CHAPTER 3

Genes, DNA, Chromosomes, and Cell Division

LEARNING OBJECTIVES

1. Describe the inheritance pattern of genes and define dominant, recessive, codominant, and sex-linked inheritance.
2. Describe how chromosomes are studied. Explain how a karyotype is determined.
3. Describe the process used by the cells to synthesize a gene product using information contained in the DNA.
4. Describe how cells utilize the genetic code within DNA chains to convey genetic information to daughter cells during cell division.
5. Compare and contrast mitosis and meiosis.
6. Compare and contrast spermatogenesis and oogenesis. Explain the implications of abnormal chromosome separations in the course of meiosis in older women.

Introduction

Transmission of genetic information was initially studied through the analysis of the inheritance of detectable traits (**phenotypic traits**) from parent through successive generations of offspring using **patterns of inheritance**. These phenotypic traits are now understood as the expression of the **genotype** of the individual; that is, the genes that are the functional manifestation of the chemical code of the **DNA** organized in **chromosomes** in the nucleus of the cell. The human **genome** contains about 20,000 genes. The information encoded by those genes in the chromosomes defines the proteins critical to the structure, metabolism, and regulation of all aspects of cellular function. This information is transmitted to each newly formed cell during cell division, either to make two identical **daughter cells** (in the process of **mitosis**) or to form sperm or ovum (**meiosis**). All nongerminal cells of the body are **diploid** (have a 2N chromosome number, 46, arranged as 23 pairs), with one of each pair of chromosomes derived from each parent during fertilization of the ovum by a sperm. Members of a chromosome pair are called **homologous chromosomes**. During the formation of ova and sperm, a **reduction division** results in the formation of a **haploid** (N chromosome number, 23, with no chromosome pairs) that recombines traits from the maternal and paternal chromosome set of the individual. Fertilization restores the diploid chromosome number and produces a unique individual with a mixture of traits derived from the maternal and paternal genome. The genome of the fertilized egg (**zygote**) also contains the blueprint for the development of the new individual,

Phenotype Collection of inherited/phenotypic traits detectable in an individual.

Phenotypic traits Characteristics apparent in the individual.

Pattern of inheritance Inheritance of detectable traits across generations in a family.

Genotype DNA/chromosome basis of inherited traits in an individual.

DNA Deoxyribonucleaic acid (DNA) is the molecule carrying the coding information for genes.

Chromosome Cell structure organizing the molecules of DNA Individual structural units of DNA which are best seen in dividing cells.

Genome The total of all the genes contained in a cell's chromosomes.

Daughter cell A cell resulting from division of a single cell (called the parent cell).

Mitosis The type of cell division of most cells in which chromosomes are duplicated in the daughter cells and are identical with those in the parent cell. The characteristic cell division found in all cells in the body except for the gametes.

Meiosis A special type of cell division occurring in *gametes* (ova and sperm) in which the number of chromosomes is reduced by one-half.

Diploid Cell containing 2N (pairs) of each chromosome.

Homologous chromosomes A matched pair of chromosomes, one derived from each parent.

Reduction division Process occurring in meiosis reducing the 2N pairs of chromosomes to 1N.

Haploid Cell containing 1N of each chromosome, a gamete.

Zygote Fertilized egg.

Autosomal chromosomes Chromosomes other than sex chromosomes. A human has 22 pairs of autosomes.

Sex chromosomes The X and Y chromosomes that determine genetic sex. A human has 1 pair of sex chromosomes.

the formation of the body plan that defines us as human. In the new individual, 22 of the chromosome pairs consist of **autosomal chromosomes** and one pair are the **sex chromosomes**, an XY pair determining a male, XX a female. The 23 pairs of chromosomes make up the **karyotype**.

Genes and Inheritance Patterns

The alternate forms of a gene found on the maternal and paternal chromosomes pair are called **alleles**. The chromosome site of a given gene is called a **locus**. Different alleles may produce easily detectable differences in the individual (such as eye color), more subtle differences (blood type, or the level of activity of a particular protein detectable only in the laboratory), or differences so subtle as to be undetectable in an individual. Deleterious alleles, the result of inherited alterations in the gene (**mutations**), can result in dysfunction and disease. The inherited differences between alleles at multiple loci (**polymorphisms**) are what define us as individuals and help define populations (which differ in the frequency of particular alleles for many genes). The much misused term "race" represents frequency differences in many polymorphic genes that define populations historically derived from different areas. Such differences are likely to be of evolutionary significance. Populations of African origin have alleles for a number of genes that result in higher levels of skin pigment, which protect against the deleterious effects of solar ultraviolet radiation. Scandinavian populations tend to have alleles resulting in lower levels of skin pigments, perhaps to capture scanty northern solar radiation important in the biosynthesis of vitamin D.

An individual is **homozygous** for a gene if both alleles are the same and **heterozygous** if the alleles are different. Alleles of different genes differ in how they are expressed. A **recessive gene** produces a detectable phenotype only in the homozygous state. An example is the ABO blood group. An individual will have blood type O only if he or she inherited the O allele for the ABO gene from each parent and is homozygous for this allele. A **dominant gene** expresses itself in either the heterozygous or the homozygous state. The A and B alleles of the ABO blood group are dominant. Individuals inheriting either one or two A or B alleles have blood types A and B, respectively. In some cases, there are detectable differences between homozygous or heterozygous expression of a dominant allele. Such **dosage effects** can result in higher levels of expression of a gene product when two doses of a dominant allele are present. Sometimes both alleles of a pair are expressed. Such alleles are called **codominant**. If one A and one B allele is present at the ABO gene locus, the individual has blood type AB, and his or her red cells will have both the A and B substance. Genes carried on sex chromosomes are called **sex-linked genes**; the effects they produce are called **sex-linked traits**. The small Y chromosome carries few genes other than those that direct male sex differentiation, but the much larger X chromosome carries many genes in addition to those concerned with sexual development. Most X-linked traits are recessive. The female carrier of a recessive X-linked trait is usually normal because the effect of the defective allele on one X chromosome is offset by the normal allele on the other X chromosome. The male, however, possesses only one X chromosome. Consequently, he can be neither heterozygous nor homozygous for X-linked genes and is called **hemizygous** for genes carried on the X chromosome. If the male receives an X chromosome containing a defective gene, the defective X-linked gene functions like a dominant gene when paired with the Y chromosome. Such is the case for the well-known disease hemophilia (resulting in excessive bleeding) because the genes responsible for the most common forms of this disease (hemophilia A and hemophilia B) are sex linked. Females who inherit an allele responsible for hemophilia are most often normal because the allele is recessive. They are, however, carriers of

the disease. If their offspring inherits the maternal chromosome having the defective hemophilia allele and also the Y chromosome from the male parent, the result is a male child who is hemizygous for the defective allele and has the disease hemophilia. It is of interest to note that female hemophiliacs do occur (although rarely) for reasons to be discussed. The inheritance pattern of genes can be traced through a family tree or **pedigree** (**FIGURE 3-1**). The pattern of appearance of a trait or disease in a pedigree offers clues to the nature of the allele studied (i.e., dominant, recessive, or sex-linked). For example, in the case of dominant inheritance, the trait (disease) is expected to appear in one of the parents of the affected child. In the recessive case, neither parent will be affected (but both will be carriers). In the case of recessive gene transmission, parents are most often related and have the same recessive allele because they share a common ancestor. An example of this is a first-cousin

Karyotype Chromosomes from a single cell arranged in pairs in descending order according to size of the chromosomes and the positions of the centromeres used to visualize the chromosome composition of an individual.

Allele One of several related forms of a single gene.

Locus The position of a gene on a chromosome.

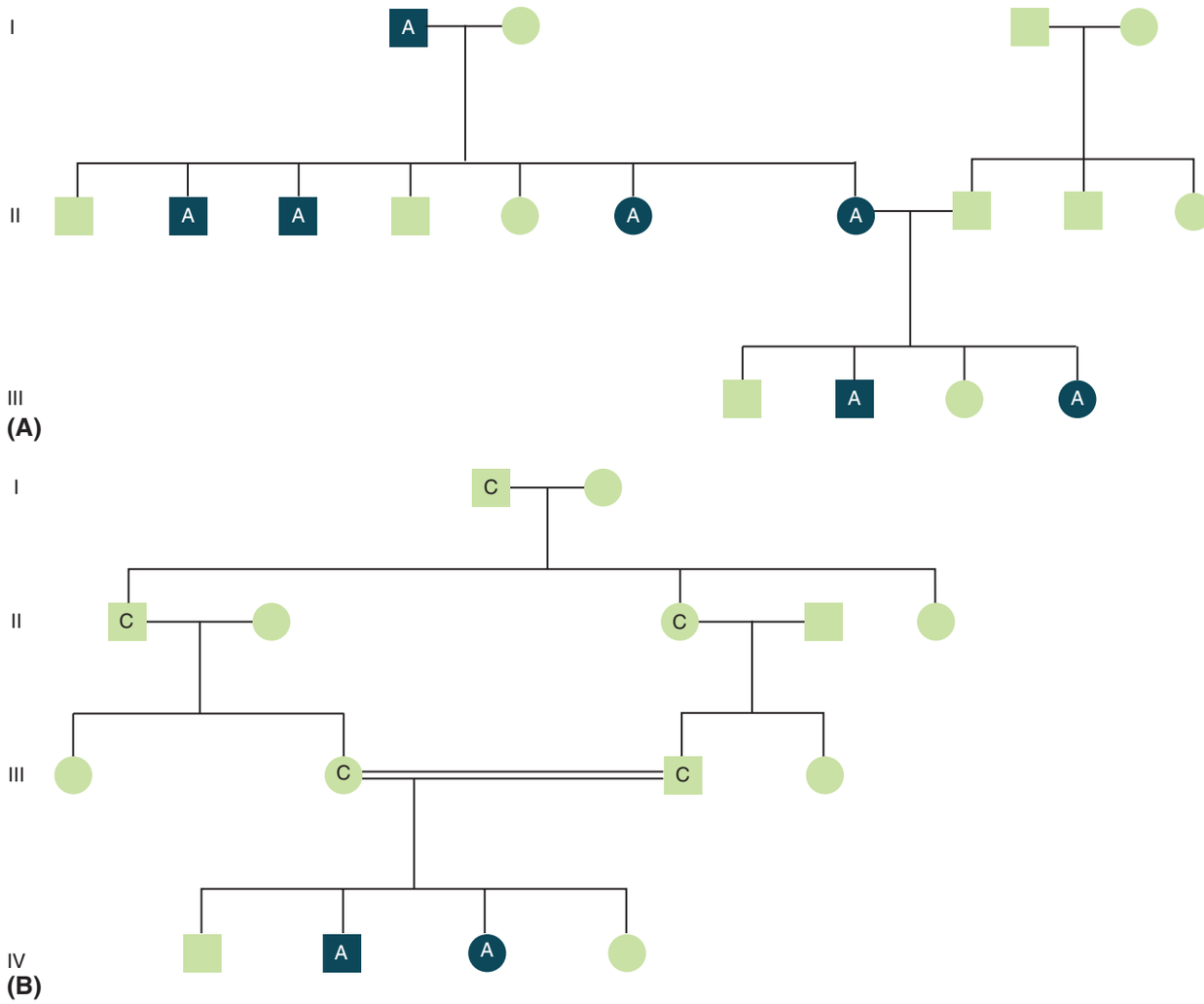


FIGURE 3-1 Examples of family trees (pedigrees) tracing dominant and recessive traits. Pedigrees use standard symbols. Males are shown by squares, females by circles. Matings are indicted by a horizontal line connecting male and female symbols. A double line indicates a mating between related individuals. Offspring of a mating are connected by vertical lines. Each generation of individuals is shown on a horizontal line that is given a Roman numeral. Affected individuals are shown by filled-in symbols (in this example containing an "A"). **(A)** Pedigree for a dominant trait. A parent of each affected individual is also affected. In the example given two nonrelated families intermarry. Only one family expresses the trait. **(B)** Pedigree for a recessive trait. The parents of affected individuals are most often not affected but are each carriers (heterozygous) for the trait. In the case of uncommon traits, the carrier parents are most often related by descent from a shared relative who carried the trait. This example shows a first-cousin marriage. Carriers of the trait are indicated by a "C" within the symbol. Sex-linked pedigrees are discussed in the case at the end of the chapter.

Courtesy of Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill.

Mutation Inherited or somatic alteration in a gene.

Polymorphism Collection of alleles for a single gene.

Homozygous Presence of the same alleles at a given gene loci on the chromosome pair.

Heterozygous Presence of two different alleles at given gene loci on the homologous pair of chromosomes.

Recessive gene A gene that expresses a trait only when present in the homozygous state.

Dominant gene A gene that expresses a trait in the heterozygous state.

Dosage effects Differing amounts of gene product in the heterozygous and homozygous states.

Codominant gene Products of both genes are expressed in the heterozygous state.

Sex-linked gene Gene present on the X or Y chromosome.

Sex-linked trait Product of gene present on X or Y chromosome.

Hemizygous A term applied to genes located on the X chromosome in the male.

Pedigree Formal pattern displaying the inheritance of traits or gene products through generations of a family.

Aneuploidy Condition of having extra or missing chromosomes.

Heterochromatin Small dense mass of condensed chromatin appearing in inactivated X chromosomes.

marriage. Sex-linked (recessive) traits appear only in males. Fathers are not affected (they donate their normal Y chromosome to their male offspring) but male uncles or grandfathers are likely to show the trait.

X CHROMOSOME INACTIVATION: THE LYON HYPOTHESIS

Having the proper number of chromosomes is critical to normal human development. Defects in the number of chromosomes, having extra or missing chromosomes (**aneuploidy**), is always associated with disease and often fatal prior to birth. In normal individuals, all autosomal chromosomes occur in pairs. This is not the case with sex chromosomes: females who are XX appear to have twice the X chromosomal DNA of XY males. Female cells, however, function as though they contained only genetic material equivalent to that of the single X chromosome of the male. The reason for this behavior is that almost all of one of the X chromosomes is inactivated and nonfunctional. In the female, the genetic activity of both X chromosomes is essential only during the first week of embryonic development. Thereafter, one of the X chromosomes in each of the developing cells is inactivated. With only rare exceptions, the inactivation occurs in a random manner (**FIGURE 3-2**). After the initial inactivation of an X chromosome has occurred, the same paternal- or maternal-derived X chromosome will also be inactivated in all descendants of the precursor cell. The inactivated X chromosome appears as a small, dense mass of condensed chromatin (**heterochromatin**) attached to the nuclear membrane of somatic cells.

The inactivated X chromosome can be identified in the cells of a normal female and is called a sex chromatin body, or **Barr body**, after the man who first described it. Because the inactivation occurs at random, females are a **mosaic** for genes that are

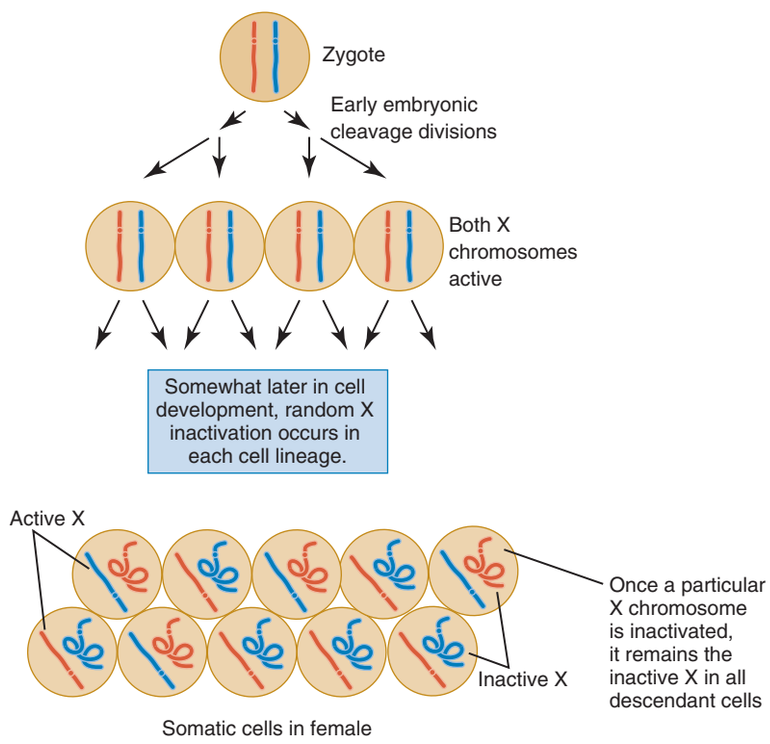


FIGURE 3-2 The two X chromosomes are shown in red and blue. An active X is depicted as a straight chromosome, an inactive X as a tangle. Each cell has just one active X. The particular X that remains active is a matter of chance, but it remains active in the progeny of that cell.

sex linked. If a female is heterozygous for a sex-linked gene (which has an "A" allele and a "B" allele), about half the cells in the women would be expected to express A and half B. The situation is actually more complex and is further described in the Case 3-1 and in later sections dealing with X-linked genetic diseases.

Barr body The inactivated X chromosome attached to the nuclear membrane in the female. Sex chromatin body.

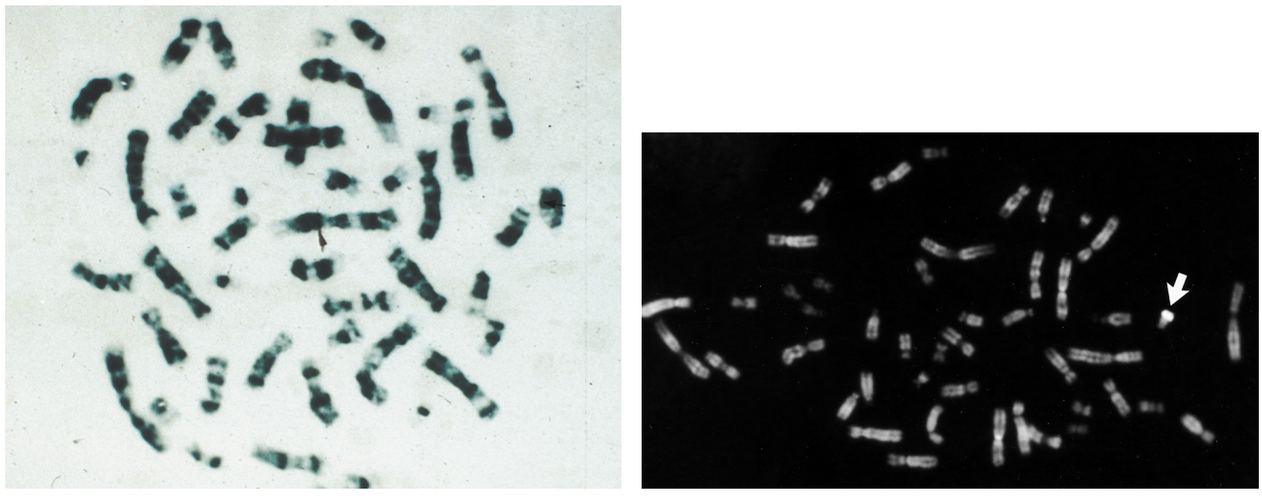
Genetic mosaic Condition of having different genes inactivated at random on the X chromosome.

Fluorescent probes Artificial pieces of DNA labeled with a dye.

CHROMOSOME ANALYSIS

The chromosome composition (karyotype) of a human can be studied with great accuracy to determine the presence of abnormalities in chromosome number or structure. This is accomplished by using a drug to arrest dividing cells (usually from the blood) at a stage in which the chromosomes are condensed and have become separate and distinct. The complex structure of the chromosomes in such cells can be established by using special stains that allow microscopic visualization of the details of chromosome structure or by using a molecular biological technique that identifies specific regions of chromosomes using **fluorescent probes**, a technique termed "chromosome painting."

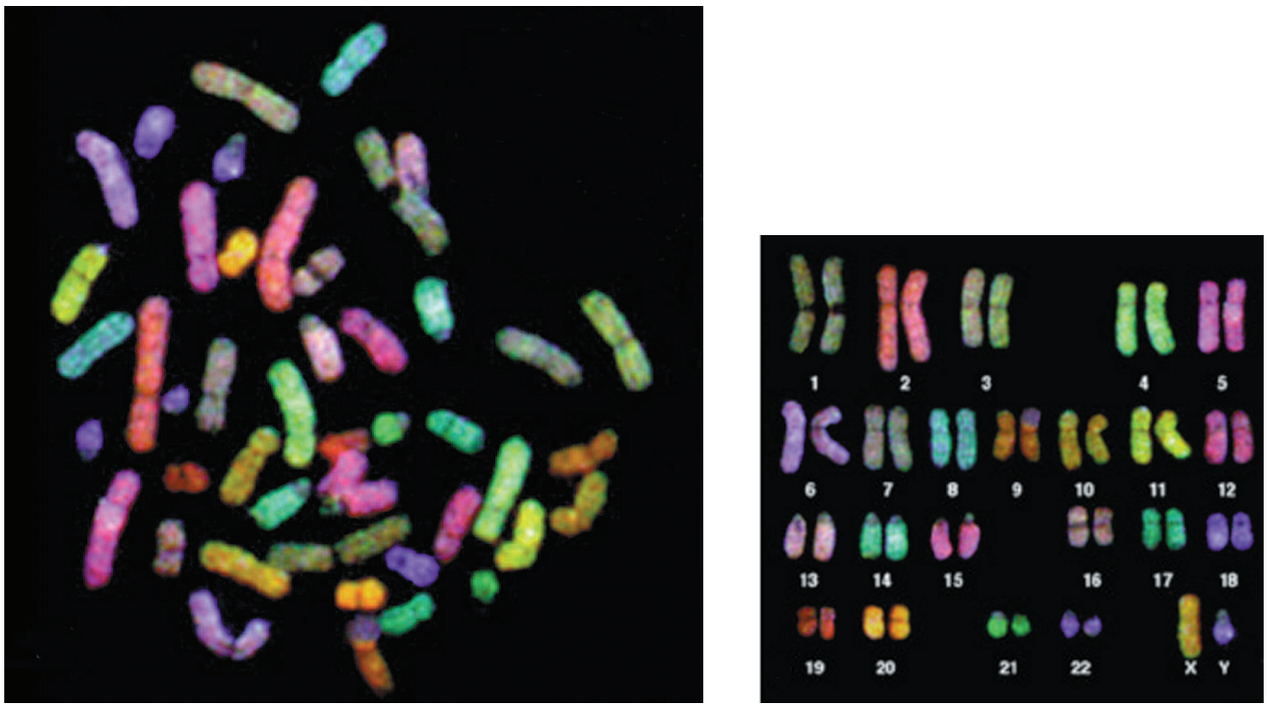
Artificially synthesized stretches of DNA that bind (hybridize) to specific chromosomes are linked to a series of fluorescent dyes in order to identify chromosomes by color. **FIGURE 3-3** illustrates the appearance of a cell arrested in mitosis with the chromosomes well separated. A normal dividing cell arrested in mitosis contains 46 chromosomes, each consisting of two chromatids joined at their centromeres. Each chromosome has its own unique structure and is classified according to size, the location of the centromere, the relative lengths of the chromatids that extend outward from the centromere (called the arms of the chromosome), and the pattern of light and dark bands along the chromosome. The separated chromosomes from a single cell are photographed and arranged in pairs in a standard pattern called a karyotype. **FIGURE 3-4** illustrates a karyotype produced using the technique of chromosome painting. Each pair of chromosomes is "painted" a specific color using a fluorescent probes.



(A) **(B)**

FIGURE 3-3 The appearance of chromosomes from a single cell arrested in mitosis, illustrating the banded pattern that facilitates the identification of individual chromosomes. The two chromatids composing each chromosome lie side by side. **(A)** Giemsa stain (photograph courtesy of Dr. Jorge Yunis). **(B)** Fluorescent stain showing intensely stained (*arrow*) Y chromosome (photograph courtesy of Patricia Crowley-Larsen).

Courtesy of Leonard V. Crowley, MD, Century College.



(A) (B)

FIGURE 3-4 Result of human chromosome painting in which each pair of chromosomes is stained a different color by hybridizing with a specific fluorescent probe. **(A)** The appearance of stained chromosomes from a single cell arrested in mitosis. Each pair can be identified by both structure and color. **(B)** A karyotype in which the chromosomes have been grouped in pairs and arranged in a conventional order depending in part on size.

The Structure of DNA in the Chromosomes

The chromosomes are composed of DNA combined with protein. The information on the DNA is referred to as the **genetic code**. The basic structural unit of DNA, called a **nucleotide**, consists of a phosphate group linked to a five-carbon sugar, deoxyribose, which in turn is joined to a nitrogen-containing compound called a base (**FIGURE 3-5**). There are two different types of DNA bases: a purine base, which contains a fused double ring of carbon and nitrogen atoms, and a pyrimidine base, which contains only a single ring. There are four different bases in DNA: the purine bases are adenine and guanine, and the pyrimidine bases are thymine and cytosine. Consequently, there are four different nucleotides in DNA, each containing a different base (**FIGURE 3-6A, B**). The nucleotides are joined together in long chains, with the nitrogen bases projecting at right angles from the long axes of the chains. A DNA molecule consists of two strands of DNA held together by weak chemical attractions between the bases of the adjacent chains. The chemical structure of the bases is such that only adenine can pair with thymine and only guanine can pair with cytosine. Bases that pair in this way are called complementary bases, and there are 3 billion pairs of complementary bases (base pairs) in the human genome. However, less than 2 percent of the base pairs code for proteins. The DNA chains are twisted into a double spiral somewhat like a spiral staircase, with the sugar and phosphate groups forming the two railings and the complementary base pairs forming the steps (**FIGURE 3-6B, C**).

Genetic code The information carried by DNA molecules in chromosomes. The DNA basis of the phenotype/genotype.

Nucleotide Basic structural unit of DNA consisting of a phosphate group linked to a five-carbon sugar, deoxyribose or ribose, which is linked to a nitrogen-containing compound called a base, either purine or pyrimidine.

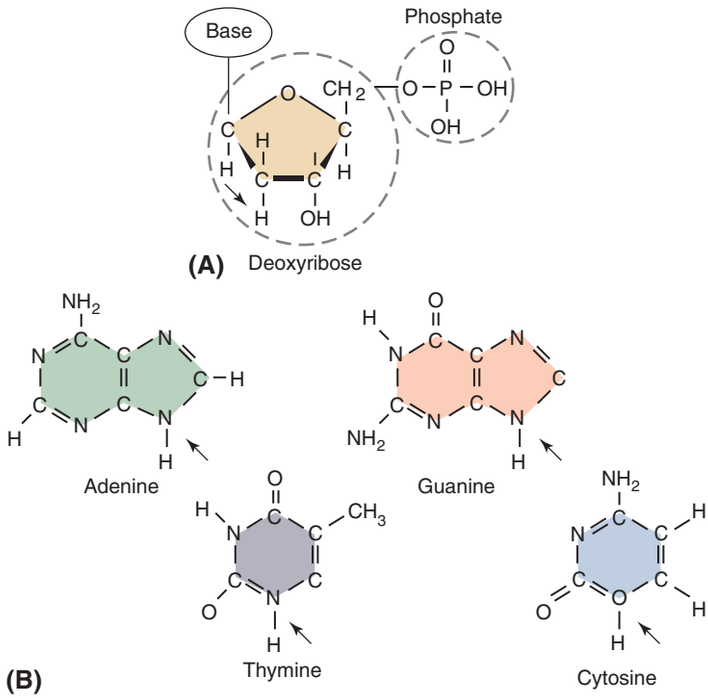


FIGURE 3-5 General structure of DNA nucleotide. **(A)** Deoxyribose is identical with ribose except for the absence of an oxygen atom (site of missing oxygen indicated by *arrow*). **(B)** Structure of the bases showing sites (*arrows*) at which bases are joined to deoxyribose.

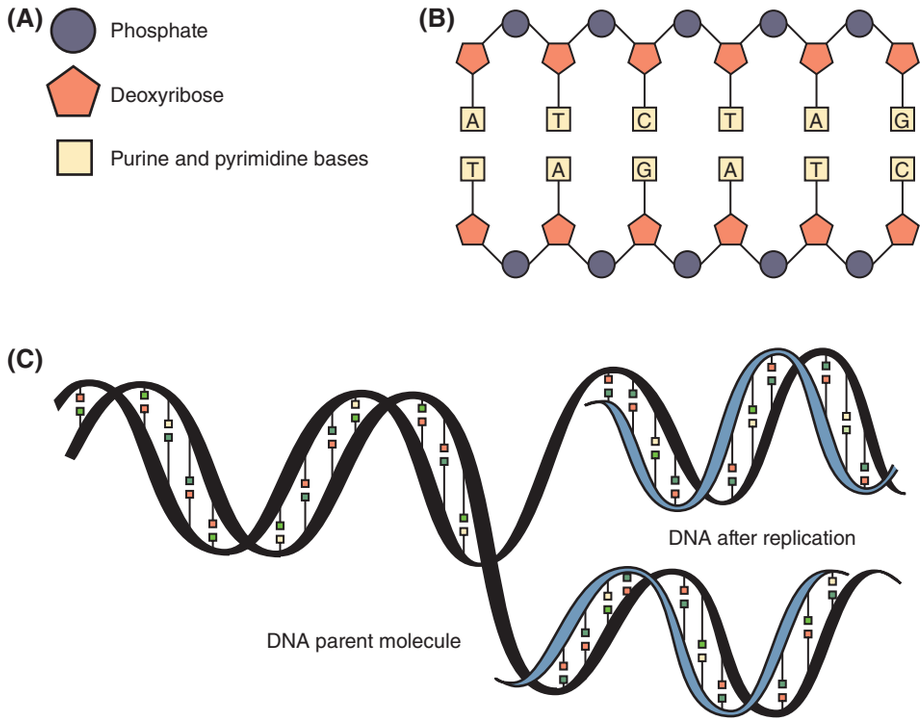


FIGURE 3-6 **(A)** Components entering into the formation of DNA molecule. **(B)** Structure of double stranded DNA. **(C)** Duplication (replication) of DNA molecule.

Semiconservative replication Production of duplicating DNA strands using complementary strands as a guide.

Codon Triplet of bases coding for one piece of information or for one amino acid.

Degenerate code Condition in which each amino acid has many different codons.

Messenger RNA (mRNA) Molecule encoding information from DNA to the ribosomes. Similar to DNA but single-stranded and contains ribose rather than deoxyribose.

Transcription Process of copying information from DNA to mRNA.

Complementary The relationship between the base sequence of the two strands in a DNA molecule. During DNA replication, either strand can make an exact copy of the other. Complementary DNA strands bind to each other by base pairing.

Exon The part of a chromosomal DNA chain that codes for a specific protein or enzyme.

Intron A noncoding part of a chromosomal DNA chain.

Transfer RNA (tRNA) RNA molecule that "picks up" appropriate amino acid to build the gene product.

Translation Process of building a gene product using information from the mRNA.

Anticodon Code complementary to the codon on the mRNA, which allows the correct amino acid to be deposited by the tRNA.

DUPLICATION (REPLICATION) OF DNA

As a cell prepares to divide, the double strands of DNA duplicate themselves. The two chains separate, and each chain serves as the model for the synthesis of a new chain (Figure 3-6C). Because adenine always pairs with thymine and guanine with cytosine, the arrangement of the nucleotides in the original chains determines how the nucleotides will reassemble to form the new chains. The process of duplication (**semiconservative replication**) forms two double complementary strands, each containing one of the original strands plus a newly formed strand. In this way, each of the two daughter cells produced by cell division receives an exact duplicate of the genetic information possessed by the chromosomes of the parent cell.

TRANSLATION OF DNA INTO PROTEINS

The DNA in the nucleus directs the synthesis of enzymes and other proteins by the ribosomes located in the cytoplasm. The information is coded into the DNA as a series of three contiguous bases (a triplet) in a DNA strand. The triplet of bases defines a **codon**. With four bases there are sixty four possible unique codons. One codon is a "start" instruction, three codons are stop (termination) instructions, and the remaining sixty codons each specify one of the amino acids that form the building blocks for the proteins expressed by the gene. There are more codons than the twenty amino acids that are coded for in DNA, so the code is said to be **degenerate**; several different codons specify the same amino acid. The "instructions" are carried by **messenger RNA (mRNA)**, so named because it carries the message encoded in the DNA to the ribosomes in the cytoplasm. Messenger RNA is quite similar to DNA but consists of only a single rather than a double strand. It also differs by containing the five-carbon sugar ribose instead of deoxyribose and a base called uracil instead of thymine. During synthesis of mRNA, the DNA chains partially separate, and the DNA serves as the model on which the mRNA is assembled. Therefore, the information **transcribed** into the mRNA strand is an exact **complement** of the genetic information possessed by the nuclear DNA because the mRNA is composed of the sequence ribose bases that are complementary to the sequence of DNA bases being copied.

At this stage the mRNA is in a precursor form (termed pre-mRNA). Before the pre-mRNA leaves the nucleus, it is modified by a number of processing steps. Most genes do not contain continuous stretches of information coding for the protein to be expressed. The information coding for functional proteins (**exons**) is separated by stretches of an "intervening sequence" (**introns**) that does not code for the expressed gene product. The pre-mRNA is processed by removal of the RNA coding for introns and splicing together the exons to produce a mature mRNA molecule containing only the coding sequences that specify the protein to be constructed. The mature mRNA strand leaves the nucleus through the pores in the nuclear membrane and becomes attached to the ribosomes in the cytoplasm, which are small nucleoprotein particles where enzymes and other proteins are constructed from individual amino acids. The combination of amino acids required to assemble the protein is determined by the information contained in the mRNA strand. The amino acids are transported to the ribosomes by means of another type of RNA called **transfer RNA (tRNA)**, so named because it "picks up" the required amino acids from the cytoplasm and transfers them to the ribosomes where they are assembled in proper order, as specified by the mRNA and **translated** into protein. The tRNA contains an **anticodon** complementary to the codon on the mRNA, which ensures that the correct amino acid is incorporated (**FIGURE 3-7**).

In addition to mRNA, tRNA, and the RNAs that form part of the structure of ribosomes, there are several classes of regulatory RNAs that control how certain

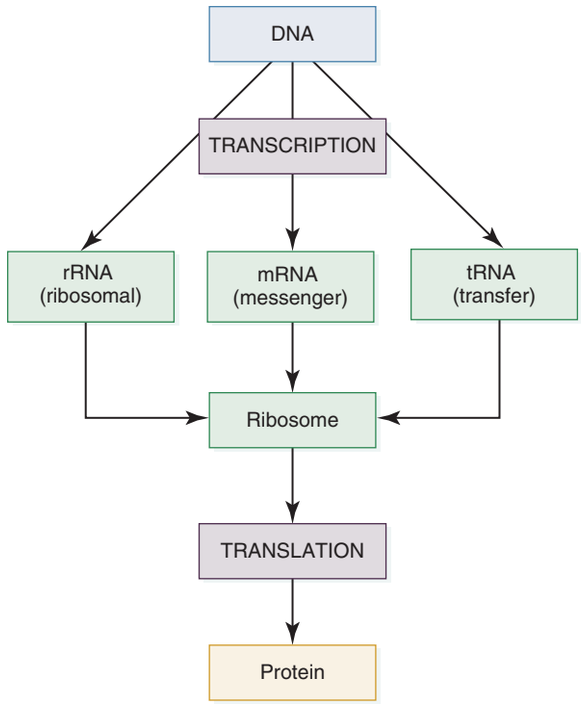


FIGURE 3-7 The central dogma of molecular genetics. DNA codes for RNA. RNA codes for protein. The DNA to RNA step is transcription. The RNA to protein step is translation and occurs on the ribosome.

genes are expressed. **MicroRNAs (miRNAs)**, so called because they are smaller than other RNA molecules, have the function of regulating the activity of groups of genes sharing certain common sequences. MiRNAs pair with the target mRNAs that have complementary sequences to "silence" them by blocking expression. About 500 different miRNA molecules have been described, each regulating the activity of a specific group of genes. In addition, there are sequences of DNA that regulate the expression of known genes and sequences whose function is currently unknown.

MicroRNA (miRNAs) Small RNA molecules that regulate the activity of sets of individual genes.

MITOCHONDRIAL GENES AND INHERITANCE

Chromosomes are not the only site where genes are located in the cell. Mitochondria (described in the chapter on cells and tissues), the site of oxidative metabolism and ATP generation in the cell, also have small amounts of DNA containing some of the genes required for synthesis of energy-generating mitochondrial proteins. The DNA in mitochondria is circular and has several similarities to the DNA found in bacteria. These similarities have led to the suggestion that mitochondria derived during evolution from trapped bacteria.

Although most of the genes involved in ATP synthesis are located on the chromosomes in the cell nuclei and are inherited like other genes, some of the ATP-generating components are coded as mitochondrial genes and are inherited differently from those on chromosomes. As mitochondria wear out, other mitochondria divide to replace them. Mutations occur frequently as the mitochondrial DNA duplicates (replicates). Because there are several mitochondrial DNA molecules in each mitochondrion

and several hundred mitochondria in an active cell, a cell may contain a mixture of normal mitochondria and mitochondria containing mutated DNA. Mitochondrial DNA mutations do not affect cell function unless there are such a large number of mitochondrial DNA mutations that the energy-generating capability of the cells is impaired.

The human ovum contains a very large number of mitochondria, but sperm contain very few. Consequently, transmission of abnormal mitochondrial DNA is almost invariably from mother to child. A number of rare diseases result from inherited abnormalities of mitochondrial DNA with the severity of the disease manifestations related in part to the number of mutated mitochondria. Although mitochondrial DNA is not unique to an individual, mitochondrial DNA has proven useful in identifying the relationship of an individual to a family. Because there are multiple copies of the mitochondrial genome in cells, useful samples are often obtainable from decomposed or skeletal remains.

Cell Division

There are two types of cell division. Mitosis is characteristic of somatic cells. Meiosis is a specialized type of cell division that occurs during the development of the eggs (ova) and sperm, a process called **gametogenesis**. In mitosis, each of the two new cells (called the daughter cells) resulting from the cell division receives the same number of chromosomes that were present in the precursor cell (called the parent cell). In meiosis, the number of chromosomes is reduced so that the daughter cells receive only half of the chromosomes possessed by the parent cell.

Gametogenesis The development of mature eggs and sperm from precursor cells.

Growth factor A soluble growth promoting substance produced by cells that attaches to receptors on the cell membrane of other cells, which activates the receptors and initiates events leading to growth or division of the target cells.

Neoplasia Dysregulated cell growth possibly leading to the development of cancer.

MITOSIS

Mitosis is characteristic of somatic cells, but not all mature cells are able to divide. Some mature cells, such as cardiac muscle cells and nerve cells, do not divide. Others, such as connective tissue cells and liver cells, divide as needed to replace lost or damaged cells or to heal an injury. Yet others divide continually, such as those lining the digestive tract and those in the bone marrow that continually replace the circulating cells in the bloodstream. Regardless of the frequency of cell division, the rate of cell division is controlled closely to match the body's needs, and excess cells are not normally produced.

Many factors regulate cell growth and cell division. Often the stimulus that induces a cell to divide does not originate within the cell itself but comes from other cells. Various soluble growth-promoting substances called **growth factors** are secreted by neighboring cells and bind to receptors on the cell membrane of the target cell, which activates the receptors. The activated receptors in turn transmit biochemical signals to the "machinery" inside the cell, which induces the cell to divide. Genes within the cell also play an important role. Some promote cell growth by directing the production of the receptors on the cell surface to which the growth factors can attach. Other genes generate inhibitory signals that suppress cell growth and division. Depending on the signals, either the cell is induced to grow and divide or its growth is inhibited. These intracellular communications allow normal cells to divide often enough to accomplish their functions and to replenish cell losses from injury or normal aging but restrain excessive proliferation. Moreover, normal cells cannot continue to divide indefinitely. They are programmed to undergo a limited number of cell divisions, and then they die. Defects in the regulation of cell division can occur and result in the process of **neoplasia** (dysregulated cell growth), which ultimately may result in cancer.

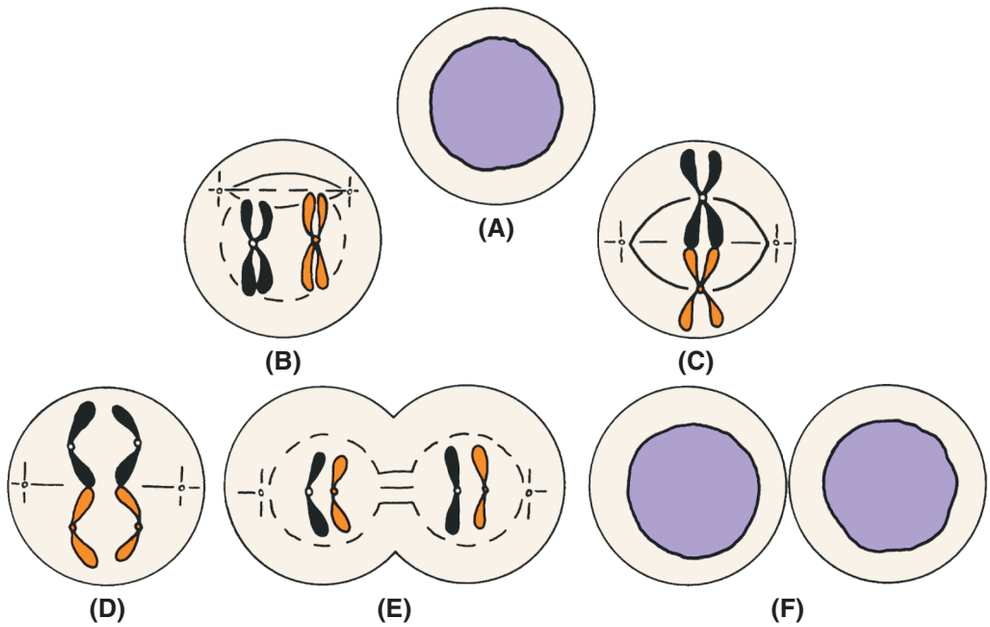


FIGURE 3-8 Stages of mitosis. The behavior of only one pair of homologous chromosomes is shown. **(A)** Prior to cell division. **(B)** Prophase. **(C)** Metaphase. **(D)** Anaphase. **(E)** Telophase. **(F)** Daughter cells resulting from mitosis, each identical to the parent cell.

Before a cell begins mitosis, its DNA chains are duplicated to form new chromosome material (the s phase prior to cell division). Each chromosome and its newly duplicated counterpart lie side by side. The two members of the pair are called **chromatids**. Mitosis is the process by which chromatids separate. (The use of the terms chromosomes and chromatids may at times be confusing. Each chromosome duplicates itself before beginning cell division. Because there are normally 46 chromosomes in each somatic cell, just prior to cell division there are actually the equivalent of 92 chromosomes in the cell [i.e., 46 × 2]. When the chromosomes condense in the course of cell division, each chromosome consists of two separate chromosomes still partially joined where the **spindle fibers** attach. The term chromatids is applied to the still-joined chromosomes at this stage. As soon as they separate, they are again called chromosomes.)

Mitosis is divided into four stages (**FIGURE 3-8**): prophase, metaphase, anaphase, and telophase.

Chromatid One of two newly formed chromosomes held together by the centromere.

Spindle fibers The structure critical for proper alignment and separation of chromosomes during mitosis and meiosis.

Centromere/centriole The structure that joins each pair of chromatids formed by chromosome duplication.

Prophase

Each chromosome thickens and shortens. The **centrioles** migrate to opposite poles of the cell and form the mitotic spindle, which consists of small fibers radiating in all directions from the centrioles. Some of these spindle fibers attach to the chromatids. The nuclear membrane breaks down toward the end of prophase.

Metaphase

The chromosomes line up in the center of the cell. At this stage, the chromatids are partially separated but still remain joined at a constricted area called the **centromere**, which is the site where the spindle fibers are attached.

Anaphase

The chromatids constituting each chromosome separate to form individual chromosomes, which are pulled to opposite poles of the cell by the spindle fibers.

Telophase

The nuclear membranes of the two daughter cells reform and the cytoplasm divides, forming two daughter cells. Each is an exact duplicate of the parent cell.

MEIOSIS

Meiotic cell division reduces the number of chromosomes by half and also leads to some intermixing of genetic material between homologous chromosomes via a **recombination** process known as **crossing over**. The process of meiosis entails two separate divisions called the first and second meiotic divisions (**FIGURE 3-9**).

Recombination/ crossover Interchange of genetic material between homologous chromosomes during synapse and meiosis. Recombination between genes can be observed.

Synapse Pairing of homologous chromosomes in meiosis.

First Meiotic Division

As in mitosis, each chromosome duplicates itself before beginning cell division, forming two chromatids. During prophase, each homologous pair of chromosomes come to lie side by side over their entire length. This association is called a **synapse**. At this stage, there is frequently some interchange of segments between homologous chromosomes, which is called a crossover. The pairing of homologous chromosomes and the interchange of genetic material during prophase is the characteristic feature of meiosis. In the female, the two X chromosomes synapse in the same way as autosomes, but in the male, the X and Y chromosomes synapse end to end and do not exchange segments. Rates of crossing over are greater in females than in males.

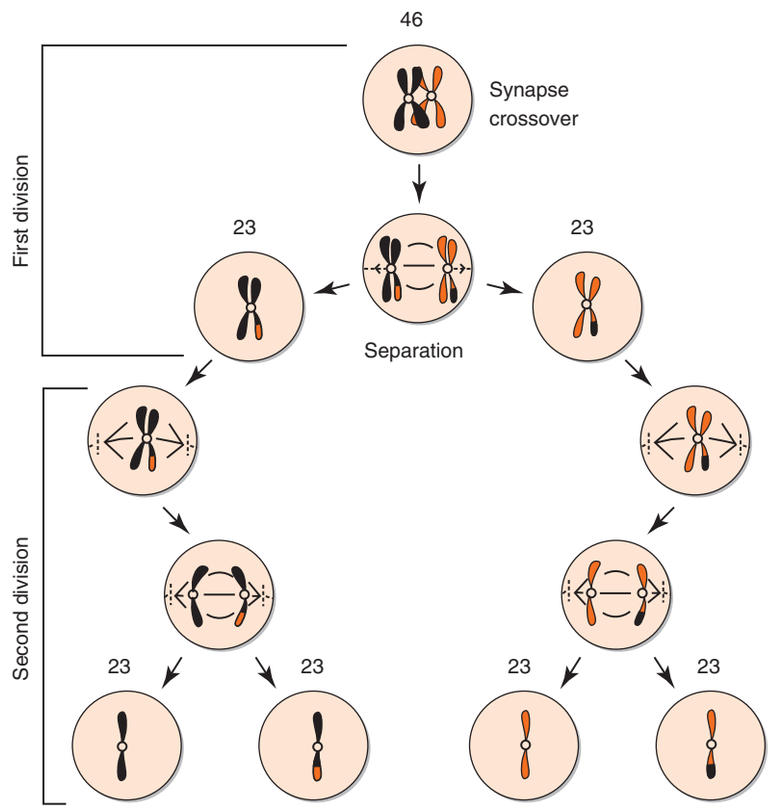


FIGURE 3-9 Stages of meiosis. The behavior of only one pair of homologous chromosomes is indicated. In the first meiotic division, each daughter cell receives only one member of each homologous pair, and the chromosomes are not exact duplicates of those in the parent cell. The second meiotic division is like a mitotic division, but each cell contains only 23 chromosomes.

In metaphase, the paired chromosomes become arranged in a plane within the middle of the cell. During anaphase, the homologous chromosomes separate and move to opposite poles of the cell. Each chromosome consists of two chromatids, but they do not separate at this stage. In telophase, two new daughter cells are formed. Each daughter cell contains only one member of each homologous pair of chromosomes; consequently, the chromosomes in each daughter cell are reduced by half. The chromosomes in the daughter cells are also different from those in the parent cell because of the interchange of genetic material during synapse.

Second Meiotic Division

The second meiotic division is similar to a mitotic division. The two chromatids composing each chromosome separate and two new daughter cells are formed, each containing half of the normal number of chromosomes.

Gametogenesis

The testes and ovaries, called **gonads**, contain precursor cells called germ cells, which are capable of developing into mature sperm or ova. The mature germ cells are called **gametes**, and the process by which they are formed is gametogenesis. The development of sperm (spermatogenesis) and of ova (oogenesis) is similar in many respects (**FIGURE 3-10**).

Gonad A general term referring to either the ovary or the testis.

Gametes Reproductive cells, eggs, and sperm, each containing 23 chromosomes, which unite during fertilization to form a zygote containing 46 chromosomes.

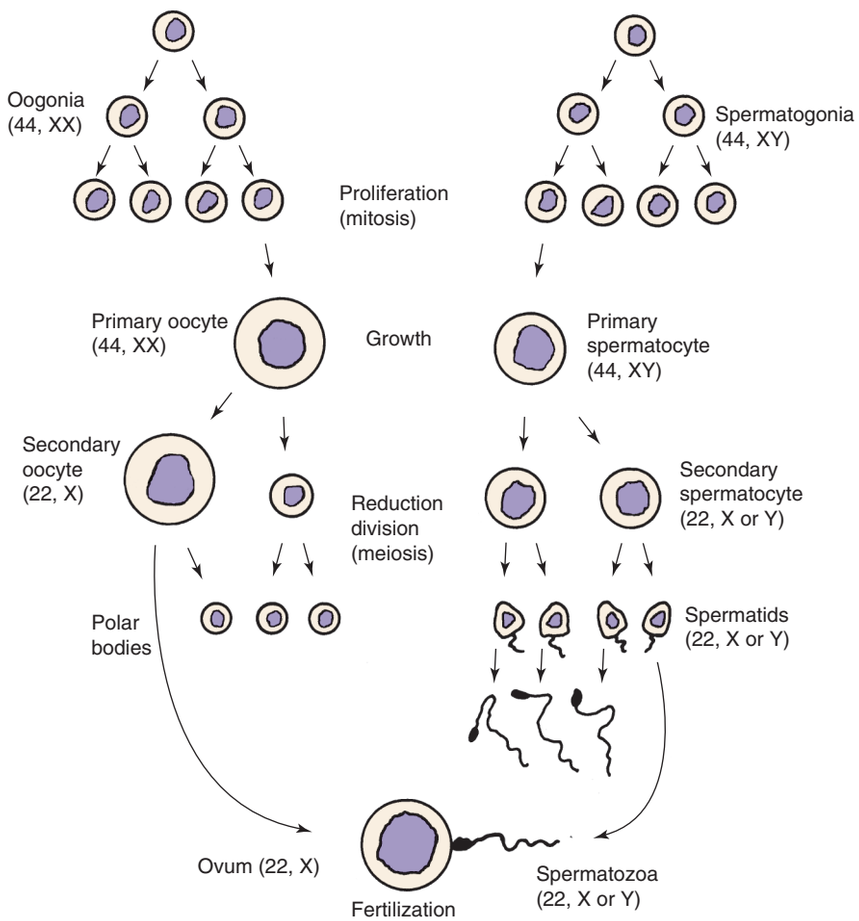


FIGURE 3-10 Sequence of events in gametogenesis. The numbers and letters in parentheses refer to the chromosomes in the cell. Numbers indicate autosomes; letters designate sex chromosomes.

SPERMATOGENESIS

Spermatogonia Precursor cells of sperm.

Spermatocytes Precursor cells to sperm formed by mitotic division of spermatogonia.

Spermatids Germ cells in a late stage of sperm development just before complete maturation to form mature sperm. *Spermatogonia* and *spermatocytes* are terms for an earlier stage.

The precursor cells in the testicular tubules are called **spermatogonia** (singular, spermatogonium). Each contains a full complement of 46 chromosomes. Spermatogonia divide by mitosis to form primary **spermatocytes**, which, like the precursor cells, contain 46 chromosomes. The primary spermatocytes then divide by meiosis. In the first meiotic division, each primary spermatocyte forms two secondary spermatocytes, each containing 23 chromosomes. Each secondary spermatocyte completes the second meiotic division and forms two **spermatids**, also containing 23 chromosomes, and the spermatids mature into sperm. The entire process of spermatogenesis takes about two months, and sperm are being produced continually after sexual maturity is reached.

OOGENESIS

Oogonia Precursor cells of oocyte.

Oocyte Germ cells in a late stage of egg development.

Granulosa cells Cells lining the ovarian follicles.

Pituitary gonadotrophic hormones Hormones controlling cyclic ovulation.

Polar body Structure extruded during the meiosis of the oocyte. Contains discarded chromosomes and a small amount of cytoplasm.

The precursors of the ova are called **oogonia** (singular, oogonium). Each contains 46 chromosomes. Oogonia divide repeatedly in the fetal ovaries before birth, forming primary **oocytes**, which contain 46 chromosomes. The oocytes then become surrounded by a single layer of cells called **granulosa cells**, or follicular cells, forming structures called primary follicles. The primary oocytes in the follicles begin the prophase of the first meiotic division during fetal life but do not carry the division through to completion. A very large number of primary follicles are formed, but many of them degenerate during infancy and childhood. Up to 20 percent of oocytes show defects in chromosome complement (aneuploidy) and are likely to represent the degenerating population. However, about half a million of the primary follicles persist into adolescence, and the loss continues throughout the woman's reproductive years. During each reproductive cycle, several oocytes begin to mature; however, usually only one is ovulated, and the others degenerate. At menopause, only a few thousand oocytes remain, and the decline continues until, eventually, there are no oocytes left in the ovaries of a postmenopausal woman.

The ovaries with their contained primary follicles remain inactive until puberty. Then cyclic ovulation begins under the influence of the **pituitary gonadotrophic hormones**, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). During each menstrual cycle, a number of primary follicles begin to grow, but normally only one follicle comes to full maturity and is ovulated. When the oocyte is discharged, it completes its first meiotic division and gives rise to two daughter cells, which are unequal in size. One daughter cell, which receives half of the chromosomes (one member of each homologous pair) and almost all of the cytoplasm, is called a secondary oocyte (23 chromosomes). The other daughter cell, which receives the remaining 23 chromosomes but almost none of the cytoplasm, is called the first **polar body** and is discarded. The newly formed secondary oocyte promptly begins its second meiotic division, which will lead to the formation of the mature ovum and a second polar body, each containing 23 chromosomes. The meiotic division is not completed, however, unless the ovum is fertilized.

COMPARISON OF SPERMATOGENESIS AND OOGENESIS

Spermatogenesis and oogenesis have two major differences. First, four spermatozoa are produced from each precursor cell in spermatogenesis, but only one ovum is formed from each precursor cell in oogenesis. The other three "daughter cells" derived from the meiotic divisions are discarded as polar bodies.

Second, spermatogenesis occurs continually and is carried through to completion in about two months. Consequently, seminal fluid always contains relatively "fresh" sperm. In contrast, the oocytes are not produced continually. All of the oocytes present

in the ovary were formed before birth and have remained in a prolonged prophase of the first meiotic division from fetal life until they are ovulated. This may be why congenital abnormalities that result from abnormal separation of chromosomes in the course of gametogenesis are more frequent in older women. The ova released late in a woman's reproductive life have been held in prophase for as long as forty-five years before they finally resume meiosis at the time of ovulation. If the chromosomes do not separate normally in meiosis, an ovum may end up with either an excess or a deficiency of chromosomes. If the abnormal ovum is fertilized, a fetus that has an abnormal number of chromosomes may be conceived. This subject is considered in the discussion on congenital and hereditary diseases.

New Research on Genes

Starting with the Human Genome Project in 1990, tremendous strides have been made in our ability to determine the entire sequence of DNA in individuals. Currently, limited regions of the genome of individuals are sequenced using automated technology on a routine basis to obtain information for clinical diagnosis of disease and as an aid in selecting therapies. Major medical centers are beginning to routinely sequence multiple genes in individuals with a variety of cancers to determine genetic lesions involved in the causation and progress of the disease. This has led to the concept of personalized therapy in which specific drugs are matched to defects in genes responsible for particular diseases (so-called drugable genes). As previously noted, defects in growth factors and receptors may lead to unregulated cell growth and cancer. In many cases, the specific defect in the genome (mutation) responsible for unregulated growth may be determined by sequencing, and, in some cases, drugs have been tailored to inhibit the defect and slow down (although not cure) the disease. Differences in how individuals respond to drugs are often related to variation in specific genes in the genome. Technology exists to detect those differences and tailor drug therapy to prevent inappropriate dosing, which can result in dangerous drug reactions.

Although still somewhat costly and time consuming, the sequence of all expressed genes (the **exome**) or even the 3 billion base pairs of a single individual, can be determined. This has led to massive databases available for study and to the development of the study of **bioinformatics**. Bioinformatics as applied to studies of the human genome (**genomics** is a commonly used term for such) involves complex mathematical comparisons between data from the genome of many individuals to find base sequences that may be associated with the susceptibility to disease (or to resistance to particular diseases). Obviously, such information can be used for the prognosis, early therapy, or, potentially, the prevention of diseases in the human population.

Exome The set of all expressed genes.

Bioinformatics Massive databases used for the study of the human genome.

Genomics Bioinformatic techniques applied to studies of human genome.

SINGLE NUCLEOTIDE POLYMORPHISMS

The sequence of base pairs in the human genome is about 99.9 percent identical between humans. (In comparison, we differ from our closest primate cousin by about 4 percent.) At the gross level, human genomes appear to be nearly identical. However, the remaining 0.1 percent of 3 billion base pairs means that average humans differ in about 3 million base pairs. In fact, as is familiar to all from popular media productions involving the science of forensics, no two individuals have exactly the same genome except identical twins. Determination of identity does not require complex sequencing of the genome because particular regions of DNA show great variation. Simple technology allows rapid typing of individual DNA samples (from body fluids or tissue such as hair) in a manner somewhat analogous to blood typing.

Single nucleotide polymorphisms (SNPs) Minor variations in the nucleotides contained in the individual genes of different individuals.

Genomewide association studies (GWAS) Process using tests of many SNPs to identify genes associated with particular diseases or traits.

Recombinant DNA technology/genetic engineering/gene splicing Methods for combining a gene from one organism, such as a gene specifying insulin synthesis, with genes from another organism, such as a bacterium.

Expression vector A set of DNA sequences necessary for the expression of a foreign gene in an organism. Often engineered to promote high levels of production of the gene product.

Monoclonal antibodies Antibodies of a unique specificity for use in treating human disease, produced in tissue culture.

Many of the minor variations in the nucleotides contained in the individual genes of different individuals may or may not have direct significance. These variations are called **single nucleotide polymorphisms (SNPs)**, usually pronounced "snips." Some SNPs may affect how the gene functions, such as how rapidly a cell enzyme inactivates a drug or environmental toxin or repairs damage to cell DNA. Genes in individuals within specific groups having risk factors for some disease or condition can be analyzed (constructing a "gene profile") to see if there is a correlation with particular SNPs. Although some SNPs lie within genes and may directly affect how a gene functions, many SNPs occur in the 98 percent of the genome that does not contain genes expressing a protein product. Such SNPs are important because their position within the genome is known and also because they easily can be typed without the need for direct DNA sequencing. This has allowed for the ever-increasing use of **genomewide association studies (GWAS)** to identify genes associated with particular diseases or traits. In such studies, the frequency of a very large number of SNPs spread across the entire genome is compared between a population with the particular condition or disease and a control population without the disease. Statistical studies are undertaken to see if the presence of particular SNPs correlates with the presence (or absence) of the disease in question. Because the position of the SNPs in the genome is known, an association suggests areas of the genome (or particular genes) that are associated with the condition. GWAS are particularly useful in studying complex chronic conditions such as heart disease and neuropsychiatric disorders that may be associated with differences in multiple genes found in different areas of the genome.

GENES AND RECOMBINANT DNA TECHNOLOGY (GENETIC ENGINEERING)

Overuse of terms such as **recombinant DNA technology** (because genes from two different sources are being recombined in a single unit), **genetic engineering** (because genes are being manipulated), or **gene splicing** (because genetic material is being reassembled in a given order) have made them both difficult to define and of limited utility. At its heart genetic engineering involves the techniques used to manipulate how and where individual genes and their products are expressed and to modify such genes to produce products of desired characteristics. The initial stages of the technology involved understanding how bacterial genes and their products could be manipulated. Currently gene products derived from viruses, bacteria, fungi and higher organisms can be manipulated to be expressed in essentially any host cell desired. When the product is to be used for human therapy (such as human growth factors, or proteins involved in coagulation used to treat deficiency diseases), the source of the gene coding for the product is most often human. This is inserted into an **expression vector**, a cassette of DNA sequences necessary for expression of the gene, which in turn is introduced into a mammalian cell grown in culture for expression. Both the gene and the expression vector are optimized to result in high levels of expression and increased stability of the desired product, which optimally will be secreted into the medium in which the mammalian cells are growing. An exception to the use of human genes is in the production of antibodies of a unique specificity (**monoclonal antibodies**) for use in treating human disease. Very often the gene may be derived from a mouse immunized to produce the desired antibody. A common example are antibodies to human inflammatory proteins used to treat diseases such as rheumatoid arthritis (which results in chronic joint inflammation). Because the use of animal proteins in a human may result in a serious immunological reaction, the mouse genes are altered to make them more "humanlike" before they are expressed.

It is also possible to introduce genes into living organisms or to control the expression of genes in an organism. Although news accounts of animals made to "glow" by the introduction fluorescent proteins trivialize this still difficult technique, the approach could be used to cure diseases associated with the deficiency of a protein related to a genetic mutation (so-called **gene therapy**). A prime example of this would be the introduction of the gene for a clotting factor into an individual with hemophilia. The area is complex and still lacks notable success. There are obvious problems with this approach;

1. One must identify and select the correct gene to insert into the cell.
2. One must choose the proper cell to receive the gene.
3. One must select an efficient means of getting the gene into the cell.
4. One must ensure that the newly inserted gene can function effectively long enough within the cell to make the therapy worthwhile and doesn't disrupt other important cell functions.

Gene therapy Introduction of genes into cells with the object of curing diseases associated with the deficiency of a protein related to a genetic mutation.

Polymerase chain reaction (PCR) Technology used to amplify stretches of DNA.

The gene may be expressed in a small segment of DNA that does not become a permanent part of the genome (and tends to be lost with time), or it may be integrated into the chromosome much as some viral DNAs to become part of the human genome. As can be imagined, insertion of DNA into the genome at random can have serious consequences by interrupting the synthesis of other genes. In summary, although many of the pieces of this technology are present, putting the pieces together for successful human therapy remains to be achieved.

Proposed gene therapy in humans targets somatic cells, not the germ cells that produce eggs and sperm. Gene therapy directed at germ cells is currently not considered to be feasible, ethical, or desirable. However, related technology has been used with great success to "knock out" genes in animals (most often mice and pigs) in such a way as to modify the animal's germ cells to produce animal strains that can be bred as models of particular human diseases. For example, there are strains of mice that exhibit "human hemophilia" and can be used as a model in studies of gene therapy.

An often ignored aspect of genetic engineering is the ability to chemically synthesize "artificial" segments of DNA or to modify segments of naturally occurring gene sequences to serve as diagnostic tools. The area is very complex but relies on the ability of DNA sequences to bind (hybridize) specifically to their complement. For example, if one wished to detect a pathogen in human samples (e.g., the TB bacteria), one would label a complementary sequence (a probe) for a common gene found in the bacterium that could detect the target organism's DNA. Thus one can label a relatively short segment of "manufactured" DNA with an indicator (such as a fluorescent dye or enzyme) and use the probe to detect a complementary DNA sequence in a sample by its ability to bind and "light up." This technique is often used in the microbiology laboratory to diagnosis the presence of an infectious agent in a human sample. Manufactured small stretches of DNA that subtend an area of the genome can be used to artificially amplify the subtended area up to millions of times, greatly increasing the sensitivity of detection. The most common amplification technology uses the **polymerase chain reaction (PCR)**. Using such technology, the AIDS viral genes can be amplified to a level where even fifty copies of the viral genome can be detected in a single ml of patient blood.

The ability to use such technology raises difficult ethical questions, not only for the molecular biologist but for all of us who potentially might benefit. How much manipulation of human embryos is appropriate? Many would say yes to techniques aimed at curing genetic disease, but what about manipulating cosmetic factors such as attempting to increase muscle mass, height, or intelligence? Such questions most certainly will occur in the future.

CASE 3-1

Hemophilia B results from a mutation of the F.IX B gene, causing a decrease in the ability of the F.IX protein to function in coagulation. At the age of eighteen months, a female child (individual II.2 in the pedigree in **FIGURE 3-11**) fell while playing with her fraternal twin sister and hit her knee on a table leg. She suffered a serious bleeding event in the injured knee joint that required hospital therapy. At that time, she was found to have a F.IX level 2 percent of normal and was diagnosed as being a female with hemophilia B. She required therapy with recombinant F.IX.

The family history indicated that her father (individual I.1 in the pedigree) also has the bleeding disease hemophilia B. Because his F.IX activity is less than 1 percent of normal levels, he requires therapy with human recombinant F.IX produced using genetic engineering technology in animal cells grown in tissue culture. If not treated, he suffers from spontaneous bleeding into his joints (**hemarthrosis**), which ultimately can result in severe joint damage. He is also at risk for life-threatening hemorrhages in his brain. His wife (individual I.2) has no bleeding tendencies and has normal levels of F.IX (about 100 percent of normal). She has no family history of bleeding diseases.

Hemarthrosis Arthritic condition caused by bleeding into the joint.

The parents have two offspring (II.1 and II.3) in addition to II.2. The older sibling (II.1) is a male with normal levels of F.IX. The younger siblings are female fraternal (not identical) twins. The sister (II.3) has not suffered from bleeding and has a F.IX level of 60 percent, which is sufficient to provide normal coagulation. (Bleeding is usually only noted when F.IX levels are below about 20 percent of normal.) As far as could be determined, the karyotype of all members of the family (including II.2) was normal.

Discussion

The normal level of F.IX in the male sibling is expected as he would have had to inherit his father's Y chromosome (to be male) and one of the mother's two normal X chromosomes. However, because hemophilia B is a recessive sex-linked disorder, all daughters of an affected father are obligate carriers (both individuals II.2 and II.3). They must inherit their father's single X chromosome, which carries the defective gene. Even as carriers, they would be expected to be normal (in terms of hemophilia B), having inherited one of their mother's normal X chromosomes as well as their father's mutated X chromosome. However, individual II.2 is not normal.

Female hemophiliacs do occur occasionally in families in which defects of the Factor IX gene are found. There are two general reasons this might occur.

1. Suppose the mother was actually not normal, but a carrier for hemophilia B. In such a case daughter II.2 would have received her father's affected X chromosome and had a 50 percent chance of inheriting her mother's single affected X chromosome. If she had, II.2 would be homozygous for a mutation in the F.IX gene and expected to be affected. In this case, this is unlikely because the mother's family did not demonstrate a history of bleeding disease and her normal level of F.IX strongly argues against her being an unknown carrier. However, females with homozygous hemophilia are known to occur (although very rarely).
2. A more likely reason for the low level of F.IX in II.2 is nonrandom X chromosome inactivation. As noted in this chapter, about half the cells in a female should have the paternally derived X chromosome inactivated and half the maternally derived chromosome. However, random inactivation does not always occur. A number of defects in the structure of the X chromosome (such as certain deletions and other chromosomal abnormalities) can result in the faulty chromosome being selectively inactivated. In such a case, if the structurally defective chromosome was from the mother (and hence normal in terms of the F.IX gene), the father's chromosome (carrying the hemophilia B gene) might be selectively expressed.

CASE 3-1 (*Continued*)

To determine whether nonrandom X inactivation did occur, the distribution of active maternal and paternal X chromosomes in the patient was examined using an X-linked SNP, which could differentiate the maternal and paternal X chromosomes of the patient. Ninety-seven percent of X chromosome product bearing the SNP in II.2 was from the paternal (affected) chromosome. In the unaffected twin sibling (II.3), the distribution was nearly random (about 40 percent was from the affected paternal chromosome).

Etiology and Pathogenesis

The most likely cause of female hemophilia in II.2 was extreme nonrandom X inactivation resulting in overrepresentation of the paternal X chromosome bearing a mutated Factor IX gene. However, this conclusion does not explain why this occurred in II.2 but not her sister, II.3. The great majority of female hemophiliacs result from unbalanced nonrandom X chromosome inactivation with no evidence of defects in the structure of the X chromosomes.

Questions

1. Suppose the mother in the family was a carrier of hemophilia B. How would this change the probability of disease in the offspring (and in future offspring)?
2. If the twins (II.2 and II.3) were identical, would this change your explanation for the occurrence of female hemophilia?
3. It is noted that recombinant human F.IX was used in therapy for affected individuals in the family. Why might recombinant human F.IX be preferable to F.IX isolated from pools of human blood?

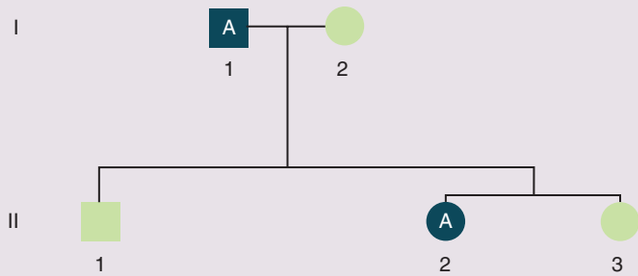


FIGURE 3-11 Case pedigree for sex-linked hemophilia B.

Courtesy of Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill.

QUESTIONS FOR REVIEW

1. What is meant by the following terms: *homologous chromosomes*, *autosomes*, *Barr body*, *gene*, *gametogenesis*, and *exome*?
2. Compare and contrast transcription and translation.
3. How does the process of mitosis compare with meiosis?
4. What are the differences between spermatogenesis and oogenesis?
5. What is a chromosome karyotype? How is it obtained? How is it used?
6. What pattern in a pedigree is typical of the inheritance of a recessive gene?
7. What is a SNP? How is it used?

SUPPLEMENTARY READINGS

Human Genome (Wikipedia). http://en.wikipedia.org/wiki/Human_genome

Introductory Genetics Online. Genetics Courses from the Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami. http://hihg.med.miami.edu/educational-programs/online-genetics-courses

All about the Human Genome project (HGP) National Institute of Health. http://www.genome.gov/10001772

- These three references provide an excellent introductory online guide to human genetics. The third reference contains an extensive list of online resources of interest to both students and teachers.

Wirth, T., Parker, N., and Yla-Herttuala, S. 2013. History of gene therapy. *Gene* 525:162–69.

- An overview of gene therapy providing an introduction to the technology used and some current applications. There is a brief discussion of ethical issues.

Fan, N., and Lai, L. 2013. Genetically modified pig models for human diseases. *Journal of Genetics and Genomics* 40:67–73.

- Genetic modification of animals to produce models of human disease has been one of the most successful applications of genetic engineering to biomedical research. The recent extension of this technology from mice to pigs has yielded disease models in a species more comparable in size and metabolism to humans.

Walters, L. 2012. Genetics and bioethics: How our thinking has changed since 1969. *Theoretical Medicine and Bioethics* 33:83–95.

Robillard, J. M., Roskams_Edris, D., Kuzeljevic, B., et al. 2014. Prevailing public perceptions of the ethics of gene therapy. *Human Gene Therapy* 25:1–7.

- These two articles review how both professional bioethicists and the public view the ethics of gene therapy.

Altman, R. B. 2012. Translational bioinformatics: Linking the molecular world to the clinical world. *Clinical Pharmacology and Therapeutics* 91:994–1000.

- Describes how bioinformatics is used to translate basic biological discoveries into the clinic.

Visscher, P. M., Brown, M. A., McCarthy, M. I., et al. 2012. Five years of GWAS discovery. *American Journal of Human Genetics* 90:7–24.

- The use of genomewide association studies (GWAS) to detect genetic associations with disease has received much attention in the press. This is a balanced overview of the role of GWAS in biomedical research examining both its strengths and weaknesses.

Okumura, K., Fujimori, Y., Takagi, A., et al. 2008. Skewed X chromosome inactivation in fraternal female twins results in moderately severe and mild haemophilia B. *Haemohilia* 14:1088–93.

Peeters, S. B., Cotton, A. M., and Brown C. J. 2014. Variable escape from X-chromosome inactivation: Identifying factors that tip the scales toward expression. *Bioessay*s 36:1–11.

- The first reference is the source on which the chapter case was modeled (in part) and contains additional details explaining how the pattern of X chromosome inactivation was determined in the family. The second reference provides additional information on the mechanism of X chromosome inactivation, which is far more interesting (and complex) than presented in the chapter.