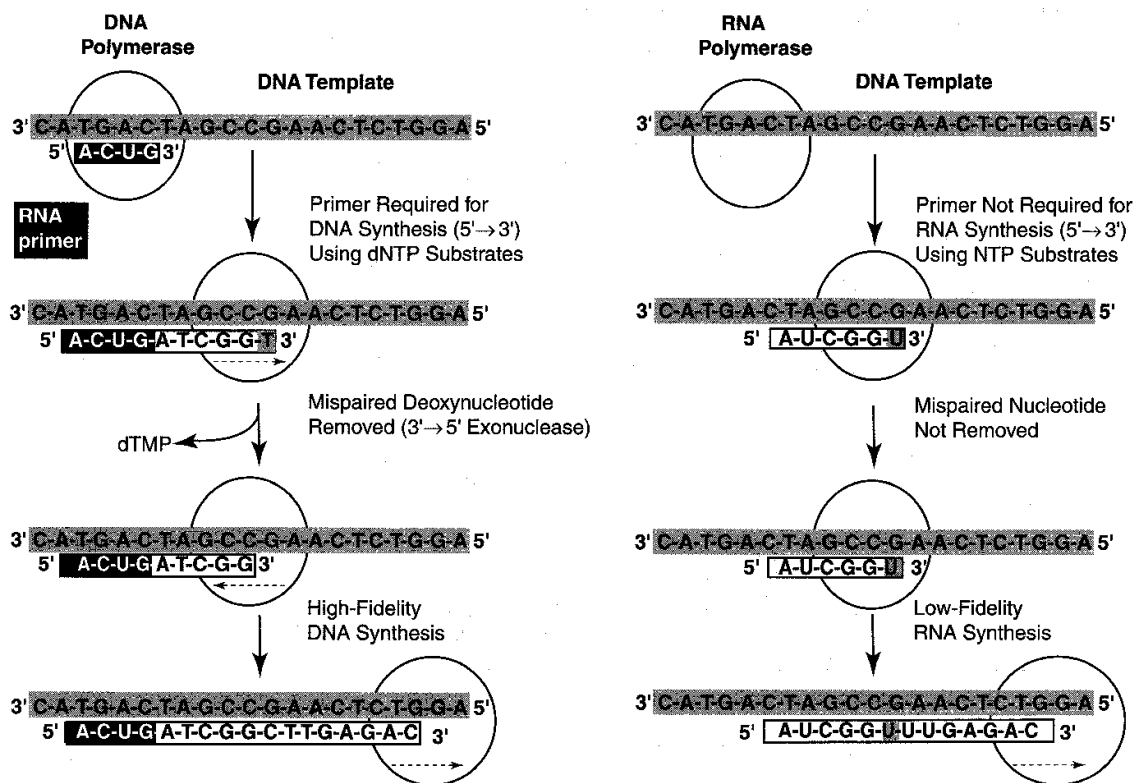


Figure I-2-2. Polymerase Enzymes Synthesize DNA and RNA

Table I-2-1. Comparison of DNA and RNA Polymerases

	DNA Polymerase	RNA Polymerase
Nucleic acid synthesized (5'→3')	DNA	RNA
Required template (copied 3'→5')	DNA*	DNA*
Required substrates	dATP, dGTP, dCTP, dTTP	ATP, GTP, CTP, UTP
Required primer	RNA (or DNA)	None
Proofreading activity (3'→5' exonuclease)	Yes	No

*Certain DNA and RNA polymerases require RNA templates. These enzymes are most commonly associated with viruses.

Similarities include:

- The newly synthesized strand is made in the 5'→3' direction.
- The template strand is scanned in the 3'→5' direction.
- The newly synthesized strand is complementary and antiparallel to the template strand.
- Each new nucleotide is added when the 3' hydroxyl group of the growing strand reacts with a nucleoside triphosphate, which is base-paired with the template strand. Pyrophosphate (PPi, the last two phosphates) is released during this reaction.

Differences include:

- The substrates for DNA synthesis are the dNTPs, whereas the substrates for RNA synthesis are the NTPs.
- DNA contains thymine, whereas RNA contains uracil.
- DNA polymerases require a primer, whereas RNA polymerases do not. That is, DNA polymerases cannot initiate strand synthesis, whereas RNA polymerases can.
- DNA polymerases can correct mistakes ("proofreading"), whereas RNA polymerases cannot. DNA polymerases have 3' → 5' exonuclease activity for proofreading.

STEPS OF DNA REPLICATION

The molecular mechanism of DNA replication as it occurs in the circular chromosome of a prokaryote such as *Escherichia coli* is shown in Figure I-2-3. The sequence of events is as follows:

1. The base sequence at the origin of replication is recognized and bound by the dna A protein. The two parental strands of DNA are pulled apart to form a "replication bubble."
2. Helicase uses energy from ATP to break the hydrogen bonds holding the base pairs together. This allows the two parental strands of DNA to begin unwinding and forms two replication forks.
3. Single-stranded DNA binding protein (SSB) binds to the single-stranded portion of each DNA strand, preventing the strands from reassociating and protecting them from degradation by nucleases.
4. Primase synthesizes a short (about 10 nucleotides) RNA primer in the 5'→3' direction, beginning at the origin on each parental strand. The parental strand is used as a template for this process. RNA primers are required because DNA polymerases are unable to initiate synthesis of DNA, but can only extend a strand from the 3' end of a preformed "primer."

5. DNA polymerase III begins synthesizing DNA in the 5'→3' direction, beginning at the 3' end of each RNA primer. The newly synthesized strand is complementary and antiparallel to the parental strand used as a template. This strand can be made continuously in one long piece and is known as the "leading strand."
 - The "lagging strand" is synthesized discontinuously as a series of small fragments (about 1,000 nucleotides long) known as Okazaki fragments. Each Okazaki fragment is initiated by the synthesis of an RNA primer by primase, and then completed by the synthesis of DNA using DNA polymerase III. Each fragment is made in the 5'→3' direction.
 - There is a leading and a lagging strand for each of the two replication forks on the chromosome.
6. RNA primers are removed by DNA polymerase I. This enzyme removes the ribonucleotides one at a time from the 5' end of the primer (5'→3' exonuclease). DNA polymerase I also fills in the resulting gaps by synthesizing DNA, beginning at the 3' end of the neighboring Okazaki fragment.
7. Both DNA polymerase I and III have the ability to "proofread" their work by means of a 3'→5' exonuclease activity. If DNA polymerase makes a mistake during DNA synthesis, the resulting unpaired base at the 3' end of the growing strand is removed before synthesis continues.
8. DNA ligase seals the "nicks" between Okazaki fragments, converting them to a continuous strand of DNA.
9. DNA gyrase (DNA topoisomerase II) provides a "swivel" in front of each replication fork. As helicase unwinds the DNA at the replication forks, the DNA ahead of it becomes overwound and positive supercoils form. DNA gyrase inserts negative supercoils by nicking both strands of DNA, passing the DNA strands through the nick, and then resealing both strands again. DNA topoisomerase I can relieve supercoiling in DNA molecules by the transient breaking and resealing of just one of the strands of DNA. Quinolones are a family of drugs that block the action of topoisomerases. Nalidixic acid kills bacteria by inhibiting DNA gyrase. Inhibitors of eukaryotic topoisomerase II (etoposide, teniposide) are becoming useful as anticancer agents.
 - Replication is completed when the two replication forks meet each other on the side of the circle opposite the origin.

The mechanism of replication in eukaryotes is believed to be very similar to this. However, the details have not yet been completely worked out. The steps and proteins involved in DNA replication in prokaryotes are compared with those used in eukaryotes in Table I-2-2.

Eukaryotic DNA Polymerases

- DNA polymerase δ synthesizes the leading strand during replication.
- DNA polymerase α synthesizes the lagging strand during replication.
- DNA polymerase γ replicates mitochondrial DNA.
- DNA polymerases β and ϵ are thought to participate primarily in DNA repair. DNA polymerase ϵ may substitute for DNA polymerase δ in certain cases.

Telomerase

Telomeres are repetitive sequences at the ends of linear DNA molecules in eukaryotic chromosomes. With each round of replication in most normal cells, the telomeres are shortened because DNA polymerase cannot complete synthesis of the 5' end of each strand. This contributes to the aging of cells, because eventually the telomeres become so short that the chromosomes cannot function properly and the cells die.

Telomerase is an enzyme in eukaryotes used to maintain the telomeres. It contains a short RNA template complementary to the DNA telomere sequence, as well as telomerase reverse transcriptase activity (hTERT). Telomerase is thus able to replace telomere sequences that would otherwise be lost during replication. Normally telomerase activity is present only in embryonic cells, germ (reproductive) cells, and stem cells, but not in somatic cells.

Cancer cells often have relatively high levels of telomerase, preventing the telomeres from becoming shortened and contributing to the immortality of malignant cells.

Table I-2-2. Steps and Proteins Involved in DNA Replication

Step in Replication	Prokaryotic Cells	Eukaryotic Cells
Recognition of origin of replication	dna A protein	Unknown
Unwinding of DNA double helix	Helicase (requires ATP)	Helicase (requires ATP)
Stabilization of unwound template strands	Single-stranded DNA-binding protein (SSB)	Single-stranded DNA-binding protein (SSB)
Synthesis of RNA primers	Primase	Primase
Synthesis of DNA Leading strand Lagging strand (Okazaki fragments)	DNA polymerase III DNA polymerase III	DNA polymerase δ DNA polymerase α
Removal of RNA primers	DNA polymerase I (5'→3' exonuclease)	Unknown
Replacement of RNA with DNA	DNA polymerase I	Unknown
Joining of Okazaki fragments	DNA ligase (requires NAD)	DNA ligase (requires ATP)
Removal of positive supercoils ahead of advancing replication forks	DNA topoisomerase II (DNA gyrase)	DNA topoisomerase II
Synthesis of telomeres	Not required	Telomerase

Reverse Transcriptase

Reverse transcriptase is an RNA-dependent DNA polymerase that requires an RNA template to direct the synthesis of new DNA. Retroviruses, most notably HIV, use this enzyme to replicate their RNA genomes. DNA synthesis by reverse transcriptase in retroviruses can be inhibited by AZT, ddC, and ddI.

Eukaryotic cells also contain reverse transcriptase activity:

- Associated with telomerase (hTERT).
- Encoded by retrotransposons (residual viral genomes permanently maintained in human DNA) that play a role in amplifying certain repetitive sequences in DNA (see Chapter 7).

Bridge to Pharmacology

Quinolones and DNA Gyrase

Quinolones and fluoroquinolones inhibit DNA gyrase (prokaryotic topoisomerase II), preventing DNA replication and transcription. These drugs, which are most active against aerobic gram-negative bacteria, include:

- Nalidixic acid
- Ciprofloxacin
- Norfloxacin

Resistance to the drugs has developed over time; current uses include treatment of gonorrhea, and upper and lower urinary tract infections in both sexes.

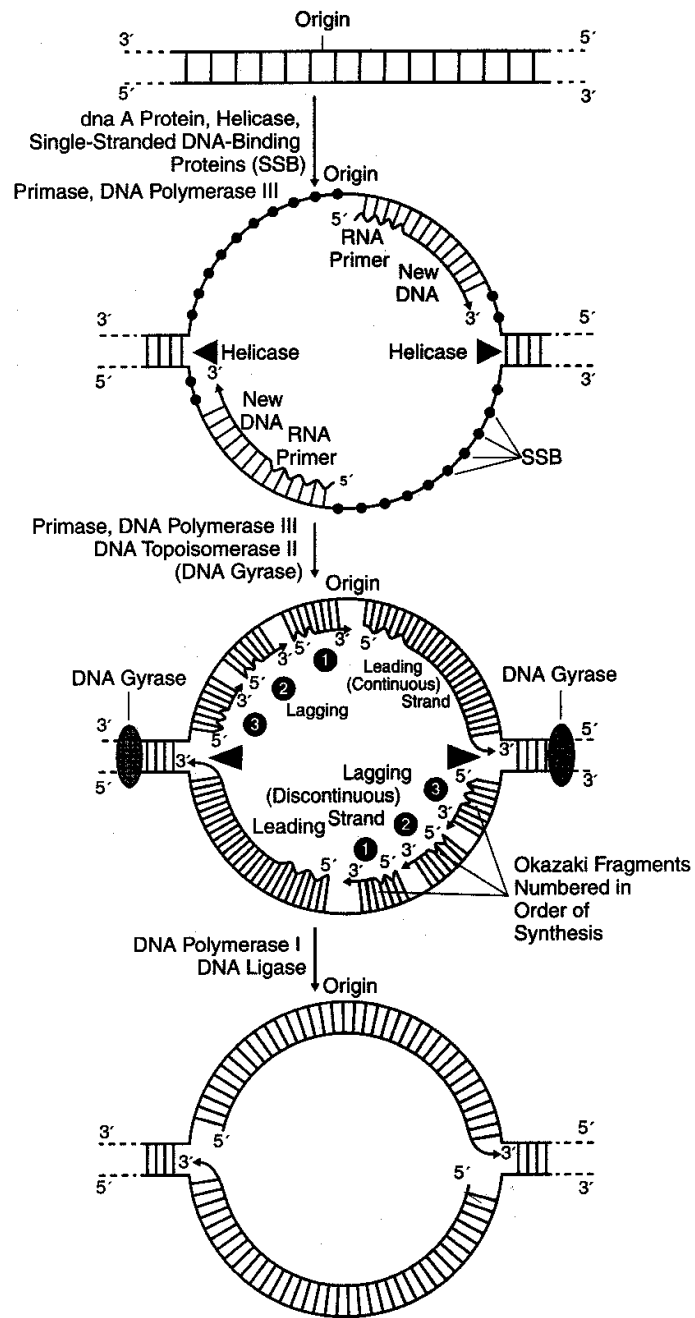


Figure I-2-3. Bacterial DNA Replication

DNA REPAIR

The structure of DNA can be damaged in a number of ways through exposure to chemicals or radiation. Incorrect bases can also be incorporated during replication. Multiple repair systems have evolved, allowing cells to maintain the sequence stability of their genomes (Table I-2-3). If cells are allowed to replicate their DNA using a damaged template, there is a high risk of introducing stable mutations into the new DNA. Thus any defect in DNA repair carries an increased risk of cancer. Most DNA repair occurs in the G₁ phase of the eukaryotic cell cycle. Mismatch repair occurs in the G₂ phase to correct replication errors.

Table I-2-3. DNA Repair

Damage	Cause	Recognition/ Excision Enzyme	Repair Enzymes
Thymine dimers (G ₁)	UV radiation	Excision endonuclease (deficient in Xeroderma pigmentosum)	DNA polymerase DNA ligase
Cytosine deamination (G ₁)	Spontaneous/ chemicals	Uracil glycosylase AP endonuclease	DNA polymerase DNA ligase
Apurination or apyrimidination (G ₁)	Spontaneous/ heat	AP endonuclease	DNA polymerase DNA ligase
Mismatched base (G ₂)	DNA replication errors	A mutation on one of two genes, hMSH2 or hMLH1, initiates defective repair of DNA mismatches, resulting in a condition known as hereditary nonpolyposis colorectal cancer—HNPCC.	DNA polymerase DNA ligase

Repair of Thymine Dimers

Ultraviolet light induces the formation of dimers between adjacent thymines in DNA (also occasionally between other adjacent pyrimidines). The formation of thymine dimers interferes with DNA replication and normal gene expression. Thymine dimers are eliminated from DNA by a nucleotide excision-repair mechanism (Figure I-2-4).

Bridge to Pathology

Tumor Suppressor Genes and DNA Repair

DNA repair may not occur properly when certain tumor suppressor genes have been inactivated through mutation or deletion:

- The *p53* gene encodes a protein that prevents a cell with damaged DNA from entering the S phase. Inactivation or deletion associated with Li Fraumeni syndrome and many solid tumors.
- *ATM* gene encodes a kinase essential for p53 activity. *ATM* is inactivated in ataxia telangiectasia, characterized by hypersensitivity to x-rays and predisposition to lymphomas.
- *BRCA-1* (breast, prostate, and ovarian cancer) and *BRCA-2* (breast cancer) required for p53 activity.

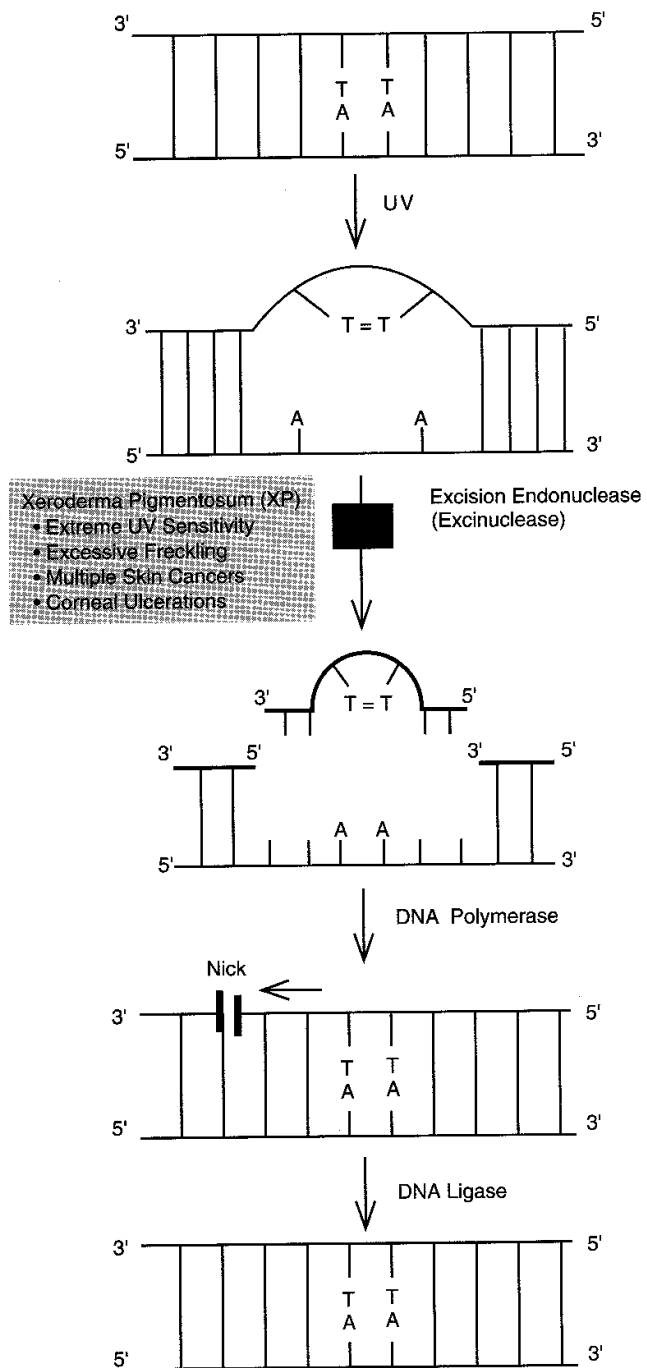


Figure I-2-4. Thymine Dimer Formation and Excision-Repair

Steps in nucleotide excision repair:

- An excision endonuclease (excinuclease) makes nicks in the phosphodiester backbone of the damaged strand on both sides of the thymine dimer and removes the defective oligonucleotide.
- DNA polymerase fills in the gap by synthesizing DNA in the 5'→3' direction, using the undamaged strand as a template.
- DNA ligase seals the nick in the repaired strand.

Repair of Deaminated and Missing Bases

Cytosine can become deaminated spontaneously or by reaction with nitrous acid to form uracil. This leaves an improper base pair (G-U), which is eliminated by a base excision repair mechanism (Figure I-2-5). Failure to repair the improper base pair can convert a normal G-C pair to an A-T pair.

Steps in base excision repair:

- A uracil glycosylase recognizes and removes the uracil base, leaving an apyrimidinic (AP) site in the DNA strand.
- An AP endonuclease nicks the backbone of the damaged strand at the missing base.
- Additional nuclease action removes a few more bases, and the gap is filled in by DNA polymerase.
- DNA ligase seals the nick in the repaired strand.

Diseases Associated With DNA Repair

Inherited mutations that result in defective DNA repair mechanisms are associated with a predisposition to the development of cancer. Some examples of such genetic diseases are:

- Xeroderma pigmentosum is an autosomal recessive disorder, characterized by extreme sensitivity to sunlight, skin freckling and ulcerations, and skin cancer. The most common deficiency occurs in the excinuclease enzyme.
- Hereditary nonpolyposis colorectal cancer results from a deficiency in the ability to repair mismatched base pairs in DNA that are accidentally introduced during replication.

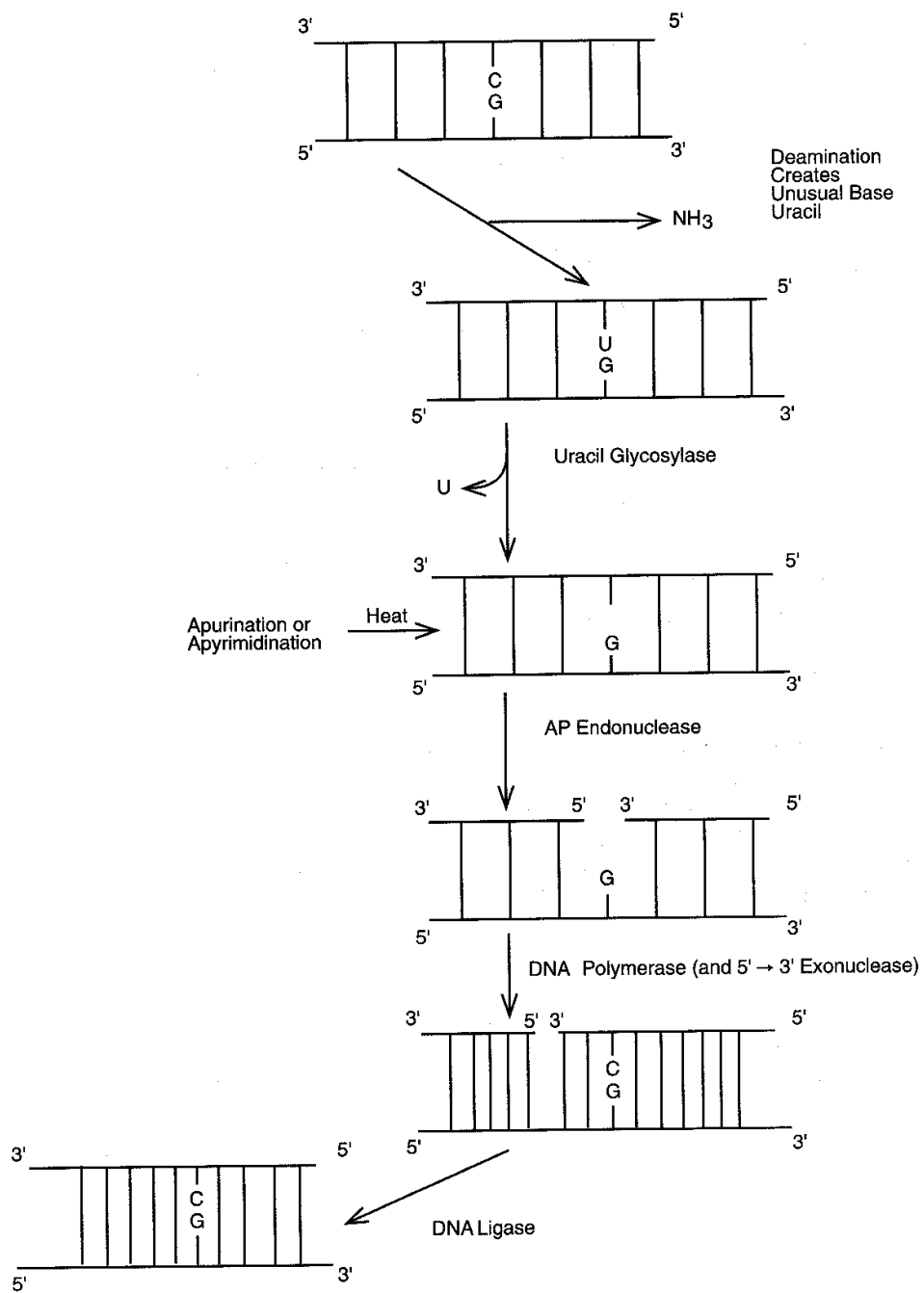


Figure I-2-5. Repair of Deaminated and Missing Bases

Chapter Summary

DNA SYNTHESIS

	Prokaryotic	Eukaryotic
Timing	Prior to cell division	S-phase
Enzymes	DnaA protein Helicase Ss DNA-binding protein Primase (an RNA polymerase) DNA pol III (leading/lagging) DNA pol I DNA ligase DNA gyrase (Topo II)	Helicase Ss DNA-binding protein Primase (an RNA polymerase) DNA pol δ (leading) DNA pol α (lagging) DNA ligase DNA topoisomerase II Telomerase

DNA REPAIR

G1 phase of eukaryotic cell cycle:

- UV radiation: thymine (pyrimidine) dimers; excinuclease
- Deaminations (C becomes U); uracil glycosylase
- Loss of purine or pyrimidine; AP endonuclease

G2 phase of eukaryotic cell cycle:

- Mismatch repair: hMSH1, hMLH2 (HPNCC)

Review Questions

Select the ONE best answer.

1. It is now believed that a substantial proportion of the single nucleotide substitutions causing human genetic disease are due to misincorporation of bases during DNA replication. Which proofreading activity is critical in determining the accuracy of nuclear DNA replication and thus the base substitution mutation rate in human chromosomes?
 - A. 3' to 5' polymerase activity of DNA polymerase δ
 - B. 3' to 5' exonuclease activity of DNA polymerase γ
 - C. Primase activity of DNA polymerase α
 - D. 5' to 3' polymerase activity of DNA polymerase III
 - E. 3' to 5' exonuclease activity of DNA polymerase δ

2. The proliferation of cytotoxic T-cells is markedly impaired upon infection with a newly discovered human immunodeficiency virus, designated HIV-V. The defect has been traced to the expression of a viral-encoded enzyme that inactivates a host-cell nuclear protein required for DNA replication. Which protein is a potential substrate for the viral enzyme?
 - A. TATA-box binding protein (TBP)
 - B. Cap binding protein (CBP)
 - C. Catabolite activator protein (CAP)
 - D. Acyl-carrier protein (ACP)
 - E. Single-strand binding protein (SBP)
3. The deficiency of an excision endonuclease may produce an exquisite sensitivity to ultraviolet radiation in Xeroderma pigmentosum. Which of the following functions would be absent in a patient deficient in this endonuclease?
 - A. Removal of introns
 - B. Removal of pyrimidine dimers
 - C. Protection against DNA viruses
 - D. Repair of mismatched bases during DNA replication
 - E. Repair of mismatched bases during transcription
4. The anti-*Pseudomonas* action of norfloxacin is related to its ability to inhibit chromosome duplication in rapidly-dividing cells. Which of the following enzymes participates in bacterial DNA replication and is directly inhibited by this antibiotic?
 - A. DNA polymerase I
 - B. DNA polymerase II
 - C. Topoisomerase I
 - D. Topoisomerase II
 - E. DNA ligase

Answers

1. **Answer: E.** The 3' to 5' exonuclease activity of DNA pol δ represents the proofreading activity of an enzyme required for the replication of human chromosomal DNA. DNA pol γ (mitochondrial) and DNA pol III (prokaryotic) do not participate in this process, short RNA primers are replaced with DNA during replication, and new DNA strands are always synthesized in the 5' to 3' direction.
2. **Answer: E.** TBP and CBP participate in eukaryotic gene transcription and mRNA translation, respectively. CAP regulates the expression of prokaryotic lactose operons. ACP is involved in fatty acid synthesis.
3. **Answer: B.** Nucleotide excision repair of thymine (pyrimidine) dimers is deficient in XP patients.
4. **Answer: D.** Norfloxacin inhibits DNA gyrase (topoisomerase II).

Transcription and RNA Processing

3

OVERVIEW OF TRANSCRIPTION

The first stage in the expression of genetic information is transcription of the information in the base sequence of a double-stranded DNA molecule to form the base sequence of a single-stranded molecule of RNA. For any particular gene, only one strand of the DNA molecule, called the template strand, is copied by RNA polymerase as it synthesizes RNA in the 5' to 3' direction. Because RNA polymerase moves in the 3' to 5' direction along the template strand of DNA, the RNA product is antiparallel and complementary to the template. RNA polymerase recognizes start signals (promoters) and stop signals (terminators) for each of the thousands of transcription units in the genome of an organism. Figure I-3-1 illustrates the arrangement and direction of transcription for several genes on a DNA molecule.

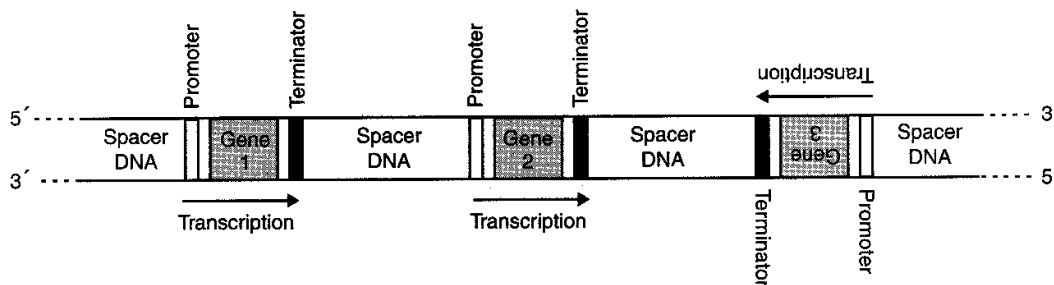


Figure I-3-1. Transcription of Several Genes on a Chromosome

TYPES OF RNA

RNA molecules play a variety of roles in the cell. The major types of RNA are:

- Ribosomal RNA (rRNA), which is the most abundant type of RNA in the cell. It is used as a structural component of the ribosome. Ribosomal RNA associates with ribosomal proteins to form the complete, functional ribosome.
- Transfer RNA (tRNA), which is the second most abundant type of RNA. Its function is to carry amino acids to the ribosome, where they will be linked together during protein synthesis.
- Messenger RNA (mRNA), which carries the information specifying the amino acid sequence of a protein to the ribosome. Messenger RNA is the only type of RNA that is translated. The mRNA population in a cell is very heterogeneous in size and base sequence, as the cell has essentially a different mRNA molecule for each of the thousands of different proteins made by that cell.

- Heterogeneous nuclear RNA (hnRNA or pre-mRNA), which is found only in the nucleus of eukaryotic cells. It represents precursors of mRNA, formed during its post-transcriptional processing.
- Small nuclear RNA (snRNA), which is also only found in the nucleus of eukaryotes. One of its major functions is to participate in splicing (removal of introns) mRNA.
- Ribozymes, which are RNA molecules with enzymatic activity. They are found in both prokaryotes and eukaryotes.

TRANSCRIPTION: IMPORTANT CONCEPTS AND TERMINOLOGY

RNA is synthesized by a DNA-dependent RNA polymerase (uses DNA as a template for the synthesis of RNA). Important terminology used when discussing transcription is illustrated in Figure I-3-2.

- RNA polymerase locates genes in DNA by searching for promoter regions. The promoter is the binding site for RNA polymerase. Binding establishes where transcription begins, which strand of DNA is used as the template, and in which direction transcription proceeds. No primer is required.
- RNA polymerase moves along the template strand in the 3' to 5' direction as it synthesizes the RNA product in the 5' to 3' direction using NTPs (ATP, GTP, CTP, UTP) as substrates. RNA polymerase does not proofread its work. The RNA product is complementary and antiparallel to the template strand.
- The coding (antitemplate) strand is not used during transcription. It is identical in sequence to the RNA molecule, except that RNA contains uracil instead of the thymine found in DNA.
- By convention, the base sequence of a gene is given from the coding strand (5'→3').
- In the vicinity of a gene, a numbering system is used to identify the location of important bases. The first base transcribed as RNA is defined as the +1 base of that gene region. To the left (5', or upstream) of this starting point for transcription, bases are -1, -2, -3, etc. To the right (3', or downstream) of this point, bases are +2, +3, etc.
- Transcription ends when RNA polymerase reaches a termination signal.

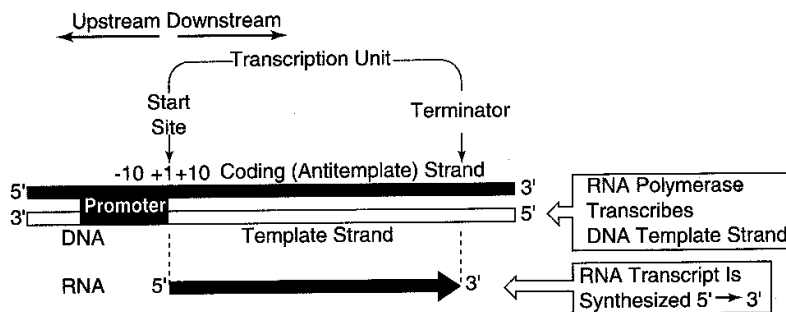


Figure I-3-2. Transcription of DNA

Flow of Genetic Information From DNA to Protein

For the case of a gene coding for a protein, the relationship among the sequences found in double-stranded DNA, single-stranded mRNA, and protein is illustrated in Figure I-3-3. Messenger RNA is synthesized in the 5' to 3' direction. It is complementary and antiparallel to the template strand of DNA. The ribosome translates the mRNA in the 5' to 3' direction, as it synthesizes the protein from the amino to the carboxyl terminus.

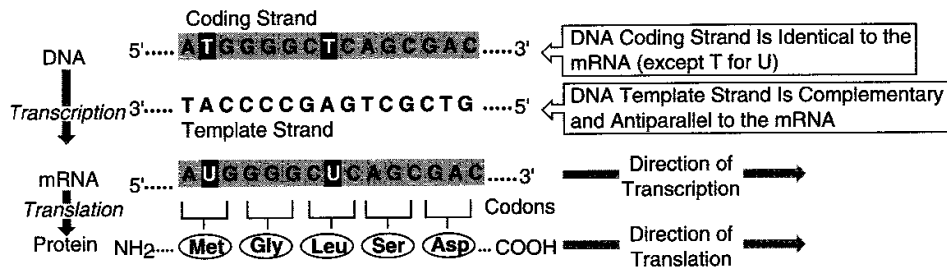


Figure I-3-3. Flow of Genetic Information From DNA to Protein

Sample Questions

- During RNA synthesis, the DNA template sequence TAGC would be transcribed to produce which of the following sequences?
 - ATCG
 - GCTA
 - CGTA
 - AUCG
 - GCUA

The answer is E. RNA is antiparallel and complementary to the template strand. Also remember that, by convention, all base sequences are written in the 5' to 3' direction regardless of the direction in which the sequence may actually be used in the cell.

Approach:

- Cross out any option with a T (RNA has U).
- Look at the 5' end of DNA (T in this case).
- What is the complement of this base? (A)

Examine the options given. A correct option will have the complement (A in this example) at the 3' end. Repeat the procedure for the 3' end of the DNA. This will usually leave only one or two options.

2. Transcription of the following sequence of the tryptophan operon occurs in the direction indicated by the arrow. What would be the base sequence of the mRNA produced?

3'...CGCCGCTGCGCG...5'

Transcription → Which product?

5'...GCGGCGACGCGC...3'

- A. 5'...GCGGCGACGCGC...3'
- B. 5'...GCGCGUCGCCGC...3'
- C. 5'...GCGCGTGCGGCG...3'
- D. 5'...GCGGCGUCGCGC...3'
- E. 5'...CGCGCTCGCCGC...3'

The answer is A. Because all nucleic acids are synthesized in the 5' to 3' direction, mRNA and the coding strand of DNA must each be oriented 5' to 3', i.e., in the direction of transcription. This means that the bottom strand in this example is the coding strand. The top strand is the template strand.

Approach:

- Cross out any option with a T.
- Identify the coding strand of DNA from the direction of transcription.
- Find the option with a sequence identical to the coding strand (remember to substitute U for T, if necessary).
- Alternatively, if you prefer to find the complement of the template strand, you will get the same answer.

RNA POLYMERASES

There is a single prokaryotic RNA polymerase that synthesizes all types of RNA in the cell. The core polymerase responsible for making the RNA molecule has the subunit structure $\alpha_2\beta\beta'$. A protein factor called sigma (σ) is required for the initiation of transcription at a promoter. Sigma factor is released immediately after initiation of transcription. Termination of transcription sometimes requires a protein called rho (ρ) factor. This enzyme is inhibited by rifampin. Actinomycin D binds to the DNA preventing transcription.

There are three eukaryotic RNA polymerases, distinguished by the particular types of RNA they produce:

- RNA polymerase I is located in the nucleolus and synthesizes 28S, 18S, and 5.8S rRNAs.
- RNA polymerase II is located in the nucleoplasm and synthesizes hnRNA/mRNA and some snRNA.
- RNA polymerase III is located in the nucleoplasm and synthesizes tRNA, some snRNA, and 5S rRNA.

Transcription factors (such as TFIID for RNA polymerase II) help to initiate transcription. The requirements for termination of transcription in eukaryotes are not well understood. All transcription can be inhibited by actinomycin D. In addition, RNA polymerase II is inhibited by α -amanitin (a toxin from certain mushrooms). These points are summarized in Table I-3-1.

Table I-3-1. Comparison of Eukaryotic and Prokaryotic RNA Polymerases

Prokaryotic	Eukaryotic
Single RNA polymerase ($\alpha_2\beta\beta'$)	RNAP 1: rRNA (nucleolus), except 5S rRNA RNAP 2: hnRNA/mRNA and some snRNA RNAP 3: tRNA, 5S rRNA
Requires sigma (σ) to initiate at a promoter	No sigma, but transcription factors (TFIID) bind before RNA polymerase
Sometimes requires rho (ρ) to terminate	No rho required
Inhibited by rifampin Actinomycin D	RNAP 2 inhibited by α -amanitin (mushrooms) Actinomycin D

PRODUCTION OF PROKARYOTIC MESSENGER RNA

The structure and expression of a typical prokaryotic gene coding for a protein is illustrated in Figure I-3-4. The following events occur during the expression of this gene:

1. With the help of sigma factor, RNA polymerase recognizes and binds to the promoter region. The bacterial promoter contains two "consensus" sequences, called the Pribnow box (or TATA box) and the -35 sequence. The promoter identifies the start site for transcription and orients the enzyme on the template strand. The RNA polymerase separates the two strands of DNA as it reads the base sequence of the template strand.
2. Transcription begins at the +1 base pair. Sigma factor is released as soon as transcription is initiated.
3. The core polymerase continues moving along the template strand in the 3' to 5' direction, synthesizing the mRNA in the 5' to 3' direction.
4. RNA polymerase eventually reaches a transcription termination signal, at which point it will stop transcription and release the completed mRNA molecule. There are two kinds of transcription terminators commonly found in prokaryotic genes:
 - Rho-independent termination occurs when the newly formed RNA folds back on itself to form a GC-rich hairpin loop closely followed by 6–8 U residues. These two structural features of the newly synthesized RNA promote dissociation of the RNA from the DNA template. This is the type of terminator shown in Figure I-3-4.
 - Rho-dependent termination requires participation of rho factor. This protein binds to the newly formed RNA and moves toward the RNA polymerase that has paused at a termination site. Rho then displaces RNA polymerase from the 3' end of the RNA.
5. Transcription and translation can occur simultaneously in bacteria. Because there is no processing of prokaryotic mRNA (no introns), ribosomes can begin translating the message even before transcription is complete. Ribosomes bind to a sequence called the Shine-Dalgarno sequence in the 5' untranslated region (UTR) of the message. Protein synthesis begins at an AUG codon at the beginning of the coding region and continues until the ribosome reaches a stop codon at the end of the coding region.
6. The ribosome translates the message in the 5' to 3' direction, synthesizing the protein from amino terminus to carboxyl terminus.

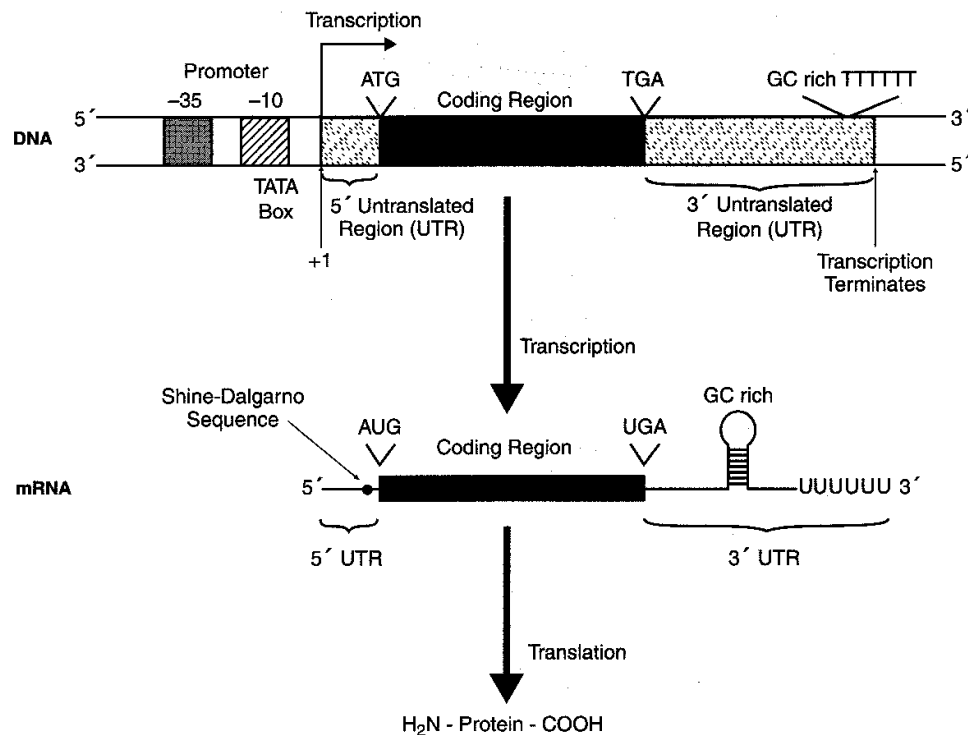


Figure I-3-4. A Prokaryotic Transcription Unit

The mRNA produced by the gene shown in Figure I-3-4 is a monocistronic message. That is, it is transcribed from a single gene and codes for only a single protein. The word *cistron* is another name for a gene. Some bacterial operons (for example, the lactose operon, Chapter 5) produce polycistronic messages. In these cases, related genes grouped together in the DNA are transcribed as one unit. The mRNA in this case contains information from several genes and codes for several different proteins (Figure I-3-5).

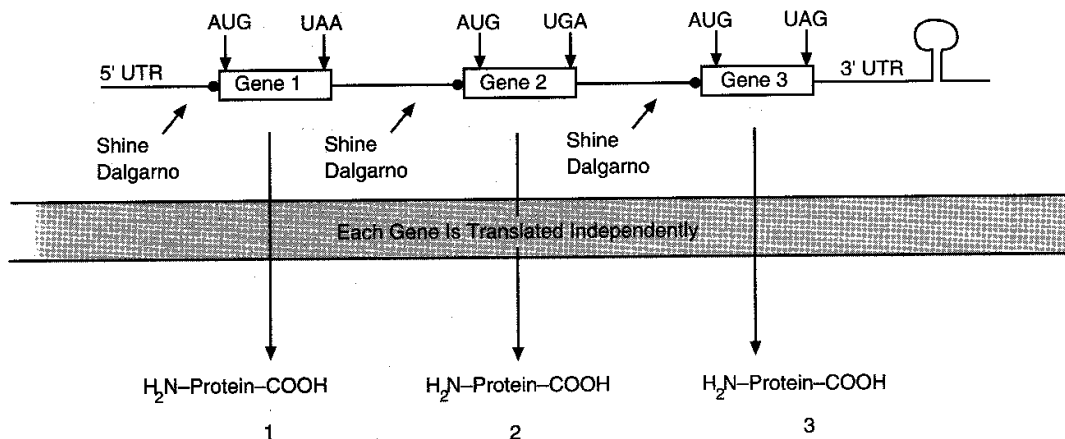


Figure I-3-5. Prokaryotic Polycistronic Message Codes for Several Different Proteins

PRODUCTION OF EUKARYOTIC MESSENGER RNA

In eukaryotes, most genes are composed of coding segments (exons) interrupted by noncoding segments (introns). Both exons and introns are transcribed in the nucleus. Introns are removed during processing of the RNA molecule in the nucleus. In eukaryotes, all mRNA is monocistronic. The mature mRNA is translated in the cytoplasm. The structure and transcription of a typical eukaryotic gene coding for a protein is illustrated in Figure I-3-6. Transcription of this gene occurs as follows:

1. With the help of proteins called transcription factors, RNA polymerase II recognizes and binds to the promoter region. The basal promoter region of eukaryotic genes usually has two consensus sequences called the TATA box (also called Hogness box) and the CAAT box.
2. RNA polymerase II separates the strands of the DNA over a short region to initiate transcription and read the DNA sequence. The template strand is read in the 3' to 5' direction as the RNA product (the primary transcript) is synthesized in the 5' to 3' direction. Both exons and introns are transcribed.
3. RNA polymerase II ends transcription when it reaches a termination signal. These signals are not well understood in eukaryotes.

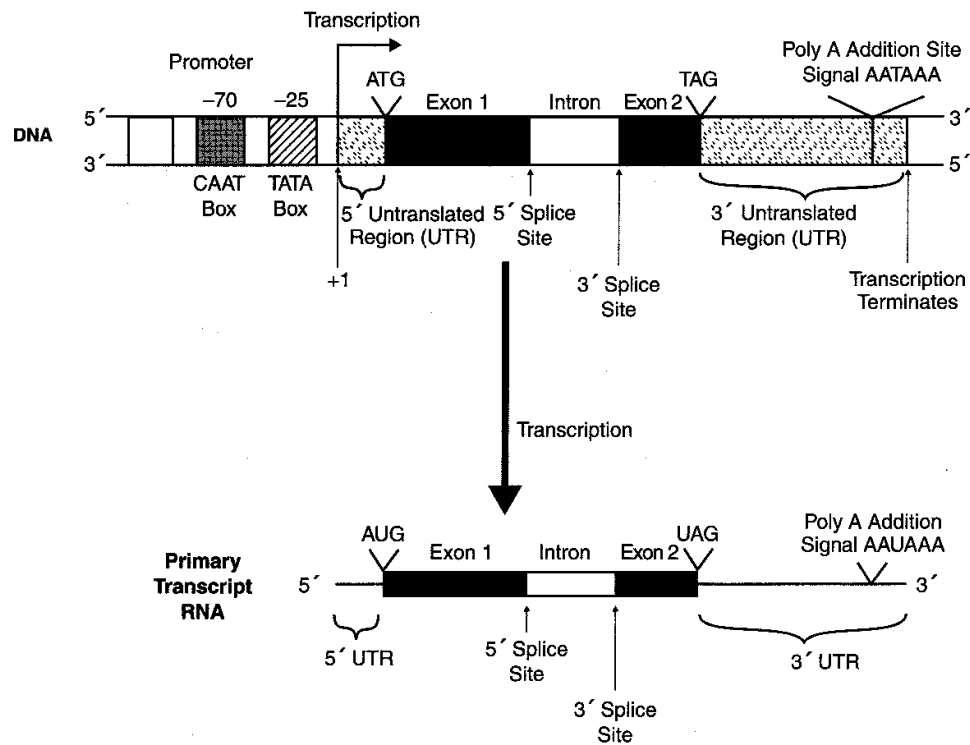


Figure I-3-6. A Eukaryotic Transcription Unit

Processing of Eukaryotic Messenger RNA

The primary transcript must undergo extensive posttranscriptional processing inside the nucleus to form the mature mRNA molecule (Figure I-3-7). These processing steps include the following:

1. A 7-methylguanosine cap is added to the 5' end while the RNA molecule is still being synthesized. The cap structure serves as a ribosome-binding site and also helps to protect the mRNA chain from degradation.
2. A poly-A tail is attached to the 3' end. In this process, an endonuclease cuts the molecule on the 3' side of the sequence AAUAAA (poly-A addition signal), then poly-A polymerase adds the poly-A tail (about 200 As) to the new 3' end. The poly-A tail protects the message against rapid degradation and aids in its transport to the cytoplasm. A few mRNAs (for example, histone mRNAs) have no poly-A tails.

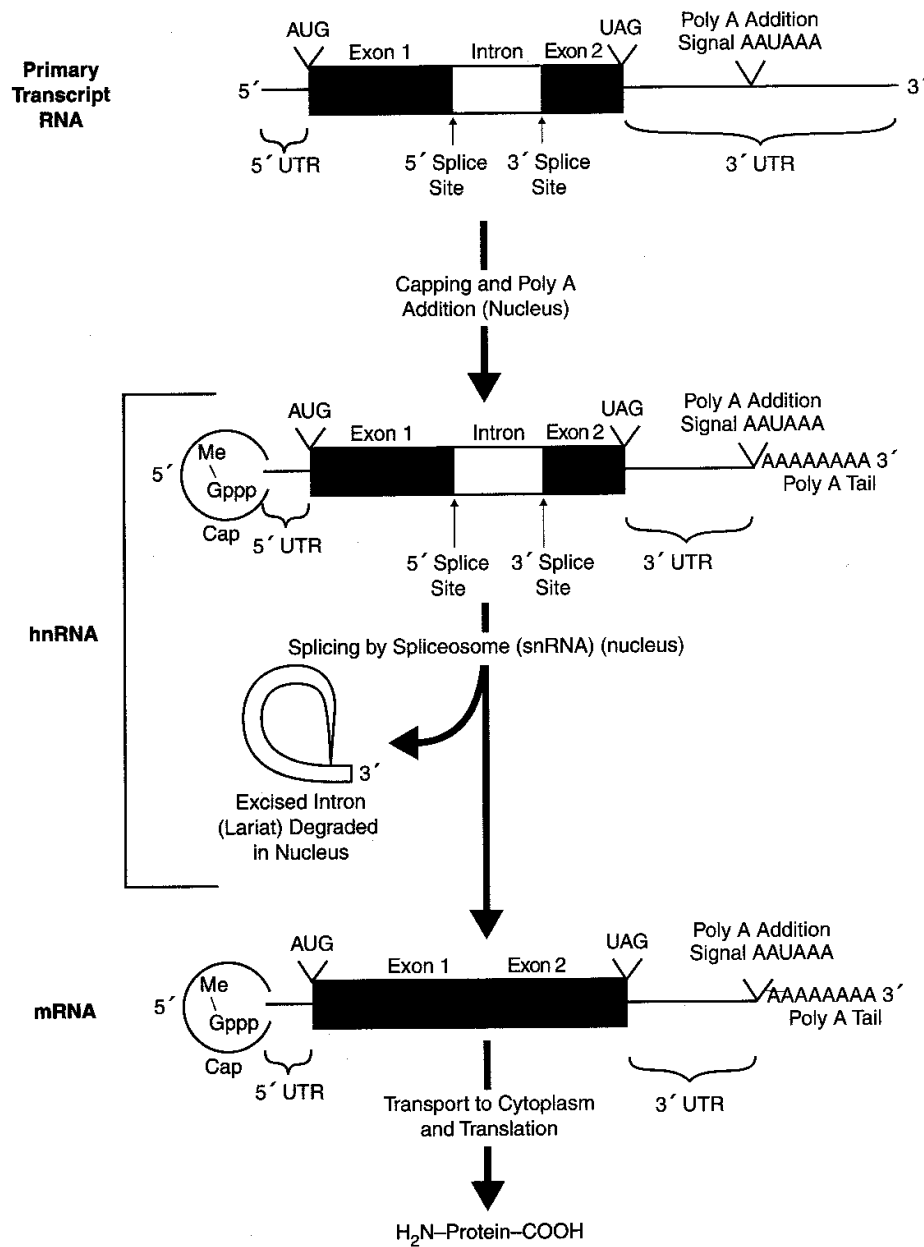


Figure I-3-7. Processing Eukaryotic mRNA

Note

Mutations in splice sites can lead to abnormal proteins. For example, mutations that interfere with proper splicing of β -globin mRNA are responsible for some cases of β -thalassemia.

3. Introns are removed from hnRNA by splicing, accomplished by spliceosomes (also known as an snRNP, or snurp), which are complexes of snRNA and protein. The hnRNA molecule is cut at splice sites at the 5' (donor) and 3' (acceptor) ends of the intron. The intron is excised in the form of a lariat structure and degraded. Neighboring exons are joined together to assemble the coding region of the mature mRNA.
4. All of the intermediates in this processing pathway are collectively known as hnRNA.
5. The mature mRNA molecule is transported to the cytoplasm, where it is translated to form a protein.

RELATIONSHIP BETWEEN EUKARYOTIC MESSENGER RNA AND GENOMIC DNA

Introns in DNA can be visualized in an electron micrograph of DNA-mRNA hybrids (Figure I-3-8). When mRNA hybridizes (base pairs) to the template strand of DNA, the introns appear as unhybridized loops in the DNA. The poly-A tail on the mRNA is also unhybridized, because it results from a posttranscriptional modification and is not encoded in the DNA.

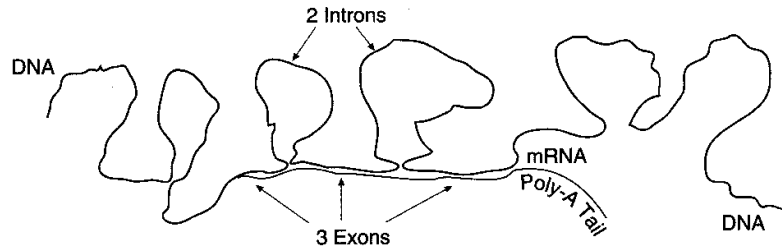


Figure I-3-8. A DNA-mRNA Hybrid

Alternative Splicing of Eukaryotic mRNA

For some genes, the primary transcript is spliced differently to produce two or more variants of a protein from the same gene. This process is known as alternative splicing and is illustrated in Figure I-3-9. Variants of the muscle proteins tropomyosin and troponin T are produced in this way. The synthesis of membrane-bound immunoglobulins by unstimulated B lymphocytes, as opposed to secreted immunoglobulins by antigen-stimulated B lymphocytes, also involves alternative splicing.

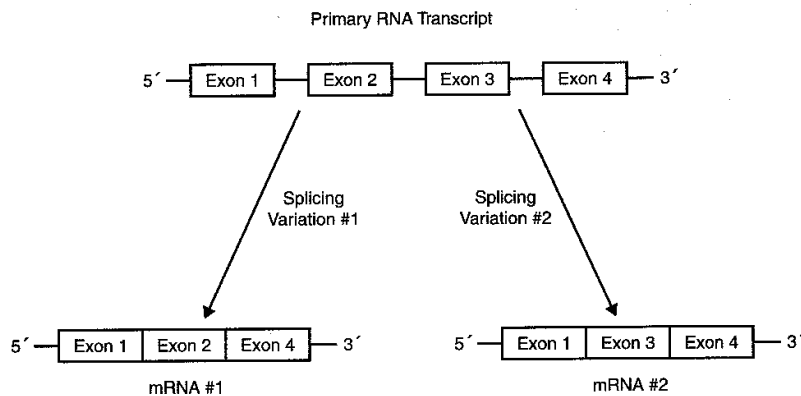


Figure I-3-9. Alternative Splicing of Eukaryotic mRNA

PRODUCTION OF OTHER CLASSES OF RNA

Genes encoding other classes of RNA are also expressed. The RNA products are not translated to produce proteins, but rather serve different roles in the process of translation.

RIBOSOMAL RNA (rRNA) IS USED TO CONSTRUCT RIBOSOMES

Figure I-3-10 shows the components of prokaryotic and eukaryotic ribosomes.

Eukaryotic ribosomal RNA is transcribed in the nucleolus by RNA polymerase I as a single piece of 45S RNA, which is subsequently cleaved to yield 28S rRNA, 18S rRNA, and 5.8S rRNA. RNA polymerase III transcribes the 5S rRNA unit from a separate gene. The ribosomal subunits assemble in the nucleolus as the rRNA pieces combine with ribosomal proteins. Eukaryotic ribosomal subunits are 60S and 40S. They join during protein synthesis to form the whole 80S ribosome.

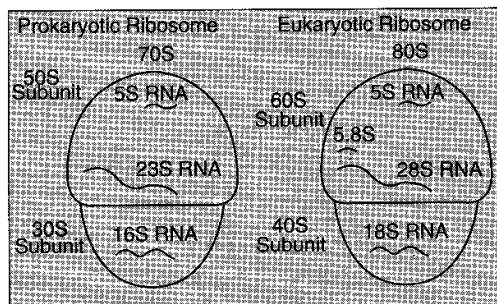


Figure I-3-10. The Composition of Prokaryotic and Eukaryotic Ribosomes

The large and small prokaryotic ribosomal subunits are 50S and 30S, respectively. The complete prokaryotic ribosome is a 70S particle. (Note: The S values are determined by behavior of the particles in an ultracentrifuge. They are a function of both size and shape, and therefore the numbers are not additive.)

TRANSFER RNA (tRNA) CARRIES ACTIVATED AMINO ACIDS FOR TRANSLATION

There are many different specific tRNAs. Each tRNA carries only one type of activated amino acid for making proteins during translation. The genes encoding these tRNAs in eukaryotic cells are transcribed by RNA polymerase III. The tRNAs enter the cytoplasm where they combine with their appropriate amino acids (see Chapter 4, Amino Acid Activation). Although all tRNAs have the same general shape shown in Figure I-3-11, small structural features distinguish among them.

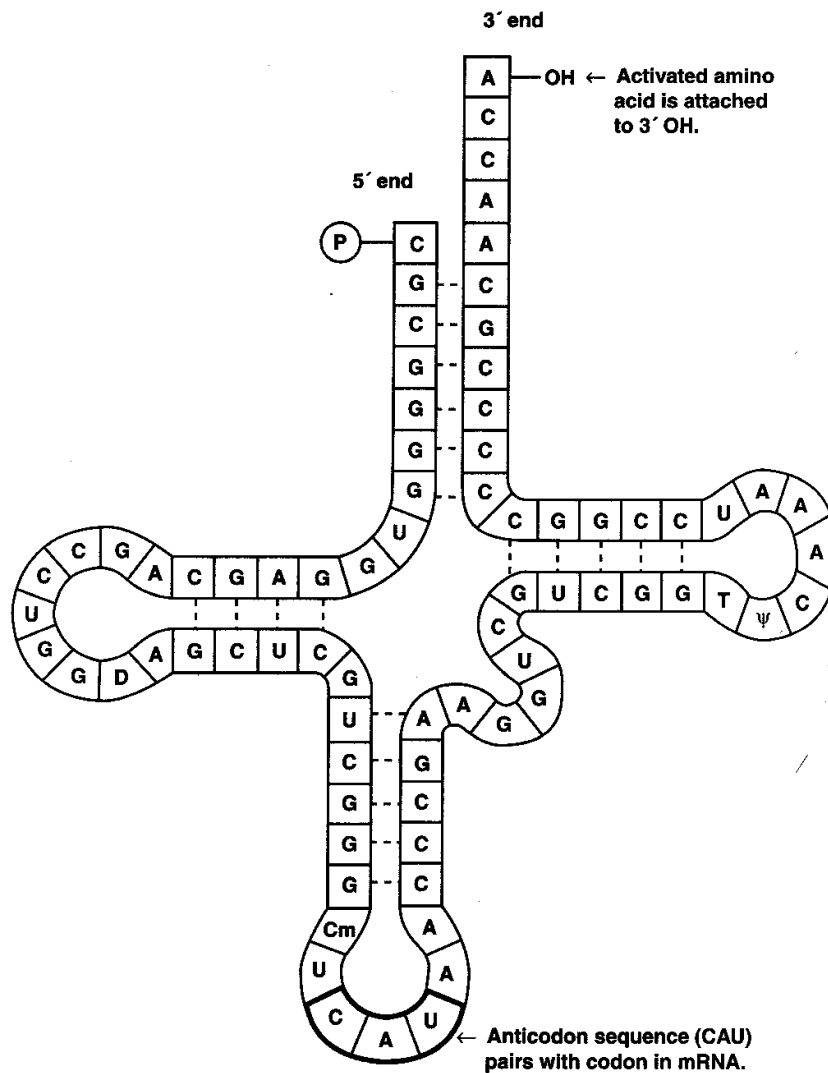


Figure I-3-11. Transfer RNA (tRNA)

Table I-3-2. Summary of Important Points About Transcription and RNA Processing

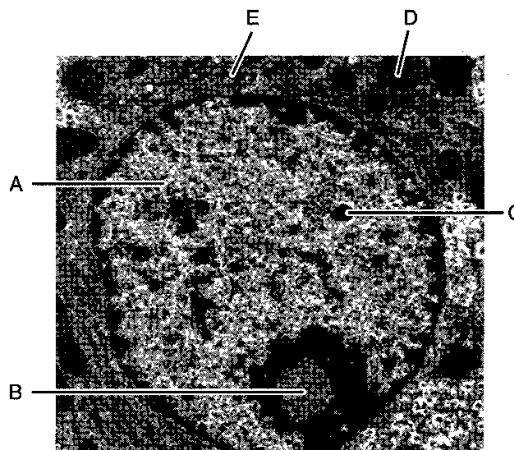
	Prokaryotic	Eukaryotic
Gene regions	May be polycistronic Genes are continuous coding regions Very little spacer (noncoding) DNA between genes	Always monocistronic Genes have exons and introns Large spacer (noncoding) DNA between genes
RNA polymerase	Core enzyme: $\alpha_2\beta\beta'$	RNA polymerase I: rRNA RNA polymerase II: mRNA; snRNA RNA polymerase III: tRNA, 5S RNA
Initiation of transcription	Promoter (–10) TATAAT and (–35) sequence Sigma initiation sub-unit required to recognize promoter	Promoter (–25) TATA and (–70) CAAT Transcription factors (TFIID) bind promoter
mRNA synthesis	Template read 3' to 5'; mRNA synthesized 5' to 3'; Gene sequence specified from coding strand 5' to 3'; Transcription begins at +1 base	
Termination of transcription	Stem and loop + UUUUU Stem and loop + rho factor	Not well characterized
Relationship of RNA transcript to DNA	RNA is antiparallel and complementary to DNA template strand; RNA is identical (except U substitutes for T) to DNA coding strand	
Posttranscriptional processing of hnRNA (pre-mRNA)	None	In nucleus: 5' cap (7-MeG) 3' tail (poly-A sequence) Removal of introns from hnRNA • Alternative splicing yields variants of protein product
Ribosomes	70S (30S and 50S) rRNA and protein	80S (40S and 60S) rRNA and protein
tRNA	Cloverleaf secondary structure • Acceptor arm (CCA) carries amino acid • Anticodon arm; anticodon complementary and antiparallel to codon in mRNA	

Review Questions

Select the ONE best answer.

- The base sequence of codons 57-58 in the cytochrome β_5 reductase gene is CAGCGC. The mRNA produced upon transcription of this gene will contain the sequence:
 - GCGCTG
 - CUGCGC
 - GCGCUG
 - CAGCGC
 - GUCGCG
- A gene encodes a protein with 150 amino acids. There is one intron of 1000 bps, a 5'-untranslated region of 100 bp and a 3'-untranslated region of 200 bp. In the final processed mRNA, how many bases lie between the start AUG codon and the final termination codon?
 - 1750
 - 750
 - 650
 - 450
 - 150

Items 3–5



- Transcription of Class 1 genes by RNA polymerase 1
- Euchromatin
- Polyadenylation of pre-mRNA by poly-A polymerase

Answers

1. **Answer: D.** Since the sequence in the stem represents the coding strand, the mRNA sequence must be identical (except U for T). No T in the DNA means no U in the mRNA.
2. **Answer: D.** Only the coding region remains to be calculated $3 \times 150 = 450$.
3. **Answer: B.** rRNA genes are transcribed by this enzyme in the nucleolus.
4. **Answer: A.** Less condensed chromatin, lighter areas in the nucleus. Darker areas are heterochromatin.
5. **Answer: A.** Polyadenylation of pre-mRNA occurs in the nucleoplasm. Generally associated with active gene expression in euchromatin.

The Genetic Code, Mutations, and Translation

4

OVERVIEW OF TRANSLATION

The second stage in gene expression is translating the nucleotide sequence of a messenger RNA molecule into the amino acid sequence of a protein. The genetic code is defined as the relationship between the sequence of nucleotides in DNA (or its RNA transcripts) and the sequence of amino acids in a protein. Each amino acid is specified by one or more nucleotide triplets (codons) in the DNA. During translation, mRNA acts as a working copy of the gene in which the codons for each amino acid in the protein have been transcribed from DNA to mRNA. tRNAs serve as adapter molecules that couple the codons in mRNA with the amino acids they each specify, thus aligning them in the appropriate sequence before peptide bond formation. Translation takes place on ribosomes, complexes of protein and rRNA that serve as the molecular machines coordinating the interactions between mRNA, tRNA, the enzymes, and the protein factors required for protein synthesis. Many proteins undergo posttranslational modifications as they prepare to assume their ultimate roles in the cell.

THE GENETIC CODE

Most genetic code tables designate the codons for amino acids as mRNA sequences (Figure I-4-1). Important features of the genetic code include:

- Each codon consists of three bases (triplet). There are 64 codons. They are all written in the 5' to 3' direction.
- 61 codons code for amino acids. The other three (UAA, UGA, UAG) are stop codons (or nonsense codons) that terminate translation.
- There is one start codon (initiation codon), AUG, coding for methionine. Protein synthesis begins with methionine (Met) in eukaryotes, and formylmethionine (fmet) in prokaryotes.
- The code is unambiguous. Each codon specifies no more than one amino acid.
- The code is degenerate. More than one codon can specify a single amino acid. All amino acids, except Met and tryptophan (Trp), have more than one codon.
- For those amino acids having more than one codon, the first two bases in the codon are usually the same. The base in the third position often varies.
- The code is universal (the same in all organisms). Some minor exceptions to this occur in mitochondria.
- The code is commaless (contiguous). There are no spacers or "commas" between codons on an mRNA.
- Neighboring codons on a message are nonoverlapping.

5' ← 1 2 3 → 3'

AUG

First Position (5' End)	Second Position				Third Position (3' End)
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Figure I-4-1. The Genetic Code

MUTATIONS

A mutation is any permanent, heritable change in the DNA base sequence of an organism. This altered DNA sequence can be reflected by changes in the base sequence of mRNA, and, sometimes, by changes in the amino acid sequence of a protein. Mutations can cause genetic diseases. They can also cause changes in enzyme activity, nutritional requirements, antibiotic susceptibility, morphology, antigenicity, and many other properties of cells.

A very common type of mutation is a single base alteration or point mutation.

- A transition is a point mutation that replaces a purine-pyrimidine base pair with a different purine-pyrimidine base pair. For example, an A-T base pair becomes a G-C base pair.
- A transversion is a point mutation that replaces a purine-pyrimidine base pair with a pyrimidine-purine base pair. For example, an A-T base pair becomes a T-A or a C-G base pair.

Mutations are often classified according to the effect they have on the structure of the gene's protein product. This change in protein structure can be predicted using the genetic code table in conjunction with the base sequence of DNA or mRNA. A variety of such mutations is listed in Table I-4-1. Point mutations and frameshifts are illustrated in more detail in Figure I-4-2.

Table I-4-1. Effect of Some Common Types of Mutations on Protein Structure

Type of Mutation	Effect on Protein
Silent: new codon specifies same amino acid	None
Missense: new codon specifies different amino acid	Possible decrease in function; variable effects
Nonsense: new codon is stop codon	Shorter than normal; usually nonfunctional
Frameshift: deletion or addition of a base	Usually nonfunctional; often shorter than normal
Large segment deletion (unequal crossover in meiosis)	Loss of function; shorter than normal or entirely missing
Splice donor or acceptor	Variable effects ranging from addition or deletion of a few amino acids to deletion of an entire exon
Triplet repeat expansion	Expansions in coding regions cause protein product to be longer than normal and unstable. Disease often shows anticipation in pedigree.

Normal	ATGGCAATTCGTTTTTACCTATAGGG... DNA coding strand	Met Ala Ile Arg Phe Leu Pro Ile Gly Amino Acid
Silent Mutation	ATGGCAATTCGTTTTTGCCTATAGGG... DNA coding strand	Met Ala Ile Arg Phe Leu Pro Ile Gly Amino Acid
Missense Mutation	ATGGCAATTCGTTTTTCACCTATAGGG... DNA coding strand	Met Ala Ile Arg Phe Ser Pro Ile Gly Amino Acid
Nonsense Mutation	ATGGCAATTCGTTTTTGACCTATAGGG... DNA coding strand	Met Ala Ile Arg Phe Stop Amino Acid
Frameshift Mutation (1bp deletion)	ATGGCAATTCGTTTTTACCTATAGGG... DNA coding strand	Met Ala Ile Arg Phe Tyr Leu Stop Amino Acid

Figure I-4-2. Some Common Types of Mutations in DNA

Large Segment Deletions

Large segments of DNA can be deleted from a chromosome during an unequal crossover in meiosis. Crossover or recombination between homologous chromosomes is a normal part of meiosis I that generates genetic diversity in reproductive cells (egg and sperm), a largely beneficial result. In a normal crossover event, the homologous maternal and paternal chromosomes exchange equivalent segments, and although the resultant chromosomes are mosaics of maternal and paternal alleles, no genetic information has been lost from either one. On rare occasions, a crossover can be unequal, and one of the two homologs loses some of its genetic information. Both normal and unequal crossing over are shown in Figure I-4-3.

α -Thalassemia is a well-known example of a genetic disease in which unequal crossover has deleted one or more α -globin genes from chromosome 16. Cri-du-chat (mental retardation, microcephaly, wide-set eyes, and a characteristic kittenlike cry) results from a terminal deletion of the short arm of chromosome 5 (see Section II, Chapter 3; Deletions).

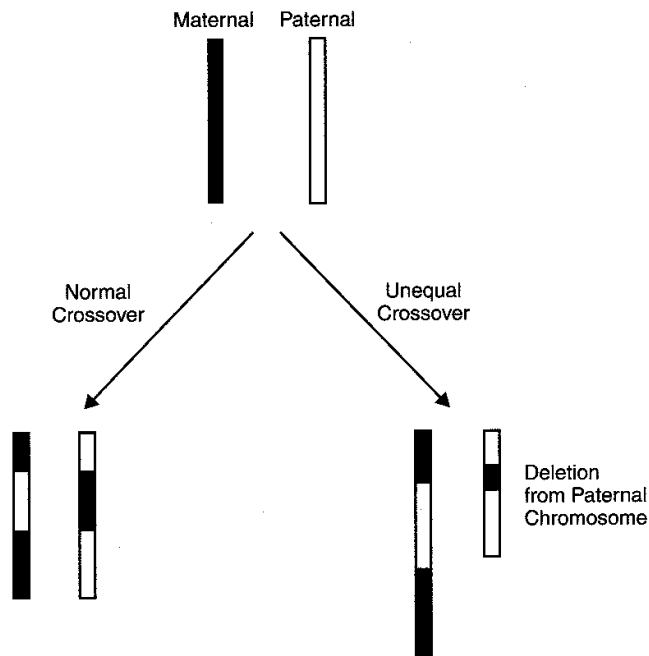


Figure I-4-3. Large Segment Deletion During Crossing-Over in Meiosis

Mutations in Splice Sites

Mutations in splice sites affect the accuracy of intron removal from hnRNA during posttranscriptional processing. As illustrated in Figure I-4-4, if a splice site is lost through mutation, spliceosomes may:

- Delete nucleotides from the adjacent exon.
- Leave nucleotides of the intron in the processed mRNA.
- Use the next normal upstream or downstream splice site, deleting an exon from the processed mRNA.

Mutations in splice sites have now been documented in many different diseases including β -thalassemia, Gaucher disease, and Tay-Sachs.

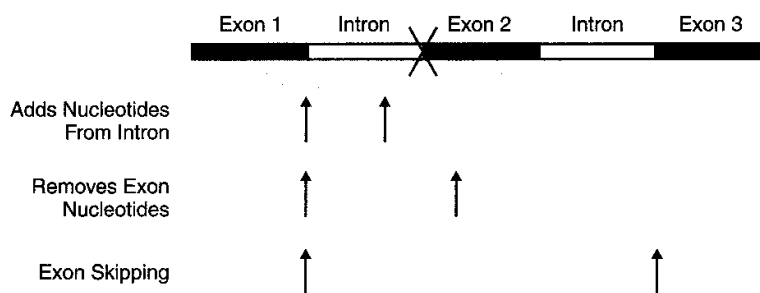


Figure I-4-4. Inaccurate Splicing After Mutation in a Splice Site

Clinical Correlate

Huntington's disease, an autosomal dominant disorder, has a mean age-of-onset of 43–48 years. Symptoms appear gradually and worsen over a period of about 15 years until death occurs. Mood disturbance, impaired memory, and hyperreflexia are often the first signs, followed by abnormal gait, chorea (loss of motor control), dystonia, dementia, and dysphagia. Cases of juvenile onset (<10 years old) are more severe and most frequently occur when the defective allele is inherited paternally. About 25% of cases have late onset, slower progression, and milder symptoms.

Trinucleotide Repeat Expansion

The mutant alleles in certain diseases, such as Huntington disease, fragile X syndrome, and myotonic dystrophy, differ from their normal counterparts only in the number of tandem copies of a trinucleotide. The expansion of the trinucleotide repeat in the mutant allele can be in a coding region (Huntington and spinobulbar muscular atrophy) or in an untranslated region of the gene (fragile X and myotonic dystrophy). In these diseases, the number of repeats often increases with successive generations and correlates with increasing severity and decreasing age of onset, a phenomenon called anticipation. In the normal Huntington allele, there are five tandem repeats of CAG in the coding region. Affected family members may have 30 to 60 of these CAG repeats. The normal protein contains five adjacent glutamine residues, whereas the proteins encoded by the disease-associated alleles have 30 or more adjacent glutamines. The long glutamine tract makes the abnormal proteins extremely unstable.

AMINO ACID ACTIVATION AND CODON TRANSLATION BY tRNAs

Inasmuch as amino acids have no direct affinity for mRNA, an adapter molecule, which recognizes an amino acid on one end and its corresponding codon on the other, is required for translation. This adapter molecule is tRNA.

Amino Acid Activation

As tRNAs enter the cytoplasm, each combines with its cognate amino acid in a two-step process called amino acid activation (Figure I-4-5).

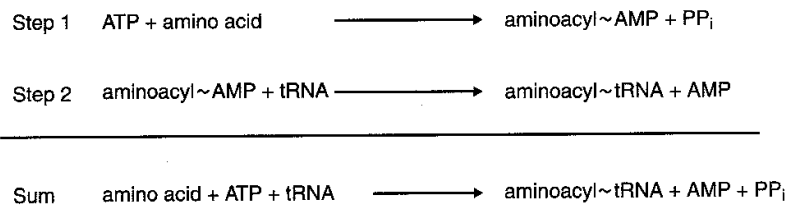


Figure I-4-5. Formation of Aminoacyl tRNA

- Each type of amino acid is activated by a different aminoacyl tRNA synthetase.
- Two high-energy bonds from an ATP are required.
- The aminoacyl tRNA synthetase transfers the activated amino acid to the 3' end of the correct tRNA.
- The amino acid is linked to its cognate tRNA with an energy-rich bond.
- This bond will later supply energy to make a peptide bond linking the amino acid into a protein.

Aminoacyl tRNA synthetases have self-checking functions to prevent incorrectly paired aminoacyl tRNAs from forming. If, however, an aminoacyl tRNA synthetase does release an incorrectly paired product (ala-tRNA^{ser}), there is no mechanism during translation to detect the error, and an incorrect amino acid will be introduced into some protein.

Codon Translation by Aminoacyl tRNAs

Each tRNA has an anticodon sequence that allows it to pair with the codon for its cognate amino acid in the mRNA. Because base pairing is involved, the orientation of this interaction will be complementary and antiparallel as shown in Figure I-4-6. The arg-tRNA^{arg} has an anticodon sequence, UCG, allowing it to pair with the arginine codon CGA.

- The anticodon sequence in tRNA is antiparallel and complementary to the codon translated in mRNA.

Wobble

Many amino acids are specified by more than one codon (redundancy). Frequently, a tRNA can translate more than one of these codons, sparing the cell from making multiple tRNAs to carry the same amino acid. For instance, in Figure I-4-6 the arg-tRNA^{arg} shown can translate both the CGA and the CGG codons that specify arginine. This phenomenon is known as "Wobble" and can be summarized as follows:

- Correct base pairing is required at the first position of the codon (third of anticodon) and the second position of the codon (second of anticodon).
- The third position of the codon does not always need to be paired with the anticodon (e.g., it is allowed to "wobble" in some cases).

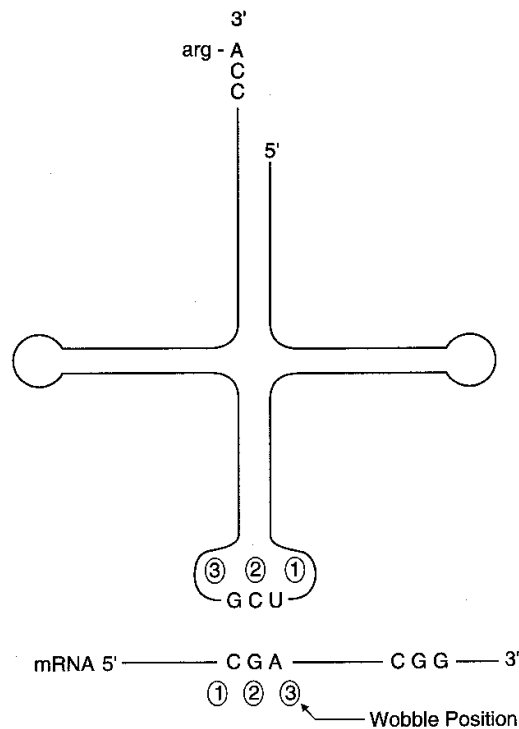


Figure I-4-6. Wobble and Protein Synthesis

TRANSLATION (PROTEIN SYNTHESIS)

Protein synthesis occurs by peptide bond formation between successive amino acids whose order is specified by a gene and thus by an mRNA. The formation of a peptide bond between the carboxyl group on one amino acid and the amino group of another is illustrated in Figure I-4-7.

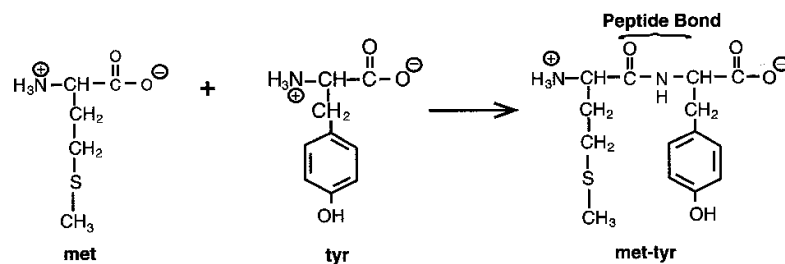


Figure I-4-7. Peptide Bond Formation

During translation, the amino acids are attached to the 3' ends of their respective tRNAs. The aminoacyl-tRNAs are situated in the P and A sites of the ribosome as shown in Figure I-4-8. Notice that the peptide bond forms between the carboxyl group of the amino acid (or growing peptide) in the P site and the amino group of the next amino acid in the A site. Proteins are synthesized from the amino to the carboxyl terminus.

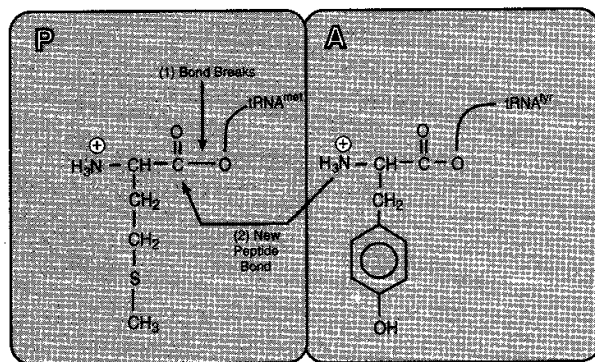


Figure I-4-8. Formation of a Peptide Bond by a Ribosome During Translation

Steps of Translation

Translation occurs in the cytoplasm of both prokaryotic (Pr) and eukaryotic (Eu) cells. In prokaryotes, ribosomes can begin translating the mRNA even before RNA polymerase completes transcription. In eukaryotes, translation and transcription are completely separated in time and space with transcription in the nucleus and translation in the cytoplasm. The process of protein synthesis occurs in three stages: initiation, elongation, and termination (Figure I-4-9). Special protein factors for initiation (IF), elongation (EF), and termination (release factors), as well as GTP, are required for each of these stages.

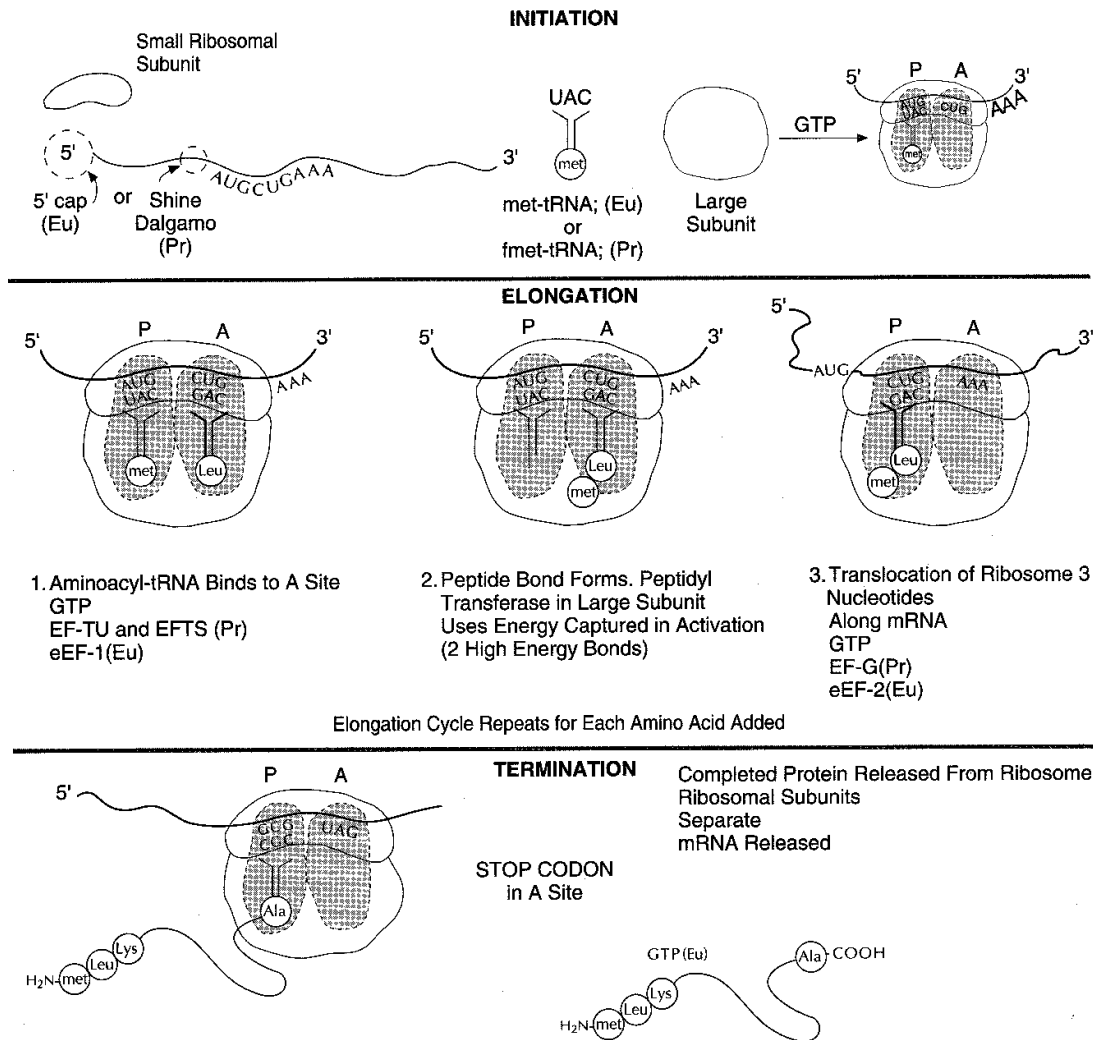


Figure I-4-9. Steps in Translation

Initiation

The small ribosomal subunit binds to the mRNA. In prokaryotes, the 16S rRNA of the small subunit binds to the Shine-Dalgarno sequence in the 5' untranslated region of the mRNA. In eukaryotes, the small subunit binds to the 5' cap structure and slides down the message to the first AUG.

The charged initiator tRNA becomes bound to the AUG start codon on the message through base pairing with its anticodon. The initiator tRNA in prokaryotes carries fmet, whereas the initiator tRNA in eukaryotes carries Met.

The large subunit binds to the small subunit, forming the completed initiation complex.

There are two important binding sites on the ribosome called the P site and the A site.

- The peptidyl site (P site) is the site on the ribosome where (f)met-tRNA_i initially binds. After formation of the first peptide bond, the P site is a binding site for the growing peptide chain.
- The aminoacyl site (A site) binds each new incoming tRNA molecule carrying an activated amino acid.

Elongation

Elongation is a three-step cycle that is repeated for each amino acid added to the protein after the initiator methionine. Each cycle uses four high-energy bonds (two from the ATP used in amino acid activation to charge the tRNA, and two from GTP). During elongation, the ribosome moves in the 5' to 3' direction along the mRNA, synthesizing the protein from amino to carboxyl terminus. The three steps are:

- A charged tRNA binds in the A site. The particular aminoacyl-tRNA is determined by the mRNA codon aligned with the A site.
- Peptidyl transferase, an enzyme that is part of the large subunit, forms the peptide bond between the new amino acid and the carboxyl end of the growing polypeptide chain. The bond linking the growing peptide to the tRNA in the P site is broken, and the growing peptide attaches to the tRNA located in the A site.
- In the translocation step, the ribosome moves exactly three nucleotides (one codon) along the message. This moves the growing peptidyl-tRNA into the P site and aligns the next codon to be translated with the empty A site.

In eukaryotic cells, elongation factor-2 (eEF-2) used in translocation is inactivated through ADP-ribosylation by *Pseudomonas* and *Diphtheria* toxins.

Termination

When any of the three stop (termination or nonsense) codons moves into the A site, peptidyl transferase (with the help of release factor) hydrolyzes the completed protein from the final tRNA in the P site. The mRNA, ribosome, tRNA, and factors can all be reused for additional protein synthesis.

POLYSOMES

Messenger RNA molecules are very long compared with the size of a ribosome, allowing room for several ribosomes to translate a message at the same time. Such a polyribosome (or polysome) is depicted in Figure I-4-10. Because ribosomes translate mRNA in the 5' to 3' direction, the ribosome closest to the 3' end has the longest nascent peptide. Polysomes are found free in the cytoplasm or attached to the rough endoplasmic reticulum (RER), depending on the protein being translated.

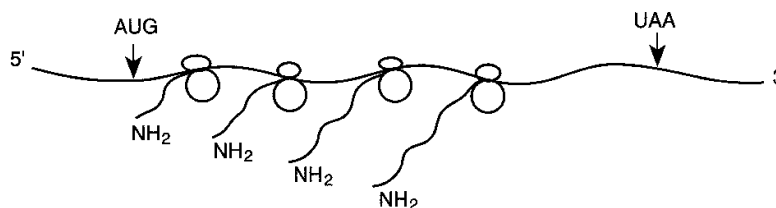


Figure I-4-10. A Polyribosome

INHIBITORS OF PROTEIN SYNTHESIS

Some well-known inhibitors of prokaryotic translation include streptomycin, erythromycin, tetracycline, and chloramphenicol. Inhibitors of eukaryotic translation include cycloheximide and *Diphtheria* and *Pseudomonas* toxins.

Puromycin inhibits both prokaryotic and eukaryotic translation by binding to the A site. Peptidyl transferase attaches the peptide to puromycin, and the peptide with puromycin attached at the C-terminus is released, prematurely terminating chain growth.

Certain antibiotics (for example, chloramphenicol) inhibit mitochondrial protein synthesis, but not cytoplasmic protein synthesis, because mitochondrial ribosomes are similar to prokaryotic ribosomes.

Clinical Correlate

Cystic Fibrosis

The majority of cases of cystic fibrosis result from deletion of phenylalanine at position 508 ($\Delta F508$), which interferes with proper protein folding and the posttranslational processing of oligosaccharide side chains. The abnormal chloride channel protein (CFTR) is degraded by the cytosolic proteasome complex rather than being translocated to the cell membrane. Other functional defects in CFTR protein that reaches the cell membrane may also contribute to the pathogenesis of cystic fibrosis.

Also see Section II, Chapter 1; Autosomal Recessive Inheritance.

PROTEIN FOLDING AND SUBUNIT ASSEMBLY

As proteins emerge from ribosomes, they fold into three-dimensional conformations that are essential for their subsequent biologic activity. Generally, four levels of protein shape are distinguished:

Primary—sequence of amino acids specified in the gene.

Secondary—folding of the amino acid chain into an energetically stable structure.

Two common examples are the α -helix and the β -pleated sheet. These shapes are reinforced by hydrogen bonds. An individual protein may contain both types of secondary structures. Some proteins, like collagen, contain neither but have their own more characteristic secondary structures.

Tertiary—positioning of the secondary structures in relation to each other to generate higher-order three-dimensional shapes (the domains of the IgG molecule are examples). Tertiary structure also includes the shape of the protein as a whole (globular, fibrous). Tertiary structures are stabilized by weak bonds (hydrogen, hydrophobic, ionic) and, in some proteins, strong, covalent disulfide bonds. Agents such as heat or urea disrupt tertiary structure to denature proteins, causing loss of function.

Quaternary—in proteins such as hemoglobin that have multiple subunits, quaternary structure describes the interactions among subunits.

TRANSLATION OCCURS ON FREE RIBOSOMES AND ON THE ROUGH ENDOPLASMIC RETICULUM

Although all translation of eukaryotic nuclear genes begins on ribosomes free in the cytoplasm, the proteins being translated may belong in other locations. For example, certain proteins are translated on ribosomes associated with the rough endoplasmic reticulum (RER), including:

- Secreted proteins
- Proteins inserted into the cell membrane
- Lysosomal enzymes

Proteins translated on free cytoplasmic ribosomes include:

- Cytoplasmic proteins
- Mitochondrial proteins (encoded by nuclear genes)

Molecular Chaperones

Proteins translated on the RER generally fold and assemble into subunits in the ER before being transferred to the Golgi apparatus. Other proteins fold in the cytoplasm. Molecular chaperones (proteins such as calnexin and BiP) assist in this process of protein folding. Proteins that are misfolded are targeted for destruction by ubiquitin and digested in cytoplasmic protein-digesting complexes called proteasomes.

Mitochondrial proteins encoded by nuclear genes are translated by ribosomes free in the cytoplasm, then folded and transferred into the mitochondria by different molecular chaperones.

Many proteins require signals to ensure delivery to the appropriate organelles. Especially important among these signals are:

- The N-terminal hydrophobic signal sequence used to ensure translation on the RER.
- Phosphorylation of mannose residues important for directing an enzyme to a lysosome.

The targeting process for these proteins is illustrated in Figure I-4-11.

Note

Proteasomes

Proteasomes are large cytoplasmic complexes that have multiple protease activities capable of sequentially digesting damaged proteins. Many proteins are marked for digestion by addition of several molecules of ubiquitin (polyubiquitination). Proteasomes may also play a role in producing antigenic peptides for presentation by class I MHC molecules.

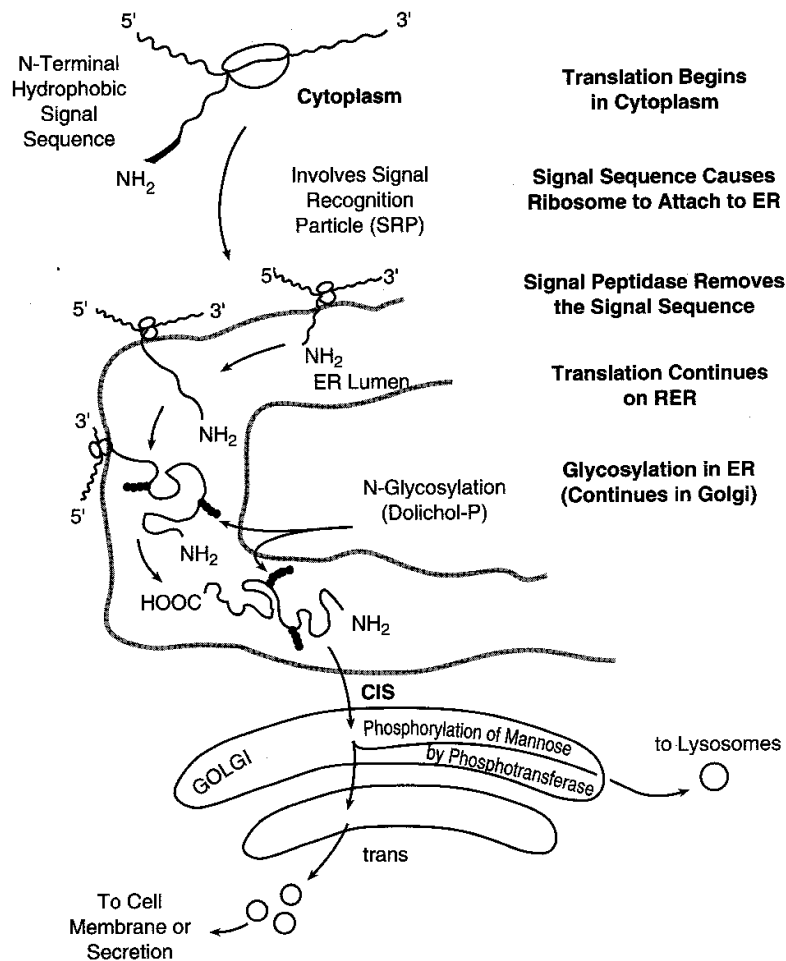


Figure I-4-11. Synthesis of Secretory, Membrane, and Lysosomal Proteins

Note

Lysosomes are organelles whose major function is to digest materials that the cell has ingested by endocytosis. Lysosomes contain multiple enzymes that, collectively, digest carbohydrates (glycosylases), lipids (lipases), and proteins (proteases). Although these organelles are especially prominent in cells such as neutrophils and macrophages, they serve this essential role in almost all cells. When a lysosomal enzyme is missing (for instance in a genetic disease like Tay-Sachs), the undigested substrate accumulates in the cell, often leading to serious consequences.

N-Terminal Hydrophobic Signal Sequence

This sequence is found on proteins destined to be secreted (insulin), placed in the cell membrane (Na^+/K^+ ATPase), or ultimately directed to the lysosome (sphingomyelinase). These proteins all require N-terminal hydrophobic signal sequences as part of their primary structure. Translation begins on free cytoplasmic ribosomes, but after translation of the signal sequence, the ribosome is positioned on the ER (now RER) with the help of a signal recognition particle. During translation, the nascent protein is fed through the membrane of the RER and captured in the lumen. The signal sequence is cleaved off in the ER, and then the protein passes into the Golgi for further modification and sorting.

In transit through the ER and Golgi, the proteins acquire oligosaccharide side chains attached commonly at serine or threonine residues (O-linked) or at asparagine residues (N-linked). N-linked glycosylation requires participation of a special lipid called dolichol phosphate.

Lysosomal Enzymes and Phosphorylation of Mannose

Lysosomal enzymes are glycosylated and modified in a characteristic way. Most importantly, when they arrive in the Golgi apparatus, specific mannose residues in their oligosaccharide chains are phosphorylated. This phosphorylation is the critical event that removes them from the secretion pathway and directs them to lysosomes. Genetic defects affecting this phosphorylation produce I-cell disease in which lysosomal enzymes are released into the extracellular space, and inclusion bodies accumulate in the cell, compromising its function.

Major Symptoms of I-Cell Disease

- Coarse facial features, gingival hyperplasia, macroglossia
- Craniofacial abnormalities, joint immobility, club-foot, claw-hand, scoliosis
- Psychomotor retardation, growth retardation
- Cardiorespiratory failure, death in first decade

CO- AND POSTTRANSLATIONAL COVALENT MODIFICATIONS

In addition to disulfide bond formation while proteins are folding, other covalent modifications include:

- Glycosylation: addition of oligosaccharide as proteins pass through the ER and Golgi apparatus
- Proteolysis: cleavage of peptide bonds to remodel proteins and activate them (proinsulin, trypsinogen, prothrombin)
- Phosphorylation: addition of phosphate by protein kinases
- γ -Carboxylation: produces Ca^{2+} binding sites
- Prenylation: addition of farnesyl or geranylgeranyl lipid groups to certain membrane-associated proteins

POSTTRANSLATIONAL MODIFICATIONS OF COLLAGEN

Collagen is an example of a protein that undergoes several important co- and posttranslational modifications. It has a somewhat unique primary structure in that much of its length is composed of a repeating tripeptide Gly-X-Y-Gly-X-Y-etc. Hydroxyproline is an amino acid unique to collagen. The hydroxyproline is produced by hydroxylation of prolyl residues at the Y positions in procollagen chains as they pass through the RER. Important points about collagen synthesis are summarized below and in Figure I-4-12.

1. Prepro- α chains containing a hydrophobic signal sequence are synthesized by ribosomes attached to the RER.
2. The hydrophobic signal sequence is removed by signal peptidase in the RER to form pro- α chains.
3. Selected prolines and lysines are hydroxylated by prolyl and lysyl hydroxylases. These enzymes, located in the RER, require ascorbate (vitamin C), deficiency of which produces scurvy.
4. Selected hydroxylysines are glycosylated.

5. Three pro- α chains assemble to form a triple helical structure (procollagen), which can now be transferred to the Golgi. Modification of oligosaccharide continues in the Golgi.
6. Procollagen is secreted from the cell.
7. The propeptides are cleaved from the ends of procollagen by proteases to form collagen molecules (also called tropocollagen).
8. Collagen molecules assemble into fibrils. Cross-linking involves lysyl oxidase, an enzyme that requires O_2 and copper.
9. Fibrils aggregate and cross-link to form collagen fibers.

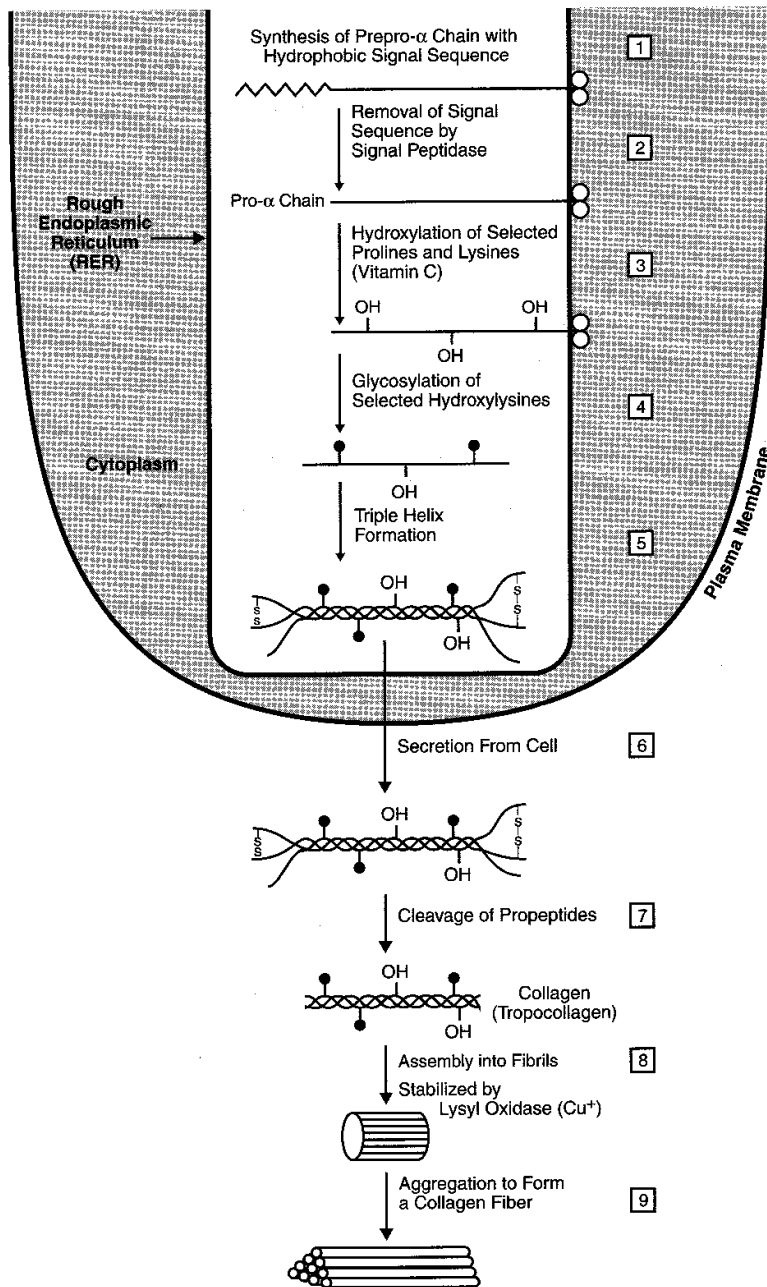


Figure I-4-12. Synthesis of Collagen

Clinical Correlate

Ehlers-Danlos (ED) Type IV represents a collection of defects in the normal synthesis and processing of collagen. Like osteogenesis imperfecta, these syndromes are a result of locus heterogeneity in which defects in several different genes (loci) can result in similar symptoms. ED Type IV, the vascular type, is an autosomal dominant disease caused by mutations in the gene for type-3 procollagen. Characteristic features include thin translucent skin, arterial, intestinal, or uterine rupture, and easy bruising.

Also see Section II, Chapter 1; Locus Heterogeneity.

Several important diseases associated with defective collagen production are shown in Table I-4-2.

Table I-4-2. Disorders of Collagen Biosynthesis

Disease	Defect	Major Symptoms
Scurvy	Deficient hydroxylation secondary to ascorbate deficiency	Petechiae, ecchymoses Loose teeth, bleeding gums Poor wound healing Poor bone development
Osteogenesis imperfecta	Mutations in collagen genes	Skeletal deformities Fractures, blue sclera
Ehlers-Danlos syndromes	Mutations in collagen genes and lysine hydroxylase gene	Hyperextensible, fragile skin Hypermobility joints, dislocations, varicose veins, ecchymoses, arterial, intestinal ruptures
Menkes disease	Deficient cross-linking secondary to functional copper deficiency	Depigmented (steely) hair Arterial tortuosity, rupture Cerebral degeneration Osteoporosis, anemia

Clinical Correlate

Menkes disease, an X-linked recessive condition, is caused by mutations in the gene encoding a Cu^{2+} efflux protein. Cells from an affected individual accumulate high concentrations of Cu^{2+} that cannot be released from the cell. The symptoms result from functional Cu^{2+} deficiency inasmuch as Cu^{2+} absorbed from the intestine becomes trapped in the intestinal epithelial cells and delivery to other tissues is inadequate.

Table I-4-3. Important Points About the Genetic Code, Mutations, and Translation

	Prokaryotic	Eukaryotic
Genetic code	Start: AUG (also codes for Met) Stop: UAG, UGA, UAA Unambiguous (1 codon = 1 amino acid) Redundant (1 amino acid >1 codon); often differ at base 3	
Mutations	Point mutations: silent, missense, nonsense Frameshift (delete 1 or 2 nucleotides; not multiple of 3) Large segment deletion	
		Mutation in splice site Trinucleotide repeat expansion
Amino acid activation	Aminoacyl-tRNA synthetase: two high-energy bonds (ATP) to link amino acid to tRNA	
Translation: Initiation	30S subunit binds to Shine-Dalgarno sequence on mRNA fMet-tRNA _i binds to P site GTP required	40S subunit associates with 5' cap on mRNA Met-tRNA _i binds to P site GTP required
Translation: Elongation	Charged aminoacyl-tRNA binds to A site (GTP) Peptide bond forms (two high-energy bonds from amino acid activation) Peptidyl synthase (50S subunit) Translocation: GTP required	Charged aminoacyl-tRNA binds to A site (GTP) Peptide bond forms (two high-energy bonds from amino acid activation) Peptidyl synthase (60S subunit) Translocation: GTP required eEF-2 inhibited by <i>Diphtheria</i> and <i>Pseudomonas</i> toxins
Termination	Release of protein; protein synthesized N to C	
Protein targeting		Secreted or membrane proteins: N-terminal hydrophobic signal sequence Lysosomal enzymes: phosphorylation of mannose by phosphotransferase in Golgi I-cell disease

Review Questions

Select the ONE best answer.

1. In the genetic code of human nuclear DNA, one of the codons specifying the amino acid tyrosine is UAC. Another codon specifying this same amino acid is:
 - A. AAC
 - B. UAG
 - C. UCC
 - D. AUG
 - E. UAU

Items 2 and 3

- A. ATGCAA...→ ATGTAA
- B. ATGAAA...→ **GT**GAAA
- C. TATAAG...→ TCTAAG
- D. CTTAAG...→ **GT**TAAG
- E. ATGAAT ...→ ATGCAT

The options above represent mutations in the DNA with base changes indicated in boldface type. For each mutation described in the questions below, choose the most closely related sequence change in the options above.

2. Nonsense mutation
3. Mutation decreasing the initiation of transcription
4. During β -globin synthesis in normal reticulocytes the sequence his-arg-pro occurs at position 165-167. How many high-energy phosphate bonds are required to insert these 3 amino acids into the β -globin polypeptide during translation?
 - A. 15
 - B. 12
 - C. 9
 - D. 6
 - E. 3
5. Accumulation of heme in reticulocytes can regulate globin synthesis by indirectly inactivating eIF-2. Which of the following steps is most directly affected by this mechanism?
 - A. Attachment of spliceosomes to pre-mRNA
 - B. Attachment of the ribosome to the endoplasmic reticulum
 - C. Met-tRNA^{Met} binding to the P-site
 - D. Translocation of mRNA on the ribosome
 - E. Attachment of RNA polymerase II to the promoter

6. A nasopharyngeal swab obtained from a 4-month-old infant with rhinitis and paroxysmal coughing tested positive upon culture for *Bordetella pertussis*. He was admitted to the hospital for therapy with an antibiotic that inhibits the translocation of peptidyl-tRNA on 70S ribosomes. This patient was most likely treated with
 - A. erythromycin
 - B. tetracycline
 - C. chloramphenicol
 - D. rifamycin
 - E. actinomycin D
7. A 25-month-old white girl has coarse facial features and gingival hyperplasia and at 2 months of age began developing multiple, progressive symptoms of mental retardation, joint contractures, hepatomegaly, and cardiomegaly. Levels of lysosomal enzymes are elevated in her serum, and fibroblasts show phase-dense inclusions in the cytoplasm. Which of the following enzyme deficiencies is most consistent with these observations?
 - A. Golgi-associated phosphotransferase
 - B. Lysosomal α -1,4-glucosidase
 - C. Endoplasmic reticulum-associated signal peptidase
 - D. Cytoplasmic α -1,4-phosphorylase
 - E. Lysosomal hexosaminidase A
8. Parahemophilia is an autosomal recessive bleeding disorder characterized by a reduced plasma concentration of the Factor V blood coagulation protein. Deficiency arises from a 12 base-pair deletion in the Factor V gene that impairs the secretion of Factor V by hepatocytes and results in an abnormal accumulation of immunoreactive Factor V antigen in the cytoplasm. In which region of the Factor V gene would this mutation most likely be located?
 - A. 5' untranslated region
 - B. First exon
 - C. Middle intron
 - D. Last exon
 - E. 3' untranslated region
9. Collagen, the most abundant protein in the human body, is present in varying amounts in many tissues. If one wished to compare the collagen content of several tissues, one could measure their content of
 - A. glycine
 - B. proline
 - C. hydroxyproline
 - D. cysteine
 - E. lysine

10. A 6-month-old infant is seen in the emergency room with a fractured rib and subdural hematoma. The child's hair is thin, colorless, and tangled. His serum copper level is 5.5 nM (normal for age, 11–12 nM). Developmental delay is prominent. A deficiency of which enzyme activity most closely relates to these symptoms?
 - A. Lysyl oxidase
 - B. Prolyl hydroxylase
 - C. γ -Glutamyl carboxylase
 - D. Phosphotransferase in Golgi
 - E. α -1, 4-glucosidase
11. Respiratory tract infections caused by *Pseudomonas aeruginosa* are associated with the secretion of exotoxin A by this organism. What effect will this toxin most likely have on eukaryotic cells?
 - A. Stimulation of nitric oxide (NO) synthesis
 - B. ADP-ribosylation of a Gs protein
 - C. ADP-ribosylation of eEF-2
 - D. ADP-ribosylation of a Gi protein
 - E. Stimulation of histamine release

Answers

1. **Answer: E.** Because of Wobble codons for the same amino acid often differ in the third base. Option B would be OK except that it is a stop codon.
2. **Answer: A.** The sequence now contains TAA which will be transcribed to UAA in the mRNA.
3. **Answer: C.** The transcription promoter TATA has been changed to TCTA. Don't choose the distractor B. The question is not about translation.
4. **Answer: B.** 4-P are required to add each amino acid ($3 \times 4 = 12$).
5. **Answer: C.** eIF-2 designates a protein factor of the initiation phase in eukaryotic translation. The only event listed that would occur during this phase is placement of initiator tRNA in the P-site.
6. **Answer: A.** Erythromycin is the antibiotic of choice for pertussis. It inhibits translocation.
7. **Answer: A.** Characteristic symptoms of I-cell disease. Note release of lysosomal enzymes into serum, which would not be seen in the other deficiencies.
8. **Answer: B.** Decreased Factor V secretion and a corresponding accumulation of cytoplasmic antigen suggest a defect in the translocation of the nascent protein to the endoplasmic reticulum. This implies a mutation in the N-terminal amino acid signal sequence required for targeting to the ER and encoded by the first exon of the gene.
9. **Answer: C.** Hydroxyproline is found uniquely in collagen. Although collagen is also rich in glycine, many other proteins contain significant amounts of glycine.

10. **Answer: A.** The child has Menkes disease, in which cellular copper transport is abnormal and produces a functional copper deficiency. Lysyl oxidase in collagen metabolism requires copper. His fragile bones and blood vessels result from weak, poorly crosslinked connective tissue.
11. **Answer: C.** *Pseudomonas* and diphtheria toxins inhibit eEF-2, the translocation factor in eukaryotic translation.

Genetic Regulation

5

OVERVIEW OF GENETIC REGULATION

Regulation of gene expression is an essential feature in maintaining the functional integrity of a cell. Increasing or decreasing the expression of a gene can occur through a variety of mechanisms, but many of the important ones involve regulating the rate of transcription. In addition to the basic transcription proteins, RNA polymerase, sigma (prokaryotes), and TFIID (eukaryotes), activator and repressor proteins help control the rate of the process. These regulatory proteins bind to specific DNA sequences associated with both prokaryotic and eukaryotic gene regions.

Other mechanisms are important, and, especially in eukaryotes, gene expression is controlled at multiple levels.

REGULATION OF PROKARYOTIC GENE EXPRESSION

Regulation of gene expression in prokaryotes usually involves either initiation or termination of transcription. In bacteria, genes are often organized into operons. An operon is a set of structural genes coding for a group of proteins required for a particular metabolic function along with the regulatory region(s) that controls the expression of the structural genes. The regulatory region is upstream (to the 5' side) from the structural genes and coordinates their regulation. An operon usually produces a polycistronic mRNA that carries the information for synthesis of all of the enzymes encoded by the structural genes.

Two examples of transcriptional control in prokaryotes are discussed:

- Regulation by activator and repressor proteins in the lactose operon
- Attenuation control in the histidine operon

The Lactose (Lac) Operon

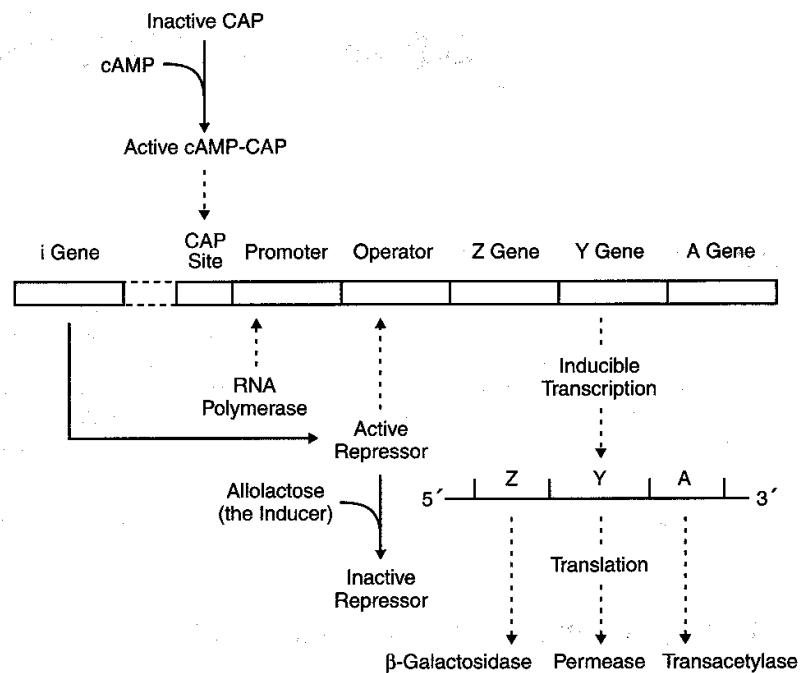


Figure I-5-1. The Lactose Operon

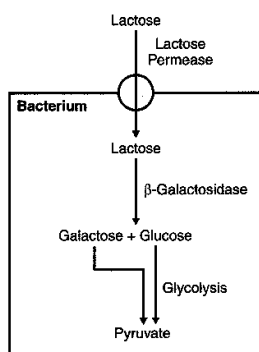


Figure I-5-2. Metabolism of Lactose

The lactose operon (Figure I-5-1) is a portion of the bacterial chromosome that controls the synthesis of three enzymes involved in the metabolism of the sugar lactose. Most bacteria carry out glycolysis, a pathway that allows glucose to be metabolized as a carbon and energy source. If glucose is unavailable, they can metabolize alternative carbohydrates, but require proteins in addition to those in glycolysis to do so. Lactose, a disaccharide of galactose and glucose, represents one alternative sugar, and the genes of the lactose operon encode the additional proteins required for its metabolism (Figure I-5-2). The cell expresses these genes only when lactose is available and glucose is not. The structural genes of the lactose operon include:

- The Z gene, which encodes a β -galactosidase (a prokaryotic lactase)
- The Y gene, which encodes a galactoside permease, the transport protein required for entry of lactose into the cell
- The A gene, which encodes a thiogalactoside transacetylase enzyme that is not essential for lactose metabolism and whose function is uncertain

In addition, the i gene, which encodes the lac repressor protein, is also considered part of the operon although it is located at a distant site in the DNA. The i gene is constitutively expressed (not regulated); thus, copies of the lac repressor protein are always in the cell.

Two gene regulatory proteins control the expression of the lac operon:

- The lac repressor (encoded by the i gene), which binds to a DNA sequence called the operator

- A cAMP-dependent activator protein, CAP, which binds to a DNA sequence called the CAP site

Glucose and lactose control the expression by different mechanisms:

- Lactose (or allolactose) induces gene expression by preventing the repressor protein binding to the operator sequence.
- Glucose represses gene expression by lowering the level of cAMP in the cell, thus preventing the cAMP-dependent activator binding to the CAP-site sequence.

Coordinate Control of the Lactose Operon by Glucose and Lactose

Full expression of the lactose operon requires that both mechanisms favor gene expression.

- The repressor protein must not bind at the operator, and
- The cAMP-dependent activator protein must bind to the CAP site.

This in turn requires that

- Lactose is present (prevents repressor binding)
- Glucose is low (to allow cAMP to increase)

Although intermediate levels of gene expression may be possible in the cell, it is convenient to simplify the situation in the following way:

- The only condition that allows high gene expression is: lactose present, glucose absent. All other combinations of these sugars result in low-level expression.

Attenuation in the Histidine Operon

The histidine operon encodes the enzymes of the histidine biosynthetic pathway. It is advantageous to the cell to produce these enzymes when histidine is not available in the surroundings, but to turn off their synthesis when histidine is readily available.

The histidine operon and several other operons for amino acid biosynthesis (e.g., tryptophan, leucine, and phenylalanine) are regulated by premature termination of transcription, a process known as attenuation. Attenuation depends on the fact that transcription and translation occur simultaneously in bacteria. In this process, transcription is constitutively initiated, and the 5' untranslated region (UTR) of the mRNA is followed by the coding region for a short (non-functional) peptide (the leader peptide). If histidine is available in the growth medium, transcription is terminated before RNA polymerase reaches the structural genes of the operon (Figure I-5-3). This form of regulation is dependent on the speed of the ribosome and the formation of two alternative secondary structures in the mRNA molecule.

In a Nutshell

Attenuation Control

- Prokaryotic cells only
- Requires simultaneous transcription and translation
- Two potential transcription termination signals

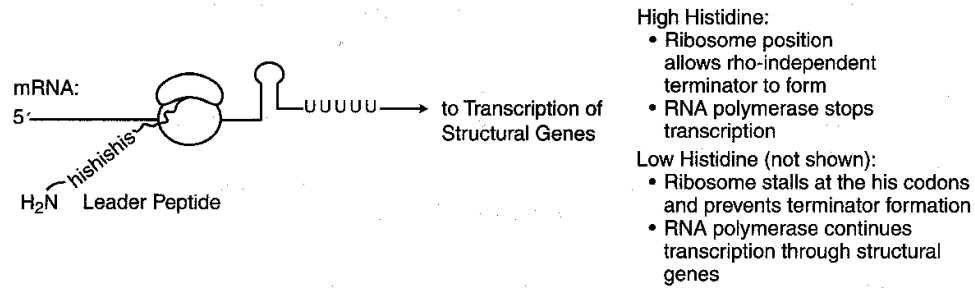


Figure I-5-3. Attenuation Control of Transcription in the Histidine Operon

At High Levels of Histidine

As soon as the Shine-Dalgarno sequence (Chapter 3) associated with the leader peptide coding region appears in the 5' UTR of the mRNA, a ribosome binds and begins translating the message. The ribosome can move quickly because it can easily find histidine to incorporate when it encounters histidine codons in the mRNA. This allows the message to fold into a rho-independent terminator of transcription (stem and loop + poly-U). RNA polymerase stops transcription before it reaches the structural genes, and no enzymes are produced.

At Low Levels of Histidine

A ribosome begins to synthesize the leader peptide, but stalls at the histidine codons because it cannot readily find histidine. Because the ribosome is covering up a different part of the mRNA, the message will not fold into the correct terminator structure, and RNA polymerase continues transcription through the structural genes of the operon. Translation of the message produces all the enzymes of the histidine biosynthetic pathway.

Attenuation is not used as a regulatory mechanism in eukaryotes, because transcription and translation are independent events and occur in different subcellular locations.

REGULATION OF EUKARYOTIC GENE EXPRESSION

In eukaryotic cells, DNA is packaged in chromatin structures, and gene expression typically requires activation to occur. Chromatin-modifying activities include:

- Histone acetylases (favor gene expression) and deacetylases (favor inactive chromatin)
- Scaffolding proteins that condense regions of the chromatin (favor inactive chromatin)
- DNA methylating enzymes (favor inactive chromatin)

Activator proteins (and a few repressors) are important in eukaryotes, as they are in prokaryotes. The DNA sequences to which activator proteins bind in eukaryotic DNA are called response elements. A few response elements are located within the promoter region (upstream promoter elements [UPE]), but most are outside the promoter and often clustered to form an enhancer region that allows control of gene expression by multiple signals (Figure I-5-4).

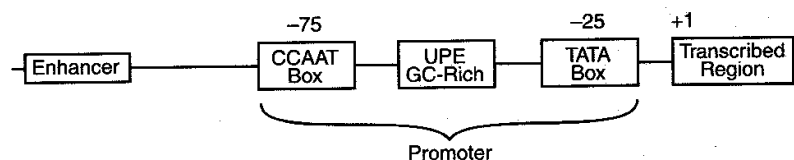


Figure I-5-4. Enhancers and Upstream Promoter Elements

Upstream Promoter Elements

Only the proximity of the upstream promoter element to the -25 sequence distinguishes it from an enhancer. Upstream promoter elements include:

- A CCAAT box (around -75) that binds a transcription factor NF-1
- A GC-rich sequence that binds a general transcription factor SP-1

Enhancers

Enhancers in the DNA are binding sites for activator proteins. Enhancers have the following characteristics:

- They may be up to 1000 base pairs away from the gene.
- They may be located upstream, downstream, or within an intron of the gene they control.
- The orientation of the enhancer sequence with respect to the gene is not important.
- Enhancers can appear to act in a tissue-specific manner if the DNA-binding proteins that interact with them are present only in certain tissues.
- Enhancers may be brought close to the basal promoter region in space by bending of the DNA molecule (Figure I-5-5).

Similar sequences that bind repressor proteins in eukaryotes are called silencers. There are fewer examples of these sequences known, and the mechanisms through which they act are not clear.

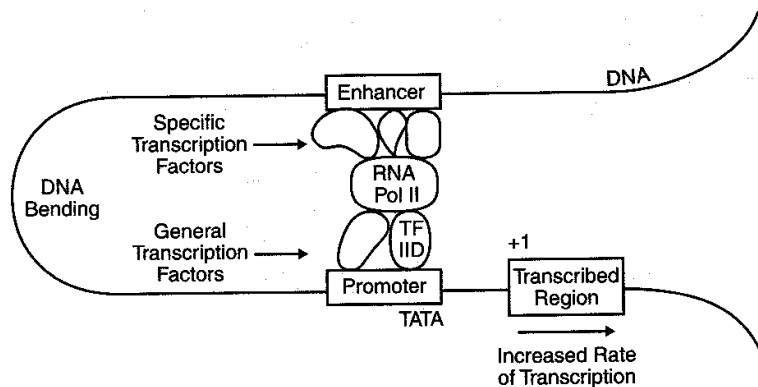


Figure I-5-5. Stimulation of Transcription by an Enhancer and Its Associated Transcription Factors

Note

The Ig heavy chain locus has an enhancer in the large intron separating the coding regions for the variable domain from the coding regions for the constant domains.

Note

Cis and *Trans* Regulatory Elements

The DNA regulatory base sequences (e.g., promoters, enhancers, response elements, and UPEs) in the vicinity of genes that serve as binding sites for proteins are often called "*cis*" regulators. Transcription factors (and the genes that code for them) are called "*trans*" regulators. *Trans* regulatory proteins can diffuse through the cell to their point of action.

Transcription Factors

The activator proteins that bind response elements are often referred to as transcription factors. Typically, transcription factors contain at least two recognizable domains, a DNA-binding domain and an activation domain.

1. The DNA-binding domain binds to a specific nucleotide sequence in the promoter or response element. Several types of DNA-binding domain motifs have been characterized and have been used to define certain families of transcription factors. Some common DNA-binding domains include:
 - Zinc fingers (steroid hormone receptors)
 - Leucine zippers (cAMP-dependent transcription factor)
 - Helix-loop-helix
 - Helix-turn-helix (homeodomain proteins encoded by homeotic/homeobox genes)
2. The activation domain allows the transcription factor to:
 - Bind to other transcription factors
 - Interact with RNA polymerase II to stabilize the formation of the initiation complex
 - Recruit chromatin-modifying proteins such as histone acetylases or deacetylases

Two types can be distinguished, general transcription factors and specific transcription factors. Examples are listed in Table I-5-1.

Table I-5-1. Properties of Some Common Transcription Factors

Transcription Factor (DNA-Binding Protein)	Response Element (Binding Site)	Function	Protein Class
SP-1	GC-rich box	Basal transcription	Zinc finger
NF-1	CCAAT box	Basal transcription	—
Steroid receptors	HRE	Steroid response	Zinc finger
cAMP response element binding (CREB) protein	CRE	Response to cAMP	Leucine zipper
Peroxisome proliferator-activated receptors (PPARs)	PPREs	Regulate multiple aspects of lipid metabolism Activated by fibrates and thiazolidinediones	Zinc finger
Homeodomain proteins	—	Regulate gene expression during development	Helix-turn-helix

General Transcription Factors

In eukaryotes, general transcription factors must bind to the promoter to allow RNA polymerase II to bind and form the initiation complex at the start site for transcription. General transcription factors are common to most genes. The general transcription factor TFIID (the TATA factor) must bind to the TATA box before RNA polymerase II can bind. Other examples include SP-1 and NF-1 that modulate basal transcription of many genes.

Specific Transcription Factors

Specific transcription factors bind to enhancer regions or, in a few cases, to silencers and modulate the formation of the initiation complex, thus regulating the rate of initiation of transcription. Each gene contains a variety of enhancer or silencer sequences in its regulatory region. The exact combination of specific transcription factors available (and active) in a particular cell at a particular time determines which genes will be transcribed at what rates. Because specific transcription factors are proteins, their expression can be cell-type specific. Additionally, hormones may regulate the activity of some specific transcription factors. Examples include steroid receptors and the CREB protein.

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that bind to DNA response elements (PPREs) and control multiple aspects of lipid metabolism. Individual members of this family of zinc-finger proteins are activated by a variety of natural and xenobiotic ligands, including:

- Fatty acids
- Prostaglandin derivatives
- Fibrates
- Thiazolidinediones

The improvement in insulin resistance seen with thiazolidinediones is thought to be mediated through their interaction with PPAR γ . Clofibrate binds PPAR α affecting different aspects of lipid metabolism than the thiazolidinediones.

Control of Gluconeogenesis by Response Elements

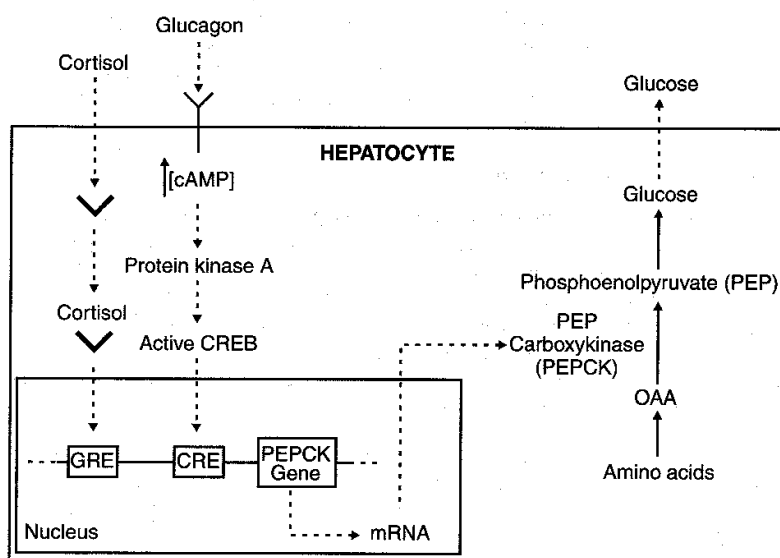


Figure I-5-6. Cortisol and Glucagon Stimulate Gluconeogenesis Through Enhancer Mechanisms

An example of how response elements affect metabolism can be seen in the pathway of gluconeogenesis (Figure I-5-6). Gluconeogenesis is a hepatic pathway whose major function is to maintain adequate glucose in the blood for tissues like the nerves (brain) and red blood cells during fasting. It also provides glucose during periods of stress. Hormones that activate the pathway include:

- Glucagon secreted in response to hypoglycemia and functioning via a membrane-associated receptor that increases cAMP concentration
- Cortisol secreted in response to stress, is permissive for glucagon in hypoglycemia and acts through an intracellular receptor, which, like other steroid receptors, is a zinc-finger DNA binding protein.

Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes a critical reaction in gluconeogenesis, which under many conditions is the rate-limiting step in the pathway. A cAMP response element (CRE) and a glucocorticoid response element (GRE) are each located upstream from the transcription start site.

Cortisol induces PEPCK gene expression by the following sequence:

- Cortisol diffuses into the hepatocyte, where it
- Binds to its receptor.
- The complex enters the nucleus, and
- Binds (through the zinc fingers) to the glucocorticoid response element (GRE) associated with the PEPCK gene, which
- Increases gene expression.

- PEPCK concentration increases in the cell.
- The rate of gluconeogenesis increases.

Glucagon induces PEPCK gene expression by the following sequence:

- Glucagon binds to a receptor in the cell membrane (Chapter 9).
- cAMP concentration increases.
- Protein kinase A becomes active, and then
- Phosphorylates and activates CREB.
- Activated CREB enters the nucleus and binds to the CRE associated with the PEPCK gene, which
- Increases gene expression.
- PEPCK concentration increases in the cell.
- The rate of gluconeogenesis increases.

These effects of CREB and the cortisol-receptor complex are not entirely independent of each other. Each contributes, along with several other transcription factors, to assembling a complex of activator proteins that ultimately determine the level of PEPCK gene expression.

Control of Cell Differentiation by Homeodomain Proteins During Development *In Utero*

Sequential and coordinated gene expression is necessary for proper tissue and cell differentiation during embryonic life. Groups of regulatory proteins called homeodomain proteins are major factors in controlling this embryonic gene expression. Each regulatory protein is responsible for activating a different set of genes at the proper time in development.

The regulatory proteins themselves are encoded by genes called homeobox (HOX) or homeotic genes. Another closely related set of genes is the PAX (paired-box) genes. Mutations in HOX or PAX genes might be expected to produce developmental errors. Klein-Waardenburg syndrome (WS-III) is one such developmental disorder resulting from a mutation in a PAX gene.

Co-Expression of Genes

Most eukaryotic cells are diploid, each chromosome being present in two homologous copies. The alleles of a gene on the two homologous chromosomes are usually co-expressed. In a person heterozygous for the alleles of a particular gene, for example a carrier of sickle cell trait, two different versions of the protein will be present in cells that express the gene. In the person heterozygous for the normal and sickle alleles, about 50% of the β -globin chains will contain glutamate and 50% valine at the variable position (specified by codon 6).

Major exceptions to this rule of codominant expression include genes:

- On the Barr body (inactivated X chromosome) in women
- In the immunoglobulin heavy and light chain loci (ensuring that one B cell makes only one specificity of antibody)
- In the T-cell receptor loci

Clinical Correlate

Klein-Waardenburg Syndrome

All of the tissues affected in Klein-Waardenburg syndrome are derived from embryonic tissue in which PAX-3 is expressed. Symptoms include:

- Dystopia canthorum (lateral displacement of the inner corner of the eye)
- Pigmentary abnormalities (frontal white blaze of hair, patchy hypopigmentation of the skin, heterochromia irides)
- Congenital deafness
- Limb abnormalities

Bridge to Medical Genetics

Genetic Imprinting in Prader-Willi Syndrome

Genetic imprinting of a few gene regions results in monoallelic expression. In some cases, this imprinting is according to the parent of origin. The gene involved in Prader-Willi syndrome is on chromosome 15 and is imprinted so that it is normally expressed only from the paternal, not the maternal, chromosome. In such a case, if one inherits a paternal chromosome in which this region has been deleted, Prader-Willi syndrome results. It can also result from uniparental (maternal) disomy of chromosome 15. Symptoms of Prader-Willi include:

- Childhood obesity and hyperphagia
- Hypogonadotrophic hypogonadism
- Small hands and feet
- Mental retardation
- Hypotonia

Other Mechanisms for Controlling Gene Expression in Eukaryotes

Table I-5-2 summarizes some of the mechanisms that control gene expression in eukaryotic cells.

Table I-5-2. Control of Eukaryotic Gene Expression

Control Point	Example
Inactivation of specific chromosomes or chromosomal regions during development	One X chromosome in each cell of a woman is inactivated by condensation to heterochromatin (Barr bodies)
Local chromatin-modifying activities	Acetylation of histones increases gene expression (many genes) Methylation of DNA silences genes in genetic imprinting (Prader-Willi and Angelman syndromes)
Gene amplification	Many oncogenes are present in multiple copies: <i>erbB</i> amplified in certain breast cancers Dihydrofolate reductase genes are amplified in some tumors, leading to drug resistance
Specific transcription factors	Steroid hormone receptors, CREB, and homeodomain proteins
Processing mRNA	Alternative splicing of mRNA in the production of membrane-bound vs. secreted antibodies
Rate of translation	Heme increases the initiation of β -globin translation
Protein modification	Proinsulin is cleaved to form active insulin
Protein degradation rate	ALA synthase has a half-life of 1 hour in the hepatocyte

Chapter Summary

PROKARYOTIC

Lactose operon:

- Repressor protein binds at operator.
- Lactose inhibits repressor protein.
- cAMP-dependent activator protein binds at CAP site.
- Glucose lowers cAMP, preventing activation.
- Genes are expressed when lactose is present and glucose is absent.

Histidine operon: attenuation control:

- Requires simultaneous transcription and translation
- Premature (upstream) termination of transcription when histidine is present
- Normal (downstream) termination of transcription when histidine is absent

EUKARYOTIC

Repressors bind silencer elements.

Activators (transcription factors) bind:

- Upstream promoter elements (general transcription factors)
- Enhancer response elements (specific transcription factors)

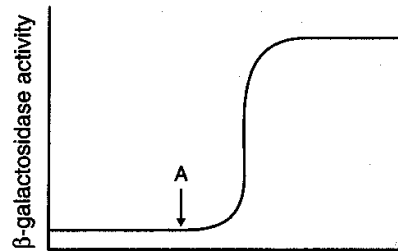
Specific transcription factors include:

- Steroid receptors (zinc finger)
- cAMP-dependent activator protein, CREB (leucine zipper)
- PPARs (zinc finger)
- Homeodomain proteins: pHOX, pPAX (helix-turn-helix)

Review Questions

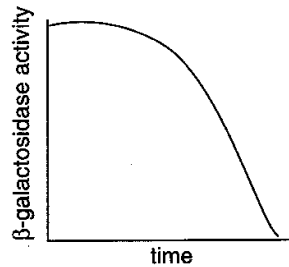
Select the ONE best answer.

1. A culture of *E. coli* is grown in a medium containing glucose and lactose. The expression of the lactose operon over time in the cells is shown in the graph below. Which statement best describes the change that occurred at point A?



- A. Lactose was added to the culture
 - B. cAMP concentration increased in the cells
 - C. Glucose was added to the culture
 - D. Repressor protein dissociated from the operator
 - E. Repressor protein became bound to the operator
2. Klein-Waardenburg syndrome is a single-gene disorder that includes dystopia canthorum (lateral displacement of the inner corner of the eye), impaired hearing, and pigmentary abnormalities. The gene involved is most likely to be a
 - A. Pseudogene
 - B. Proto-oncogene
 - C. Transgene
 - D. Homeotic gene
 - E. Tumor suppressor gene

3. *Escherichia coli* cells grown in a medium with lactose as the only carbon source are monitored for β -galactosidase activity over time with the results shown below.



Which intracellular event would most likely be associated with the change in enzyme activity observed?

- A. Decreased synthesis of cytosolic cAMP
- B. Activation of a repressor protein by lactose
- C. Increased synthesis of a repressor protein
- D. Dissociation of a cAMP-CAP complex from a CAP-binding sequence
- E. Binding of a repressor protein to an operator sequence

Answers

1. **Answer: B.** Gene expression is initially low because glucose is present and keeps the cAMP level low. At point A, glucose was depleted (answer not given as option), allowing the cAMP to increase.
2. **Answer: D.** Multiple developmental abnormalities due to mutation in a single gene.
3. **Answer: E.** The decrease in enzyme activity is caused by the depletion of lactose, its dissociation from the repressor protein, and binding of the repressor to the operator control region.

Recombinant DNA

6

OVERVIEW OF RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology allows a DNA fragment from any source to be joined *in vitro* with a nucleic acid vector that can replicate autonomously in microorganisms. This provides a means of analyzing and altering genes and proteins. It provides the reagents necessary for genetic testing for carrier detection and prenatal diagnosis of genetic diseases and for gene therapy. Additionally, this technology can provide a source of a specific protein, such as recombinant human insulin, in almost unlimited quantities.

Two approaches to producing recombinant DNA for cloning have been developed for use with somewhat different applications:

- Cloning restriction fragments of cellular DNA
- Cloning cDNA produced by reverse transcription of cellular mRNA

Cloning Restriction Fragments of Cellular DNA

Cloning DNA restriction fragments is useful in the following applications:

- Sequencing DNA (Human Genome Project, Genetic diagnosis)
- Producing restriction maps for gene mapping (Chapter 4, Medical Genetics)
- Studies involving non-expressed DNA sequences

The first step in this procedure is to produce restriction fragments of DNA using restriction endonucleases.

Restriction Endonucleases

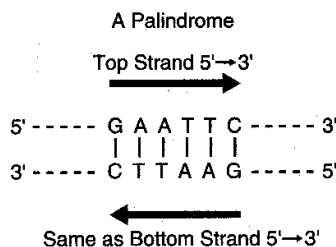
These enzymes are isolated from bacteria, their natural source. There are many different restriction endonucleases isolated from a variety of bacteria that are now readily available commercially. In bacteria they act as part of a restriction/modification system that protects the bacteria from infection by DNA viruses.

Restriction endonucleases recognize double-stranded DNA sequences called palindromes (inverted repeats) usually of four to eight base pairs in length. For example, Figure I-6-1 shows the recognition site for *EcoRI*, a restriction endonuclease isolated from *Escherichia coli*. A palindrome can be identified by examining the sequence of only one strand. Draw a line through the center of the sequence (through the central base for palindromes with an odd number of nucleotides). If the sequence is folded along this line the bases should pair.

In A Nutshell

Prokaryotic Restriction Modification Systems

- Provide defense against infecting DNA viruses
- Methylase enzyme modifies and protects palindromes in bacterial DNA.
- Unmethylated palindromes of infecting viral DNA are recognized by restriction endonuclease.
- Viral DNA is fragmented and destroyed.



This is the DNA sequence recognized by the restriction endonuclease *EcoRI*.

Figure I-6-1. *EcoRI* Recognition Sequence

DNA from a source to be cloned is mixed with a particular restriction endonuclease such as *EcoRI*, producing DNA restriction fragments. Some restriction endonucleases such as *EcoRI* produce asymmetric cuts within the palindrome yielding "sticky ends" on the fragments. Sticky ends are advantageous in facilitating the recombination of a restriction fragment with the vector DNA. Others, like *HaeIII*, cut both strands in the same location yielding "blunt ends" on the restriction fragments. These examples are shown in Figure I-6-2.

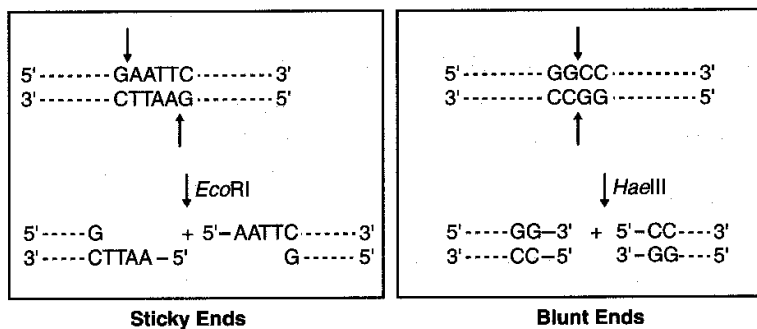


Figure I-6-2. Examples of Restriction Endonucleases

Cloning Restriction Fragments Using Vectors

To clone the restriction fragments, they must each be inserted into a vector. A vector is a piece of DNA (plasmid, viral chromosome, yeast chromosome) capable of autonomous replication in a host cell, for instance, the plasmid pBR322 shown in Figure I-6-3. The DNA used as a vector usually has

- At least one type of palindrome recognized by a restriction endonuclease
- An origin for autonomous replication
- At least one gene for resistance to an antibiotic

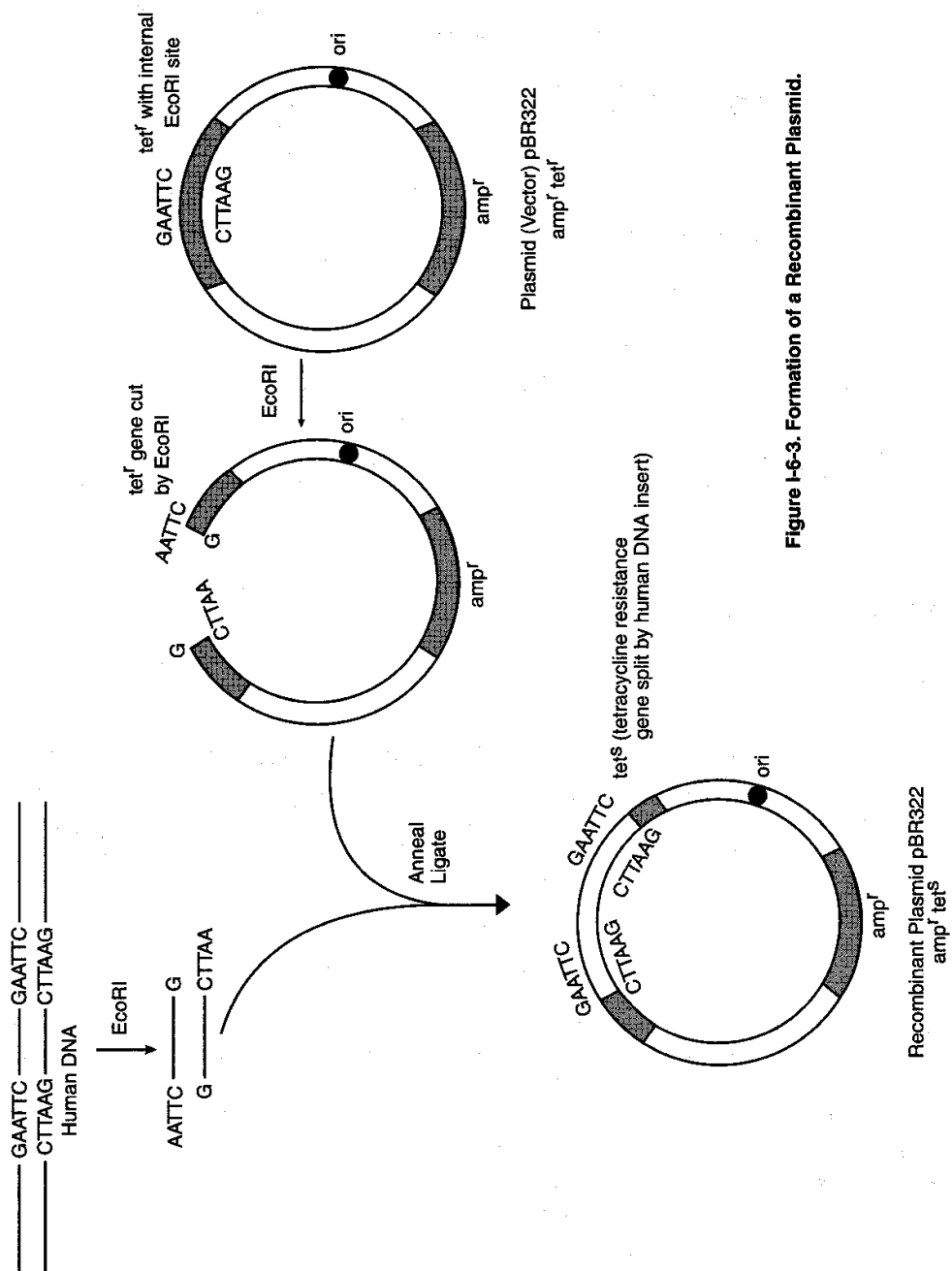


Figure I-6-3. Formation of a Recombinant Plasmid.

In a Nutshell

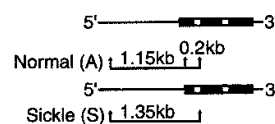
Uses of Genomic Libraries

- Sequence DNA (Human Genome Project)
- Construct restriction maps of DNA
- Identify genetic markers (microsatellites, etc.)
- Study non-expressed DNA

Bridge to Medical Genetics

Restriction Maps

- Line drawings of DNA identifying sites cut by restriction endonucleases.
- Identify potential RFLP markers for genetic diagnosis.
- Example: Restriction site polymorphism for *MstII* may be used to identify individuals with the sickle cell mutation (see Chapter 7, Genetic Testing).



The vector is cut with the restriction endonuclease and mixed with the DNA restriction fragments to be cloned. As shown in Figure I-6-3, once the vectors have combined with one of the restriction fragments, DNA ligase is used to form permanent PDE bonds between the fragment and the vector. This produces recombinant DNA.

Once the recombinant vectors have been produced, they are used to transform host cells. In the example of the plasmid pBR322, the host cells are bacteria. Once transformed, the bacteria are plated on selective media so that bacteria transformed with a recombinant plasmid can be easily identified. In the case of plasmid pBR322 shown in Figure I-6-3, bacteria with recombinant plasmids would be resistant to ampicillin but sensitive to tetracycline.

The collection of colonies produced is referred to as a genomic DNA library. The library must be screened with a radioactive probe to identify the colony with the desired restriction fragment (see Screening DNA Libraries).

Uses of Genomic Libraries

Large quantities of each clone can be grown for DNA sequencing studies, similar to what is being done in the Human Genome Project.

By producing genomic libraries using different restriction endonucleases (or allowing one type of restriction endonuclease to digest a DNA sample for different times), regions of overlap can be identified and the fragments ordered, producing DNA restriction maps useful for genetic testing and sequencing.

Other genetic markers may be identified in this way, such as minisatellite and microsatellite sequences (see Chapter 7).

Genomic libraries are also useful to clone and study DNA sequences that are not expressed in cells (response elements, introns, promoters).

Cloning cDNA Produced by Reverse Transcription of Cellular mRNA

If the end goal of cloning is to have a cloned gene expressed in a cell, the entire coding sequence must be cloned intact. Furthermore, if a cloned eukaryotic gene is to be expressed in bacteria (to make recombinant proteins), the gene must not contain introns, which could not be processed in a prokaryotic cell. In these cases it is more convenient to clone cDNA rather than DNA restriction fragments.

Producing cDNA by Reverse Transcription of mRNA

Cytoplasmic mRNA is isolated from a cell known to express the desired gene. Reverse transcriptase, along with other components (Figure I-6-4), is used in vitro to produce double-stranded cDNA that is subsequently recombined with a chosen vector to produce the recombinant DNA for cloning. In this approach:

- All genes expressed will be cloned along with the desired gene.
- None of the non-expressed DNA in the cell will be cloned.
- Each cDNA represents the complete coding sequence of a gene.
- The cDNAs have no introns.
- An expression library is produced at the end of the cloning procedure.

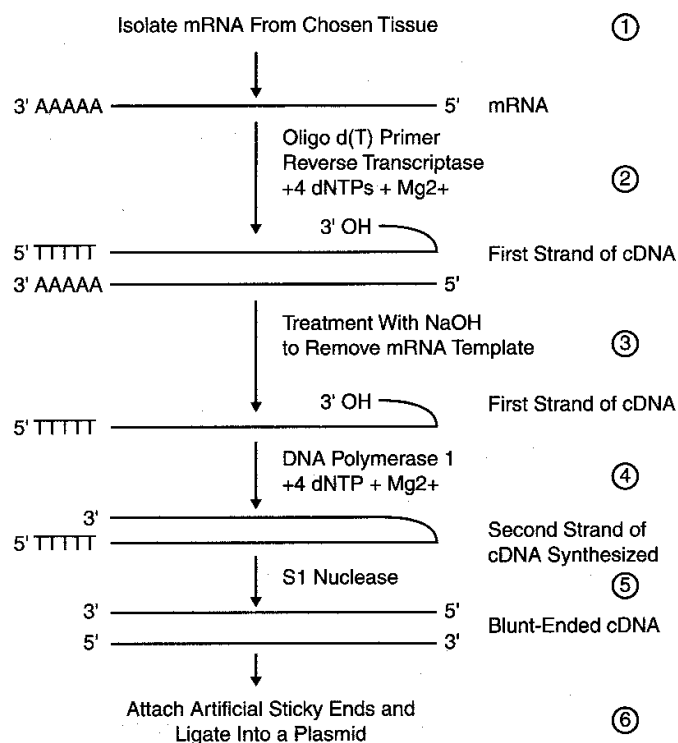


Figure I-6-4. Synthesis of cDNA *In Vitro*

Expression Vectors

If the goal of the cloning procedure is to obtain a recombinant protein, appropriate sequences required for transcription and translation in the cloning host cell must be provided because they will not be part of the cDNA. For instance, to produce recombinant human insulin in bacteria, a bacterial promoter and a Shine-Dalgarno sequence must be included in the cloning plasmid near the insertion site for the cDNA. Figure I-6-5 shows an example of an expression vector, pUC. In some expression vectors, other regulatory sequences such as operators are added to allow expression of the cloned gene to be controlled.

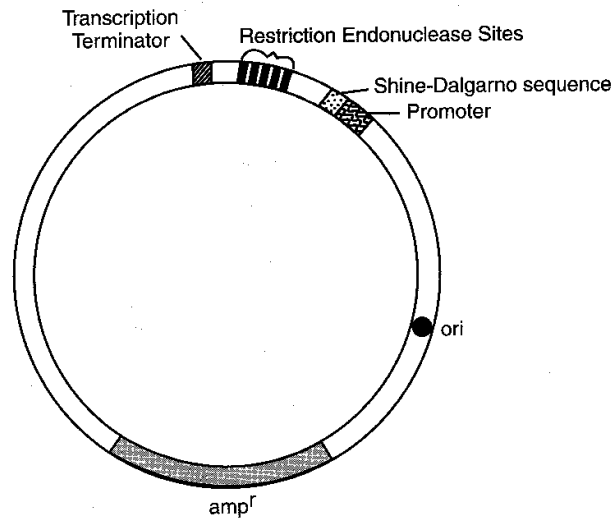


Figure I-6-5. An Expression Vector (pUC)

In a Nutshell

Uses of cDNA (Expression) Libraries

- Sequence specific coding regions
- Produce recombinant proteins (Factor VIII, HBsAg, insulin)
- Gene therapy
- Transgenic animals

Uses of cDNA (Expression) Libraries

Once the recombinant expression vectors containing the cDNA inserts are produced, they are used to transform bacteria (or other host cells) and produce cDNA (expression) libraries. Expression libraries may be used to produce recombinant proteins that in some cases have significant advantages over isolating them from natural sources:

- Larger quantities may be produced. Recombinant human insulin used to treat diabetics.
- Natural source may carry risk of infection. Recombinant Factor VIII used to treat hemophilia A has helped reduce the incidence of HIV infection in hemophiliacs. Recombinant HbsAg is now used to immunize against hepatitis B, eliminating the risk of introducing a viral infection during vaccination.

Genes cloned as cDNA are used for gene therapy protocols and for producing transgenic animals.

cDNA probes, for many of the blotting techniques, are produced by cloning.

Screening Libraries for a Specific DNA Sequence

Figure I-6-6 shows how libraries are screened to identify a desired DNA sequence. The top circle represents either a genomic library or an expression library on a growth plate.

- A blot is made from the plate.
- Colonies on the blot are lysed and treated with a radioactive probe specific for the DNA sequence (^{32}P -DNA) or recombinant protein (^{125}I -antibody reactive with the recombinant protein).
- An autoradiogram of the probed blot is produced and the radioactive colony identified.

Once the corresponding colony has been identified, a sample can be used to inoculate a large broth culture from which one can isolate the cloned DNA or the recombinant protein.

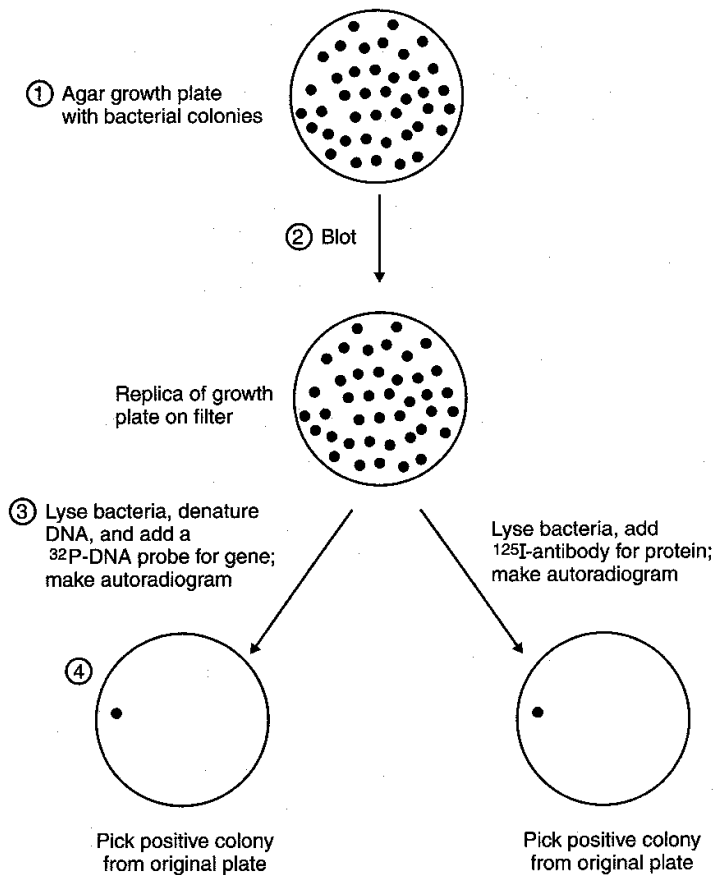


Figure I-6-6. Screening a DNA Library

Common Sources of ^{32}P -DNA probes

- cDNA can be used as a probe to locate a genomic clone
- A cloned gene from another species can be used to locate the human homolog.
- An approximate DNA sequence can be deduced and synthesized in the laboratory provided the amino acid sequence of the protein is known.

Gene Therapy and Transgenic Animals

Gene therapy now offers potential cures for individuals with inherited diseases. The initial goal is to introduce a normal copy of the gene that is defective into the tissues that give rise to the pathology of the genetic disease. For instance, about 50% of the children with severe combined immunodeficiency have a mutation in the gene encoding the γ chain common to several of the interleukin receptors. Recently, cDNA from a normal γ -chain gene was used to transduce autologous cells from infants with X-linked SCID with subsequent correction of the defects in their

Bridge to Medical Genetics

Gene Therapy

When using gene therapy to correct genetic deficiencies in humans, the cloned normal gene is targeted only to the tissues giving rise to the major symptoms. For instance, about 50% of the cases of severe combined immunodeficiency (SCID) are caused by mutations in the gene for a subunit common to several interleukin receptors. The approach to gene therapy has been to introduce a normal cloned gene into the patient's bone marrow cells that will subsequently divide and differentiate to produce T and B lymphocytes expressing the gene. These patients subsequently have improved immune function. Because the cloned normal gene has not been introduced into reproductive tissues, any children of the patient would inherit the defective allele.

T-cells and natural killer cells (Figure I-6-7; also see Chapter 13, Immunology and Chapter 6, Medical Genetics).

- Gene transfer requires a delivery vector (retrovirus, adenovirus, liposome).
- Only tissues giving rise to the disease pathology are targeted for gene therapy.
- Normal gene is not inherited by offspring.

Transgenic Animals

Transgenic animals are produced by transferring cDNA into the pronucleus of a fertilized ovum. The resultant transgenic animal has the new gene (transgene) in all of its cells including its reproductive tissues. Transgenic animals are now widely used as experimental models in which to study human diseases.

A variation of this technique produces a knockout animal, in which a normal gene has been functionally eliminated. This may be done by site-specific mutagenesis.

- Transgenic animals have a new gene (transgene) introduced into their germline.
- All cells of a transgenic animal contain the transgene.
- Transgene is inherited by offspring.
- Knockout animals have a normal gene intentionally inactivated/destroyed.
- Transgenic and knockout animals are used as models of human disease.

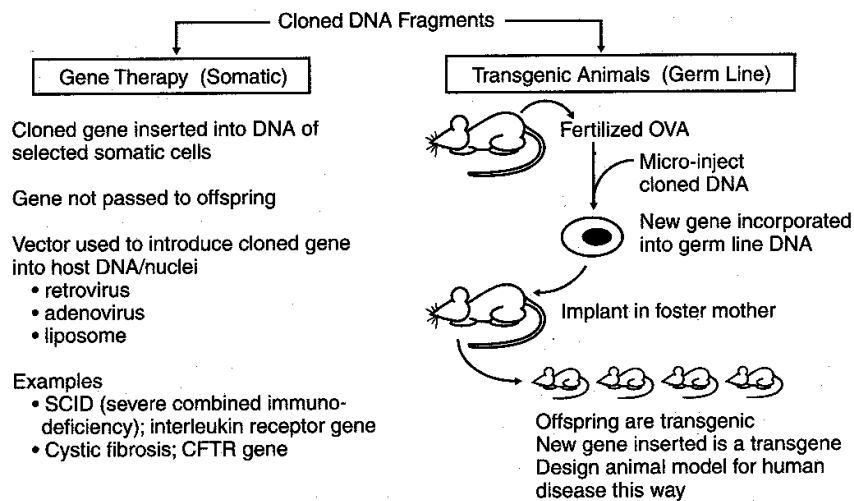


Figure I-6-7. Incorporation of Cloned DNA Into Humans and Other Animals

Table I-6-1. Summary of Important Points About Recombinant DNA

Restriction endonucleases	<p>Recognize palindromes in dsDNA: 5' --- G A A T T C --- 3' 3' --- C T T A A G --- 5'</p> <p>Cut leaving sticky ends: 5' --- G A A T T C --- 3' 3' --- C T T A A G --- 5'</p> <p>Used to make restriction maps of DNA Produce fragments for genetic analysis Produce fragments for making recombinant DNA and cloning DNA sequences</p>
Vectors for recombinant DNA and cloning	<p>Plasmid pBR322:</p> <ul style="list-style-type: none"> • Restriction site • Replication origin • Resistance to antibiotic(s) <p>Expression vector also requires:</p> <ul style="list-style-type: none"> • Promoter • Shine-Dalgarno sequence <p>Other vectors: phage, YACs</p>
Approaches to cloning DNA	<p>Genomic DNA</p> <ul style="list-style-type: none"> • Restriction endonucleases fragment DNA • Total nuclear DNA cloned • Genes contain introns <p>cDNA</p> <ul style="list-style-type: none"> • Reverse transcription of mRNAs from cell • Genes expressed cloned • Genes have no introns
Cloning procedure (in bacteria)	<p>Recombinant plasmids transfected into bacteria</p> <p>Antibiotic resistance used to select bacteria with recombinant plasmids</p> <p>Blot growth plate and probe with ^{32}P-DNA for gene, or with ^{125}I-antibody for protein</p>
Uses of cloned genes	<p>Produce recombinant proteins</p> <p>Gene therapy (somatic)</p> <p>Transgenic animals (germ line)</p> <p>Produce cDNA probes for blots</p>

Review Questions

Select the ONE best answer.

1. If a cystic fibrosis patient were to be treated by gene therapy, which type of cells should be targeted as host cells?
 - A. Germ cells
 - B. Epithelial cells
 - C. T cells
 - D. Hemopoietic stem cells

2. A pharmaceutical firm is interested in the bacterial production of thymidylate synthase in large quantities for drug-targeting studies. An important step in the overall cloning strategy involves the ligation of synthase cDNA into a plasmid vector containing a replication origin, an antibiotic resistance gene, and a promoter sequence. Which additional nucleotide sequence should be included in this vector to ensure optimal production of the thymidylate synthase?
 - A. Operator sequence
 - B. Poly A sequence
 - C. Shine-Dalgarno sequence
 - D. Attenuator sequence
 - E. 3'-splice acceptor sequence

3. Restriction fragment length polymorphisms may be produced by mutations in the sites for restriction endonucleases. For instance, a single base change in the site for the nuclease *SalI* produces the sequence GTGGAC, which can no longer be recognized by the enzyme. The original sequence recognized by *SalI* was:
 - A. GTAGAC
 - B. GCGGAC
 - C. CTGGAC
 - D. GTCGAC
 - E. GTGTAC

4. Scientists studying a common mutation in the LDL receptor gene have inserted the defective gene into fertilized murine ova. The altered ova are implanted in a foster mother and the progeny are used to study the effects of the mutant allele. The mice produced in this procedure would be referred to as:
 - A. Knockout mice
 - B. Transgenic mice
 - C. Allogenic mice
 - D. Cloned mice
 - E. Somatic-cell engineered mice

Answers

1. **Answer: B.** The pathogenesis of CF is related to defective chloride transport in epithelial cells.
2. **Answer: C.** Incorporation of a Shine-Dalgarno sequence into the expression vector will promote ribosome binding to the translation start site on the mRNA produced by transcription of the cDNA insert.
3. **Answer: D.** All options represent single-base changes in the mutant sequence in the stem, but only choice D reestablishes a palindrome.
4. **Answer: B.** A gene introduced into the germline of mice (a transgene) would produce transgenic animals.

Genetic Testing

7

OVERVIEW OF GENETIC TESTING

Genetic diseases often depend on the genotype of an individual at a single locus. Traits inherited in this manner are said to follow a Mendelian inheritance pattern. The mode of inheritance of a trait within a family can help suggest a diagnosis. Recombinant DNA technology has provided valuable probes for the diagnosis of existing diseases and the prediction of the risk of developing a genetic disease. Similar techniques have applications in forensic medicine, paternity testing, and epidemiology.

PEDIGREE ANALYSIS

Pedigree analysis is a method of recording family information to trace the passage of a gene through generations. A more extensive discussion of pedigrees and related concepts is presented in Section II, Chapter 1. Only the basic patterns are emphasized in this section. Figure I-7-1 summarizes basic pedigree nomenclature.

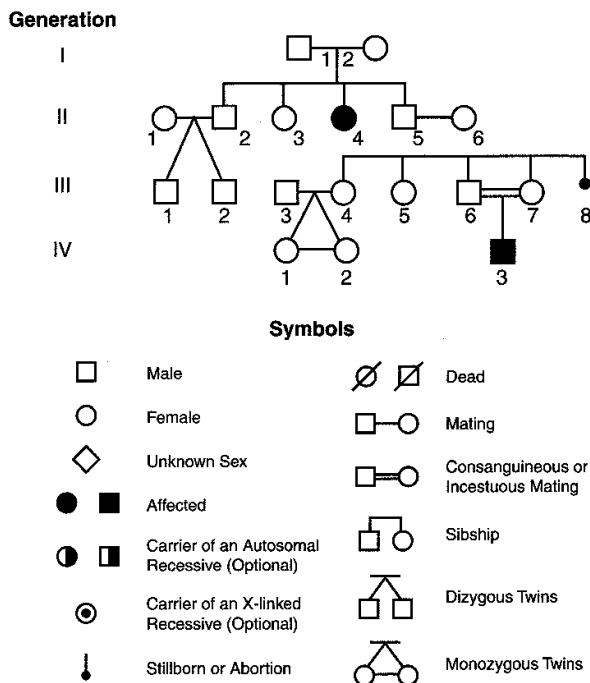


Figure I-7-1. Pedigree Nomenclature

The pattern of inheritance one finds in a family depends on whether the mutant gene is located on an autosome, the X chromosome, or mitochondrial DNA. It also depends on whether the mutant gene is dominant or recessive.

Note**Autosomal Dominant Diseases**

- Familial hypercholesterolemia (LDL receptor deficiency)
- Huntington disease
- Neurofibromatosis type 1
- Marfan syndrome
- Acute intermittent porphyria

Autosomal Dominant

Autosomal dominant traits (Figure I-7-2) have the following characteristics:

- Only one mutant allele need be present for the disease to be expressed.
- An affected person has at least one affected parent (vertical appearance).
- Either sex can be affected.
- Male-to-male transmission of the mutant allele may be present.

Autosomal dominant diseases are more likely to show late onset of symptoms. The genes involved often encode noncatalytic proteins, and may occasionally show incomplete penetrance or variable expression in a pedigree.

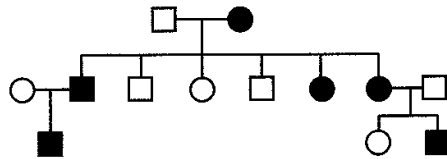


Figure I-7-2. Autosomal Dominant Inheritance Pattern

Autosomal Recessive

Autosomal recessive traits (Figure I-7-3) have the following characteristics:

- Two mutant alleles must be present for the disease to be expressed.
- An affected person is usually born to unaffected parents.
- Either sex can be affected.
- Both parents of an affected child are obligate carriers.
- Male-to-male transmission of the mutant allele may be present.

Autosomal recessive diseases often show early onset of symptoms, and the genes involved usually encode catalytic proteins.

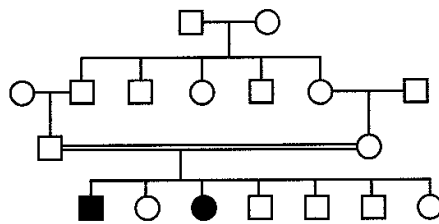


Figure I-7-3. Autosomal Recessive Inheritance Pattern

X-Linked Dominant

X-linked dominant traits (Figure I-7-4) have the following characteristics:

- Only one mutant allele need be present for the disease to be expressed.
- Either sex can be affected.
- There is no male-to-male transmission.
- An affected male parent passes the trait to all of his daughters, but none of his sons.
- An affected female parent can pass the trait to both sons and daughters.

Affected females often have more mild and variable symptoms than affected males. There are very few diseases with X-linked dominant inheritance.

Note

Autosomal Recessive Diseases

Examples include:

- Sickle cell anemia
- Cystic fibrosis
- Phenylketonuria (PKU)
- Tay-Sachs disease (hexosaminidase A deficiency)

Note

X-Linked Dominant Disease

- Hypophosphatemic rickets
- Fragile X syndrome

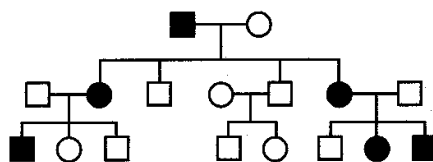


Figure I-7-4. X-Linked Dominant Inheritance Pattern

Note

X-Linked Recessive Diseases

- Duchenne muscular dystrophy
- Lesch-Nyhan syndrome (hypoxanthine-guanine phosphoribosyltransferase [HGPRT] deficiency)
- Glucose-6-phosphate dehydrogenase deficiency
- Hemophilia A and B

X-Linked Recessive

X-linked recessive traits (Figure I-7-5) have the following characteristics:

- Usually affects only males (females usually have a normal X from an unaffected parent).
- Affected males usually have unaffected parents (usually the mother is an unaffected carrier).
- There is no male-to-male transmission of the mutant allele.

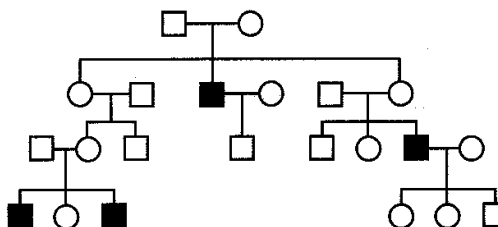


Figure I-7-5. X-Linked Recessive Inheritance Pattern

Note

Mitochondrial Diseases

- Leber hereditary optic neuropathy
- MELAS: mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
- Myoclonic epilepsy with ragged red muscle fibers

Mitochondrial Inheritance

Mitochondrial traits (Figure I-7-6) are inherited in a nonmendelian fashion because they are carried on mitochondrial DNA. They have the following characteristics:

- The disease is inherited only maternally, since only the mother contributes mitochondrial DNA to the progeny.
- Both males and females can be affected by the disease. All offspring of an affected female are affected, whereas there is no inheritance of the disease from an affected male.
- Mitochondrial diseases are often expressed as neuropathies and myopathies because brain and muscle are highly dependent on oxidative phosphorylation. Mitochondrial genes code for some of the components of the electron transport chain and oxidative phosphorylation, as well as some mitochondrial tRNA molecules.

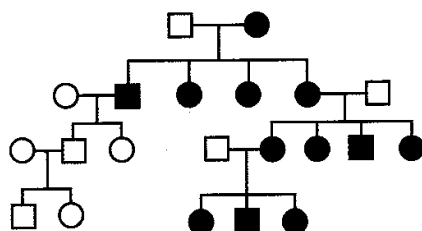


Figure I-7-6. Mitochondrial Inheritance Pattern

BLOTTING TECHNIQUES

Blotting techniques have been developed to detect and visualize specific DNA, RNA, and protein among complex mixtures of contaminating molecules. These techniques have allowed the identification and characterization of the genes involved in numerous inherited diseases. The general method for performing a blotting technique is illustrated in Figure I-7-7.

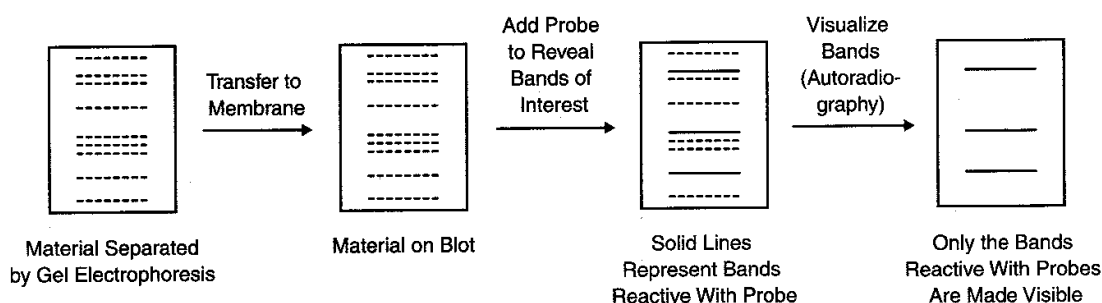


Figure I-7-7. Blotting Technique

The fragments in the material to be analyzed (DNA, RNA, or protein) are separated by gel electrophoresis. The smaller molecules travel faster and appear nearer the bottom of the gel. The bands of material in the gel are transferred or blotted to the surface of a membrane. The membrane is incubated with a (usually radioactive) labeled probe that will specifically bind to the molecules of interest. Visualization of the labeled probe (usually by autoradiography) will reveal which band interacted with the probe. The most common types of blots are compared in Table I-7-1. Most typically, DNA restriction fragments are analyzed on a Southern blot.

Table I-7-1. Types of Blots

Blot Type	Material Analyzed	Electrophoresis Required	Probe Used	Purpose
Southern	DNA	Yes	^{32}P -DNA	To determine which restriction fragments of DNA are associated with a particular gene
Northern	RNA	Yes	^{32}P -DNA	To measure sizes and amounts of specific mRNA molecules to answer questions about gene expression
Western	Protein	Yes	^{125}I - or enzyme-linked antibody	To measure amount of antigen (proteins) or antibody
Dot (slot)	RNA, DNA, or protein	No	Same as for blots above	To detect specific DNA, RNA, protein, or antibody

DNA probes are radioactively labeled single-stranded DNA molecules that are able to specifically hybridize (anneal) to particular denatured DNA sequences. Different kinds of probes have been developed for the recognition of particular genes (Table I-7-2 and Figure I-7-8).

- Single-gene probes are relatively long DNA probes that bind to only one gene or to a genetic marker located near the gene of interest. They are not able to differentiate between different alleles of that gene. They are most useful for the analysis of Southern blots.
- Allele-specific oligonucleotides (ASO) are relatively short DNA probes that under stringent conditions can differentiate between alleles of a gene. To design an ASO, one must know the mutation involved in the disease. An ASO is most useful if it is specific for the particular mutation that accounts for most cases of the disease. They are usually used to probe dot blots.

Table I-7-2. Types of DNA Probes

Probe Type	Recognizes	Application
Single gene	Single gene; does not differentiate among alleles	Genetic counseling
Allele-specific oligonucleotide	Specific alleles of a gene	Genetic counseling and DNA fingerprinting

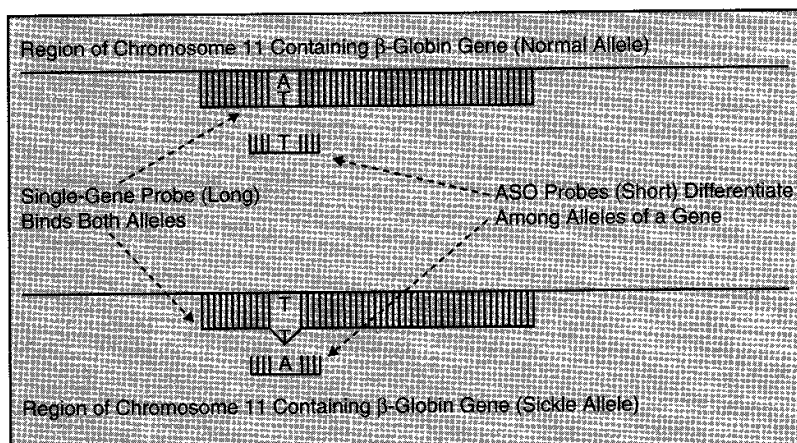


Figure I-7-8. Comparison of Probes: Single-Gene Versus Allele-Specific Oligonucleotide (ASO)

Southern Blot Analysis of Restriction Fragment Length Polymorphisms (RFLPs) for Genetic Testing

Southern blots analyzing DNA have been very useful in identifying the genes involved in many genetic diseases, Huntington disease being a prominent example. These analyses are based on restriction fragment length polymorphisms (RFLPs) that have arisen in human DNA over thousands of years of evolution. Mechanisms that have created this diversity within the human genome include:

- Mutations in restriction sites (the recognition sites for restriction endonucleases such as *MstII*)
- Expansion of repetitive sequences (minisatellites or VNTRs, variable number of tandem repeats) within the DNA

The initial goal is to identify an RFLP that is closely linked to the gene involved in the disease. Once such markers have been identified, Southern blots may be used to predict which members within a family have inherited the allele associated with the disease. An example of an RFLP analysis based on a mutation in a restriction site is presented here.

Carrier Detection and Prenatal Diagnosis of Sickle Cell Disease

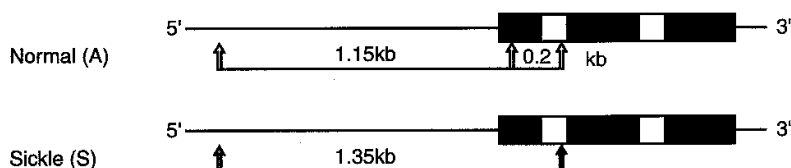
Figure I-7-9 shows the pedigree of a family in which the autosomal recessive disorder sickle cell disease is segregating. The father and mother are both carriers, and they have produced an affected son. *MstII* restriction maps of the normal (A) and mutant (S) β -globin genes and Southern blots from each member of the family are also given. The probe used binds to the 5' flanking region.

Note

Highly Repetitive Sequences in DNA

Several types of repetitive sequences have been identified in chromosomes. Generally, but not always, these are found in the noncoding (spacer) DNA. The following classes are distinguished:

- **Satellites**—the repeated unit typically ranges from 20 to 175 bp, and the length of the entire repeat is from 0.1 to 1 Mb. Satellite DNA is clustered in centromeric regions and is rarely used in genetic testing.
- **Minisatellites**—the repeated unit typically ranges from 20 to 70 bp, and the length of the entire repeat may reach 20 kb. This is the class most often referred to as VNTRs and contributes to RFLP patterns on Southern blots.
- **Microsatellites**—the repeated units are typically 2–4 bp, and the length of the entire repeat is generally less than 150 bp. This class often referred to as STRs (simple tandem repeats) is most typically amplified by a PCR for paternity testing, forensic cases, or gene linkage analysis (see Section II, Chapter 4; Polymorphic Markers and Linkage Analysis).

A. *Mst* II Restriction Map of the β -Globin Gene

B. Pedigree Analysis

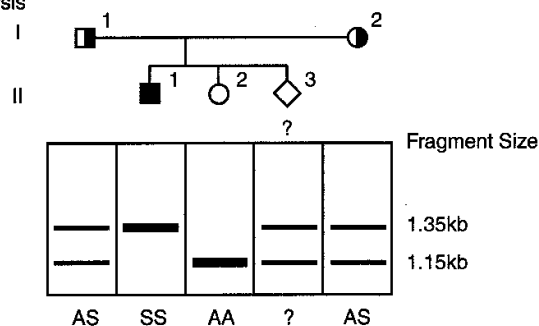


Figure I-7-9. Prenatal Diagnosis of Sickle Cell Disease

Question: What is the genotype and phenotype of fetus II-3?

Recurrence risk analysis based on pedigree alone:

- 25% chance that II-3 will have sickle-cell disease (SS)
- 50% chance that II-3 will be a carrier (AS)
- 25% chance that II-3 will be homozygous normal (AA)

Analysis of Southern blots: The restriction maps show that the normal (A) gene produces a 1.15-kb fragment, whereas the mutant (S) gene produces a 1.35-kb fragment. This difference occurs because the sickle cell mutation destroys the middle *Mst*II recognition site. The father and mother each yield two bands on their Southern blots, because they each carry one normal and one mutant gene. Affected son II-1 has only the larger band, because he has two copies of the mutant gene. Daughter II-2 shows only the smaller band. She must have two normal genes (homozygous normal). A Southern blot of fetal DNA obtained from II-3 by amniocentesis or chorionic villus sampling shows both bands. The fetus therefore has one normal and one mutant gene and is destined to be a carrier of the disease, but will not have symptoms.

The example using sickle cell disease is unusual in that the disease-causing mutation also produces a restriction site polymorphism. Usually in an RFLP analysis, the RFLP polymorphism and the disease-producing mutation have occurred as two independent events but are located so close together that the presence of the RFLP can serve as a surrogate marker for the disease mutation. This linkage phase (see Medical Genetics, Chapter 4) must be established within each affected family member by examining DNA from as many family members as possible. In analyzing these cases, it is helpful to remember the following points:

- The disease-causing mutation will always be associated with the same size restriction fragment (unless a rare crossover has occurred).
- The normal allele may be associated with many different size fragments because normal alleles are introduced into a family every time a marriage occurs. (Occasionally the normal allele may even be associated by chance with the same size fragment as the disease-producing allele.)

One should always first determine which RFLP is associated with the disease-producing mutation (the linkage phase) in a given family. For example, see Questions 3 and 4 at the end of this chapter.

RFLP Diagnosis of Myotonic Dystrophy

With the advent of the polymerase chain reaction (PCR), faster, more-sensitive tests have been developed, and therefore Southern blots have largely been replaced by these PCR-based tests in clinical practice. RFLP analysis is still useful in a few cases in which polymorphisms are too large to conveniently amplify with a PCR. One such case is myotonic dystrophy, in which the expanded sequence is within the gene region itself (a CTG in the 3' untranslated region [UTR]). This disease shows anticipation, and family members with a severe form of myotonic dystrophy may have several thousand copies of this repeat. As shown in Figure I-7-10, when *Eco*RI digests are analyzed by Southern blotting, a probe reveals 9- to 10-kb fragments in unaffected individuals. The size of the fragment can reach 20 kb in severely affected individuals.

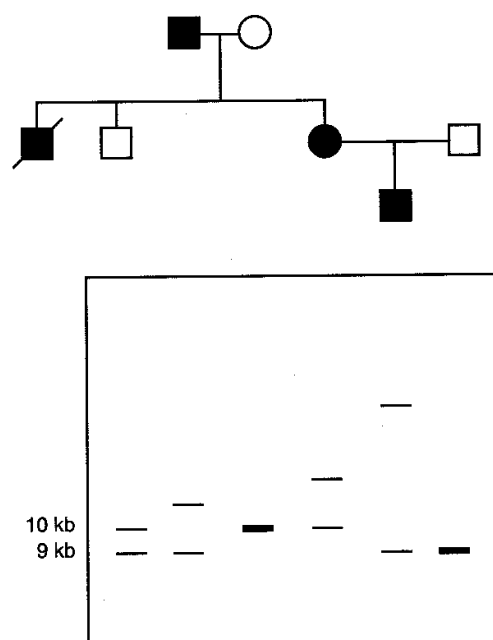


Figure I-7-10. *Eco*RI RFLP Analysis of a Family With Myotonic Dystrophy

Clinical Correlate

Myotonic Dystrophy

The gene involved in myotonic dystrophy encodes a protein kinase whose function is still uncharacterized. The disease is characterized by progressive muscle deterioration, cardiac arrhythmia, frontal baldness, cataracts, and testicular atrophy. Because the disease shows anticipation, symptoms range from mild to a severe neonatal condition.

PCR FOR GENETIC TESTING

PCR is a technique in which a short sequence of DNA can be amplified more than a million-fold within a few hours. PCR allows very small amounts of DNA to be analyzed without cloning and without the need for Southern blotting. The procedure is illustrated in Figure I-7-11. The DNA to be amplified is flanked by two short oligonucleotide primers that hybridize to opposite strands of the target sequence. Synthesis of the DNA target sequence is achieved by the addition of *Taq* polymerase (isolated from *Thermus aquaticus*), which is heat stable, and the four deoxynucleotides. The primers are oriented in such a way that at the end of each cycle, the two new strands of DNA are complementary, and together they constitute a new copy of the target sequence. Repeated cycles of heat denaturation, hybridization with primers, and polymerization by *Taq* polymerase results in the exponential amplification of the target sequence. Within a few hours, millions of copies of the starting sequence can be generated.

Note

Polymerase Chain Reaction (PCR)

Important steps in the PCR reaction include:

- Addition of short (10–20 bases) primers complementary to the 3' regions bordering the sequence to be amplified
- Addition of a heat-stable DNA polymerase and dNTPs
- Thermal cycling to melt the target DNA, allow primers to bind, and initiate DNA synthesis
- Detection of amplified DNA by expected size (gel electrophoresis) or by sequence (probing a dot blot)

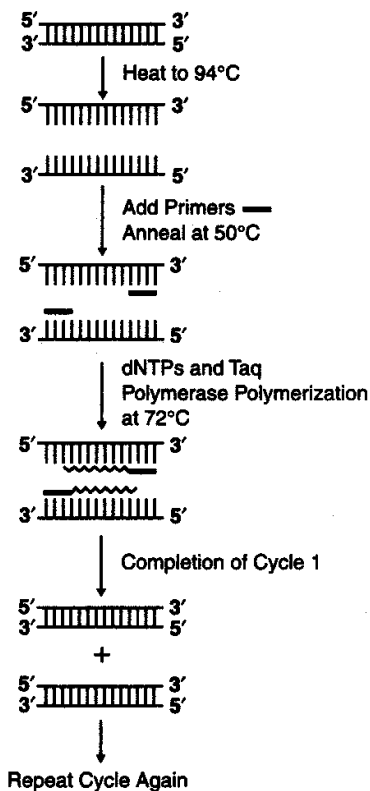


Figure I-7-11. Polymerase Chain Reaction

Genetic Testing of Cystic Fibrosis Patients Using PCR

Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder among American whites. The disease is caused by a mutation in the cystic fibrosis transmembrane regulator (CFTR), which provides and regulates a chloride channel through epithelial membranes. The most common CF mutation is $\Delta F508$, a three-base deletion that results in the loss of the amino acid phenylalanine (F) at position 508 in the CF protein. This particular mutation is found in about 70% of families with cystic fibrosis. PCR can be used to distinguish between individuals who are homozygous normal, homozygous affected, and heterozygous carriers (Figure I-7-12). The test is based on the fact that the PCR product from the mutant gene is three bases shorter than the product from the normal gene. Note that this specific test works only for those families with $\Delta F508$, and not for those with other mutations.

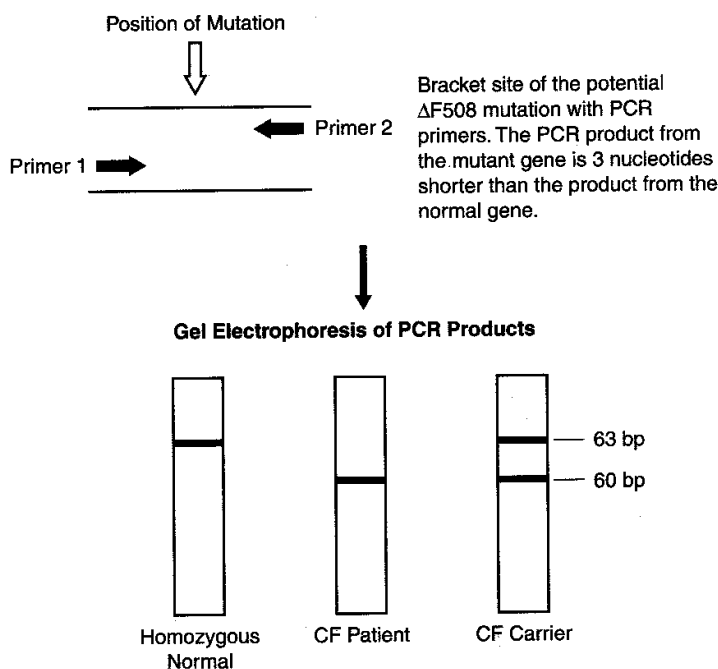


Figure I-7-12. Genetic Testing of Cystic Fibrosis Patients Using PCR

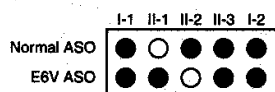
ASO Probes and Cystic Fibrosis

PCR can be combined with ASOs to rapidly analyze individuals at risk for cystic fibrosis for the presence or absence of the $\Delta F508$ mutation (Figure I-7-13). DNA representing the CFTR gene from the individuals to be tested is amplified by PCR and spotted onto a filter (dot blot or slot blot). Two labeled ASO probes are synthesized in the laboratory, each specific enough to react with only one allele of the gene. One probe binds only to the normal CFTR gene, the other binds only to the defective gene with the $\Delta F508$ mutation. Analysis of the dot blot readily

Note

PCR Testing for Sickle Cell Disease with ASO Probes

RFLP analysis of the β -globin gene for genetic testing has been replaced by PCR in combination with ASO probes on dot blots. The blot shown here corresponds to the family whose pedigree is shown in Figure I-7-9. In the mutant allele, glutamate (E) at codon 6 is replaced by valine (V).



reveals which alleles each individual carries. ASOs are also available for other mutant cystic fibrosis alleles as well as for other diseases, including sickle cell disease and phenylketonuria.

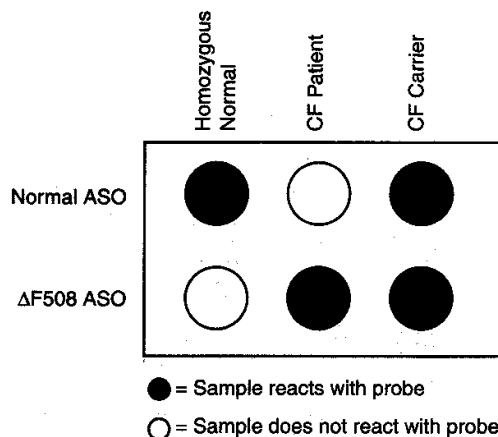


Figure I-7-13. The Use of ASO Probes for Cystic Fibrosis

Genetic Fingerprinting Using PCR Amplification of Microsatellite Sequences

Most repetitive sequences are not in coding regions. Because expansion of these sequences in spacer DNA rarely affects any function, they become highly polymorphic in the population and can be used to develop a genetic fingerprint. Such fingerprints are important in paternity testing and forensic medicine. Very small samples containing dried tissue can be analyzed by this technique.

Paternity Testing Using PCR Amplification of Microsatellite Sequences

Although microsatellite sequences are distributed throughout the DNA, a single region may be selectively amplified by using primers that overlap the 3' flanking regions adjacent to the repeat analyzed. Such primers amplify "single-locus" sequences, which are highly polymorphic within the population. Because humans have pairs of chromosomes, each individual will have a maximum of two bands, one from the father and one from the mother. An example is shown in Figure I-7-14.

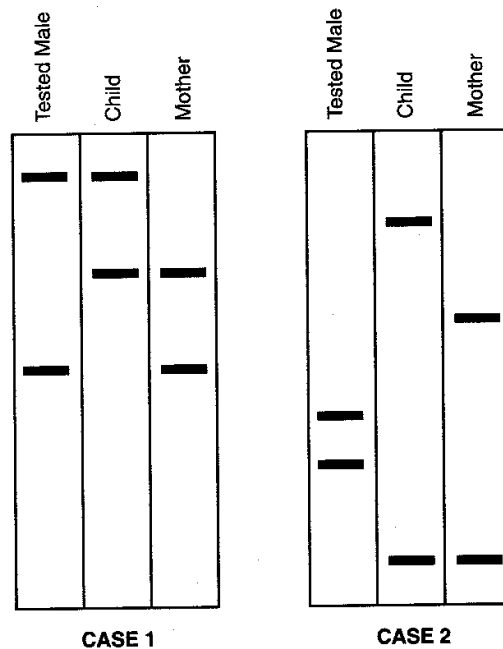


Figure I-7-14. Paternity Testing

Question: Are the tested males (Figure I-7-14) in case 1 and case 2 the fathers of the children?

Approach:

- Identify the child's band in common with the mother. The other band must be from the father.
- Does the tested male have a band matching the band from the father?
- Draw conclusion.

Case 1: The tested male in case 1 may be the father, as he shares a band with the child. We cannot be certain, however, because many other men in the population could have this same band. Matches are required at several different loci to indicate with high probability that he is the father.

Case 2: The tested male in case 2 cannot be the father, as neither of his bands is shared with the child.

In practice, 4–10 different polymorphisms are necessary to indicate a match.

DETECTING HIV INFECTION

ELISA and Western Blot (Immunoblot)

Patients and blood donors are routinely screened for exposure to HIV by means of ELISA and Western blot assays of blood samples (Figure I-7-15). The assays are designed to detect antibodies to HIV in the blood of the test subject. The ELISA is used as the primary screening assay because it is very sensitive. Because the reference interval for the test is set to include everyone with antibodies to HIV, it also gives false positives and thus has a rather low positive predictive value, especially in low-risk populations. The Western blot (or immunoblot) is used as the confirmatory test for HIV exposure. In the Western blot technique, specific HIV proteins are separated by gel electrophoresis and blotted to a filter. The filter is incubated with the test sample. If the sample contains antibodies to HIV, they will bind to the proteins on the filter. The filter is next washed and incubated with a labeled goat anti-human IgG to visualize any bound human antibodies. The Western blot is highly specific. The combination of an ELISA and Western blot has a positive predictive value of greater than 99%.

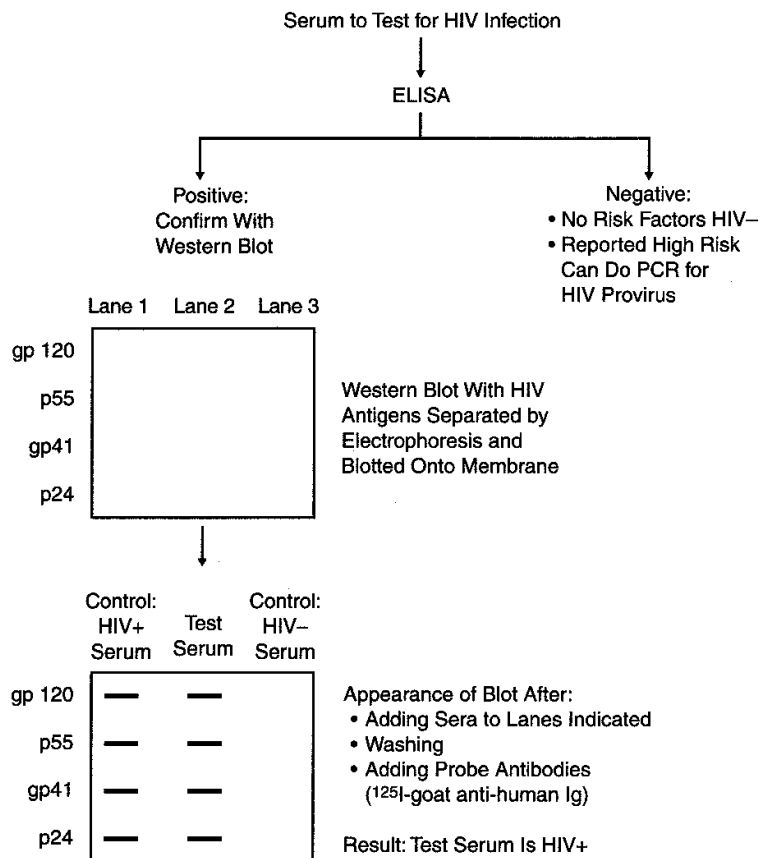


Figure I-7-15. ELISA and Western Blot (Immunoblot) for Detecting Antibodies to HIV

PCR Amplification of the HIV Proviral DNA

Both the ELISA and Western blot suffer from the problem that antibodies may not appear in an exposed individual's blood until months after the initial exposure. Methods for using PCR to screen blood samples for HIV are being developed. PCR amplification of the HIV proviral DNA provides the ability to detect HIV at earlier stages of infection, because the viral nucleic acid is present immediately upon exposure. It is used to detect HIV infection in newborns whose mothers are HIV positive.

Reverse Transcriptase PCR (RT-PCR)

An RT-PCR is a modified version of the PCR technique in which cDNA produced from an RNA sample using reverse transcriptase is amplified. RT-PCR is used to measure the quantity of HIV (an RNA virus) circulating in the blood (viral load) when monitoring the response to drugs or the status of infection in patients with HIV infection.

Northern Blotting

Northern blots analyze RNA extracted from a tissue and are typically used to determine which genes are being expressed. One example is shown in Figure I-7-16. The goal is to determine which tissues express the *FMR1* gene involved in fragile X syndrome. RNA samples from multiple tissues have been separated by electrophoresis, blotted, and probed with a ^{32}P -cDNA probe from the *FMR1* gene. The results are consistent with high-level expression (a 4.4-kb transcript) of this gene in brain and testis and lower level expression in the lung. In the heart, the gene is also expressed, but the transcripts are only 1.4 kb long.

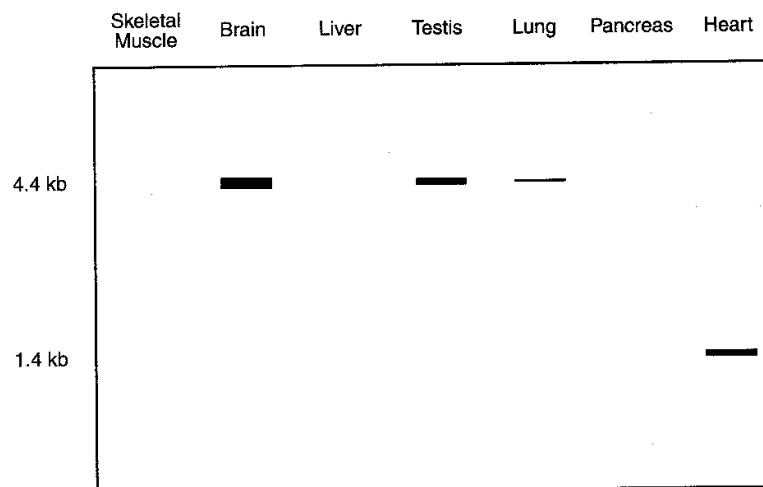


Figure I-7-16. Northern Blot to Determine Pattern of *FMR1* Expression

Clinical Correlate

Fragile X Syndrome

Fragile X syndrome is the leading known cause of inherited mental retardation. Other symptoms include large ears, elongated face, hypermobile joints, and macroorchidism in postpubertal males. The gene involved, *FMR1*, maps to the long arm of the X chromosome. See Section II, Chapter 1, for a further discussion of this single-gene disorder.

Chapter Summary

Southern blot: DNA restriction fragments (RFLP analysis)

- Diversity: mutations in RE sites and expansion of repetitive sequences
- RE sites serve as surrogate probes for genetic analysis.
- Genetic diagnosis usually direct and requires pedigree.
- Infrequently used in medical diagnosis

Northern blots: RNA

- Gene expression

Western blot: protein

- Often used to detect antibodies or antigens
- Confirmation of positive ELISA for HIV infection (more specific test for anti-HIV antibodies)

Polymerase chain reaction (PCR):

- Amplifies a DNA sequence selected by primers complementary to 3' borders
- Uses heat-stable DNA polymerase (Taq polymerase)
- PCR-amplified DNA sequences tested for (1) length difference (electrophoresis) and (2) sequence difference (allele-specific probes, ASOs)

Reverse transcriptase (RT)-PCR:

- Amplifies RNA
- RNA-directed DNA synthesis (reverse transcription) produces cDNA.
- cDNA amplified with PCR
- Can be quantitated for viral-load measurement in HIV patients

Review Questions

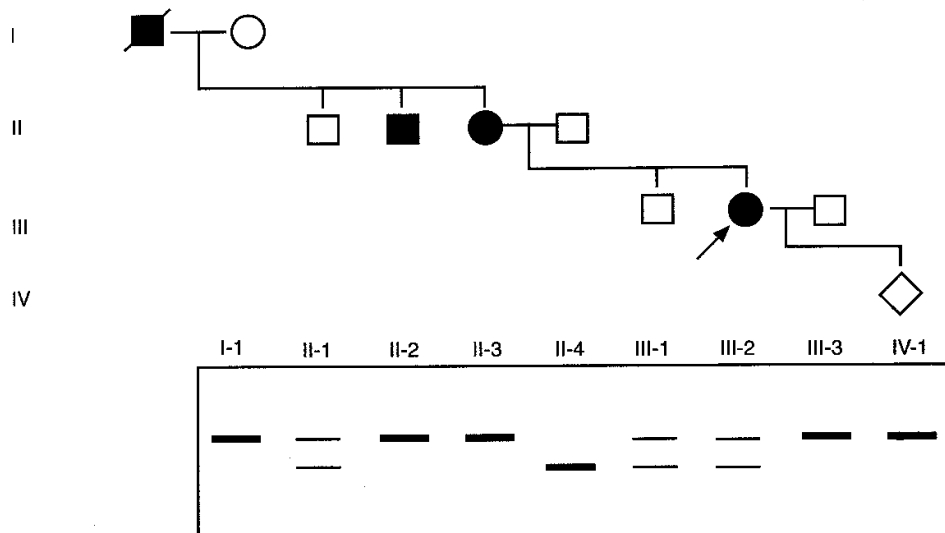
Select the ONE best answer.

1. A genetic progressive neurodegenerative disease is inherited only from affected females. Symptoms include encephalomyopathy, lactic acidosis, and strokelike episodes. The most likely genetic defect is in the gene encoding
 - A. hepatic glucose 6-phosphatase
 - B. mitochondrial tRNA^{leu}
 - C. cytoplasmic carnitine acyltransferase
 - D. hepatic glycogen phosphorylase
 - E. microsomal cytochrome P₄₅₀

2. Paternal relationship between a man and infant can best be determined by the technique commonly referred to as DNA fingerprinting. Which of the following sequences is most conveniently analyzed in a DNA fingerprint?
- Histocompatibility loci
 - Centromeres
 - Microsatellite tandem repeats (STRs)
 - Restriction enzyme sites
 - Single-copy sequences

Items 3–4

3. A 22-year-old woman with Marfan syndrome (a dominant genetic disorder) is referred to a prenatal genetics clinic during her 10th week of pregnancy. Her family pedigree is shown below along with a Taq I RFLP analysis of genomic DNA from family members. Using a probe for the *Fibrillin* gene, two restriction fragments are seen in this family.



3. Which of the following represents the best conclusion about the fetus (IV-1)? The fetus:
- Will develop Marfan syndrome
 - Has a 25% chance of developing Marfan syndrome
 - Has a 50% chance of developing Marfan syndrome
 - Will not develop Marfan syndrome but will be a carrier of the disease allele
 - Will not develop Marfan syndrome

4. Molecular analysis of the fibrillin gene in these family members identified the mutation associated with Marfan syndrome as a single base substitution at the 3' end of exon 2. The result of this substitution was a deletion of the entire exon 2 from the mRNA, and consequently 41 amino acids from the fibrillin protein. The mutation was most likely a
- missense mutation
 - nonsense mutation
 - frameshift mutation
 - splice mutation
 - silent mutation

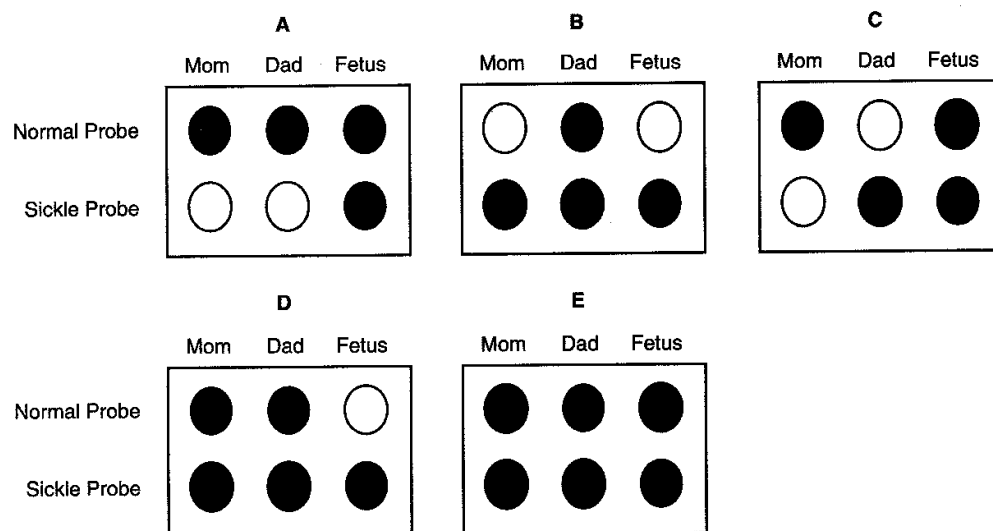
Items 5–6

Sickle cell anemia is caused by a missense mutation in codon 6 of the β -globin gene.

	Codon number			
	5	6	7	8
Normal allele	CCT	GAG	GAG	AAG
Mutant allele	CCT	GTG	GAG	AAG

A man with sickle cell disease and his phenotypically normal wife request genetic testing because they are concerned about the risk for their unborn child. DNA samples from the man and the woman and from fetal cells obtained by amniocentesis are analyzed using the PCR to amplify exon 1 of the β -globin gene.

5. When the amplified material was tested under stringent conditions using allele-specific probes, the results indicated that the child was heterozygous at the β -globin locus. Which dot blot shown below best represents the results from this family?



4. Which 12-base oligonucleotide sequence was most likely used as a specific probe complementary to the coding strand of the sickle cell allele?
- CCTCACCTCAGG
 - CCTGTGGAGAAG
 - GGACACCTCTTC
 - CTTCTCCACAGG
 - CTTCTCCTCAGG

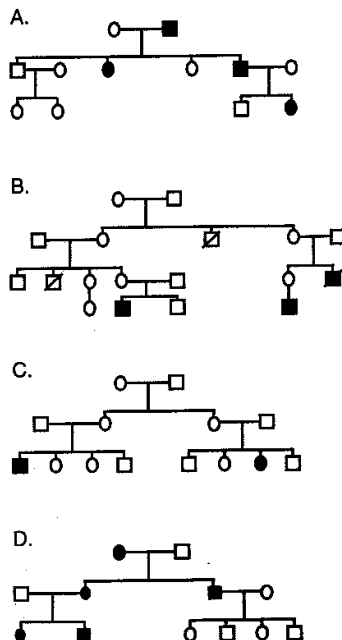
Items 7-8

A 4-year-old toddler with cystic fibrosis (CF) is seen by his physician for an upper respiratory infection with *Pseudomonas aeruginosa*. He is started on oral norfloxacin and referred to a CF center as a potential candidate for gene therapy. Prior genetic testing of the patient identified the mutation causing cystic fibrosis as a 3-base-pair deletion in exon 10 of the CF gene. The nucleotide sequences of codons 506-511 in this region of the normal and mutant alleles are compared below.

Codon Number	506	507	508	509	510	511
Normal Gene	ATC	ATC	TTT	GGT	GTT	TCC
Mutant Gene	ATC	AT*	TT	GGT	GTT	TCC

3-base deletion

7. Which of the following pedigrees best illustrates the mode of inheritance of this patient's genetic disease?



8. What effect will this patient's mutation have on the amino-acid sequence of the protein encoded by the CF gene?

	U	C	A	G	
U	UUU } Phe UUC } UUA } Leu UUG }	UCU } Ser UCC } UCA } UCG }	UAU } Tyr UAC } UAA } Stop UAG }	UGU } Cys UGC } UGA } Stop UGG } Trp	U C A G
C	CUU } CUC } CUA } CUG }	CCU } CCC } CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } CGA } CGG }	U C A G
A	AUU } AUC } AUA } AUG } Met	ACU } ACC } ACA } ACG }	AAU } Asn AAC } AAA } AAG } Lys	AGU } Ser AGC } AGA } AGG } Arg	U C A G
G	GUU } GUC } GUA } GUG }	GCU } GCC } GCA } GCG }	GAU } Asp GAC } GAA } GAG } Glu	GGU } GGC } GGA } GGG }	U C A G

- A. Deletion of a phenylalanine residue with no change in C-terminal sequence
 B. Deletion of a leucine residue causing a change in the C-terminal sequence
 C. Deletion of a phenylalanine residue causing a change in the C-terminal sequence
 D. Deletion of a leucine residue with no change in C-terminal sequence

Items 9–10

Spinobulbar muscular atrophy (SMA) is an X-linked recessive motor neuron disease, arising from a mutation in the androgen receptor gene. Shown in the diagram below are results of DNA sequencing studies of the receptor gene from healthy individuals and SMA patients. The normal allele of the gene has 10 adjacent glutamine codons (CAG) in exon 1, whereas the SMA allele has 40 of these CAG repeats in the same region (a mutation referred to as triplet repeat expansion).

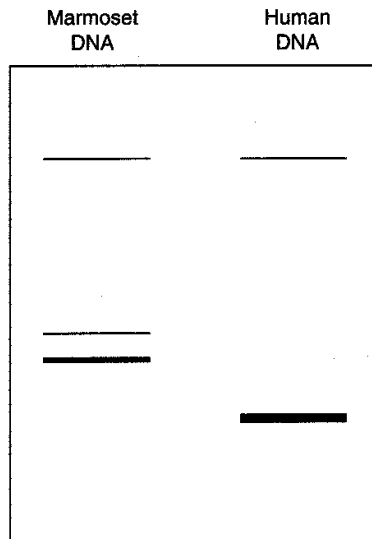
Normal gene...5'UTR...CAG(CAG)₈CAG...

SMA allele...5'UTR...CAG(CAG)₃₈CAG...

9. The most appropriate DNA-based test to detect this mutation would utilize PCR primers:
- A. Complementary to the CAG repeat followed by gel electrophoresis of the PCR products.
 B. Flanking the CAG repeat followed by a dot-blot using a CAG repeat-specific DNA probe.
 C. Flanking the CAG repeat followed by gel electrophoresis of the PCR products.
 D. Complementary to the CAG repeat followed by a dot-blot using a CAG repeat-specific DNA probe.

10. The absence of appropriate androgen signaling in patients with SMA diminishes protein synthesis, especially in muscle, contributing to decreased muscle mass. The product normally encoded by the SMA gene would best be described as a
- A. Tyrosine kinase
 - B. Zinc-finger protein
 - C. 5α -Steroid reductase
 - D. Serine-threonine kinase
 - E. Trimeric G protein
11. A couple is expecting their first child and have consulted a genetic counselor because the woman had a brother who died of sickle cell anemia. There is also a history of disease in the man's family. Fetal cells are obtained by amniocentesis. Which test would best determine whether the fetus would be born with the disease?
- A. Western blot
 - B. Hemoglobin electrophoresis
 - C. Northern blot
 - D. PCR with allele-specific probes on a dot-blot

12. mRNA encoding glucose 6-phosphatase was isolated from baboon liver and used to make a ^{32}P -cDNA probe. DNA was then isolated from marmoset and from human tissue, digested with a restriction endonuclease, Southern blotted, and probed with the ^{32}P -cDNA. Which of the following conclusions can be drawn from the results of this analysis shown below?



- A. The glucose 6-phosphatase gene is present in baboon, marmoset and human liver.
- B. Both marmoset and human liver express the glucose 6-phosphatase gene.
- C. There are two glucose 6-phosphatase genes in the human liver.
- D. The glucose 6-phosphatase gene is on different chromosomes in the marmoset and in the human.
- E. The human and marmoset tissue used in this experiment is from liver.

Answers

1. **Answer: B.** "Inheritance only from affected females" strongly suggests a defect in a mitochondrial gene. Defects in mitochondrial gene expression phenotypically present as problems in nerves and/or muscles, tissues with high aerobic metabolism.
2. **Answer: C.** VNTR sequences are amplified using a PCR and analyzed by gel electrophoresis. Although RFLP analysis could potentially be used for this purpose, it is not the method of choice.
3. **Answer: A.** Comparison of the RFLP patterns of II-3 and III-2 indicates that the upper (larger) fragment is associated with the disease-producing allele. This can be confirmed by looking at other affected family members. Because the fetus, IV-1, has inherited this fragment from the mother and the disease is dominant, the fetus will develop Marfan syndrome.
4. **Answer: D.** At the 3' border of exon 2 with intron 2, a splice donor site is present. A mutation could cause exon skipping. Choices A, B, and C would not affect transcription or mRNA processing.
5. **Answer: C.** This is the only pattern showing a father homozygous for the sickle-cell allele. Mom is homozygous normal, and the fetus is heterozygous.
6. **Answer: D.** The complementary probe will be antiparallel to the coding strand of the mutant allele, with all sequences written 5' → 3'.
7. **Answer: C.** CF is an autosomal recessive disease. Choices A, B, and D best illustrate AD, XR and mitochondrial pedigrees, respectively.
8. **Answer: A.** Deletion of CTT results only in the loss of phe 508; ile 507 and the C-terminal sequence are unaltered since ATC and ATT both code for ile (the coding sequence is unchanged).
9. **Answer: C.** PCR primers flanking the mutation, as well as Southern blotting, would be needed to detect the extent of the triplet repeat expansion.
10. **Answer: B.** All intracellular receptors for steroid hormones are zinc-finger proteins.
11. **Answer: D.** DNA-based test would have to be used on the cells obtained from amniocentesis. They would not express the β -globin gene.
12. **Answer: A.** All three tissues contain the gene (the probe was produced from baboon mRNA, implying the gene is also there).

Amino Acids, Proteins, and Enzymes

8

AMINO ACIDS

General Structure

All amino acids have a central carbon atom attached to a carboxyl group, an amino group, and a hydrogen atom. The amino acids differ from one another only in the chemical nature of the side chain (R).

Classification

The amino acids can be classified as either hydrophobic or hydrophilic, depending on the ease with which their side chains interact with water. In general, proteins fold so that amino acids with hydrophobic side chains are in the interior of the molecule where they are protected from water and those with hydrophilic side chains are on the surface.

Hydrophobic amino acids are shown in Figure I-8-1. Additional points about some of these amino acids include:

- Phenylalanine and tyrosine are precursors for catecholamines.
- Tryptophan can form serotonin and niacin.
- Valine, leucine, and isoleucine are branched-chain amino acids whose metabolism is abnormal in maple syrup urine disease (discussed in Chapter 17).
- Proline is a secondary amine whose presence in a protein disrupts normal secondary structure.

Hydrophilic amino acids have side chains that contain O or N atoms. Some of the hydrophilic side chains are charged at physiologic pH. The acidic amino acids (aspartic and glutamic acids) have carboxyl groups that are negatively charged, whereas the basic amino acids (lysine, arginine, and histidine) have nitrogen atoms that are positively charged. The structures of the hydrophilic amino acids are shown in Figure I-8-2. Additional points about some of these amino acids include:

- Serine and threonine are sites for O-linked glycosylation of proteins, a posttranslational modification that should be associated with the Golgi apparatus.
- Asparagine is a site for N-linked glycosylation of proteins, a posttranslational modification that should be associated with the endoplasmic reticulum.
- Cysteine contains sulfur and can form disulfide bonds to stabilize the shape (tertiary structure) of proteins. Destroying disulfide bonds denatures proteins.
- Methionine, another sulfur-containing amino acid, is part of S-adenosylmethionine (SAM), a methyl donor in biochemical pathways.

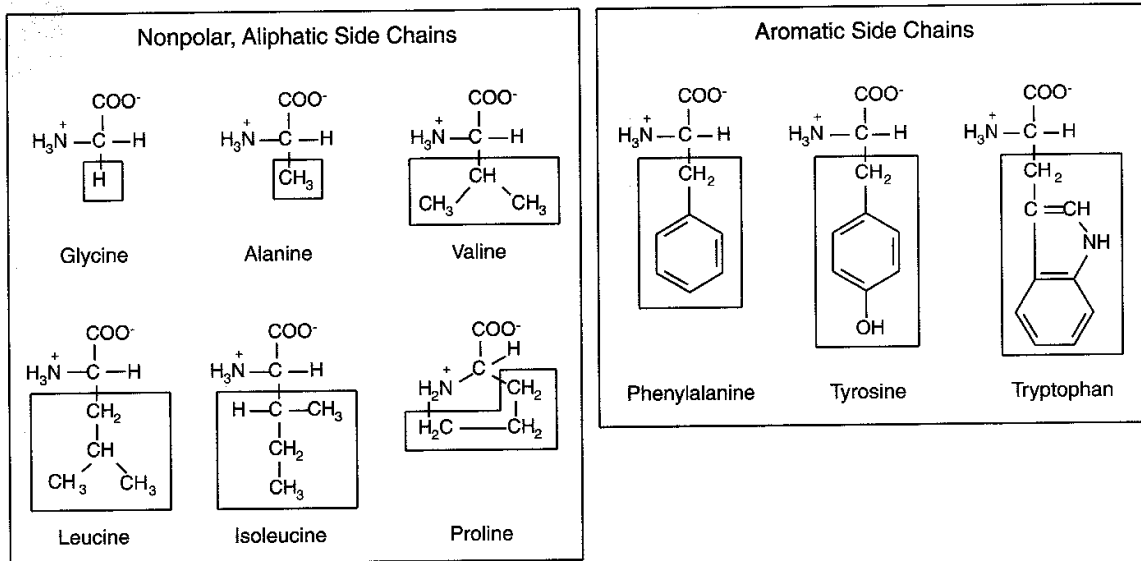


Figure I-8-1. The Hydrophobic Amino Acids

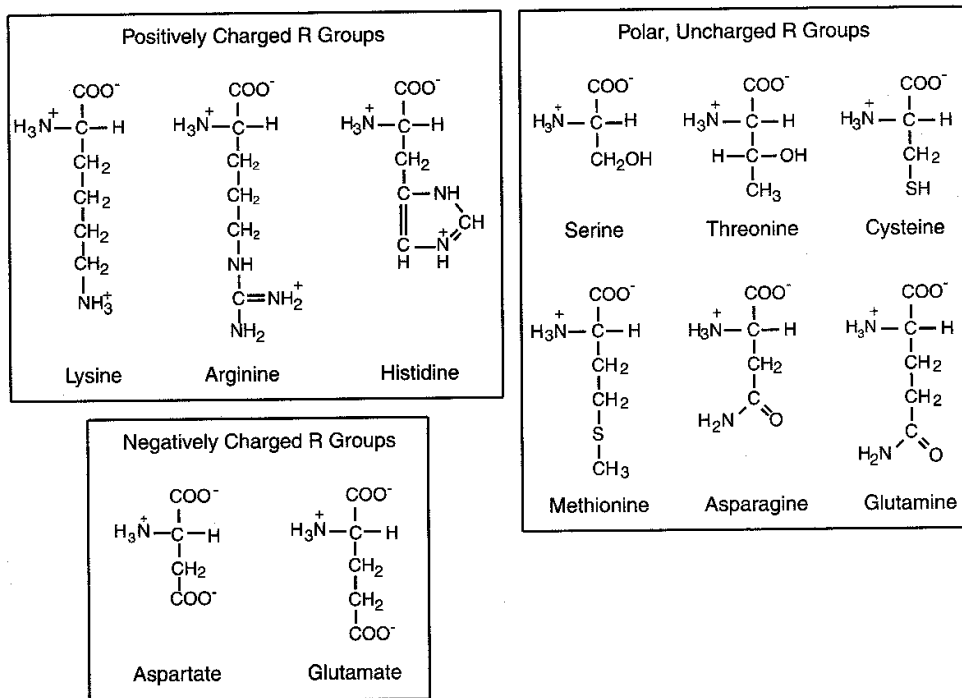


Figure I-8-2. The Hydrophilic Amino Acids

ACID-BASE PROPERTIES OF AMINO ACIDS AND PROTEINS

Amino acids and proteins have groups that can release and bind protons (H^+); in other words, they have acid-base character. The groups with acid-base character can be generally represented in one of the two ways shown below.

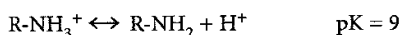
Structures with a carboxyl group:

Acid (A) Base (B)



Structures with an amino group:

Acid (A) Base (B)



The pK value indicates the pH range in which the dissociation of the H^+ occurs. More specifically, the pK is the pH at which the group is 50% dissociated, or half of it is in the acid form and half in the base form. These values of pK are used in determining the net charge a substance (amino acid, protein, drug) will have in the body and which ones will act as good buffers to keep the pH constant, and to understand pathologic states of acidosis and alkalosis.

Classifying Acidic and Basic Amino Acids

Amino acids are classified as acidic or basic according to their R groups because in proteins, these are the only groups that can dissociate. The α -amino and α -carboxyl groups are in peptide bonds and lose their acid-base character. This system of classification can be confusing since the words "acid" and "base" are used in a way slightly different than discussed in the section above.

If an amino acid has a carboxyl in its R group, that amino acid is said to be acidic because the carboxyl dissociates in the acidic (pH < 7) range of the pH scale. Therefore, aspartic acid (pK 4) and glutamic acid (pK 4) are acidic amino acids. At physiologic pH:

- Their R groups are completely dissociated.
- They carry a charge, e.g., are ionized.
- They contribute negative charge to proteins that contain them.

If an amino acid has an amino group as part of the R structure, that amino acid is said to be basic because the amino group dissociates in the basic (pH > 7) range of the pH scale. Therefore lysine (pK 10) and arginine (pK 13) are basic amino acids. At physiologic pH:

- Their R groups are completely undissociated.
- They carry a charge, e.g., are ionized.
- They contribute positive charge to proteins that contain them.

Histidine is often classified as a basic amino acid because it has a version of an amino group in the imidazole ring of its R group. However, its pK of 6.5–7 is close to neutral. Nevertheless, at physiologic pH, a (variable) percentage of the histidine in proteins will carry a positive charge and so it best fits that classification.

From this discussion, one could deduce that proteins, such as histones, that have an abundance of lysine and arginine would carry substantial positive charge at physiologic pH. Histones are found in nucleosomes, where they package negatively charged DNA. If the pH of the nucleus became too basic, the histones would lose this positive charge and their ability to function. The proper function of proteins is highly dependent on maintaining physiologic pH within a very narrow range.

Bridge to Pharmacology

Aspirin (acetylsalicylic acid) has a carboxyl group and hence is classified as an acidic drug. To increase excretion of aspirin, the pH of the urine is raised to produce the ionized form, in this case the dissociated form.

Bridge to Pathology

In ischemic episodes such as occur in myocardial infarction, lack of oxygen forces cells to rely on anaerobic glycolysis, which increases production of lactic acid. The consequent intracellular acidosis can cause proteins to denature and precipitate, leading to coagulation necrosis.

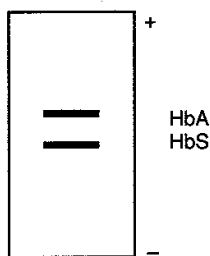
In a Nutshell

Buffers

Buffers resist changes in pH. Substances can act as buffers at their pK values. In proteins, the amino acids with ionizable R-groups can act as buffers, although the only amino acid that is useful in maintaining physiologic pH (7.2–7.4) is histidine with an R-group pK near 7. Hemoglobin can act as an intracellular buffer in red blood cells because it contains histidyl residues.

Note**Hemoglobin Electrophoresis**

Hemoglobin electrophoresis takes advantage of the differences in pI values between HbA and HbS (Glu6Val; glutamate at position 6 has been replaced by valine).



Hemoglobin electrophoresis at pH 8.4

Determining the Charge on an Amino Acid or Protein at Physiologic pH

When assessing the charge on a structure at physiologic pH, the isoelectric point (pI) is a useful reference. The pI is the pH at which the structure carries no net charge. For instance, Figure I-8-3 shows the structure of aspartic acid at four different pH values.

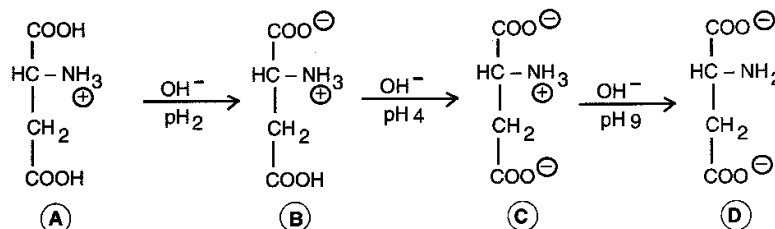


Figure I-8-3. Aspartic Acid

Only structure B has no net charge, this is the isoelectric structure. Most of the aspartic acid would be found in this form at pH 3, and therefore the pI for aspartic acid is 3 ($pI = 3$). It's not difficult to determine the pI for a simple structure like a free amino acid, although there is no simple way of quickly determining the pI for a protein. If the pI is known, however, one can easily deduce whether the protein will have a negative, positive, or zero net charge at any pH:

- If the pH is more acidic than the pI (e.g., if the pH is a smaller number than the pI), the protein will have a net positive charge.
- If the pH is more basic than the pI (e.g., if the pH is a larger number than the pI), the protein will have a net negative charge.
- The larger the magnitude of the difference between pH and pI, the more charge on the protein.

These same concepts apply to any acid-base structure, not just to proteins.

PROTEIN TURNOVER AND NITROGEN BALANCE

When older proteins are broken down in the body, they must be replaced. This concept is called protein turnover, and different types of proteins have very different turnover rates. Protein synthesis occurs during the process of translation on ribosomes. Protein breakdown occurs generally in two cellular locations:

- Lysosomal proteases digest endocytosed proteins.
- Cytoplasmic complexes, called proteasomes, digest older or abnormal proteins that have been tagged with ubiquitin for destruction.

Essential Amino Acids

All 20 types of amino acids are required for protein synthesis. These amino acids can be derived from digesting dietary protein and absorbing their constituent amino acids or, alternatively, by synthesizing them *de novo*.

The 10 amino acids listed in Table I-8-1 cannot be synthesized in humans and therefore must be provided from dietary sources. These are called the essential amino acids. Arginine is required only during periods of growth, or positive nitrogen balance.

Table I-8-1. Essential Amino Acids

Arginine*	Methionine
Histidine	Phenylalanine
Isoleucine	Threonine
Leucine	Tryptophan
Lysine	Valine

*Essential only during periods of positive nitrogen balance.

Nitrogen Balance

Nitrogen balance is the (normal) condition in which the amount of nitrogen incorporated into the body each day exactly equals the amount excreted.

Negative nitrogen balance occurs when nitrogen loss exceeds incorporation and is associated with:

- Protein malnutrition (kwashiorkor)
- A dietary deficiency of even one essential amino acid
- Starvation
- Uncontrolled diabetes
- Infection

Positive nitrogen balance occurs when the amount of nitrogen incorporated exceeds the amount excreted and is associated with:

- Growth
- Pregnancy
- Recovery phase of injury or surgery
- Recovery from condition associated with negative nitrogen balance

BIOCHEMICAL REACTIONS

Chemical reactions have two independent properties, their energy and their rate. Table I-8-2 compares these two properties. ΔG represents the amount of energy released or required per mole of reactant. The amount or sign of ΔG indicates nothing about the rate of the reaction.

Table I-8-2. Comparison of Energy and Rate

Energy (ΔG)	Rate (v)
Not affected by enzymes	Increased by enzymes
$\Delta G < 0$, thermodynamically spontaneous (energy released, often irreversible) $\Delta G > 0$, thermodynamically nonspontaneous (energy required) $\Delta G = 0$, reaction at equilibrium (freely reversible) ΔG^0 = energy involved under standardized conditions	Decrease energy of activation, ΔG^\ddagger

The rate of the reaction is determined by the energy of activation (ΔG^\ddagger), which is the energy required to initiate the reaction. ΔG and ΔG^\ddagger are represented in Figure I-8-4. Enzymes lower the energy of activation for a reaction; they do not affect the value of ΔG or the equilibrium constant for the reaction, K_{eq} .

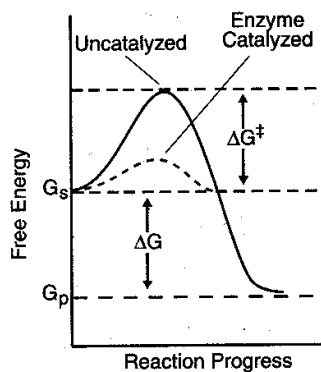
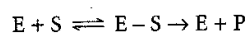


Figure I-8-4. Energy Profile for a Catalyzed and Uncatalyzed Reaction

Michaelis-Menten Equation

The Michaelis-Menten equation describes how the rate of the reaction, V , depends on the concentration of both the enzyme $[E]$ and the substrate $[S]$, which forms product $[P]$.



$$V = \frac{k_2 [E] [S]}{K_m + [S]} \text{ or, with } [E] \text{ held constant, } V = \frac{V_{\max} [S]}{K_m + [S]} \quad \text{Note: } V_{\max} = k_2 [E]$$

V_{\max} is the maximum rate possible to achieve with a given amount of enzyme. The only way to increase V_{\max} is by increasing the $[E]$. In the cell, this can be accomplished by inducing the expression of the gene encoding the enzyme.

The other constant in the equation, K_m is often used to compare enzymes. K_m is the substrate concentration required to produce half the maximum velocity. Under certain conditions, K_m is a measure of the affinity of the enzyme for its substrate. When comparing two enzymes, the one with the higher K_m has a lower affinity for its substrate. The K_m value is an intrinsic property of the enzyme-substrate system and cannot be altered by changing $[S]$ or $[E]$.

When the relationship between $[S]$ and V is determined in the presence of constant enzyme, many enzymes yield the graph shown in Figure I-8-5, a hyperbola.

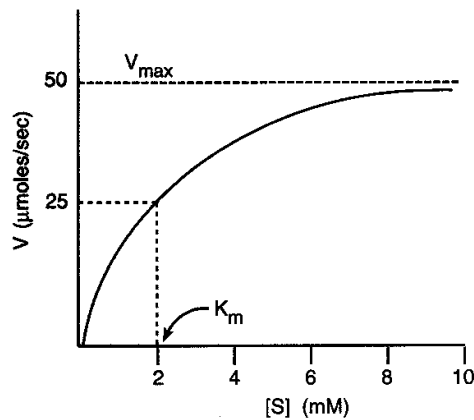


Figure I-8-5. Michaelis-Menten Plot

Lineweaver-Burk Equation

The Lineweaver-Burk equation is a reciprocal form of the Michaelis-Menten equation. The same data graphed in this way yield a straight line as shown in Figure I-8-6. The actual data are represented by the portion of the graph to the right of the y -axis, but the line is extrapolated into the left quadrant to determine its intercept with the x -axis. The intercept of the line with the x -axis gives the value of $-1/K_m$. The intercept of the line with the y -axis gives the value of $1/V_{\max}$.

$$\frac{1}{V} = \frac{K_m}{V_{\max}} \frac{1}{[S]} + \frac{1}{V_{\max}}$$

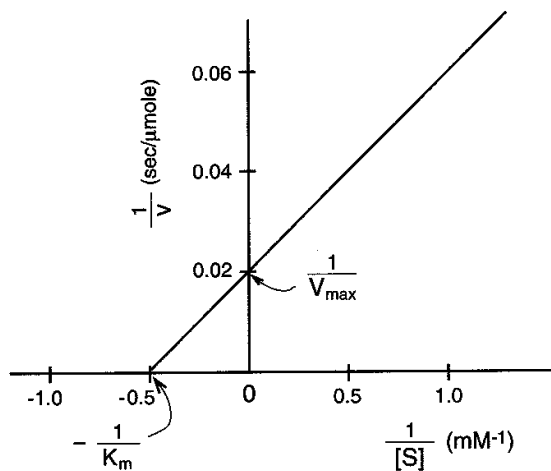


Figure I-8-6. Lineweaver-Burk Plot

Note**Drugs That Competitively Inhibit Enzymes**

Many drugs are competitive inhibitors of key enzymes in pathways. The statin drugs (lovastatin, simvastatin), used to control blood cholesterol levels, competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase in cholesterol biosynthesis. Methotrexate, an antineoplastic drug, competitively inhibits dihydrofolate reductase, depriving the cell of active folate needed for purine and deoxythymidine synthesis, thus interfering with DNA replication during S phase.

Inhibitors and Activators

Three important classes of inhibitors are shown in Table I-8-3. Competitive inhibitors resemble the substrate and compete for binding to the active site of the enzyme. Noncompetitive inhibitors do not bind at the active site. They bind to regulatory sites on the enzyme. Irreversible inhibitors inactivate the enzyme similar to removing enzyme from the assay.

Table I-8-3. Important Classes of Enzyme Inhibitors

Class of Inhibitor	K_m	V_{max}
Competitive, reversible	Increase	No effect
Noncompetitive, reversible	No effect	Decrease
Irreversible (inactivator)	No effect	Decrease

The effects of these classes of inhibitors on Lineweaver-Burk kinetics are shown in Figures I-8-7 and I-8-8. Notice that on a Lineweaver-Burk graph, inhibitors always lie above the control on the right side of the y-axis. A line below the control might represent the addition of an activator.

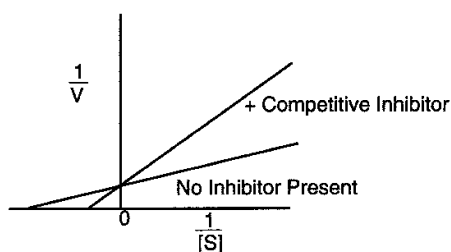


Figure I-8-7. Lineweaver-Burk Plot of Competitive Inhibition

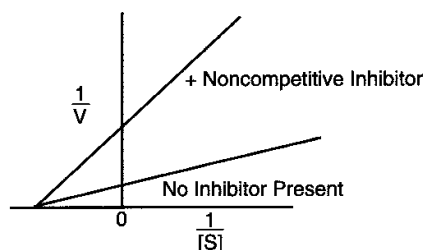


Figure I-8-8. Lineweaver-Burk Plot of Noncompetitive Inhibition

Cooperative Enzyme Kinetics

Certain enzymes do not show the normal hyperbola when graphed on a Michaelis-Menton plot ($[S]$ versus V), but rather show sigmoid kinetics owing to cooperativity among substrate binding sites (Figure I-8-9). Cooperative enzymes have multiple subunits and multiple active sites. Enzymes showing cooperative kinetics are often regulatory enzymes in pathways (for example, phosphofructokinase-1 [PFK-1] in glycolysis).

In addition to their active sites, these enzymes often have multiple sites for a variety of activators and inhibitors (e.g., AMP, ATP, citrate, fructose-2,6-bisphosphate [F2,6-BP]). Cooperative enzymes are sometimes referred to as allosteric enzymes because of the shape changes that are induced or stabilized by binding substrates, inhibitors, and activators.

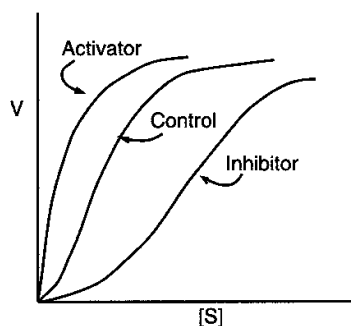


Figure I-8-9. Cooperative Kinetics

Chapter Summary

Amino acids:

- pK is pH at which a group is 50% dissociated.
- Basic amino acids have "R-groups" with $pK > 7$ (lysine, arginine).
- Acidic amino acids have "R-groups" with $pK < 7$ (aspartic acid, glutamic acid).
- Histidine buffers well in proteins ($pK = 7$).
- pI is pH at which there is no net charge.

Protein turnover and nitrogen balance:

- Essential amino acids: phe, val, trp, thr, ile, met, his, lys, leu, arg (only during positive N-balance)
- Negative: nitrogen lost > nitrogen gained (illness, protein malnutrition, deficiency of an essential amino acid)
- Positive: nitrogen lost < nitrogen gained (growth, pregnancy, convalescence)

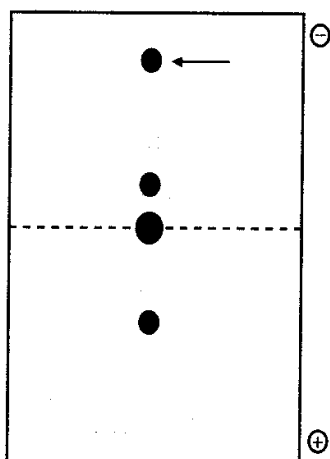
Enzyme kinetics:

- Enzymes do not affect energy of reaction, ΔG .
- Enzymes lower energy of activation, ΔG^\ddagger .
- V_{max} = maximum velocity with a specified amount of enzyme
- K_m = [substrate] required to produce half of the V_{max}
- Inhibitors include (1) competitive (increases K_m), (2) noncompetitive (decreases V_{max}), (3) irreversible (decreases V_{max}).
- Rate-limiting enzymes may show cooperative kinetics.

Review Questions

Select the ONE best answer.

1. The peptide ala-arg-his-gly-glu is treated with peptidases to release all of the amino acids. The solution is adjusted to pH 7, and electrophoresis is performed. In the electrophoretogram depicted below, the amino acid indicated by the arrow is most likely to be



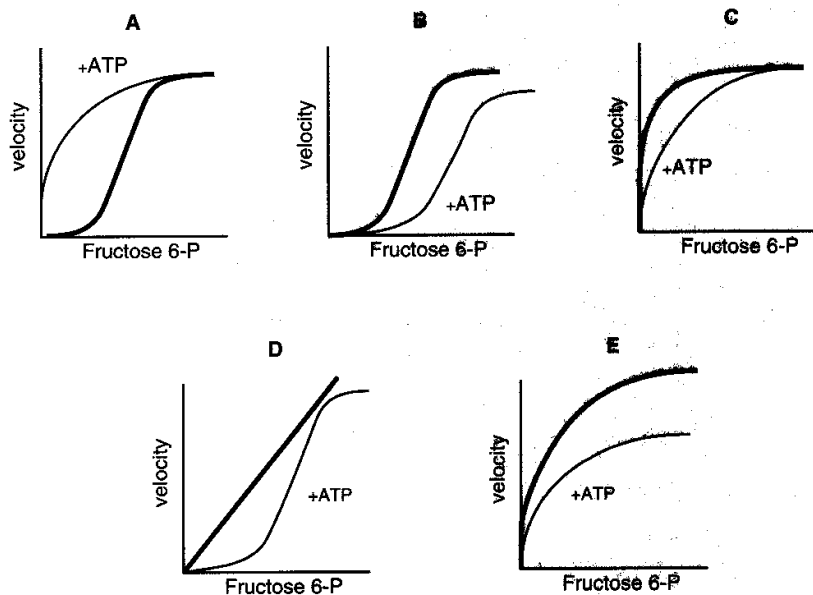
- A. glycine
B. arginine
C. glutamate
D. histidine
E. alanine
2. The reaction catalyzed by hepatic phosphofructokinase-1 has a ΔG^0 value of -3.5 kcal/mol. This value indicates that under standard conditions this reaction
- A. is reversible
B. occurs very slowly
C. produces an activator of pyruvate kinase
D. is inhibited by ATP
E. has a low energy of activation
F. will decrease in activity as the pH decreases
G. cannot be used for gluconeogenesis
H. shows cooperative substrate binding
I. is indirectly inhibited by glucagon
J. is stimulated by fructose 2,6-bisphosphate

3. The activity of an enzyme is measured at several different substrate concentrations, and the data are shown in the table below.

[S] (mM)	v_o (mmole/sec)
0.010	2.0
0.050	9.1
0.100	17
0.500	50
1.00	67
5.00	91
10.0	95
50.0	99
100.0	100

K_m for this enzyme is approximately

- A. 50.0
 B. 10.0
 C. 5.0
 D. 1.0
 E. 0.5
4. Which of the diagrams illustrated below best represents the effect of ATP on hepatic phosphofructokinase-1 (PFK-1)?



Answers

1. **Answer: B.** Arginine is the most basic of the amino acids ($pI \sim 11$) and would have the largest positive charge at pH 7.
2. **Answer: G.** The negative ΔG^0 value indicates the reaction is thermodynamically favorable (irreversible), requiring a different bypass reaction for conversion of F1, 6BP to F6P in the gluconeogenic pathway.
3. **Answer: E.** Since the apparent V_{\max} is near 100 mmole/sec, $V_{\max}/2$ equals 50 mmole/sec. The substrate concentration giving this rate is 0.50 mM.
4. **Answer: B.** Sigmoidal control curve with ATP inhibiting and shifting curve to the right is needed.

Hormones

9

HORMONES AND SIGNAL TRANSDUCTION

Broadly speaking, a hormone is any compound produced by a cell, which by binding to its cognate receptor alters the metabolism of the cell bearing the hormone-receptor complex. Although a few hormones bind to receptors on the cell that produces them (autoregulation or autocrine function), hormones are more commonly thought of as acting on some other cell, either close by (paracrine) or at a distant site (telecrine). Paracrine hormones are secreted into the interstitial space and generally have a very short half-life. These include the prostaglandins and the neurotransmitters. The paracrine hormones are discussed in the various Lecture Notes, as relevant to the specific topic under consideration. Telecrine hormones are secreted into the bloodstream, generally have a longer half-life, and include the endocrine and gastrointestinal (GI) hormones. The endocrine hormones are the classic ones, and it is sometimes implied that reference is being made to endocrine hormones when the word hormones is used in a general sense. The GI and endocrine hormones are discussed in detail in the GI and endocrinology chapters in the Physiology Lecture Notes. Although there is some overlap, this chapter presents basic mechanistic concepts applicable to all hormones, whereas coverage in the Physiology notes emphasizes the physiologic consequences of hormonal action.

Hormones are divided into two major categories, those that are water soluble (hydrophilic) and those that are lipid soluble (lipophilic, also known as hydrophobic). Important properties of these two classes are shown in Table I-9-1.

Table I-9-1. Two Classes of Hormones

Water Soluble	Lipid Soluble
Receptor in cell membrane	Receptor inside cell
Second messengers often involved Protein kinases activated	Hormone-receptor complex binds hormone response elements (HRE, of enhancer regions) in DNA
Protein phosphorylation to modify activity of enzymes (requires minutes)	—
Control of gene expression through proteins like cAMP response element binding (CREB) protein (requires hours)	Control of gene expression (requires hours)
Examples: <ul style="list-style-type: none">• Insulin• Glucagon• Catecholamines	Examples: <ul style="list-style-type: none">• Steroids• Calcitriol• Thyroxines• Retinoic acid

MECHANISM OF WATER SOLUBLE HORMONES

Water-soluble hormones must transmit signals to affect metabolism and gene expression without themselves entering the cytoplasm. They often do so via second messenger systems that, in turn, activate protein kinases.

Protein Kinases

A protein kinase is an enzyme that phosphorylates many other proteins, changing their activity (e.g., phosphorylation of acetyl CoA carboxylase inhibits it). Examples of protein kinases are listed in Table I-9-2 along with the second messengers that activate them.

Table I-9-2. Summary of Signal Transduction by Water-Soluble Hormones

Pathway	G-Protein	Enzyme	Second Messenger(s)	Protein Kinase	Examples
cAMP	G _s (G _i)	Adenyl cyclase	cAMP	Protein kinase A	Glucagon Epinephrine (α_2 and β)
PIP ₂	G _q	Phospholipase C	DAG, IP ₃ , Ca ²⁺	Protein kinase C	Vasopressin Epinephrine (α_1)
cGMP	None	Guanyl cyclase	cGMP	Protein kinase G	Atrial natriuretic factor (ANF) Nitric oxide (NO)
Insulin, growth factors	Monomeric p21 ^{ras}	—	—	Tyrosine kinase activity of receptor	Insulin Insulin-like growth factor (IGF) Platelet-derived growth factor (PDGF) Epidermal growth factor (EGF)

Some water-soluble hormones bind to receptors with intrinsic protein kinase activity (often tyrosine kinases). In this case, no second messenger is required for protein kinase activation. The insulin receptor is an example of a tyrosine kinase receptor.

Activation of a protein kinase causes:

- Phosphorylation of enzymes to rapidly increase or decrease their activity.
- Phosphorylation of gene regulatory proteins such as CREB to control gene expression, usually over several hours. The typical result is to add more enzyme to the cell. CREB induces the phosphoenolpyruvate carboxykinase (PEPCK) gene.

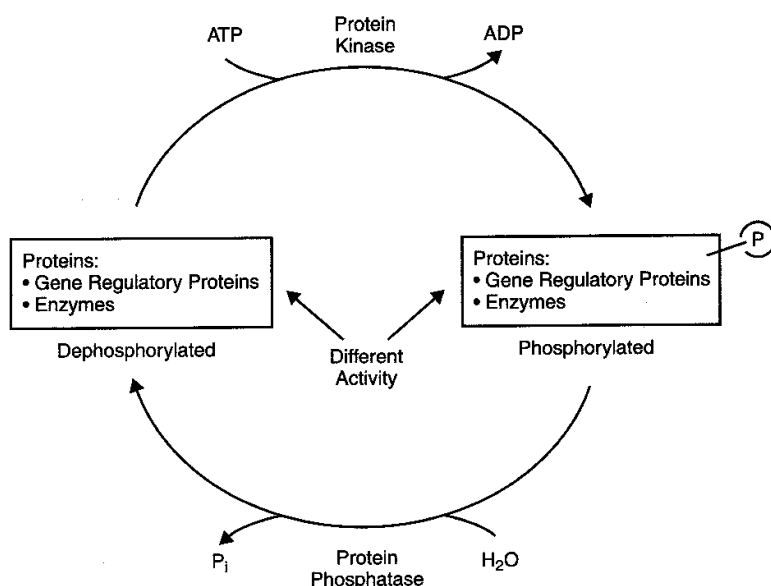


Figure I-9-1. Protein Kinases and Phosphatases

Both represent strategies to control metabolism. The action of protein kinases is reversed by protein phosphatases (Figure I-9-1).

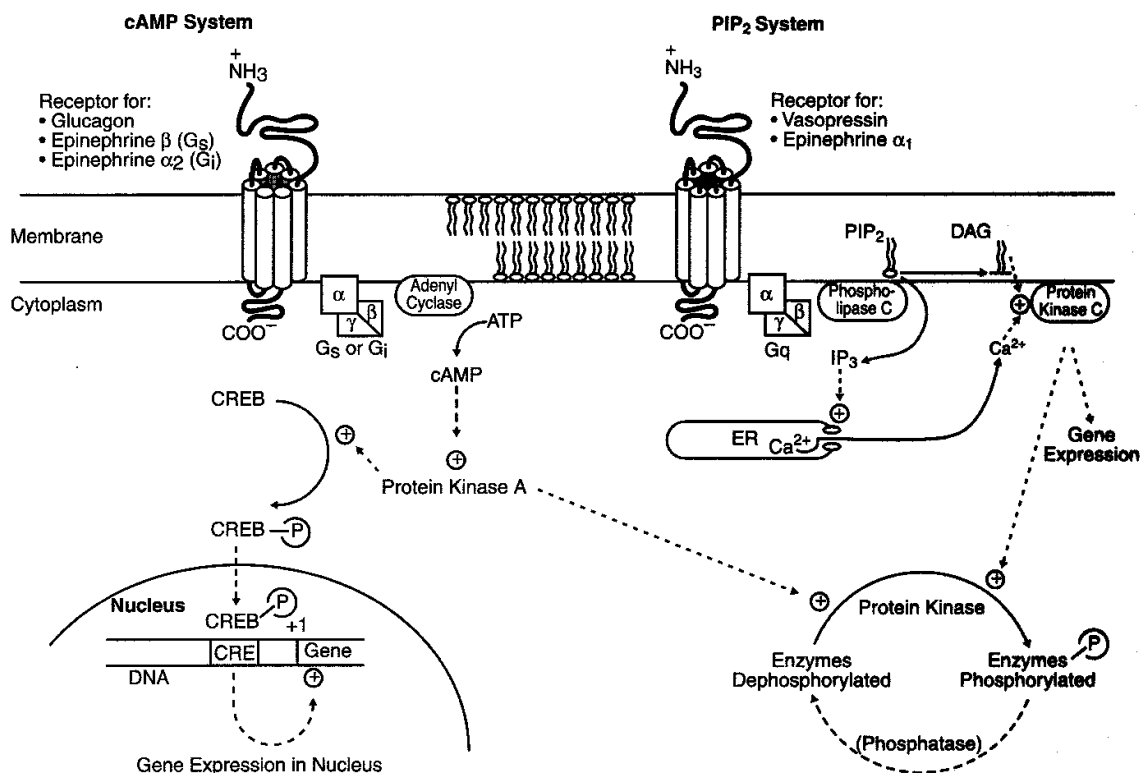
Sequence of Events From Receptor to Protein Kinase

Cyclic AMP (cAMP) and Phosphatidylinositol Bisphosphate (PIP₂)

Receptors in these pathways are coupled through trimeric G proteins in the membrane. The receptors all have characteristic 7-helix membrane-spanning domains.

The sequence of events (illustrated in Figure I-9-2) leading from receptor to activation of the protein kinase via the cAMP and PIP₂ second messenger systems is as follows:

- Hormone binds receptor
- Trimeric G protein in membrane is engaged
- Enzyme (adenylate cyclase or phospholipase)
- Second messenger
- Protein kinase
- Protein phosphorylation (minutes) and gene expression (hours)

Figure I-9-2. Cyclic AMP and Phosphatidylinositol Bisphosphate (PIP₂)**cGMP**

Atrial natriuretic factor (ANF), produced by cells in the atrium of the heart in response to distension, binds the ANF receptor in vascular smooth muscle and in the kidney. The ANF receptor spans the membrane and has guanylate cyclase activity associated with the cytoplasmic domain. It causes relaxation of vascular smooth muscle, resulting in vasodilation, and in the kidney it promotes sodium and water excretion.

Nitric oxide (NO) is synthesized by vascular endothelium in response to vasodilators. It diffuses into the surrounding vascular smooth muscle, where it directly binds the heme group of soluble guanylate cyclase, activating the enzyme.

Both the ANF receptor and the soluble guanylate cyclase are associated with the same vascular smooth muscle cells. These cGMP systems are shown in Figure I-9-3.

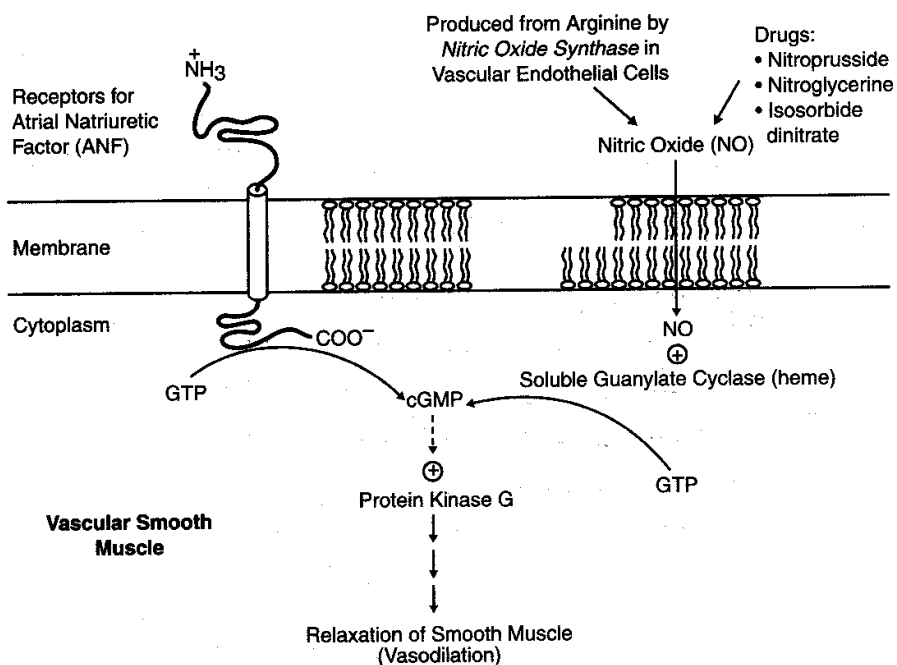


Figure I-9-3. Cyclic GMP

The sequence from receptor to protein kinase is quite similar to the one above for cAMP with two important variations:

- The ANF receptor has intrinsic guanylate cyclase activity. Because no G protein is required in the membrane, the receptor lacks the 7-helix membrane-spanning domain.
- Nitric oxide diffuses into the cell and directly activates a soluble, cytoplasmic guanylate cyclase, so no receptor or G protein is required.

The Insulin Receptor: A Tyrosine Kinase

Insulin binding activates the tyrosine kinase activity associated with the cytoplasmic domain of its receptor as shown in Figure I-9-4. There is no trimeric G protein, enzyme, or second messenger required to activate this protein tyrosine kinase activity:

- Hormone binds receptor
- Receptor tyrosine kinase (protein kinase) is activated
- Protein phosphorylation (autophosphorylation and activation of other proteins)

Once autophosphorylation begins, a complex of other events ensues. An insulin receptor substrate (IRS-1) binds the receptor and is phosphorylated on tyrosine residues, allowing proteins with SH2 (*src* homology) domains to bind to the phosphotyrosine residues on IRS-1 and become active. In this way, the receptor activates several enzyme cascades, which involve:

- Activation of phosphatidylinositol-3 kinase (PI-3 kinase), one of whose effects in adipose and muscle tissues is to increase GLUT-4 in the membrane
- Activation of protein phosphatases. Paradoxically, insulin stimulation via its tyrosine kinase receptor ultimately may lead to dephosphorylating enzymes
- Stimulation of the monomeric G protein (p21^{ras}) encoded by the normal *ras* gene

All these mechanisms can be involved in controlling gene expression although the pathways by which this occurs have not yet been completely characterized.

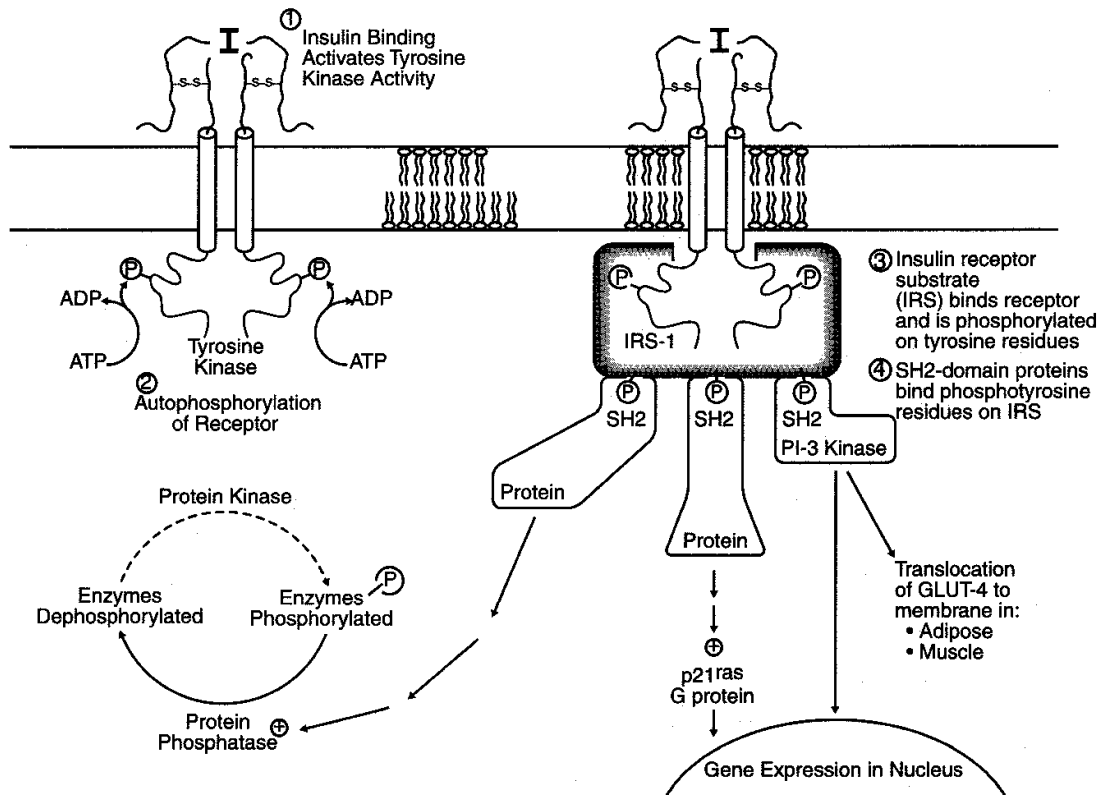


Figure I-9-4. Insulin Receptor

Tyrosine kinase receptors are also involved in signaling by several growth factors, including platelet-derived growth factor (PDGF) and epidermal growth factor (EGF).

Functional Relationship of Glucagon and Insulin

Insulin, associated with well-fed, absorptive metabolism, and glucagon, associated with fasting and postabsorptive metabolism, usually oppose each other with respect to pathways of energy metabolism. Glucagon works through the cAMP system to activate protein kinase A favoring phosphorylation of rate-limiting enzymes, whereas insulin often activates protein phosphatases that dephosphorylate many of the same enzymes. An example of this opposition in glycogen metabolism is shown in Figure I-9-5. Glucagon promotes phosphorylation of both rate-limiting enzymes (glycogen phosphorylase for glycogenolysis and glycogen synthase for glycogen synthesis). The result is twofold in that synthesis slows and degradation increases, but both effects contribute to the same physiologic outcome, release of glucose from the liver during hypoglycemia. Insulin reverses this pattern, promoting glucose storage after a meal.

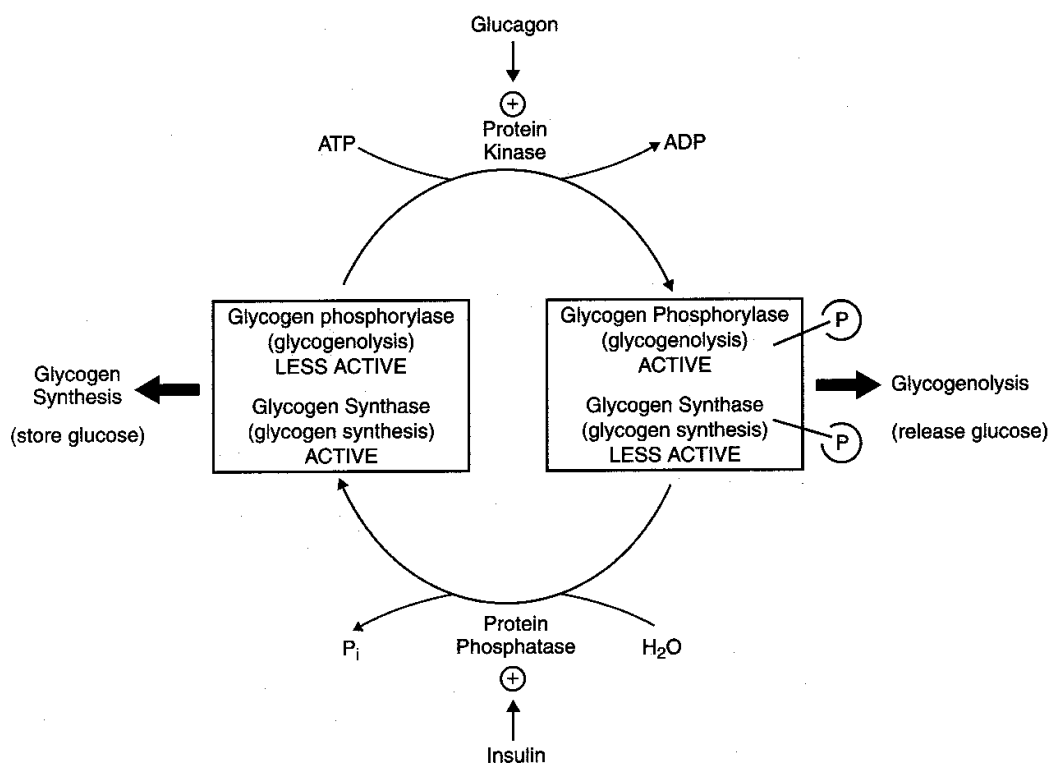


Figure I-9-5. Opposing Activities of Insulin and Glucagon

G PROTEINS IN SIGNAL TRANSDUCTION

Table I-9-2 is a summary of the major components of water-soluble hormone pathways reviewed in this section. There are several different G proteins (GTP-binding) involved. Trimeric G proteins include G_s , G_p , G_q , and in the photoreceptor pathway reviewed in Chapter 10, G_t (transducin). Receptors that engage these all have the 7-helix membrane-spanning structure. Receptor stimulation causes the $G\alpha$ subunit to bind GTP and become active. The $G\alpha$ subunit subsequently hydrolyzes the GTP to GDP, terminating the signal. The p21^{ras} G protein is monomeric.

G protein defects can cause disease in several ways, some of which are summarized in Table I-9-3.

Table I-9-3. Abnormal G Proteins and Disease

Defect	Example	Disease
ADP-ribosylation by: <ul style="list-style-type: none"> • <i>Cholera</i> toxin • <i>E. coli</i> toxin • <i>Pertussis</i> toxin 	$G_s\alpha$ $G_s\alpha$ $G_i\alpha$	Diarrhea of cholera Traveler's diarrhea Pertussis (whooping cough)
Oncogenic mutations	P21 ^{ras} (<i>ras</i>)	Colon, lung, breast, bladder tumors
—	$G_s\alpha$ (<i>gsp</i>)	Pituitary tumors, adenomas, endocrine ovarian tumors

ADP-Ribosylation by Bacterial Toxins

Certain bacterial exotoxins are enzymes that attach the adenosine diphosphate (ADP)-ribose residue of NAD to $G\alpha$ subunits, an activity known as ADP-ribosylation:

- *Vibrio cholera* exotoxin ADP-ribosylates $G_s\alpha$, leading to an increase in cAMP and subsequently chloride secretion from intestinal mucosal cells, causing the diarrhea of cholera.
- Certain strains of *Escherichia coli* release toxins similar to cholera toxin, producing traveler's diarrhea.
- *Bordetella pertussis* exotoxin ADP-ribosylates $G_i\alpha$, dramatically reducing its responsiveness to the receptor, thus increasing cAMP. It is not known how this relates to the persistent paroxysmal coughing symptomatic of pertussis (whooping cough).

Activating Mutations in $G\alpha$

Mutations that increase G protein activity may be oncogenic. Examples of oncogenes with activating gain-of-function mutations include *ras* (p21 monomeric G protein) and *gsp* ($G_s\alpha$).

LIPID-SOLUBLE HORMONES

Lipid-soluble hormones diffuse through the cell membrane, where they bind to their respective receptors inside the cell. The receptors have a DNA-binding domain (usually Zn-fingers) and interact with specific response elements in enhancer (or possibly silencer) regions associated with certain genes. For example, the cortisol receptor binds to its response element in the enhancer region of the phosphoenolpyruvate carboxykinase (PEPCK) gene. By increasing the amount of PEPCK in the hepatocyte, cortisol can increase the capacity for gluconeogenesis, one of its mechanisms for responding to chronic stress often associated with injury. The enhancer mechanism was reviewed in Chapter 5.

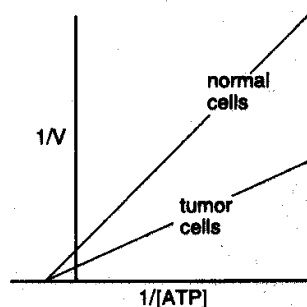
Review Questions

1. A patient with manic depressive disorder is treated with lithium, which slows the turnover of inositol phosphates and the phosphatidyl inositol derivatives in cells. Which of the following protein kinases is most directly affected by this drug?
 - A. Protein kinase C
 - B. Receptor tyrosine kinase
 - C. Protein kinase G
 - D. Protein kinase A
 - E. Protein kinase M

Items 2 and 3

Tumor cells from a person with leukemia have been analyzed to determine which oncogene is involved in the transformation. After partial sequencing of the gene, the predicted gene product is identified as a tyrosine kinase.

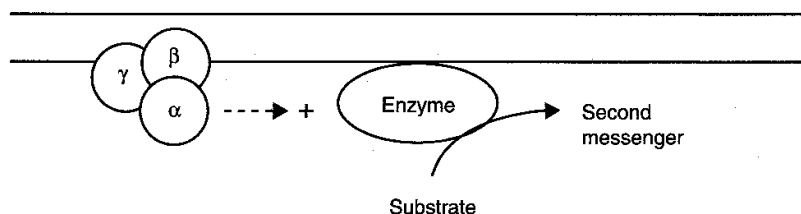
2. Which of the following proteins would most likely be encoded by an oncogene and exhibit tyrosine kinase activity?
 - A. Nuclear transcriptional activator
 - B. Epidermal growth factor
 - C. Membrane-associated G protein
 - D. Platelet-derived growth factor
 - E. Growth factor receptor
3. A kinetic analysis of the tyrosine kinase activities in normal and transformed cells is shown below.



Which of the following conclusions is best supported by these results?

- A. The tumor cell kinase has a higher-than-normal affinity for ATP.
- B. A kinase gene has been deleted from the tumor cell genome.
- C. A noncompetitive inhibitor has been synthesized in the tumor cells.
- D. A kinase gene has been amplified in the tumor cell genome.
- E. The tumor cell kinase has a lower-than-normal affinity for ATP.

4. In a DNA sequencing project, an open reading frame (ORF) has been identified. The nucleotide sequence includes a coding region for an SH2 domain in the protein product. This potential protein is most likely to
- bind to an enhancer region in DNA
 - be a transmembrane hormone receptor
 - transmit signals from a tyrosine kinase receptor
 - bind to an upstream promoter element
 - activate a soluble guanyl cyclase enzyme in vascular smooth muscle



5. The diagram above represents a signal transduction pathway associated with hormone X. The receptor for hormone X is most likely to be characterized as a(n)
- seven-helix transmembrane domain receptor
 - intracellular receptor with a zinc finger domain
 - helix-turn-helix transmembrane domain receptor
 - transmembrane receptor with a guanyl cyclase domain
 - tyrosine kinase domain receptor
6. A 58-year-old man with a history of angina for which he occasionally takes isosorbide dinitrate is having erectile dysfunction. He confides in a colleague who suggests that sildenafil might help and gives him three tablets from his own prescription. The potentially lethal combination of these drugs relates to

Isosorbide Dinitrate	Sildenafil
A. Activates nitric oxide synthase in vascular endothelium	Inhibits guanyl cyclase in vascular smooth muscle
B. Activates nitric oxide synthase in vascular endothelium	Inhibits guanyl cyclase in corpora cavernosa smooth muscle
C. Releases cyanide as a byproduct	Inhibits cGMP phosphodiesterase in corpora cavernosa smooth muscle
D. Activates guanyl cyclase in vascular smooth muscle	Inhibits cGMP phosphodiesterase in vascular smooth muscle
E. Activates the ANF receptor in vascular smooth muscle	Inhibits protein kinase G in vascular smooth muscle

Answers

1. **Answer: A.** The description best fits the PIP_2 system in which protein kinase C is activated.
2. **Answer: E.** Although any of the listed options might be encoded by an oncogene, the "tyrosine kinase" description suggests it is likely to be a growth factor receptor.
3. **Answer: D.** An increase in V_{\max} with no change in K_m (ATP affinity) is consistent with expansion of the kinase gene and inconsistent with the other choices listed.
4. **Answer: C.** Proteins with SH2 domains might bind to the insulin receptor substrate-1 (IRS-1) to transmit signals from the insulin receptor, a tyrosine kinase type of receptor. PI-3 kinase is an example of an SH2 domain protein. SH2 domains are not involved in DNA binding (**choices A and D**). Examples of protein domains that bind DNA include zinc fingers (steroid receptors), leucine zippers (CREB protein), and helix-turn-helix proteins (homeodomain proteins).
5. **Answer: A.** The diagram indicates that the receptor activates a trimeric G-protein associated with the inner face of the membrane and that the G-protein subsequently signals an enzyme catalyzing a reaction producing a second messenger. Receptors that activate trimeric G-proteins have a characteristic seven-helix transmembrane domain. The other categories of receptors do not transmit signals through trimeric G-proteins.
6. **Answer: D.** Nitrates may be metabolized to nitric oxide (NO) that activates a soluble guanyl cyclase in vascular smooth muscle. The increase in cGMP activates protein kinase G and subsequently leads to vasodilation. Sildenafil inhibits cGMP phosphodiesterase (PDE), potentiating vasodilation that can lead to shock and sudden death. Although sildenafil has much higher potency for the cGMP PDE isozyme in the corpora cavernosa, it can also inhibit the cGMP PDE in vascular smooth muscle. Nitric oxide synthase (**choices A and B**) is the physiologic source of nitric oxide in response to vasodilators such as acetylcholine, bradykinin, histamine, and serotonin.

Vitamins

10

OVERVIEW OF VITAMINS

Vitamins have historically been classified as either water soluble or lipid soluble. Water-soluble vitamins are precursors for coenzymes and are reviewed in the context of the reactions for which they are important. A summary of these vitamins is shown in Table I-10-1.

Table I-10-1. Water-Soluble Vitamins

Vitamin or Coenzyme	Enzyme	Pathway	Deficiency
Biotin	Pyruvate carboxylase Acetyl CoA carboxylase Propionyl CoA carboxylase	Gluconeogenesis Fatty acid synthesis Odd-carbon fatty acids, Val, Met, Ile, Thr	MCC* (rare): excessive consumption of raw eggs (contain avidin, a biotin-binding protein) Alopecia (hair loss), bowel inflammation, muscle pain
Thiamine (B ₁)	Pyruvate dehydrogenase α -Ketoglutarate dehydrogenase Transketolase	PDH TCA cycle HMP shunt	MCC: alcoholism (alcohol interferes with absorption) Wernicke (ataxia, nystagmus, ophthalmoplegia) Korsakoff (confabulation, psychosis) High-output cardiac failure (wet beri-beri)
Niacin (B ₃) NAD(H) NADP(H)	Dehydrogenases	Many	Pellagra: diarrhea, dementia, dermatitis, and, if not treated, death Pellagra may also be related to deficiency of tryptophan (corn major dietary staple), which supplies a portion of the niacin requirement.
Folic acid THF	Thymidylate synthase Enzymes in purine synthesis need not be memorized	Thymidine (pyrimidine) synthesis Purine synthesis	MCC: alcoholics and pregnancy (body stores depleted in 3 months) Homocystinemia with risk of deep vein thrombosis and atherosclerosis Megaloblastic (macrocytic) anemia Deficiency in early pregnancy causes neural tube defects in fetus

*MCC, most common cause.

(Continued)

Table I-10-1. Water-Soluble Vitamins (*continued*)

Vitamin or Coenzyme	Enzyme	Pathway	Deficiency
Cyanocobalamin (B ₁₂)	Homocysteine methyltransferase Methylmalonyl CoA mutase	Methionine, SAM Odd-carbon fatty acids, Val, Met, Ile, Thr	MCC: pernicious anemia. Also in aging, especially with poor nutrition, bacterial overgrowth of terminal ileum, resection of the terminal ileum secondary to Crohn disease, chronic pancreatitis, and, rarely, vegans, or infection with <i>D. latum</i> Megaloblastic (macrocytic) anemia Progressive peripheral neuropathy
Pyridoxine (B ₆) Pyridoxal-P (PLP)	Aminotransferases (transaminase): AST (GOT), ALT (GPT) δ -Aminolevulinate synthase	Protein catabolism Heme synthesis	MCC: isoniazid therapy Sideroblastic anemia Cheilosis or stomatitis (cracking or scaling of lip borders and corners of the mouth) Convulsions
Riboflavin (B ₂) FAD(H ₂)	Dehydrogenases	Many	Corneal neovascularization Cheilosis or stomatitis (cracking or scaling of lip borders and corners of the mouth) Magenta-colored tongue
Ascorbate (C)	Prolyl and lysyl hydroxylases Dopamine hydroxylase	Collagen synthesis Catecholamine synthesis Absorption of iron in GI tract	MCC: diet deficient in citrus fruits and green vegetables Scurvy: poor wound healing, easy bruising (perifollicular hemorrhage), bleeding gums, increased bleeding time, painful glossitis, anemia
Pantothenic acid CoA	Fatty acid synthase Fatty acyl CoA synthetase Pyruvate dehydrogenase α -Ketoglutarate dehydrogenase	Fatty acid metabolism PDH TCA cycle	Rare

There are four important lipid-soluble vitamins, D, A, K, and E. Two of these vitamins, A and D, work through enhancer mechanisms similar to those for lipid-soluble hormones. In addition, all four lipid-soluble vitamins have more specialized mechanisms through which they act. Table I-10-2 lists their major functions.

Table I-10-2. Lipid-Soluble Vitamins

Vitamin	Important Functions
D (cholecalciferol)	In response to hypocalcemia, helps normalize serum calcium levels
A (carotene)	Retinoic acid and retinol act as growth regulators, especially in epithelium Retinal is important in rod and cone cells for vision
K	Carboxylation of glutamic acid residues in many Ca^{2+} -binding proteins, importantly coagulation factors II, VII, IX, and X, as well as protein C and protein S
E (α -tocopherol)	Antioxidant in the lipid phase. Protects membrane lipids from peroxidation and helps prevent oxidation of LDL particles thought to be involved in atherosclerotic plaque formation

VITAMIN D AND CALCIUM HOMEOSTASIS

Hypocalcemia (below-normal blood calcium) stimulates release of parathyroid hormone (PTH), which in turn binds to receptors on cells of the renal proximal tubules. The receptors are coupled through cAMP to activation of a 1α -hydroxylase important for the final, rate-limiting step in the conversion of vitamin D to 1,25-DHCC (dihydroxycholecalciferol or calcitriol).

Once formed, 1,25-DHCC acts on duodenal epithelial cells as a lipid-soluble hormone. Its intracellular receptor (a Zn-finger protein) binds to response elements in enhancer regions of DNA to induce the synthesis of calcium-binding proteins thought to play a role in stimulating calcium uptake from the GI tract.

1,25-DHCC also facilitates calcium reabsorption in the kidney and mobilizes calcium from bone when PTH is also present. All these actions help bring blood calcium levels back within the normal range.

The relation of vitamin D to calcium homeostasis and its *in vivo* activation are shown in Figure I-10-1.

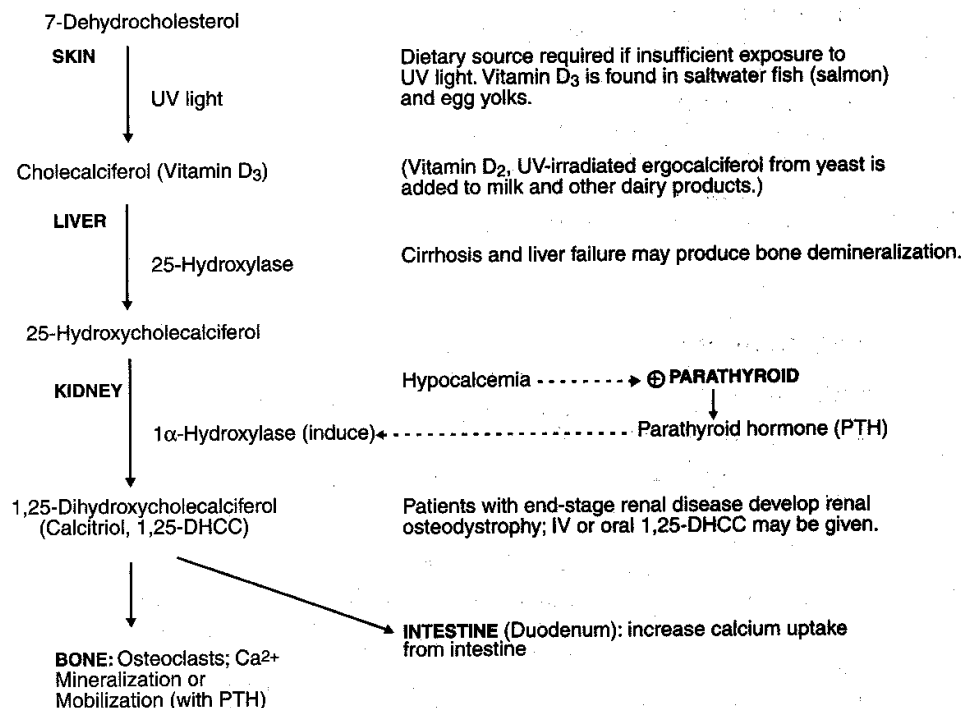


Figure I-10-1. Synthesis and Activation of Vitamin D

Synthesis of 1,25-Dihydroxycholecalciferol (Calcitriol)

Humans can synthesize calcitriol from 7-dehydrocholesterol derived from cholesterol in the liver. Three steps are involved, each occurring in a different tissue:

1. Activation of 7-dehydrocholesterol by UV light in the skin produces cholecalciferol (vitamin D₃). This step is insufficient in many people in cold, cloudy climates, and vitamin D₃ supplementation is necessary.
2. 25-Hydroxylation in the liver (patients with severe liver disease may need to be given 25-DHCC or 1,25-DHCC).
3. 1α-Hydroxylation in the proximal renal tubule cells in response to PTH. Genetic deficiencies or patients with end-stage renal disease develop renal osteodystrophy because of insufficiency of 1,25-DHCC and must be given 1,25-DHCC or a drug analog that does not require metabolism in the kidney. Such patients include those with:
 - End-stage renal disease secondary to diabetes mellitus
 - Fanconi renal syndrome (renal proximal tubule defect)
 - Genetic deficiency of the 1α-hydroxylase (vitamin D-resistant rickets)

Vitamin D Deficiency

Deficiency of vitamin D in childhood produces rickets, a constellation of skeletal abnormalities most strikingly seen as deformities of the legs, but many other developing bones are affected. Muscle weakness is common.

Vitamin D deficiency after epiphyseal fusion causes osteomalacia, which produces less deformity than rickets. Osteomalacia may present as bone pain and muscle weakness.

VITAMIN A

Vitamin A (carotene) is converted to several active forms in the body associated with two important functions, maintenance of healthy epithelium and vision.

Maintenance of Epithelium

Retinol and retinoic acid are required for the growth, differentiation, and maintenance of epithelial cells. In this capacity they bind intracellular receptors, which are in the family of Zn-finger proteins, and they regulate transcription through specific response elements.

Vision

cis-Retinal acts as a cofactor for the protein opsin to form rhodopsin. It functions similarly in rod and cone cells. A diagram of the signal transduction pathway for light-activated rhodopsin in the rod cell is shown in Figure I-10-2, along with the relationship of this pathway to rod cell anatomy and changes in the membrane potential. Note the following points:

- Rhodopsin is a 7-pass receptor coupled to the trimeric G protein transducin (G_t).
- The pathway activates cGMP phosphodiesterase, which lowers cGMP in response to light.
- Rhodopsin and transducin are embedded in the disk membranes in the outer rod segment.
- cGMP-gated Na^+ channels in the cell membrane of the outer rod segment respond to the decrease in cGMP by closing and hyperpolarizing the membrane.
- The rod cell is unusual for an excitable cell in that the membrane is partially depolarized (~ -30 mV) at rest (in darkness) and hyperpolarizes on stimulation.

Because the membrane is partially depolarized in the dark, its neurotransmitter glutamate is continuously released. Glutamate inhibits the optic nerve bipolar cells with which the rod cells synapse. By hyperpolarizing the rod cell membrane, light stops the release of glutamate, relieving inhibition of the optic nerve bipolar cell and thus initiating a signal into the brain.

Clinical Correlate

Isotretinoin, a form of retinoic acid, is used in the treatment of acne. It is teratogenic (malformations of the craniofacial, cardiac, thymic, and CNS structures) and is therefore absolutely contraindicated in pregnant women and used with caution in women of childbearing age.

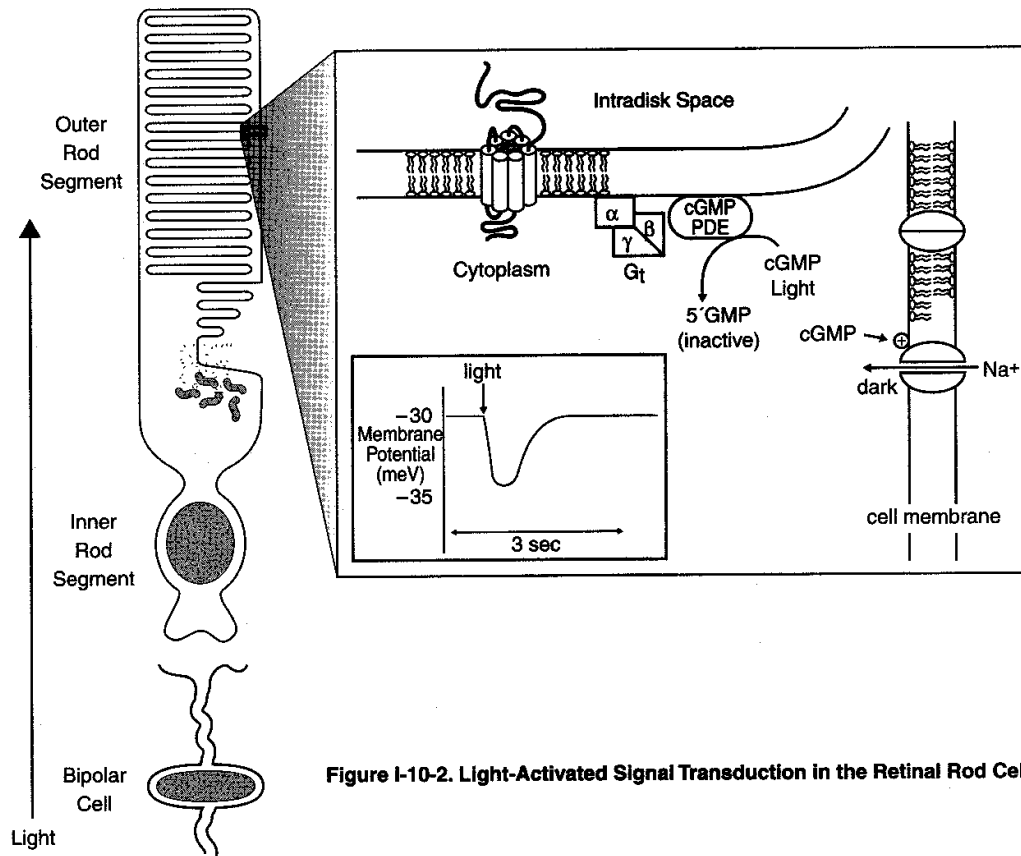


Figure I-10-2. Light-Activated Signal Transduction in the Retinal Rod Cell

Vitamin A Deficiency

Deficiency of vitamin A results in night blindness (rod cells are responsible for vision in low light), metaplasia of the corneal epithelium, dry eyes, bronchitis, pneumonia, and follicular hyperkeratosis.

VITAMIN K

Vitamin K is required to introduce Ca^{2+} binding sites on several calcium-dependent proteins. The modification that introduces the Ca^{2+} binding site is a γ -carboxylation of glutamyl residue(s) in these proteins, often identified simply as the γ -carboxylation of glutamic acid. Nevertheless, this vitamin K-dependent carboxylation (Figure I-10-3) is a cotranslational modification occurring as the proteins are synthesized on ribosomes during translation.

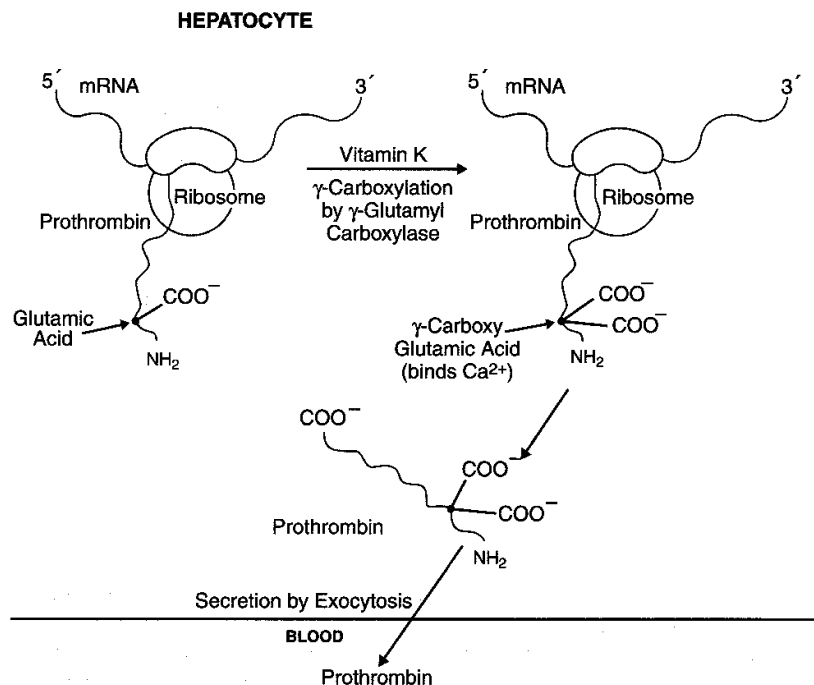


Figure I-10-3. Vitamin K-Dependent γ -Carboxylation of Prothrombin During Translation

Examples of proteins undergoing this vitamin K-dependent carboxylation include the coagulation factors II (prothrombin), VII, IX, and X, as well as the anticoagulant proteins C and S. All these proteins require Ca^{2+} for their function. Vitamin K deficiencies produce prolonged bleeding, easy bruising, and potentially fatal hemorrhagic disease. Conditions predisposing to a vitamin K deficiency include:

- Fat malabsorption (bile duct occlusion)
- Prolonged treatment with broad-spectrum antibiotics (eliminate intestinal bacteria that supply vitamin K)
- Breast-fed newborns (little intestinal flora, breast milk very low in vitamin K), especially in a home-birth where a postnatal injection of vitamin K may not be given
- Infants whose mothers have been treated with certain anticonvulsants during pregnancy (phenylhydantoins)

Vitamin K deficiency should be distinguished from vitamin C deficiency. Table I-10-3 summarizes some of these differences.

Table I-10-3. Vitamin K and Vitamin C Deficiencies Compared

Vitamin K Deficiency	Vitamin C Deficiency
Easy bruising, bleeding	Easy bruising, bleeding
Normal bleeding time	Increased bleeding time
Increased PT	Normal PT
Hemorrhagic disease with no connective tissue problems	Gum hyperplasia, inflammation, loss of teeth Skeletal deformity in children Poor wound healing Anemia
Associated with: • Fat malabsorption • Long-term antibiotic therapy • Breast-fed newborns • Infant whose mother was taking anticonvulsant therapy during pregnancy	Associated with: • Diet deficient in citrus fruit, green vegetables

Anticoagulant Therapy

Warfarin and dicoumarol antagonize the γ -carboxylation activity of vitamin K and thus act as anticoagulants. They interfere with the cotranslational modification during synthesis of the precoagulation factors. Once these proteins have been released into the bloodstream, vitamin K is no longer important for their subsequent activation and function. Related to this are two important points:

- Warfarin and dicoumarol prevent coagulation only *in vivo* and cannot prevent coagulation of blood *in vitro* (drawn from a patient into a test tube).
- When warfarin and dicoumarol are given to a patient, 2–3 days are required to see their full anticoagulant activity. Heparin is often given to provide short-term anticoagulant activity.

VITAMIN E

Vitamin E (α -tocopherol) is an antioxidant. As a lipid-soluble compound, it is especially important for protecting other lipids from oxidative damage.

It prevents peroxidation of fatty acids in cell membranes, helping to maintain their normal fluidity. Deficiency can lead to hemolysis, neurologic problems, and retinitis pigmentosa.

It prevents oxidation of LDL particles. Oxidized LDLs are strongly associated with atherosclerosis and coronary artery disease.

Review Questions

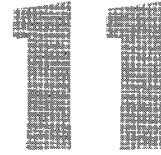
Select the ONE best answer.

1. Retinitis pigmentosa (RP) is a genetically heterogeneous disease characterized by progressive photoreceptor degeneration and ultimately blindness. Mutations in more than 20 different genes have been identified in clinically affected patients. Recent studies have mapped an RP locus to the chromosomal location of a new candidate gene at 5q31. One might expect this gene to encode a polypeptide required for the activity of a
 - A. receptor tyrosine kinase
 - B. cGMP phosphodiesterase
 - C. phospholipase C
 - D. adenylyl cyclase
 - E. protein kinase C
2. A 27-year-old woman with epilepsy has been taking phenytoin to control her seizures. She is now pregnant, and her physician is considering changing her medication to prevent potential bleeding episodes in the infant. What biochemical activity might be deficient in the infant if her medication is continued?
 - A. Hydroxylation of proline
 - B. Glucuronidation of bilirubin
 - C. Reduction of glutathione
 - D. γ -Carboxylation of glutamate
 - E. Oxidation of lysine
3. A 75-year-old woman is seen in the emergency room with a fractured arm. Physical examination revealed multiple bruises and perifollicular hemorrhages, periodontitis, and painful gums. Her diet consists predominately of weak coffee, bouillon, rolls, and plain pasta. Lab results indicated mild microcytic anemia. Which of the following enzymes should be less active than normal in this patient?
 - A. Homocysteine methyltransferase
 - B. γ -Glutamyl carboxylase
 - C. Dihydrofolate reductase
 - D. ALA synthase
 - E. Prolyl hydroxylase

Answers

1. **Answer: B.** Only phosphodiesterase participates as a signaling molecule in the visual cycle of photoreceptor cells.
2. **Answer: D.** Phenyl hydantoins decrease the activity of vitamin K, which is required for the γ -carboxylation of coagulation factors (II, VII, IX, X), as well as proteins C and S.
3. **Answer: E.** The patient has many signs of scurvy from a vitamin C deficiency. The diet, which contains no fruits or vegetables, provides little vitamin C. Prolyl hydroxylase requires vitamin C, and in the absence of hydroxylation, the collagen α -chains do not form stable, mature collagen. The anemia may be due to poor iron absorption in the absence of ascorbate.

Overview of Energy Metabolism



METABOLIC SOURCES OF ENERGY

Energy is extracted from food via oxidation, resulting in the end products carbon dioxide and water. This process occurs in the four stages shown in Figure I-11-1.

In the first stage, metabolic fuels are hydrolyzed in the gastrointestinal (GI) tract to a diverse set of monomeric building blocks (glucose, amino acids, and fatty acids) and absorbed.

In the second stage, the building blocks are degraded by various pathways in tissues to a common metabolic intermediate, acetyl CoA. Most of the energy contained in metabolic fuels is conserved in the chemical bonds (electrons) of acetyl CoA. A smaller portion is conserved in reducing nicotinamide adenine dinucleotide (NAD) to NADH or flavin adenine dinucleotide (FAD) to FADH₂. Reduction indicates the addition of electrons that may be free, part of a hydrogen atom (H), or a hydride ion (H⁻).

In stage three, the citric acid (Krebs, or tricarboxylic acid [TCA]) cycle oxidizes acetyl CoA to CO₂. The energy released in this process is primarily conserved by reducing NAD to NADH or FAD to FADH₂.

The final stage in the extraction of energy from food is oxidative phosphorylation, in which the energy of NADH and FADH₂ is released via the electron transport chain (ETC) and used by an ATP synthase to produce ATP. This process requires O₂.

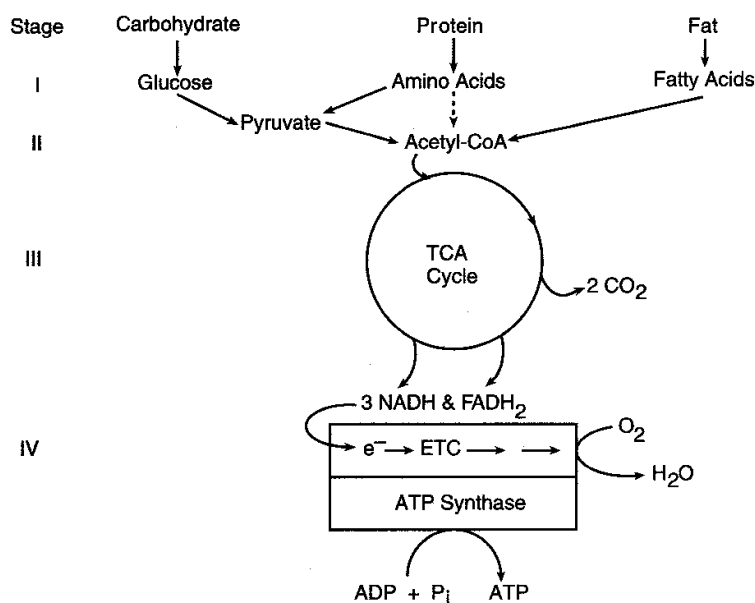


Figure I-11-1. Energy From Metabolic Fuels

METABOLIC ENERGY STORAGE

ATP is a form of circulating energy currency in cells. It is formed in catabolic pathways by phosphorylation of ADP and may provide energy for biosynthesis (anabolic pathways). There is a limited amount of ATP in circulation. Most of the excess energy from the diet is stored as fatty acids (a reduced polymer of acetyl CoA) and glycogen (a polymer of glucose). Although proteins can be mobilized for energy in a prolonged fast, they are normally more important for other functions (contractile elements in muscle, enzymes, intracellular matrix, etc.).

In addition to energy reserves, many other types of biochemicals are required to maintain an organism. Cholesterol is required for cell membrane structure, proteins for muscle contraction, and polysaccharides for the intracellular matrix, to name just a few examples. These substances may be produced from transformed dietary components.

REGULATION OF FUEL METABOLISM

The pathways that are operational in fuel metabolism depend on the nutritional status of the organism. Shifts between storage and mobilization of a particular fuel, as well as shifts among the types of fuel being used, are very pronounced in going from the well-fed state to an overnight fast, and finally to a prolonged state of starvation. The shifting metabolic patterns are regulated mainly by the insulin/glucagon ratio. Insulin is an anabolic hormone that promotes fuel storage. Its action is opposed by a number of hormones, including glucagon, epinephrine, cortisol, and growth hormone. The major function of glucagon is to respond rapidly to decreased blood glucose levels by promoting the synthesis and release of glucose into the circulation. Anabolic and catabolic pathways are controlled at three important levels:

- Allosteric inhibitors and activators of rate-limiting enzymes
- Control of gene expression by insulin and glucagon
- Phosphorylation (glucagon) and dephosphorylation (insulin) of rate-limiting enzymes

Well-Fed (Absorptive) State

Immediately after a meal, the blood glucose level rises and stimulates the release of insulin. The three major target tissues for insulin are liver, muscle, and adipose tissue (Figure I-11-2). Insulin promotes glycogen synthesis in liver and muscle. After the glycogen stores are filled, the liver converts excess glucose to fatty acids and triglycerides. Insulin promotes triglyceride synthesis in adipose tissue and protein synthesis in muscle, as well as glucose entry into both tissues. After a meal, most of the energy needs of the liver are met by the oxidation of excess amino acids.

Two tissues, brain and red blood cells (Figure I-11-2), are insensitive to insulin (are insulin independent). The brain and other nerves derive energy from oxidizing glucose to CO_2 and water in both the well-fed and normal fasting states. Only in prolonged fasting does this situation change. Under all conditions, red blood cells use glucose anaerobically for all their energy needs.

Postabsorptive State

Glucagon and epinephrine levels rise during an overnight fast. These hormones exert their effects on skeletal muscle, adipose tissue, and liver. In liver, glycogen degradation and the release of glucose into the blood are stimulated (Figure I-11-3). Hepatic gluconeogenesis is also stimulated by glucagon, but the response is slower than that of glycogenolysis. The release of amino acids from skeletal muscle and fatty acids from adipose tissue are both stimulated by the decrease in insulin and by an increase in epinephrine. The amino acids and fatty acids are taken up by the liver, where the amino acids provide the carbon skeletons and the oxidation of fatty acids provides the ATP necessary for gluconeogenesis.

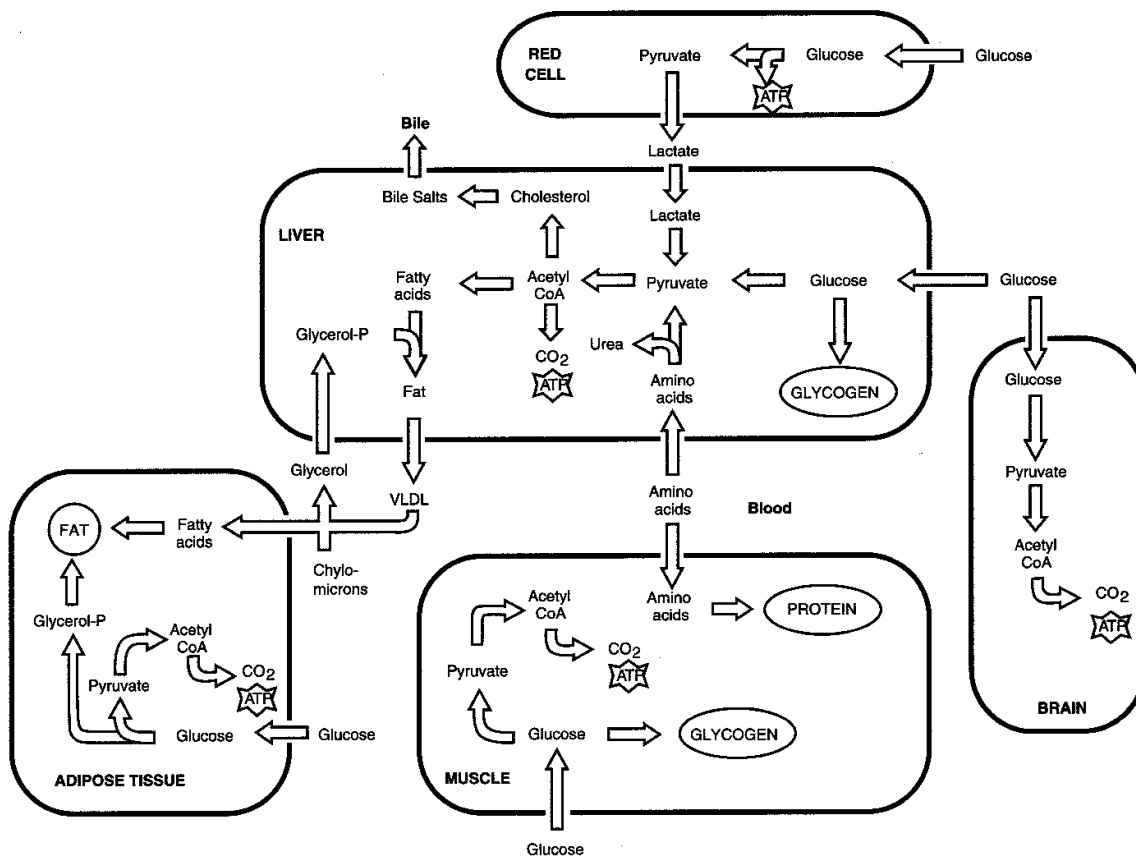


Figure I-11-2. Metabolic Profile of the Well-Fed (Absorptive) State

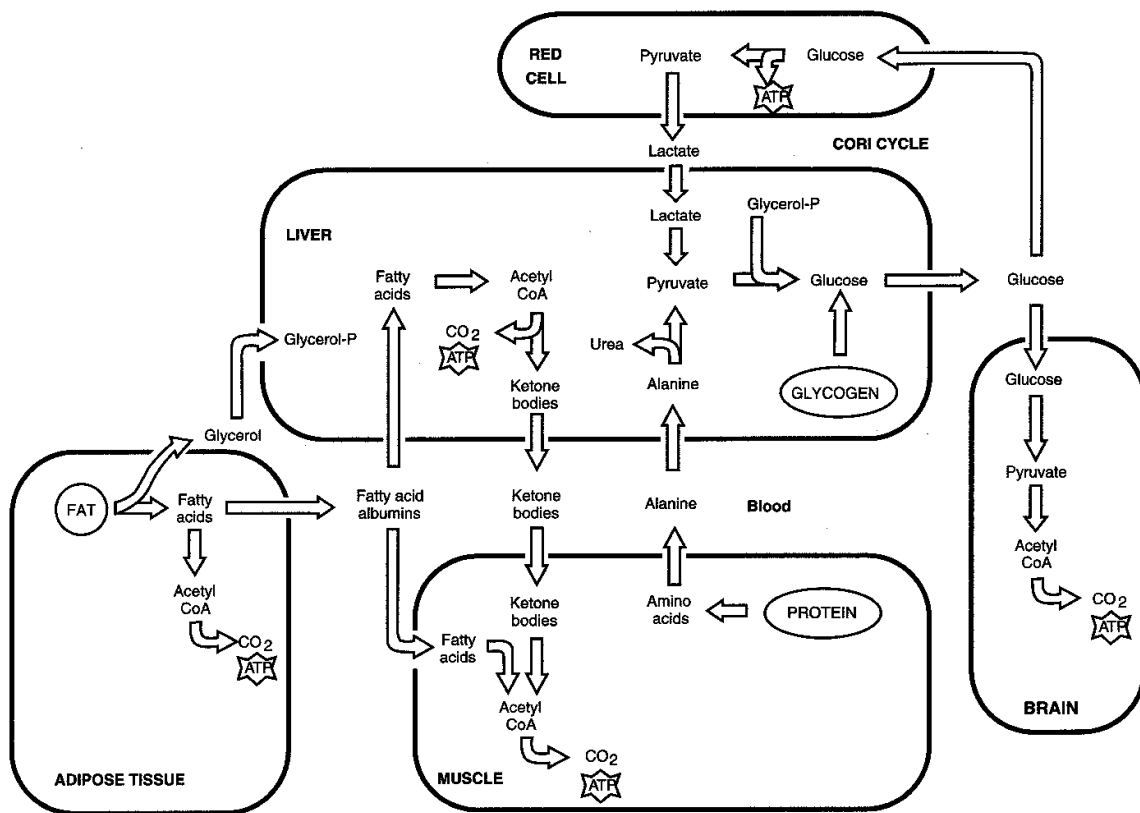


Figure I-11-3. Metabolic Profile of the Postabsorptive State

Note**Diet**

A recommended 2,100 kcal diet consisting of 58% carbohydrate, 12% protein, and 30% fat content:

305 g of carbohydrate

$0.58 \times 2,100 \text{ kcal} = 1,218 \text{ kcal}$;
 $1,218 \text{ kcal} / 4 \text{ kcal/g} = 305 \text{ g}$

63 g of protein

$0.12 \times 2,100 = 252 \text{ kcal}$; $252 \text{ kcal} / 4 \text{ kcal/g} = 63 \text{ g}$

70 g of fat

$0.30 \times 2,100 = 630 \text{ kcal}$; $630 \text{ kcal} / 9 \text{ kcal/g} = 70 \text{ g}$

Prolonged Fast (Starvation)

Levels of glucagon and epinephrine are markedly elevated during starvation. Lipolysis is rapid, resulting in excess acetyl CoA that is used for ketone synthesis. Levels of both lipids and ketones are therefore increased in the blood. Muscle uses fatty acids as the major fuel, and the brain adapts to using ketones for some of its energy. After several weeks of fasting, the brain derives approximately two thirds of its energy from ketones and one third from glucose. The shift from glucose to ketones as the major fuel diminishes the amount of protein that must be degraded to support gluconeogenesis. There is no "energy-storage form" for protein because each protein has a specific function in the cell. Therefore, the shift from using glucose to ketones during starvation spares protein, which is essential for these other functions. Red blood cells (and renal medullary cells) that have few, if any, mitochondria continue to be dependent on glucose for their energy.

PATTERNS OF FUEL METABOLISM IN TISSUES

Fats are much more energy-rich than carbohydrates, proteins, or ketones. Complete combustion of fat results in 9 kcal/g compared with 4 kcal/g derived from carbohydrate, protein, and ketones. The storage capacity and pathways for utilization of fuels varies with different organs and with the nutritional status of the organism as a whole. The organ-specific patterns of fuel utilization in the well-fed and fasting states are summarized in Table I-11-1.

Table I-11-1. Preferred Fuels in the Well-Fed and Fasting States

Organ	Well-Fed	Fasting
Liver	Glucose and amino acids	Fatty acids
Resting skeletal muscle	Glucose	Fatty acids, ketones
Cardiac muscle	Fatty acids	Fatty acids, ketones
Adipose tissue	Glucose	Fatty acids
Brain	Glucose	Glucose (ketones in prolonged fast)
Red blood cells	Glucose	Glucose

Liver

Two major roles of liver in fuel metabolism are to maintain a constant level of blood glucose under a wide range of conditions and to synthesize ketones when excess fatty acids are being oxidized. After a meal, the glucose concentration in the portal blood is elevated. The liver extracts excess glucose and uses it to replenish its glycogen stores. Any glucose remaining in the liver is then converted to acetyl CoA and used for fatty acid synthesis. The increase in insulin after a meal stimulates both glycogen synthesis and fatty acid synthesis in liver. The fatty acids are converted to triglycerides and released into the blood as very low-density lipoproteins (VLDLs). In the well-fed state, the liver derives most of its energy from the oxidation of excess amino acids.

Between meals and during prolonged fasts, the liver releases glucose into the blood. The increase in glucagon during fasting promotes both glycogen degradation and gluconeogenesis. Lactate, glycerol, and amino acids provide carbon skeletons for glucose synthesis.

Adipose Tissue

After a meal, the elevated insulin stimulates glucose uptake by adipose tissue. Insulin also stimulates fatty acid release from VLDL and chylomicron triglyceride (triglyceride is also known as triacylglycerol). Lipoprotein lipase, an enzyme found in the capillary bed of adipose tissue, is induced by insulin. The fatty acids that are released from lipoproteins are taken up by adipose tissue and re-esterified to triglyceride for storage. The glycerol phosphate required for triglyceride synthesis comes from glucose metabolized in the adipocyte. Insulin is also very effective in suppressing the release of fatty acids from adipose tissue.

During fasting, the decrease in insulin and the increase in epinephrine activate hormone-sensitive lipase in fat cells, allowing fatty acids to be released into the circulation.

Skeletal Muscle

Resting Muscle

The major fuels of skeletal muscle are glucose and fatty acids. Because of the enormous bulk, skeletal muscle is the body's major consumer of fuel. After a meal, under the influence of insulin, skeletal muscle takes up glucose to replenish glycogen stores and amino acids that are used for protein synthesis. Both excess glucose and amino acids can also be oxidized for energy.

In the fasting state, resting muscle uses fatty acids derived from free fatty acids in the blood. Ketones may be used if the fasting state is prolonged.

Active Muscle

The primary fuel used to support muscle contraction depends on the magnitude and duration of exercise as well as the major fibers involved. Skeletal muscle has stores of both glycogen and some triglycerides. Blood glucose and free fatty acids also may be used.

Fast-twitch muscle fibers have a high capacity for anaerobic glycolysis but are quick to fatigue. They are involved primarily in short-term, high-intensity exercise. Slow-twitch muscle fibers in arm and leg muscles are well vascularized and primarily oxidative. They are used during prolonged, low-to-moderate intensity exercise and resist fatigue. Slow-twitch fibers and the number of their mitochondria increase dramatically in trained endurance athletes.

Short bursts of high-intensity exercise are supported by anaerobic glycolysis drawing on stored muscle glycogen.

During moderately high, continuous exercise, oxidation of glucose and fatty acids are both important, but after 1 to 3 hours of continuous exercise at this level, muscle glycogen stores become depleted, and the intensity of exercise declines to a rate that can be supported by oxidation of fatty acids.

During low-intensity exercise, fat oxidation predominates as the energy source with some contribution by glucose.

Cardiac Muscle

During fetal life cardiac muscle primarily uses glucose as an energy source, but in the postnatal period there is a major switch to β -oxidation of fatty acids. Thus, in humans fatty acids serve as the major fuel for cardiac myocytes. When ketones are present during prolonged fasting, they are also used. Thus, not surprisingly, cardiac myocytes most closely parallel the skeletal muscle during extended periods of exercise.

In patients with cardiac hypertrophy, this situation reverses to some extent. In the failing heart glucose oxidation increases, and β -oxidation falls.

Brain

Glucose is the primary fuel for the brain. Fatty acids cannot cross the blood-brain barrier and are therefore not used at all. Between meals, the brain relies on blood glucose supplied by either hepatic glycogenolysis or gluconeogenesis. Only in prolonged fasts does the brain gain the capacity to use ketones for energy, and even then ketones supply only approximately two thirds of the fuel; the remainder is glucose.

Review Questions

Select the ONE best answer.

- Two weeks after an episode of the flu, an 8-year-old boy with IDDM is brought to the emergency room in a coma. His breathing is rapid and deep, and his breath has a fruity odor. His blood glucose is 36.5 mM (normal: 4–8 mM). The physician administers IV fluids, insulin, and potassium chloride. A rapid effect of insulin in this situation is to stimulate
 - gluconeogenesis in the liver
 - fatty acid release from adipose
 - glucose transport in muscle
 - ketone utilization in the brain
 - glycogenolysis in the liver
- An alcoholic has been on a 2-week drinking binge during which time she has eaten little and has become severely hypoglycemic. Which additional condition may develop in response to chronic, severe hypoglycemia?
 - Glycogen accumulation in the liver with cirrhosis
 - Thiamine deficiency
 - Ketoacidosis
 - Folate deficiency
 - Hyperuricemia

Answers

- Answer: C.** Insulin increases glucose transport in only two tissues, adipose and muscle. The major site of glucose uptake is muscle, which decreases hyperglycemia. Glucose and ketone transport and metabolism are insulin independent in the brain (**choice D**). Insulin would slow gluconeogenesis (**choice A**) and fatty acid release from adipose (**choice B**). Insulin would inhibit glycogenolysis in the liver (**choice E**).
- Answer: C.** Severe hypoglycemia lowers the insulin level and increases glucagon. This would favor fatty acid release from the adipose and ketogenesis in the liver.

Glycolysis and Pyruvate Dehydrogenase

12

OVERVIEW

All cells can carry out glycolysis. In a few tissues, most importantly red blood cells, glycolysis represents the only energy-yielding pathway available. Glucose is the major monosaccharide that enters the pathway, but others such as galactose and fructose can also be used. The first steps in glucose metabolism in any cell are transport across the membrane and phosphorylation by kinase enzymes inside the cell to prevent it from leaving via the transporter.

GLUCOSE TRANSPORT

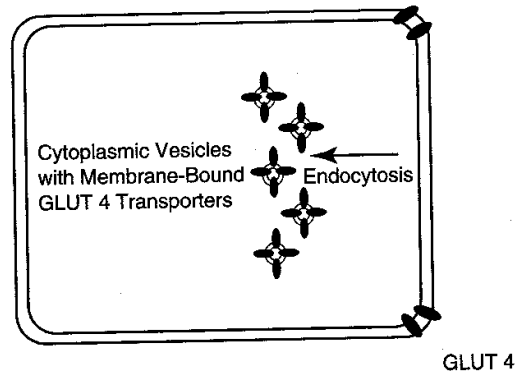
Glucose entry into most cells is concentration driven and independent of sodium. Four glucose transporters (GLUT) are listed in Table I-12-1. They have different affinities for glucose coinciding with their respective physiologic roles. Normal glucose concentration in peripheral blood is 4–8 mM (70–140 mg/dL).

- GLUT 1 and GLUT 3 mediate basal glucose uptake in most tissues, including brain, nerves, and red blood cells. Their high affinities for glucose ensure glucose entry even during periods of relative hypoglycemia. At normal glucose concentration, GLUT 1 and GLUT 3 are at V_{\max} .
- GLUT 2, a low-affinity transporter, is in hepatocytes. After a meal, portal blood from the intestine is rich in glucose. GLUT 2 captures the excess glucose primarily for storage. When the glucose concentration drops below the K_m for the transporter, much of the remainder leaves the liver and enters the peripheral circulation.
- GLUT 4 is in adipose tissue and muscle and responds to the glucose concentration in peripheral blood. The rate of glucose transport in these two tissues is increased by insulin, which stimulates the movement of additional GLUT 4 transporters to the membrane by a mechanism involving exocytosis (Figure I-12-1).

Bridge to Physiology

GLUT 4 translocation to the cell membrane in skeletal muscle is stimulated by exercise. This effect, which is independent of insulin, involves a 5' AMP-activated kinase.

Decreased insulin decreases the number of plasma membrane GLUT 4 transporters



Increased insulin increases the number of plasma membrane GLUT 4 transporters

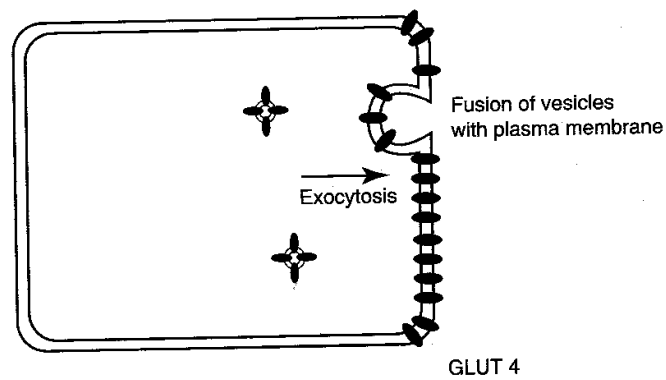


Figure I-12-1. Insulin Regulation of Glucose Transport in Muscle and Adipose Cells

Although basal transport occurs in all cells independently of insulin, the transport rate increases in adipose tissue and muscle when insulin levels rise. Muscle stores excess glucose as glycogen, and adipose tissue requires glucose to form dihydroxyacetone phosphate (DHAP), which is converted to glycerol phosphate used to store incoming fatty acids as triglyceride (TGL, three fatty acids attached to glycerol).

Table I-12-1. Major Glucose Transporters in Human Cells

Name	Tissues	K_m , glucose	Functions
GLUT 1	Most tissues (brain, red cells)	~1 mM	Basal uptake of glucose
GLUT 2	Liver Pancreatic β -cells	~15 mM	Uptake and release of glucose by the liver β -cell glucose sensor
GLUT 3	Most tissues	~1 mM	Basal uptake
GLUT 4	Skeletal muscle Adipose tissue	~5 mM	Insulin-stimulated glucose uptake

Normal blood glucose concentration is 4–8 mM.

GLYCOLYSIS

Glycolysis is a cytoplasmic pathway that converts glucose into two pyruvates, releasing a modest amount of energy captured in two substrate-level phosphorylations and one oxidation reaction. If a cell has mitochondria and oxygen, glycolysis is aerobic. If either mitochondria or oxygen is lacking, glycolysis may occur anaerobically (erythrocytes, exercising skeletal muscle), although some of the available energy is lost.

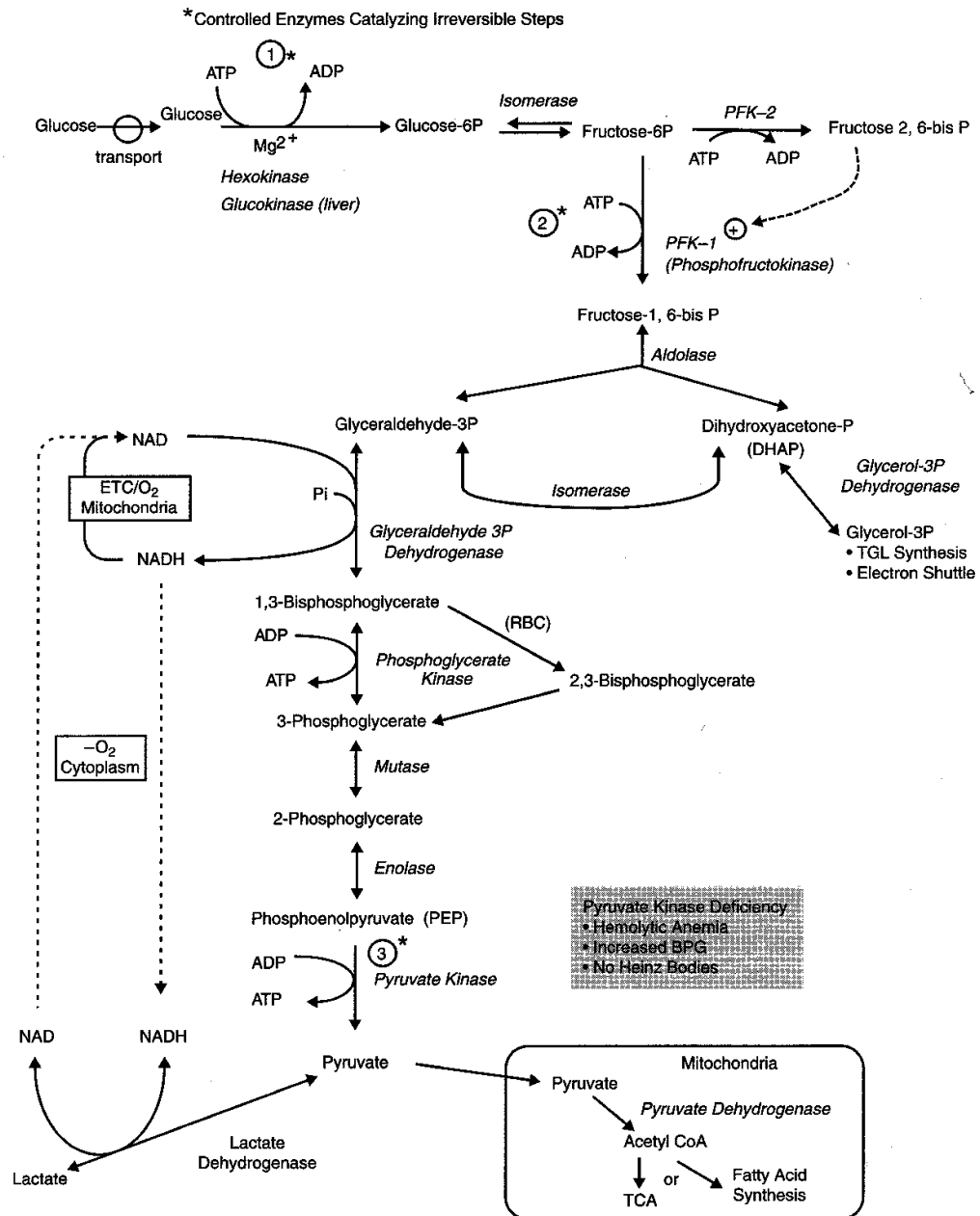


Figure I-12-2. Glycolysis

Glycolysis also provides intermediates for other pathways. In the liver, glycolysis is part of the process by which excess glucose is converted to fatty acids for storage. Glycolysis is shown in Figure I-12-2. Important enzymes in glycolysis include:

1. **Hexokinase/glucokinase:** glucose entering the cell is trapped by phosphorylation using ATP. Hexokinase is widely distributed in tissues, whereas glucokinase is found only in hepatocytes and pancreatic β -islet cells. Table I-12-2 identifies the differences in their respective K_m and V_{max} values. These coincide with the differences in K_m values for the glucose transporters in these tissues listed in Table I-12-1.

Table I-12-2. Comparison of Hexokinase and Glucokinase

Hexokinase	Glucokinase
Most tissues	Hepatocytes and pancreatic β -islet cells
Low K_m (0.05 mM in erythrocytes)	High K_m (10 mM)
Inhibited by glucose 6-phosphate	Induced by insulin in hepatocytes

2. **Phosphofructokinases (PFK-1 and PFK-2):** PFK-1 is the rate-limiting enzyme and main control point in glycolysis. In this reaction, fructose 6-phosphate is phosphorylated to fructose 1,6-bisphosphate using ATP.
 - PFK-1 is inhibited by ATP and citrate, and activated by AMP.
 - Insulin stimulates and glucagon inhibits PFK-1 in hepatocytes by an indirect mechanism involving PFK-2 and fructose 2,6-bisphosphate (Figure I-12-3).

Insulin activates PFK-2 (via the tyrosine kinase receptor and activation of protein phosphatases), which converts a tiny amount of fructose 6-phosphate to fructose 2,6-bisphosphate (F2,6-BP). F2,6-BP activates PFK-1. Glucagon inhibits PFK-2 (via cAMP-dependent protein kinase A), lowering F2,6 BP and thereby inhibiting PFK-1.

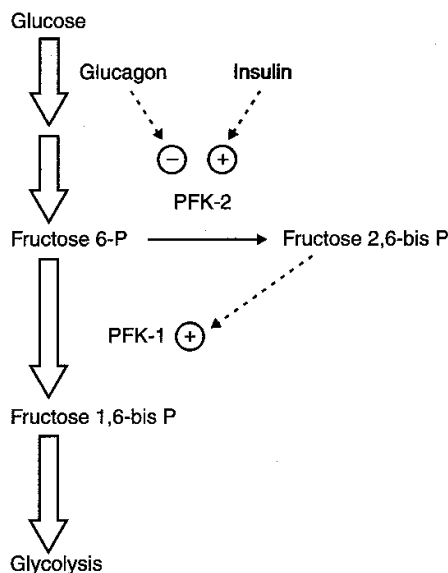


Figure I-12-3. PFK-2/PFK-1 Control by Insulin and Glucagon

3. **Glyceraldehyde 3-phosphate dehydrogenase:** catalyzes an oxidation and addition of inorganic phosphate (P_i) to its substrate. This results in the production of a high-energy intermediate 1,3-bisphosphoglycerate and the reduction of NAD to NADH. If glycolysis is aerobic, the NADH can be reoxidized (indirectly) by the mitochondrial electron transport chain, providing energy for ATP synthesis by oxidative phosphorylation.
4. **3-Phosphoglycerate kinase:** transfers the high-energy phosphate from 1,3-bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate. This type of reaction in which ADP is directly phosphorylated to ATP using a high-energy intermediate is referred to as a substrate-level phosphorylation. In contrast to oxidative phosphorylation in mitochondria, substrate-level phosphorylations are not dependent on oxygen, and are the only means of ATP generation in an anaerobic tissue.
5. **Pyruvate kinase:** the last enzyme in aerobic glycolysis, it catalyzes a substrate-level phosphorylation of ADP using the high-energy substrate phosphoenolpyruvate (PEP). Pyruvate kinase is activated by fructose 1,6-bisphosphate from the PFK-1 reaction (feed-forward activation).
6. **Lactate dehydrogenase:** is used only in anaerobic glycolysis. It reoxidizes NADH to NAD, replenishing the oxidized coenzyme for glyceraldehyde 3-phosphate dehydrogenase. Without mitochondria and oxygen, glycolysis would stop when all the available NAD had been reduced to NADH. By reducing pyruvate to lactate and oxidizing NADH to NAD, lactate dehydrogenase prevents this potential problem from developing. In aerobic tissues, lactate does not normally form in significant amounts. However, when oxygenation is poor (skeletal muscle during strenuous exercise, myocardial infarction), most cellular ATP is generated by anaerobic glycolysis, and lactate production increases.

Important Intermediates of Glycolysis

- Dihydroxyacetone phosphate (DHAP) is used in liver and adipose tissue for triglyceride synthesis.
- 1,3-Bisphosphoglycerate and phosphoenolpyruvate (PEP) are high-energy intermediates used to generate ATP by substrate-level phosphorylation.

Glycolysis Is Irreversible

Three enzymes in the pathway catalyze reactions that are irreversible. When the liver produces glucose, different reactions, and therefore different enzymes, must be used at these three points:

- Glucokinase/hexokinase
- PFK-1
- Pyruvate kinase

Glycolysis in the Erythrocyte

In red blood cells, anaerobic glycolysis represents the only pathway for ATP production, yielding a net 2 ATP/glucose.

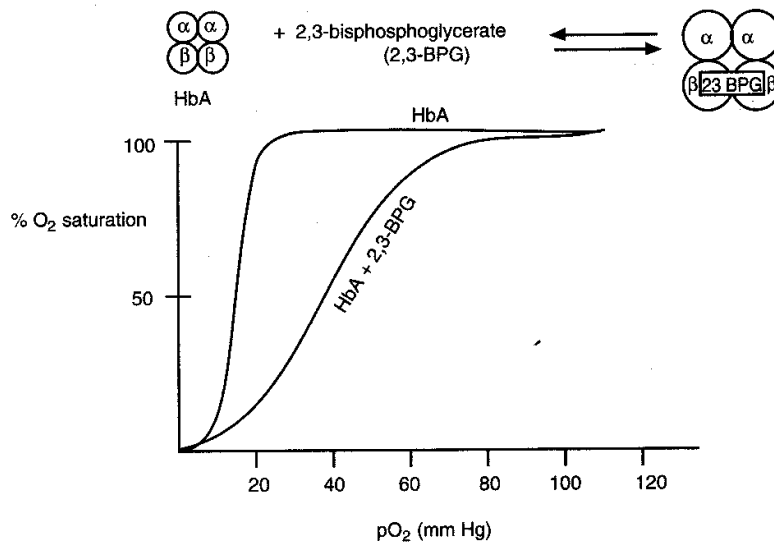


Figure I-12-4. The Effect of 2,3-Bisphosphoglycerate on Hemoglobin A

Erythrocytes have bisphosphoglycerate mutase, which produces 2,3-bisphosphoglycerate (BPG) from 1,3-BPG in glycolysis. 2,3-BPG binds to the β -chains of hemoglobin A (HbA) and decreases its affinity for oxygen. This effect of 2,3-BPG is seen in the oxygen dissociation curve for HbA shown in Figure I-12-4. The rightward shift in the curve is sufficient to allow unloading of oxygen in tissues, but still allows 100% saturation in the lungs. An abnormal increase in erythrocyte 2,3-BPG might shift the curve far enough so HbA is not fully saturated in the lungs.

Bridge to Physiology

Adaptation to high altitudes (low P_{O_2}) involves:

- Increased respiration
- Respiratory alkalosis
- Lower P_{50} for hemoglobin (initial)
- Increased rate of glycolysis
- Increased [2,3-BPG] in RBC (12–24 hours)
- Normal P_{50} for hemoglobin restored by the increased level of 2,3-BPG
- Increased hemoglobin and hematocrit (days–weeks)

Although 2,3-BPG binds to HbA, it does not bind well to HbF ($\alpha_2\gamma_2$), with the result that HbF has a higher affinity for oxygen than maternal HbA, allowing transplacental passage of oxygen from mother to fetus.

Pyruvate Kinase Deficiency

Pyruvate kinase deficiency is the second most common genetic deficiency that causes a hemolytic anemia (glucose 6-phosphate dehydrogenase, G6PDH, is the most common). Characteristics include:

- Chronic hemolysis
- Increased 2,3-BPG and thereby a lower-than-normal oxygen affinity of HbA
- Absence of Heinz bodies (Heinz bodies are more characteristic of G6PDH deficiency)

The red blood cell has no mitochondria and is totally dependent on anaerobic glycolysis for ATP. In pyruvate kinase deficiency, the decrease in ATP causes the erythrocyte to lose its characteristic biconcave shape and signals its destruction in the spleen. In addition, decreased ion pumping by Na^+/K^+ -ATPase results in loss of ion balance and causes osmotic fragility, leading to swelling and lysis.

ATP Production and Electron Shuttles

Anaerobic glycolysis yields 2 ATP/glucose by substrate-level phosphorylation. Aerobic glycolysis yields these 2 ATP/glucose plus 2 NADH/glucose that can be utilized for ATP production in the mitochondria; however, the inner membrane is impermeable to NADH. Cytoplasmic NADH is reoxidized to NAD and delivers its electrons to one of two electron shuttles in the inner membrane. In the malate shuttle, electrons are passed to mitochondrial NADH and then to the electron transport chain. In the glycerol phosphate shuttle, electrons are passed to mitochondrial FADH_2 . The two shuttles are diagrammed in Figure I-12-5; important points include:

- Cytoplasmic NADH oxidized using the malate shuttle produces a mitochondrial NADH and yields approximately 3 ATP by oxidative phosphorylation.
- Cytoplasmic NADH oxidized by the glycerol phosphate shuttle produces a mitochondrial FADH_2 and yields approximately 2 ATP by oxidative phosphorylation.

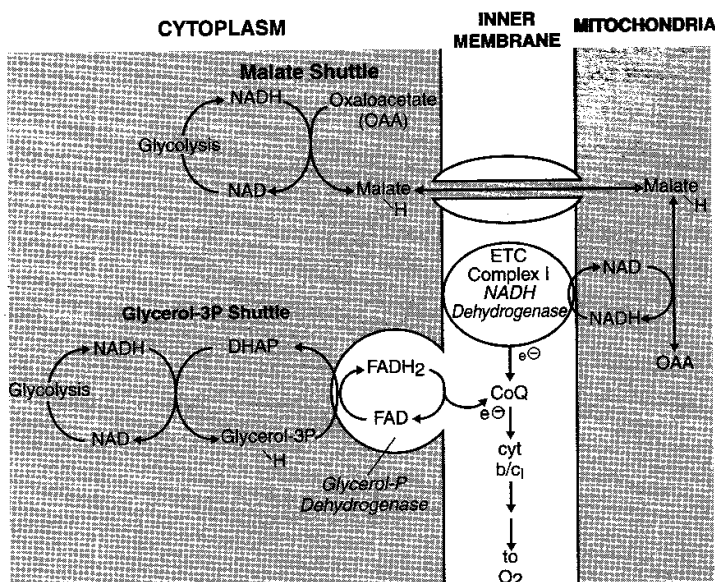


Figure I-12-5. Oxidation of Cytoplasmic NADH in the ETC Involves Two Electron Shuttles

The total ATP from aerobic glycolysis will depend on which shuttle is used. These numbers are approximations:

- 6 ATP/glucose (glycerol phosphate shuttle)
- 8 ATP/glucose (malate shuttle)

GALACTOSE METABOLISM

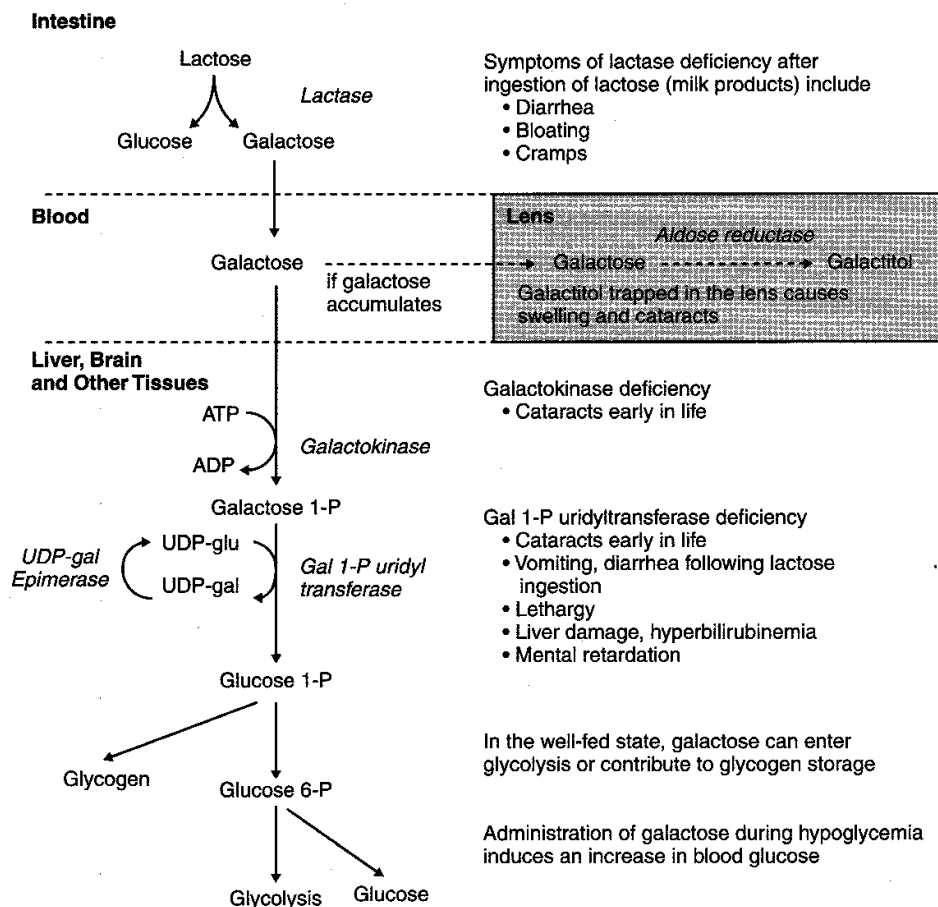


Figure I-12-6. Galactose Metabolism

An important source of galactose in the diet is the disaccharide lactose present in milk. Lactose is hydrolyzed to galactose and glucose by lactase associated with the brush border membrane of the small intestine. Along with other monosaccharides, galactose reaches the liver through the portal blood.

Once transported into tissues, galactose is phosphorylated (galactokinase), trapping it in the cell. Galactose 1-phosphate is converted to glucose 1-phosphate by galactose 1-P uridyltransferase and an epimerase. The pathway is shown in Figure I-12-6; important enzymes to remember are:

- Galactokinase
- Galactose 1-phosphate uridyltransferase

Genetic deficiencies of these enzymes produce galactosemia. **Cataracts, a characteristic finding in patients with galactosemia, result from conversion of the excess galactose in peripheral blood to galactitol in the lens of the eye, which has aldose reductase. Accumulation of galactitol in the lens causes osmotic damage and cataracts.**

The same mechanism accounts for the cataracts in diabetics because aldose reductase also converts glucose to sorbitol, which causes osmotic damage.

Deficiency of galactose 1-phosphate uridylyltransferase produces a more severe disease because, in addition to galactosemia, galactose 1-P accumulates in the liver, brain, and other tissues. The two enzyme deficiencies are compared in Table I-12-3.

Table I-12-3. Comparison of Galactokinase and Galactose 1-Phosphate Uridylyltransferase Deficiencies

Galactokinase Deficiency	Galactose 1-Phosphate Uridylyltransferase Deficiency
Galactosemia	Galactosemia
Galactosuria	Galactosuria
Cataracts in early childhood	Cataracts often within a few days of birth Vomiting and diarrhea after milk ingestion Jaundice and hyperbilirubinemia Hypoglycemia may be present Liver disease and cirrhosis Lethargy, hypotonia Mental retardation
Treatment: eliminate sources of galactose from diet	Treatment: eliminate sources of galactose from diet

Clinical Correlate

Primary lactose intolerance is caused by a hereditary deficiency of lactase, most commonly found in persons of Asian and African descent. Secondary lactose intolerance can be precipitated at any age by gastrointestinal disturbances such as celiac sprue, colitis, or viral-induced damage to intestinal mucosa. Common symptoms of lactose intolerance include vomiting, bloating, explosive and watery diarrhea, cramps, and dehydration. The symptoms can be attributed to bacterial fermentation of lactose to a mixture of CH_4 , H_2 , and small organic acids. The acids are osmotically active and result in the movement of water into the intestinal lumen. Diagnosis is based on a positive hydrogen breath test after an oral lactose load. Treatment is by dietary restriction of milk and milk products (except unpasteurized yogurt, which contains active *Lactobacillus*) or by lactase pills.

FRUCTOSE METABOLISM

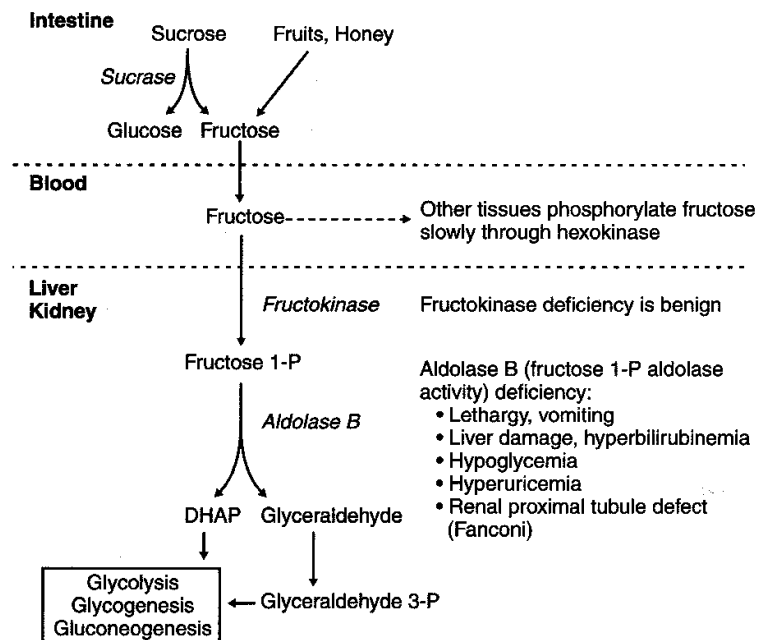


Figure I-12-7. Fructose Metabolism

Fructose is found in honey and fruit and as part of the disaccharide sucrose (common table sugar). Sucrose is hydrolyzed by intestinal brush border sucrase, and the resulting monosaccharides, glucose and fructose, are absorbed into the portal blood. The liver phosphorylates fructose and cleaves it into glyceraldehyde and DHAP. Smaller amounts are metabolized in renal proximal tubules. The pathway is shown in Figure I-12-7; important enzymes to remember are:

- Fructokinase
- Fructose 1-P aldolase (aldolase B)

Genetic deficiency of fructokinase is benign and often detected incidentally when the urine is checked for glucose with a dipstick. Fructose 1-phosphate aldolase deficiency is a severe disease because of accumulation of fructose 1-phosphate in the liver and renal proximal tubules. Table I-12-4 compares the two conditions. Symptoms are reversed after removing fructose and sucrose from the diet.

Cataracts are not a feature of this disease because fructose is not an aldose sugar and therefore not a substrate for aldose reductase in the lens.

Table I-12-4. Comparison of Fructokinase and Fructose 1-Phosphate Aldolase Deficiencies

Fructokinase Deficiency (Essential Fructosuria)	Fructose 1-Phosphate Aldolase (Aldolase B) Deficiency (Hereditary Fructose Intolerance)
Fructosuria	Fructosuria
Benign	Not evident while sole nutrition is breast milk Severe hypoglycemia and lactic acidosis after fructose ingestion Vomiting, apathy, diarrhea Liver damage and jaundice Proximal renal tubule disorder resembling Fanconi syndrome Treatment: eliminate sources of fructose from diet

PYRUVATE DEHYDROGENASE

Pyruvate from aerobic glycolysis enters mitochondria, where it may be converted to acetyl CoA for entry into the citric acid cycle if ATP is needed, or for fatty acid synthesis if sufficient ATP is present. The pyruvate dehydrogenase (PDH) reaction (Figure I-12-8) is irreversible and cannot be used to convert acetyl CoA to pyruvate or to glucose. Pyruvate dehydrogenase in the liver is activated by insulin, whereas in the brain and nerves the enzyme is not responsive to hormones.

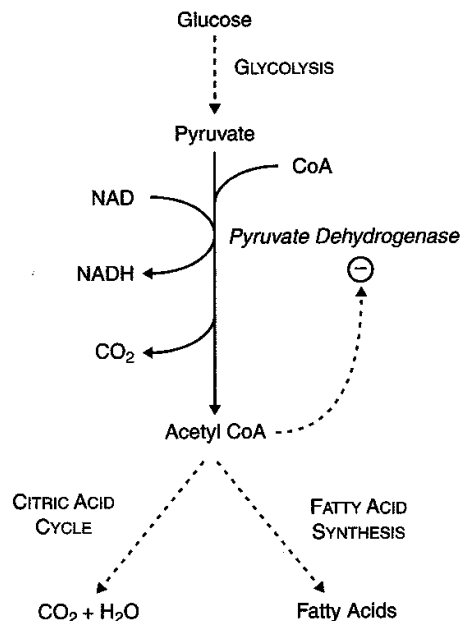


Figure I-12-8. Pyruvate Dehydrogenase

Cofactors and coenzymes used by pyruvate dehydrogenase include:

- Thiamine pyrophosphate (TPP) from the vitamin thiamine
- Lipoic acid
- Coenzyme A (CoA) from pantothenate
- FAD(H₂) from riboflavin
- NAD(H) from niacin (some may be synthesized from tryptophan)

Pyruvate dehydrogenase is inhibited by its product acetyl CoA. This control is important in several contexts and should be considered along with pyruvate carboxylase, the other mitochondrial enzyme that uses pyruvate (introduced in gluconeogenesis, Chapter 14, Figure I-14-5).

Thiamine Deficiency: Wernicke-Korsakoff Syndrome

Thiamine deficiency is commonly seen in alcoholics, who may develop a complex of symptoms associated with Wernicke peripheral neuropathy and Korsakoff psychosis. Alcohol interferes with thiamine absorption from the intestine. Symptoms include:

- Ataxia
- Ophthalmoplegia, nystagmus
- Memory loss and confabulation
- Cerebral hemorrhage

Congestive heart failure may be a complication (wet beri-beri) owing to inadequate ATP and accumulation of ketoacids in the cardiac muscle.

Two other enzyme complexes similar to pyruvate dehydrogenase that use thiamine are:

- α -Ketoglutarate dehydrogenase (citric acid cycle)
- Branched-chain ketoacid dehydrogenase (metabolism of branched-chain amino acids)

Insufficient thiamine significantly impairs glucose oxidation, causing highly aerobic tissues, such as brain and cardiac muscle, to fail first. In addition, branched-chain amino acids are sources of energy in brain and muscle.

Chapter Summary

Glycolysis

Glucose Transport

- GLUT 2: High K_m ; liver (storage) and β -islet (glucose sensor)
- GLUT 4: Lower K_m ; insulin-stimulated; adipose and muscle

Important Enzymes

Glucokinase (induced by insulin in liver), hexokinase (peripheral tissues)

PFK-1 (rate-limiting)

- Inhibitors: ATP, citrate
- Activators: AMP, fructose 2,6-bisphosphate (F 2,6-bisP)

PFK-2 responds to insulin (activated) and glucagon (inhibited).

- Produces F 2,6-bisP that activates PFK-1

Enzymes Catalyzing Irreversible Reactions

Glucokinase/hexokinase, PFK, pyruvate kinase

Aerobic Glycolysis

NADH reoxidized by mitochondrial electron transport chain

(Continued)

Chapter Summary (continued)

Anaerobic Glycolysis

NADH reoxidized by cytoplasmic lactate dehydrogenase

- Lactate released from tissue
- RBC, skeletal muscle (short intense burst of exercise)
- Any cell deprived of oxygen

ATP Yield

Anaerobic: 2 ATP/glucose (substrate level phosphorylations)

Aerobic: 6 to 8 ATP (substrate level phosphorylations and oxidation of NADH)

Genetic Deficiency

Pyruvate kinase

- Hemolytic anemia
- Possible decrease in hemoglobin affinity for oxygen due to increased RBC 2,3-BPG
- Heinz bodies rarely seen
- Autosomal recessive

Important Intermediates

Dihydroxyacetone phosphate (DHAP): forms glycerol 3-P for triglyceride synthesis.

Pyruvate Dehydrogenase (PDH)

- Mitochondrial
- Insulin-stimulated

Coenzymes: Thiamine, lipoic acid, CoA, FAD, NAD

Disease Association:

Wernicke-Korsakoff

- Most common in alcoholics
- Thiamine deficiency
- Neuropathy (ataxia, nystagmus, ophthalmoplegia)
- Memory loss and confabulation
- Psychosis

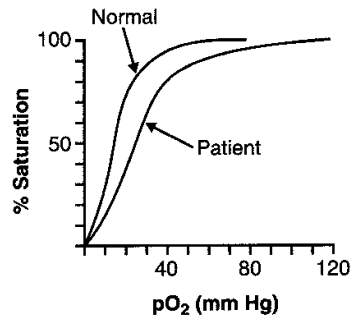
High-output cardiac failure

- Chronic, prolonged thiamine deficiency

Review Questions

Select the ONE best answer.

1. A 10-month-old child is being evaluated for the underlying cause of a hemolytic anemia. In the diagram shown below, the oxygen dissociation curve for hemoglobin in his erythrocytes is compared with the curve obtained with normal red cells.



- A deficiency of which enzyme is most likely to account for the hemolytic anemia in this patient?
- Glucokinase
 - Glucose 6-P dehydrogenase
 - Pyruvate carboxylase
 - Glutathione reductase
 - Pyruvate kinase
2. A breast-fed infant begins to vomit frequently and lose weight. Several days later she is jaundiced, her liver is enlarged, and cataracts are noticed in her lenses. These symptoms are most likely caused by a deficiency of
- galactose 1-P uridyltransferase
 - lactase
 - glucose 6-phosphatase
 - galactokinase
 - aldolase B
3. Following an early-morning run, a 29-year-old man consumes an all-American breakfast consisting of cereal, eggs, bacon, sausage, pancakes with maple syrup, doughnuts, and coffee with cream and sugar. Which of the following proteins will most likely be activated in his liver after breakfast?
- Cytoplasmic PEP carboxykinase
 - Plasma membrane GLUT-4 transporter
 - Cytoplasmic phosphofructokinase-2
 - Mitochondrial carnitine transporter
 - Cytoplasmic glycogen phosphorylase

Items 4 and 5

A 55-year-old alcoholic was brought to the emergency department by his friends. During their usual nightly gathering at the local bar, he had passed out and they had been unable to revive him. The physician ordered an injection of thiamine followed by overnight parenteral glucose. The next morning the patient was alert and coherent, serum thiamine was normal, and blood glucose was 73 mg/dL (4 mM). The IV line was removed, and he was taken home.

4. Which of the following enzymes is thiamine-dependent and essential for glucose oxidation in the brain?
 - A. Transketolase
 - B. Transaldolase
 - C. Succinyl-CoA thiokinase
 - D. Acetyl-CoA carboxylase
 - E. Pyruvate dehydrogenase
5. At the time of discharge from the hospital, which of the following proteins would have no significant physiologic activity in this patient?
 - A. Malate dehydrogenase
 - B. Glucokinase
 - C. α -Ketoglutarate dehydrogenase
 - D. GLUT 1 transporter
 - E. Phosphofructokinase-1

Answers

1. **Answer: E.** A right-shift in the O_2 binding curve is indicative of abnormally elevated 2,3-BPG secondary to a defect in red cell anaerobic glycolysis. Only pyruvate kinase participates in this pathway.
2. **Answer: A.** Cataracts + liver disease in a milk-fed infant = classic galactosemia.
3. **Answer: C.** Only PFK-2 will be insulin-activated in the postprandial period.
4. **Answer: E.** Most important TPP-dependent enzymes include pyruvate dehydrogenase, α -keto-glutarate dehydrogenase, and transketolase. Transketolase is in the HMP shunt and is not strictly essential for glucose oxidation.
5. **Answer: B.** After an overnight fast (plasma glucose 73 mg/dL), the liver is producing glucose and glucokinase activity would be insignificant (high K_m , low insulin). The other proteins would be needed for aerobic glucose oxidation in the brain or for hepatic gluconeogenesis.

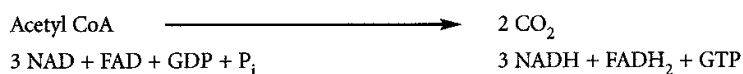
Citric Acid Cycle and Oxidative Phosphorylation

13

CITRIC ACID CYCLE

The citric acid cycle, also called the Krebs cycle or the tricarboxylic acid (TCA) cycle, is in the mitochondria. Although oxygen is not directly required in the cycle, the pathway will not occur anaerobically because NADH and FADH₂ will accumulate if oxygen is not available for the electron transport chain.

The primary function of the citric acid cycle is oxidation of acetyl CoA to carbon dioxide. The energy released from this oxidation is saved as NADH, FADH₂, and guanosine triphosphate (GTP). The overall result of the cycle is represented by the following reaction:



Notice that none of the intermediates of the citric acid cycle appear in this reaction, not as reactants or as products. This emphasizes an important (and frequently misunderstood) point about the cycle. It does not represent a pathway for the net conversion of acetyl CoA to citrate, to malate, or to any other intermediate of the cycle. The only fate of acetyl CoA in this pathway is its oxidation to CO₂. Therefore, the citric acid cycle does not represent a pathway by which there can be net synthesis of glucose from acetyl CoA.

The cycle is central to the oxidation of any fuel that yields acetyl CoA, including glucose, fatty acids, ketone bodies, ketogenic amino acids, and alcohol. There is no hormonal control of the cycle, as activity is necessary irrespective of the fed or fasting state. Control is exerted by the energy status of the cell.

The citric acid cycle is shown in Figure I-13-1. All the enzymes are in the matrix of the mitochondria except succinate dehydrogenase, which is in the inner membrane.

Key points:

1. Isocitrate dehydrogenase, the major control enzyme, is inhibited by NADH and activated by ADP.
2. α -Ketoglutarate dehydrogenase is similar to the pyruvate dehydrogenase complex. It requires thiamine, lipoic acid, CoA, FAD, and NAD. Lack of thiamine slows oxidation of acetyl CoA in the citric acid cycle.
3. Succinyl CoA synthetase (succinate thiokinase) catalyzes a substrate-level phosphorylation of GDP to GTP.
4. Succinate dehydrogenase is on the inner mitochondrial membrane, where it also functions as complex II of the electron transport chain.

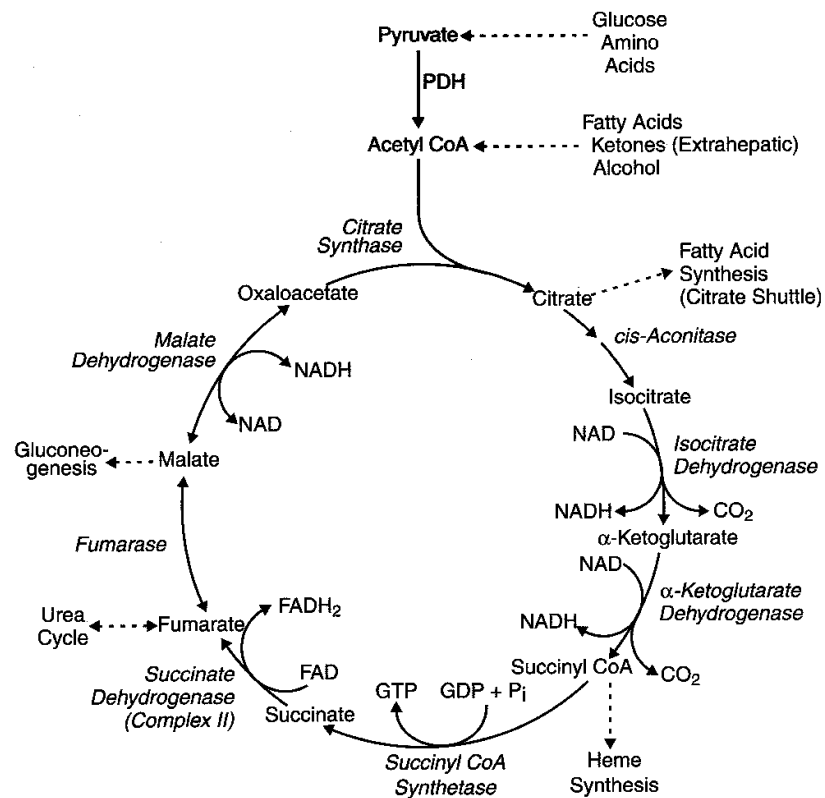


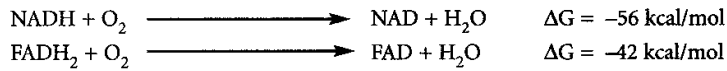
Figure I-13-1. The Citric Acid Cycle

Several intermediates of the cycle may serve other functions:

- Citrate may leave the mitochondria (citrate shuttle) to deliver acetyl CoA into the cytoplasm for fatty acid synthesis.
- Succinyl CoA is a high-energy intermediate that can be used for heme synthesis and to activate ketone bodies in extrahepatic tissues.
- Malate can leave the mitochondria (malate shuttle) for gluconeogenesis.

ELECTRON TRANSPORT CHAIN AND OXIDATIVE PHOSPHORYLATION

The mitochondrial electron transport chain (ETC) carries out the following two reactions:



Although the value of ΔG should not be memorized, it does indicate the large amount of energy released by both reactions. The electron transport chain is a device to capture this energy in a form useful for doing work.

Sources of NADH, FADH₂, and O₂

Many enzymes in the mitochondria, including those of the citric acid cycle and pyruvate dehydrogenase, produce NADH, all of which can be oxidized in the electron transport chain and in the process, capture energy for ATP synthesis by oxidative phosphorylation. If NADH is produced in the cytoplasm, either the malate shuttle or the α -glycerol phosphate shuttle can transfer the electrons into the mitochondria for delivery to the ETC. Once NADH has been oxidized, the NAD can again be used by enzymes that require it.

FADH₂ is produced by succinate dehydrogenase in the citric acid cycle and by the α -glycerol phosphate shuttle. Both enzymes are located in the inner membrane and can reoxidize FADH₂ directly by transferring electrons into the ETC. Once FADH₂ has been oxidized, the FAD can be made available once again for use by the enzyme.

O₂ is delivered to tissues by hemoglobin. The majority of oxygen required in a tissue is consumed in the ETC. Its function is to accept electrons at the end of the chain, and the water formed is added to the cellular water. This scheme is shown in Figure I-13-2.

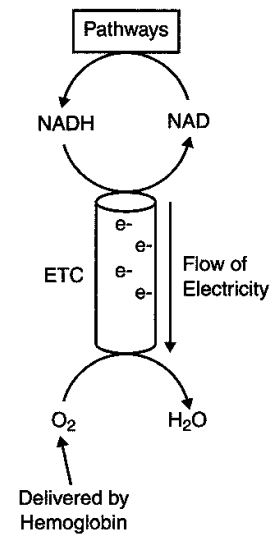


Figure I-13-2. Overview of the Electron Transport Chain

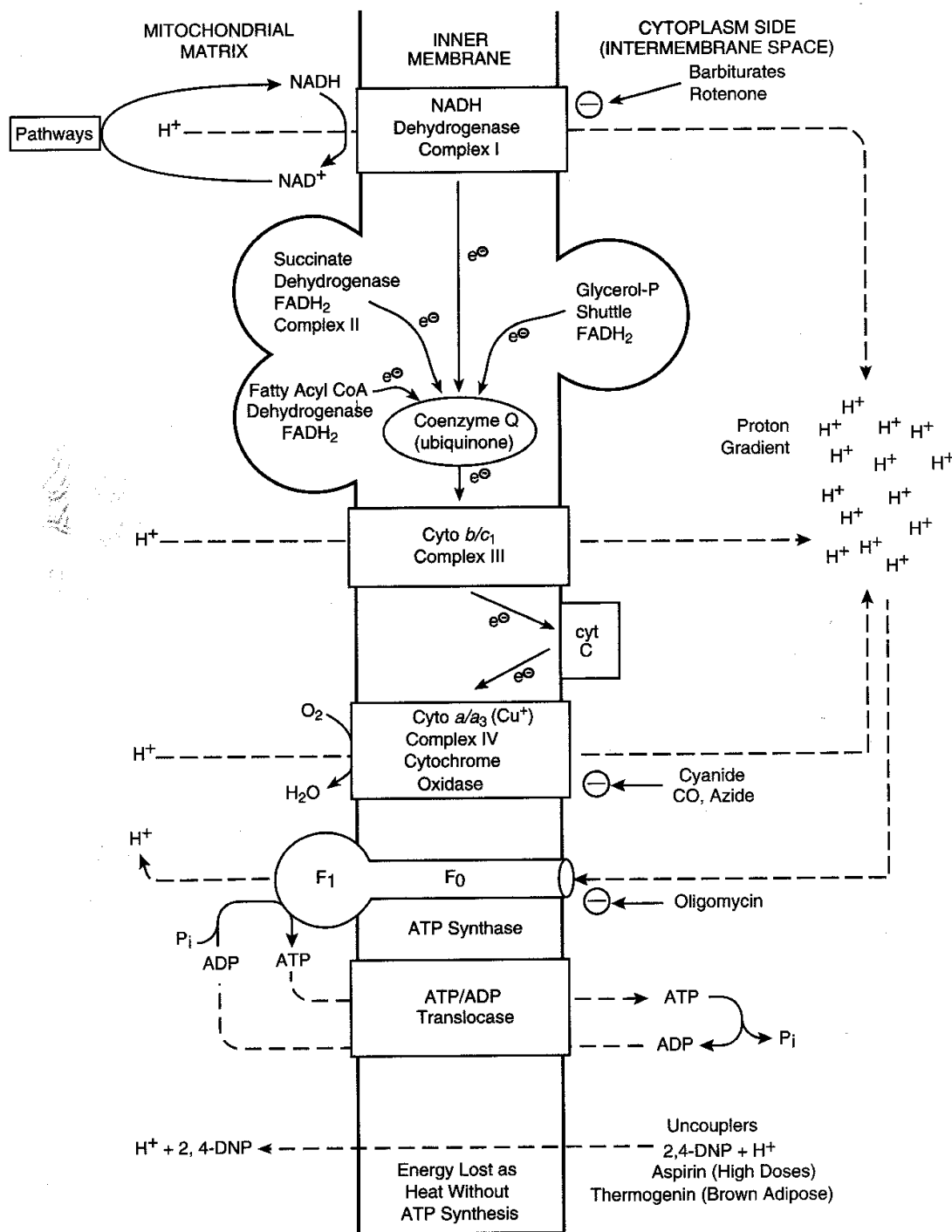


Figure I-13-3. Oxidative Phosphorylation

Capturing Chemical Energy as Electricity

The mitochondrial electron transport chain works like a chemical battery. In one location, an oxidation reaction is poised to release electrons at very high energy; in another location, a potential electron acceptor waits to be reduced. Because the two components are physically separated, nothing happens. Once the two terminals of the battery are connected by a wire, electrons flow from one compartment to the other through the wire, producing an electrical current or electricity. A light bulb or an electrical pump inserted into the circuit will run on the electricity generated. If no electrical device is in the circuit, all the energy is released as heat. The mitochondrial electron transport chain operates according to the same principle.

Electron Transport Chain

NADH is oxidized by NADH dehydrogenase (complex I), delivering its electrons into the chain and returning as NAD to enzymes that require it. The electrons are passed along a series of protein and lipid carriers that serve as the wire. These include, in order:

- NADH dehydrogenase (complex I) accepts electrons from NADH
- Coenzyme Q (a lipid)
- Cytochrome *b/c*₁ (an Fe/heme protein; complex III)
- Cytochrome *c* (an Fe/heme protein)
- Cytochrome *a/a*₃ (a Cu/heme protein; cytochrome oxidase, complex IV) transfers electrons to oxygen

All these components are in the inner membrane of the mitochondria as shown in Figure I-13-3. Succinate dehydrogenase and the α -glycerol phosphate shuttle enzymes reoxidize their FADH_2 and pass electrons directly to CoQ.

Proton Gradient

The electricity generated by the ETC is used to run proton pumps (translocators), which drive protons from the matrix space across the inner membrane into the intermembrane space, creating a small proton (or pH) gradient. This is similar to pumping any ion, such as Na^+ , across a membrane to create a gradient. The three major complexes I, III, and IV (NADH dehydrogenase, cytochrome *b/c*₁, and cytochrome *a/a*₃) each translocate protons in this way as the electricity passes through them. The end result is that a proton gradient is normally maintained across the mitochondrial inner membrane. If proton channels open, the protons run back into the matrix. Such proton channels are part of the oxidative phosphorylation complex.

Oxidative Phosphorylation

ATP synthesis by oxidative phosphorylation uses the energy of the proton gradient and is carried out by the F_0F_1 ATP synthase complex, which spans the inner membrane as shown in Figure I-13-3. As protons flow into the mitochondria through the F_0 component, their energy is used by the F_1 component (ATP synthase) to phosphorylate ADP using P_i . On average, when an NADH is oxidized in the ETC, sufficient energy is contributed to the proton gradient for the phosphorylation of 3 ATP by F_0F_1 ATP synthase. FADH_2 oxidation provides enough energy for approximately 2 ATP. These figures are referred to as the P/O ratios.

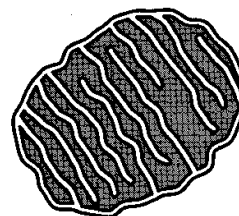


Figure I-13-4.
Mitochondrion

Bridge to Pathology**Ischemic Chest Pain**

Patients with chest pain whose symptoms are suggestive of acute myocardial infarction (AMI) are evaluated by electrocardiogram (EKG) and by serial measurements of cardiac enzymes. Although myocardial specific CK-MB has been used as an early indicator of an AMI, measurements of troponin levels are rapidly replacing it. Troponin I and troponin T are sensitive and specific markers that appear three to six hours after the onset of symptoms, peak by 16 hours, and remain elevated for nearly a week. In the absence of ST-segment elevation on the EKG, elevated troponin I and troponin T are useful indicators of those patients at high risk for evolving myocardial infarction. LDH isozyme analysis may be helpful if a patient reports chest pain that occurred several days previously because this change ($\text{LDH}_1 > \text{LDH}_2$) peaks two to three days following an AMI.

Tissue Hypoxia

Hypoxia deprives the ETC of sufficient oxygen, decreasing the rate of ETC and ATP production. When ATP levels fall, glycolysis increases and, in the absence of oxygen, will produce lactate (lactic acidosis). Anaerobic glycolysis is not able to meet the demand of most tissues for ATP, especially in highly aerobic tissues like nerves and cardiac muscle.

In a myocardial infarction (MI), myocytes swell as the membrane potential collapses and the cell gets leaky. Enzymes are released from the damaged tissue, and lactic acidosis contributes to protein precipitation and coagulation necrosis.

Inhibitors

The ETC is coupled to oxidative phosphorylation so that their activities rise and fall together. Inhibitors of any step effectively inhibit the whole coupled process, resulting in:

- Decreased oxygen consumption
- Increased intracellular NADH/NAD and FADH_2/FAD ratios
- Decreased ATP

Important inhibitors include cyanide and carbon monoxide.

Cyanide

Cyanide is a deadly poison because it binds irreversibly to cytochrome a/a_3 , preventing electron transfer to oxygen, producing many of the same changes seen in tissue hypoxia. Sources of cyanide include:

- Burning polyurethane (foam stuffing in furniture and mattresses)
- Byproduct of nitroprusside (released slowly; thiosulfate can be used to destroy the cyanide)

Nitrites may be used as an antidote for cyanide poisoning if given rapidly. They convert hemoglobin to methemoglobin, which binds cyanide in the blood before reaching the tissues. Oxygen is also given if possible.

Carbon Monoxide

Carbon monoxide binds to cytochrome a/a_3 but less tightly than cyanide. It also binds to hemoglobin, displacing oxygen. Symptoms include headache, nausea, tachycardia, and tachypnea. Lips and cheeks turn a cherry-red color. Respiratory depression and coma result in death if not treated by giving oxygen. Sources of carbon monoxide include:

- Propane heaters and gas grills
- Vehicle exhaust
- Tobacco smoke
- House fires
- Methylene chloride-based paint strippers

Other inhibitors include antimycin (cytochrome b/c_1), doxorubicin (CoQ), and oligomycin (F_0).

Uncouplers

Uncouplers decrease the proton gradient, causing:

- Decreased ATP synthesis
- Increased oxygen consumption
- Increased oxidation of NADH

Because the rate of the ETC increases, with no ATP synthesis, energy is released as heat. Important uncouplers include 2,4-dinitrophenol (2,4-DNP) and aspirin (and other salicylates). Brown adipose tissue contains a natural uncoupling protein (UCP, formerly called thermogenin), which allows energy loss as heat to maintain a basal temperature around the kidneys, neck, breastplate, and scapulae in newborns.

Reactive Oxygen Species

When molecular oxygen (O_2) is partially reduced, unstable products called reactive oxygen species (ROS) are formed. These react rapidly with lipids to cause peroxidation, with proteins, and with other substrates, resulting in denaturation and precipitation in tissues. Reactive oxygen species include:

- Superoxide ($O_2^{\cdot-}$)
- Hydrogen peroxide (H_2O_2)
- Hydroxyl radical (OH^{\cdot})

The polymorphonuclear neutrophil produces these substances to kill bacteria in the protective space of the phagolysosome during the oxidative burst accompanying phagocytosis. Production of these same ROS can occur at a slower rate wherever there is oxygen in high concentration. Small quantities of ROS are inevitable by-products of the electron transport chain in mitochondria. These small quantities are normally destroyed by protective enzymes such as catalase. The rate of ROS production can increase dramatically under certain conditions, such as reperfusion injury in a tissue that has been temporarily deprived of oxygen. ATP levels will be low and NADH levels high in a tissue deprived of oxygen (as in an MI). When oxygen is suddenly introduced, there is a burst of activity in the ETC, generating incompletely reduced ROS.

Defenses against ROS accumulation include the enzymes superoxide dismutase, glutathione peroxidase, and catalase.

Mutations in Mitochondrial DNA

The circular mitochondrial chromosome encodes 13 of the more than 80 proteins that comprise the major complexes of oxidative phosphorylation as well as 22 tRNAs and 2 rRNAs. Mutations in these genes affect highly aerobic tissues (nerves, muscle), and the diseases exhibit characteristic mitochondrial pedigrees (maternal inheritance).

- Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)
- Leber hereditary optic neuropathy
- Ragged red muscle fiber disease

Bridge to Pharmacology

Aspirin in doses used to treat rheumatoid arthritis can result in uncoupling of oxidative phosphorylation, increased oxygen consumption, depletion of hepatic glycogen, and the pyretic effect of toxic doses of salicylate. Depending on the degree of salicylate intoxication, the symptoms can vary from tinnitus to pronounced CNS and acid-base disturbance.

Coordinate Regulation of the Citric Acid Cycle and Oxidative Phosphorylation

The rates of oxidative phosphorylation and the citric acid cycle are closely coordinated, and are dependent mainly on the availability of O_2 and ADP. If O_2 is limited, the rate of oxidative phosphorylation decreases, and the concentrations of NADH and $FADH_2$ increase. The accumulation of NADH, in turn, inhibits the citric acid cycle. The coordinated regulation of these pathways is known as “respiratory control.”

In the presence of adequate O_2 , the rate of oxidative phosphorylation is dependent on the availability of ADP. The concentrations of ADP and ATP are reciprocally related; an accumulation of ADP is accompanied by a decrease in ATP and the amount of energy available to the cell. Therefore, ADP accumulation signals the need for ATP synthesis. ADP allosterically activates isocitrate dehydrogenase, thereby increasing the rate of the citric acid cycle and the production of NADH and $FADH_2$. The elevated levels of these reduced coenzymes, in turn, increase the rate of electron transport and ATP synthesis.

Chapter Summary

Citric Acid Cycle

Mitochondria

Function

Acetyl (CoA) is completely oxidized to carbon dioxide.

- Energy saved as $FADH_2$, NADH, GTP
- Cycle functions catalytically; no net synthesis of intermediates from acetyl CoA

Controlled Step

Isocitrate dehydrogenase inhibited by NADH (causing the citric acid cycle to stop when the ETC stops in the anaerobic cell).

Other Important Enzymes

α -Ketoglutarate dehydrogenase (thiamine, lipoic acid, CoA, FAD, NAD)

Links Between Cycle Intermediates and Other Pathways

- Citrate carries acetyl CoA into cytoplasm for fatty acid synthesis.
- Succinyl CoA used for heme synthesis
- OAA from pyruvate in gluconeogenesis
- Gluconeogenesis from several amino acids uses the malate shuttle.

(Continued)

Chapter Summary (continued)

Electron Transport and Oxidative Phosphorylation

Mitochondrial inner membrane (cell membrane in prokaryotes)

Function

- Oxidizes NADH and FADH_2
- Generates electrical energy by passing electrons through the ETC to O_2
- Creates a proton gradient across the inner membrane: $[\text{H}^+]_{\text{in}} < [\text{H}^+]_{\text{out}}$
- Proton gradient drives phosphorylation of ADP to ATP

Inhibitors

ATP synthesis decreases, ETC decreases, O_2 consumption decreases

- Cyanide (Complex IV, cytochrome oxidase)
- Barbiturates (Complex I, NADH dehydrogenase)
- Oligomycin (F_0 component of F_0F_1 ATP synthase)

Uncouplers

ATP synthesis decreases, ETC decreases, O_2 consumption decreases

- Destroy the proton gradient
- Produce heat rather than ATP
- 2,4-DNP
- Aspirin in high doses
- Uncoupling proteins (thermogenin)

Important Patients

Myocardial (or other) infarction

Genetic Deficiencies

Mitochondrial pedigrees (neuropathies/myopathies)

Review Questions

Select the ONE best answer.

1. During a myocardial infarction, the oxygen supply to an area of the heart is dramatically reduced, forcing the cardiac myocytes to switch to anaerobic metabolism. Under these conditions, which of the following enzymes would be activated by increasing intracellular AMP?
 - A. Succinate dehydrogenase
 - B. Phosphofructokinase-1
 - C. Glucokinase
 - D. Pyruvate dehydrogenase
 - E. Lactate dehydrogenase

Items 2 and 3

A 40-year-old black man is seen in the emergency room for a severe headache. His blood pressure is 180/110 mm Hg, and he has evidence of retinal hemorrhage. An infusion of nitroprusside is given.

2. Which of the following enzymes is affected most directly by the active metabolite of this drug?
 - A. Phospholipase A2
 - B. Cyclic AMP phosphodiesterase
 - C. Guanylate cyclase
 - D. Cyclic GMP phosphodiesterase
 - E. Phospholipase C
3. When nitroprusside is given in higher than usual doses, it may be accompanied by the administration of thiosulfate to reduce potential toxic side effects. Which complex associated with electron transport or oxidative phosphorylation is most sensitive to the toxic byproduct that may accumulate with high doses of nitroprusside?
 - A. NADH dehydrogenase
 - B. Succinate dehydrogenase
 - C. Cytochrome b/c_1
 - D. Cytochrome a/a_3
 - E. F_0F_1 ATP synthase
4. A patient has been exposed to a toxic compound that increases the permeability of mitochondrial membranes for protons. Which of the following events in liver cells would you expect to occur?
 - A. Increased ATP levels
 - B. Increased F_1F_0 ATP synthase activity
 - C. Increased oxygen utilization
 - D. Decreased malate-aspartate shuttle activity
 - E. Decreased pyruvate dehydrogenase activity

Items 5 and 6

- A. Citrate shuttle
 - B. Glycerolphosphate shuttle
 - C. Malate-aspartate shuttle
 - D. Carnitine shuttle
 - E. Adenine nucleotide shuttle
5. Delivers electrons from FADH_2 to coenzyme Q.
6. Required for the hepatic conversion of pyruvate to glucose.

Answers

1. **Answer: B.** Both PFK-1 and LDH participate in extrahepatic anaerobic glycolysis, but only PFK-1 is regulated by allosteric effectors.
2. **Answer: C.** Nitroprusside is metabolized to produce nitric oxide. NO, normally produced by the vascular endothelium, stimulates the cyclase in vascular smooth muscle to increase cGMP, activate protein kinase G, and cause relaxation.
3. **Answer: D.** In addition to NO, metabolism of nitroprusside also releases small quantities of cyanide, a potent and potentially lethal inhibitor of $\text{cyt } a/a_3$ (Complex IV). Thiosulfate is a common antidote for CN poisoning.
4. **Answer: C.** The toxic agent (example, 2,4-dinitrophenol) would uncouple oxidative phosphorylation, leading to a fall in ATP levels, increased respiration, and increased substrate utilization.
5. **Answer: B.** The glycerol-P shuttle oxidizes cytoplasmic NADH and reduces FAD. The resultant FADH_2 is subsequently oxidized and the electrons passed to CoQ (Figure I-12-5).
6. **Answer: C.** Oxaloacetate, produced from pyruvate, exits the mitochondrion after conversion to malate.

Glycogen, Gluconeogenesis, and the Hexose Monophosphate Shunt

14

GLYCOGENESIS AND GLYCOGENOLYSIS

Glycogen, a branched polymer of glucose, represents a storage form of glucose. Glycogen synthesis and degradation occur primarily in liver and skeletal muscle, although other tissues, including cardiac muscle and the kidney, store smaller quantities.

Glycogen is stored in the cytoplasm as either single granules (skeletal muscle) or as clusters of granules (liver). The granule has a central protein core with polyglucose chains radiating outward to form a sphere (Figure I-14-1). Glycogen granules composed entirely of linear chains have the highest *density* of glucose near the core. If the chains are branched, the glucose *density* is highest at the periphery of the granule, allowing more rapid release of glucose on demand.

Glycogen stored in the liver is a source of glucose mobilized during hypoglycemia. Muscle glycogen is stored as an energy reserve for muscle contraction. In white (fast-twitch) muscle fibers, the glucose is converted primarily to lactate, whereas in red (slow-twitch) muscle fibers, the glucose is completely oxidized.

GLYCOGEN SYNTHESIS

Synthesis of glycogen granules begins with a core protein glycogenin. Glucose addition to a granule, shown in Figure I-14-2, begins with glucose 6-phosphate, which is converted to glucose 1-phosphate and activated to UDP-glucose for addition to the glycogen chain by glycogen synthase. Glycogen synthase is the rate-limiting enzyme of glycogen synthesis.

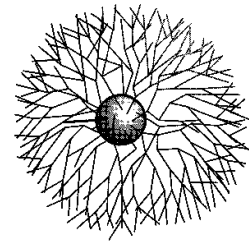


Figure I-14-1.
A Glycogen Granule

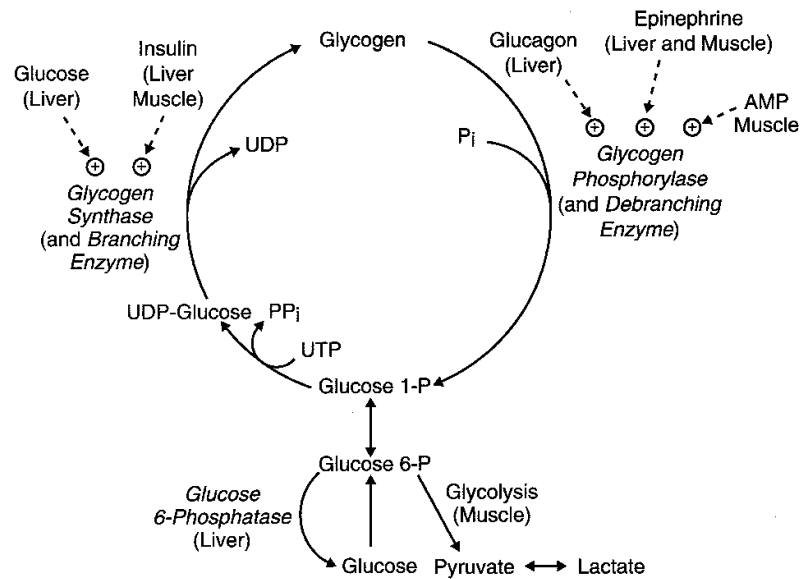


Figure I-14-2. Glycogen Metabolism

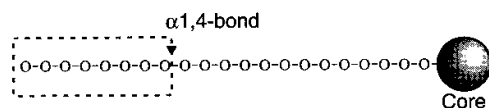
Glycogen Synthase

Glycogen synthase forms the α -1,4 glycosidic bond found in the linear glucose chains of the granule. Table I-14-1 shows the control of glycogen synthase in liver and skeletal muscle.

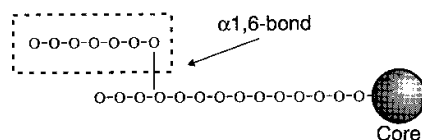
Table I-14-1. Comparison of Glycogen Synthase in Liver and Muscle

Glycogen Synthase	Liver	Skeletal Muscle
Activated by	Insulin Glucose	Insulin
Inhibited by	Glucagon Epinephrine	Epinephrine

Branching Enzyme (Glycosyl α -1,4: α -1,6 Transferase)



1. Glycogen synthase makes a linear α 1,4-linked polyglucose chain (o-o-o-o-o).
2. Branching enzyme hydrolyses an α 1,4-bond.



3. Transfers the oligoglucose unit and attaches it with an α 1,6-bond to create a branch.
4. Glycogen synthase extends both branches.

Figure I-14-3. Branching Enzyme

Branching enzyme is responsible for introducing α -1,6 linked branches into the granule as it grows. The process by which the branch is introduced is shown schematically in Figure I-14-3. Branching enzyme:

- Hydrolyzes one of the α -1,4 bonds to release a block of oligoglucose, which is then moved and added in a slightly different location.
- Forms an α -1,6 bond to create a branch.

GLYCOGENOLYSIS

The rate-limiting enzyme of glycogenolysis is glycogen phosphorylase (in contrast to a hydrolase, a phosphorylase breaks bonds using P_i rather than H_2O). The glucose 1-phosphate formed is converted to glucose 6-phosphate by the same mutase used in glycogen synthesis (Figure I-14-2).

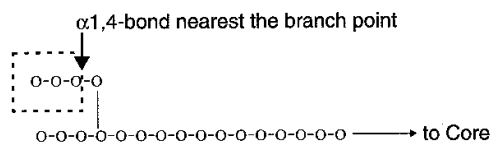
Glycogen Phosphorylase

Glycogen phosphorylase breaks α -1,4 glycosidic bonds, releasing glucose 1-phosphate from the periphery of the granule. Control of the enzyme in liver and muscle is compared in Table I-14-2.

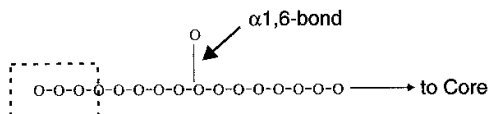
Table I-14-2. Comparison of Glycogen Phosphorylase in Liver and Muscle

Glycogen Phosphorylase	Liver	Skeletal Muscle
Activated by	Epinephrine Glucagon	Epinephrine AMP Ca ²⁺ (through calmodulin)
Inhibited by	Insulin	Insulin ATP

Glycogen phosphorylase cannot break α -1,6 bonds and therefore stops when it nears the outermost branch points.



1. Glycogen phosphorylase releases glucose 1-P from the periphery of the granule until it encounters the first branch points.
2. Debranching enzyme hydrolyzes the $\alpha 1,4$ -bond nearest the branch point as shown.



3. Transfers the oligoglucose unit to the end of another chain, then
4. Hydrolyzes the α 1,6-bond releasing the single glucose from the former branch.

Figure I-14-4. Debranching Enzyme

Debranching Enzyme (Glucosyl α -1,4: α -1,4 Transferase and α -1,6 Glucosidase)

Debranching enzyme deconstructs the branches in glycogen that have been exposed by glycogen phosphorylase. The two-step process by which this occurs is diagrammed in Figure I-14-4.

Debranching enzyme:

- Breaks an α -1,4 bond adjacent to the branch point and moves the small oligoglucose chain released to the exposed end of the other chain.
- Forms a new α -1,4 bond.
- Hydrolyzes the α -1,6 bond, releasing the single residue at the branch point as free glucose. This represents the only free glucose produced directly in glycogenolysis.

GENETIC DEFICIENCIES OF ENZYMES IN GLYCOGEN METABOLISM

Important genetic deficiencies, listed in Table I-14-3, are classed as glycogen storage diseases because all are characterized by accumulation of glycogen in one or more tissues.

Table I-14-3. Glycogen Storage Diseases

Type	Deficient Enzyme	Cardinal Clinical Features	Glycogen Structure
I: von Gierke	Glucose-6-phosphatase	Severe hypoglycemia, lactic acidosis, hepatomegaly, hyperlipidemia, hyperuricemia, short stature	Normal
II: Pompe	Lysosomal α -1,4-glucosidase	Cardiomegaly, muscle weakness, death by 2 years	Glycogen-like material in inclusion bodies
III: Cori	Glycogen debranching enzyme	Mild hypoglycemia, liver enlargement	Short outer branches Single glucose residue at outer branch
IV: Andersen (amylopectinosis)	Branching enzyme	Infantile hypotonia, cirrhosis, death by 2 years	Very few branches, especially toward periphery
V: McArdle	Muscle glycogen phosphorylase	Muscle cramps and weakness on exercise	Normal
VI: Hers	Hepatic glycogen phosphorylase	Mild fasting hypoglycemia, hepatomegaly, cirrhosis	Normal

Glucose 6-Phosphatase Deficiency (von Gierke Disease)

Deficiency of hepatic glucose 6-phosphatase produces a profound fasting hypoglycemia, lactic acidosis, and hepatomegaly. Additional symptoms include:

- Glycogen deposits in the liver (glucose 6-P stimulates glycogen synthesis, and glycogenolysis is inhibited)
- Hyperuricemia predisposing to gout. Decreased P_i causes increased AMP, which is degraded to uric acid. Lactate slows uric acid excretion in the kidney.
- Hyperlipidemia with skin xanthomas; elevation of triglycerides (VLDL)
- Fatty liver

In a person with glucose 6-phosphatase deficiency, ingestion of galactose or fructose causes no increase in blood glucose, nor does administration of glucagon or epinephrine.

Hepatic Glycogen Phosphorylase Deficiency (Hers Disease)

Hepatic glycogen phosphorylase deficiency is usually a relatively mild disease because gluconeogenesis compensates for the lack of glycogenolysis (Figure I-14-5). If present, hypoglycemia, hyperlipidemia, and hyperketosis are mild. Hepatomegaly and growth retardation may be present in early childhood, although hepatomegaly may improve with age.

Lysosomal α -1,4 Glucosidase Deficiency (Pompe Disease)

Pompe disease is different from the other diseases in Table I-14-3 because the enzyme missing is not one in the normal process of glycogenolysis described in this chapter. The deficient enzyme normally resides in the lysosome and is responsible for digesting glycogen-like material accumulating in endosomes. In this respect, it is more similar to diseases like Tay-Sachs or even I-cell disease in which indigestible substrates accumulate in inclusion bodies. In Pompe disease, the tissues most severely affected are those that normally have glycogen stores. With infantile onset, massive cardiomegaly is usually the cause of death, which occurs before 2 years of age.

GLUCONEOGENESIS

The liver maintains glucose levels in blood during fasting through either glycogenolysis or gluconeogenesis. These pathways are promoted by glucagon and epinephrine and inhibited by insulin. In fasting, glycogen reserves drop dramatically in the first 12 hours, during which time gluconeogenesis increases. After 24 hours, it represents the sole source of glucose. Important substrates for gluconeogenesis are:

- Gluconeogenic amino acids (protein from muscle)
- Lactate (from anaerobic glycolysis)
- Glycerol 3-phosphate (from triacylglycerol in adipose)

Dietary fructose and galactose can also be converted to glucose in the liver.

In humans, it is not possible to convert acetyl CoA to glucose. Inasmuch as most fatty acids are metabolized solely to acetyl CoA, they are not a major source of glucose either. One minor exception are odd-number carbon fatty acids (e.g., C17), which yield a small amount of propionyl CoA that is gluconeogenic.

The pathway of gluconeogenesis is diagrammed in Figure I-14-5. Lactate is oxidized to pyruvate by lactate dehydrogenase. The important gluconeogenic amino acid alanine is converted to pyruvate by alanine aminotransferase (ALT or GPT). Glycerol 3-phosphate is oxidized to dihydroxyacetone phosphate (DHAP) by glycerol 3-phosphate dehydrogenase. Most steps represent a reversal of glycolysis, and several of these have been omitted from the diagram. The four important enzymes are those required to catalyze reactions that circumvent the irreversible steps:

Glycogen, Gluconeogenesis, and the Hexose Monophosphate Shunt

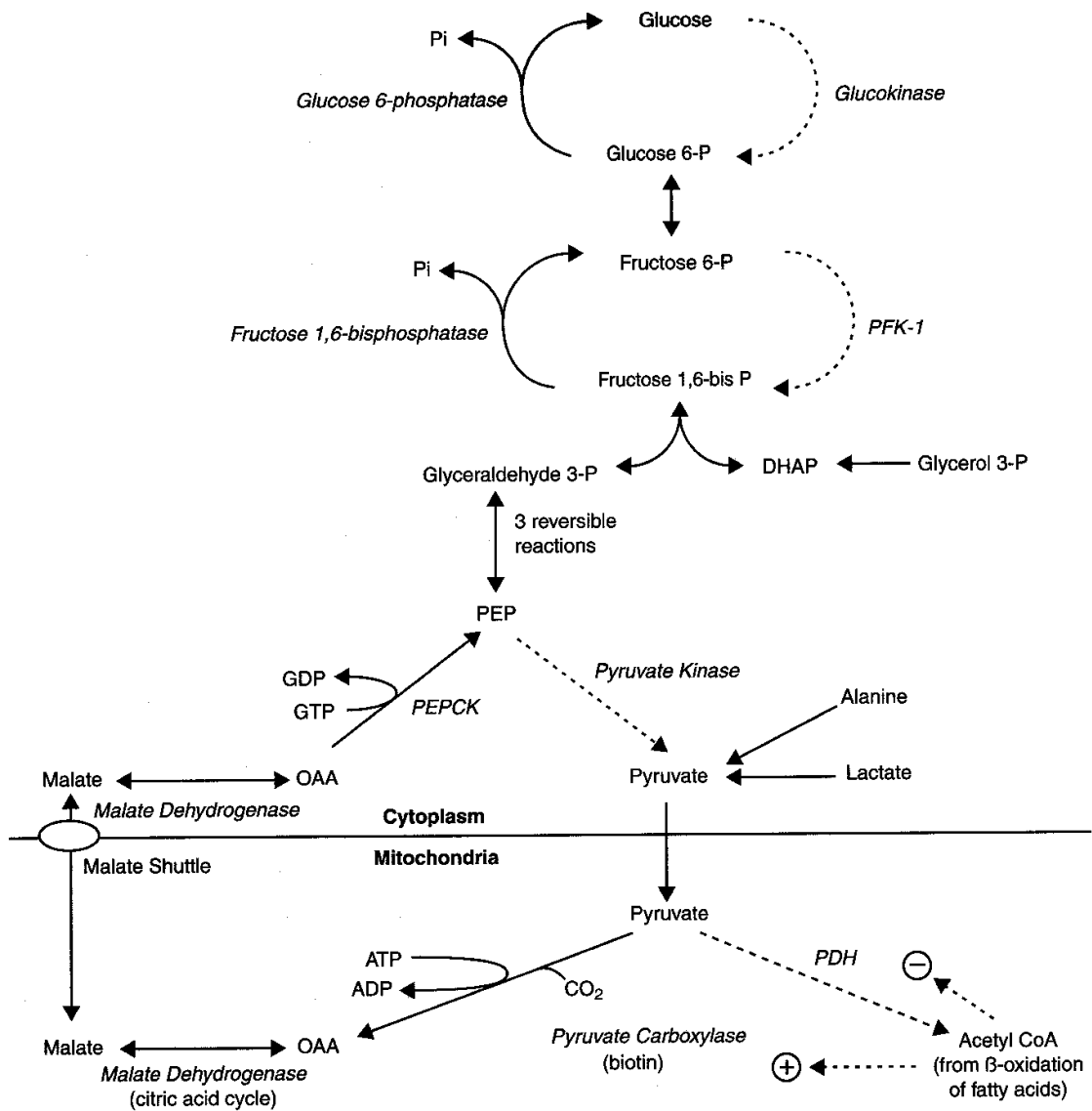


Figure I-14-5. Gluconeogenesis

1. **Pyruvate carboxylase** is a mitochondrial enzyme requiring biotin. It is activated by acetyl CoA (from β oxidation). The product **oxaloacetate (OAA)**, a citric acid cycle intermediate, cannot leave the mitochondria but is **reduced to malate** that can leave via the **malate shuttle**. In the cytoplasm, malate is reoxidized to OAA.
2. **Phosphoenolpyruvate carboxykinase (PEPCK)** in the cytoplasm is induced by glucagon and cortisol. It converts OAA to phosphoenolpyruvate (PEP) in a reaction that requires GTP. PEP continues in the pathway to fructose 1,6-bisphosphate.
3. **Fructose 1,6-bisphosphatase** in the cytoplasm is a key control point of gluconeogenesis. It hydrolyzes phosphate from fructose 1,6-bisphosphate rather than using it to generate ATP from ADP. A common pattern to note is that phosphatases oppose kinases. Fructose 1,6-bisphosphatase is activated by ATP and inhibited by AMP and fructose 2,6-bisphosphate. Fructose 2,6-bisphosphate, produced by PFK-2, controls both gluconeogenesis and glycolysis (in the liver). Recall from the earlier discussion of this enzyme (see Chapter 12, Figure I-12-3) that PFK-2 is activated by insulin and inhibited by glucagon. Thus, glucagon will lower F 2,6-BP and stimulate gluconeogenesis, whereas insulin will increase F 2,6-BP and inhibit gluconeogenesis.
4. **Glucose 6-phosphatase** is in the lumen of the endoplasmic reticulum. Glucose 6-phosphate is transported into the ER, and free glucose is transported back into the cytoplasm from which it leaves the cell. Glucose 6-phosphatase is only in the liver. The absence of glucose 6-phosphatase in skeletal muscle accounts for the fact that muscle glycogen cannot serve as a source of blood glucose (see Chapter 17, Figure I-17-3).

Although alanine is the major gluconeogenic amino acid, 18 of the 20 (all but leucine and lysine) are also gluconeogenic. Most of these are converted by individual pathways to citric acid cycle intermediates, then to malate, following the same path from there to glucose.

It is important to notice that glucose produced by hepatic gluconeogenesis does not represent an energy source for the liver. Gluconeogenesis requires expenditure of ATP, provided by β -oxidation of fatty acids. Therefore, hepatic gluconeogenesis is always dependent on β -oxidation of fatty acids in the liver. During hypoglycemia, adipose tissue releases these fatty acids by breaking down triglyceride.

Although the acetyl CoA from fatty acids cannot be converted to glucose, it can be converted to ketone bodies as an alternative fuel for cells, including the brain. Chronic hypoglycemia is thus often accompanied physiologically by an increase in ketone bodies.

Coordinate Regulation of Pyruvate Carboxylase and Pyruvate Dehydrogenase by Acetyl CoA

The two major mitochondrial enzymes (Figure I-14-5) that use pyruvate, pyruvate carboxylase and pyruvate dehydrogenase, are both regulated by acetyl CoA. This control is important in these contexts:

- Between meals when fatty acids are oxidized in the liver for energy, accumulating acetyl CoA activates pyruvate carboxylase and gluconeogenesis and inhibits PDH, thus preventing conversion of lactate and alanine to acetyl CoA.
- In the well-fed, absorptive state (insulin), accumulating acetyl CoA is shuttled into the cytoplasm for fatty acid synthesis. OAA is necessary for this transport, and acetyl CoA can stimulate its formation from pyruvate (see Chapter 15, Figure I-15-1).

Cori Cycle and Alanine Cycle

During fasting, lactate from red blood cells (and possibly exercising skeletal muscle) is converted in the liver to glucose that can be returned to the red blood cell or muscle. This is called the Cori cycle. The alanine cycle is a slightly different version of the Cori cycle, in which muscle releases alanine, delivering both a gluconeogenic substrate (pyruvate) and an amino group for urea synthesis.

Alcoholism and Hypoglycemia

Alcoholics are very susceptible to hypoglycemia. In addition to poor nutrition and the fact that alcohol is metabolized to acetate (acetyl CoA), the high amounts of cytoplasmic NADH formed by alcohol dehydrogenase and acetaldehyde dehydrogenase interfere with gluconeogenesis. High NADH favors the formation of:

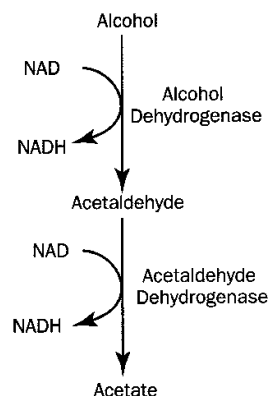
- Lactate from pyruvate
- Malate from OAA in the cytoplasm
- Glycerol 3-phosphate from DHAP

The effect is to divert important gluconeogenic substrates from entering the pathway.

Accumulation of cytoplasmic NADH and glycerol 3-P may also contribute to lipid accumulation in alcoholic liver disease. Free fatty acids released from adipose in part enter the liver where β -oxidation is very slow (high NADH). In the presence of high glycerol-3P, fatty acids are inappropriately stored in the liver as triglyceride.

Bridge to Pharmacology

Alcohol metabolism increases the NADH/NAD ratio in the cytoplasm.



HEXOSE MONOPHOSPHATE SHUNT

The hexose monophosphate (HMP) shunt (pentose phosphate pathway) occurs in the cytoplasm of all cells, where it serves two major functions:

- NADPH production
- Source of ribose 5-phosphate for nucleotide synthesis

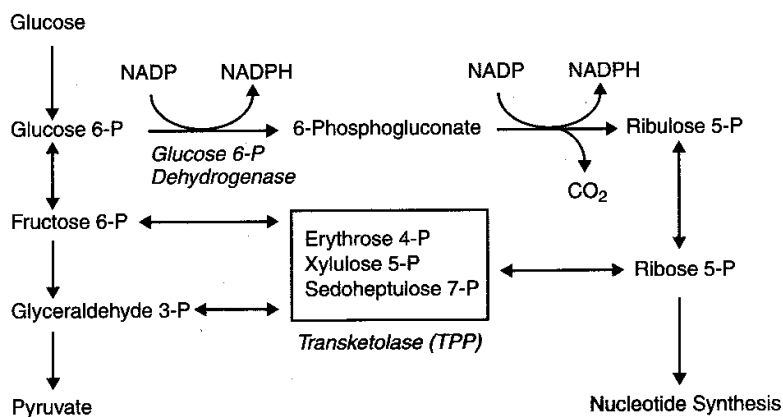


Figure I-14-6. The Hexose Monophosphate Shunt

An abbreviated diagram of the pathway is shown in Figure I-14-6. The first part of the HMP shunt begins with glucose 6-phosphate and ends with ribulose 5-phosphate and is irreversible. This part produces NADPH and involves the important rate-limiting enzyme glucose 6-phosphate dehydrogenase (G6PDH). G6PDH is induced by insulin, inhibited by NADPH, and activated by NADP.

The second part of the pathway, beginning with ribulose 5-phosphate, represents a series of reversible reactions that produce an equilibrated pool of sugars for biosynthesis, including ribose 5-phosphate for nucleotide synthesis. Because fructose 6-phosphate and glyceraldehyde 3-phosphate are among the sugars produced, intermediates can feed back into glycolysis; conversely, pentoses can be made from glycolytic intermediates without going through the G6PDH reaction. Transketolase, a thiamine-requiring enzyme, is important for these interconversions. Transketolase is the only thiamine enzyme in red blood cells.

Functions of NADPH

Cells require NADPH for a variety of functions, including:

- Biosynthesis
- Maintenance of a supply of reduced glutathione to protect against reactive oxygen species (ROS)
- Bactericidal activity in polymorphonuclear leukocytes (PMN)

These important roles are shown in Figure I-14-7.

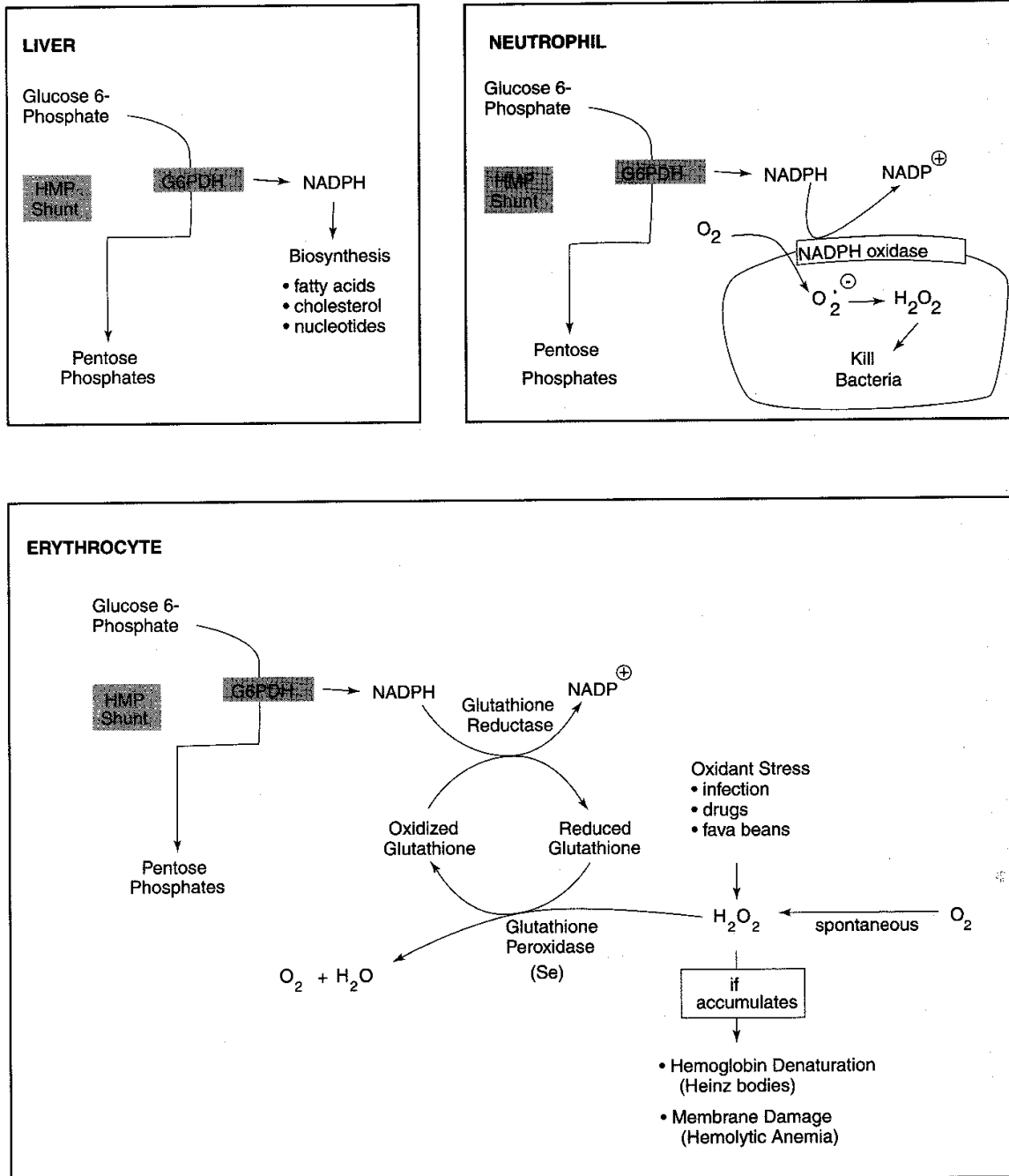


Figure I-14-7. Role of the HMP Shunt in Hepatocytes, Phagocytes, and Erythrocytes

Clinical Correlate**Favism**

Broad beans, commonly called fava beans, are common to diets in Mediterranean countries (Greece, Italy, Spain, Portugal, and Turkey), in which their ingestion may cause severe hemolysis in G6PDH individuals. Clinically, the condition presents as pallor, hemoglobinuria, jaundice, and severe anemia 24–48 hours after ingestion of the beans.

Clinical Correlate**CGD**

Chronic granulomatous disease is most frequently caused by genetic deficiency of NADPH oxidase in the PMN. Patients are susceptible to infection by catalase-positive organisms such as *Staphylococcus aureus*, *Klebsiella*, *Escherichia coli*, *Candida*, and *Aspergillus*. A negative nitroblue tetrazolium test is useful in confirming the diagnosis.

Glucose 6-Phosphate Dehydrogenase Deficiency

Deficiency of G6PDH may result in hemolytic anemia and, in rare cases, symptoms resembling chronic granulomatous disease (CGD). The disease shows significant allelic heterogeneity (over 400 different mutations in the G6PDH gene are known). The major symptom is either an acute episodic or (rarely) a chronic hemolysis. The disease is X-linked recessive. Female heterozygous for G6PDH deficiency have increased resistance to malaria. Consequently, the deficiency is seen more commonly in families from regions where malaria is endemic.

Because red blood cells contain a large amount of oxygen, they are prone to spontaneously generate ROS that damage protein and lipid in the cell. In the presence of ROS, hemoglobin may precipitate (Heinz bodies) and membrane lipids may undergo peroxidation, weakening the membrane and causing hemolysis. As peroxides form, they are rapidly destroyed by the glutathione peroxidase/glutathione reductase system in the red blood cell, thus avoiding these complications. These enzymes are shown in the red blood cell diagram in Figure I-14-7. NADPH required by glutathione reductase is supplied by the HMP shunt in the erythrocyte.

Persons with mutations that partially destroy G6PDH activity may develop an acute, episodic hemolysis. Certain mutations affect the stability of G6PDH, and, because erythrocytes cannot synthesize proteins, the enzyme is gradually lost over time and older red blood cells lyse. This process is accelerated by certain drugs and, in a subset of patients, ingestion of fava beans. In the United States, the most likely cause of a hemolytic episode in these patients is overwhelming infection, often pneumonia (viral and bacterial) or infectious hepatitis.

In rare instances, a mutation may decrease the activity of G6PDH sufficiently to cause chronic nonspherocytic hemolytic anemia. Symptoms of CGD may also develop if there is insufficient activity of G6PDH (<5% of normal) in the PMN to generate NADPH for the NADPH oxidase bactericidal system.

Chapter Summary**Glycogen Metabolism**

Cytoplasm

Rate-Limiting Enzymes

Glycogen synthesis: glycogen synthase

- Activated by insulin in liver and muscle
- Stimulated by glucose in liver

Glycogenolysis: glycogen phosphorylase

- Activated by glucagon in liver (hypoglycemia)
- Activated by epinephrine and AMP in skeletal muscle (exercise)

Other Enzymes

Glucose 6-phosphatase releases free glucose; only in liver

(Continued)

Chapter Summary (continued)

Genetic Deficiencies

- Glucose 6-phosphatase deficiency
- Hepatic glycogen phosphorylase deficiency
- Muscle glycogen phosphorylase deficiency
- Lysosomal α 1,4-glucosidase deficiency

Gluconeogenesis

Cytoplasm and mitochondria; predominantly in liver

Controlled Enzyme

Fructose 1,6-bisphosphate

- Cytoplasm
- Activated by ATP
- Inhibited by AMP and fructose 2,6-bisP
- Insulin (inhibits) glucagon (activates) by their control of PFK-2 (produces fructose 2,6-bisP)

Other Enzymes

Pyruvate carboxylase

- Activated by acetyl CoA from β -oxidation
- Biotin
- Mitochondria

Phosphoenolpyruvate carboxykinase (PEPCK)

- Cytoplasm
- Induced by glucagon and cortisol

Glucose 6-phosphatase (endoplasmic reticulum)

- Only in liver
- Required to release free glucose from tissue

Important Patients

Alcoholic hypoglycemia (high NADH)

Glucose 6-phosphatase deficiency

Defects in β -oxidation susceptible to hypoglycemic episodes

(Continued)

Chapter Summary (continued)

HMP Shunt

Cytoplasm of most cells

Functions

- Generates NADPH
- Produces sugars for biosynthesis (ribose 5-P for nucleotides)

Rate-Limiting Enzymes

Glucose 6-phosphate dehydrogenase

- Inhibited by NADPH
- Induced by insulin in liver

Genetic Deficiency

Glucose 6-phosphate dehydrogenase

- Episodic hemolytic anemia (most common) induced by infection and drugs
- Chronic hemolysis, CGD-like symptoms (very rare)

Review Questions

Select the ONE best answer.

1. A liver biopsy is done on a child with hepatomegaly and mild fasting hypoglycemia. Hepatocytes show accumulation of glycogen granules with single glucose residues remaining at the branch points near the periphery of the granule. The most likely genetic defect is in the gene encoding a(n):
 - A. α -1,4 phosphorylase
 - B. α -1,4: α -1,4 transferase
 - C. phosphoglucomutase
 - D. α -1,6 glucosidase
 - E. lysosomal α -1,4 glucosidase
2. When fatty acid β -oxidation predominates in the liver, mitochondrial pyruvate is most likely to be
 - A. carboxylated to phosphoenolpyruvate for entry into gluconeogenesis
 - B. oxidatively decarboxylated to acetyl CoA for entry into ketogenesis
 - C. reduced to lactate for entry into gluconeogenesis
 - D. oxidatively decarboxylated to acetyl CoA for oxidation in Krebs cycle
 - E. carboxylated to oxaloacetate for entry into gluconeogenesis

Items 3 and 4

A 44-year-old Algerian man living in the United States and receiving antibiotic therapy for a urinary tract infection has a self-limiting episode of hemolysis, back pain, and jaundice. The peripheral blood smear reveals a nonspherocytic, normocytic anemia, and Heinz bodies are seen in some of his erythrocytes.

3. Which of the following genetic deficiencies is most likely related to his hemolytic episode?
 - A. Homocysteine methyltransferase
 - B. Pyruvate kinase
 - C. Dihydrofolate reductase
 - D. Ferrochelatase
 - E. Glucose 6-phosphate dehydrogenase
4. Which of the following sets of laboratory test results would most likely have been obtained for this patient?

	Direct Bilirubin	Indirect Bilirubin	Urinary Bilirubin
A.	Increased	Increased	Absent
B.	Increased	Increased	Present
C.	Normal	Increased	Absent
D.	Normal	Decreased	Present
E.	Increased	Decreased	Present

Answers

1. **Answer: D.** This activity of the debranching enzyme removes 1,6-linked glucose residues from the branch points during glycogenolysis.
2. **Answer: E.** Hepatic fatty acid oxidation generates energy in the postabsorptive period when pyruvate is being converted to OAA for glucose biosynthesis.
3. **Answer: E.** Only option E is consistent with the constellation of clinical findings presented. Major clue is the positive Heinz body preparation.
4. **Answer: C.** Only option C is characteristic of hemolytic jaundice; indirect hyperbilirubinemia with no spillover of the water-insoluble unconjugated form into the urine.

Lipid Synthesis and Storage

15

FATTY ACID NOMENCLATURE

Fatty acids are long-chain carboxylic acids. The carboxyl carbon is number 1, and carbon number 2 is referred to as the α carbon. When designating a fatty acid, the number of carbons is given along with the number of double bonds (carbons:double bonds). Saturated fatty acids have no double bonds. Palmitic acid (palmitate) is the primary end product of fatty acid synthesis.

Palmitic C16:0 or 16:0

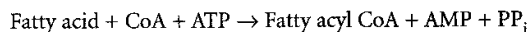
Unsaturated fatty acids have one or more double bonds. Humans can synthesize only a few of the unsaturated fatty acids *de novo*; the rest must be made from essential fatty acids in the diet transported from the intestine in chylomicrons. Two important essential fatty acids are linolenic and linoleic acids.

The omega (ω) numbering system is also used for unsaturated fatty acids. The ω -family describes the position of the last double bond relative to the end of the chain. The omega designation identifies the major precursor fatty acid, e.g., arachidonic acid is formed from linoleic acid (ω -6 family). Arachidonic acid is itself an important precursor for prostaglandins, thromboxanes, and leukotrienes.

Linoleic	C18:2 (9,12) or 18 $\Delta^{9,12}$	ω -6 family (18 - 12 = 6)
Linolenic	C18:3 (9,12,15) or 18 $\Delta^{9,12,15}$	ω -3 family
Arachidonic	C20:4 (5,8,11,14) or 20 $\Delta^{5,8,11,14}$	ω -6 family

Activation of Fatty Acids

When fatty acids are used in metabolism, they are first activated by attaching coenzyme A (CoA); fatty acyl CoA synthetase catalyzes this activation step. The product is generically referred to as a fatty acyl CoA or sometimes just acyl CoA. Specific examples would be acetyl CoA with a 2-carbon acyl group, or palmitoyl CoA with a 16-carbon acyl group.



FATTY ACID BIOSYNTHESIS

Excess dietary glucose can be converted to fatty acids in the liver and subsequently sent to the adipose tissue for storage. Adipose tissue synthesizes smaller quantities of fatty acids. The pathway is shown in Figure I-15-1. Insulin promotes many steps in the conversion of glucose to acetyl CoA in the liver:

Clinical Correlate

Cardioprotective Effects of Omega-3 Fatty Acids

Omega-3 fatty acids in the diet are correlated with a decreased risk of cardiovascular disease. These appear to replace some of the arachidonic acid (an omega-6 fatty acid) in platelet membranes and may lower the production of thromboxane and the tendency of the platelets to aggregate. A diet high in omega-3 fatty acids has also been associated with a decrease in serum triglycerides. Omega-3 fatty acids are found in cold-water fish, such as salmon, tuna, and herring, as well as in some nuts (walnuts) and seeds (flax seed).

- Glucokinase (induced)
- PFK-2/PFK-1 (PFK-2 dephosphorylated)
- Pyruvate dehydrogenase (dephosphorylated)

Both of the major enzymes of fatty acid synthesis are also affected by insulin:

- Acetyl CoA carboxylase (dephosphorylated)
- Fatty acid synthase (induced)

Citrate Shuttle and Malic Enzyme

The citrate shuttle transports acetyl CoA groups from the mitochondria to the cytoplasm for fatty acid synthesis. Acetyl CoA combines with oxaloacetate in the mitochondria to form citrate, but rather than continuing in the citric acid cycle, citrate is transported into the cytoplasm. Factors that indirectly promote this process include insulin and high-energy status.

In the cytoplasm, citrate lyase splits citrate back into acetyl CoA and oxaloacetate. The oxaloacetate returns to the mitochondria to transport additional acetyl CoA. This process is shown in Figure I-15-1 and includes the important malic enzyme. This reaction represents an additional source of cytoplasmic NADPH in liver and adipose tissue, supplementing that from the HMP shunt.

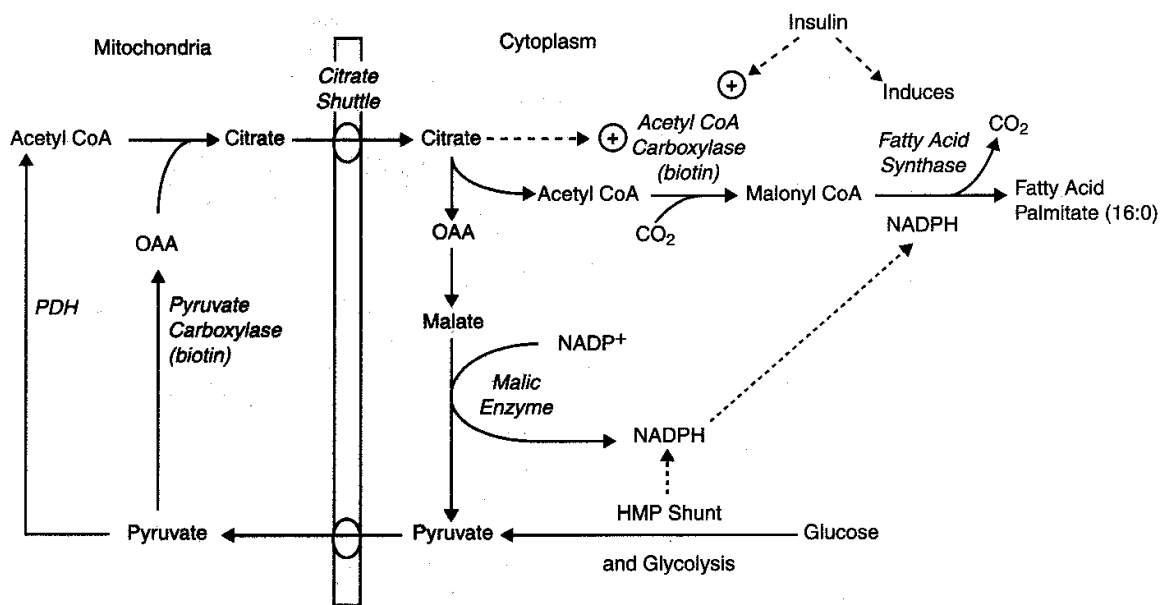


Figure I-15-1. Synthesis of Palmitate From Glucose

Acetyl CoA Carboxylase

Acetyl CoA is activated in the cytoplasm for incorporation into fatty acids by acetyl CoA carboxylase, the rate-limiting enzyme of fatty acid biosynthesis. Acetyl CoA carboxylase requires biotin, ATP, and CO_2 . Controls include:

- Activation by insulin (dephosphorylated)
- Activation by citrate

The CO_2 added to form malonyl CoA is never incorporated into the fatty acid because it is removed by fatty acid synthase during the addition of the acetyl group to the fatty acid.

Fatty Acid Synthase

Fatty acid synthase is a large multienzyme complex in the cytoplasm that is rapidly induced in the liver after a meal by high carbohydrate and the concomitant rise in insulin levels. It contains an acyl carrier protein (ACP) that requires the vitamin pantothenic acid. Although malonyl CoA is the substrate used by fatty acid synthase, only the carbons from the acetyl CoA portion are actually incorporated into the fatty acid produced. Therefore, the fatty acid is derived entirely from acetyl CoA.

NADPH is required to reduce the acetyl groups added to the fatty acid. Eight acetyl CoA groups are required to produce palmitate (16:0).

Fatty acyl CoA may be elongated and desaturated (to a limited extent in humans) using enzymes associated with the smooth endoplasmic reticulum (SER). Cytochrome b_5 is involved in the desaturation reactions. These enzymes cannot introduce double bonds past position 9 in the fatty acid.

TRIGLYCERIDE (TRIACYLGLYCEROL) SYNTHESIS

Triglycerides

Triglycerides, the storage form of fatty acids, are formed by attaching three fatty acids (as fatty acyl CoA) to glycerol. Triglyceride formation from fatty acids and glycerol 3-phosphate occurs primarily in liver and adipose tissue.

Liver sends triglycerides to adipose tissue packaged as very low-density lipoproteins (VLDL; reviewed later in this chapter). A small amount of triglyceride may be stored in the liver. Accumulation of significant triglyceride in tissues other than adipose tissue usually indicates a pathologic state.

Sources of Glycerol 3-Phosphate for Synthesis of Triglycerides

There are two sources of glycerol 3-P for triglyceride synthesis:

- Reduction of dihydroxyacetone phosphate (DHAP) from glycolysis by glycerol 3-P dehydrogenase, an enzyme in both adipose tissue and liver
- Phosphorylation of free glycerol by glycerol kinase, an enzyme found in liver but not in adipose tissue

Glycerol kinase allows the liver to recycle the glycerol released during VLDL metabolism (insulin) back into new triglyceride synthesis. During fasting (glucagon), this same enzyme allows the liver to trap glycerol released into the blood from lipolysis in adipose tissue for subsequent conversion to glucose.

Adipose tissue lacks glycerol kinase and is strictly dependent on glucose uptake to produce DHAP for triglyceride synthesis. In adipose tissue, the GLUT 4 transporter is stimulated by insulin, ensuring a good supply of DHAP for triglyceride synthesis. The roles of glycerol kinase and glycerol 3-P dehydrogenase during triglyceride synthesis and storage are shown in Figure I-15-2.

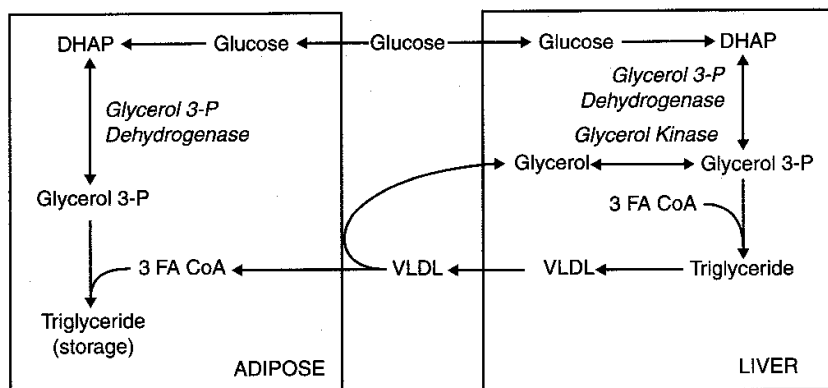


Figure I-15-2. Glycerol 3-P Dehydrogenase and Glycerol Kinase in Triglyceride Synthesis and Storage

Glycerophospholipids

Glycerophospholipids are used for membrane synthesis and for producing a hydrophilic surface layer on lipoproteins such as VLDL. In cell membranes, they also serve as a reservoir of second messengers such as diacylglycerol, inositol 1,4,5-triphosphate, and arachidonic acid. Their structure is similar to triglycerides, except that the last fatty acid is replaced by phosphate and a water-soluble group such as choline (phosphatidylcholine, lecithin) or inositol (phosphatidylinositol).

A comparison of the structures is diagrammed in Figure I-15-3.

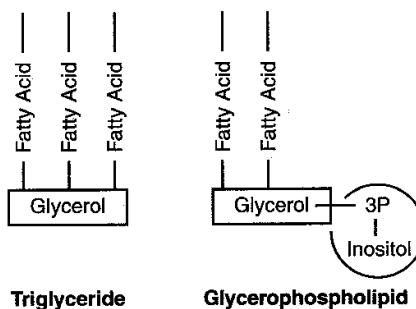


Figure I-15-3. Triglycerides and Glycerophospholipids

LIPOPROTEIN METABOLISM

Triglycerides and cholesterol are transported in the blood as lipoproteins. Lipoproteins are named according to their density, which increases with the percentage of protein in the particle. From least dense to most dense:

chylomicrons < VLDL < IDL (intermediate-density lipoproteins) < LDL (low-density lipoproteins) < HDL (high-density lipoproteins)

The classes of lipoproteins and the important apoproteins associated with their functions are summarized in Table I-15-1 and Figure I-15-4.

Table I-15-1. Classes of Lipoproteins and Important Apoproteins

Lipoprotein	Functions	Apoproteins	Functions
Chylomicrons	Transport dietary triglyceride and cholesterol from intestine to tissues	apoB-48 apoC-II apoE	Secreted by epithelial cells Activates lipoprotein lipase Uptake by liver
VLDL	Transports triglyceride from liver to tissues	apoB-100 apoC-II apoE	Secreted by liver Activates lipoprotein lipase Uptake of remnants by liver
LDL	Delivers cholesterol into cells	apoB-100	Uptake by liver and other tissues via LDL receptor (apoB-100 receptor)
IDL (VLDL remnants)	Picks up cholesterol from HDL to become LDL Picked up by liver	apoE	Uptake by liver
HDL	Picks up cholesterol accumulating in blood vessels Delivers cholesterol to liver and steroidogenic tissues via scavenger receptor (SR-B1) Shuttles apoC-II and apoE in blood	apoA-1	Activates lecithin cholesterol acyltransferase (LCAT) to produce cholesterol esters

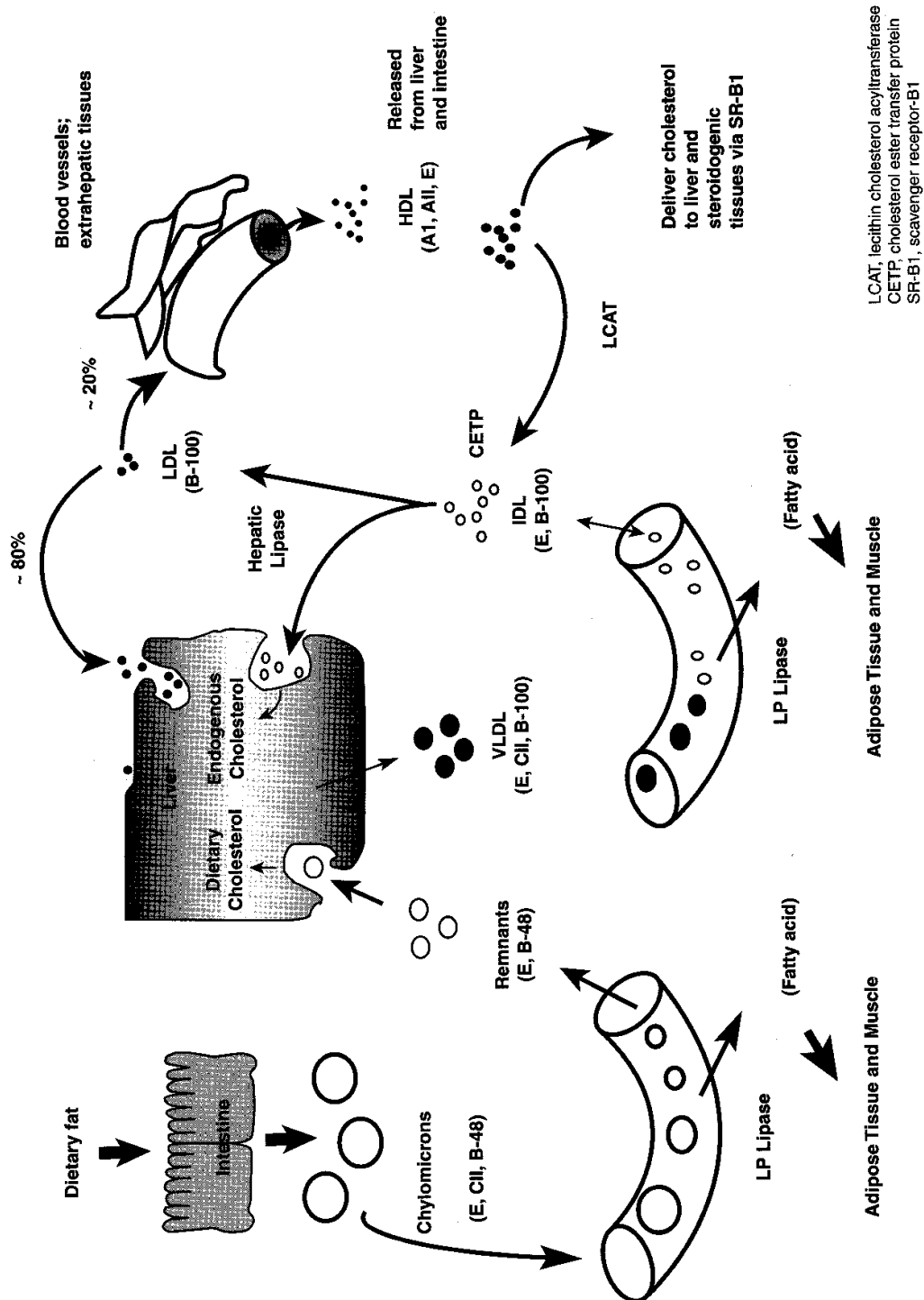


Figure I-15-4. Overview of Lipoprotein Metabolism

Chylomicrons, VLDL, and IDL (VLDL Remnants)

Chylomicrons and VLDL are primarily triglyceride particles, although they each have small quantities of cholesterol esters. Chylomicrons transport dietary triglyceride to adipose tissue and muscle, whereas VLDL transport triglyceride synthesized in the liver to these same tissues. Both chylomicrons and VLDL have apoC-II, apoE, and apoB (apoB-48 on chylomicrons and apoB-100 on VLDL). The metabolism of these particles is shown in Figure I-15-5.

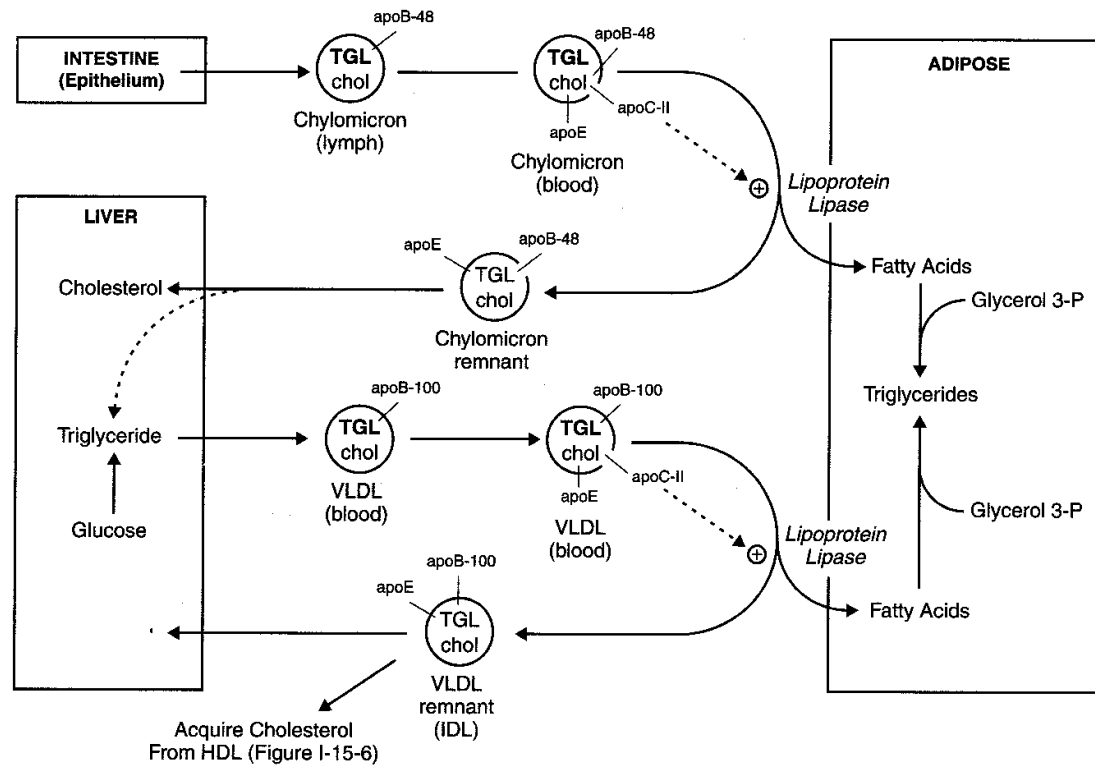


Figure I-15-5. Chylomicron and VLDL Metabolism

Lipoprotein Lipase

Lipoprotein (LPLase) is required for the metabolism of both chylomicrons and VLDL. This enzyme is induced by insulin and transported to the luminal surface of capillary endothelium where it is in direct contact with the blood. Lipoprotein lipase hydrolyzes the fatty acids from triglycerides carried by chylomicrons and VLDL and is activated by apoC-II.

Chylomicrons

Chylomicrons are assembled from dietary triglyceride (containing predominantly the longer-chain fatty acids) and cholesterol esters by intestinal epithelial cells. The core lipid is surrounded by phospholipids similar to those found in cell membranes, which increase the solubility of chylomicrons in lymph and blood. ApoB-48 is attached and required for release from the epithelial cells into the lymphatics.

Chylomicrons leave the lymph and enter the peripheral blood where the thoracic duct joins the left subclavian vein, thus initially bypassing the liver. After a high-fat meal, chylomicrons cause serum to become turbid or milky. While in the blood, chylomicrons acquire apoC-II and apoE from HDL particles.

In capillaries of adipose tissue (and muscle), apoC-II activates lipoprotein lipase, the fatty acids released enter the tissue for storage, and the glycerol is retrieved by the liver, which has glycerol kinase. The chylomicron remnant is picked up by hepatocytes through the apoE receptor; thus, dietary cholesterol, as well as any remaining triglyceride, is released in the hepatocyte.

VLDL (Very Low-Density Lipoprotein)

The metabolism of VLDL is very similar to that of chylomicrons, the major difference being that VLDL are assembled in hepatocytes to transport triglyceride containing fatty acids newly synthesized from excess glucose, or retrieved from the chylomicron remnants, to adipose tissue and muscle. ApoB-100 is added in the hepatocytes to mediate release into the blood. Like chylomicrons, VLDL acquire apoC-II and apoE from HDL in the blood, and are metabolized by lipoprotein lipase in adipose tissue and muscle.

VLDL Remnants (IDL, Intermediate-Density Lipoprotein)

After triglyceride is removed from the VLDL, the resulting particle is referred to as either a VLDL remnant or as an IDL. A portion of the IDLs are picked up by hepatocytes through their apoE receptor, but some of the IDLs remain in the blood, where they are further metabolized. These IDLs are transition particles between triglyceride and cholesterol transport. In the blood, they can acquire cholesterol transferred from HDL particles and thus become converted into LDLs, as shown in Figure I-15-6.

LDL, HDL, and Atherosclerosis

LDL (Low-Density Lipoprotein)

Although both LDL and HDL are primarily cholesterol particles, most of the cholesterol measured in the blood is associated with LDL. The normal role of LDL is to deliver cholesterol to tissues for biosynthesis. When a cell is repairing membrane or dividing, the cholesterol is required for membrane synthesis. Bile acids and salts are made from cholesterol in the liver, and many other tissues require some cholesterol for steroid synthesis. As shown in Figure I-15-6, about 80% of LDL are picked up by hepatocytes, the remainder by peripheral tissues. ApoB-100 is the only apoprotein on LDL, and endocytosis of LDL is mediated by apoB-100 receptors (LDL receptors) clustered in areas of cell membranes lined with the protein clathrin.

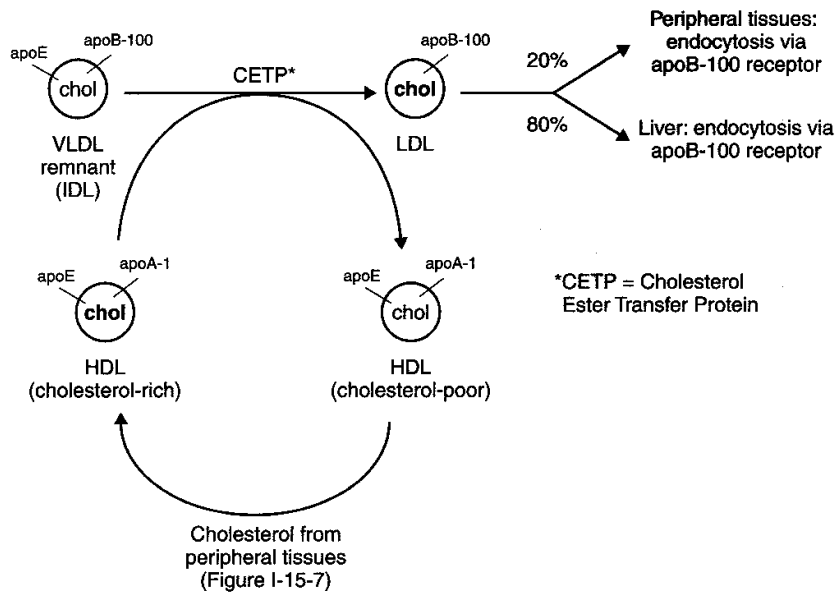


Figure I-15-6. IDL and LDL Metabolism

Endocytosis involves:

- Formation of a coated pit, which further invaginates to become an endosome
- Fusion of the endosome with a lysosome, accompanied by acidification and activation of lysosomal enzymes
- Release of LDL from the LDL receptor

The receptor may recycle to the surface, the LDL is degraded, and cholesterol is released into the cell. Expression of the gene for LDL receptors (apoB-100 receptor) is regulated by the cholesterol level within the cell. High cholesterol decreases expression of this gene.

HDL (High-Density Lipoprotein)

HDL is synthesized in the liver and intestines and released as dense protein-rich particles into the blood. They contain apoA-1 used for cholesterol recovery from fatty streaks in the blood vessels. HDL also carry apoE and apoC-II, but those apoproteins are primarily to donate temporarily to chylomicrons and VLDL.

Lecithin-Cholesterol Acyltransferase (LCAT)

LCAT (or PCAT, phosphatidylcholine-cholesterol acyltransferase) is an enzyme in the blood that is activated by apoA-1 on HDL. LCAT adds a fatty acid to cholesterol, producing cholesterol esters, which dissolve in the core of the HDL, allowing HDL to transport cholesterol from the periphery to the liver. This process of reverse cholesterol transport is shown in Figure I-15-7.

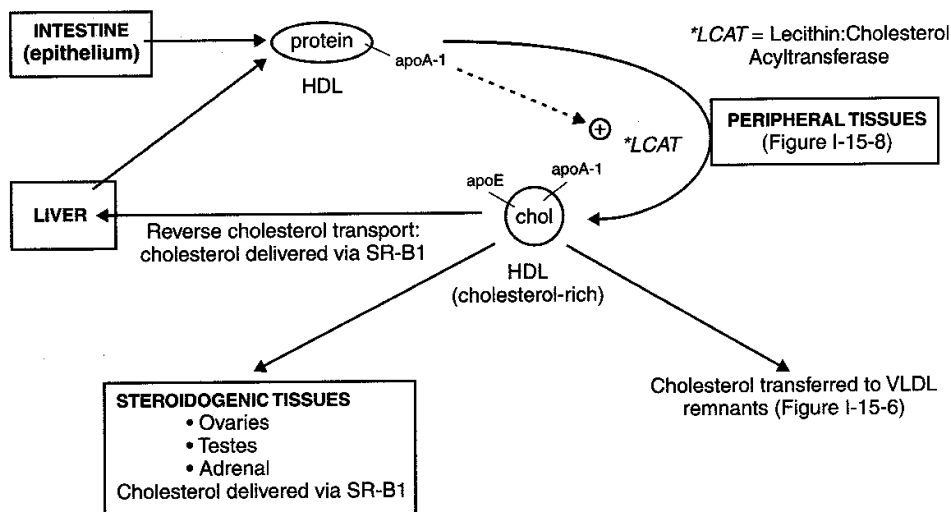


Figure I-15-7. HDL Metabolism

Cholesterol Ester Transfer Protein (CETP)

HDL cholesterol picked up in the periphery can be distributed to other lipoprotein particles such as VLDL remnants (IDL), converting them to LDL. The cholesterol ester transfer protein facilitates this transfer, shown in Figure I-15-6.

Scavenger Receptors (SR-B1)

HDL cholesterol picked up in the periphery can also enter cells through a scavenger receptor, SR-B1. This receptor is expressed at high levels in hepatocytes and the steroidogenic tissues, including ovaries, testes, and areas of the adrenal glands. This receptor does not mediate endocytosis of the HDL, but rather transfer of cholesterol into the cell by a mechanism not yet clearly defined. This process is shown in Figure I-15-7.

Atherosclerosis

The metabolism of LDL and HDL intersects in the production and control of fatty streaks and potential plaques in blood vessels. Figure I-15-8 illustrates one model of atherosclerosis involving HDL and LDL at the site of endothelial cell injury. Damage to the endothelium may be related to many factors, including normal turbulence of the blood, elevated LDL, especially modified or oxidized LDL, free radicals from cigarette smoking, homocystinemia (Chapter 17), diabetes (glycation of LDL), and hypertension. The atherosclerotic lesion represents an inflammatory response sharing several characteristics with granuloma formation, and not simple deposition of cholesterol in the blood vessel.

- Endothelial dysfunction increases adhesiveness and permeability of the endothelium for platelets and leukocytes. Infiltrations involve monocytes and T cells. Damaged endothelium has procoagulant rather than anticoagulant properties. In some cases, the endothelial lining may become partially denuded.
- Local inflammation recruits monocytes and macrophages with subsequent production of reactive oxygen species. LDL can become oxidized and then taken up, along with

other inflammatory debris, by macrophages, which can become laden with cholesterol (foam cells). Initially the subendothelial accumulation of cholesterol-laden macrophages produces fatty streaks.

- As the fatty streak enlarges over time, necrotic tissue and free lipid accumulates, surrounded by epithelioid cells and eventually smooth muscle cells, an advanced plaque with a fibrous cap. The plaque eventually begins to occlude the blood vessel, causing ischemia and infarction in the heart, brain, or extremities.
- Eventually the fibrous cap may thin, and the plaque becomes unstable, leading to rupture and thrombosis.
- HDL may be protective by picking up accumulating cholesterol before the advanced lesion forms. Apo-1 activates LCAT, which in turn adds a fatty acid to cholesterol to produce a cholesterol ester that dissolves in the core of the HDL.
- The HDL may subsequently be picked up by the liver through the apoE receptor or deliver cholesterol through the scavenger receptor SR-B1 (reverse cholesterol transport from the periphery to the liver). The HDL may also transfer the cholesterol to an IDL reforming a normal, unoxidized LDL particle.

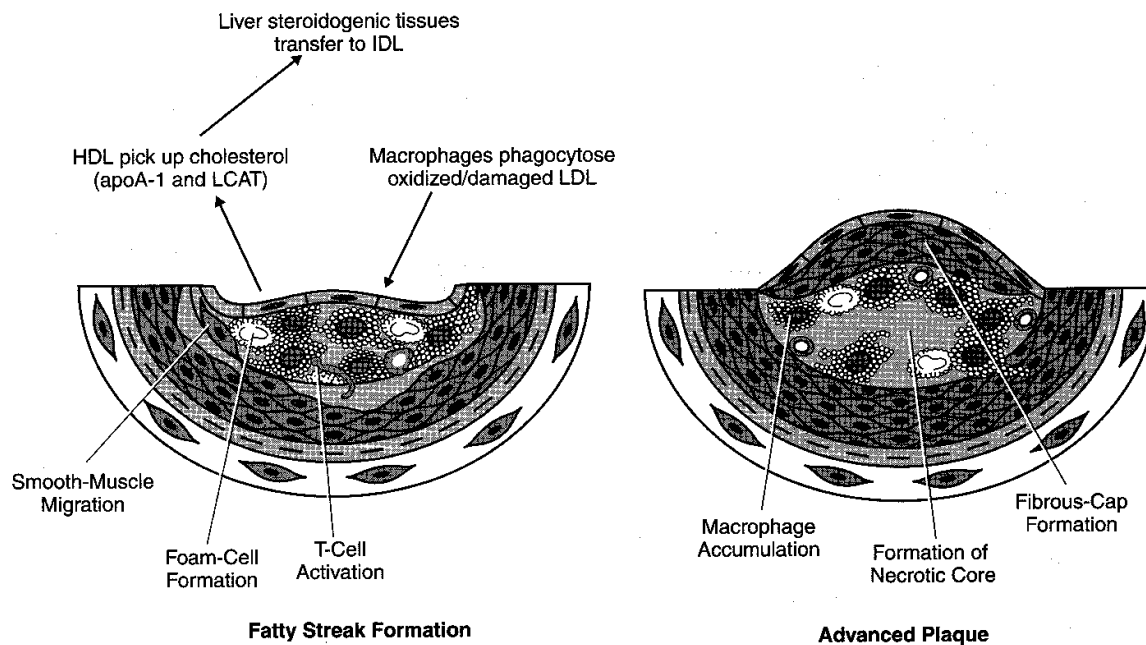


Figure I-15-8. LDL, HDL, and Atherogenesis

Role of Vitamin E

The oxidation of LDL at sites of endothelial damage is thought to be a major stimulus for uptake by macrophages. Some studies have shown a protective role of vitamin E in this process. Vitamin E is a lipid-soluble vitamin that acts as an antioxidant in the lipid phase. In addition to protecting LDL from oxidation, it may also prevent peroxidation of membrane lipids. Vitamins C and A lack this protective effect despite their antioxidant properties.

HYPERLIPIDEMIAS

Excess lipid in the blood can result from primary genetic deficiencies, most of which are rare, or as a secondary consequence of another disease. Two primary hyperlipidemias, type I hypertriglyceridemia and type IIA hypercholesterolemia, are summarized in Table I-15-2.

Table I-15-2. Primary Hyperlipidemias

Type	Deficiency	Lipid Elevated in Blood	Lipoprotein Elevated in Blood	Comments
I	Familial lipoprotein lipase (rare) apoC-II (rare) Autosomal recessive	Triglyceride	Chylomicrons	Red-orange eruptive xanthomas Fatty liver Acute pancreatitis Abdominal pain after fatty meal
IIa	Familial hypercholesterolemia Autosomal dominant (Aa 1/500, AA 1/10 ⁶)	Cholesterol	LDL	High risk of atherosclerosis and coronary artery disease Homozygous condition usually death <20 years Xanthomas of the Achilles tendon Tuberous xanthomas on elbows Xanthelasma Corneal arcus

Type I Hypertriglyceridemia

Rare genetic absence of lipoprotein lipase results in excess triglyceride in the blood and its deposition in several tissues, including liver, skin, and pancreas. Orange-red eruptive xanthomas over the mucous membranes and skin may be seen. Abdominal pain and acute pancreatitis may occur. Fasting chylomicronemia produces a milky turbidity in the serum or plasma.

Diabetes, alcoholism, and glucose 6-phosphatase deficiency all can produce less severe hypertriglyceridemia with an increase in VLDL and chylomicrons. Factors contributing to the hyperlipidemia are:

- Decreased glucose uptake in adipose tissue
- Overactive hormone sensitive lipase (Chapter 16, Figure I-16-1)
- Underactive lipoprotein lipase

Type IIA Hypercholesterolemia (LDL Receptor Deficiency)

This is a dominant genetic disease affecting 1/500 (heterozygous) individuals in the United States. It is characterized by elevated LDL cholesterol and increased risk for atherosclerosis and coronary artery disease. Cholesterol deposits may be seen as:

- Xanthomas of the Achilles tendon
- Subcutaneous tuberous xanthomas over the elbows
- Xanthelasma (lipid in the eyelid)
- Corneal arcus

Homozygous individuals (1/10⁶) often have myocardial infarctions before 20 years of age.

CHOLESTEROL METABOLISM

Cholesterol is required for membrane synthesis, steroid synthesis, and in the liver, bile acid synthesis. Most cells derive their cholesterol from LDL or HDL, but some cholesterol may be synthesized *de novo*. Most *de novo* synthesis occurs in the liver, where cholesterol is synthesized from acetyl CoA in the cytoplasm. The citrate shuttle carries mitochondrial acetyl CoA into the cytoplasm, and NADPH is provided by the HMP shunt and malic enzyme. Important points are noted in Figure I-15-9.

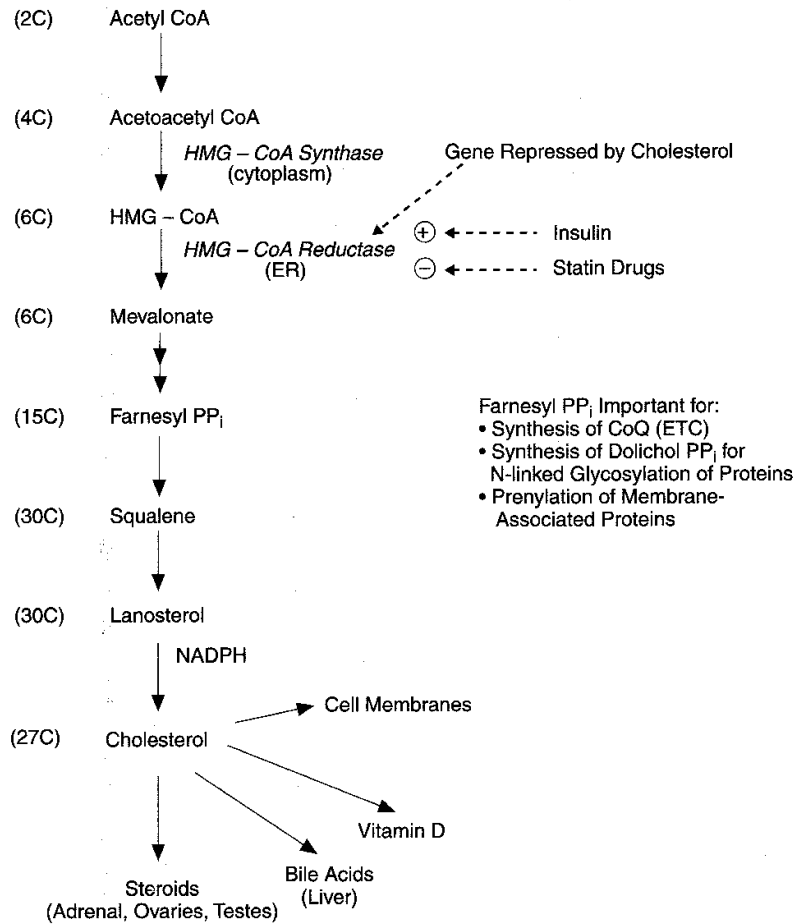


Figure I-15-9. Synthesis of Cholesterol

3-Hydroxy-3-methylglutaryl (HMG)-CoA reductase on the smooth endoplasmic reticulum (SER) is the rate-limiting enzyme. Insulin activates the enzyme (dephosphorylation), and glucagon inhibits it. Mevalonate is the product, and the statin drugs competitively inhibit the enzyme. Cholesterol represses the expression of the HMG-CoA reductase gene and also increases degradation of the enzyme.

Bridge to Pharmacology

Treatment of Hypercholesterolemia

Cholestyramine and other drugs that increase elimination of bile salts force the liver to increase their synthesis from cholesterol, thus lowering the internal level of cholesterol in the hepatocytes. Decreased cholesterol within the cell increases LDL receptor expression, allowing the hepatocyte to remove more LDL cholesterol from the blood. HMG-CoA reductase inhibitors such as lovastatin and simvastatin inhibit *de novo* cholesterol synthesis in the hepatocyte, which subsequently increases LDL receptor expression.

Farnesyl pyrophosphate, an intermediate in the pathway, may also be used for:

- Synthesis of CoQ for the mitochondrial electron transport chain
- Synthesis of dolichol pyrophosphate, a required cofactor in N-linked glycosylation of proteins in the endoplasmic reticulum
- Prenylation of proteins (a posttranslational modification) that need to be held in the cell membrane by a lipid tail. An example is the p21^{ras} G protein in the insulin and growth factor pathways.

Regulation of the Cholesterol Level in Hepatocytes

The liver has multiple pathways for acquiring cholesterol, including:

- *De novo* synthesis
- Endocytosis of LDL
- Transfer of cholesterol from HDL via the SR-B1 receptor
- Endocytosis of chylomicron remnants with residual dietary cholesterol

Increased cholesterol in the hepatocytes inhibits further accumulation by repressing the expression of the genes for HMG-CoA reductase, the LDL receptor, and the SR-B1 receptor.

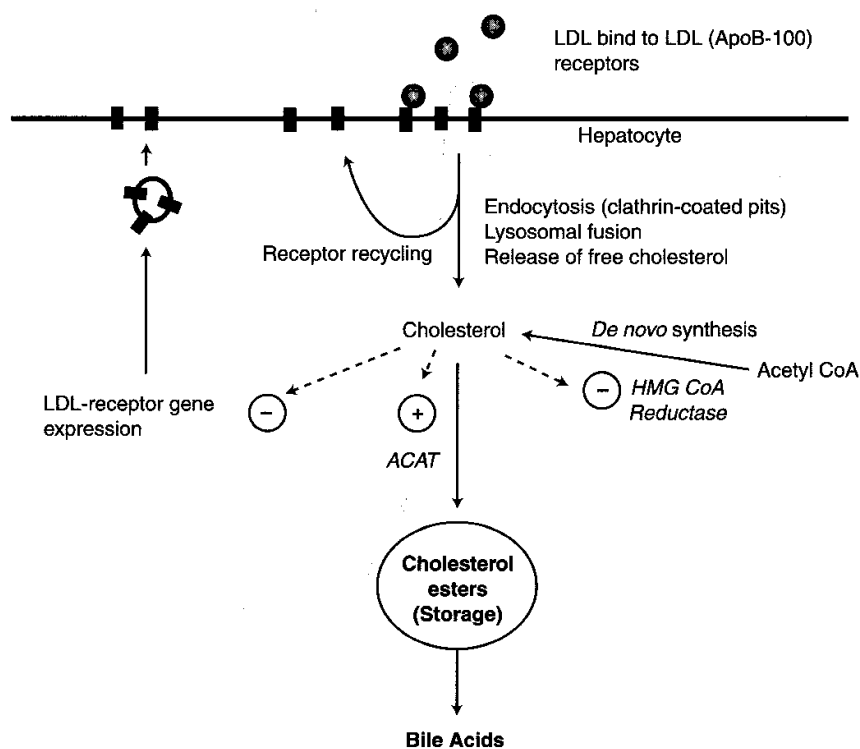


Figure I-15-10. Regulation of Cholesterol Level in Hepatocytes.

Chapter Summary

Fatty Acid Synthesis

Cytoplasm

Citrate shuttle

Acetyl CoA carboxylase (biotin)

- Rate-limiting: Citrate activates, insulin activates

Fatty acid synthase (requires NADPH)

- Induced by insulin

Malonyl CoA is intermediate

Triglyceride Synthesis

Glycerol 3-P dehydrogenase (liver and adipose)

Glycerol kinase (liver)

Lipoproteins

See Table I-15-1.

Type I: Lipoprotein lipase deficiency (triglycerides)

Type IIa: LDL (B100) receptor deficiency (cholesterol)

Cholesterol Synthesis—Rate-Limiting Enzyme

Most occurs in liver

HMG CoA reductase

- Inhibited by statin drugs

Precursor for

- Vitamin D
- Cell membranes
- Bile salts/acids

Review Questions

Select the ONE best answer.

1. What is the most positive activator of the process shown below?
 $8 \text{ acetyl CoA} + n \text{ ATP} + 14 \text{ NADPH} \rightarrow \text{palmitate} + 8 \text{ CoASH} + n\text{ADP} + n\text{Pi} + 14 \text{ NADP}$
 - A. Acetyl CoA
 - B. Citrate
 - C. Malonyl CoA
 - D. Malate
 - E. Oxaloacetate
2. When adipose tissue stores triglyceride arriving from the liver or intestine, glycolysis must also occur in the adipocyte. Which of the following products or intermediates of glycolysis is required for fat storage?
 - A. Glycerol
 - B. Glucose 6-phosphate
 - C. Pyruvate
 - D. Acetyl CoA
 - E. Dihydroxyacetone phosphate

Items 3 and 4

Abetalipoproteinemia is a genetic disorder characterized by malabsorption of dietary lipid, steatorrhea (fatty stools), accumulation of intestinal triglyceride, and hypolipoproteinemia.

3. A deficiency in the production of which apoprotein would most likely account for this clinical presentation?
 - A. ApoB-100
 - B. ApoB-48
 - C. ApoC-II
 - D. ApoA-I
 - E. ApoE
4. Patients with abetalipoproteinemia exhibit membrane abnormalities in their erythrocytes with production of acanthocytes (thorny-appearing cells). This unusual red cell morphology would most likely result from malabsorption of
 - A. palmitic acid
 - B. ascorbic acid
 - C. arachidonic acid
 - D. folic acid
 - E. linoleic acid

5. A patient with a history of recurring attacks of pancreatitis, eruptive xanthomas, and increased plasma triglyceride levels (2000 mg/dL) associated with chylomicrons, most likely has a deficiency in
- A. lipoprotein lipase
 - B. LDL receptors
 - C. HMG-CoA reductase
 - D. apoB-48
 - E. apoB-100 receptor
6. Uncontrolled phagocytosis of oxidized LDL particles is a major stimulus for the development of foam cells and fatty streaks in the vascular subendothelium. This process may be inhibited by increased dietary intake of
- A. vitamin E
 - B. vitamin B₆
 - C. vitamin D
 - D. vitamin B₁₂
 - E. vitamin K

Items 7–9

A 42-year-old man presents with a chief complaint of intermittent claudication during exercise. His family history is significant for the presence of cardiovascular disease on his father's side, but not on his mother's side. Physical exam reveals xanthelasmas and bilateral tendon xanthomas. A plasma lipid profile reveals a cholesterol level of 340 mg/dL, with a high LDL/HDL ratio. He is given instructions for dietary modifications and a prescription for Zocor (simvastatin).

7. The clinical findings noted in this patient are most likely caused by deficient production of
- A. lethicin cholesterol acyltransferase
 - B. apoB-100 receptors
 - C. fatty acyl-CoA synthetase
 - D. VLDL from LDL
 - E. cholesterol ester transfer protein
8. The anticholesterolemic action of simvastatin is based on its effectiveness as a competitive inhibitor of the rate-limiting enzyme in cholesterol biosynthesis. The reaction product normally produced by this enzyme is
- A. squalene
 - B. methylmalonate
 - C. lanosterol
 - D. mevalonate
 - E. acetoacetate

9. From a Lineweaver-Burk plot, the K_m and V_{max} of this rate-limiting enzyme were calculated to be 4×10^{-3} M and 8×10^2 mmol/h, respectively. If the above experiment is repeated in the presence of simvastatin, which of the following values would be obtained?

	K_m (M)	V_{max} (mmol/h)
A.	4×10^{-3}	3×10^2
B.	2×10^{-3}	1×10^2
C.	4×10^{-3}	9×10^2
D.	8×10^{-3}	8×10^2
E.	8×10^{-3}	9×10^2

Answers

- Answer: B.** Citrate is a potent activator of acetyl CoA carboxylase for fatty acid synthesis.
- Answer: E.** To reform triglycerides from the incoming fatty acids, glycerol 3-P must be available. The adipose can produce this only from DHAP in glycolysis.
- Answer: B.** Apo B-48 is required for intestinal absorption of dietary fat in the form of chylomicrons. Apo B-100 formation is also impaired in these patients, but this would not explain the clinical symptoms described.
- Answer: E.** The genetic defect would result in malabsorption of the 3 fatty acids listed, but only linoleate is strictly essential in the diet. Absorption of water-soluble ascorbate and folate would not be significantly affected.
- Answer: A.** These are the clinical features of lipoprotein lipase deficiency (Type I lipoproteinemia). LDL receptor defects would result in elevated LDLs. HMG-CoA reductase and ApoB-100 have no direct relationship to chylomicrons. ApoB-48 deficiency would result in decreased production of chylomicrons.
- Answer: A.** Only vitamin E is an antioxidant.
- Answer: B.** The findings are indicative of heterozygous Type IIa familial hypercholesterolemia, an autosomal dominant disease. Deficient CETP, LCAT or fatty acid CoA synthetase would not elevate LDL cholesterol. VLDL are not produced from LDL.
- Answer: D.** Must know that mevalonate precedes squalene and lanosterol in the pathway, and that methylmalonate and acetoacetate are not associated with cholesterolgenesis.
- Answer: D.** With a competitive inhibitor, there will be an increase in K_m with no change in V_{max} . Option A would be for a noncompetitive inhibitor (V_{max} decreased, K_m unaltered).

Lipid Mobilization and Catabolism

16

LIPID MOBILIZATION

In the postabsorptive state, fatty acids can be released from adipose tissue to be used for energy. Although human adipose tissue does not respond directly to glucagon, the fall in insulin activates a hormone-sensitive triacylglycerol lipase (HSL) that hydrolyzes triglycerides yielding fatty acids and glycerol. Epinephrine and cortisol also activate HSL. These steps are shown in Figure I-16-1.

Glycerol may be picked up by liver and converted to dihydroxyacetone phosphate (DHAP) for gluconeogenesis, and the fatty acids are distributed to tissues that can use them. Free fatty acids are transported through the blood in association with serum albumin.

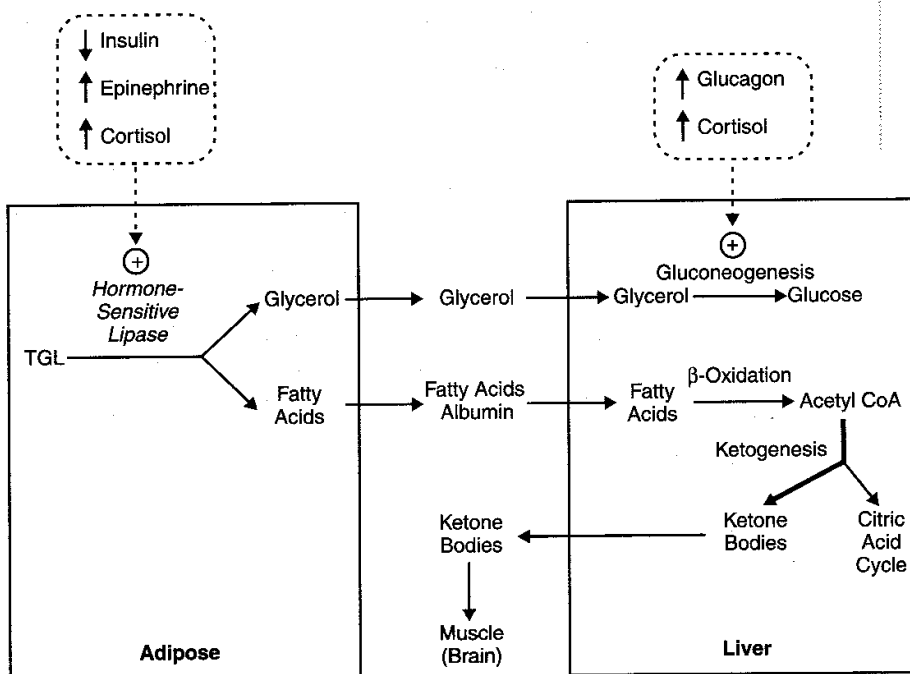


Figure I-16-1. Lipolysis of Triglyceride in Response to Hypoglycemia and Stress

FATTY ACID OXIDATION

Fatty acids are oxidized in several tissues, including liver, muscle, and adipose tissue, by the pathway of β -oxidation. Neither erythrocytes nor brain can use fatty acids, and so continue to rely on glucose during normal periods of fasting. Erythrocytes lack mitochondria, and fatty acids do not cross the blood-brain barrier efficiently.

Fatty Acid Entry Into Mitochondria

Long-chain fatty acids must be activated and transported into the mitochondria. Fatty acyl CoA synthetase, on the outer mitochondrial membrane, activates the fatty acids by attaching CoA. The fatty acyl portion is then transferred onto carnitine by carnitine acyltransferase-I for transport into the mitochondria. The sequence of events is shown in Figure I-16-2 and includes the following steps:

- Fatty acyl synthetase activates the fatty acid (outer mitochondrial membrane).
- Carnitine acyltransferase-1 transfers the fatty acyl group to carnitine (outer mitochondrial membrane).
- Fatty acylcarnitine is shuttled across the inner membrane.
- Carnitine acyltransferase-2 transfers the fatty acyl group back to a CoA (mitochondrial matrix).

Carnitine acyltransferase-1 is inhibited by malonyl CoA from fatty acid synthesis and thereby prevents newly synthesized fatty acids from entering the mitochondria. Insulin indirectly inhibits β -oxidation by activating acetyl CoA carboxylase (fatty acid synthesis) and increasing the malonyl CoA concentration in the cytoplasm. Glucagon reverses this process.

β -Oxidation in Mitochondria

β -oxidation reverses the process of fatty acid synthesis by oxidizing (rather than reducing) and releasing (rather than linking) units of acetyl CoA. The pathway is a repetition of four steps and is shown in Figure I-16-2. Each four-step cycle releases one acetyl CoA and reduces NAD and FAD (producing NADH and FADH₂).

The FADH₂ and NADH are oxidized in the electron transport chain, providing ATP. In muscle and adipose tissue, the acetyl CoA enters the citric acid cycle. In liver, the ATP may be used for gluconeogenesis, and the acetyl CoA (which cannot be converted to glucose) stimulates gluconeogenesis by activating pyruvate carboxylase.

In a fasting state, the liver produces more acetyl CoA from β oxidation than is used in the citric acid cycle. Much of the acetyl CoA is used to synthesize ketone bodies (essentially two acetyl CoA groups linked together) that are released into the blood for other tissues.

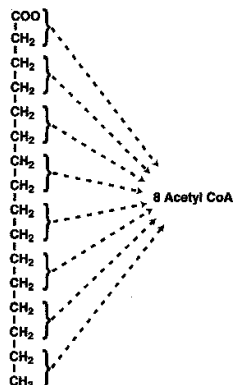
In a Nutshell

Carnitine Acyltransferases

Carnitine acyltransferase-1 (CAT-1) and carnitine acyltransferase-2 (CAT-2) are also referred to as carnitine palmitoyl transferase-1 (CPT-1) and carnitine palmitoyl transferase-2 (CPT-2). The carnitine transport system is most important for allowing long-chain fatty acids to enter into the mitochondria.

In a Nutshell

β -oxidation of palmitate (16:0) yields 8 acetyl CoA.



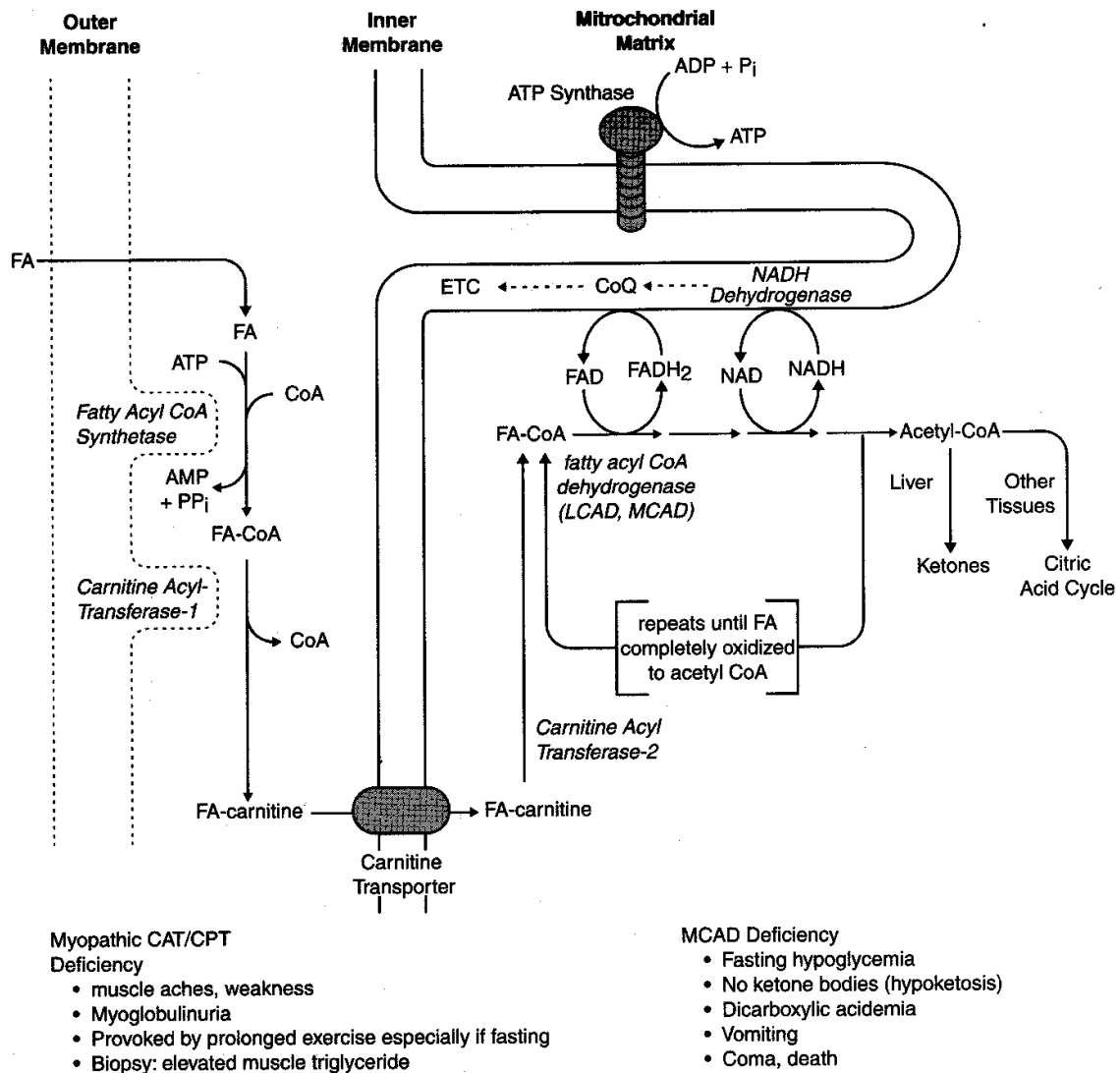


Figure I-16-2. Fatty Acid Activation, Transport, and β -Oxidation

Genetic Deficiencies of Fatty Acid Oxidation

Two of the most common genetic deficiencies affecting fatty acid oxidation are:

- Medium chain acyl CoA dehydrogenase (MCAD) deficiency, primary etiology hepatic
- Myopathic carnitine acyltransferase (CAT/CPT) deficiency, primary etiology myopathic

Medium Chain Acyl CoA Dehydrogenase (MCAD) Deficiency. Non-ketotic hypoglycemia should be strongly associated with a block in hepatic β -oxidation. During fasting, hypoglycemia can become profound due to lack of ATP to support gluconeogenesis. Decreased acetyl-CoA lowers pyruvate carboxylase activity and also limits ketogenesis. Hallmarks of MCAD deficiency include:

- Profound fasting hypoglycemia
- Low to absent ketones
- Lethargy, coma, death if untreated
- Dicarboxylic acidemia
- Episode may be provoked by overnight fast in an infant
- In older child, often provoked by illness (flu) that causes loss of appetite and vomiting
- Primary treatment: IV glucose
- Prevention: frequent feeding, high-carbohydrate, low-fat diet

Carnitine Acyltransferase (CAT/CPT) Deficiency (Myopathic Form). Although all tissues with mitochondria contain carnitine acyltransferase, the most common form of this genetic deficiency is myopathic and due to a defect in the muscle-specific CAT/CPT gene. Hallmarks of this disease include:

- Muscle aches; mild to severe weakness
- Rhabdomyolysis, myoglobinuria, red urine
- Episode provoked by prolonged exercise especially after fasting, cold, or associated stress
- Symptoms may be exacerbated by high-fat, low-carbohydrate diet
- Muscle biopsy shows elevated muscle triglyceride detected as lipid droplets in cytoplasm
- Primary treatment: cease muscle activity; give glucose

A somewhat similar syndrome can be produced by muscle carnitine deficiency secondary to a defect in the transport system for carnitine in muscle.

Propionic Acid Pathway

Fatty acids with an odd number of carbon atoms are oxidized by β -oxidation identically to even-carbon fatty acids. The difference results only from the final cycle, in which even-carbon fatty acids yield two acetyl CoA (from the 4-carbon fragment remaining) but odd-carbon fatty acids yield one acetyl CoA and one propionyl CoA (from the 5-carbon fragment remaining).

Propionyl CoA is converted to succinyl CoA, a citric acid cycle intermediate, in the two-step propionic acid pathway. Because this extra succinyl CoA can form malate and enter the cytoplasm and gluconeogenesis, odd-carbon fatty acids represent an exception to the rule that fatty

acids cannot be converted to glucose in humans. The propionic acid pathway is shown in Figure I-16-3 and includes two important enzymes, both in the mitochondria:

- Propionyl CoA carboxylase requires biotin.
- Methylmalonyl CoA mutase requires vitamin B₁₂, cobalamin.

Vitamin B₁₂ deficiency can cause a megaloblastic anemia of the same type seen in folate deficiency (discussed in Chapter 17). In a patient with megaloblastic anemia, it is important to determine the underlying cause because B₁₂ deficiency, if not corrected, produces a peripheral neuropathy owing to aberrant fatty acid incorporation into the myelin sheets associated with inadequate methylmalonyl CoA mutase activity. Excretion of methylmalonic acid indicates a vitamin B₁₂ deficiency rather than folate.

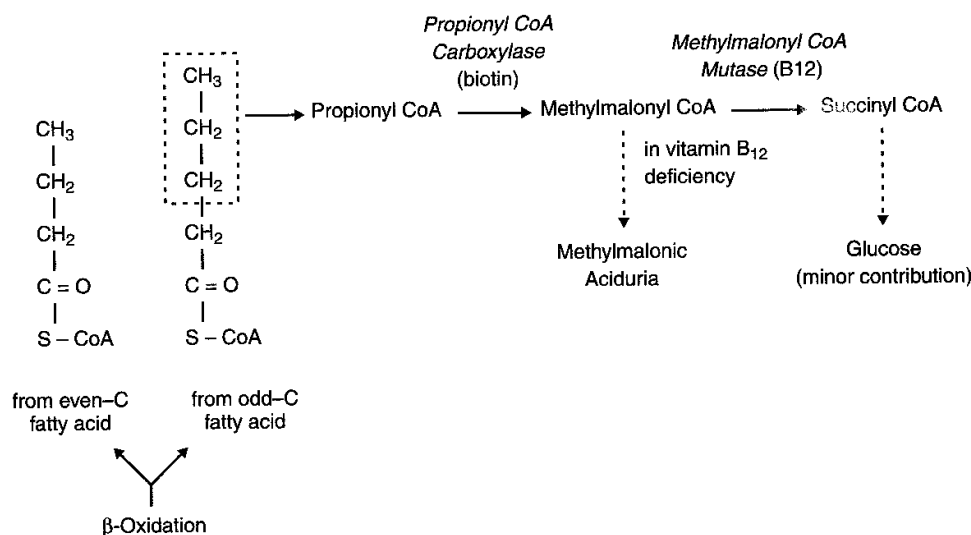


Figure I-16-3. The Propionic Acid Pathway

KETONE BODY METABOLISM

In the fasting state, the liver converts excess acetyl CoA from β -oxidation of fatty acids into ketone bodies, acetoacetate and 3-hydroxybutyrate (β -hydroxybutyrate), which are used by extrahepatic tissues. Cardiac and skeletal muscles and renal cortex metabolize acetoacetate and 3-hydroxybutyrate to acetyl CoA. Normally during a fast, muscle metabolizes ketones as rapidly as the liver releases them, preventing their accumulation in blood. After a week of fasting, ketones reach a concentration in blood high enough for the brain to begin metabolizing them. If ketones increase sufficiently in the blood, they can lead to ketoacidosis. Ketogenesis and ketogenolysis are shown in Figure I-16-4.

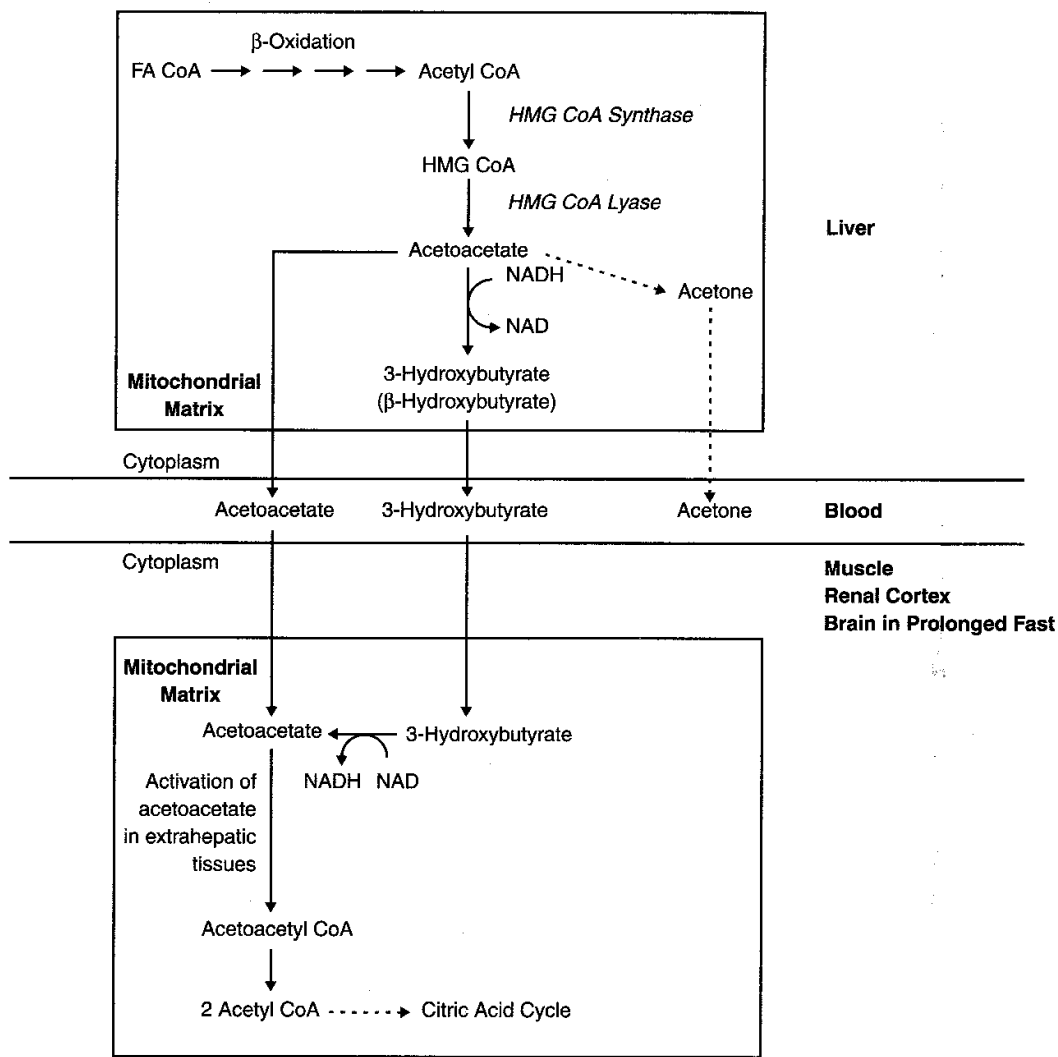


Figure I-16-4. Ketogenesis (Liver) and Ketogenolysis (Extrahepatic)

Ketogenesis

Ketogenesis occurs in mitochondria of hepatocytes when excess acetyl CoA accumulates in the fasting state. HMG-CoA synthase forms HMG-CoA, and HMG-CoA lyase breaks HMG-CoA into acetoacetate, which can subsequently be reduced to 3-hydroxybutyrate. Acetone is a minor side product formed nonenzymatically but is not used as a fuel in tissues. It does, however, impart a strong odor (sweet or fruity) to the breath, which is almost diagnostic for ketoacidosis.

Ketogenesis

Acetoacetate picked up from the blood is activated in the mitochondria by succinyl CoA acetoacetyl CoA transferase (common name thiophorase), an enzyme present only in extrahepatic tissues; 3-hydroxybutyrate is first oxidized to acetoacetate. Because the liver lacks this enzyme, it cannot metabolize the ketone bodies.

Ketogenesis in Brain

Figure I-16-5 shows the major pathways producing fuel for the brain. Note the important times at which the brain switches from:

- Glucose derived from liver glycogenolysis to glucose derived from gluconeogenesis (~12 hours)
- Glucose derived from gluconeogenesis to ketones derived from fatty acids (~1 week)

In the brain, when ketones are metabolized to acetyl CoA, pyruvate dehydrogenase is inhibited. Glycolysis and subsequently glucose uptake in brain decreases. This important switch spares body protein (which otherwise would be catabolized to form glucose by gluconeogenesis in the liver) by allowing the brain to indirectly metabolize fatty acids as ketone bodies.

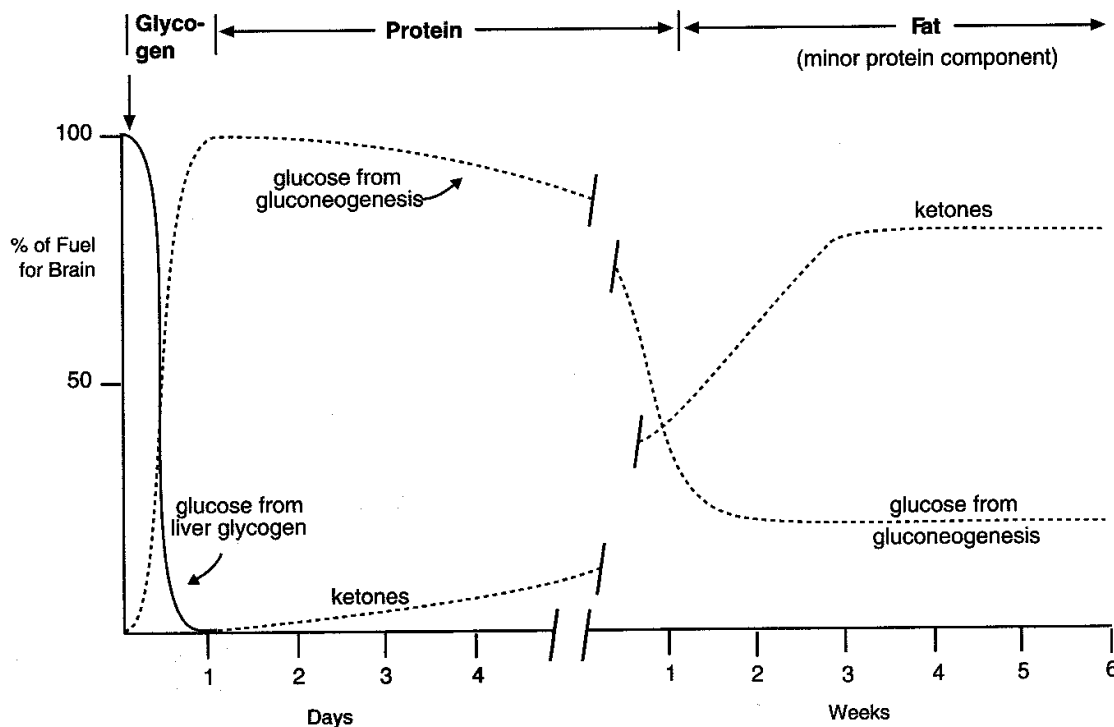


Figure 1-16-5. Fuel Use in the Brain During Fasting and Starvation

Ketoacidosis

In patients with type 1 insulin-dependent diabetes mellitus not adequately treated with insulin, fatty acid release from adipose tissue and ketone synthesis in the liver exceed the ability of other tissues to metabolize them, and a profound, life-threatening ketoacidosis may occur. An infection or trauma (causing an increase in cortisol or epinephrine) may precipitate an episode of ketoacidosis. Patients with type 2 non-insulin-dependent diabetes mellitus (NIDDM) are much less likely to show ketoacidosis. The basis for this observation is not completely understood, although type 2 disease has a much slower, insidious onset, and insulin resistance in the periphery is usually not complete. Type 2 diabetics can develop ketoacidosis after an infection or trauma. In certain populations with NIDDM, ketoacidosis is much more common than previously appreciated.

Alcoholics can also develop ketoacidosis. In alcoholic ketoacidosis, 3-hydroxybutyrate is the major ketone body produced because there is usually a high NADH/NAD ratio in the liver. The urinary nitroprusside test detects only acetoacetate and may dramatically underestimate the extent of ketosis in an alcoholic. 3-Hydroxybutyrate levels (β -hydroxybutyrate) should always be measured in these patients.

Associated with ketoacidosis:

- Polyuria, dehydration, and thirst (exacerbated by hyperglycemia and osmotic diuresis)
- CNS depression and coma
- Potential depletion of K^+ (although loss may be masked by a mild hyperkalemia)
- Decreased plasma bicarbonate
- Breath with a sweet or fruity odor, acetone

SPHINGOLIPIDS

Sphingolipids are important constituents of cell membranes as shown in Figure I-16-6. Although sphingolipids contain no glycerol, they are similar in structure to the glycerophospholipids in that they have a hydrophilic region and two fatty acid-derived hydrophobic tails. The various classes of sphingolipids shown in Figure I-16-7 differ primarily in the nature of the hydrophilic region.

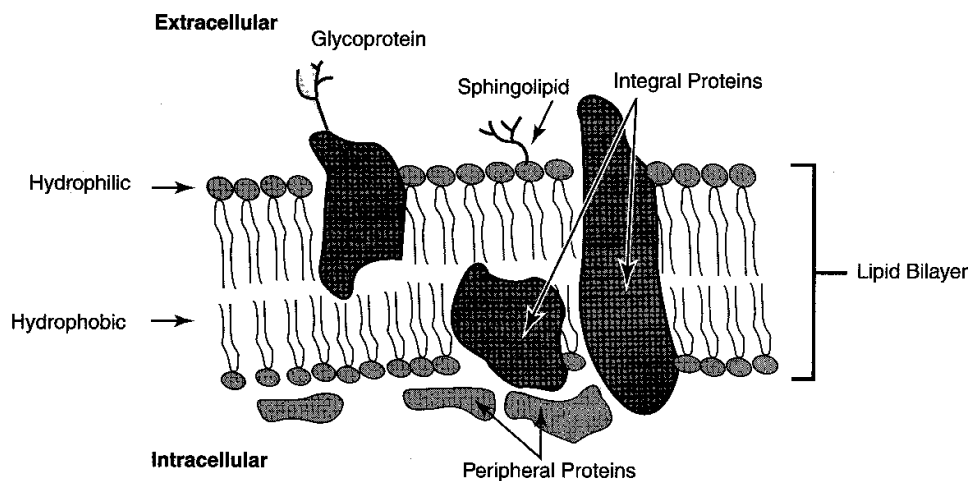


Figure I-16-6. Plasma Membrane

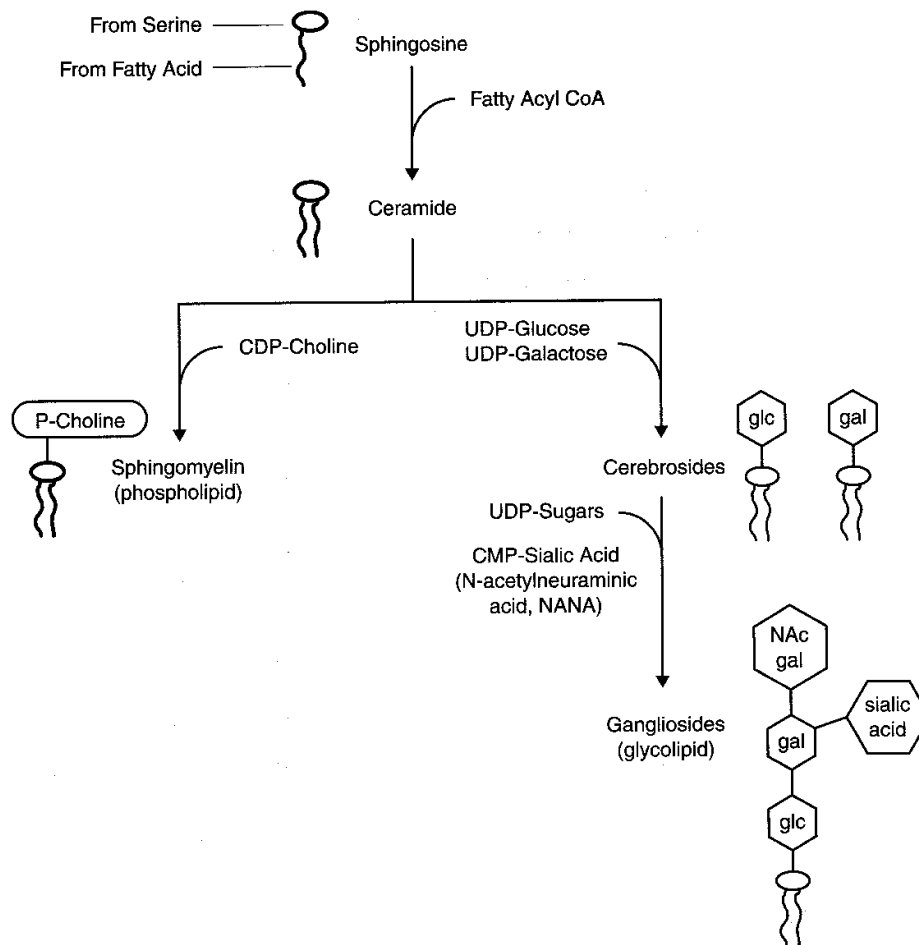


Figure I-16-7. Synthesis of Sphingolipids

Classes of sphingolipids and their hydrophilic groups include:

- Sphingomyelin: phosphorylcholine
- Cerebrosides: galactose or glucose
- Gangliosides: branched oligosaccharide chains terminating in the 9-carbon sugar, sialic acid (N-acetylneuraminic acid, NANA)

Genetic Deficiencies of Enzymes in Sphingolipid Catabolism

Sphingolipids released when membrane is degraded are digested in endosomes after fusion with lysosomes. Lysosomes contain many enzymes, each of which removes specific groups from individual sphingolipids. Genetic deficiencies of many of these enzymes are known, and the diseases share some of the characteristics of I-cell disease discussed in Chapter 4. Table I-16-1 summarizes these.

Table I-16-1. Genetic Deficiencies of Sphingolipid Catabolism

Disease	Lysosomal Enzyme Missing	Substrate Accumulating in Inclusion Body	Symptoms
Tay-Sachs	Hexosaminidase A	Ganglioside GM ₂	Cherry red spots in macula Blindness Psychomotor retardation Death usually <2 years
Gaucher	Glucocerebrosidase	Glucocerebroside	Type 1: Adult Hepatosplenomegaly Erosion of bones, fractures Pancytopenia or thrombocytopenia Characteristic macrophages (crumpled paper inclusions)
Niemann-Pick	Sphingomyelinase	Sphingomyelin	Hepatosplenomegaly Microcephaly, severe mental retardation Zebra bodies in inclusions Characteristic foamy macrophages Early death

Chapter Summary

β-Oxidation

Fat Release From Adipose

Hormone-sensitive lipase

- Activated by decreased insulin, increased epinephrine
- Induced by cortisol

Transport of Fatty Acids Into Mitochondria of Target Tissues

Carnitine shuttle

(Continued)

Chapter Summary (continued)

Rate-Limiting Enzyme

Carnitine acyltransferase-1 (CAT-1, CPT-1)

- Inhibited by malonyl CoA (increases during fatty acid synthesis)

Important Deficiencies

Medium-chain acyl CoA dehydrogenase (MCAD)

- Profound fasting hypoglycemia
- Hypoketosis
- Dicarboxylic acidemia

Myopathic CAT-1 deficiency

- Extreme muscle weakness associated with endurance exercise and/or exercise after prolonged fasting
- Rhabdomyolysis and myoglobinuria

Myopathic carnitine deficiency

- Similar to CAT-1 deficiency but less severe

Odd-Carbon Fatty Acid Oxidation

Propionyl CoA/B12 required

Ketone Bodies

Formed from excess hepatic acetyl CoA during fasting: acetoacetate, 3-hydroxybutyrate, and acetone (not metabolized further)

Oxidized in cardiac skeletal muscle, renal cortex, and brain (prolonged fast)

Sphingolipids

Constituents of lipid bilayer membranes

- Sphingomyelin (ceramide + P and choline)
- Cerebrosides (ceramide + glc or gal)
- Gangliosides/glycolipids (ceramide + oligosaccharides + sialic acid)

Genetic Deficiencies

Tay-Sachs (hexosaminidase A)

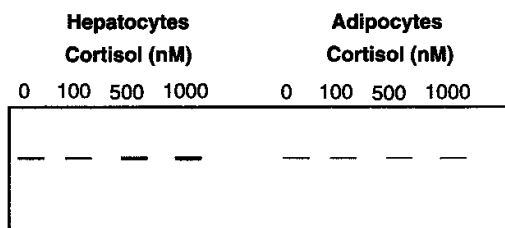
Niemann-Pick (sphingomyelinase)

Gaucher (glucocerebrosidase)

Review Questions

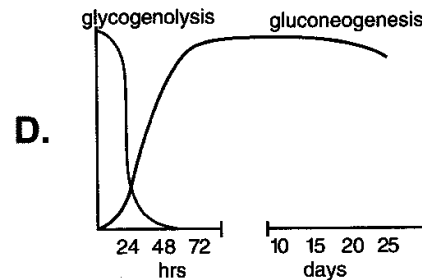
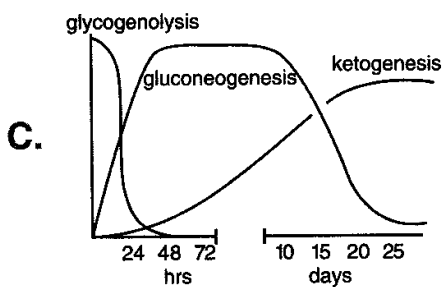
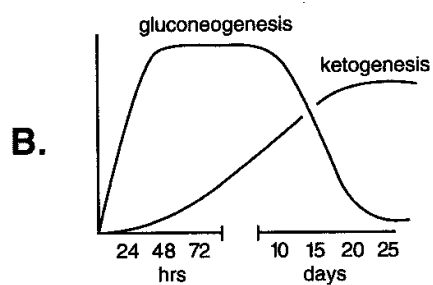
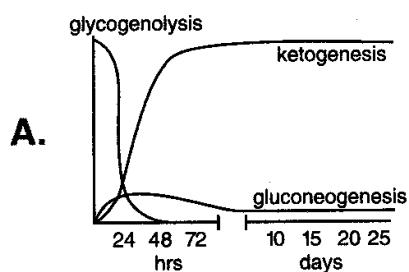
Select the ONE best answer.

1. As part of a study to quantify contributors of stress to hyperglycemia and ketosis in diabetes, normal hepatocytes and adipocytes in tissue culture were treated with cortisol and analyzed by Northern blotting using a gene-specific probe. The results of one experiment are shown below.



- The ^{32}P -probe used in this experiment most likely binds to a mRNA encoding
- A. phosphoenolpyruvate carboxykinase
 - B. lipoprotein lipase
 - C. glucokinase
 - D. hormone-sensitive lipase
 - E. acetyl-CoA carboxylase
2. A child is diagnosed with a congenital deficiency of medium-chain acyl-CoA dehydrogenase activity. Which of the following signs or symptoms would most likely occur upon fasting in this child?
- A. Hypolacticacidemia
 - B. Ketoacidosis
 - C. Hyperglycemia
 - D. Dicarboxylic acidosis
 - E. Hyperchylomicronemia
3. A 14-year-old boy has been experiencing progressive onset of muscle fatigue and cramping. His physician finds no evidence for hypoglycemia, and fatty acids are released appropriately in response to a glucagon challenge. A muscle biopsy reveals unusual lipid-filled vacuoles in the cytoplasm of his myocytes. On analysis the vacuoles are found to contain triglyceride. The most likely cause of these symptoms is a deficiency of
- A. muscle phosphorylase
 - B. hepatic phosphorylase
 - C. debranching enzyme
 - D. muscle carnitine
 - E. apoB-100 receptor

Items 4–6



In the options above, each graph depicts the primary source of fuel used by the brain during fasting/starvation. For each condition listed below, select the most closely matched graph.

4. Liver phosphorylase deficiency
5. Hepatic fructose 1,6-bisphosphatase deficiency
6. Normal individual

Items 7–9

A 54-year-old man with type I (IDDM) diabetes is referred to an ophthalmologist for evaluation of developing cataracts. Pre-appointment blood work was requested and the results are shown below:

Fasting blood glucose	180 mg/dl
Hemoglobin A	15 gm/dl
Hemoglobin A _{1c}	10% of total Hb
Urine ketones	positive
Urine glucose	positive

7. Which of the following enzymes is most strongly associated with cataract formation in this patient?
 - A. Galactokinase
 - B. Aldose reductase
 - C. Glucokinase
 - D. Galactose 1-P uridyl transferase
 - E. Aldolase B

8. Which of the following best indicates the blood glucose in this patient has been elevated over a period of weeks?
 - A. Presence of ketone bodies
 - B. Hyperglycemia
 - C. Lipemia
 - D. Elevated HbA_{1c}
 - E. Lipoprotein lipase

9. Which of the following enzymes would be more active in this patient than in a normal control subject?
 - A. Hormone sensitive lipase
 - B. Glucokinase
 - C. Fatty acid synthase
 - D. Glycogen synthase
 - E. Lipoprotein lipase

10. A 40-year-old woman with a history of bleeding and pancytopenia now presents with leg pain. She describes a deep, dull pain of increasing severity that required pain medication. Computed tomography examination reveals erosion and thinning of the femoral head. A bone marrow biopsy is performed to confirm a diagnosis of Gaucher disease. What material would be found abnormally accumulating in the lysosomes of her cells?
 - A. Mucopolysaccharide
 - B. Ganglioside
 - C. Ceramide
 - D. Cerebroside
 - E. Sulfatide
11. An underweight 4-year-old boy presents semi-comatose in the emergency room at 10 A.M. Plasma glucose, urea, and glutamine are abnormally low; acetoacetate is elevated; and lactate is normal. He is admitted to the ICU, where an increase in blood glucose was achieved by controlled infusion of glucagon or alanine. Which metabolic pathway is most likely deficient in this child?
 - A. Hepatic gluconeogenesis
 - B. Skeletal muscle glycogenolysis
 - C. Adipose tissue lipolysis
 - D. Skeletal muscle proteolysis
 - E. Hepatic glycogenolysis

Answers

1. **Answer: A.** Cortisol stimulates transcription of the PEP carboxykinase gene in the liver but not in adipose tissue.
2. **Answer: D.** Fasted MCAD patients typically present with nonketotic hypoglycemia, lactic acidosis, and plasma dicarboxylates.
3. **Answer: D.** Triglyceride accumulation in muscle is not normal and indicates fatty acids are not entering the mitochondria normally.
4. **Answer: B.** Glycogen would not be mobilized from the liver.
5. **Answer: A.** Gluconeogenesis from proteins would be severely restricted without this enzyme.
6. **Answer: C.** Glycogen depleted around 18 hours, gluconeogenesis from protein begins to drop gradually, and by two weeks, ketones have become the more important fuel for the brain.
7. **Answer: B.** Aldose reductase is rich in lens and nerve tissue (among others) and converts glucose to sorbitol, which causes the osmotic damage. In galactosemia, this same enzyme converts galactose to galactitol, also creating cataracts.

8. **Answer: D.** HbA1c is glycosylated HbA and is produced slowly whenever the glucose in blood is elevated. It persists until the RBC is destroyed and the Hb degraded and so is useful as a long-term indicator of glucose level.
9. **Answer: A.** Since the diabetes is not being well controlled, assume the response to insulin is low and the man would have over-stimulated glucagon pathways.
10. **Answer: D.** Glucocerebrosides would accumulate in the cells since the missing enzyme is glucosylcerebrosidase.
11. **Answer: D.** The patient is hypoglycemic because of deficient release of gluconeogenic amino acid precursors from muscle (low urea and glutamine, alanine and glucagon challenge tests). These results plus normal lactate and hyperketonemia eliminate deficiencies in glycogenolysis, gluconeogenesis, and lipolysis as possibilities; defective muscle glycogenolysis would not produce hypoglycemia.

Amino Acid Metabolism

17

OVERVIEW

Protein obtained from the diet or from body protein during prolonged fasting or starvation may be used as an energy source. Body protein is catabolized primarily in muscle and in liver. Amino acids released from proteins usually lose their amino group through transamination or deamination. The carbon skeletons can be converted in the liver to glucose (glucogenic amino acids), acetyl CoA, and ketone bodies (ketogenic), or in a few cases both may be produced (glucogenic and ketogenic).

REMOVAL AND EXCRETION OF AMINO GROUPS

Excess nitrogen is eliminated from the body in the urine. The kidney adds small quantities of ammonium ion to the urine in part to regulate acid-base balance, but nitrogen is also eliminated in this process. Most excess nitrogen is converted to urea in the liver and goes through the blood to the kidney, where it is eliminated in urine.

Amino groups released by deamination reactions form ammonium ion (NH_4^+), which must not escape into the peripheral blood. An elevated concentration of ammonium ion in the blood, hyperammonemia, has toxic effects in the brain (cerebral edema, convulsions, coma, and death). Most tissues add excess nitrogen to the blood as glutamine. Muscle sends nitrogen to the liver as alanine and smaller quantities of other amino acids, in addition to glutamine. Figure I-17-1 summarizes the flow of nitrogen from tissues to either the liver or kidney for excretion. The reactions catalyzed by four major enzymes or classes of enzymes involved in this process are summarized in Table I-17-1.

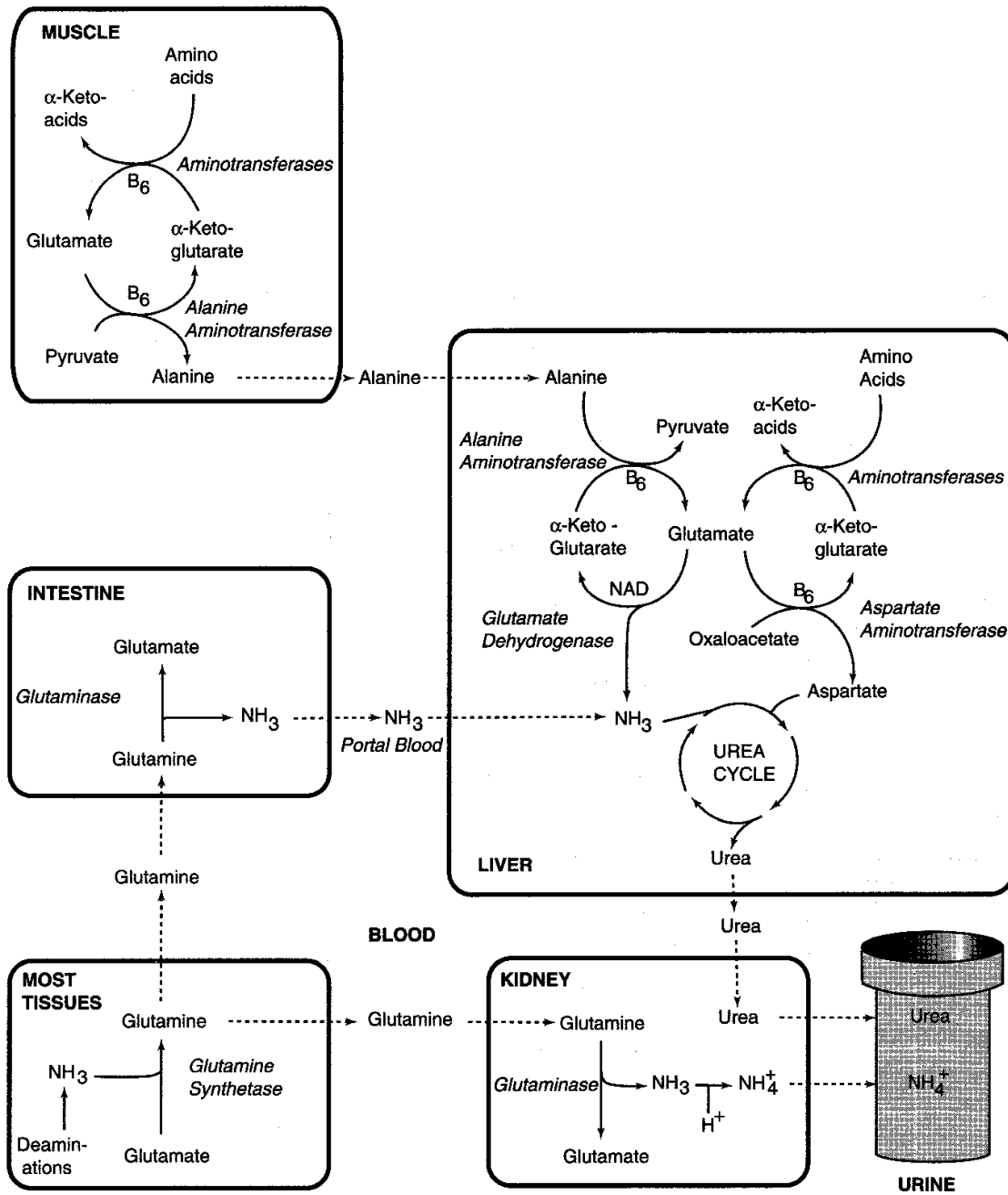


Figure I-17-1. Amino Group Removal for Elimination as Urea and Ammonia

Table I-17-1. Enzymes for Removal and Elimination of Nitrogen

Enzyme or Class of Enzyme and Reaction Catalyzed
Glutamine synthetase $\text{Glutamate} + \text{NH}_4^+ + \text{ATP} \rightarrow \text{Glutamine} + \text{H}_2\text{O} + \text{ADP} + \text{P}_i$
Glutaminase $\text{Glutamine} + \text{H}_2\text{O} \rightarrow \text{Glutamate} + \text{NH}_4^+$
Aminotransferases (Transaminases) Require pyridoxal phosphate (PLP) derived from vitamin B ₆ Generic reaction $\text{Amino acid} + \alpha\text{-Ketoglutarate} \rightleftharpoons \alpha\text{-Ketoacid} + \text{Glutamate}$ $\text{Alanine} + \alpha\text{-Ketoglutarate} \xrightleftharpoons{\text{ALT (GPT)}} \text{Pyruvate} + \text{Glutamate}$ $\text{Aspartate} + \alpha\text{-Ketoglutarate} \xrightleftharpoons{\text{AST (GOT)}} \text{Oxaloacetate} + \text{Glutamate}$
Glutamate dehydrogenase $\text{Glutamate} + \text{NAD}^+ + \text{H}_2\text{O} \rightleftharpoons \alpha\text{-Ketoglutarate} + \text{NH}_4^+ + \text{NADH}$

ALT = alanine aminotransferase (newer name); GPT = glutamate-pyruvate transaminase (older name); AST = aspartate aminotransferase (newer name); GOT = glutamate-oxaloacetate transaminase (older name)

Glutamine Synthetase

Most tissues, including muscle, have glutamine synthetase, which captures excess nitrogen by aminating glutamate to form glutamine. The reaction is irreversible. Glutamine, a relatively nontoxic substance, is the major carrier of excess nitrogen from tissues.

Glutaminase

The kidney contains glutaminase, allowing it to deaminate glutamine arriving in the blood and to eliminate the amino group as ammonium ion in urine. The reaction is irreversible. Kidney glutaminase is induced by chronic acidosis, in which excretion of ammonium may become the major defense mechanism. The liver has only small quantities of glutaminase; however, levels of the enzyme are high in the intestine where the ammonium ion from deamination can be sent directly to the liver via the portal blood and used for urea synthesis.

Aminotransferases (Transaminases)

Both muscle and liver have aminotransferases, which, unlike deaminases, do not release the amino groups as free ammonium ion. This class of enzymes transfers the amino group from one carbon skeleton (an amino acid) to another (usually α -ketoglutarate, a citric acid cycle intermediate). Pyridoxal phosphate (PLP) derived from vitamin B₆ is required to mediate the transfer.

Aminotransferases are named according to the amino acid donating the amino group to α -ketoglutarate. Two important examples are **alanine aminotransferase** (ALT, formerly GPT) and **aspartate aminotransferase** (AST, formerly GOT). Although the aminotransferases are in liver and muscle, in pathologic conditions these enzymes may leak into the blood where they are useful clinical indicators of damage to liver or muscle.

The reactions catalyzed by aminotransferases are reversible and play several roles in metabolism:

- During protein catabolism in muscle, they move the amino groups from many of the different amino acids onto glutamate, thus pooling it for transport. A portion of the glutamate may be aminated by glutamine synthetase (as in other tissues) or may transfer the amino group to pyruvate, forming alanine using the aminotransferase ALT.
- In liver, aminotransferases ALT and AST can move the amino group from alanine arriving from muscle into aspartate, a direct donor of nitrogen into the urea cycle.

Glutamate Dehydrogenase

This enzyme is found in many tissues, where it catalyzes the reversible oxidative deamination of the amino acid glutamate. It produces the citric acid cycle intermediate α -ketoglutarate, which serves as an entry point to the cycle for a group of glucogenic amino acids. Its role in urea synthesis and nitrogen removal is still controversial, but has been included in Figure I-17-1 and Table I-17-1.

UREA CYCLE

Urea, which contains two nitrogens, is synthesized in the liver from aspartate and carbamoyl phosphate, which in turn is produced from ammonium ion and carbon dioxide by mitochondrial carbamoyl phosphate synthetase. The urea cycle and the carbamoyl phosphate synthetase reaction are shown in Figure I-17-2.

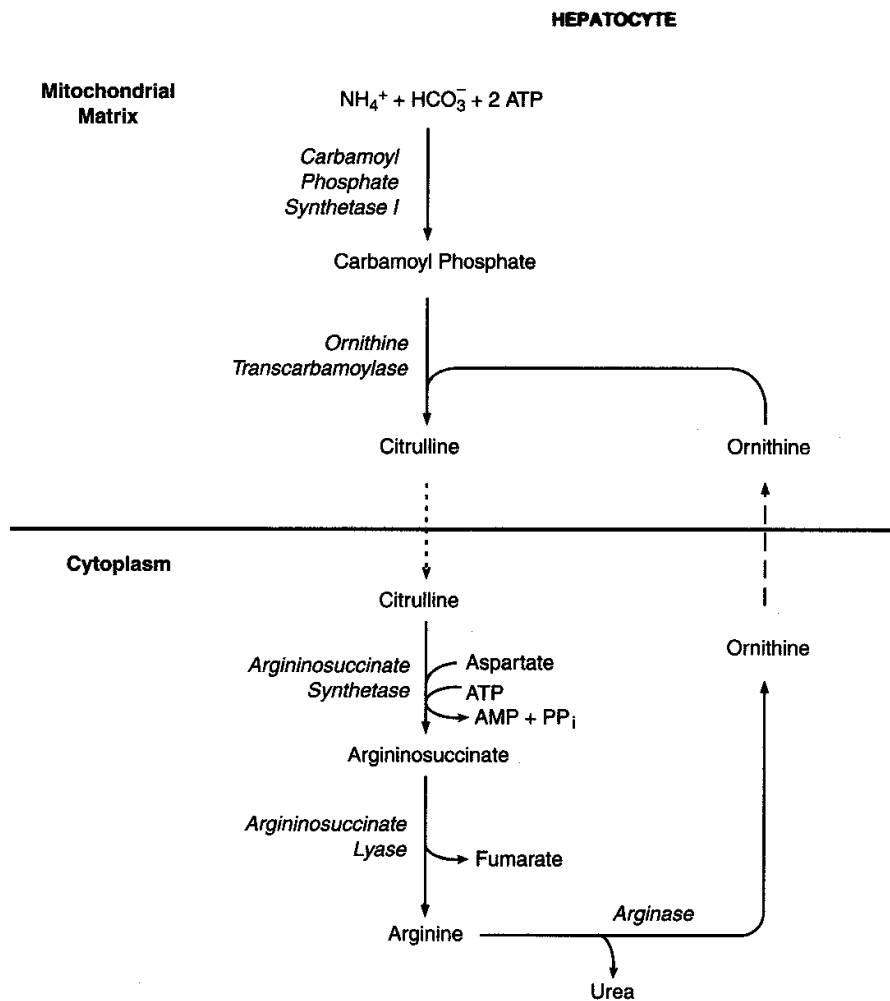


Figure I-17-2. The Urea Cycle in the Liver

The urea cycle, like the citric acid cycle, acts catalytically. Small quantities of the intermediates are sufficient to synthesize large amounts of urea from aspartate and carbamoyl phosphate. The cycle occurs partially in the mitochondria and partially in the cytoplasm.

- Citrulline enters the cytoplasm, and ornithine returns to the mitochondria.
- Carbamoyl phosphate synthetase and ornithine transcarbamoylase are mitochondrial enzymes.
- Aspartate enters the cycle in the cytoplasm and leaves the cycle (minus its amino group) as fumarate. If gluconeogenesis is active, fumarate can be converted to glucose.
- The product urea is formed in the cytoplasm and enters the blood for delivery to the kidney.

Genetic Deficiencies of the Urea Cycle

A combination of hyperammonemia, elevated blood glutamine, and decreased blood urea nitrogen (BUN) suggests a defect in the urea cycle. With neonatal onset, infants typically appear normal for the first 24 hours. Sometime during the 24- to 72-hour postnatal period, symptoms of lethargy, vomiting, and hyperventilation begin and, if not treated, progress to coma, respiratory failure, and death. Table I-17-2 compares the deficiencies of the two mitochondrial enzymes in the urea cycle, carbamoyl phosphate synthetase and ornithine transcarbamoylase.

The two conditions can be distinguished by an increase in orotic acid and uracil, which occurs in ornithine transcarbamoylase deficiency, but not in the deficiency of carbamoyl phosphate synthetase. Orotic acid and uracil are intermediates in pyrimidine synthesis (see Chapter 18). This pathway is stimulated by the accumulation of carbamoyl phosphate, the substrate for ornithine transcarbamoylase in the urea cycle and for aspartate transcarbamoylase in pyrimidine synthesis.

These conditions can be treated with a low protein diet and administration of sodium benzoate or phenylpyruvate to provide an alternative route for capturing and excreting excess nitrogen.

Table I-17-2. Genetic Deficiencies of Urea Synthesis

Carbamoyl Phosphate Synthetase	Ornithine Transcarbamoylase
↑ $[\text{NH}_4^+]$; hyperammonemia	↑ $[\text{NH}_4^+]$; hyperammonemia
Blood glutamine is increased	Blood glutamine is increased
BUN is decreased	BUN is decreased
No increase in uracil or orotic acid	Uracil and orotic acid increase in blood and urine
Cerebral edema	Cerebral edema
Lethargy, convulsions, coma, death	Lethargy, convulsions, coma, death

Metabolism of Amino Acids to Glucose or Ketones

After removal of the α -amino group from the amino acids, the remaining ketoacids may be metabolized for energy or used for hepatic synthesis of glucose or ketone bodies. Table I-17-3 shows this classification.

Table I-17-3. Glucogenic and Ketogenic Amino Acids

Ketogenic	Ketogenic and Glucogenic	Glucogenic
Leucine Lysine	Phenylalanine Tyrosine Tryptophan Isoleucine Threonine	All others

DISORDERS OF AMINO ACID METABOLISM

Figure I-17-3 presents a diagram of pathways in which selected amino acids are converted to citric acid cycle intermediates (and glucose) or to acetyl CoA (and ketones). Important genetic deficiencies are identified on the diagram.

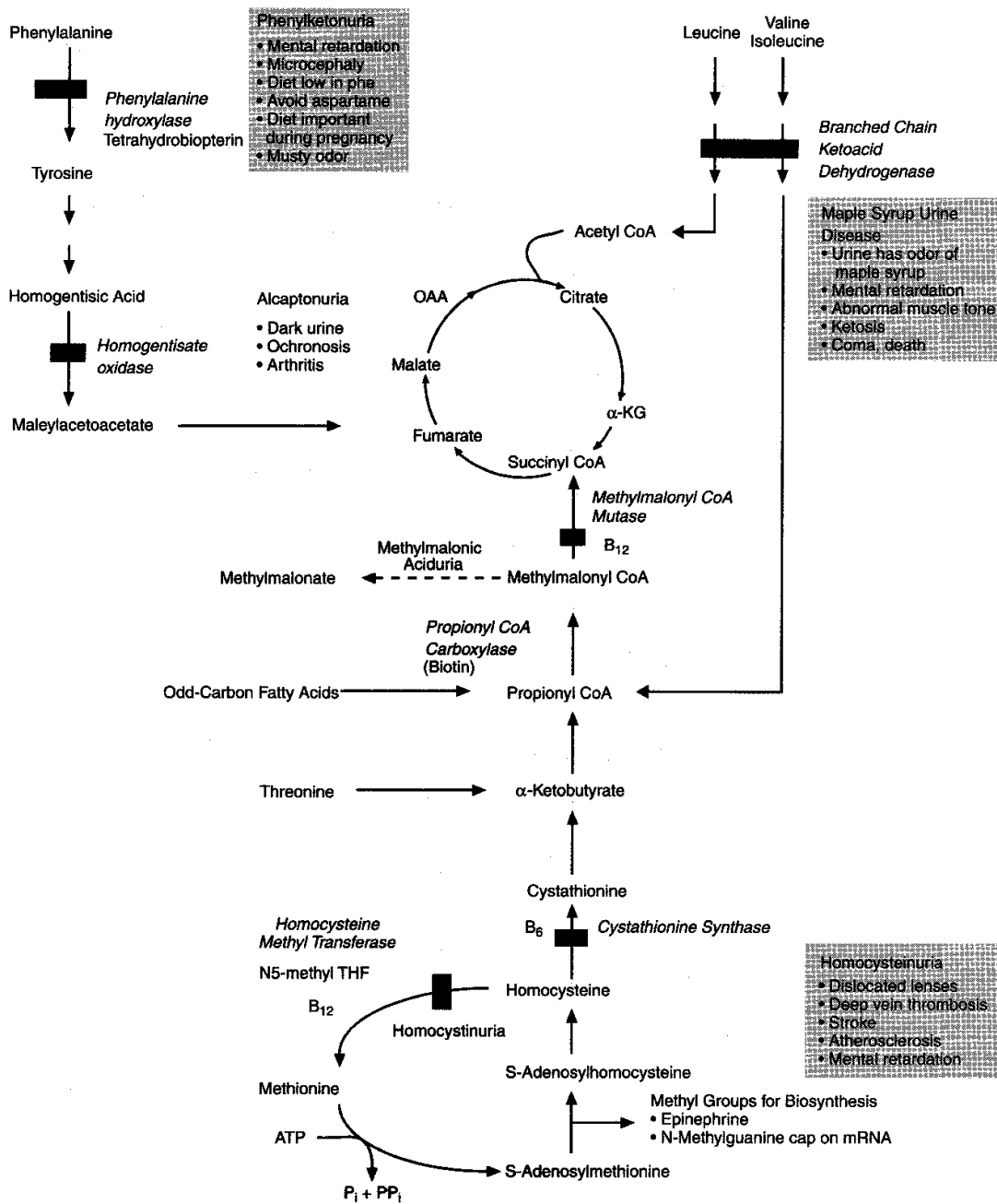


Figure I-17-3. Genetic Deficiencies of Amino Acid Metabolism

Phenylalanine Hydroxylase Deficiency (Phenylketonuria)

Infants with classic phenylketonuria (PKU) are normal at birth but if untreated show slow development, severe mental retardation, autistic symptoms, and loss of motor control. Children may have pale skin and white-blond hair. The neurotoxic effects relate to high levels of phenylalanine and not to the phenylketones from which the name of the disease derives. Infants are routinely screened a few days after birth for blood phenylalanine level. Treatment consists of a life-long semisynthetic diet restricted in phenylalanine (small quantities are necessary because it is an essential amino acid). Aspartame (*N*-aspartyl-phenylalanine methyl ester), which is widely used as an artificial sweetener, must be strictly avoided by phenylketonurics.

Women with PKU who become pregnant must be especially careful about the phenylalanine level in their blood so as not to adversely affect neurologic development in the fetus. Infants whose phenylketonuric mothers have not maintained adequate metabolic control during pregnancy have a high risk for mental retardation (although less profound than in a child with untreated PKU), microcephaly, and low birth weight.

Homogentisate Oxidase Deficiency (Alcaptonuria)

Accumulation of homogentisic acid in the blood causes its excretion in urine, after which it gradually darkens upon exposure to air. This sign of alcaptonuria is not present in all patients with the enzyme deficiency. The dark pigment also accumulates over years in the cartilage (ochronosis), and most patients develop arthritis in adulthood.

Branched-Chain Ketoacid Dehydrogenase Deficiency (Maple Syrup Urine Disease)

Branched-chain ketoacid dehydrogenase, an enzyme similar to α -ketoglutarate dehydrogenase (thiamine, lipoic acid, CoA, FAD, NAD^+), metabolizes branched-chain ketoacids produced from their cognate amino acids, valine, leucine, and isoleucine. In the classic form of the disease, infants are normal for the first few days of life, after which they become progressively lethargic, lose weight, and have alternating episodes of hypertonia and hypotonia, and the urine develops a characteristic odor of maple syrup. Ketosis, coma, and death ensue if not treated. Treatment requires restricting dietary valine, leucine, and isoleucine.

Propionyl CoA Carboxylase and Methylmalonyl CoA Mutase Deficiencies

Valine, methionine, isoleucine, and threonine are all metabolized through the propionic acid pathway (also used for odd-carbon fatty acids). Deficiency of either enzyme results in neonatal ketoacidosis from failure to metabolize ketoacids produced from these four amino acids. The deficiencies may be distinguished based on whether methylmalonic aciduria is present. A diet low in protein or a semisynthetic diet with low amounts of valine, methionine, isoleucine, and threonine is used to treat both deficiencies.

Homocystinemia/Homocystinuria

Accumulation of homocystine in blood is associated with cardiovascular disease; deep vein thrombosis, thromboembolism, and stroke; dislocation of the lens (ectopic lens); and mental retardation. Homocystinemia caused by an enzyme deficiency is a rare, but severe, condition in which atherosclerosis in childhood is a prominent finding. These children often have myocardial infarctions before 20 years of age. All patients excrete high levels of homocystine in the urine. Treatment includes a diet low in methionine. The two major enzyme deficiencies producing homocystinemia are:

- Cystathionine β -synthase deficiency
- Methyltetrahydrofolate-homocysteine methyltransferase deficiency

Homocystinemia From Vitamin Deficiencies

Vitamin deficiencies may produce a more mild form of homocystinemia. Mild homocystinemia is associated with increased risk for atherosclerosis, deep vein thrombosis, and stroke. The vitamin deficiencies causing homocystinemia include:

- Folate deficiency: The recommended dietary intake of folate has been increased (also protects against neural tube defects in the fetus), and additional folate is now added to flour (bread, pasta, and other products made from flour)
- Vitamin B₁₂
- Vitamin B₆

S-ADENOSYLMETHIONINE, FOLATE, AND COBALAMIN

One-Carbon Units in Biochemical Reactions

One-carbon units in different oxidation states are required in the pathways producing purines, thymidine, and many other compounds. When a biochemical reaction requires a methyl group (methylation), S-adenosylmethionine (SAM) is generally the methyl donor. If a one-carbon unit in another oxidation state is required (methylene, methenyl, formyl), tetrahydrofolate (THF) typically serves as its donor.

S-Adenosylmethionine

Important pathways requiring SAM include synthesis of epinephrine and of the 7-methylguanine cap on eukaryotic mRNA. Synthesis of SAM from methionine is shown in Figure I-17-3. After donating the methyl group, SAM is converted to homocysteine and remethylated in a reaction catalyzed by N-methyl THF-homocysteine methyltransferase requiring both vitamin B₁₂ and N-methyl-THF. The methionine produced is once again used to make SAM.

Tetrahydrofolate

THF is formed from the vitamin folate through two reductions catalyzed by dihydrofolate reductase shown in Figure I-17-4. It picks up a one-carbon unit from a variety of donors and enters the active one-carbon pool. Important pathways requiring forms of THF from this pool include the synthesis of all purines and thymidine, which in turn are used for DNA and RNA synthesis during cell growth and division.

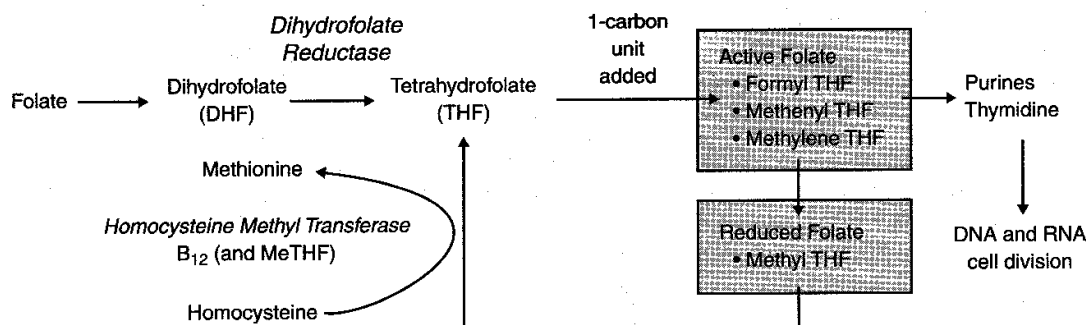


Figure I-17-4. Folate Metabolism

Megaloblastic anemia results from insufficient active THF to support cell division in the bone marrow. Methotrexate inhibits DHF reductase, making it a useful antineoplastic drug. Folate deficiencies may be seen during pregnancy and in alcoholism.

Additional folate may be stored as the highly reduced N^5 -methyl-THF. This form is referred to as the storage pool as there is only one known enzyme that uses it, and in turn moves it back into the active pool. This enzyme is N -methyl THF-homocysteine methyltransferase, discussed above, which also requires vitamin B_{12} and is involved in regenerating SAM as a methyl donor for reactions.

Cobalamin

The vitamin cobalamin (vitamin B_{12}) is reduced and activated in the body to two forms, adenosylcobalamin, used by methylmalonyl CoA mutase, and methylcobalamin, formed from N^5 -methyl-THF in the N -methyl THF-homocysteine methyltransferase reaction. These are the only two enzymes that use vitamin B_{12} (other than the enzymes that reduce and add an adenosyl group to it).

Cobalamin deficiency can create a secondary deficiency of active THF by preventing its release from the storage pool through the N -methyl THF-homocysteine methyltransferase reaction, and thus also result in megaloblastic anemia. Progressive peripheral neuropathy also results from cobalamin deficiency. Treating a cobalamin deficiency with folate corrects the megaloblastic anemia but does not halt the neuropathy.

The most likely reason for cobalamin deficiency is pernicious anemia (failure to absorb vitamin B_{12} in the absence of intrinsic factor from parietal cells). Vitamin B_{12} absorption also decreases with aging and in individuals with chronic pancreatitis. Less common reasons for B_{12} deficiency include a long-term completely vegetarian diet (plants don't contain vitamin B_{12}) and infection with *Diphyllobothrium latum*, a parasite found in raw fish. Excess vitamin B_{12} is stored in the body, so deficiencies develop slowly.

Deficiencies of folate and cobalamin are compared in Table I-17-4.

Table I-17-4. Comparison of Folate and Vitamin B₁₂ Deficiencies

Folate Deficiency	Vitamin B ₁₂ (Cobalamin) Deficiency
Megaloblastic anemia	Megaloblastic anemia
Homocystinemia with risk for cardiovascular disease	Progressive peripheral neuropathy
Deficiency develops in 3–4 months	Homocystinemia with risk for cardiovascular disease
Risk factors for deficiency:	Methylmalonic aciduria
• Pregnancy (neural tube defects in fetus may result)	Deficiency develops in years
• Alcoholism	Risk factors for deficiency:
• Severe malnutrition	• Pernicious anemia
	• Gastric resection
	• Chronic pancreatitis
	• Severe malnutrition
	• Vegan
	• Infection with <i>D. latum</i>

Clinical Correlate**Megaloblastic Anemia**

- Hypersegmented neutrophils
- Large RBCs
- Megaloblasts in bone marrow

SPECIALIZED PRODUCTS DERIVED FROM AMINO ACIDS

Table I-17-5 identifies some important products formed from amino acids.

Table I-17-5. Products of Amino Acids

Amino Acid	Products
Tyrosine	Thyroid hormones T ₃ and T ₄ Melanin Catecholamines
Tryptophan	Serotonin NAD, NADP
Arginine	Nitric oxide (NO)
Glutamate	γ-Aminobutyric acid (GABA)
Histidine	Histamine

HEME SYNTHESIS

Heme synthesis occurs in almost all tissues because heme proteins include not only hemoglobin and myoglobin, but all the cytochromes (electron transport chain, cytochrome P-450, cytochrome b₅), as well as the enzymes catalase, peroxidase, and the soluble guanylate cyclase stimulated by nitric oxide. The pathway producing heme, shown in Figure I-17-5, is controlled independently in different tissues. In liver, the rate-limiting enzyme δ-aminolevulinate synthase (ALA) is repressed by heme.

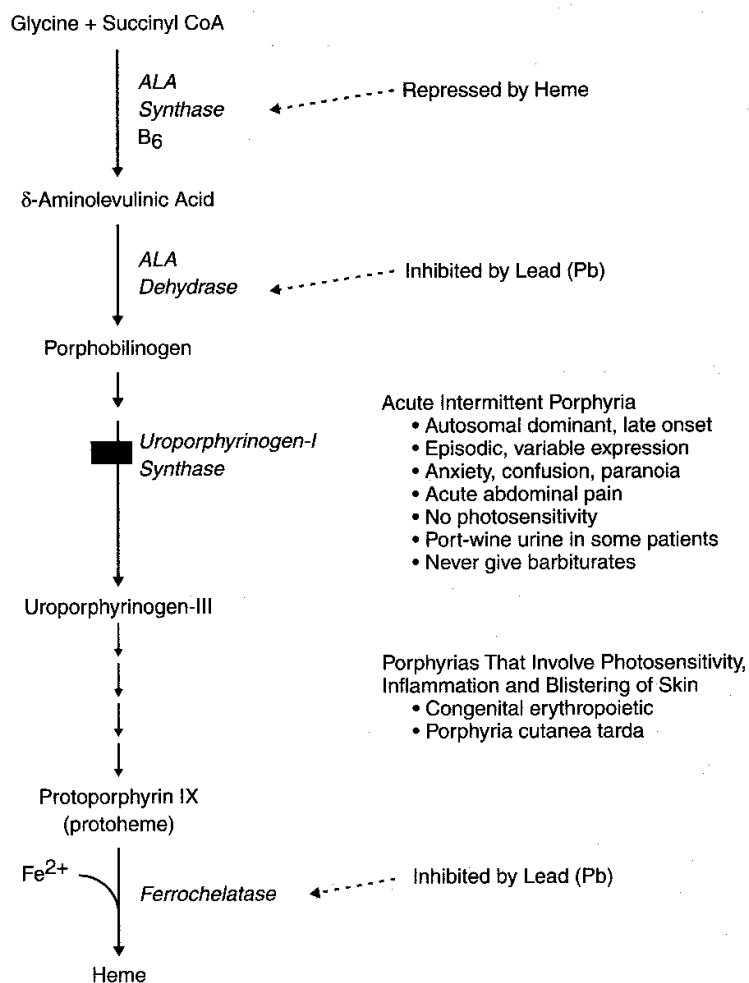


Figure I-17-5. Heme Synthesis

Acute Intermittent Porphyria: Uroporphyrinogen-I Synthase Deficiency

This late-onset autosomal dominant disease exhibits variable expression. Many heterozygotes remain symptom-free throughout their lives. Signs and symptoms, when present, include:

- Abdominal pain, often resulting in multiple laparoscopies (scars on abdomen)
- Neuropathy
- Anxiety, paranoia, and depression
- Excretion of ALA (δ-aminolevulinic) and PBG (porphobilinogen)
- In severe cases, dark port-wine color to urine on standing

Some of these individuals are incorrectly diagnosed and placed in psychiatric institutions. Episodes may be induced by hormonal changes and by many drugs, including barbiturates.

Other Porphyrrias

Deficiencies of other enzymes in the heme pathway produce porphyrias in which photosensitivity is a common finding. Chronic inflammation to overt blistering and shearing in exposed areas of the skin characterize these porphyrias. The most common is porphyria cutanea tarda (deficiency of uroporphyrinogen decarboxylase).

Vitamin B₆ Deficiency

ALA synthase, the rate-limiting enzyme, requires pyridoxine (vitamin B₆). Deficiency of pyridoxine is associated with isoniazid therapy for tuberculosis and may cause sideroblastic anemia with ringed sideroblasts.

Iron Deficiency

The last enzyme in the pathway, heme synthase (ferrochelatase), introduces the Fe²⁺ into the heme ring. Deficiency of iron produces a microcytic hypochromic anemia.

Lead Poisoning

Lead inactivates many enzymes including ALA dehydrase and ferrochelatase (heme synthase), and can produce a microcytic sideroblastic anemia with ringed sideroblasts in the bone marrow. Other symptoms include:

- Coarse basophilic stippling of erythrocytes
- Headache, nausea, memory loss
- Abdominal pain, diarrhea (lead colic)
- Lead lines in gums
- Lead deposits in abdomen and epiphyses of bone seen on radiograph
- Neuropathy (claw hand, wrist-drop)
- Increased urinary ALA
- Increased free erythrocyte protoporphyrin

Vitamin B₆ deficiency, iron deficiency, and lead poisoning all can cause anemia. These three conditions are summarized and compared in Table I-17-6.

Table I-17-6. Comparison of Vitamin B₆ Deficiency, Iron Deficiency, and Lead Poisoning

Vitamin B ₆ (Pyridoxine) Deficiency	Iron Deficiency	Lead Poisoning
Microcytic Ringed sideroblasts in bone marrow Protoporphyrin: ↓ δ-ALA: ↓ Ferritin: ↑ Serum iron: ↑ Isoniazid for tuberculosis	Microcytic Protoporphyrin: ↑ δ-ALA: Normal Ferritin: ↓ Serum iron: ↓ Dietary iron insuf- ficient to compensate for normal loss	Microcytic Coarse basophilic stippling in erythrocytes Ringed sideroblasts in bone marrow Protoporphyrin: ↑ δ-ALA: ↑ Ferritin: ↑ Serum iron: ↑ Lead paints Pottery glaze Batteries (Diagnose by measur- ing blood lead level)

BILIRUBIN METABOLISM

Subsequent to lysis of older erythrocytes in the spleen, heme released from hemoglobin is converted to bilirubin in the histiocytes. This sequence is shown in Figure I-17-6.

- Bilirubin is not water soluble and is therefore transported in the blood attached to serum albumin.
- Hepatocytes conjugate bilirubin with glucuronic acid, increasing its water solubility.
- Conjugated bilirubin is secreted into the bile.
- Intestinal bacteria convert conjugated bilirubin into urobilinogen.
- A portion of the urobilinogen is further converted to bile pigments (stercobilin) and excreted in the feces producing their characteristic red-brown color. Bile duct obstruction results in clay-colored stools.
- Some of the urobilinogen is converted to urobilin (yellow) and excreted in urine.

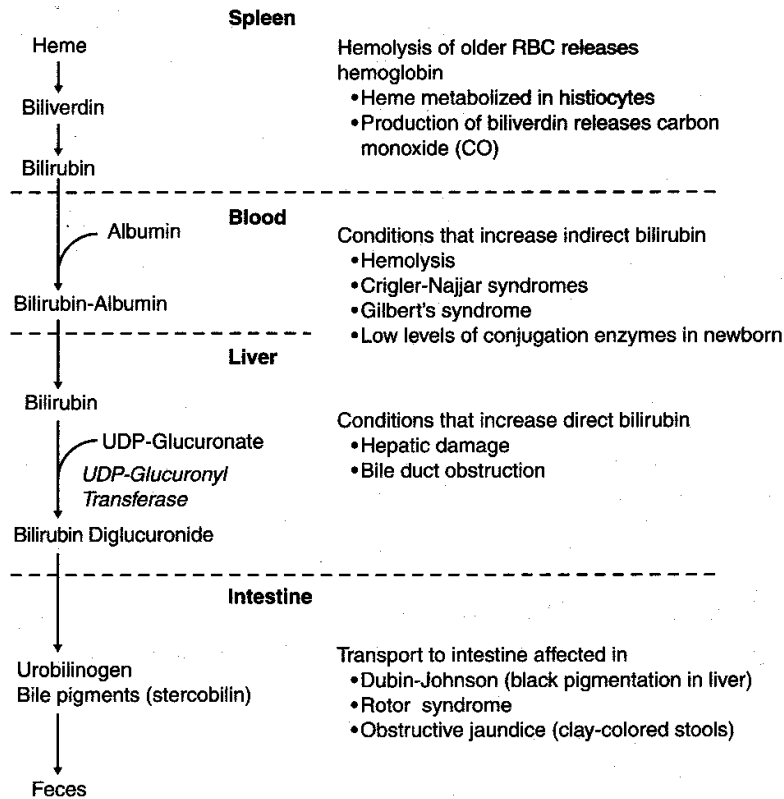


Figure I-17-6. Heme Catabolism and Bilirubin

Bilirubin and Jaundice

Jaundice (yellow color of skin, whites of the eyes) may occur when blood levels of bilirubin exceed normal (icterus). Jaundice may be characterized by an increase in unconjugated (indirect) bilirubin, conjugated (direct) bilirubin, or both. Accumulation of bilirubin (usually unconjugated) in the brain (kernicterus) may result in death. When conjugated bilirubin increases, it may be excreted, giving a deep yellow-red color to the urine. Examples of conditions associated with increased bilirubin and jaundice include the following.

Hemolytic Crisis

With severe hemolysis, more bilirubin is released into the blood than can be transported on albumin and conjugated in the liver. Unconjugated and total bilirubin increase and may produce jaundice and kernicterus. Examples include:

- Episode of hemolysis in G6PDH deficiency
- Sickle cell crisis
- Rh disease of newborn

Hemolytic crisis may be confirmed by **low hemoglobin and elevated reticulocyte counts**.

UDP-Glucuronyl Transferase Deficiency

When bilirubin conjugation is low because of genetic or functional deficiency of the glucuronyl transferase system, unconjugated and total bilirubin increase. Examples include:

- Crigler-Najjar syndromes
- Gilbert syndrome
- Physiologic jaundice in the newborn, especially premature infants (enzymes may not be fully induced)

Hepatic Damage

Viral hepatitis or cirrhosis produces an increase in both direct and indirect bilirubin. Aminotransferase levels will also be elevated.

- Alcoholic liver disease, AST increases more than ALT
- Viral hepatitis, ALT increases more than AST

Bile Duct Occlusion

Occlusion of the bile duct (gallstone, primary biliary cirrhosis, pancreatic cancer) prevents conjugated bilirubin from leaving the liver. Conjugated bilirubin increases in blood and may also appear in urine. Feces are light-colored.

IRON TRANSPORT AND STORAGE

Iron (Fe^{3+}) released from hemoglobin in the histiocytes is bound to ferritin and then transported in the blood by transferrin, which can deliver it to tissues for synthesis of heme. Important proteins in this context are:

- Ferroxidase (also known as ceruloplasmin, a Cu^{2+} protein) oxidizes Fe^{2+} to Fe^{3+} for transport and storage.
- Transferrin carries Fe^{3+} in blood.
- Ferritin stores normal amounts of Fe^{3+} in tissues.
- Hemosiderin binds excess Fe^{3+} to prevent escape of free Fe^{3+} into the blood where it is toxic.

Chapter Summary

Amino Acid Metabolism

Major transport forms of excess nitrogen from tissues

- Muscle: alanine
- Other tissues: glutamine

Enzymes

Glutamate synthetase (most tissues)

Glutaminase (kidney, intestine)

(Continued)

Chapter Summary (continued)**Aminotransferases (transaminases)**

- Muscle and liver
- Require vitamin B6, pyridoxine
- AST (GOT), ALT (GPT)

Urea Cycle

Liver (mitochondria and cytoplasm)

Rate-limiting enzyme: carbamoyl phosphate synthetase-1 (activated by N-acetylglutamate)

Urea Cycle Deficiencies

Most result in hyperammonemia and cerebral edema, decreased BUN, increased blood glutamine

- Carbamoyl phosphate synthetase (no increase in orotic acid or uracil)
- Ornithine transcarbamoylase (increase in uracil and orotic acid)

Other Genetic Diseases Associated With Amino Acid Metabolism

- Phenylketonuria (phenylalanine hydroxylase)
- Alcaptonuria (homogentisate oxidase)
- Maple syrup urine disease (branched-chain ketoacid dehydrogenase)
- Homocysteinuria (cystathionine synthase or homocysteine methyl transferase)

Vitamin Deficiencies

- Homocysteinemia (folate, B12, B6)
- Megaloblastic anemia (folate, B12)

Heme Synthesis**Rate-limiting enzyme**

δ -aminolevulinic acid synthase (B6)

Repressed by heme

Anemias

Differentiate microcytic anemias due to

- Iron deficiency
- B6 deficiency
- Lead poisoning

Porphyrias

Acute intermittent porphyria (urobilinogen-dehydratase)

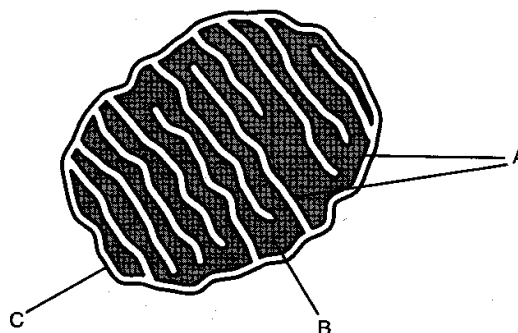
- Neurologic and hepatic
- May show port-wine urine during episode

Other porphyrias mainly cutaneous symptoms

Review Questions

Select the ONE best answer.

Items 1–4



For each item listed below, select the appropriate location from the drawing shown above.

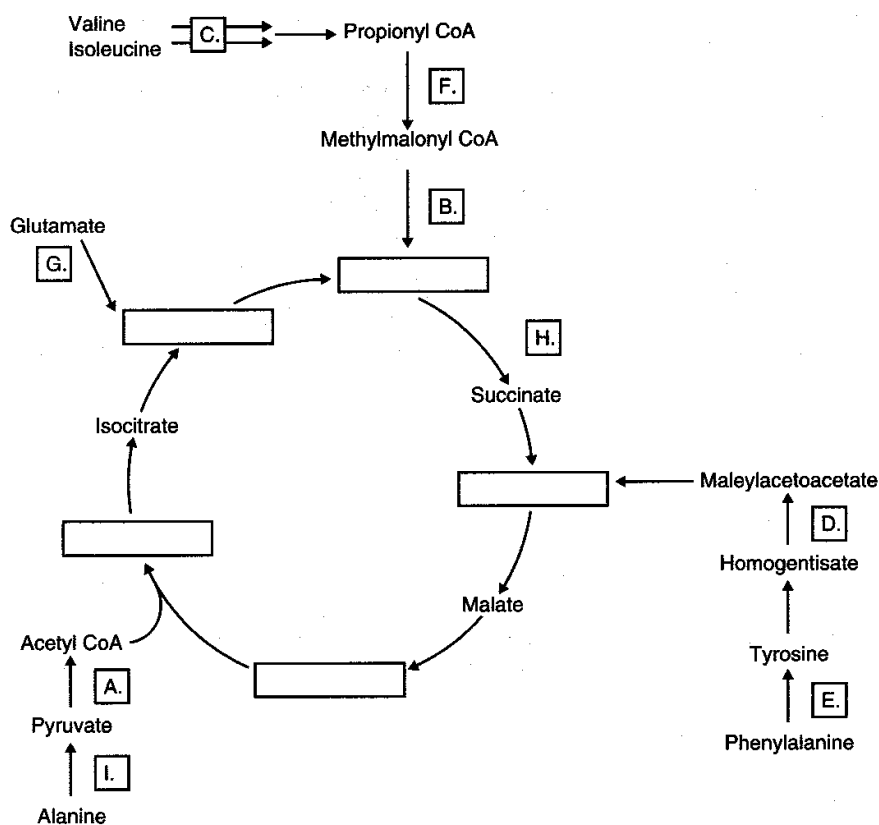
1. Carnitine shuttle
2. F_0F_1 ATP synthase
3. HMG CoA lyase
4. Carnitine acyltransferase-1
5. Which enzymes are responsible for producing the direct donors of nitrogen into the pathway producing urea?
 - A. Arginase and argininosuccinate lyase
 - B. Xanthine oxidase and guanine deaminase
 - C. Glutamate dehydrogenase and glutaminase
 - D. Argininosuccinate synthetase and ornithine transcarbamoylase
 - E. Aspartate aminotransferase and carbamoyl phosphate synthetase
6. Two days after a full-term normal delivery, a neonate begins to hyperventilate, develops hypothermia and cerebral edema, and becomes comatose. Urinalysis reveals high levels of glutamine and orotic acid. The BUN is below normal. Which enzyme is most likely to be deficient in this child?
 - A. Cytoplasmic glutaminase
 - B. Cytoplasmic carbamoyl phosphate synthetase
 - C. Cytoplasmic orotidylate decarboxylase
 - D. Mitochondrial carbamoyl phosphate synthetase
 - E. Mitochondrial ornithine transcarbamoylase

Items 7–8

A 49-year-old man with a rare recessive condition is at high risk for deep vein thrombosis and stroke and has had replacement of ectopic lenses. He has a normal hematocrit and no evidence of megaloblastic anemia.

7. A mutation in the gene encoding which of the following is most likely to cause this disease?
 - A. Cystathionine synthase
 - B. Homocysteine methyltransferase
 - C. Fibrillin
 - D. Lysyl oxidase
 - E. Branched chain α -ketoacid dehydrogenase
8. Amino acid analysis of this patient's plasma would most likely reveal an abnormally elevated level of
 - A. lysine
 - B. leucine
 - C. methionine
 - D. ornithine
 - E. cysteine
9. A 56-year-old man with a history of genetic disease undergoes hip replacement surgery for arthritis. During the operation the surgeon notes a dark pigmentation (ochronosis) in the man's cartilage. His ochronotic arthritis is most likely caused by oxidation and polymerization of excess tissue
 - A. homogentisic acid
 - B. orotic acid
 - C. methylmalonic acid
 - D. uric acid
 - E. ascorbic acid

Items 10–12



10. A 9-week-old boy, healthy at birth, begins to develop symptoms of ketoacidosis, vomiting, lethargy, seizures and hypertonia. Urine has characteristic odor of maple syrup.
11. A child with white-blond hair, blue eyes, and pale complexion is on a special diet in which one of the essential amino acids is severely restricted. He has been told to avoid foods artificially sweetened with aspartame.
12. A chronically ill patient on long-term (home) parenteral nutrition develops metabolic acidosis, a grayish pallor, scaly dermatitis, and alopecia (hair loss). These symptoms subside upon addition of the B vitamin biotin to the alimentation fluid.

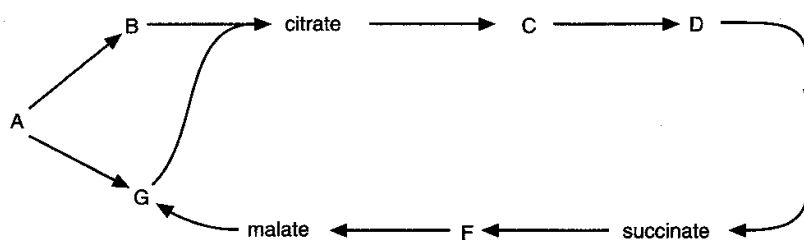
13. A woman 7 months pregnant with her first child develops anemia. Laboratory evaluation indicates an increased mean cell volume (MVC), hypersegmented neutrophils, and altered morphology of several other cell types. The most likely underlying cause of this woman's anemia is
- A. folate deficiency
 - B. iron deficiency
 - C. glucose 6-phosphate dehydrogenase deficiency
 - D. cyanocobalamin (B_{12}) deficiency
 - E. lead poisoning

Items 14–15

A 64-year-old woman is seen by a hematologist for evaluation of a macrocytic anemia. The woman was severely malnourished. Both homocysteine and methylmalonate were elevated in her blood and urine, and the transketolase level in her erythrocytes was below normal.

14. What is the best evidence cited that the anemia is due to a primary deficiency of cyanocobalamin (B_{12})?
- A. Macrocytic anemia
 - B. Elevated methylmalonate
 - C. Low transketolase activity
 - D. Elevated homocysteine
 - E. Severe malnutrition
15. In response to a B_{12} deficiency, which of the additional conditions may develop in this patient if she is not treated?
- A. Progressive peripheral neuropathy
 - B. Gout
 - C. Wernicke-Korsakoff
 - D. Destruction of parietal cells
 - E. Bleeding gums and loose teeth

Items 16–19



16. Allosteric activator of hepatic pyruvate carboxylase in the postabsorptive state.
17. Product formed by argininosuccinate lyase during urea synthesis.
18. Substrate and energy source for synthesis of δ -aminolevulinate in the heme pathway.
19. Converted to glutamate in a reaction requiring the coenzyme form of pyridoxine (B_6)
20. A 62-year-old man being treated for tuberculosis develops a microcytic, hypochromic anemia. Ferritin levels are increased, and marked sideroblastosis is present. A decrease in which of the following enzyme activities is most directly responsible for the anemia in this man?
 - A. Cytochrome oxidase
 - B. Cytochrome P_{450} oxidase
 - C. Pyruvate kinase
 - D. δ -Aminolevulinate synthase
 - E. Lysyl oxidase
21. A 48-year-old man developed abdominal colic, muscle pain, and fatigue. Following a 3-week hospitalization, acute intermittent porphyria was initially diagnosed based on a high level of urinary δ -aminolevulinic acid. Subsequent analysis of the patient's circulating red blood cells revealed that 70% contained elevated levels of zinc protoporphyrin, and the diagnosis was corrected. The correct diagnosis is most likely to be
 - A. protoporphyrin
 - B. congenital erythropoietic porphyria
 - C. lead poisoning
 - D. barbiturate addiction
 - E. iron deficiency

Answers

1. **Answer: A.** Needed for transport of fatty acids across the **mitochondrial** inner membrane.
2. **Answer: A.** Mitochondrial inner membrane.
3. **Answer: B.** Mitochondrial matrix (ketogenesis).
4. **Answer: C.** CAT-1 (CPT-1) and fatty acyl synthetase are among the few enzymes associated with the outer mitochondrial membrane.
5. **Answer: E.** Aspartate is produced by AST and carbamoyl phosphate by CPS-I.
6. **Answer: E.** Given these symptoms, the defect is in the urea cycle and the elevated orotate suggests deficiency of ornithine transcarbamoylase.
7. **Answer: A.** Homocysteine, the substrate for the enzyme, accumulates increasing the risk of deep vein thrombosis and disrupting the normal crosslinking of fibrillin. Deficiency of homocysteine methyl transferase would cause homocystinuria, but would also predispose to megaloblastic anemia.
8. **Answer: C.** Only methionine is degraded via the homocysteine/cystathionine pathway and would be elevated in the plasma of a cystathionine synthase-deficient patient via activation of homocysteine methyl-transferase by excess substrate.
9. **Answer: A.** Adults with alcaptonuria show a high prevalence of ochronotic arthritis due to deficiency of homogentisate oxidase.
10. **Answer: C.** Maple syrup urine disease; substrates are branched chain α -ketoacids derived from the branched chain amino acids.
11. **Answer: E.** The child has PKU; aspartame contains phenylalanine. These children may be blond, blue eyed, and pale complected because of deficient melanin production from tyrosine.
12. **Answer: F.** The only biotin-dependent reaction in the diagram. The enzyme is propionyl CoA carboxylase.
13. **Answer: A.** Pregnant woman with megaloblastic anemia and elevated serum homocysteine strongly suggests folate deficiency. Iron deficiency presents as microcytic, hypochromic anemia and would not elevate homocysteine. B₁₂ deficiency is not most likely in this presentation.
14. **Answer: B.** Methylmalonyl CoA mutase requires B₁₂ but not folate for activity. Macrocytic anemia, elevated homocysteine, and macrocytic anemia can be caused by either B₁₂ or folate deficiency.
15. **Answer: A.** Progressive peripheral neuropathy. A distractor may be D, but this would be the cause of a B₁₂ deficiency, not a result of it.
16. **Answer: B.** Acetyl CoA activates pyruvate **carboxylase** and gluconeogenesis during fasting.
17. **Answer: F.** Fumarate.

18. **Answer: E.** Succinyl CoA.
19. **Answer: D.** Glutamate is produced by B₆-dependent transamination of α -ketoglutarate.
20. **Answer: D.** Sideroblastic anemia in a person being treated for tuberculosis (with isoniazid) is most likely due to vitamin B₆ deficiency. δ -Aminolevulinate synthase, the first enzyme in heme synthesis requires vitamin B₆ (pyridoxine).
21. **Answer: C.** Lead inhibits both ferrochelatase (increasing the zinc protoporphyrin) and ALA dehydrase (increasing δ -ALA).

Purine and Pyrimidine Metabolism

18

OVERVIEW

Nucleotides are needed for DNA and RNA synthesis (DNA replication and transcription) and for energy transfer. Nucleoside triphosphates (ATP and GTP) provide energy for reactions that would otherwise be extremely unfavorable in the cell.

Ribose 5-phosphate for nucleotide synthesis is derived from the hexose monophosphate shunt and is activated by the addition of pyrophosphate from ATP, forming phosphoribosyl pyrophosphate (PRPP) using PRPP synthetase (Figure I-18-1). Cells synthesize nucleotides in two ways, *de novo* synthesis and salvage pathways (Figure I-18-1). In *de novo* synthesis, which occurs predominantly in the liver, purines and pyrimidines are synthesized from smaller precursors, and PRPP is added to the pathway at some point. In the salvage pathways, preformed purine and pyrimidine bases can be converted into nucleotides by salvage enzymes distinct from those of *de novo* synthesis. Purine and pyrimidine bases for salvage enzymes may arise from:

- Synthesis in the liver and transport to other tissues
- Digestion of endogenous nucleic acids (cell death, RNA turnover)

In many cells, the capacity for *de novo* synthesis to supply purines and pyrimidines is insufficient, and the salvage pathway is essential for adequate nucleotide synthesis. In patients with Lesch-Nyhan disease, an enzyme for purine salvage (hypoxanthine guanine phosphoribosyl pyrophosphate transferase, HPRT) is absent. People with this genetic deficiency have CNS deterioration, mental retardation, and spastic cerebral palsy associated with compulsive self-mutilation. Cells in the basal ganglia of the brain (fine motor control) normally have very high HPRT activity. These patients also all have hyperuricemia because purines cannot be salvaged.

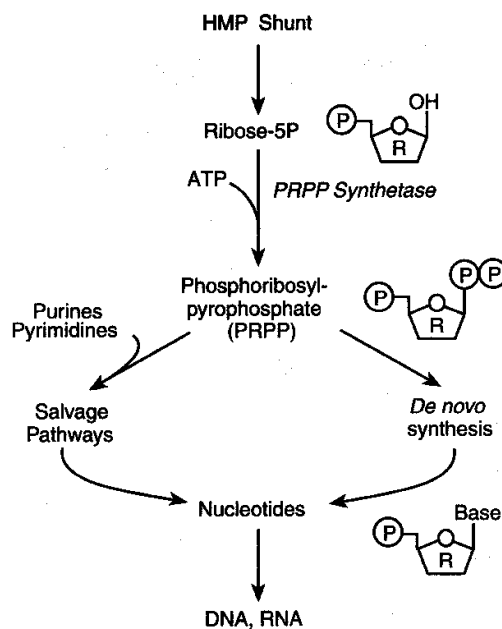


Figure I-18-1. Nucleotide Synthesis by Salvage and *De Novo* Pathways

PYRIMIDINE SYNTHESIS

Pyrimidines are synthesized *de novo* in the cytoplasm from aspartate, CO_2 , and glutamine as shown in Figure I-18-2. Synthesis involves a cytoplasmic carbamoyl phosphate synthetase that differs from the mitochondrial enzyme with the same name used in the urea cycle.

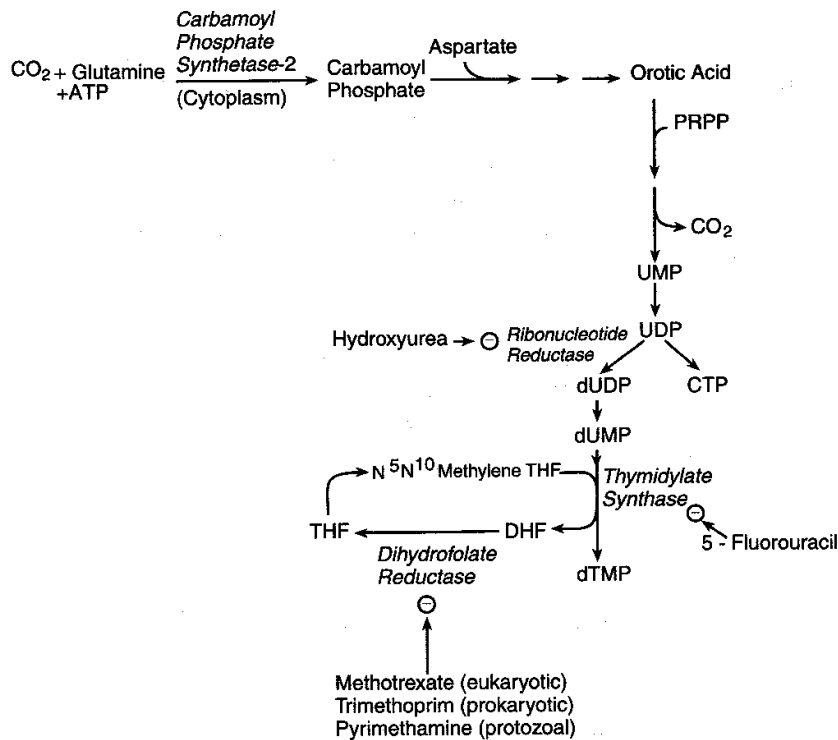


Figure I-18-2. *De Novo* Pyrimidine Synthesis

The primary end product is uridine monophosphate (UMP). In the conversion of UMP to dTMP, three important enzymes are ribonucleotide reductase, thymidylate synthase, and dihydrofolate reductase. All three enzymes are targets of antineoplastic drugs and are summarized in Table I-18-1.

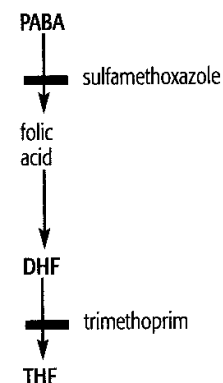
Table I-18-1. Important Enzymes of Pyrimidine Synthesis

Enzyme	Function	Drug
Ribonucleotide reductase	Reduces all NTPs to dNTPs for DNA synthesis	Hydroxyurea (S phase)
Thymidylate synthase	Methylates dUMP to dTMP Requires THF	5-Fluorouracil (S phase)
Dihydrofolate reductase (DHFR)	Converts DHF to THF Without DHFR, thymidylate synthesis will eventually stop	Methotrexate (eukaryotic) (S phase) Trimethoprim (prokaryotic) Pyrimethamine (protozoal)

Bridge to Pharmacology

Cotrimoxazole

Cotrimoxazole contains the synergistic antibiotics sulfamethoxazole and trimethoprim, which inhibit different steps in the prokaryotic synthesis of tetrahydrofolate.



Ribonucleotide Reductase

Ribonucleotide reductase is required for the formation of the deoxyribonucleotides for DNA synthesis. Figure I-18-2 shows its role in dTMP synthesis, and Figure I-18-3 shows all four nucleotide substrates:

- All four nucleotide substrates must be diphosphates.
- dADP strongly inhibits ribonucleotide reductase.
- Hydroxyurea, an anticancer drug, blocks DNA synthesis indirectly by inhibiting ribonucleotide reductase.

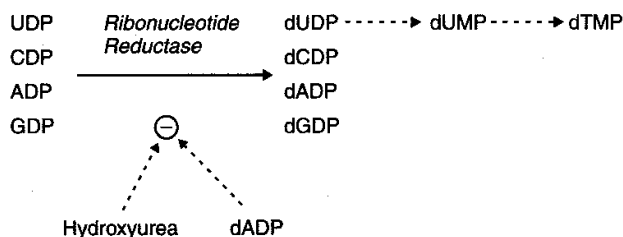


Figure I-18-3. Ribonucleotide Reductase

PYRIMIDINE CATABOLISM

Pyrimidines may be completely catabolized (NH_4^+ is produced) or recycled by pyrimidine salvage enzymes.

PURINE SYNTHESIS

Purines are synthesized *de novo* beginning with PRPP as shown in Figure I-18-4. The most important enzyme is PRPP amidotransferase, which catalyzes the first and rate-limiting reaction of the pathway. It is inhibited by the three purine nucleotide end products AMP, GMP, and IMP.

The drugs allopurinol (used for gout) and 6-mercaptopurine (antineoplastic) also inhibit PRPP amidotransferase. These drugs are purine analogs that must be converted to their respective nucleotides by HPRT within cells. Also note that:

- The amino acids glycine, aspartate, and glutamine are used in purine synthesis.
- Tetrahydrofolate is required for synthesis of all the purines.
- Inosine monophosphate (contains the purine base hypoxanthine) is the precursor for AMP and GMP.

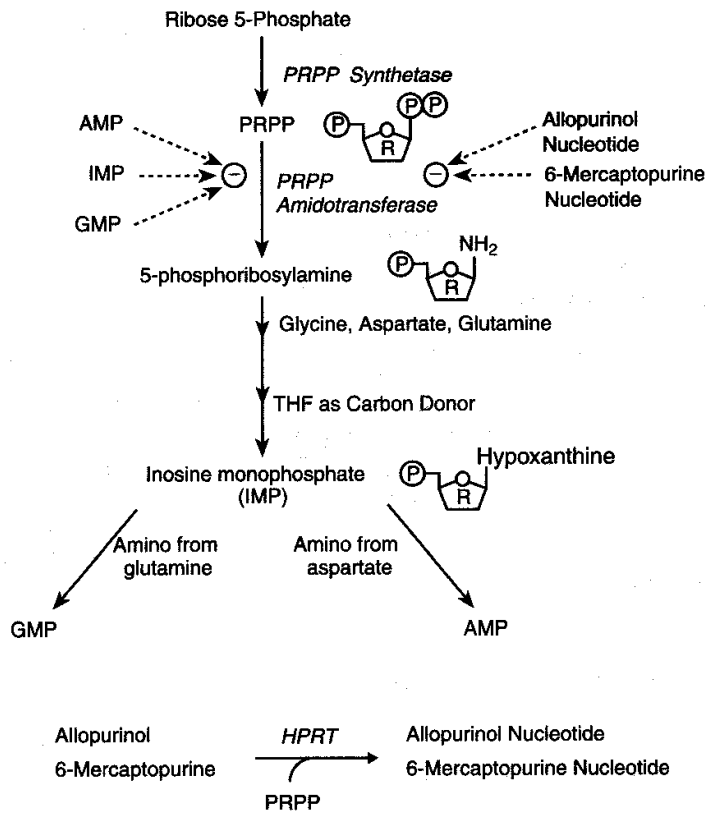


Figure I-18-4. *De Novo* Purine Synthesis

PURINE CATABOLISM AND THE SALVAGE ENZYME HPRT

Excess purine nucleotides or those released from DNA and RNA by nucleases are catabolized first to nucleosides (loss of P_i) and then to free purine bases (release of ribose or deoxyribose). Excess nucleoside monophosphates may accumulate when:

- RNA is normally digested by nucleases (mRNAs and other types of RNAs are continuously turned over in normal cells).
- Dying cells release DNA and RNA, which is digested by nucleases.
- The concentration of free P_i decreases as it may in galactosemia, hereditary fructose intolerance, and glucose 6-phosphatase deficiency.

Salvage enzymes recycle normally about 90% of these purines, and 10% are converted to uric acid and excreted in urine. When purine catabolism is increased significantly, a person is at risk for developing hyperuricemia and potentially gout.

Purine catabolism to uric acid and salvage of the purine bases hypoxanthine (derived from adenosine) and guanine are shown in Figure I-18-5.

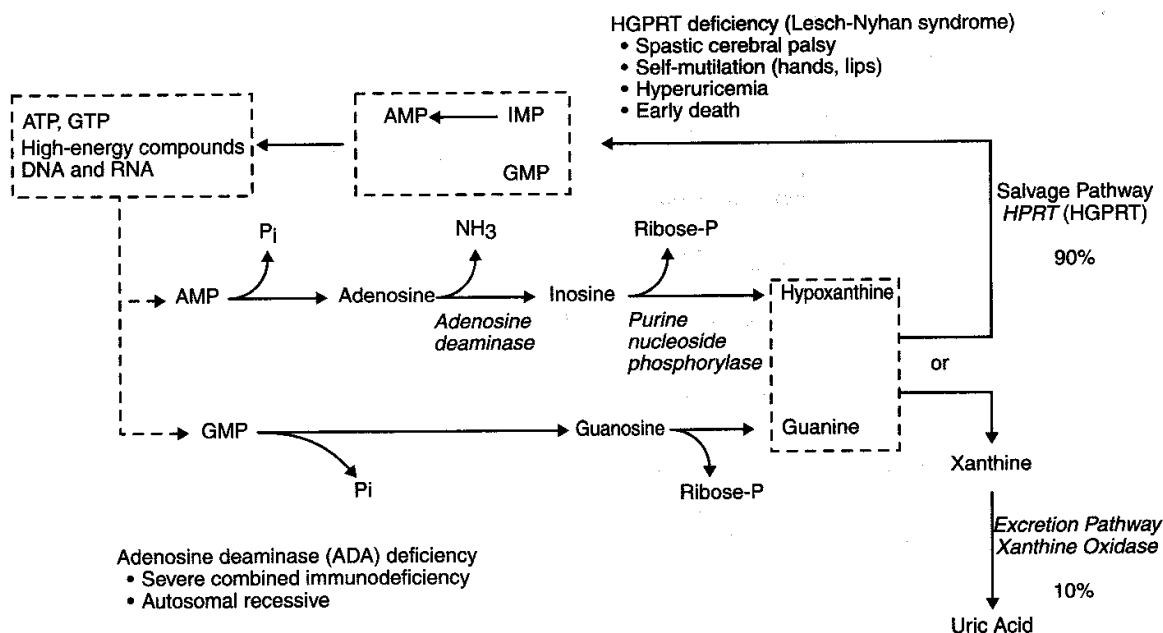


Figure I-18-5. Purine Excretion and Salvage Pathways

Adenosine Deaminase Deficiency

Adenosine deaminase (ADA) deficiency, an autosomal recessive disorder, produces severe combined immunodeficiency (SCID). Lacking both B-cell and T-cell function, children are multiply infected with many organisms (*Pneumocystis carinii*, *Candida*) and do not survive without treatment. Enzyme replacement therapy and bone marrow transplantation may be used. Experimental gene therapy trials have not yet yielded completely successful cures.

Hyperuricemia and Gout

Hyperuricemia may be produced by overproduction of uric acid or under-excretion of uric acid by the kidneys. Hyperuricemia may progress to acute and chronic gouty arthritis if uric acid (monosodium urate) is deposited in joints and surrounding soft tissue, where it causes inflammation. Uric acid is produced from excess endogenous purines as shown in Figure I-18-5, and is also produced from dietary purines (digestion of nucleic acid in the intestine) by intestinal epithelia. Both sources of uric acid are transported in the blood to the kidneys for excretion in urine.

Allopurinol inhibits xanthine oxidase and also can reduce purine synthesis by inhibiting PRPP amidotransferase (provided HPRT is active). Hyperuricemia and gout often accompany the following conditions:

- Lesch-Nyhan syndrome (no purine salvage)
- Partial deficiency of HPRT
- Alcoholism (lactate and urate compete for same transport system in the kidney)
- Glucose 6-phosphatase deficiency

- Hereditary fructose intolerance
- Galactose 1-phosphate uridyl transferase deficiency (galactosemia)

In the last three diseases, phosphorylated sugars accumulate, decreasing the available Pi and increasing AMP (which cannot be phosphorylated to ADP and ATP). The excess AMP is converted to uric acid.

Lesch-Nyhan Syndrome

Lesch-Nyhan syndrome is an X-linked recessive condition involving:

- Near-complete deficiency of HPRT activity
- Mental retardation
- Spastic cerebral palsy with compulsive biting of hands and lips
- Hyperuricemia
- Death often in first decade

Chapter Summary

Nucleotide Synthesis

Ribose 5-P from HMP shunt

PRPP synthetase activates

Salvage pathway

Utilizes pre-formed purine or pyrimidine

De novo synthesis pathway

Includes synthesis of purine or pyrimidine

Pyrimidine De Novo Synthesis

UMP, CMP, dTMP

Important Vitamin

Folate for dTMP synthesis

Amino Acids Used

Aspartate and glutamine

Important Enzymes

Ribonucleotide reductase

- Inhibited by hydroxyurea

Thymidylate synthase

- Inhibited by 5-fluoracil

Dihydrofolate reductase

- Inhibited by methotrexate (euk), trimethoprim (prok), pyrimethamine (protozoal)

(Continued)

Clinical Correlate

Gout

Acute gouty arthritis, seen most commonly in males, results from precipitation of monosodium urate crystals in joints. The crystals, identified as negatively birefringent and needle-shaped, initiate neutrophil-mediated acute inflammation, often first affecting the big toe. Chronic gout may manifest over time as tophi (deposits of monosodium urate) develop in soft tissue around joints, leading to chronic inflammation involving granulomas.

- Acute attacks of gout are treated with colchicine or indomethacin to reduce the inflammation.
- Chronic hyperuricemia, because of under-excretion, is treated with a uricosuric drug (probenecid).
- Overproduction of uric acid and chronic gout are treated with allopurinol.

Chapter Summary (continued)

Purine De Novo Synthesis

GMP, AMP (IMP)

Important Vitamin

Folate

Amino Acids Used

Aspartate, glutamine, glycine

Rate-Limiting Enzyme

PRPP amidotransferase

- Inhibited by GMP, AMP, and IMP
- Inhibited by allopurinol (nucleotide) and 6-mercaptopurine (nucleotide)

Purine Salvage Pathway Enzyme

HGPRT

Genetic Deficiency

Lesch-Nyhan

Purine Catabolism

End Product

Uric acid

Causes of Hyperuricemia

Excessive cell death

Excessive alcohol consumption

Excessive dietary nucleic acid

Secondary to genetic disease:

- Lesch-Nyhan
- Glucose 6-phosphatase deficiency
- Galactose uridyltransferase deficiency
- Fructose 1-P aldolase deficiency

Underexcretion by kidney

Review Questions

Select the ONE best answer.

1. A 6-month-old boy becomes progressively lethargic and pale and shows delayed motor development. Laboratory evaluation reveals normal blood urea nitrogen (BUN), low serum iron, hemoglobin 4.6 g/dL, and leukopenia. His bone marrow shows marked megaloblastosis, which did not respond to treatment with iron, folic acid, vitamin B₁₂, or pyridoxine. His urine developed abundant white precipitate identified as orotic acid. The underlying defect causing the megaloblastic anemia in this child is most likely in which of the following pathways?
 - A. Homocysteine metabolism
 - B. Pyrimidine synthesis
 - C. Urea synthesis
 - D. Uric acid synthesis
 - E. Heme synthesis
2. Patients with Lesch-Nyhan syndrome have hyperuricemia, indicating an increased biosynthesis of purine nucleotides, and markedly decreased levels of hypoxanthine phosphoribosyl transferase (HPRT). The hyperuricemia can be explained on the basis of a decrease in which regulator of purine biosynthesis?
 - A. ATP
 - B. GDP
 - C. Glutamine
 - D. IMP
 - E. PRPP
3. A 12-week-old infant with a history of persistent diarrhea and candidiasis is seen for a respiratory tract infection with *Pneumocystis carinii*. A chest x-ray confirms pneumonia and reveals absence of a thymic shadow. Trace IgG is present in his serum, but IgA and IgM are absent. His red blood cells completely lack an essential enzyme in purine degradation. The product normally formed by this enzyme is
 - A. guanine monophosphate
 - B. hypoxanthine
 - C. inosine
 - D. xanthine
 - E. xanthine monophosphate

Items 4–5

The anticancer drug 6-mercaptopurine is deactivated by the enzyme xanthine oxidase. A cancer patient being treated with 6-mercaptopurine develops hyperuricemia, and the physician decides to give the patient allopurinol.

4. What effect will allopurinol have on the activity of 6-mercaptopurine?
 - A. Enhanced deactivation of 6-mercaptopurine
 - B. Enhanced elimination of 6-mercaptopurine as uric acid
 - C. Enhanced retention and potentiation of activity
 - D. Decreased inhibition of PRPP glutamylamidotransferase
5. Resistance of neoplastic cells to the chemotherapeutic effect of 6-mercaptopurine would most likely involve loss or inactivation of a gene encoding
 - A. thymidylate synthase
 - B. hypoxanthine phosphoribosyltransferase
 - C. purine nucleoside pyrophosphorylase
 - D. orotic acid phosphoribosyltransferase
 - E. adenosine deaminase

Answers

1. **Answer: B.** Accumulation of orotic acid indicates megaloblastic anemia arises since pyrimidines are required for DNA synthesis.
2. **Answer: D.** IMP is a feedback inhibitor of PRPP amidophosphoribosyl transferase, the first reaction in the biosynthesis of purines. IMP is formed by the HPRT reaction in the salvage of hypoxanthine.
3. **Answer: C.** The child most likely has severe combined immunodeficiency caused by adenosine deaminase deficiency. This enzyme deaminates adenosine (a nucleoside) to form inosine (another nucleoside). Hypoxanthine and xanthine are both purine bases, and the monophosphates are nucleotides.
4. **Answer: C.** Since allopurinol inhibits xanthine oxidase, the 6-mercaptopurine will not be deactivated as rapidly.
5. **Answer: B.** HPRT is required for activation of 6-mercaptopurine to its ribonucleotide and inhibition of purine synthesis. The other enzymes listed are not targets for this drug.

SECTION II

Medical Genetics

Single-Gene Disorders



BASIC DEFINITIONS

A **gene** is a hereditary factor, transmitted from parents to offspring, that influences traits among the offspring. Physically, a gene consists of a sequence of DNA bases that encode a specific protein. The physical location of a gene on a chromosome is termed a **locus**. Variation in the DNA sequence at a locus produces different forms of a gene, called **alleles**. Some alleles may result in a missing or abnormal protein, causing disease. When a specific site on a chromosome has multiple alleles in a population, it is termed a **polymorphism** ("multiple forms").

The body's cells can be divided into two groups: **gametes** (sperm and egg cells), which are transmitted to offspring, and **somatic cells** (cells other than gametes), which are not transmitted. Nearly all somatic cells are **diploid**, containing 23 pairs of chromosomes (one member of the pair from the father, the other member from the mother). These 23 pairs consist of 22 pairs of **autosomes** and one pair of **sex chromosomes** (the X and Y chromosomes). Gametes are **haploid**, having derived only one member of each chromosome pair during meiosis (see the Histology and Cytology section in the Anatomy notes).

The specific DNA sequence at a locus is termed a **genotype**. In diploid somatic cells, a genotype may be **homozygous (homozygote)** at a given locus, indicating that the individual inherited the same allele from both parents. If he or she inherited different alleles from each parent, the genotype is **heterozygous (heterozygote)**. The genotype is observed physically as a **phenotype**, which reflects the interaction of the genotype with the environment and with genes at other loci.

If only one copy of an allele is required for its phenotypic expression, the allele is **dominant** (i.e., it is observable in the heterozygous state). If two copies of the allele are required for its expression (i.e., the disease phenotype is observable only in the homozygous state), it is **recessive**. The expression of the recessive allele is thus "hidden" in the heterozygote. The terms dominant and recessive provide a convenient classification of genetic diseases, as seen below. If two different alleles are both phenotypically expressed in a heterozygous genotype, the alleles are said to be **codominant**.

A **mutation** is an alteration in DNA sequence (thus, mutations produce new alleles). When mutations occur in cells giving rise to gametes, they can be transmitted to future generations. **Missense** mutations result in the substitution of a single amino acid in the polypeptide chain (e.g., sickle cell disease is caused by a missense mutation that produces a substitution of valine for glutamic acid in the β -globin polypeptide). **Nonsense** mutations produce a stop codon, resulting in premature termination of translation and a truncated protein. Nucleotide bases may be inserted or deleted. When the number of inserted or deleted bases is a multiple of three, the mutation is said to be **in-frame**. If not a multiple of three, the mutation is a **frameshift**, which alters all codons downstream of the mutation, typically producing a truncated or severely altered protein product. Mutations can occur in promoter and other regulatory regions or in genes for transcription factors that bind to these regions. This can decrease or increase the amount of gene product produced in the cell. For a complete description of these and other mutations, see Section I, Chapter 4: Translation; Mutations.

Note

- **Gene**—basic unit of inheritance
- **Locus**—location of a gene on a chromosome
- **Allele**—different forms of a gene
- **Genotype**—alleles found at a locus
- **Phenotype**—physically observable features
- **Homozygote**—alleles at a locus are the same
- **Heterozygote**—alleles at a locus are different
- **Dominant**—requires only one copy of the mutation to produce disease
- **Recessive**—requires two copies of the mutation to produce disease

Note

Major types of single-gene mutations are:

- Missense
- Nonsense
- Deletion
- Insertion
- Frameshift

Mutations can also be classified according to their phenotypic effects. Mutations that cause a missing or decreased protein product are termed **loss-of-function**. Those that produce a protein product with a novel or abnormal function are termed **gain-of-function**.

The **recurrence risk** is the probability that the offspring of a couple will express a genetic disease. For example, in the mating of a normal homozygote with a heterozygote who has a dominant disease-causing allele, the recurrence risk for each offspring is 1/2, or 50%. It is important to remember that each reproductive event is statistically independent of all previous events. Therefore, the recurrence risk remains the same regardless of the number of previously affected or unaffected offspring. Determining the mode of inheritance of a disease (e.g., autosomal dominant versus autosomal recessive) enables one to assign an appropriate recurrence risk for a family.

A patient's family history is diagrammed in a **pedigree** (see symbols in Fig II-1-1). The first affected individual to be identified in the family is termed the **proband**.

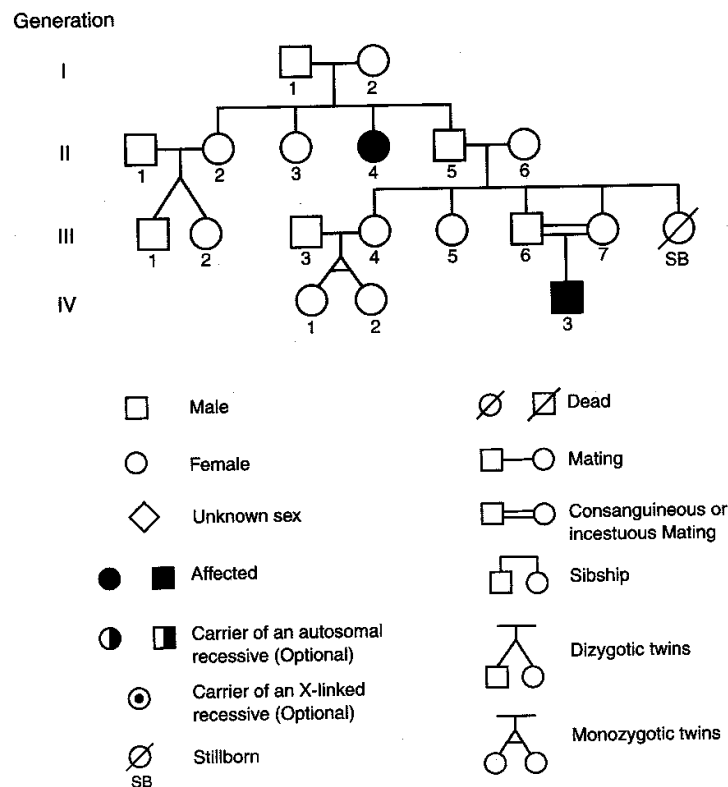


Figure II-1-1. Pedigree Nomenclature

MAJOR MODES OF INHERITANCE

Autosomal Dominant Inheritance

A number of features in a pedigree help to identify autosomal dominant inheritance:

- Because affected individuals must receive a disease-causing gene from an affected parent, the disease is typically observed in multiple generations of a pedigree (Fig II-1-2).
- Skipped generations are not typically seen because two unaffected parents cannot transmit a disease-causing allele to their offspring (an exception occurs when there is reduced penetrance, discussed below).
- Because these genes are located on autosomes, males and females are affected in roughly equal frequencies.

Autosomal dominant alleles are relatively rare in populations, so the typical mating pattern is a heterozygous affected individual (Aa genotype) mating with a homozygous normal individual (aa genotype), as shown in Figure II-1-3. Note that, by convention, the dominant allele is shown in upper case (A) and the recessive allele is shown in lower case (a). The recurrence risk is thus 50%, and half the children, on average, will be affected with the disease. If both parents are heterozygous, the recurrence risk is 75%.

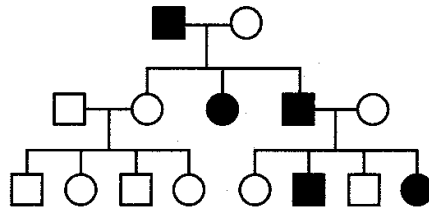


Figure II-1-2. Autosomal Dominant Inheritance

	A	a
a	Aa	aa
a	Aa	aa

Affected offspring (Aa) are shaded.

Figure II-1-3. Recurrence Risk for the Mating of Affected Individual (Aa) with a Homozygous Unaffected Individual (aa)

Note

Major modes of inheritance of single-gene mutations are:

- Autosomal dominant
- Autosomal recessive
- X-linked dominant
- X-linked recessive
- Mitochondrial

Disease Example: Achondroplasia

Achondroplasia is the most common of the reduced-stature conditions, occurring in approximately 1 in 10,000 live births. It is an autosomal dominant condition characterized by a mature height usually less than 4 feet, prominent forehead, low nasal root, and lumbar lordosis. Achondroplasia is the result of a gain-of-function mutation in a fibroblast growth factor receptor gene (*FGFR3*) on chromosome 4. The *FGFR3* receptor is expressed in resting chondrocytes, where it restricts chondrocyte proliferation and thus bone growth. The mutation results in overexpression of *FGFR3* and excess inhibition of bone growth, particularly in the long bones.

Autosomal Recessive Inheritance

Because autosomal recessive alleles are clinically expressed only in the homozygous state, the offspring must inherit one copy of the disease-causing allele from each parent.

Most commonly, a homozygote is produced by the union of two heterozygous (**carrier**) parents. The recurrence risk for offspring of such matings is 25% (Fig II-1-4). Occasionally, an affected homozygote mates with a heterozygote; in this case the recurrence risk for the offspring is 50%.

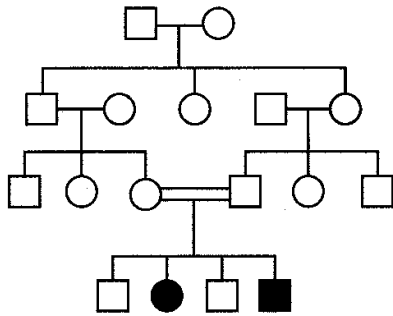
In contrast to autosomal dominant diseases, autosomal recessive diseases are typically seen in only one generation of a pedigree (Fig II-1-5).

Consanguinity (the mating of related individuals) is sometimes seen in recessive pedigrees because individuals who share common ancestors are more likely to carry the same recessive disease-causing alleles.

	A	a
A	AA	Aa
a	Aa	aa

The affected genotype (*aa*) is shaded.

Figure II-1-4. Recurrence Risk for the Mating of Two Heterozygous Carriers (*Aa*) of a Recessive Mutation



A consanguineous mating has produced two affected offspring.

Figure II-1-5. Pedigree for an Autosomal Recessive Disease

Disease Example: Cystic Fibrosis

Cystic fibrosis is one of the most common autosomal recessive diseases among whites, affecting approximately 1 in 2,500. It is considerably rarer in other ethnic groups. Cystic fibrosis is characterized by abnormally elevated sweat chloride levels (providing the basis for a common diagnostic test), pancreatic insufficiency in 85% of patients (requiring pancreatic enzyme replacement to avoid malnutrition), and blockage of the airway by heavy, thick mucus. The latter contributes to chronic airway infections (typically *Staphylococcus* or *Pseudomonas aeruginosa*). More than 95% of males are sterile as a result of absence of the vas deferens. Death caused by pulmonary disease occurs in more than 90% of cystic fibrosis patients, and roughly half die before the age of 30. Identification of the gene responsible for this disease demonstrated that it encodes a chloride ion channel present in some specialized epithelial cells, such as those that line the airway. Mutations in the gene result in absent or abnormal expression of the chloride channel as well as misregulation of a sodium channel. The resulting salt and fluid imbalance contributes to the accumulation of heavy secretions in the airway and pancreas. Treatment includes pancreatic enzyme replacement therapy, chest physical therapy, and antibiotics to combat airway infections. Gene therapy (see Chapter 6) is being attempted in a small number of patients.

X-Linked Recessive Inheritance

X Inactivation

Normal males inherit an X chromosome from their mother and a Y chromosome from their father, whereas normal females inherit an X chromosome from each parent. Because the Y chromosome carries only about 30 protein-coding genes and the X chromosome carries hundreds of protein-coding genes, a mechanism must exist to equalize the amount of protein encoded by X chromosomes in males and females. This mechanism, termed X inactivation, occurs very early in the development of female embryos. When an X chromosome is inactivated, its DNA is not transcribed into mRNA, and it is visualized under the microscope as a highly condensed **Barr body** in the nuclei of interphase cells. X inactivation has several important characteristics:

- It is *random*—in some cells of the female embryo, the X chromosome inherited from the father is inactivated, and in others the X chromosome inherited from the mother is inactivated. Like coin tossing, this is a random process.

Note

X inactivation occurs early in the female embryo and is random, fixed, and incomplete. In a cell, all X chromosomes but one are inactivated.

- It is *fixed*—once inactivation of an X chromosome occurs in a cell, the same X chromosome is inactivated in all descendants of the cell.
- X-inactivation is *incomplete*—there are regions throughout the X chromosome, including the tips of both the long and short arms, that are not inactivated.
- All X chromosomes in a cell are inactivated except one. For example, females with three X chromosomes in each cell (see Chapter 3) have two X chromosomes inactivated in each cell (thus two Barr bodies can be visualized in an interphase cell).
- X inactivation is associated with DNA methylation (the attachment of methyl groups to cytosine bases).

A gene called *XIST* has been identified as the primary gene that causes X inactivation. *XIST* produces an RNA product that coats the chromosome, helping to produce its inactivation.

Properties of X-Linked Recessive Inheritance

Because males have only one copy of the X chromosome, they are said to be **hemizygous** (hemi = “half”) for the X chromosome. If a recessive disease-causing mutation occurs on the X chromosome, a male will be affected with the disease.

Normal females have two copies of the X chromosome, so they usually require two copies of the mutation to express the disease. However, because X inactivation is a random process, a heterozygous female will occasionally express an X-linked recessive mutation because, by random chance, most of the X chromosomes carrying the normal allele have been inactivated. Such females are termed **manifesting heterozygotes**. Because they usually have at least a small population of active X chromosomes carrying the normal allele, their disease expression is typically milder than that of a hemizygous male.

Because males require only one copy of the mutation to express the disease, whereas females require two copies, X-linked recessive diseases are seen much more commonly in males than in females (Fig II-1-6).

Skipped generations are commonly seen because an affected male can transmit the disease-causing mutation to a heterozygous daughter, who is unaffected but who can transmit the disease-causing allele to her sons.

Male-to-male transmission is not seen in X-linked inheritance; this helps to distinguish it from autosomal inheritance.

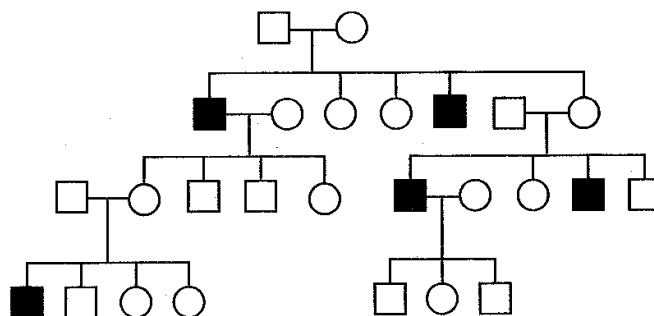


Figure II-1-6. X-Linked Recessive Inheritance

Recurrence Risks

Figure II-1-7 shows the recurrence risks for X-linked recessive disease.

- Affected male—homozygous normal female: all of the daughters will be heterozygous carriers; all of the sons will be homozygous normal
- Carrier female—normal male: on average, half of the sons will be affected, and half of the daughters will be carriers
- Affected male—heterozygous carrier female: on average, half of the sons will be affected and half will be normal; on average, half of the daughters will be affected and half will be heterozygous carriers

	x	Y
X	Xx	XY
X	Xx	XY
A		

	X	Y
x	XX	XY
x	Xx	XY
B		

	x	Y
X	Xx	XY
x	xx	XY
C		

- A. Affected male—normal female (X chromosome with mutation is in lower case)
 B. Normal male—carrier female
 C. Affected male—carrier female

Figure II-1-7. Recurrence Risks for X-Linked Recessive Diseases

Disease Example: Hemophilia A

Hemophilia A is caused by a deficiency of clotting factor VIII, a protein that is encoded by a gene on the long arm of the X chromosome. It affects approximately 1 in 10,000 males worldwide. As a result of impaired coagulation, hemophiliacs can suffer prolonged bleeding episodes, including intracranial hemorrhage (a common cause of death) and hemarthrosis (bleeding into the joints, a painful condition that produces swelling and a loss of joint mobility). This disease can be treated by infusions of purified factor VIII. Formerly, donor-derived factor VIII was often contaminated with HIV, leading to a high incidence of AIDS among hemophiliacs. AIDS continues to be the leading cause of death in this population. Factor VIII can also be produced through recombinant DNA methods; this form is free of contaminants.

Hemophilia B, another X-linked recessive bleeding disorder, is caused by a deficiency of clotting factor IX.

Disease Example: Duchenne Muscular Dystrophy

Of the more than one dozen forms of muscular dystrophy, Duchenne muscular dystrophy is the most severe and the most common (affecting approximately 1 in 3,500 males worldwide). Duchenne muscular dystrophy is caused by mutations in a gene that encodes dystrophin, a rare but vital muscle protein that connects actin with the membrane-spanning sarcoglycan–dystroglycan complex. Muscle cells lacking dystrophin eventually disintegrate and die, leading to the progressive muscle wastage observed in this disease. Patients are typically wheelchair-bound by age 10–12, and survival beyond the age of 20 or so is rare. The heart and respiratory musculature are increasingly impaired, leading to death from cardiorespiratory failure. Approximately two thirds of the mutations causing Duchenne muscular dystrophy are insertions or deletions. They are usually frameshift mutations, with the frameshift producing a truncated and nonfunctional protein. In-frame deletions or insertions in the same gene produce an aberrant but partially functional dystrophin, resulting in the milder Becker form of muscular dystrophy.

X-Linked Dominant Inheritance

As in X-linked recessive inheritance, male–male transmission of the disease-causing mutation is not seen (Fig II-1-8).

Heterozygous females are affected. Because females have two X chromosomes (and thus two chances to inherit an X-linked disease-causing mutation), whereas males have only one, X-linked dominant diseases are seen about twice as often in females as in males.

As in autosomal dominant inheritance, the disease phenotype is seen in multiple generations of a pedigree; skipped generations are relatively unusual.

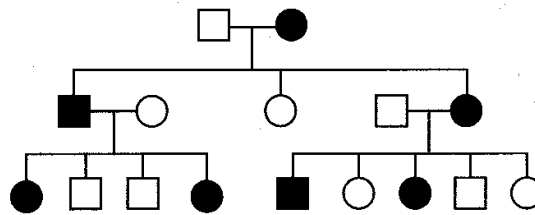
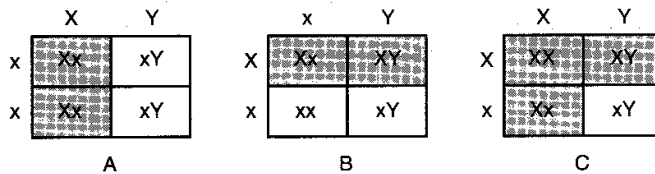


Figure II-1-8. X-Linked Dominant Inheritance

Recurrence Risks

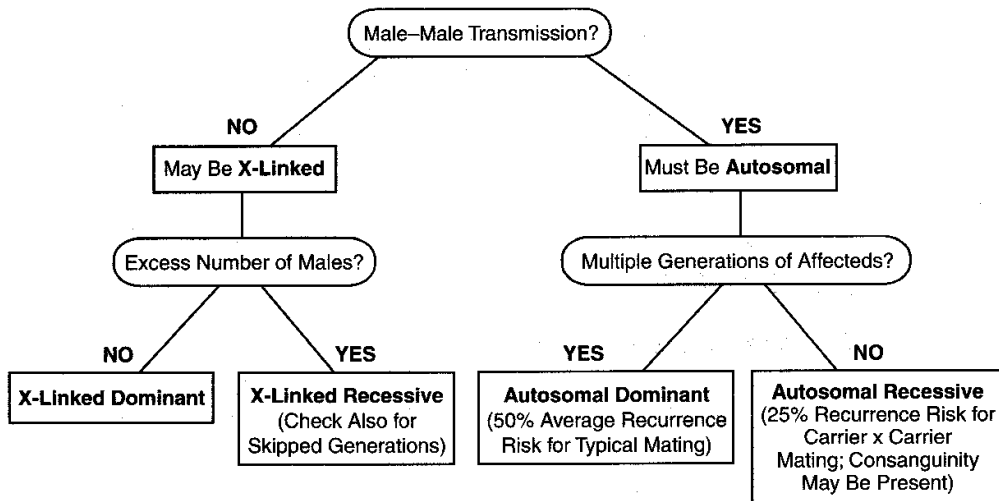
Figure II-1-9 shows the recurrence risks for X-linked dominant inheritance.

- Affected male—homozygous normal female: none of the sons are affected; all of the daughters are affected
- Heterozygous female—normal male: on average; 50% of sons are affected and 50% of daughters are affected
- Affected male—heterozygous carrier female: on average, half of the sons are affected and half are normal; on average, half of the daughters are affected heterozygotes and half are affected homozygotes



- A. Affected male—homozygous normal female
 (the mutation-carrying chromosome is upper case)
 B. Normal male—heterozygous affected female
 C. Affected male—heterozygous affected female

Figure II-1-9. Recurrence Risks for X-Linked Dominant Inheritance



Note: If transmission occurs only through affected mothers and never through affected sons, the pedigree is likely to reflect mitochondrial inheritance.

Figure II-1-10. A Basic Decision Tree for Determining the Mode of Inheritance in a Pedigree

Mitochondrial Inheritance

Mitochondria, which are cytoplasmic organelles involved in cellular respiration, have their own chromosome, which contains 16,569 DNA base pairs (bp) arranged in a circular molecule. This DNA encodes 13 proteins that are subunits of complexes in the electron transport and oxidative phosphorylation processes (see Section I, Chapter 13). In addition, mitochondrial DNA encodes 22 transfer RNAs and two ribosomal RNAs.

Because a sperm cell contributes no mitochondria to the egg cell during fertilization, mitochondrial DNA is inherited exclusively through females. Pedigrees for mitochondrial diseases thus display a distinct mode of inheritance: diseases are transmitted only from affected females to their offspring (Fig II-1-11).

A typical cell contains hundreds of mitochondria in its cytoplasm. Sometimes a specific mutation is seen in only some of the mitochondria, a condition known as **heteroplasmy**. Variations in heteroplasmy can result in substantial variation in the severity of expression of mitochondrial diseases.

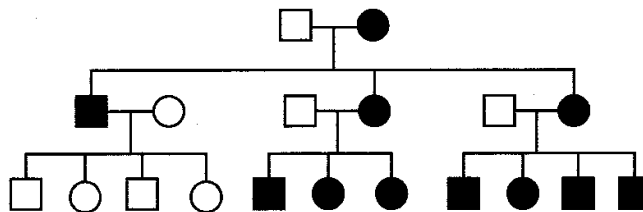


Figure II-1-11. Pedigree for a Mitochondrial Disease

Disease Example: Leber Hereditary Optic Neuropathy

This form of hereditary blindness is caused by mutations in protein-coding mitochondrial DNA. Typically, a rapid, irreversible loss of vision in the central visual field begins in the third decade of life. The cause of vision loss is optic nerve death. Heteroplasmy is relatively uncommon for this mitochondrial disease, so affected individuals tend to have similar levels of expression.

IMPORTANT PRINCIPLES THAT CAN CHARACTERIZE SINGLE-GENE DISEASES

Variable Expression

Most genetic diseases vary in the degree of phenotypic expression: some individuals may be severely affected, whereas others are more mildly affected. This can be the result of several factors:

- Environmental influences (e.g., the autosomal recessive disease xeroderma pigmentosum will be expressed more severely in individuals who are exposed more frequently to ultraviolet radiation)
- Different mutations in the disease-causing locus may cause more or less severe expression. For example, mis-sense mutations in the factor VIII gene tend to produce less severe hemophilia than do nonsense mutations, which result in a truncated protein product and little, if any, expression of factor VIII. The presence of different mutations, or alleles, in the same locus is termed **allelic heterogeneity**.
- Disease expression may be affected by the action of other loci, termed **modifier loci**.

Disease Example: Neurofibromatosis Type 1

This disease is seen in approximately 1 in 3,000 individuals, making it one of the more common autosomal dominant disorders. Patients typically have multiple neurofibromas (benign peripheral nerve tumors), café-au-lait spots (describing the color of hyperpigmented regions of the skin), axillary freckling, Lisch nodules (benign hamartomas of the iris), and various other features. Malignancies resulting from the disease condition are seen in a small percentage of patients. A hallmark of neurofibromatosis type 1 is its highly variable expression: Even within the same family, different individuals may be severely affected, with dozens or hundreds of neurofibromas, or so mildly affected that they are unaware they have the disease-causing mutation. This disease is caused by a mutation in a gene on chromosome 17 that in its normal state down-regulates the ras protein, helping to regulate the cell cycle. It is an example of a tumor suppressor gene (see Chapter 5).

Neurofibromatosis type 2 is characterized by acoustic neuromas and café-au-lait spots. It is caused by mutations in a tumor suppressor gene on chromosome 22.

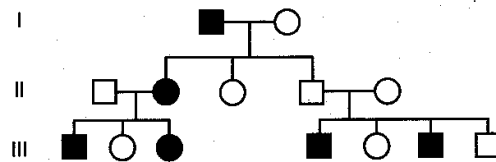
Note

Important principles related to single-gene disorders:

- Variable expression
- Incomplete penetrance
- Delayed age of onset
- Locus heterogeneity
- Pleiotropy
- Anticipation
- Imprinting

Incomplete Penetrance

A disease-causing mutation is said to have incomplete penetrance when some individuals who have the disease genotype (e.g., one copy of the mutation for an autosomal dominant disease or two copies for an autosomal recessive disease) do not display the disease phenotype (Fig II-1-12). Incomplete penetrance is distinguished from variable expression in that the nonpenetrant gene has *no* phenotypic expression at all. The penetrance of a disease-causing mutation is quantified by estimating the proportion of **obligate carriers** (individuals who are known to have the disease-causing genotype) who display the disease phenotype.



The unaffected male in generation II has an affected father, sister, and two sons. He is therefore an obligate carrier in whom the mutation has incomplete penetrance.

Figure II-1-12. Incomplete Penetrance for an Autosomal Dominant Disease

Disease Example: Hereditary Hemochromatosis

Hereditary hemochromatosis, an autosomal recessive disease, is one of the most common genetic diseases: approximately 1 in 300 whites is a homozygote. Patients with this disease absorb excess iron in the gut, leading to a gradual increase of iron stores in the body. This iron is deposited in the liver, kidney, heart, pancreas, and joints and eventually damages these structures. Hepatic cirrhosis, cardiomyopathy, joint pain, and, in some cases, hepatocellular carcinoma are the results. Although 1 in 300 whites inherits the homozygous genotype, a much smaller percentage of individuals develop the disease (approximately 1 in 1,000–2,000). This incomplete penetrance is caused in part by periodic iron loss, particularly in women (menstruation, pregnancy, and lactation all leading to depletion of iron stores). This disease is easily diagnosed by identification of the mutation, and periodic phlebotomy provides a completely effective treatment.

Other examples of incomplete penetrance include familial breast cancer and retinoblastoma (see Chapter 5).

Delayed Age of Onset

Many individuals who carry a disease-causing mutation do not manifest the phenotype until later in life. This can complicate the interpretation of a pedigree because it may be difficult to distinguish genetically normal individuals from those who have inherited the mutation but have not yet displayed the phenotype.

Disease Example: Huntington Disease

This autosomal dominant condition affects approximately 1 in 20,000 individuals. Features of the disease include progressive dementia, loss of motor control, and affective disorder. This is a slowly progressing disease, with an average duration of approximately 15 years. Common causes of death include aspiration pneumonia, head trauma (resulting from loss of motor control), and suicide. Most patients first develop symptoms in their 30s or 40s, so this is a good example of a disease with delayed age of onset. The condition results from a gain-of-function mutation on chromosome 4 and is an example of a trinucleotide repeat expansion disorder (see Anticipation, below). The mutation produces a buildup of toxic protein aggregates in neurons, eventually resulting in neuronal death.

Other examples of single-gene diseases with delayed age of onset include familial breast cancer, familial colon cancer, adult polycystic kidney disease, and hemochromatosis.

Pleiotropy

Pleiotropy exists when a single disease-causing mutation affects multiple organ systems. Pleiotropy is a common feature of genetic diseases.

Disease Example: Marfan Syndrome

Marfan syndrome is an autosomal dominant disease that affects approximately 1 in 10,000 individuals. It is characterized by skeletal abnormalities (thin, elongated limbs, pectus excavatum, pectus carinatum), hypermobile joints, ocular abnormalities (frequent myopia and detached lens), and, most importantly, cardiovascular disease (mitral valve prolapse and aortic aneurysm). Dilatation of the ascending aorta is seen in 90% of patients and frequently leads to aortic rupture or congestive heart failure. Although the features of this disease seem rather disparate, they are all caused by a mutation in the gene that encodes fibrillin, a key component of connective tissue. Fibrillin is expressed in the periosteum and perichondrium, the suspensory ligament of the eye, and the aorta. Defective fibrillin causes the connective tissue to be “stretchy” and leads to all of the observed disease features. Marfan syndrome thus provides a good example of the principle of pleiotropy.

Locus Heterogeneity

Locus heterogeneity exists when the same disease phenotype can be caused by mutations in different loci. Locus heterogeneity becomes especially important when genetic testing is performed by testing for mutations at specific loci.

Disease Example: Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) is a disease of bone development that affects approximately 1 in 10,000 individuals. It results from a defect in the collagen protein, a major component of the bone matrix. Four major forms of OI have been identified. The severe perinatal form (type II) is the result of a defect in type 1 collagen, a trimeric molecule that has a triple helix structure. Two members of the trimer are encoded by a gene on chromosome 17, and the third is encoded by a gene on chromosome 7. Mutations in either of these genes give rise to a faulty collagen molecule, causing type II OI. Often, patients with chromosome 17 mutations are clinically indistinguishable from those with chromosome 7 mutations. This exemplifies the principle of locus heterogeneity.

New Mutation

In many genetic diseases, particularly those in which the mortality rate is high or the fertility rate is low, a large proportion of cases are caused by a new mutation transmitted from an unaffected parent to an affected offspring. There is thus no family history of the disease (for example, 80% of individuals with achondroplasia, discussed above, are the result of a new mutation in the family, as are 50% of cases of neurofibromatosis type 1). Because the mutation occurred in only one parental gamete, the recurrence risk for other offspring of the parents remains very low. However, the recurrence risk for future offspring of the affected individual would be the same as that of any individual who has inherited the disease-causing mutation.

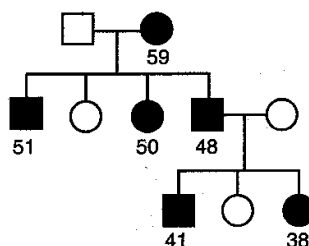
Anticipation

Anticipation refers to a pattern of inheritance in which individuals in the most recent generations of a pedigree develop a disease at an earlier age or with greater severity than do those in earlier generations (Fig II-1-13). For a number of genetic diseases, this phenomenon can be attributed to the gradual expansion of **trinucleotide repeat polymorphisms** within or near a coding gene. For example, the 3' untranslated region (UTR) of a gene on chromosome 19 contains a trinucleotide repeat in which the sequence CTG is repeated 5 to 37 times in unaffected individuals (e.g., a chromosome with five repeats would contain the sequence CTGCTGCTGCTGCTG). An individual with 50 to 100 repeats may be mildly affected with the disease myotonic dystrophy (see disease example) at a relatively late age. This individual may then transmit an expanded number of repeats to his or her offspring (e.g., between 100 and several thousand repeats) who may be severely affected and may present at an early age. Thus, the number of repeats, which can increase through the generations, accounts for the pattern of anticipation in some genetic diseases.

Note

Diseases caused by trinucleotide repeat expansion that may show anticipation in a pedigree:

- Myotonic dystrophy
- Fragile X syndrome
- Huntington disease



The age of onset (shown below each affected individual) is earlier in more recent generations.

Figure II-1-13. Anticipation for an Autosomal Dominant Disease

Disease Example: Myotonic Dystrophy

This autosomal dominant disorder, which affects approximately 1 in 8,000 individuals, is characterized by progressive muscle deterioration, cardiac arrhythmia, testicular atrophy, frontal baldness, and cataracts. As noted above, most cases are caused by a trinucleotide repeat expansion in the 3' UTR of a gene that encodes a protein kinase. Larger repeat numbers lead to earlier and more severe expression of the disease (anticipation). Especially large expansions sometimes occur in maternal transmission of the trinucleotide repeat, resulting in a severe neonatal form of the disorder.

Also see Section I, Chapter 7: Genetic Testing; RFLP Analysis of a Family With Myotonic Dystrophy.

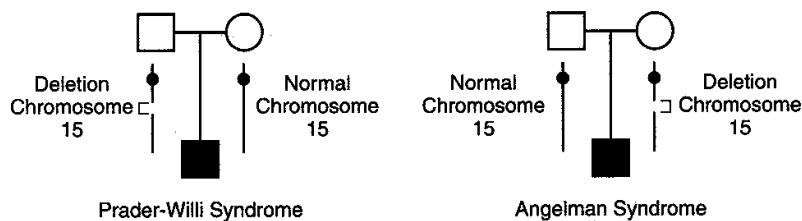
Disease Example: Fragile X Syndrome

Fragile X syndrome receives its name because the long arms of X chromosomes in individuals with this disorder often display a characteristic elongation when the cells are cultured in a folate-deficient medium. A major feature of fragile X syndrome is mild to moderate mental retardation. Affecting approximately 1 in 4,000 males and 1 in 8,000 females, this X-linked dominant disease is the leading known cause of inherited mental retardation. Other features of fragile X syndrome include large ears, elongated face, hypermobile joints, and macro-orchidism (increased testicular volume) in postpubertal males. The lower prevalence (and penetrance) in females is thought to be related to X inactivation. The major mutation responsible for this disease is an expanded CGG repeat in the 5' untranslated region of the *FMRI* gene. As with the trinucleotide repeat involved in myotonic dystrophy, this trinucleotide repeat tends to expand from one generation to the next, leading to more affected cases in more recent generations of fragile X syndrome families. Expansions to the disease-causing range (i.e., more than 200 CGG repeats) occur almost exclusively when the X chromosome carrying the "pre-mutation" (50–200 repeats) is transmitted by a female carrier (fewer than 50 repeats is considered the normal range).

Imprinting

The phenomenon of imprinting refers to the fact that a small number of human genes are transcriptionally active only when transmitted by one of the two sexes. A good example is provided by Prader-Willi syndrome, a disorder characterized by moderate mental retardation, hypogonadism, small hands and feet, and obesity. Most cases of Prader-Willi syndrome are caused by a deletion of 4 million bases (4 megabases or Mb) on the long arm of chromosome 15. However, the deletion produces this syndrome *only* if it is transmitted by the father. If the same deletion is

instead transmitted by the mother, an entirely different disease called Angelman syndrome results (severe mental retardation, seizures, and ataxic gait). The reason for this difference is that specific genes (including the one responsible for Prader-Willi syndrome) in this region are transcriptionally active only when they are inherited from the father, whereas other genes (including the one responsible for Angelman syndrome) are active only when inherited from the mother. It is thus normal to have only one active copy of some of the genes in this region. When a deletion occurs in a chromosome containing the single active copy, the individual is left with no active copies of the gene, and disease results. Because each parent transmits different active genes, a deletion of this region produces different diseases depending on which parent transmitted the deletion (Fig II-1-14). The biochemical nature of the imprint is not yet completely understood, but transcriptional inactivation (i.e., the "imprint") is associated with **methylation** (i.e., the attachment of methyl groups to DNA in this region). Methylation is thought to render the DNA less receptive to transcription factors.



The inheritance of a deletion on chromosome 15 from a male produces Prader-Willi syndrome, whereas inheritance of the same deletion from a female produces Angelman syndrome.

Figure II-1-14. Genomic Imprinting

Chapter Summary

Single-gene diseases have clear inheritance patterns.

Modes of inheritance:

- Autosomal dominant
- Autosomal recessive
- X-linked dominant
- X-linked recessive
- Mitochondrial (maternal)

Recurrence risks can be predicted by drawing Punnett squares.

Principles that can characterize single gene diseases:

- Variable expression in severity of symptoms
- Incomplete penetrance (individuals with the disease genotype don't have the disease phenotype)
- Delayed age of onset for diseases that don't appear until later in life
- Locus heterogeneity for diseases that can be caused by mutations in two or more different genes
- New mutations (not inherited from a parent)
- Anticipation caused by trinucleotide repeat expansion
- Imprinting (symptoms depend on whether the mutant gene was inherited from the father or mother)

Review Questions

1. A 25-year-old woman has mild expression of hemophilia A. A genetic diagnosis reveals that she is a heterozygous carrier of a mutation in the X-linked factor VIII gene. What is the most likely explanation for mild expression of the disease in this individual?
 - A. A high proportion of the X chromosomes carrying the mutation are active in this woman
 - B. Her father is affected, and her mother is a heterozygous carrier
 - C. Nonsense mutation causing truncated protein
 - D. One of her X chromosomes carries the SRY gene
 - E. X inactivation does not affect the entire chromosome

2. A 20-year-old man has had no retinoblastomas but has produced two offspring with multiple retinoblastomas. In addition, his father had two retinoblastomas as a young child, and one of his siblings has had three retinoblastomas. What is the most likely explanation for the absence of retinoblastomas in this individual?
 - A. A new mutation in the unaffected individual, which has corrected the disease-causing mutation
 - B. Highly variable expression of the disorder
 - C. Incomplete penetrance
 - D. Multiple new mutations in other family members
 - E. Pleiotropy
3. A 30-year-old man is phenotypically normal, but two of his siblings died from infantile Tay-Sachs disease, an autosomal recessive condition that is lethal by the age of five. What is the risk that this man is a heterozygous carrier of the disease-causing mutation?
 - A. $1/4$
 - B. $1/2$
 - C. $2/3$
 - D. $3/4$
 - E. Not elevated above that of the general population
4. A large, three-generation family in whom multiple members are affected with a rare, undiagnosed disease is being studied. Affected males never produce affected children, but affected females do produce affected children of both sexes when they mate with unaffected males. What is the most likely mode of inheritance?
 - A. Autosomal dominant, with expression limited to females
 - B. Y-linked
 - C. Mitochondrial
 - D. X-linked dominant
 - E. X-linked recessive
5. A man who is affected with hemophilia A (X-linked recessive) mates with a woman who is a heterozygous carrier of this disorder. What proportion of this couple's daughters will be affected, and what proportion of the daughters will be heterozygous carriers?
 - A. 0%; 50%
 - B. 100%; 0%
 - C. 0%; 100%
 - D. 50%; 50%
 - E. $2/3$; $1/3$

6. The clinical progression of Becker muscular dystrophy is typically much slower than that of Duchenne muscular dystrophy. This is usually the result of
 - A. gain-of-function mutations in the Duchenne form; loss-of-function mutations in the Becker form
 - B. in-frame deletions or insertions in the Becker form; frameshift deletions or insertions in the Duchenne form
 - C. mis-sense mutations in the Becker form; nonsense mutations in the Duchenne form
 - D. mutations at two distinct loci for these two forms of muscular dystrophy
 - E. nonsense mutations in the Becker form; mis-sense mutations in the Duchenne form
7. A 10-year-old girl is diagnosed with Marfan syndrome, an autosomal dominant condition. An extensive review of her pedigree indicates no previous family history of this disorder. The most likely explanation for this pattern is
 - A. highly variable expression of the disease phenotype
 - B. incomplete penetrance
 - C. mitochondrial compensation in the mother
 - D. new mutation transmitted by one of the parents to the affected girl
 - E. pleiotropy
8. In assessing a patient with osteogenesis imperfecta, a history of bone fractures, as well as blue sclerae, are noted. These findings are an example of
 - A. allelic heterogeneity
 - B. gain-of-function mutation
 - C. locus heterogeneity
 - D. multiple mutations
 - E. pleiotropy
9. In studying a large number of families with a small deletion in a specific chromosome region, it is noted that the disease phenotype is distinctly different when the deletion is inherited from the mother as opposed to the father. What is the most likely explanation?
 - A. Imprinting
 - B. Mitochondrial inheritance
 - C. Sex-dependent penetrance
 - D. X-linked dominant inheritance
 - E. X-linked recessive inheritance

10. A man and woman are both affected by an autosomal dominant disorder that has 80% penetrance in all affected individuals. They are both heterozygotes for the disease-causing mutation. What is the probability that they will produce phenotypically normal offspring?
- A. 20%
 - B. 25%
 - C. 40%
 - D. 60%
 - E. 80%
11. The severe form of alpha-1 antitrypsin deficiency is the result of a single nucleotide substitution that produces a single amino acid substitution. This is best described as a
- A. frameshift mutation
 - B. in-frame mutation
 - C. mis-sense mutation
 - D. nonsense mutation
 - E. splice-site mutation

Answers

1. **Answer: A.** The most likely explanation for mild expression in a heterozygous carrier is that when X inactivation occurred in the affected individual, the random process happened to inactivate most of the X chromosomes that carried the normal version of the factor VIII gene. Thus, most of the active X chromosomes in this individual would carry the mutation and would not produce factor VIII, leading to a clinically expressed deficiency.
- If the woman's father is affected and her mother is a carrier (**choice B**), she has a 50% chance of being an affected homozygote, but her expression is more likely to be severe.
- A nonsense mutation (**choice C**) is likely to produce severe expression if it is inherited from both the mother and the father.
- The SRY gene (**choice D**) is involved in sex determination and would not affect factor VIII expression.
- Although it is true that X inactivation does not affect the entire X chromosome (**choice E**), it consistently affects the factor VIII gene and thus could not explain the status of this woman.
2. **Answer: C.** Because multiple family members are affected and because mutations at the retinoblastoma gene are known to be sometimes nonpenetrant, the man in question is most likely an obligate carrier of the mutation who did not experience a second mutation in this gene during his fetal development.
- A new mutation correcting the defect could occur (**choice A**), but then the man's two sons would both have experienced new mutations. The combination of three mutations affecting three different individuals in the family is highly unlikely.
- Variable expression (**choice B**) refers to differences in the severity of a disorder but does not refer to the complete absence of the disorder, which is incomplete penetrance.

The number of affected individuals in this family (four) makes multiple new mutations in so many individuals extremely unlikely (**choice D**). Remember that inherited mutations are rare events.

Pleiotropy is observed in retinoblastoma (**choice E**), in that mutation carriers can develop other cancers, such as osteosarcoma. This, however, does not explain the lack of a tumor in the 20-year-old man.

3. **Answer: C.** Because two of the man's siblings had Tay-Sachs disease, his parents must both be carriers. This clearly elevates his risk above the general population and excludes **choice E**. He is not affected, so this excludes **choice A**, which is the probability of inheriting two copies of the disease allele. His risk of inheriting one copy of the disease gene at conception is 1/2 (**choice B**). However, the fact that he is phenotypically normal at age 30 means that he cannot have inherited copies of the disease gene from both parents. Only three possibilities remain: Either he inherited no copies of the mutation, he inherited a copy from his father, or he inherited a copy from his mother. Each of these three possibilities is equally likely, and two of them lead to heterozygosity. Thus, the risk that he is a carrier is 2/3.

4. **Answer: C.** This is a pattern expected of mitochondrial inheritance because only females transmit mitochondrial DNA to their offspring. Thus, an affected female can transmit the mutation to her offspring of both sexes, but an affected male cannot transmit it.

Answer A is excluded because, although the disease is not transmitted by males, it is seen in them.

Under Y-linked inheritance (**choice B**), affected males would transmit the mutation and would transmit it only to their sons.

X-linked dominant inheritance (**choice D**) is excluded because affected males can transmit X-linked dominant mutations to their daughters.

X-linked recessive inheritance (**choice E**) could explain this pattern because affected males typically produce only heterozygous carrier daughters and unaffected sons (unless they mate with a carrier female). However, affected homozygous females, who will produce affected sons, would produce an affected daughter only if they mated with an affected male.

5. **Answer: D.** Because the man transmits his X chromosome to all of his daughters, all of the daughters must carry at least one copy of the mutation. The mother will transmit a mutation-carrying X chromosome half the time and a normal X chromosome half the time. Thus, half of the daughters will be heterozygous carriers, and half will be affected homozygotes, having received a mutation from both parents.

6. **Answer: B.** In-frame deletions or insertions typically produce an altered protein product (dystrophin), but the alteration is mild enough so that Becker muscular dystrophy results. Frame-shifts usually produce a truncated protein because a stop codon is eventually encountered. The truncated protein is degraded, resulting in an absence of dystrophin and a more severe disease phenotype.

Both types of muscular dystrophy are X-linked recessive mutations, making a gain-of-function highly unlikely for either type (**choice A**).

Because approximately 2/3 of all mutations leading to these diseases are insertions or deletions, differences in single-base mutations (i.e., mis-sense or nonsense mutations) would not be the most likely explanation, excluding **choice C** and **choice E**.

These two forms of muscular dystrophy are known to be encoded by the same locus, so locus heterogeneity (**choice D**) is excluded.

7. **Answer: D.** For an autosomal dominant condition, the first occurrence in a family is usually the result of a new mutation that occurred in one of the gametes transmitted by a parent of the affected individual.

Although variable expression (**choice A**) is a characteristic of this disease, other family members (including a parent) would be likely to manifest at least mild expression of the disorder.

The penetrance of Marfan mutations is high, so it is highly unlikely that all other gene carriers in the family would be nonpenetrant carriers (**choice B**).

Mitochondrial genes are not known to affect the expression of Marfan syndrome (**choice C**).

Marfan syndrome is an excellent example of pleiotropy (**choice E**), but this principle refers to the fact that a single mutation can affect multiple aspects of the phenotype, so it would not explain the pattern observed in this pedigree.

8. **Answer: E.** Pleiotropy refers to the multiple effects exerted by a single mutation and thus describes the two features observed in this patient.

Allelic heterogeneity is observed in osteogenesis imperfecta (**choice A**), but allelic heterogeneity causes variable expression in patients and is not the principle described here.

Osteogenesis imperfecta is a good example of a disease in which locus heterogeneity (**choice C**) is observed, but this principle refers to the fact that a mutation in either the type 1 procollagen gene on chromosome 7 or the type 1 procollagen gene on chromosome 17 can result in imperfect formation of the trimeric protein. This principle does not explain the co-occurrence of fractures and blue sclerae.

A single mutation at either the chromosome 7 or chromosome 17 locus is sufficient to cause the disease, so multiple mutations (**choice D**) do not explain the pattern.

9. **Answer: A.** Imprinting refers to the differential transcriptional activity of genes inherited from the father versus the mother.

Under mitochondrial inheritance (**choice B**), only an affected mother can transmit the disease phenotype; the offspring of affected males are always unaffected.

The other modes of inheritance can influence the relative proportions of affected individuals who belong to one gender or the other (e.g., more affected males under X-linked recessive inheritance, more affected females under X-linked dominant inheritance), but they do not involve any differences in expression depending on the transmitting parent.

10. **Answer: C.** If both parents are heterozygotes, there is a 75% chance that their offspring will receive one or two copies of the disease-causing gene (i.e., a 50% chance that the offspring will receive one copy and a 25% chance that the offspring will receive two copies). With 80% penetrance, the probability that the offspring will be affected is 0.75×0.8 , or 0.6 (60%). The probability that the offspring will be phenotypically normal is $1 - 0.60 = 0.40$, or 40%.

11. **Answer: C.** A mis-sense mutation results in the change of only a single amino acid.

Frameshift mutations (**choice A**) are the result of the deletion or insertion of a series of nucleotides that are not a multiple of three (thus altering the reading frame). Although the insertion or deletion of a single nucleotide would produce a frameshift, it is highly unlikely that it would alter only a single amino acid. The shift in the reading frame typically alters a number of amino acids subsequent to the insertion or deletion site.

An in-frame mutation (**choice B**) is the insertion or deletion of a multiple of three nucleotides, so this single-nucleotide substitution cannot be an in-frame mutation.

A nonsense mutation (**choice D**) is a single nucleotide substitution that produces a stop codon and thus truncation of the polypeptide. Therefore, it typically alters more than a single amino acid.

Splice-site mutations (**choice E**) occur at intron-exon boundaries and typically result in the loss of an exon or the inclusion of part of an intron in the coding sequence. Thus, more than a single amino acid would be altered in a typical splice-site mutation.

Population Genetics

2

DEFINITION

Population genetics is the study of genetic variation in populations. Basic concepts of population genetics allow us to understand how and why the prevalence of various genetic diseases differs among populations.

GENE AND GENOTYPE FREQUENCIES

An essential step in understanding genetic variation is to measure it in populations. This is done by estimating genotype and gene frequencies.

Genotype Frequencies

For a given locus, the genotype frequency measures the proportion of each genotype in a population. For example, suppose that a population of 100 individuals has been assayed for an autosomal restriction length fragment polymorphism (RFLP; see Chapter 7 in Section I). If the RFLP has two possible alleles, labeled 1 and 2, there are three possible genotypes: 1-1, 1-2, and 2-2. Visualization of a Southern blot allows us to determine the genotype of each individual in our population, and we find that the genotypes are distributed as follows:

Genotype	Count
1-1	49
1-2	42
2-2	9
Total	100

The genotype frequency is then obtained by dividing the count for each genotype by the total number of individuals. Thus, the frequency of genotype 1-1 is $49/100 = 0.49$, and the frequencies of genotypes 1-2 and 2-2 are 0.42 and 0.09, respectively.

Gene Frequencies

The gene frequency measures the proportion of chromosomes that contain a specific allele. To continue the RFLP example given above, we wish to estimate the frequencies of alleles 1 and 2 in our population. Each individual with the 1-1 genotype has two copies of allele 1, and each heterozygote (1-2 genotype) has one copy of allele 1. Because each diploid somatic cell contains

two copies of each autosome, our denominator is 200. Thus, the gene frequency of allele 1 in the population is:

$$\frac{(2 \times 49) + 42}{200} = 0.7$$

The same approach can be used to estimate the frequency of allele 2, which is 0.3. A convenient shortcut is to remember that the gene frequencies for all of the alleles of a given locus must add up to 1. Therefore, we can obtain the frequency of allele 2 simply by subtracting the frequency of allele 1 (0.7) from 1.

This "allele counting" approach can be used for a locus that has any number of alleles. If, for example, our locus has three possible alleles, and if each genotype can be observed directly, we can still count the alleles within each genotype to obtain a gene frequency. Suppose the following genotype counts are observed:

Genotype	Count
1-1	10
1-2	25
1-3	5
2-2	20
2-3	15
3-3	25
Total	100

Then the frequency of allele 1 is given by: $\frac{(2 \times 10) + 25 + 5}{200} = 0.25$

The frequency of allele 2 is given by: $\frac{25 + (2 \times 20) + 15}{200} = 0.40$

And the frequency of allele 3 is given by: $\frac{5 + 15 + (2 \times 25)}{200} = 0.35$

Note

Genotype frequencies measure the proportion of each genotype in a population. Gene frequencies measure the proportion of chromosomes that contain a specific allele (gene).

HARDY-WEINBERG EQUILIBRIUM

If a population is large, and if individuals mate at random with respect to their genotypes at a locus, the population should be in Hardy-Weinberg equilibrium, which means that there is a constant and predictable relationship between genotype frequencies and gene frequencies. This allows us to estimate genotype frequencies if we know gene frequencies, and vice versa.

Suppose the gene frequency of allele 1, denoted p , is 0.4. If the locus has two alleles, then the frequency of allele 2, denoted q , is $1 - p = 1 - 0.4 = 0.6$. Knowing these frequencies, and assuming Hardy-Weinberg equilibrium, we can predict the frequencies of the three possible genotypes in our population. The assumption of random mating allows us to estimate the probability (frequency) that an individual has genotype 1-1 by simply multiplying the frequency of allele 1 by itself (i.e., this is the probability that the individual inherited a copy of allele 1 from his father and from his mother). Thus, the frequency of the 1-1 genotype is given by $p^2 = 0.4 \times 0.4 = 0.16$. Similarly the frequency of genotype 2-2 is given by $q^2 = 0.6 \times 0.6 = 0.36$. The frequency of the heterozygous genotype, 1-2, is given by $2pq$. Here, we are multiplying the frequency of p and q and taking into account the fact that the individual could have obtained the heterozygous genotype in two ways: either the mother transmitted allele 1 and the father transmitted allele 2, or the mother transmitted allele 2 and the father transmitted allele 1. Because each of these events has a probability of pq , we multiply by 2 to account for the occurrence of either event (in essence the 2 reflects that fact that our cells are diploid, containing two copies of each autosome). To summarize the gene frequencies and the predicted frequencies of each genotype under Hardy-Weinberg equilibrium:

$$\text{Frequency of allele 1 } (p) = 0.4$$

$$\text{Frequency of allele 2 } (q) = 0.6$$

$$\text{Frequency of genotype 1-1} = p^2 = 0.4^2 = 0.16$$

$$\text{Frequency of genotype 1-2} = 2pq = 2 \times 0.4 \times 0.6 = 0.48$$

$$\text{Frequency of genotype 2-2} = q^2 = 0.6^2 = 0.36$$

$$\text{Note that the genotype frequencies must add to 1: } p^2 + 2pq + q^2 = 1$$

Although human populations are typically in Hardy-Weinberg equilibrium for most loci, deviations from equilibrium can be produced by nonrandom mating (i.e., inbreeding; see below) or by the action of natural selection, genetic drift, or gene flow (see below).

A Practical Application of the Hardy-Weinberg Principle

The Hardy-Weinberg equilibrium principle has a number of useful applications. One of them involves the estimation of genotype frequencies when all genotypes are not readily observable in a population. Consider the autosomal recessive disease phenylketonuria (PKU, see Clinical Correlate). The prevalence of PKU is the frequency of the homozygous recessive genotype, q^2 (i.e., one must have the homozygous recessive genotype to be affected with the disease).¹ We can measure q^2 by ascertaining the prevalence of PKU among live births (approximately 1 in 10,000). We cannot, however, estimate the genotype frequencies of the normal homozygotes and the heterozygotes because these are not clinically distinguishable from one another. Here we can apply the Hardy-Weinberg principle:

$$\text{If } q^2 = 1/10,000 \text{ then } q = \sqrt{(1/10,000)} = 1/100 = 0.01$$

$$\text{We know that } p + q = 1, \text{ so } p = 1 - q = 0.99$$

¹By convention, p denotes the frequency of the dominant allele, and q denotes the frequency of the recessive allele.

Note

Assuming random mating, the Hardy-Weinberg principle specifies a predictable relationship between gene and genotype frequencies in populations. It can be applied to estimate the frequency of heterozygous carriers of an autosomal recessive mutation.

Note

Hardy-Weinberg Equilibrium in Phenylketonuria (PKU)

- Prevalence of PKU is 1/10,000 live births
- Gene frequency = $\sqrt{(1/10,000)} = 1/100 = 0.01$
- Carrier frequency = $2(1/100) = 1/50$

Clinical Correlate

Classical phenylketonuria (PKU) is an autosomal recessive disease seen in approximately 1 in 10,000 individuals of European descent. These individuals lack the enzyme phenylalanine hydroxylase, so they cannot metabolize the amino acid phenylalanine. The buildup of phenylalanine and toxic metabolites affects the central nervous system, causing a severe decline in IQ during the first year or so of life (as many as 1–2 IQ points may be lost each week). Fortunately, a simple screening test, applied in all 50 states, detects PKU at birth. PKU babies are placed on a low-phenylalanine diet, and they develop normally. Because excess phenylalanine can adversely affect the brain throughout life, it is now recommended that individuals with PKU maintain the low-phenylalanine diet permanently. It is especially important that women with PKU maintain a low-phenylalanine diet during pregnancy because high phenylalanine levels are highly toxic to a fetus.

Applying the Hardy-Weinberg principle, we can now estimate the frequencies of the other two genotypes: $p^2 = 0.99^2 = 0.98$; $2pq = 2 \times 0.01 \times 0.99 = 0.02$ (in estimating $2pq$, a handy shortcut is to note that p is so close to 1 that it can be eliminated from the equation, leaving only $2q$ to be estimated).

This exercise demonstrates two important points:

- The Hardy-Weinberg principle can be applied to estimate the prevalence of heterozygous carriers in populations when we know only the prevalence of the recessive disease.
- For autosomal recessive diseases, such as PKU, the prevalence of heterozygous carriers is much higher than the prevalence of affected homozygotes. In effect, the vast majority of recessive genes are “hidden” in the heterozygotes.

SEX CHROMOSOMES AND GENE FREQUENCIES

Because males have only one X chromosome, gene frequency estimation for X-linked traits differs from that of autosomal traits. Consider hemophilia A (Chapter 1), which is an X-linked recessive disease. If a male's X chromosome has a factor VIII mutation, he will have hemophilia A. If his X chromosome does not have the mutation, he will not develop the disease. Thus, the gene frequency for hemophilia A is obtained simply by counting the proportion of affected males in the population (i.e., the proportion of X chromosomes containing the mutation). Approximately one in 10,000 males has hemophilia A. Thus, the gene frequency for this disease, q , is 1/10,000.

Females have two X chromosomes, so they must inherit two copies of the mutated chromosome to develop hemophilia A. Gene frequencies are similar in males and females, so we can use the gene frequency estimated in males to predict the genotype frequencies in females. Assuming Hardy-Weinberg equilibrium, the frequency of affected females is given by

$$q^2 = \left(\frac{1}{10^4}\right)^2 \text{ or } \frac{1}{10^8}$$

In other words, one in 100 million females is affected.

The female heterozygote frequency is $2pq = \frac{2}{10^4}$ or $\frac{1}{5,000}$

This exercise demonstrates that:

- As with autosomal recessive traits, the majority of X-linked recessive genes are hidden in female heterozygous carriers (although a considerable number are seen in affected males).
- X-linked recessive traits will be seen much more commonly in males than in females, an observation also made in Chapter 1.

FACTORS RESPONSIBLE FOR GENETIC VARIATION IN POPULATIONS

Several evolutionary forces are responsible for gene frequency variation in populations:

Mutation

Mutation, discussed previously, is ultimately the source of all new genetic variation in populations. In general, mutation rates do not differ very much from population to population.

Natural Selection

Natural selection acts upon genetic variation, increasing the frequencies of genes that promote survival or fertility (referred to as **fitness**) and decreasing the frequencies of genes that reduce fitness. The reduced fitness of most disease genes helps to explain why most genetic diseases are relatively rare. Dominant diseases, in which the gene is more readily exposed to the effects of natural selection, tend to have lower gene frequencies than do recessive diseases, where the gene is typically hidden in heterozygotes. An interesting example of natural selection in a human disease is given by sickle cell disease (see Clinical Correlate), which affects 1 in 600 African Americans and up to 1 in 50 individuals in some parts Africa. How could this highly deleterious disease-causing mutation become so frequent, especially in Africa? The answer lies in the fact the falciparum malaria parasite, which has been common in much of Africa, does not survive well in the erythrocytes of sickle cell heterozygotes. These individuals, who have no clinical signs of sickle cell disease, are thus protected against the lethal effects of malaria. Consequently, there is a **heterozygote advantage** for the sickle cell mutation, and it maintains a relatively high frequency in some African populations. There is now evidence for heterozygote advantages for several other recessive diseases that are relatively common in some populations (e.g., cystic fibrosis [heterozygote resistance to typhoid fever] and hemochromatosis [heterozygote advantage in iron-poor environments]).

Genetic Drift

Genetic drift refers to gene frequency change caused by finite population size. This change can be quite rapid in very small populations. To understand genetic drift, consider a coin-tossing experiment in which only a small number of coins are tossed (10 or 20). Although it is expected that half of the tosses will produce heads and half will produce tails, substantial deviations can occur when only a small number of coins are tossed. The same is true when a small number of gene copies are being transmitted from generation to generation. Genetic drift helps to explain why some genetic diseases are unusually common in small, isolated populations (e.g., more cases of the rare recessive Ellis van Creveld disease² have been seen in the Old Order Amish than in the rest of the world).

Gene Flow

Gene flow refers to the exchange of genes among populations. Because of gene flow, populations located close to one another often tend to have similar gene frequencies. Gene flow can also cause gene frequencies to change through time: the frequency of sickle cell disease is lower in African Americans in part because of gene flow from neighboring populations that do not carry the disease-causing mutation; in addition, the heterozygote advantage for the sickle cell mutation (see Clinical Correlate) has disappeared because malaria has been rare in North America.

²This disease is characterized by reduced stature, polydactyly, and congenital heart defects.

Clinical Correlate

Sickle cell disease is an autosomal recessive disorder seen in approximately 1/600 African American births. It is even more common in some parts of Africa, where up to 1/50 live births are affected. This disease is caused by a mutation in the β -globin gene on chromosome 11. The mutation alters the hemoglobin molecule such that erythrocytes assume a characteristic "sickle" shape under conditions of low oxygen tension. These erythrocytes cause vascular obstruction because they cannot squeeze through capillaries efficiently. This in turn produces localized hypoxemia (lack of oxygen) and infarctions of various tissues (bone, kidneys, lungs, and spleen). Sickle-shaped erythrocytes are destroyed prematurely, leading to anemia. Individuals with sickle cell disease are susceptible to recurrent infections (especially pneumonia), and 15% of children with sickle cell disease in the United States die before the age of 5 years. This percentage is decreasing with the prophylactic administration of antibiotics. In African populations, the mortality rate for sickle cell homozygotes is considerably higher.

Note

The four evolutionary factors responsible for genetic variation in populations are:

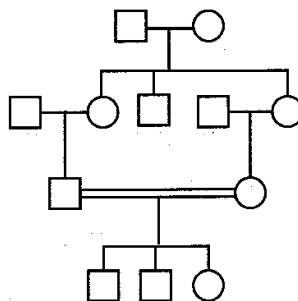
- Mutation
- Natural selection
- Genetic drift
- Gene flow

Note

Consanguineous matings are more likely to produce offspring affected with recessive diseases because individuals who share common ancestors are more liable to share disease-causing mutations.

CONSANGUINITY AND ITS HEALTH CONSEQUENCES

As briefly mentioned in Chapter 1, consanguinity refers to the mating of individuals who are related to one another (typically, a union is considered to be consanguineous if it occurs between individuals related at the second cousin level or closer). Figure II-2-1 illustrates a pedigree for a consanguineous union. Because of their mutual descent from a common ancestor, relatives are more likely to share the same disease-causing genes. For example, siblings share one half of their genes, on average; uncles and nieces share one fourth; first cousins share one eighth; and so on (the proportion of shared genes for a pair of relatives is termed the coefficient of relationship). Thus, if the carrier of an autosomal recessive disease gene mates with his or her first cousin, there is a probability of one eighth that the first cousin carries the same gene. Consequently, there is an increased risk of genetic disease in the offspring of consanguineous matings. Dozens of empirical studies have examined the health consequences of consanguinity, particularly first-cousin matings. These studies show that the offspring of first-cousin matings are approximately twice as likely to present with a genetic disease than are the offspring of unrelated matings. The frequency of genetic disease increases further in the offspring of closer unions (e.g., uncle–niece or brother–sister matings).



In this example, a first-cousin mating is shown.

Figure II-2-1. A Pedigree Illustrating Consanguinity, the Mating of Related Individuals

Although first-cousin matings are now relatively rare in the United States, they do occur occasionally. Such matings are common in some populations, including much of the Mideast and South Asia.

Chapter Summary

Population genetics allows predictions about the prevalence of diseases in populations.

Genotype frequency measures the proportion of each genotype in a population.

Gene (allele) frequency measures the proportion of each allele at a particular locus.

Hardy-Weinberg equilibrium:

- $p^2 + 2pq + q^2 = 1$
- p and q are the allele frequencies at a locus
- allows calculations of carrier frequency and prevalence of genetic diseases
- p^2 = homozygous normal; $2pq$ = heterozygous carrier; q^2 = homozygous affected (for autosomal recessive diseases)

Factors responsible for genetic variation:

- Mutation is the source of new genetic variations
- Natural selection increases or decreases allele frequencies, depending on their survival value (e.g., heterozygote advantage for the sickle cell mutation)
- Genetic drift can change allele frequencies in small populations by chance
- Gene flow occurs when populations exchange genes with each other

Consanguinity (mating of related individuals) increases the likelihood of genetic disease in the offspring.

Review Questions

1. A population has been assayed for a four-allele polymorphism, and the following genotype counts have been obtained:

Genotype	Count
1,1	4
1,3	8
1,4	3
2,3	5
2,4	9
3,3	4
3,4	6
4,4	11

On the basis of these genotype counts, what are the gene frequencies of alleles 1 and 2?

- A. 0.38, 0.28
- B. 0.19, 0.14
- C. 0.095, 0.07
- D. 0.25, 0.25
- E. 0.38, 0.20

2. Which of the following best characterizes Hardy-Weinberg equilibrium?
 - A. Consanguinity has no effect on Hardy-Weinberg equilibrium.
 - B. Genotype frequencies can be estimated from gene frequencies, but the reverse is not true.
 - C. Natural selection has no effect on Hardy-Weinberg equilibrium.
 - D. Once a population deviates from Hardy-Weinberg equilibrium, it takes many generations to return to equilibrium.
 - E. The frequency of heterozygous carriers of an autosomal recessive mutation can be estimated if one knows the incidence of affected homozygotes in the population.
3. In a genetic counseling session, a healthy couple has revealed that they are first cousins and that they are concerned about health risks for their offspring. Which of the following best characterizes these risks?
 - A. Because the couple shares approximately half of their genes, most of the offspring are likely to be affected with some type of genetic disorder.
 - B. The couple has an increased risk of producing a child with an autosomal dominant disease.
 - C. The couple has an increased risk of producing a child with an autosomal recessive disease.
 - D. The couple has an increased risk of producing a child with Down syndrome.
 - E. There is no known increase in risk for the offspring.
4. An African American couple has produced two children with sickle cell disease. They have asked why this disease seems to be more common in the African American population than in other U.S. populations. Which of the following factors provides the best explanation?
 - A. Consanguinity
 - B. Genetic drift
 - C. Increased gene flow in this population
 - D. Increased mutation rate in this population
 - E. Natural selection
5. If the incidence of cystic fibrosis is $1/2,500$ among a population of Europeans, what is the predicted incidence of heterozygous carriers of a cystic fibrosis mutation in this population?
 - A. $1/25$
 - B. $1/50$
 - C. $2/2,500$
 - D. $1/2,500$
 - E. $(1/2,500)^2$

6. A man is a known heterozygous carrier of a mutation that causes hemochromatosis (autosomal recessive disease). Suppose that 1% of the general population consists of homozygotes for this mutation. If the man mates with somebody from the general population, what is the **probability** that he and his mate will produce a child who is an affected homozygote?
- A. 0.025
B. 0.045
C. 0.09
D. 0.10
E. 0.25
7. The incidence of Duchenne muscular dystrophy in North America is about 1/3,000 males. On the basis for this figure, what is the gene frequency of this X-linked recessive mutation?
- A. 1/3,000
B. 2/3,000
C. $(1/3,000)^2$
D. $.(1/3,000)$
E. $2 \times \%(1/3,000)$

Answers

1. **Answer: B.** The denominator of the gene frequency is 100, which is obtained by adding the number of genotyped individuals (50) and multiplying by 2 (because each individual has two alleles at the locus). The numerator is obtained by counting the number of alleles of each type: the 4 homozygotes with the 1,1 genotype contribute 8 copies of allele 1; the 1,3 heterozygotes contribute another 8 alleles; and the 1,4 heterozygotes contribute 3 alleles. Adding these together, we obtain 19 copies of allele 1. Dividing by 100, this yields a gene frequency of 0.19 for allele 1. For allele 2, there are two classes of heterozygotes that have a copy of the allele: those with the 2,3 and 2,4 genotypes. These 2 genotypes yield 5 and 9 copies of allele 2, respectively, for a frequency of $14/100 = 0.14$.
2. **Answer: E.** The incidence of affected homozygotes permits the estimation of the frequency of the recessive mutation in the population. Using the Hardy-Weinberg equilibrium relationship between gene frequency and genotype frequency, the gene frequency can then be used to estimate the frequency of the heterozygous genotype in the population.

Consanguinity (**choice A**) affects Hardy-Weinberg equilibrium by increasing the number of homozygotes in the population above the equilibrium expectation (i.e., consanguinity results in a violation of the assumption of random mating).

Genotype frequencies can be estimated from gene frequencies (**choice B**), but gene frequencies can also be estimated from genotype frequencies (as in **choice A**).

By eliminating a specific genotype from the population (e.g., affected homozygotes), natural selection can cause deviations from equilibrium (**choice C**).

Only one generation of random mating is required to return a population to equilibrium (**D**).

3. **Answer: C.** Because the couple shares common ancestors (i.e., one set of grandparents), they are more likely to be heterozygous carriers of the same autosomal recessive disease-causing mutations. Thus, their risk of producing a child with an autosomal recessive disease is elevated above that of the general population.

First cousins share approximately 1/8 of their genes, not 1/2 (**choice A**).

Because both members of the couple are healthy, neither one is likely to harbor a dominant disease-causing mutation (**choice B**). In addition, consanguinity itself does not elevate the probability of producing a child with a dominant disease because only one copy of the disease-causing allele is needed to cause the disease.

Down syndrome (**choice D**) typically is the result of a new mutation. When it is transmitted by an affected female, it acts like a dominant mutation and thus would not be affected by consanguinity.

Empirical studies indicate that the risk of genetic disease in the offspring of first cousin couples is approximately double that of the general population (**choice E**).

4. **Answer: E.** The frequency of sickle cell disease is elevated in many African populations because heterozygous carriers of the sickle cell mutation are resistant to malarial infection but do not develop sickle cell disease, which is autosomal recessive. Thus, there is a selective advantage for the mutation in heterozygous carriers, elevating its frequency in the population.

Consanguinity (**choice A**) could elevate the incidence of this autosomal recessive disease in a specific family, but it does not account for the elevated incidence of this specific disease in the African American population in general.

The African American population is large and consequently would not be expected to have experienced elevated levels of genetic drift (**choice B**).

Although there has been gene flow (**choice C**) from other populations into the African American population, this would be expected to decrease, rather than increase, the frequency of sickle cell disease because the frequency of this disease is highest in some African populations.

There is no evidence that the mutation rate (**choice D**) is elevated in this population. In contrast, the evidence for natural selection is very strong.

5. **Answer: A.** This answer is obtained by taking the square root of the incidence (i.e., the frequency of affected homozygotes) to get a gene frequency for the disease-causing mutation (q) of 1/50 (0.02). The carrier frequency is given by $2pq$, or approximately $2q$, or 1/25.
6. **Answer: B.** One must first determine the probability that the man's mate will also be a heterozygous carrier. This is done by applying the Hardy-Weinberg rule. If the frequency of affected homozygotes is 1%, then the gene frequency of the hemochromatosis mutation is the square root of 1%, or 0.10. Then the estimated frequency of heterozygous carriers in the population, $2pq$, is $2 \times 0.10 \times 0.9 = 0.18$ (notice that we do not use the $2q$ approximation in this case because p is not approximately equal to 1). The probability that two heterozygous carriers will produce an affected offspring is 1/4, so the probability that the man mates with a carrier and the probability that they will in turn produce an affected offspring is obtained by multiplying the two probabilities together: $1/4 \times 0.18 = 0.045$.
7. **Answer: A.** Because males have only a single X chromosome, each affected male has one copy of the disease-causing recessive mutation. Thus, the incidence of an X-linked recessive disease in the male portion of a population is a direct estimate of the gene frequency in the population.

Cytogenetics

3

OVERVIEW

This chapter reviews diseases that are caused by microscopically observable alterations in chromosomes. These alterations may involve the presence of extra chromosomes or the loss of chromosomes. They may also consist of structural alterations of chromosomes. Chromosome abnormalities are seen in approximately 1 in 150 live births and are the leading known cause of mental retardation. The vast majority of chromosome abnormalities are lost prenatally: chromosome abnormalities are seen in 50% of spontaneous fetal losses during the first trimester of pregnancy, and they are seen in 20% of fetuses lost during the second trimester. Thus, chromosome abnormalities are the leading known cause of pregnancy loss.

BASIC DEFINITIONS AND TERMINOLOGY

Chromosomes are most easily visualized during the metaphase stage of mitosis, when they are maximally condensed. They are photographed under the microscope to create a **karyotype**, an ordered display of the 23 pairs of human chromosomes in a typical somatic cell (Fig II-3-1). Chromosomes are ordered according to size, with the sex chromosomes (X and Y) placed in the lower right portion of the karyotype.

□ Negative or pale Staining 'Q' and 'G' Bands
 Positive 'R' Bands
 ■ Positive 'Q' and 'G' Bands
 Negative 'R' Bands
 ▨ Variable Bands

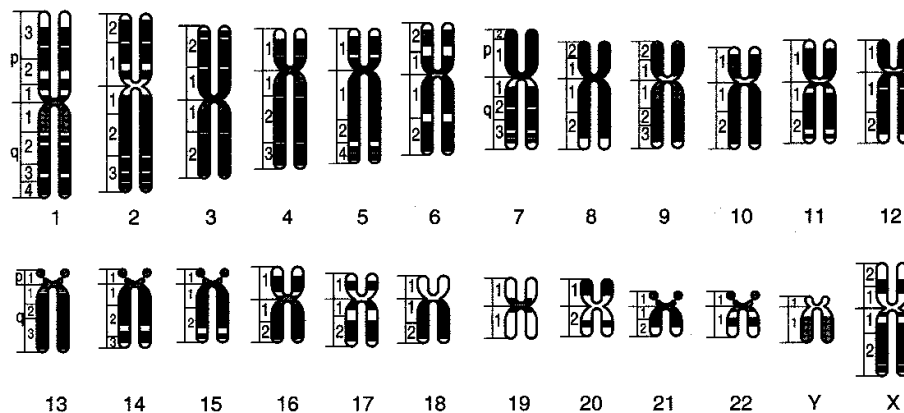


Figure II-3-1a. Karyotype—Idealized Drawing



To visualize chromosomes more accurately, various **stains** are applied so that the **bands** in chromosomes can be seen (Fig II-3-1). The bands tend to **reflect differences** in chromosome structure or composition (e.g., regions rich in CG bases versus those rich in AT bases).

A chromosome consists of a constricted area termed a **centromere** and an arm on either side of the centromere. During mitosis and meiosis, the centromere is the site of attachment of spindle fibers that pull apart either homologous chromosomes or sister chromatids. The tips of the chromosomes are termed **telomeres**. The **long arm** is labeled **q**, and the **short arm** is labeled **p**. Chromosome arms are subdivided further by regions and by bands within the regions. Thus, the designation 14q32 refers to the second band within region 3 of the long arm of chromosome 14. When the centromere is near the middle of the chromosome, the arms are of roughly equal length, and the chromosome is termed **metacentric**. **Acrocentric** chromosomes have the centromere near one telomere, and in **submetacentric** chromosomes, the centromere is found between the middle and the telomere.

A normal male karyotype is labeled 46,XY (46 chromosomes, including one X and one Y). Similarly, a normal female karyotype is labeled 46,XX. A complex nomenclature has been devised to describe deviations from these karyotypes. This nomenclature is given in Table II-3-1.

Table II-3-1. Common Symbols Used in Karyotype Nomenclature

1-22	Autosome number
X, Y	The sex chromosomes
(+) or (-)	When placed before an autosomal number, indicates that chromosome is extra or missing
cen	Centromere
dic	Dicentric
inv	Inversion
p	Short arm of the chromosome
q	Long arm of the chromosome
t	Translocation
del	Deletion
ins	Insertion
dup	Duplication
ter	Terminal or end (pter = end of the short arm; qter = end of the long arm)
:	Break (no reunion, as in a terminal deletion)
::	Break and join
→	From-to
mos	Mosaic

Note

Euploid Cells (multiple of 23 chromosomes)

- Haploid (23 chromosomes): gametes
- Diploid (46 chromosomes): most somatic cells
- Triploid (69 chromosomes): rare lethal condition
- Tetraploid (92 chromosomes): very rare lethal condition

NUMERICAL CHROMOSOME ABNORMALITIES**Euploidy**

When a cell has a multiple of 23 chromosomes, it is said to be **euploid**. Gametes (sperm and egg cells) are euploid cells that have 23 chromosomes (one member of each pair); they are said to be **haploid**. Most somatic cells are **diploid**, containing both members of each pair, or 46 chromosomes. Two types of euploid cells with abnormal numbers of chromosomes are seen in humans, triploidy and tetraploidy.

Triploidy

Triploidy refers to cells that contain three copies of each chromosome (69 total). Triploidy, which usually occurs as a result of the fertilization of an ovum by two sperm cells, is common at conception, but the vast majority of these conceptions are lost prenatally. However, about 1 in 10,000 live births is a triploid. These babies have multiple defects of the heart and central nervous system, and they do not survive.

Tetraploidy

Tetraploidy refers to cells that contain four copies of each chromosome (92 total). This lethal condition is much rarer than triploidy among live births: only a few cases have been described.

Aneuploidy

Aneuploidy, which indicates a deviation from the euploid number of chromosomes, is the term used to describe the loss or gain of specific chromosomes. Two major types of aneuploidy are observed: **trisomy** (three copies of a specific chromosome) and **monosomy** (one copy of a specific chromosome). Monosomies and trisomies are usually caused by **nondisjunction** (the failure of the two members of the chromosome pair to disjoin or separate) during meiosis (Fig II-3-2). All autosomal monosomies are lethal, but trisomies of three different autosomes (13, 18, and 21) are compatible with survival to term in at least some cases. This difference illustrates the fact that the body tolerates extra genetic material more successfully than a loss of genetic material.

Trisomy 21

Trisomy 21 (47,XY,+21; 47,XX,+21) is the most common autosomal trisomy, and it causes Down syndrome. This well known syndrome is seen in approximately 1 in 800 live births and is characterized by mental retardation (IQ ranging typically from 20 to 70), short stature, hypotonia, characteristic facial features (depressed nasal bridge, upslanting palpebral fissures, epicanthic fold), congenital heart defects (in about 40% of cases), increased risk of leukemia, frequent respiratory infections, and the development of Alzheimer disease by the fifth or sixth decade of life. At least three fourths of Down syndrome conceptions are spontaneously lost during pregnancy. As a result of surgical correction of congenital heart defects and more advanced treatment of leukemia and infections, the survival rate of babies with Down syndrome has increased substantially: about 80% now survive to age 10 and beyond.

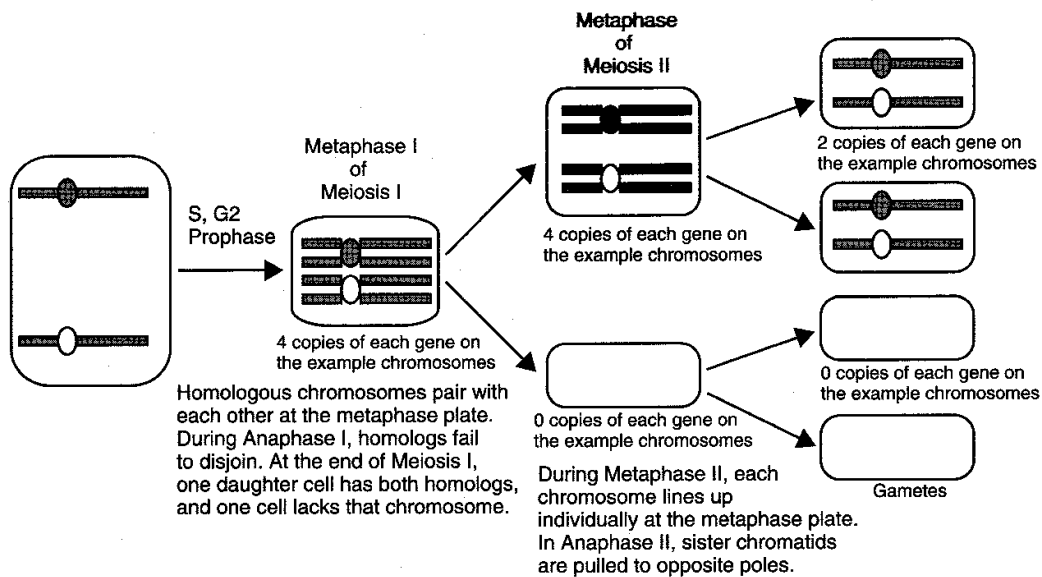


Figure II-3-2a. Nondisjunction During Meiosis I

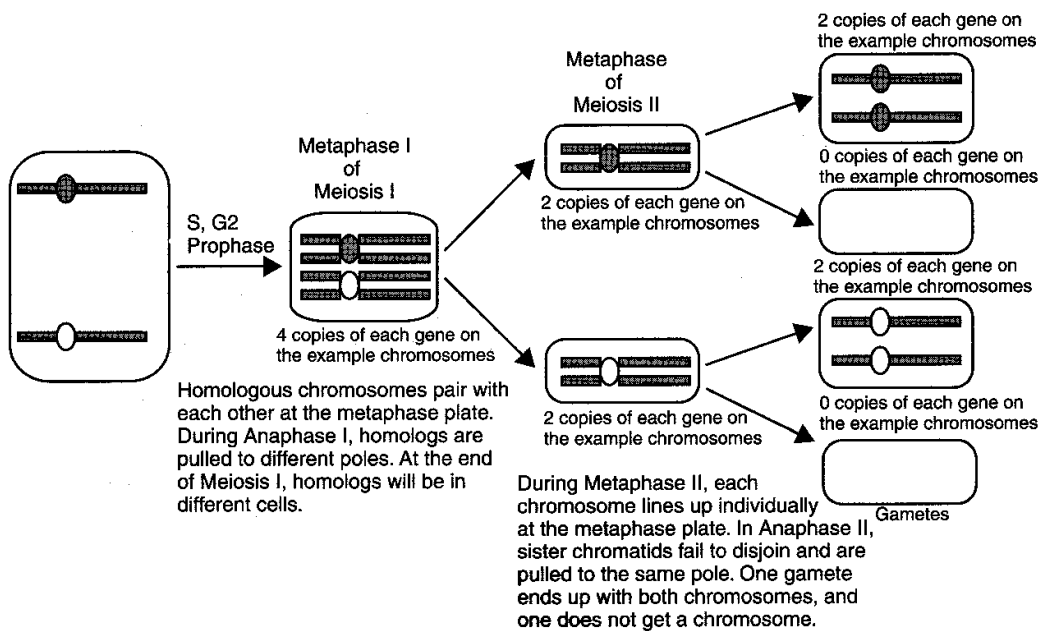


Figure II-3-2b. Nondisjunction During Meiosis II

Note**Aneuploid Cells**
(deviation from euploid numbers)

- Trisomy 21: Down syndrome (47,XY,+21; or 47,XX,+21)
- Trisomy 18: Edwards syndrome (47,XY,+18; or 47,XX,+18)
- Trisomy 13: Patau syndrome (47,XY,+13; or 47,XX,+13)

Note**Sex Chromosome Aneuploidy**

- Klinefelter syndrome (47,XXY)
- Turner syndrome (45,X)
- Other: 47,XYY; 47,XXX

Trisomy 18

Trisomy 18 (Edwards syndrome; 47,XY,+18; 47,XX,+18) is seen in about 1 in 6,000 live births. More than 95% of conceptions with trisomy 18 are spontaneously aborted during pregnancy. Among those who survive to term, more than 90% die during the first year of life. Typical features in newborns include small size for gestational age, small mouth and ears, clenched fists with overlapping fingers, congenital heart defects, and omphalocele (protrusion of the bowel into the umbilical cord).

Trisomy 13

Trisomy 13 (Patau syndrome, 47,XY,+13; 47,XX,+13) is seen in approximately 1 in 10,000 live births. More than 90% of conceptions are lost prenatally, and more than 90% of those who survive to term do not survive to 1 year of age. Common disease features include oral-facial clefts, microphthalmia (small eyes), renal defects, and polydactyly (extra fingers). Central nervous system malformations and heart defects are common and contribute to mortality.

Sex Chromosome Aneuploidy

Aneuploidy affecting the sex chromosomes is relatively common and tends to have less severe consequences than does autosomal aneuploidy (although at least one X chromosome must be present for survival). Two aneuploidies of the sex chromosomes are clinically significant.

Klinefelter Syndrome

Klinefelter syndrome (47,XXY) is seen in approximately 1 in 1,000 males (all individuals with this condition have a male phenotype because they have a Y chromosome). Klinefelter males are nearly always sterile because of atrophy of the seminiferous tubules. They tend to be taller than average, with abnormally long arms and legs. Breast development (gynecomastia) is seen in about one third of cases, and the IQ is on average 10–15 points below that of unaffected siblings. Individuals have also been seen with 48,XXXY and 49,XXXXY karyotypes; the additional X chromosomes produce a more severely affected phenotype.

Turner Syndrome

Turner syndrome (45,X) affects approximately 1 in 2,500 live-born females. Features include reduced stature, webbed neck, lymphedema of the ankles and wrists (especially at birth), diminished spatial perception, shield-shaped chest with widely spaced nipples, and sterility as a result of gonadal dysgenesis. Although Turner syndrome is relatively uncommon among live births, it is found in approximately 2% of conceptions. Thus, more than 99% of Turner conceptions are spontaneously aborted. Among those who survive to term, **mosaicism** is quite common (i.e., the presence of more than one genetically distinct cell type). The most common mosaicism in Turner syndrome is the presence of some 45,X cells and some normal 46,XX cells. Turner females with 45,X; 46,XY mosaicism are highly susceptible to gonadoblastoma. Mosaicism is an occasional feature of many chromosome abnormalities (Down syndrome, trisomies 13 and 18) and is sometimes associated with milder expression of the disease.

Other common, but less clinically significant, sex chromosome aneuploidies are the 47,XXX (1/1,000 females) and 47,XYY (1/1,000 males) karyotypes. The former karyotype is associated with increased stature and a mild decrease in IQ, and the latter is associated with increased stature, mild decrease in IQ, acne, and a susceptibility to impulsive behavior (early studies suggesting a propensity to violent criminal behavior have not been substantiated).

Clinical Correlate: Maternal Age, Risk of Down Syndrome, and Prenatal Diagnosis

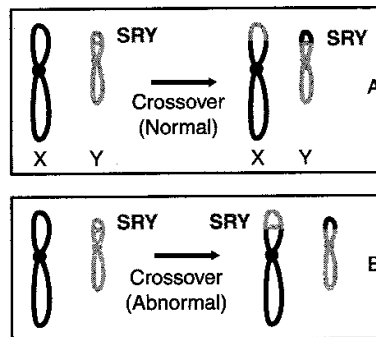
Surveys of babies with trisomy 21 show that approximately 90% to 95% of the time, the extra copy of the chromosome is contributed by the mother (similar figures are obtained for trisomies of the 18th and 13th chromosomes). The increased risk of Down syndrome with maternal age is well documented. The risk of bearing a child with Down syndrome is less than 1/1,000 for women younger than 30. The risk increases to about 1/400 at age 35, 1/100 at age 40, and 3–4% or more after age 45. This increase reflects an elevated rate of nondisjunction in older ova (recall that all of a woman's egg cells are formed during her fetal development, and they remain suspended in prophase I until ovulation). There is no corresponding increase in risk with advanced paternal age; sperm cells are generated continuously throughout the life of the male.

The increased risk of trisomy with advanced maternal age motivates more than half of pregnant women in North America to undergo prenatal diagnosis (most commonly, amniocentesis or chorionic villus sampling, discussed in Chapter 6). Down syndrome can also be screened by assaying maternal serum levels of α -fetoprotein, chorionic gonadotropin, and unconjugated estriol. This so-called **triple screen** can detect approximately 70% of fetuses with Down syndrome.

XX Males, XY Females, and Human Sex Determination

Much has been learned about the genetic basis of human sex determination since the identification of the SRY (sex-determining region on the Y) gene on the human Y chromosome. SRY encodes a product that blocks the action of DAX1, a gene whose protein product inhibits genes that lead to male differentiation. In the absence of DAX1 action, male differentiation genes are activated, leading to Sertoli cell differentiation, secretion of Müllerian inhibiting substance, and thus to a male phenotype.

The SRY gene is located on Yp, just proximal to the boundary of the **pseudoautosomal region** (Fig II-3-3). This 2.5-Mb region undergoes crossing over with the distal Xp in male meiosis (hence the term pseudoautosomal). Approximately 1 in 20,000 males has a 46,XX karyotype. The phenotype is similar to that of a male with Klinefelter syndrome. Close examination of the paternally transmitted X chromosome shows that it contains the SRY gene as a result of a crossover that occurs closer to the centromere than it should, such that the resulting X chromosome receives the SRY gene (Fig II-3-3). Conversely, if the offspring receives the Y chromosome that results from this incorrect crossover event, an XY female is produced. These females have gonadal dysgenesis and poorly developed secondary sexual characteristics.



*A. Normal crossover between the pseudoautosomal regions of X and Y produces a Y chromosome that retains the SRY gene.
 B. The crossover has occurred below the SRY gene, transferring it to the X chromosome. This will produce XX male or XY female offspring.*

Figure II-3-3. X-Y Crossover and the SRY Gene

STRUCTURAL CHROMOSOME ABNORMALITIES

Structural alterations of chromosomes occur when chromosomes are broken by agents termed **clastogens** (e.g., radiation, some viruses, and some chemicals). Some alterations may result in a loss or gain of genetic material and are called **unbalanced** alterations; **balanced** alterations do not result in a gain or loss of genetic material and usually have fewer clinical consequences. As with other types of mutations, structural alterations can occur either in the germ line or in somatic cells. The former can be transmitted to offspring. The latter, although not transmitted to offspring, can alter genetic material such that the cell can give rise to cancer.

Translocations

Translocations occur when chromosomes are broken and the broken elements reattach to other chromosomes. Translocations can be classified into two major types, reciprocal and Robertsonian.

Reciprocal Translocation

As its name implies, a translocation is reciprocal when genetic material is exchanged between two chromosomes. For example, parts of the short arms of chromosomes 2 and 8 could be exchanged (Fig II-3-4). The individual who carries the reciprocal translocation (46,XX,t[2p;8p]) will not usually be affected clinically because he or she has the normal complement of genetic material. However, his or her offspring can inherit unbalanced chromosome material (e.g., a copy of the normal 8 and a copy of chromosome 2 that contains the translocated piece of 8, resulting in a **partial trisomy** of the 8th chromosome and **partial monosomy** of the second chromosome [Fig II-3-4]).

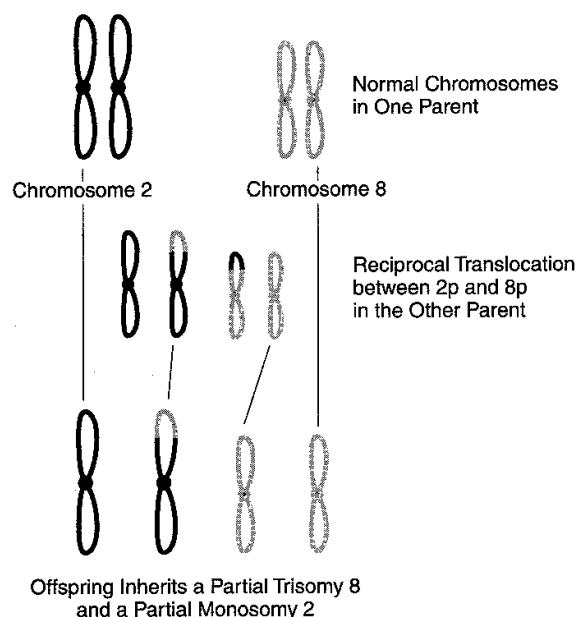


Figure II-3-4. Results of a Reciprocal Translocation

Robertsonian Translocation

These translocations are much more common than reciprocal translocations and are estimated to occur in approximately 1 in 1,000 live births. They occur only in the acrocentric chromosomes (13, 14, 15, 21, and 22) and involve the loss of the short arms of two of the chromosomes and subsequent fusion of the long arms. An example of a Robertsonian translocation involving chromosomes 14 and 21 is shown in Figure II-3-5. Because the short arms of the acrocentric chromosomes contain no essential genetic material, their loss produces no clinical consequences, and the translocation carrier is not clinically affected. When the carrier's germ cells are formed

Clinical Correlate

Translocations and Cancer

Although most of our discussion deals with inherited chromosome alterations, rearrangements in somatic cells can lead to the formation of cancers by altering the genetic control of cellular proliferation. A classic example is a reciprocal translocation of the long arms of chromosomes 9 and 22, termed the **Philadelphia chromosome**. This translocation alters the activity of the *abl* proto-oncogene (proto-oncogenes can lead to cancer; see Chapter 5). When this alteration occurs in hematopoietic cells, it can result in chronic myelogenous leukemia. More than 100 different chromosome rearrangements involving nearly every chromosome have been observed in more than 40 types of cancer.

Note**Translocations**

- Robertsonian (fusion of long arms of 13, 14, 15, 21, or 22)
- Reciprocal (exchange of material between nonhomologous chromosomes)

through meiosis, the translocated chromosome must pair with its homologs. If **alternate segregation** occurs, the offspring will inherit either a normal chromosome complement or will be a normal carrier like the parent (Fig II-3-6). If **adjacent segregation** occurs, the offspring will have an unbalanced chromosome complement (an extra or missing copy of the long arm of chromosome 21 or 14). Because only the long arms of these chromosomes contain genetically important material, the effect is equivalent to a trisomy or monosomy.

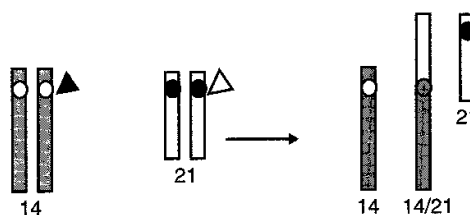
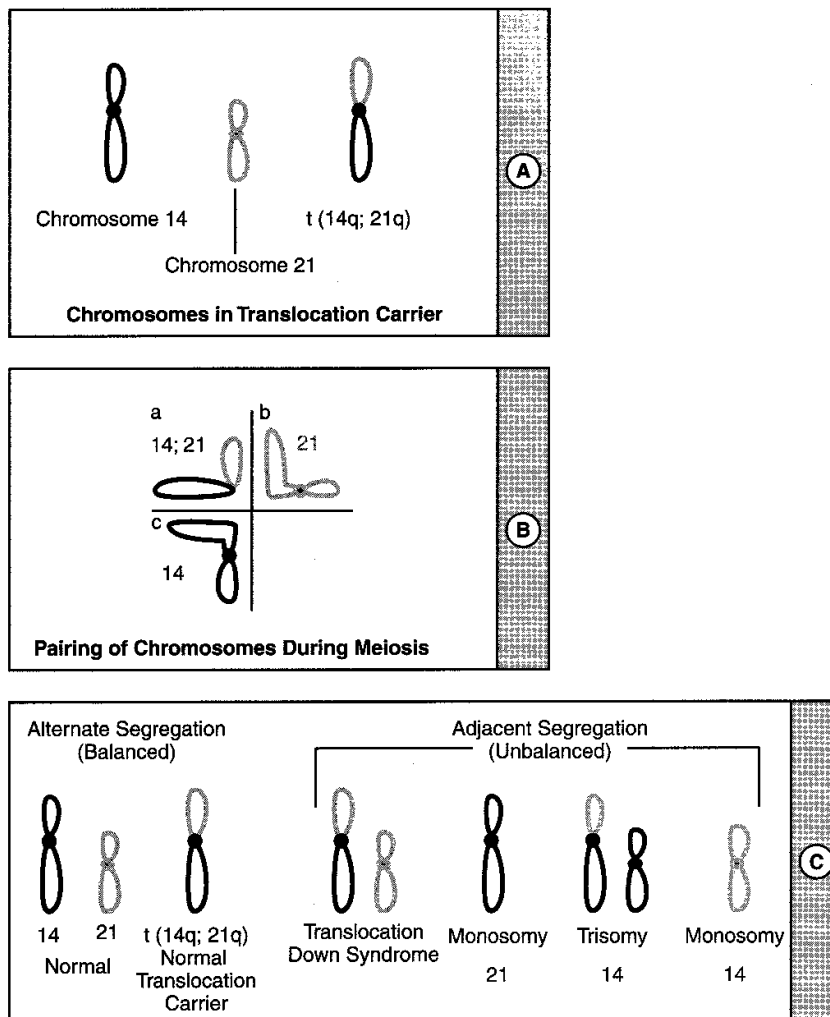


Figure II-3-5. Robertsonian Translocation

Approximately 5% of Down syndrome cases are the result of a Robertsonian translocation affecting chromosome 21 and chromosome 14. A male carrier of this translocation would be designated 45,XY,-14,-21,+t(14q;21q), indicating that he is missing one normal chromosome 14 and one normal chromosome 21, in addition to having the translocation. Because the full complement of genetic material is present in this carrier (the absence of 14p and 21p on the translocated chromosome being without effect), the carrier is phenotypically normal. The same would be true of a female translocation carrier, designated 45,XX,-14,-21,+t(14q;21q). A male child with Down syndrome as a result of such a Robertsonian translocation would have the karyotype 46,XY,-14,+t(14q;21q). Because a parent who carries the translocation may transmit unbalanced chromosome material to each offspring, the recurrence risk of Down syndrome is significantly elevated: approximately 10–15% for female translocation carriers and 1–2% for male carriers (the reason for this difference is not well understood, but it may reflect selection against sperm cells bearing unbalanced chromosome material). In contrast, for mothers younger than 30 who are not translocation carriers and who have produced a baby with trisomy 21, the recurrence risk is only about 1%. The elevated recurrence risk for translocation carriers versus noncarriers demonstrates the importance of ordering a chromosome study when Down syndrome is suspected in a newborn.



A translocation carrier (A) produces aberrant chromosome pairing during meiosis. Alternate segregation (B) (quadrant b with quadrant c or quadrant a alone) produces a balanced chromosome complement in the offspring. Adjacent segregation (C) (quadrant a with b or quadrant a with c) produces four possible unbalanced chromosome complements in the offspring.

Figure II-3-6. Consequences of a Robertsonian Translocation

Clinical Correlate

The **cri-du-chat syndrome**, which is the result of a terminal deletion of the short arm of chromosome 5 (karyotype 46,XY,del[5p]), is a classic example of a chromosome deletion. The name is derived from the French term "cry of the cat," which denotes the characteristic kittenlike cry of babies with this condition. It is rare, affecting approximately 1 in 50,000 newborns. Characteristic features include mental retardation (average IQ of 35), microcephaly, and wide-set eyes. Most children born with this condition do not survive to adulthood. Other important diseases caused by deletions include Williams syndrome, Wolf-Hirschhorn syndrome, DiGeorge syndrome, Wilms tumor, and the Prader-Willi and Angelman syndromes (see Chapter 1).

Deletions. A deletion occurs when a chromosome is broken and the material is lost. A **terminal deletion** is a single break in which the lost material includes the telomere. An **interstitial deletion** occurs when there are two breaks on a chromosome and the material between the breaks is lost (Fig II-3-7). Deletions can also be produced by **unequal crossover** during meiosis (i.e., homologous chromosomes exchange unequal amounts of material, resulting in the loss of genetic material on one chromosome and the gain of material [a **duplication**] on the other). Because the body is sensitive to a loss of genetic material, deletions often have important clinical consequences.

Also see Section I, Chapter 4: Translation; Mutations.

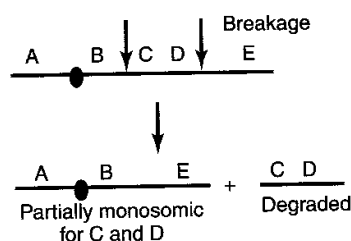


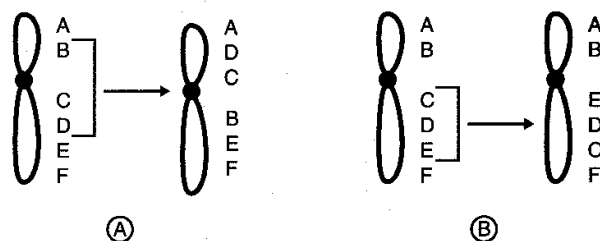
Figure II-3-7. Interstitial Deletion

Other Chromosome Abnormalities

Several other types of structural abnormalities are seen in human karyotypes. In general, their frequency and clinical consequences tend to be less than those of translocations and deletions.

Inversions

Inversions occur when the chromosome segment between two breaks is reinserted in the same location but in reverse order (Fig II-3-8). Inversions that include the centromere are termed **pericentric**, whereas those that do not include the centromere are termed **paracentric**. An example of a karyotype of an inversion extending from 3p21 to 3q13 is 46,XY,inv(3)(p21;q13). Inversion carriers still retain all of their genetic material, so they are usually unaffected (although an inversion may interrupt or otherwise affect a specific gene and thus cause disease). Because homologous chromosomes must line up during meiosis, inverted chromosomes will form loops that may result in a gamete that contains a deletion or a duplication, which may then be transmitted to the offspring.



A. Pericentric inversions include the centromere.
B. Paracentric inversions do not include the centromere.

Figure II-3-8. Chromosome Inversions

Ring Chromosome

A ring chromosome can form when a deletion occurs on both tips of a chromosome and the remaining chromosome ends fuse together (the karyotype for a female with a ring chromosome X would be 46,X,r[X]). Ring chromosomes are often lost, resulting in a monosomy (e.g., loss of a ring X chromosome would produce Turner syndrome). They have been observed at least once for each human chromosome.

Isochromosome

When a chromosome divides along the axis perpendicular to its normal axis of division, an **isochromosome** is created (i.e., two copies of one arm but no copy of the other). Because of the lethality of autosomal isochromosomes, most isochromosomes observed in live births involve the X chromosome. The karyotype of an isochromosome for the long arm of the X would be 46,X,i(Xq); this karyotype results in an individual with Turner syndrome, indicating that most of the critical genes responsible for the Turner phenotype are on Xp.

ADVANCES IN MOLECULAR CYTOGENETICS

Although chromosome abnormalities are still commonly visualized by examining metaphase chromosomes under a microscope, several powerful new techniques combine cytogenetics with modern molecular methods. Two of the most important are described here.

Fluorescence *In Situ* Hybridization

In fluorescence *in situ* hybridization (FISH), a chromosome-specific DNA segment is labeled with a fluorescent tag to create a **probe**. This probe is then hybridized with the patient's chromosomes, which are visualized under a fluorescence microscope. Because the probe will hybridize only with a complementary DNA sequence, it will mark the presence of the chromosome segment being tested. For example, a probe that is specific for chromosome 21 will hybridize in three places in the cells of a trisomy 21 patient, providing a diagnosis of Down syndrome. FISH is also used commonly to detect deletions: an analysis using a probe that hybridizes to the region of 15q corresponding to Prader-Willi syndrome (Chapter 1) will show only a single signal in a patient, confirming the diagnosis of this deletion syndrome. An advantage of FISH is that chromosomes do not have to be in the metaphase stage for accurate diagnosis: even though interphase or prophase chromosomes cannot be clearly visualized themselves, the number of hybridization signals can still be counted accurately.

Note

Structural Abnormalities

- Translocations (Robertsonian and reciprocal)
- Deletions and duplications
- Inversions (pericentric and paracentric)
- Ring chromosomes
- Isochromosomes

Spectral Karyotyping

Spectral karyotyping involves the use of five different fluorescent probes that hybridize differentially to different sets of chromosomes. In combination with special cameras and image-processing software, this technique produces a karyotype in which every chromosome is "painted" a different color. This allows the ready visualization of chromosome rearrangements such as small translocations (e.g., the Philadelphia chromosome rearrangement discussed above).

Chapter Summary

Cytogenetics is the study of microscopically observable chromosomal abnormalities.

Diseases can be caused by abnormalities in chromosome number or structure.

Numerical chromosome abnormalities

Euploidy (multiple of 23 chromosomes):

- Haploid (23, normal gametes)
- Diploid (46, normal somatic cells)
- Triploid (69, lethal)
- Tetraploid (92, lethal)

Aneuploidy (loss or gain of specific chromosomes, usually caused by nondisjunction during meiosis):

- Trisomy 21 (Down syndrome)
- Trisomy 18 (Edwards syndrome)
- Trisomy 13 (Patau syndrome)
- 47,XXY (Klinefelter syndrome, male)
- 45,X (Turner syndrome, female)

Structural chromosome abnormalities

Translocations:

- Reciprocal (chronic myelogenous leukemia)
- Robertsonian (5% of Down syndrome cases)

Deletions (cri-du-chat syndrome)

Inversions

Ring chromosomes

Isochromosomes

New methods for studying chromosomes:

- FISH
- Spectral karyotyping

Review Questions

1. A 26-year-old woman has produced two children with Down syndrome, and she has also had two miscarriages. Which of the following would be the best explanation?
 - A. Her first cousin has Down syndrome.
 - B. Her husband is 62 years old.
 - C. She carries a Robertsonian translocation involving chromosomes 14 and 21.
 - D. She carries a reciprocal translocation involving chromosomes 14 and 18.
 - E. She was exposed to multiple X-rays as a child.
2. A 6-year-old boy has a family history of mental retardation and has developmental delay and some unusual facial features. He is being evaluated for possible Fragile X syndrome. Which of the following would be most useful in helping to establish the diagnosis?
 - A. Genetic test for a trinucleotide repeat expansion in the fragile X gene
 - B. IQ test
 - C. Karyotype of the child's chromosomes
 - D. Karyotype of the father's chromosomes
 - E. Measurement of testicular volume

Answers

1. **Answer: C.** As a translocation carrier, it is possible that she can transmit the translocated chromosome, containing the long arms of both 14 and 21, to each of her offspring. If she also transmits her normal copy of chromosome 21, then she will effectively transmit two copies of chromosome 21. When this egg cell is fertilized by a sperm cell carrying another copy of chromosome 21, the zygote will receive three copies of the long arm of chromosome 21. The miscarriages may represent fetuses that inherited three copies of the long arm and were spontaneously aborted during pregnancy.

Although the risk for Down syndrome increases if a woman has had a previous child, there is no evidence that the risk increases if a more distant relative, such as a first cousin, is affected (**choice A**).

Although there is conclusive evidence for an increased risk of Down syndrome with advanced maternal age, there is little or no evidence for a paternal age effect on Down syndrome risk (**choice B**).

An extra copy of material from chromosome 14 or 18 (**choice D**) could result in a miscarriage, but neither would produce children with Down syndrome, which is caused by an extra copy of the long arm of chromosome 21.

Heavy irradiation has been shown to induce nondisjunction in some experimental animals, but there is no good evidence for a detectable effect on human trisomy (**choice E**).

2. **Answer: A.** The presence of an expanded trinucleotide repeat in 5' untranslated region of the gene is an accurate test for fragile X syndrome.

An IQ test (**choice B**) would be useful because the IQ is typically much lower than average in Fragile X syndrome patients. However, many other syndromes also include mental retardation as a feature, so this would not be a specific test.

A karyotype of the child's chromosomes (**choice C**) might reveal X chromosomes with the decondensed long arm characteristic of this syndrome, but not all X chromosomes have this appearance in affected individuals. Thus, the karyotype may yield a false-negative diagnosis.

The father's chromosomes (**choice D**) will not be relevant because this is an X-linked disorder.

Testicular volume (**choice E**) is increased in males with fragile X syndrome, but this is observed in postpubertal males.

Gene Mapping and Cloning

4

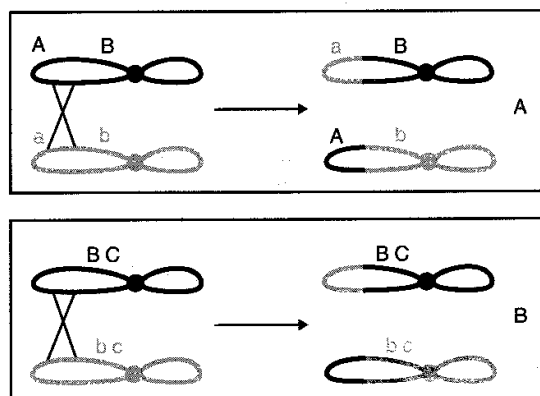
OVERVIEW

An important step in understanding the basis of an inherited disease is to locate the gene(s) responsible for the disease. This chapter provides an overview of the techniques that have been used to map and clone thousands of human genes.

GENE MAPPING: LINKAGE ANALYSIS

Crossing Over, Recombination, and Linkage

Haploid germ cells (sperm and ova) are produced during meiosis. During prophase I of meiosis, homologous chromosomes line up and occasionally exchange portions of their DNA. This process is termed **crossover**. When a crossover event occurs between two loci, A and B, the resulting chromosomes may contain a new combination of alleles at loci A and B (Fig II-4-1). When a new combination occurs, the crossover has produced a **recombination**. Because crossover events occur more or less randomly across chromosomes, loci that are located farther apart are more likely to experience an intervening crossover and thus a recombination of alleles. This provides a means of assessing the distance between loci on chromosomes, a key goal of gene mapping.



*A. A crossover event occurs between loci A and B, producing a recombination of alleles in the chromosomes transmitted to an offspring.
B. Loci B and C are more closely linked, so the crossover does not produce a recombination of their alleles.*

Figure II-4-1. A Crossover Event

Note

- Loci located on the same chromosome are syntenic.
- Loci located close together on the same chromosome are linked.
- A crossover that occurs between two linked loci can produce a recombination of alleles.
- The farther apart two loci, the more likely a recombination will be seen.

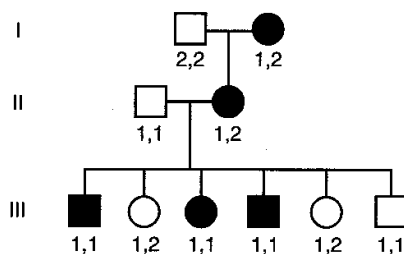
Alleles of loci that are close together on the same chromosome are likely to be inherited together; these loci are said to be **linked**. If two loci are on different chromosomes, or if they are far apart on the same chromosome, their alleles will be transmitted independently. This means that if an allele at one locus is transmitted, there is a 50% chance (as in coin tossing) that a given allele at the other locus will also be transmitted. Linked loci are close enough together so that the chance of a recombination is less than 50%. Thus, their inheritance is not independent.

Loci that are on the same chromosome are said to be **syntenic** ("same thread"). Linked loci are always on the same chromosome, so they are always syntenic. However, not all syntenic loci are linked (i.e., loci located far apart on a chromosome may experience 50% recombination and thus be unlinked).

Recombination Frequencies and Gene Mapping

The distance between two loci can be inferred by estimating the frequency with which crossovers occur between them. Because this is done by looking at recombinations in families, we refer to this as a recombination frequency.

A brief example illustrates the concept of recombination frequency. Consider the pedigree for neurofibromatosis type 1 shown in Figure II-4-2. Each family member has been assayed for a polymorphism that has two alleles, labeled 1 and 2. (This polymorphism, often termed a **marker**, could be an RFLP or, more commonly, a short tandem repeat polymorphism; see below.) The genotype of each individual is shown in the pedigree. We wish to determine whether the marker locus is linked to the neurofibromatosis gene, and, if so, how close the two loci are to one another. Examination of generations I and II allows us to determine that, under the hypothesis of linkage, the mother in generation I transmitted a chromosome to her daughter that contains allele 1 of the marker locus and the neurofibromatosis mutation. The chromosome the daughter received from her father does not contain the disease-causing mutation because he is not affected, and it must contain allele 2 of the marker because the father is homozygous for the marker locus.



A family in which a mutation causing neurofibromatosis type 1 is transmitted in three generations. The genotype of a marker locus is shown for each pedigree member.

Figure II-4-2. Pedigree for Neurofibromatosis Type 1

Note

A **haplotype** designates the specific alleles of multiple loci on a single chromosome

We now know the **linkage phase** in the daughter: the chromosome containing the neurofibromatosis mutation has allele 1 of the marker, whereas the chromosome containing the normal allele has allele 2 of the marker. We can then designate the daughter's two **haplotypes** (from "haploid genotype," this term designates the genotypes of multiple loci on each member of a chromosome pair). If the disease-causing and normal alleles are designated N and n, respectively, then the

daughter's two haplotypes are N1 and n2, or N1/n2. We can predict that, under linkage, the offspring in generation 3 who receive marker 1 from their mother will also receive the disease-causing mutation (i.e., the N1 haplotype), whereas the offspring who receive marker 2 from their mother will receive the normal allele (the n2 haplotype) and will thus be unaffected. Because the mother mates with an individual who has the 1,1 genotype for the marker, offspring with a 1,1 genotype should be affected, whereas offspring with a 1,2 genotype should be unaffected.

Examination of the offspring in generation 3 shows that our prediction is met in all cases but one: the last son in the pedigree has a 1,1 genotype but is unaffected. We infer that a crossover occurred in the chromosome transmitted from the mother to this offspring. Thus, on the basis of the limited information provided by this family, we estimate the recombination frequency to be one sixth, or about 17%. In other words, we predict that, about 17% of the time, a crossover will occur between the marker locus and the disease locus. In practice, a much larger sample of families is used to provide a more accurate estimate of the recombination frequency. If the recombination frequency is estimated to be 50%, the two loci are unlinked and may be on different chromosomes.

The recombination frequency provides a measure of the **genetic distance** between any pair of linked loci. Genetic distances are often expressed in **centiMorgans (cM)**. One centiMorgan is equal to a 1% recombination frequency between two loci (for example, two loci that are 10 cM apart would have a recombination frequency of 10%). Physically, 1 cM is approximately equal to 1 million base pairs of DNA (1 Mb). This relationship is only approximate, however, because crossover frequencies vary somewhat throughout the genome (e.g., crossovers are less common near centromeres and more common near telomeres).

LOD Scores

In the example given above, it is quite possible that because of the small size of our sample, the two loci only appear to be linked at 17 cM—the result could easily have been obtained simply by chance. To estimate the likelihood that two loci are truly linked with a specific recombination frequency, a LOD score is used. The LOD (“log of the odds”) is estimated as:

$$\text{LOD} = \log_{10} \frac{P(\text{recombination frequency} = \theta)}{P(\text{recombination frequency} = 50\%)}$$

In this simple equation, the probability, P , of a recombination frequency of θ is compared with the probability of a recombination frequency of 50% (no linkage). We thus compare the odds of linkage at a given distance against no linkage, and then take the common logarithm, to derive the LOD score. In practice, LOD scores are estimated using computers, and the details of this procedure are beyond the scope of this review, but the equation shows us that the LOD score increases as the probability in the numerator becomes larger than the probability in the denominator (i.e., linkage becomes more likely than nonlinkage). A logarithm is used because it allows LOD scores from individual families to be added together to obtain an overall LOD score. A LOD score greater than 3 is usually interpreted as statistical evidence of linkage (i.e., the numerator is 1,000 times greater than the denominator, indicating that linkage is 1,000 times more likely than nonlinkage). If the numerator is less than the denominator, the LOD score becomes negative, indicating that nonlinkage is more likely than linkage. Conventionally a LOD score of -2 or less is taken as evidence that two loci are not linked (i.e., nonlinkage is 100 times more likely than linkage).

In searching for disease-causing loci, family data are collected, and then several hundred marker loci are typed throughout the genome (there are now more than 20,000 polymorphic markers in the genome, each with a known position on a specific chromosome). As each marker is typed in family members, it is tested for linkage using the LOD score approach. When a LOD score of 3 or greater is reached, the approximate location of the disease-causing locus has been identified by its linkage with a marker polymorphism: the gene has been mapped.

Note

For Linked Loci:

- 1 centiMorgan (cM) = 1% recombination frequency
- 1 cM \approx 1 million base pairs

Note

- A LOD score > 3 indicates linkage; a LOD score < -2 indicates no linkage.
- The value of θ at which the highest LOD score is seen is the most likely estimate of the recombination frequency.
- For any pair of loci, LOD scores obtained from different families can be added together.

Clinical Correlate**Adult Polycystic Kidney Disease and Locus Heterogeneity**

Adult polycystic kidney disease (APKD) is one of the most common autosomal dominant diseases, affecting about 1/1,000 whites. The key feature of this disease is the progressive accumulation of renal cysts, which ultimately culminate in kidney failure. APKD is responsible for approximately 10% of end-stage renal disease in North America. Patients may also have hypertension, cerebral aneurysms, liver cysts, and cardiac valvular defects. Linkage analysis has identified a disease-causing gene on chromosome 16, as well as a second disease-causing gene on chromosome 4 (locus heterogeneity, see Chapter 1). In some families, mutations in the chromosome 16 gene cause APKD, whereas in others, the disease is caused by mutations in the chromosome 4 gene. The disease states produced by mutations in the two different genes are sometimes clinically indistinguishable. By identifying the two distinct loci that can cause APKD, linkage analysis provides evidence for locus heterogeneity and thus paves the way for a more accurate genetic diagnosis in families.

Gene mapping by linkage analysis serves several important functions:

- As demonstrated already, it can define the approximate location of a disease-causing gene.
- Linked markers can be used to determine whether a family member has received the chromosome containing the disease-causing mutation or the other (unaffected) member of the chromosome pair from a carrier or affected parent (this form of genetic diagnosis is discussed further in Chapter 6).
- Linkage analysis can identify locus heterogeneity (see Chapter 1), which must be identified to perform an accurate genetic diagnosis (see Clinical Correlate).

Linkage Disequilibrium

Two linked loci are said to be in **linkage disequilibrium** if specific combinations of alleles at the loci are seen together on chromosomes more often than expected by chance. For example, if the frequency of allele 1 at locus A (A1) is 0.7 in a population and the frequency of allele 1 at locus B (B1) is 0.6, then the expected proportion of chromosomes bearing the combination A1/B1 is given by the product of the frequencies of A1 and B1: $0.7 \times 0.6 = 0.42$. If we examine a series of chromosomes in a population and find that A1 and B1 are found on the same chromosome significantly more often or less often than expected (e.g., suppose the frequency of chromosomes containing both alleles is only 0.3), then the two loci are in linkage disequilibrium. Through time, recombination between two loci tends to distribute alleles so that they are in linkage equilibrium. Because recombination is rare for very closely linked loci, such loci are more likely to exhibit linkage disequilibrium (Fig II-4-3). Thus, the measurement of linkage disequilibrium in populations can be useful in gene mapping.

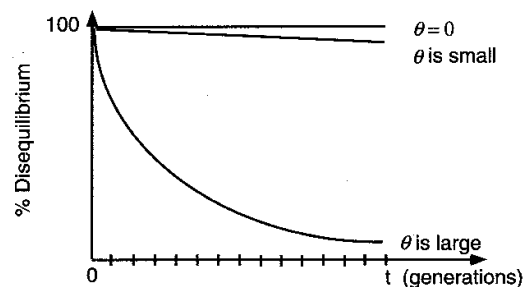


Figure II-4-3. Duration of Linkage Disequilibrium as a Function of Time for Loci Showing Different Recombination Frequencies (θ)

Polymorphic Markers and Linkage Analysis

A prerequisite for successful linkage analysis is the availability of a large number of highly polymorphic markers dispersed throughout the genome. Several major types of markers are now available (Fig II-4-4). These are also discussed in Section I, Chapter 7: Genetic Testing.

- RFLPs (restriction fragment length polymorphisms). These are polymorphisms in which the presence or absence of a restriction site produces fragments of varying lengths that can be visualized on a Southern blot.
- VNTRs (variable number of tandem repeats). These polymorphisms are the result of varying numbers of minisatellite repeats in a specific region of a chromosome. The repeat units typically range in size from 20 to 70 bases each. The repeat is flanked on both sides by a restriction site, and variation in the number of repeats produces restriction fragments of varying size.
- Short tandem repeat polymorphisms (STRPs). Polymorphism is produced by variation in the number of microsatellite repeats, which range in size from 2 to 6 bases. These blocks of repeats are much shorter in length than those of VNTRs, and they are amplified through PCR (Section I, Chapter 7: Genetic Testing; PCR), with PCR primers designed to flank the repeat block. Variation in the number of repeats produces PCR products of varying size, which are then visualized on an agarose gel or in an automated genotyping machine.
- Single nucleotide polymorphisms (SNPs). These single-base polymorphisms can be assayed by DNA sequencing or through the use of DNA chips (see Chapter 6).

Note

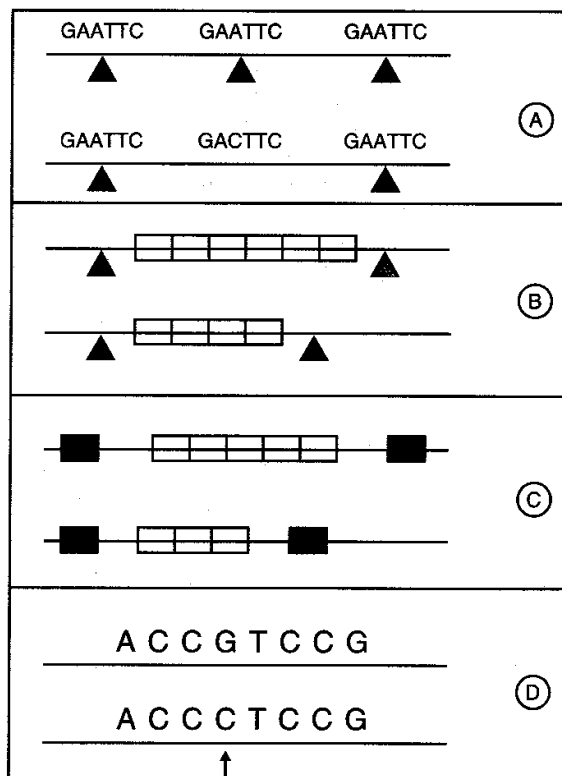
Linkage Disequilibrium

- Specific alleles at two linked loci are observed together as a haplotype more frequently than predicted by chance alone.
- May be used as a mapping tool.
- The greater the observed linkage disequilibrium, the closer together the linked loci.

Note

Major types of polymorphic markers include:

- RFLPs (restriction fragment length polymorphisms)
- VNTRs (variable number of tandem repeats)
- STRPs (short tandem repeat polymorphisms)
- SNPs (single nucleotide polymorphisms)



- A. In an RFLP, the presence or absence of a restriction site (▲) produces DNA fragments of varying lengths, reflecting sequence variation.
- B. In a VNTR, variation in fragment lengths is produced by differences in the number of tandem repeats located between two restriction sites (▲).
- C. In an STRP, variation in fragment lengths is produced by differences in the number of microsatellite repeats found between two PCR primer sites (■).
- D. SNPs are single differences in a nucleotide sequence.

Figure II-4-4. Different Types of DNA Polymorphisms

GENE CLONING

Although linkage analysis is invaluable for determining the approximate location of a gene, it seldom pinpoints the gene itself. In this section, we review some basic approaches for the pinpointing, or cloning, of genes. A **clone** is an identical copy. When a gene is cloned, it has been uniquely identified, such that copies of it can be made for various applications (e.g., DNA sequencing, insertion into vectors for gene therapy). For a discussion of gene cloning techniques, see Molecular Biology and Biochemistry, Chapter 6: Recombinant DNA and Gene Cloning.

Positional Candidates

Typically, linkage analysis may localize a gene to a region of one to several million bases. Dozens of genes may be located within a region of this size. As the Human Genome Project nears its completion (see note), it becomes more and more likely that some of the genes within a region have been identified. These are potential **candidates** within the region. If a candidate's protein product is known, or if it has a high degree of sequence similarity to another known gene, the investigator can assess the likelihood that the candidate would be responsible for the inherited disease in question (e.g., if the disease involves liver defects, one would expect the gene's protein product to be related in some way to the liver).

Once a candidate has been identified, the DNA sequence of this gene can be compared in affected individuals and unaffected controls. If specific mutations are found within the DNA sequence in affected cases but not in controls, it is highly likely that the gene is responsible for the disease.

Positional Cloning

Often, an identified region does not contain any plausible candidates. Several methods can be used to identify all of the genes within a region and to assess their potential involvement in a given disease.

CG Islands

The great majority of CG dinucleotides in the human genome are methylated. About 60% of human genes, however, have a series of unmethylated CG dinucleotides, termed CG islands, in their 5' region. These can serve as the "signature" of a gene and thus help to distinguish coding from noncoding sequence.

Cross-Species Conservation

This is another approach for identifying coding versus noncoding DNA. Because coding DNA serves an essential function in the individual, it is much more likely to be similar across a variety of species than is noncoding DNA (which is more free to mutate and thus diverge rapidly among species). A labeled probe is constructed from the DNA sequence being analyzed, and it is tested for hybridization with the DNA of a series of different organisms (e.g., human, ape, mouse, etc.). This can be done, for example, using a Southern blot (see Section I, Chapter 7, Blotting Techniques). If the human DNA sequence in the probe hybridizes with DNA from other organisms, it is more likely to represent coding DNA and thus to be part of a gene.

Computer Analysis

Increasingly, DNA sequences are being analyzed by computer algorithms to help decide whether they contain genes. Computers can often identify the characteristic "signatures" of genes (e.g., the DNA bases that are typically seen at the beginning or end of a gene, or those that are seen at intron-exon boundaries).

Animal Models

A human DNA sequence can be tested for similarity with known genes in experimental animals (e.g., mouse). If the human DNA sequence is similar to an animal gene that encodes a protein product related to the disease in question, then the DNA sequence becomes a possible candidate.

Note

The Human Genome Project

The major goal of the Human Genome Project, initiated in 1991, is the identification of the entire 3 billion-base pair human DNA sequence. As of mid-2000, this goal is 90% complete; the finished sequence should be available within 2–3 years. By identifying the entire human DNA sequence, it will be much easier to identify each of the estimated 50,000–100,000 protein-coding genes located within the sequence. Additionally, important regulatory sequences, located outside the coding DNA, will be identified.

Mutation Screening

As with positional candidates, a critically important test (once coding DNA has been identified) is the isolation of mutations in the DNA sequence that are specific to affected individuals.

Gene Expression

The DNA sequence being analyzed can be used in a Northern blot (see biochemistry review) to determine whether it is expressed in tissues known to be affected by the disease.

Chapter Summary

Linked genes are likely to be inherited together because they are close together on the same chromosome. Crossovers that occur between two linked loci during meiosis can produce a recombination of alleles. The frequency of recombination gives a measure of the distance between two genes.

LOD scores:

- > 3 indicates linkage
- < -2 indicates no linkage

Types of polymorphic markers:

- RFLPs (restriction fragment length polymorphisms)
- VNTRs (variable number of tandem repeats, minisatellites)
- STRs (short tandem repeats, microsatellites)
- SNPs (single nucleotide polymorphisms)

Linkage analysis aids in the cloning of genes by identifying positional candidates.

Protein-coding genes within a chromosomal region can be identified by finding:

- Promoter regions
- Exon-intron boundaries
- CG islands
- Mutations specific to affected individuals

Genetics of Common Diseases

5

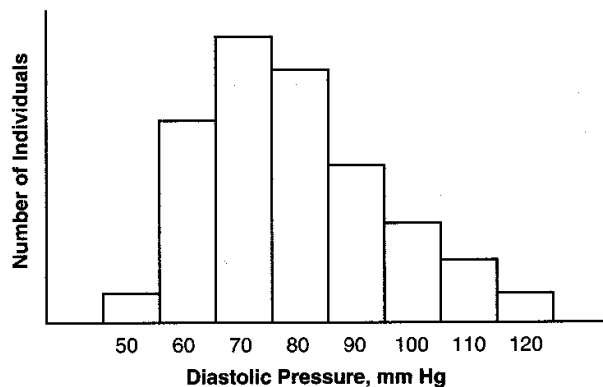
OVERVIEW

Previous discussion has dealt with diseases caused by an alteration in a single gene or in a specific chromosome. Most common diseases (heart disease, cancer, diabetes, etc.) have substantial genetic components, but their causation is complex. This chapter reviews some basic principles of the genetics of common, complex diseases.

MULTIFACTORIAL INHERITANCE

Multifactorial Model

The term **multifactorial** refers to the fact that most common diseases are caused by multiple factors: their expression is influenced simultaneously by multiple genes (i.e., a **polygenic** component) and by environmental factors. Because multiple factors are involved in the causation of these traits, they tend to follow a normal (“bell-shaped”) distribution. An example would be the distribution of diastolic blood pressures in a population (Fig II-5-1). Other traits that have multiple genetic and environmental components include height, weight, and IQ.

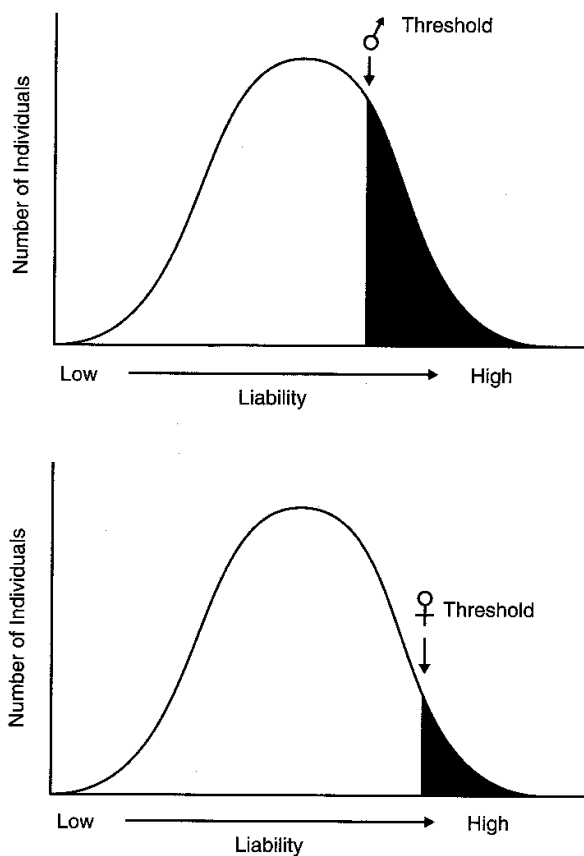


The effects of multiple factors tend to produce a normal distribution.

Figure II-5-1. The Distribution of Diastolic Blood Pressure in a Population of Individuals

Multifactorial Threshold Model

Many disease traits are either present or absent in the individual (e.g., cleft lip and/or palate, club foot, congenital heart defects, cancer, diabetes). These traits are also thought to correspond to the multifactorial model, but the distribution here refers to an underlying **liability** to develop disease. Expression of the disease occurs only when a specific **threshold** is reached (Fig II-5-2). For some multifactorial diseases, the threshold differs in males and females.



In the example, the male threshold is lower than the female threshold, so the prevalence of the disease is higher in males than in females.

Figure II-5-2. A Liability Distribution for a Multifactorial Disease

Patterns of Multifactorial Inheritance

The inheritance patterns of multifactorial diseases differ from those of single-gene disorders in several important ways.

- For single-gene disorders, the mechanism of gene action is understood (e.g., cystic fibrosis is caused by an autosomal recessive mutation, neurofibromatosis is produced by an autosomal dominant mutation, etc.), and recurrence risks can be derived based on known principles of inheritance. In contrast, the genes and environmental factors underlying multifactorial traits have not been identified specifically. Consequently, empirical recurrence risks (i.e., based on direct observation of data) must be derived. For example, if we wish to know the recurrence risk for siblings of cases with cleft lip and/or palate, we ascertain a large cohort of individuals with cleft lip and/or palate and then measure the proportion of their siblings who are also affected with cleft lip and/or palate (in this case, the sibling recurrence risk is approximately 3%, which is considerably higher than the general population prevalence of 0.1%).
- Recurrence risks for single-gene traits remain the same regardless of the number of affected individuals in the family (e.g., the recurrence risk for cystic fibrosis is 25% in a carrier-by-carrier mating even if several previous siblings have all been affected). Multifactorial recurrence risks increase as the number of affected relatives (e.g., siblings) increases. This does not mean that the true risk has changed; rather, it reflects the fact that additional affected individuals provide more information about the true risk. The presence of multiple affected individuals indicates that the family is located higher on the liability distribution (i.e., they likely have more genetic and environmental risk factors). For example, one study showed that sibling recurrence risk for a neural tube defect (spina bifida or anencephaly; see Clinical Correlate) was 3% if one sibling was affected, 12% if two were affected, and 25% if three were affected.
- Recurrence risks for multifactorial disorders increase if the affected individual has a more severe expression of the disease. Again, this reflects the fact that the individual and his or her relatives are located higher on the liability distribution.
- The recurrence risk is higher if the affected individual (i.e., the proband) is a member of the less commonly affected sex. This principle follows from the fact that an affected individual of the less commonly affected sex will be, on average, higher on the liability distribution (Fig II-5-2). For example, the prevalence of pyloric stenosis (congenital constriction of the pylorus) is approximately 1/1,000 for females and 1/200 for males. Thus, the average affected female is likely to be located higher on the liability distribution than is an affected male (i.e., the female has more genetic and environmental risk factors). The presence of more risk factors implies that the affected female's relatives are more likely to be affected than are the affected male's relatives.
- For multifactorial diseases, the recurrence risk decreases rapidly for more remotely related relatives. For example, one study of autism reported a sibling risk of 4.5%, an uncle-niece risk of 0.1%, and a first-cousin risk of 0.05%. In contrast, the risk of carrying a single-gene mutation decreases by only 1/2 with each successive degree of relationship (i.e., 50% chance for siblings, 25% for uncle-niece relationships, and 12.5% for first cousins).
- Although the recurrence risk for a single-gene disorder remains the same regardless of the prevalence of the disease in a population, the empirical risk for multifactorial diseases increases as the population prevalence increases. This is because populations with higher prevalence rates have a higher preponderance of genetic and environmental risk factors. This in turn raises the risk for relatives of affected individuals.

Note

Recurrence Risks for Multifactorial Diseases

- Are estimated empirically
- Increase as the number of affected relatives increases
- Increase as the severity of the disease expression increases
- Increase if the proband is a member of the lower-risk gender
- Decrease very rapidly for more remotely related relatives
- Increase as the prevalence of the disease increases in a population

Clinical Correlate: Neural Tube Defects

Neural tube defects (NTDs: anencephaly, spina bifida, and encephalocele) are one of the most common congenital malformations and are seen in approximately 1 in 1,000 births in the United States. Anencephaly (partial or complete absence of the brain) usually leads to a stillbirth, and anencephalics that survive to term do not live for more than a few days. Spina bifida, a protrusion of spinal tissue through the vertebral column, produces secondary hydrocephalus in 75% of cases and often produces some degree of paralysis. Improved intervention strategies have increased survival rates substantially for this condition, with more than two thirds of patients now surviving beyond 10 years of age. The sibling recurrence risk for NTDs is estimated to be 2–5%, which is much higher than the population prevalence. Thus, the disorder clusters in families. Recent epidemiologic studies show that 50–70% of NTDs can be prevented by periconceptional dietary folic acid supplementation. Folic acid deficiency is likely to be present in successive pregnancies, providing a nongenetic explanation for some of the familial clustering of this disease. However, there is also evidence for genetic variation in the ability to metabolize folic acid. Thus, NTDs provide an example in which familial clustering is likely related to both genetic and nongenetic factors.

NATURE VERSUS NURTURE: TWIN AND ADOPTION STUDIES**Twin Studies**

It is a major challenge to disentangle the effects of common genes and common environment when studying the causes of multifactorial diseases. A classic means of doing so is the twin study. **Monozygotic** (MZ, or “identical”) twins are formed when an embryo is cleaved during early development. The result is two genetically identical embryos. **Dizygotic** (DZ, or “fraternal”) twins are the result of the fertilization of two different ova by two different sperm cells. DZ twins are genetically the same as siblings, sharing 50% of their genes.

If we wish to gauge the relative effect of genetic inheritance on a trait, we can compare the **concordance** of a trait in MZ and DZ twins (two individuals are concordant if they share the same trait; if they do not share the trait, they are discordant). For a trait that is “determined” completely by genes, we would expect a concordance of 100% for MZ twins because they share all of their genes. The concordance rates for DZ twins should be only about 50%. In contrast, a trait that has no genetic basis should have equivalent concordance rates for MZ and DZ twins. Table II-5-1 illustrates concordance rates for some common genetic diseases. As this table demonstrates, twin studies indicate that genes play a role in the causation of most common diseases. Although twin studies have been very useful for estimating the relative effects of genes and environment, several potential biases exist:

- It is assumed that the environments of MZ and DZ twins are equally similar. However, MZ twins are often treated more similarly than are like-sexed DZ twins.
- Somatic mutations may occur in MZ twins after the cleavage event that forms them, causing “identical” twins to be at least somewhat different genetically.
- The uterine environments of MZ twins will be more or less similar depending on whether there are two amnions and two chorions, two amnions and a shared chorion, or a shared amnion and chorion.

Table II-5-1. Examples of Concordance Rates in MZ and DZ Twins

Disease or Trait	MZ Twin Concordance	DZ Twin Concordance
Affective disorder (bipolar)	0.79	0.24
Affective disorder (unipolar)	0.54	0.19
Club foot	0.32	0.03
Diabetes mellitus (type 1)	0.35–0.50	0.05–0.10
Diabetes mellitus (type 2)	0.70–0.90	0.25–0.40
Epilepsy (idiopathic)	0.69	0.14
Height*	0.94	0.44
IQ*	0.76	0.51
Measles	0.95	0.87
Multiple sclerosis	0.28	0.03
Myocardial infarction (male)	0.39	0.26
Myocardial infarction (female)	0.44	0.14
Schizophrenia	0.47	0.12
Spina bifida	0.72	0.33

*Because these are quantitative traits, correlation coefficients, rather than concordance rates, are given.

Heritability

Keeping these biases in mind, data from twin studies can be used to estimate the **heritability** of a trait (i.e., the proportion of variation in a trait that can be attributed to genetic factors). One simple measure of heritability is $(C_{MZ} - C_{DZ}) / (1 - C_{DZ})$, where C_{MZ} is the concordance rate for MZ twins and C_{DZ} is the concordance rate for DZ twins. For example, studies of alcoholism show that the concordance rate for MZ twins tends to be 0.6 or higher, whereas it is 0.3 or lower for DZ twins. If these figures are used in the equation, the heritability of alcoholism is estimated to be 0.43.

Adoption Studies

Another way of assessing the relative effects of genes and environment is to measure the incidence of a trait in individuals whose biological parent has the trait but who were adopted by parents who do not have the trait. Thus, the individual has received the predisposing genes (if any) from the affected parent, but he or she does not share environmental factors with the affected biological parent. For example, the population prevalence of schizophrenia is approximately 1%. For those who have one schizophrenic parent, the risk of developing the disease is approximately 10% if they are reared by that parent. For those who have an affected parent but are adopted by unaffected parent, the risk of developing schizophrenia has been estimated as 8%—a slight decrease, but much higher than the **general population** risk of 1%. This result supports the involvement of genes in the etiology of **schizophrenia**.

Note

Concordance rates are compared in monozygotic (identical) and dizygotic (fraternal) twins to assess the contribution of genes to a trait. These rates can be used to estimate heritability, the proportion of variation in a trait caused by genes.

Clinical Correlate**Key Features of LDL Receptor-Associated Familial Hypercholesterolemia**

- Elevated LDL cholesterol
- Accelerated atherosclerosis and increased risk of myocardial infarction
- Xanthomas (fatty deposits) in skin, eyelids, and tendons

COMMON DISEASES: EXAMPLES**Coronary Heart Disease**

Coronary heart disease accounts for approximately 25% of all deaths in the United States. This disease clusters in families: an individual with a positive family history of coronary heart disease is 2–7 times more likely to have heart disease than is an individual with no family history. The risk tends to be higher if more family members are affected, if the affected members are female (the less commonly affected sex), and if the age of onset in relatives is early (e.g., before age 55).

A number of genes have been shown to play a role in the causation of coronary heart disease, including more than a dozen that encode proteins involved in lipid metabolism and transport. The best known of these genes encodes the receptor for low-density lipoprotein (LDL). Mutations in the LDL receptor produce a deficit in receptor abundance or activity, resulting in increased circulating LDL levels and familial hypercholesterolemia. This autosomal dominant disease affects approximately 1 in 500 individuals and results in cholesterol levels that are approximately twice the normal level (i.e., roughly 300–400 mg/dl). The consequence is a 3- to 4-fold increase in the risk of coronary heart disease in heterozygotes. Homozygotes, who have a prevalence of approximately 1 in 1,000,000, present with cholesterol levels that are several times higher than normal. If untreated, most die of myocardial infarction before the age of 30.

Cancer

Cancer is the second leading cause of death in the United States. It is estimated that one third of cancer cases can be attributed to cigarette smoking and another one third to dietary factors. Like heart disease, the common cancers (e.g., breast, colon, prostate) cluster in families. Family history is thus another important risk factor, and genes play a role in all of the common cancers. In addition, a number of **inherited cancer syndromes** have been identified in which family members have a high degree of predisposition to a cancer as a result of a single-gene mutation.

Inherited Cancer Syndromes, Tumor Suppressors, and Oncogenes

Inherited cancer syndromes are usually the result of a germline mutation in a **tumor suppressor** gene or in a **proto-oncogene**. Tumor suppressor genes encode proteins whose normal function helps to prevent tumor formation (e.g., the protein may be involved in regulating the cell cycle). Proto-oncogenes encode proteins involved in various aspects of cellular growth and proliferation (e.g., cellular growth factors, growth factor receptors, signal transduction factors, nuclear transcription factors). When mutated, a proto-oncogene can become an oncogene, which produces a protein that can lead to tumor formation (e.g., an overactive growth factor receptor can lead to cellular proliferation). Mutations in tumor suppressor genes and proto-oncogenes can result in inherited cancer syndromes when they arise in the germline and are then potentially passed on to successive generations.

Table II-5-2. Key Features of Oncogenes and Tumor Suppressor Genes

Oncogenes	Tumor Suppressor Genes
Normal version (proto-oncogene) is usually involved in promoting cell growth/proliferation	Normal version of gene is typically involved in controlling cell proliferation (e.g., cell cycle control, cell adhesion control)
Mutation is typically a gain of function	Mutation is typically a loss of function
A mutation of one copy of the gene in a cell is sufficient to promote tumor growth	Mutations of both copies (two hits) of the gene are typically necessary to promote tumor growth

Disease Example: Retinoblastoma

An example of a cancer caused by a tumor suppressor mutation is retinoblastoma. This is the most common childhood eye tumor, affecting approximately 1/20,000 children. About one half of retinoblastoma cases are familial, with the phenotype following an autosomal dominant inheritance pattern. Penetrance for heterozygous gene carriers is approximately 90%. The predisposition to retinoblastoma is caused by the inheritance of a mutation of *RB1*, a tumor suppressor gene on chromosome 13; however, inheritance of this mutation alone is not sufficient to produce a tumor. For this to happen, the developing fetus must experience a second mutation in the other copy of *RB1* in a specific retinoblast (i.e., a somatic mutation). Normally, the *RB1* protein product regulates the cell cycle to cause cells to divide in a controlled manner. Once both copies of the tumor suppressor gene are mutated in a cell, cell cycle control is lost, and the cell can divide uncontrollably, leading to tumor formation (Fig II-5-3). This model of carcinogenesis is termed the **two-hit model**. The first "hit," or mutation, is inherited, but a second mutation must occur in a retinoblast to cause tumor formation. This model accounts for reduced penetrance: those individuals who do not develop a retinoblastoma were fortunate enough to not experience a somatic mutation in any of their retinoblasts.

The two-hit model applies to a number of other inherited neoplasias, including familial breast cancer, familial colon cancer, familial melanoma, and neurofibromatosis.

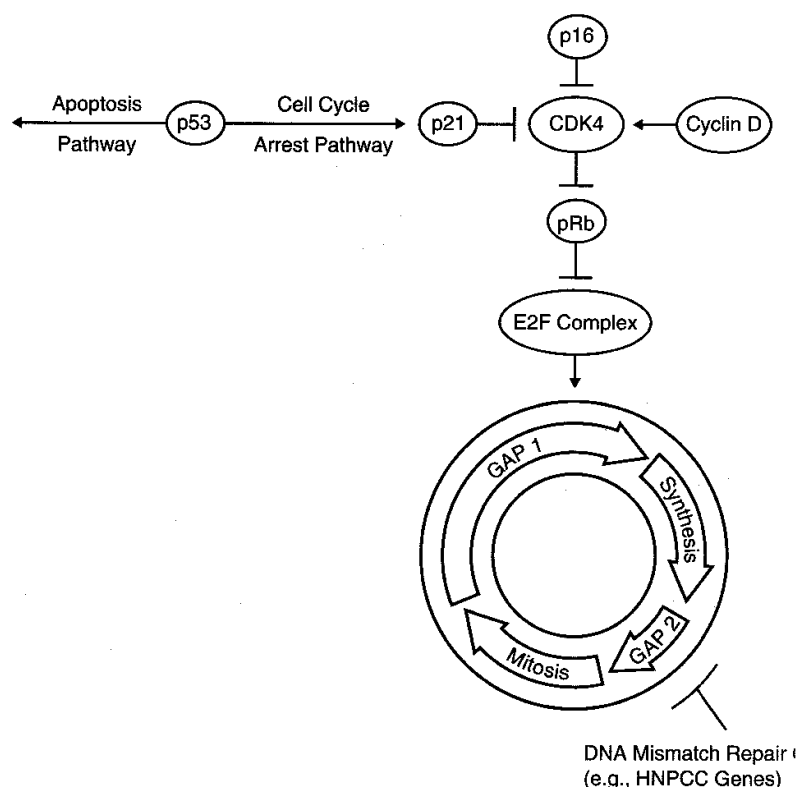


Figure II-5-3. Cell-Cycle Regulation Accomplished by Complex Interactions Among Activators and Repressors of the Cycle

Inherited Cancer Syndromes Resulting from Mutations in Proto-Oncogenes

Several inherited cancer syndromes are also known to result from mutations in proto-oncogenes. An example is given by the *RET* proto-oncogene. Depending on the type of mutation and on which part of the gene is affected, *RET* mutations can lead to multiple endocrine neoplasia 2A or 2B or familial medullary thyroid carcinoma. These familial cancers are inherited in autosomal dominant fashion. A second example is the *CDK4* proto-oncogene, which when mutated can cause familial melanoma.

Unlike tumor suppressors, in which loss-of-function mutations are required in both copies of the gene in a specific cell, a single gain-of-function mutation in a proto-oncogene is usually sufficient to give rise to cancer.

Genes, Environment, and Cancer

Although the study of inherited cancer syndromes has led to the identification of a number of tumor suppressor genes and oncogenes, the inherited cancer syndromes are thought to account for only about 1% of all cancers. However, somatic (as opposed to germline) mutations in many of these tumor suppressors and proto-oncogenes play a key role in the causation of noninher-

ited, common cancers such as most breast and colon tumors. It is important to keep in mind that many of these somatic mutations can be caused by environmental factors. For example, cigarette smoke can cause somatic mutations in the *p53* gene, which normally encodes a protein that halts the cell cycle to allow DNA repair in damaged cells. A disabled *p53* results in the persistence of damaged cells, which can lead to the formation of tumors, including lung cancers. This example illustrates the link between genes, environment, and cancer.

Common Cancers

Breast Cancer

A woman's lifetime risk of breast cancer is approximately 1 in 8. Breast cancer clusters in families: the risk of breast cancer doubles for a woman with one affected first-degree relative (e.g., mother, sister, or daughter). The risk increases further if there are multiple affected relatives or if the relatives developed early-onset breast cancer.

About 5% of breast cancer cases are inherited in autosomal dominant fashion, and most of these cases are the result of mutations in either the *BRCA1* gene (chromosome 17) or the *BRCA2* gene (chromosome 13). Women who inherit one of these mutations have an approximately 60% chance of developing a breast tumor. Women with a *BRCA1* mutation also have at least a 20% chance of developing ovarian cancer. Both *BRCA1* and *BRCA2* are involved in the DNA repair process. Faulty DNA repair leads to an accumulation of harmful mutations in cells, ultimately causing a tumor to form. A small proportion of breast cancer cases are the result of mutations in the *p53* tumor suppressor gene. These mutations cause the autosomal dominant Li-Fraumeni syndrome, in which breast cancer is a major feature.

Colon Cancer

Approximately 1 in 20 Americans will develop colon cancer, and currently about one third of those who are diagnosed with this cancer will die of it. Like breast cancer, colon cancer clusters in families, with a 2- to 3-fold increase in risk when a first-degree relative is affected. A small proportion of colon cancers result from inherited mutations in the *APC* (adenomatous polyposis coli) gene on chromosome 5 (i.e., about 1 in 8,000 individuals has this form of colon cancer). *APC* is a tumor suppressor gene that encodes a protein involved in regulating cell adhesion and signaling to the nucleus. Individuals who inherit an *APC* mutation develop hundreds of colonic polyps and have a very high risk of developing colon cancer. The two-hit model applies to this inherited cancer syndrome: a first hit is inherited, but a second hit in a colonic epithelial cell is required to produce a cell capable of tumor formation. In addition to its role in this inherited cancer syndrome, *APC* is an important part of the complex pathway to common noninherited colon cancer. About 85% of all colon tumor cells have somatic mutations in their *APC* gene.

A second group of inherited colon cancers are termed **hereditary nonpolyposis colorectal cancer** (HNPCC). HNPCC may account for 5% of all colon cancer cases and can be caused by mutations in any of five different genes. All of these genes encode proteins involved in DNA mismatch repair¹ (Fig II-5-3). As with inherited breast cancer, faulty DNA repair leads to mutated cells capable of producing tumors.

¹ DNA mismatches occur when a base on one strand of a double-stranded DNA molecule is not complementary to the corresponding base on the other strand.

Note

Genes Responsible for Inherited Cancer Syndromes

- Breast cancer: *BRCA1* and *BRCA2* (DNA repair genes)
- Colon cancer: *APC* (cell adhesion and nuclear signaling) and several HNPCC genes (DNA repair)
- Melanoma: *p16* (tumor suppressor) and *CDK4* (proto-oncogene, see Fig II-5-3)

Melanoma

The incidence of melanoma increased by 20 times in the United States during the 20th century, with nearly 40,000 new cases each year. One's risk of developing this cancer is doubled if a first-degree relative is affected. Another obvious risk factor is exposure to sunlight. Approximately 5–10% of melanoma cases are inherited in autosomal dominant fashion. These cases are the result of mutations in either the *p16* tumor suppressor gene or the *CDK4* proto-oncogene. Both of these genes encode proteins that are involved in regulating the cell cycle.

Diabetes

Diabetes is another common disease, affecting at least 10,000,000 Americans. Diabetes clusters in families and is conventionally classified into two major types.

Type 1 Diabetes

Type 1 diabetes affects approximately 1 in 200 Americans, with a sibling recurrence risk of about 6%. It is an example of an autoimmune disease (see Immunology Lecture Notes), in which self-reactive T cells infiltrate the pancreas to destroy insulin-producing islet cells. Mutations in the class II major histocompatibility locus (MHC) region are estimated to contribute about one third of the risk of developing type 1 diabetes. Mutations in or near the insulin gene itself are responsible for another 10–15% of the risk.

Type 2 Diabetes

Type 2 diabetes is nearly 10 times as common as type 1 diabetes and tends to be seen more often in older individuals. It typically begins with insulin resistance and may later lead to a lack of insulin production. Although type 2 diabetes clusters in families more strongly than does type 1 (Table II-5-1), no specific genes causing this form of diabetes have yet been conclusively identified. A small proportion (2–5%) of type 2 diabetes cases are seen in individuals younger than 25 years. This form is termed **maturity onset diabetes of the young (MODY)**. This form is usually inherited in autosomal dominant fashion and is caused by mutations in any of several different genes (glucokinase, insulin promoter factor, and two hepatic nuclear factors).

Table II-5-3. A Summary of Features of Type 1 and Type 2 Diabetes

Feature	Type 1	Type 2
Insulin production	None	Partial, decreasing through time
Insulin resistance	No	Yes
Age of onset	Usually <40 years	Usually >40 years (except for MODY subset)
Autoimmunity	Yes	No
Obesity	Usually no	Usually yes, helps lead to insulin resistance
Heritability (see Table II-5-1)	Moderate	High

Alzheimer Disease

Alzheimer disease is estimated to affect 10% of Americans older than 65 and as many as half of those older than 85. As with many other common diseases, a subset of Alzheimer cases are familial, and mutations in specific genes have been shown to be responsible for some of these familial cases. Approximately 5–10% of Alzheimer disease cases have early onset (before age 60) and are inherited in autosomal dominant fashion. A small proportion of familial cases are the result of mutations in the chromosome 21 gene that encodes β -amyloid precursor protein (BAPP). These mutations lead to improper processing of BAPP and to the buildup of β -amyloid plaques in the brain (recall that Alzheimer disease is a consistent feature in Down syndrome patients; this appears to be caused by the presence of three copies of the *BAPP* gene in these individuals). Amyloid plaques are thought to be toxic to neurons, resulting in the disease phenotype. Mutations in either of two *presenilin* genes (one on chromosome 1 and one on chromosome 14) have been shown to cause about half of inherited early onset cases. These genes encode products (secretases) that are involved in the cleavage of BAPP; defects in cleavage again lead to a buildup of neurotoxic amyloid plaques. The E4 allele of the apolipoprotein E (*APOE*) gene on chromosome 19 is a risk factor for late-onset Alzheimer disease. Among whites, those who have one copy of the allele are approximately three times more likely to develop late-onset Alzheimer disease, and those who have two copies are 15 times more likely to develop late-onset disease.

Clinical Correlate

Familial Alzheimer Disease Mutations

- β -Amyloid precursor protein gene (*BAPP*): mutations in *BAPP* gene itself affect BAPP protein processing, produces early onset form
- *Presenilin* genes: encode enzymes involved in cleaving BAPP protein; mutations produce early onset form
- Apolipoprotein E: mutations increase susceptibility to late-onset form; apoE may be involved in amyloid clearance in the brain

GENETICS OF COMMON DISEASES: SUMMARY OF PRINCIPLES

Several key principles should emerge from this review of the genetics of common diseases:

- Common diseases generally have both genetic and environmental components.
- For many common diseases, subsets of cases exist in which genetic factors play an especially important role. These subsets tend to develop disease early in life (e.g., *BRCA1* and *BRCA2* mutations in breast cancer), and they often tend to have a more severe expression of the disease (e.g., *APC* mutations in colon cancer).
- Often, genes involved in the less common, strongly inherited subsets of common diseases are also involved in the common noninherited cases (but in different ways, such as a somatic mutation instead of germline mutation).

Chapter Summary

Many common diseases exhibit multifactorial inheritance.

Recurrence risks for multifactorial diseases are estimated empirically.

Twin and adoption studies are performed to determine the relative effects of genetics and environment on diseases.

Coronary heart disease can be caused by mutations in the LDL receptor (familial hypercholesterolemia).

Inherited cancer syndromes can result from mutations in:

- Oncogenes (promote cell growth, gain of function, one hit)
- Tumor suppressor genes (control cell growth, loss of function, two hits)

Inherited cancer syndromes:

- Retinoblastoma (Rb)
- Breast cancer (BRCA1, BRCA2)
- Colon cancer (APC, HNPCC)
- Melanoma (p16, CDK4)

Familial Alzheimer disease mutations:

- β -Amyloid precursor protein (BAPP)
- Presenilis
- Apolipoprotein E

Genetic Diagnosis and Gene Therapy

6

OVERVIEW

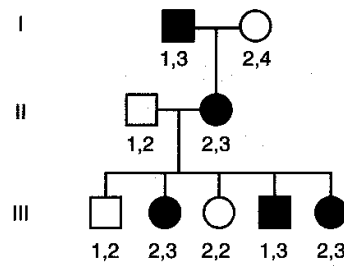
In this chapter, we review some of the practical clinical applications of genetic research. Once a gene is identified, it becomes feasible to diagnose the associated genetic disease in at-risk individuals. In addition, gene therapy, the correction of mutations in cells, becomes a possibility.

GENETIC DIAGNOSIS

The goal of genetic diagnosis is to determine whether an at-risk individual has inherited a disease-causing gene. Two major types of genetic diagnosis can be distinguished: **direct diagnosis**, in which the mutation itself is examined, and **indirect diagnosis**, in which linked markers are used to infer whether the individual has inherited the chromosome segment containing the disease-causing mutation.

Indirect Genetic Diagnosis

In indirect diagnosis, the principles of linkage analysis are used to determine whether a parent who carries a mutation has transmitted it to his or her offspring. An example will illustrate the procedure. Figure II-6-1 portrays a three-generation pedigree in which Marfan syndrome is being transmitted. Each family member has been typed for a four-allele microsatellite polymorphism that is closely linked to the disease locus (by closely linked, we conventionally mean that the marker and the disease locus are less than 1 cM [1% recombination frequency] apart). The affected father in generation 1 transmitted the disease-causing mutation to his daughter, and he also transmitted allele 3 of the marker polymorphism. This allows us to establish linkage phase in this family. Because of the close linkage between the marker and the disease locus, we can predict accurately that the offspring in generation 3 who receive allele 3 from their mother will also receive the disease-causing mutation. Thus, the risk for each child, instead of being the standard 50% recurrence risk for an autosomal dominant disease, is much more definitive: nearly 100% or nearly 0%.



The genotype of a closely linked marker locus is shown below each individual.

Figure II-6-1. A Three-Generation Family in Which Marfan Syndrome Is Being Transmitted

Indirect diagnosis can also be performed in a two-generation family if there are multiple affected individuals (e.g., an affected parent and an affected offspring for an autosomal dominant disease).

The advantages of indirect diagnosis are:

- The disease-causing mutation(s) does not need to be identified. We need identify only a closely linked marker polymorphism.
- Indirect diagnosis is especially useful when there are many possible disease-causing mutations at a locus, rendering direct identification of each possible mutation relatively costly and time consuming.

The disadvantages of indirect diagnosis are:

- Even for closely linked markers, there is a small possibility of a recombination and thus an incorrect diagnosis.
- Multiple family members must be typed to perform a diagnosis (i.e., to establish linkage phase).
- Polymorphic markers are not always “informative” about linkage phase. For example, what would happen if the parents in generation I of Figure II-6-1 both had the 1,3 genotype? (A moment’s thought should reveal that we cannot determine linkage phase in this case.)

Direct Genetic Diagnosis

Direct genetic diagnosis, in which the disease-causing mutation itself is assayed, can be accomplished in a variety of ways. Here we review some of the most common techniques.

Allele-Specific Oligonucleotide (ASO) Probes

This technique, described in Section I, involves the construction of short DNA sequences (oligonucleotides) of approximately 20 bp that exactly complement either the mutated sequence or the normal sequence (hence, “allele-specific”). The labeled oligonucleotide is then used to probe DNA from the individual in question. This DNA may be placed, for example, on a dot blot. Successful hybridization of the ASO containing the mutation indicates presence of the mutation, whereas hybridization of the normal ASO indicates presence of the normal sequence. Hybridization of both probes would be seen in a heterozygote. Because a different probe must be constructed for each mutation, this technique is practical when a limited number of mutations

cause most or all cases of disease (as in sickle cell disease, hemochromatosis, or α_1 -antitrypsin deficiency [see Clinical Correlate]).

For additional discussion of allele-specific oligonucleotide (ASO) probes and dot blots, see Section I, Chapter 7: Genetic Testing.

Direct DNA Sequencing

Sequencing of the entire gene (or at least the exons and intron–exon boundaries) is relatively time consuming and expensive. However, it is sometimes necessary if no specific set of mutations is responsible for most cases of disease (e.g., familial breast cancer caused by any of several hundred mutations of the *BRCA1* or *BRCA2* genes). DNA sequencing is typically done using automated sequencing machines.

DNA Chips

This relatively new approach involves embedding thousands of different oligonucleotides, representing various mutations and normal sequences, on a silicone chip. Patient DNA from specific regions is amplified by PCR, tagged with a fluorescent label, and exposed to the oligonucleotides on the chip. The sites of hybridization on the chip are recorded by a computer. This approach has the advantages of ready computerization and miniaturization (hundreds of thousands of oligonucleotides can be embedded on a single 2-cm² chip).

The advantages of direct diagnosis are:

- Because the mutation itself is detected, family data are not necessary. Direct diagnosis can be performed on any individual.
- Error caused by recombination is not a factor, so diagnostic accuracy is potentially higher than in indirect diagnosis.

The disadvantages of direct diagnosis are:

- The disease gene, or at least the mutated sequence, must be pinpointed before this technique can be used (in contrast, indirect analysis can be used only if linkage with a marker has been established and the disease-causing gene itself has not been pinpointed).
- ASO techniques, which are generally cheaper than direct sequencing, are limited to diseases in which most cases are caused by relatively few mutations.

With advancing technology and the completion of the human DNA sequence, these disadvantages are becoming less important.

Clinical Correlate

α_1 -Antitrypsin deficiency, an autosomal recessive disorder, affects approximately 1 in 2,500 whites. In the lower respiratory tract, α_1 -antitrypsin binds strongly to neutrophil elastase, a protease produced by neutrophils in response to infections and irritants. Its binding is necessary to prevent the elastase from digesting the lungs' alveolar septi. Individuals deficient in α_1 -antitrypsin develop pulmonary emphysema by the age of 30–50. Because cigarette smoke is both an irritant in the lung and an inactivator of α_1 -antitrypsin, smokers with this disease develop emphysema at a substantially earlier age. Most clinically significant cases of α_1 -antitrypsin deficiency are caused by a single missense mutation, termed the "Z" variant. This single-base substitution can be easily assayed using the ASO technique, providing reliable genetic diagnosis.

Clinical Correlate

Infantile Tay-Sachs disease is an autosomal recessive condition that results from a lack of the lysosomal enzyme, β -hexosaminidase A. Lacking this enzyme, patients experience an accumulation of the substrate GM2 ganglioside in their neuronal lysosomes. (Tay-Sachs disease is thus an example of a "lysosomal storage disease.") As a result of progressive neuronal damage, patients have blindness, seizures, and hypotonia, and typically death occurs by age 5. This disease is rare in most populations but affects approximately 1/3,600 individuals of Ashkenazi Jewish descent (application of the Hardy-Weinberg principle shows that the carrier frequency would then be 1/30). Because of the high prevalence of Tay-Sachs disease, and because genetic diagnosis is possible through direct mutation testing, carrier screening and prenatal diagnosis have both become common in the Ashkenazi Jewish population. The result has been a 90% reduction in the prevalence of this disease among Ashkenazi Jews in the United States during the past 2 decades.

Table II-6-1. Key Features of Indirect and Direct Genetic Diagnosis

	Indirect Diagnosis	Direct Diagnosis
Family information needed	Yes	No
Errors possible because of recombination	Yes	No
Markers may be uninformative	Yes	No
Multiple mutations can be assayed with a single test	Yes	No
Disease-causing mutation itself must be known	No	Yes

APPLICATIONS OF GENETIC DIAGNOSIS

Genetic diagnosis, which is becoming increasingly common, can be applied in a number of ways. Principal examples are discussed here.

Carrier Diagnosis

Individuals with a family history of an autosomal or X-linked recessive disease may wish to know whether they are a heterozygous carrier of the disease. This can be established by genetic diagnosis (e.g., for cystic fibrosis, hemochromatosis, PKU, or albinism). In some specific cases, a population at high risk for a specific disease may be screened for carrier status using genetic diagnosis (e.g., Tay-Sachs disease in the Jewish population [see Clinical Correlate]).

Presymptomatic Diagnosis

Individuals at risk for developing a genetic disease with a delayed age of onset may wish to learn whether they have inherited a disease-causing mutation (e.g., Huntington disease, familial breast cancer, hemochromatosis, adenomatous polyposis coli). In some cases, presymptomatic diagnosis can be highly useful in preventing serious disease consequences before they occur (e.g., phlebotomy for hemochromatosis, early tumor detection for familial breast cancer).

Prenatal Diagnosis

Prenatal diagnosis is one of the most common applications of genetic diagnosis. Diagnosis of a genetic disease in a fetus may aid in making an informed decision regarding pregnancy termination, and it often aids parents in preparing emotionally and medically for the birth of an affected child. There are a variety of types of prenatal diagnosis.

Ultrasonography

This is now a routine part of prenatal care, and it will detect some malformations and genetic diseases (e.g., nearly all cases of anencephaly, most cases of open spina bifida, some types of reduced stature conditions, many congenital heart defects). However, the sensitivity of ultrasound diagnosis is low for many conditions (e.g., Down syndrome), and it fails to detect many genetic and biochemical abnormalities.

Amniocentesis

A small sample of amniotic fluid (10–20 ml) is collected at approximately 16 weeks' gestation. Fetal cells are present in the amniotic fluid and can be used to diagnose single-gene disorders, chromosome abnormalities, and some biochemical disorders. Elevated α -fetoprotein levels indicate a fetus with a neural tube defect. The risk of fetal demise because of amniocentesis is estimated to be approximately 1/200.

Chorionic Villus Sampling

This technique, typically performed at 10–12 weeks' gestation, involves the removal of a small sample of chorionic villus material (either a transcervical or transabdominal approach may be used). The villi are of fetal origin and thus provide a large sample of actively dividing fetal cells for diagnosis. This technique has the advantage of providing a diagnosis earlier in the pregnancy. Disadvantages are a higher fetal mortality rate than with amniocentesis (about 1/100) and a small possibility of diagnostic error because of placental mosaicism (i.e., multiple cell types in the villi).

In Vitro Fertilization Diagnosis

Embryos derived from *in vitro* fertilization can be diagnosed by removing a single cell, typically from the eight-cell stage (this does not harm the embryo). DNA is PCR amplified and used to make a genetic diagnosis. The advantage of this technique is that pregnancy termination need not be considered: only embryos without the mutation are implanted. The primary disadvantage is potential diagnostic error as a result of PCR amplification from a single cell.

Diagnosis of Fetal Cells in Maternal Circulation

A small number of fetal cells cross the placental barrier and circulate in the mother's bloodstream. These can be isolated from a sample of the mother's blood using cell-sorting techniques, and DNA can be amplified by PCR for genetic diagnosis. Although still experimental, this technique offers the advantage that there is no risk of fetal loss as a result of the procedure.

GENE THERAPY

Gene therapy is the genetic alteration of cells to correct the effects of a disease-causing mutation. In humans, gene therapy is performed on somatic cells (**somatic cell gene therapy**). Because only somatic cells are affected, the alteration is not transmitted to offspring. Another form, **germline gene therapy**, affects all cells of the body, resulting in transmission of the alteration to offspring. This form of gene therapy is not currently practiced with humans.

In somatic cell gene therapy, a DNA sequence is inserted into a somatic cell to correct a mutation. Cells may be removed from the patient for manipulation and subsequent reinsertion (**ex vivo therapy**), or they may be manipulated without removal from the patient (**in vivo therapy**). Ideally, cells with a very long life span (e.g., bone marrow stem cells) are treated, but other cells (e.g., lymphocytes) are sometimes more practical targets.

Gene Replacement Therapy

Most gene therapy protocols currently under way involve the replacement of a missing gene product in a cell (e.g., the replacement of clotting factor VIII in a hemophilia A patient). Recombinant DNA techniques (see Section I, Chapter 6: Recombinant DNA and Gene Cloning) are used to insert a normal DNA sequence into a **vector**, which then carries the DNA into the patient's cells, where it supplies a template for the **normal gene product** (Fig II-6-2).

Note

Applications of Genetic Diagnosis

Presymptomatic diagnosis

Carrier diagnosis

Prenatal diagnosis

- Ultrasonography
- Amniocentesis
- Chorionic villus sampling (CVS)
- *In vitro* fertilization (IVF) diagnosis
- Diagnosis of fetal cells in maternal circulation

Note

Somatic cell gene therapy involves the use of recombinant DNA techniques to introduce a therapeutic DNA sequence into a somatic (noninherited) cell.

Germline gene therapy is the introduction of a DNA sequence into a fertilized egg, resulting in incorporation of the DNA into all cells of the recipient.

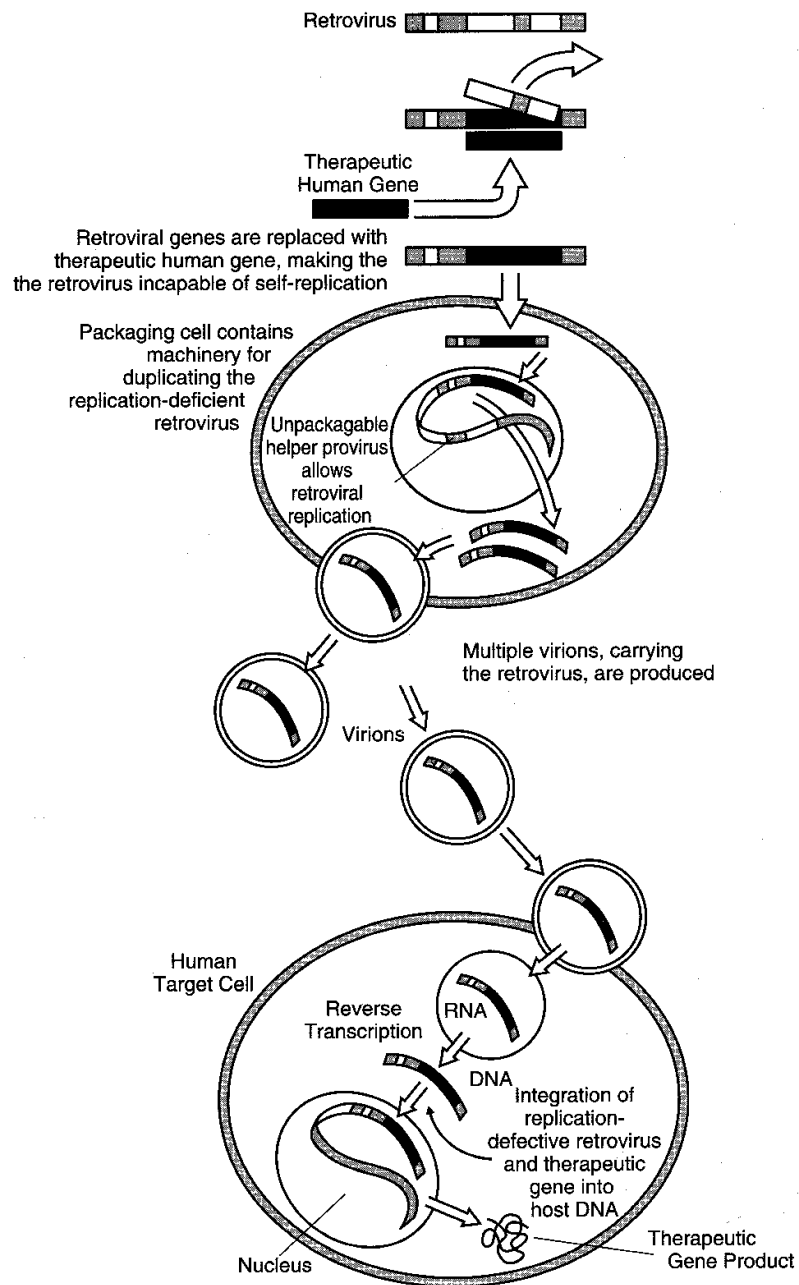


Figure II-6-2. Retroviral Gene Therapy

Vectors

A variety of different vectors are used to deliver genes into cells. Because viruses naturally invade cells to insert their own genetic material, most vectors now in use are modified viruses (i.e., the genes the virus would use to cause an infection are removed and replaced with the desired normal human DNA sequence). The principal types of vectors include the following.

Retroviruses

One of the most commonly used vectors, these viruses naturally insert their RNA, in the form of reverse-transcribed DNA, into the chromosomes of dividing cells. Most retroviruses can traverse the nuclear membranes of dividing cells only; an exception is the lentivirus (of which HIV is an example).

Adenoviruses

These DNA viruses can invade cells that are not dividing, providing an advantage over retroviruses. Disadvantages include the fact that adenoviruses do not insert their DNA into the DNA sequence of the host, so the inserted gene is eventually lost. Repeated administrations are therefore necessary. Adenoviruses, although modified, may still provoke an immune reaction.

Adeno-Associated Viruses

These vectors integrate stably into the host's DNA, and they are not known to cause disease in humans. Their disadvantage is that they accept only a relatively small inserted DNA sequence (about 5 kb, as opposed to 8 kb in a retrovirus and 30 kb in an adenovirus).

Liposomes

These are an example of a nonviral vector that can accept large DNA inserts. Because the liposome lacks peptides, it does not provoke an immune response. The primary disadvantage is that, unlike viruses, the integration rate of liposome-contained DNA is very low.

Remaining Challenges

Although much progress has been made in gene replacement therapy, significant challenges remain. These include:

- Limitations on the size of the insert. In some cases the needed DNA sequence is too large to fit in the viral vectors.
- Low levels of expression or transient gene expression. Largely because the DNA sequence is inserted randomly into the host's DNA, the regulatory sequences necessary for full and stable expression are at least partially lacking, leading to under-expression or transient expression.
- Difficulties in reaching the affected tissue. Some tissue types (e.g., brain) may be difficult to reach with most existing vectors.

Gene-Blocking Therapy

Gene replacement therapy is ineffective in countering the effects of gain-of-function mutations (e.g., the mutation that causes Huntington disease). Instead, the harmful effects of these mutations must somehow be blocked. Gene-blocking strategies, which in general are not as well developed as gene replacement techniques, include the following.

Antisense Therapy

An oligonucleotide is created whose DNA sequence is complementary to that of the mRNA of the mutated DNA sequence. The oligonucleotide then hybridizes with the mRNA, blocking its translation into an abnormal protein product.

Note

Somatic cell therapy can be classified into two forms:

- For loss-of-function mutations: gene replacement therapy using retroviruses, adenoviruses, adeno-associated viruses, or liposomes as vectors
- For gain-of-function mutations: gene-blocking therapy, using antisense oligonucleotides or ribozymes to block gene expression

Clinical Correlate

Severe combined immune deficiency (SCID) is a rare inherited disorder in which multiple components of the immune system are affected. About 50% of SCID cases are caused by **X-linked recessive mutations** in the gene encoding a subunit of a receptor for interleukins 2, 4, 7, 9, and 15. The lack of normal receptors for these cellular growth signals compromises early lymphoid growth and differentiation, and patients with X-linked SCID have a serious deficiency of T lymphocytes and natural killer cells (the B cell count is normal or elevated). Without treatment (bone marrow transplants or isolation), the condition is uniformly fatal in infancy. Some of these patients have been isolated in plastic "bubbles." In recent gene therapy trials, retroviruses containing the normal gene have been placed in the patients' bone marrow stem cells, resulting in normal levels of natural killer, B, and T cells. Some treated patients demonstrate normal immune function and normal development, providing good evidence for therapeutic success.

Ribozyme Therapy

Ribozymes are enzymes that, in some cases, can cleave mRNA. A ribozyme is engineered so that it cleaves the mutant mRNA sequence, preventing its translation into a polypeptide.

Applications of Somatic Cell Therapy

Currently, more than 400 human somatic cell gene therapy protocols are being tested. Most of these involve the use of genetically modified cells to treat *noninherited* diseases. For example, normal copies of the p53 tumor suppressor gene are inserted into lung tumors to halt tumor progression, and genetically modified cells have been used to create new coronary vessels in patients with coronary heart disease. Success has also been achieved in the treatment of hereditary disease (most notably, the recent successful treatment of X-linked severe combined immune deficiency; see Clinical Correlate).

Germline Gene Therapy

This type of therapy typically consists of injecting DNA into a pronucleus. The DNA can then be incorporated into all cells of the organism, including the gametes. This type of therapy was first used successfully in the mouse nearly 20 years ago, and in many ways it is technically easier than somatic cell therapy. However, most injected embryos die, and some develop tumors and various malformations. In addition, there are ethical concerns about the permanent modification of our genetic legacy. Consequently, germline therapy is not currently practiced in humans.

Genes are introduced, however, into the germlines of experimental animals such as mice to serve as models of human disease. These animals are called **transgenic animals**, and the introduced genes are termed **transgenes**.

Chapter Summary

Genetic diagnosis can be used to determine whether an individual has inherited a disease-causing gene. It is often used for carrier detection and prenatal diagnosis.

Indirect genetic diagnosis utilizes markers (RFLPs, STRs) linked to the disease-causing gene in question.

Direct genetic diagnosis is accomplished by looking directly at the disease-causing mutation (ASOs, DNA chips, DNA sequencing).

Gene therapy is used to correct the effects of a disease-causing mutation. Somatic cell gene therapy is used in humans because the alteration is not transmitted to offspring. Germline gene therapy (not practiced in humans) alters all cells in the body and results in transmission of the alteration to offspring.

Two forms of somatic gene therapy:

- Gene replacement therapy for loss of function mutations
- Gene-blocking therapy for gain of function mutations

