Chromosomes in the prometaphase stage of mitosis, derived from a cell in the flower of *Haemanthus*.

2

Mitosis and Meiosis

CHAPTER CONCEPTS

- Genetic continuity between generations of cells and between generations of sexually reproducing organisms is maintained through the processes of mitosis and meiosis, respectively.

- Diploid eukaryotic cells contain their genetic information in pairs of homologous chromosomes, with one member of each pair being derived from the maternal parent and one from the paternal parent.

- Mitosis provides a mechanism by which chromosomes, having been duplicated, are distributed into progeny cells during cell reproduction.

- Mitosis converts a diploid cell into two diploid daughter cells.

- The process of meiosis distributes one member of each homologous pair of chromosomes into each gamete or spore, thus reducing the diploid chromosome number to the haploid chromosome number.

- Meiosis generates genetic variability by distributing various combinations of maternal and paternal members of each homologous pair of chromosomes into gametes or spores.

- During the stages of mitosis and meiosis, the genetic material is condensed into discrete structures called chromosomes.

E very living thing contains a substance described as the genetic material. Except in certain viruses, this material is composed of the nucleic acid, DNA. DNA has an underlying linear structure possessing segments called genes, the products of which direct the metabolic activities of cells. An organism's DNA, with its arrays of genes, is organized into structures called **chromosomes**, which serve as vehicles for transmitting genetic information. The manner in which chromosomes are transmitted from one generation of cells to the next and from organisms to their descendants must be exceedingly precise. In this chapter we consider exactly how genetic continuity is maintained between cells and organisms.

Two major processes are involved in the genetic continuity of nucleated cells: **mitosis** and **meiosis**. Although the mechanisms of the two processes are similar in many ways, the outcomes are quite different. Mitosis leads to the production of two cells, each with the same number of chromosomes as the parent cell. In contrast, meiosis reduces the genetic content and the number of chromosomes by precisely half. This reduction is essential if sexual reproduction is to occur without doubling the amount of genetic material in each new generation. Strictly speaking, mitosis is that portion of the cell cycle during which the hereditary components are equally partitioned into daughter cells. Meiosis is part of a special type of cell division that leads to the production of sex cells: **gametes** or **spores**. This process is an essential step in the transmission of genetic information from an organism to its offspring.

Normally, chromosomes are visible only during mitosis and meiosis. When cells are not undergoing division, the genetic material making up chromosomes unfolds and uncoils into a diffuse network within the nucleus, generally referred to as **chromatin**. Before describing mitosis and meiosis, we will briefly review the structure of cells, emphasizing components that are of particular significance to genetic function. We will also compare the structural differences between the prokaryotic (nonnucleated) cells of bacteria and the eukaryotic cells of higher organisms. We then devote the remainder of the chapter to the behavior of chromosomes during cell division.

2.1
## Cell Structure Is Closely Tied to Genetic Function

Before 1940, our knowledge of cell structure was limited to what we could see with the light microscope. Around 1940, the transmission electron microscope was in its early stages of development, and by 1950, many details of cell ultrastructure had emerged. Under the electron microscope, cells were seen as highly varied, highly organized structures whose form and function are dependent on specific genetic expression by each cell type. A new world of whorled membranes, organelles, microtubules, granules, and filaments was revealed. These discoveries revolutionized thinking in the entire field of biology. Many cell components, such as the nucleolus, ribosome, and centriole, are involved directly or indirectly with genetic processes. Other components—the mitochondria and chloroplasts—contain their own unique genetic information. Here, we will focus primarily on those aspects of cell structure that relate to genetic study. The generalized animal cell shown in **Figure 2–1** illustrates most of the structures we will discuss.

All cells are surrounded by a **plasma membrane**, an outer covering that defines the cell boundary and delimits the cell from its immediate external environment. This membrane is not passive but instead actively controls the movement of materials into and out of the cell. In addition to this membrane, plant cells have an outer covering called the **cell wall** whose major component is a polysaccharide called *cellulose.*

Many, if not most, animal cells have a covering over the plasma membrane, referred to as the **glycocalyx**, or **cell coat**. Consisting of glycoproteins and polysaccharides, this covering has a chemical composition that differs from comparable structures in either plants or bacteria. The glycocalyx, among other functions, provides biochemical identity at the surface of cells, and the components of the coat that establish cellular identity are under genetic control. For example, various cell-identity markers that you may have heard of—the AB, Rh, and MN antigens—are found on the surface of red blood cells, among other cell types. On the surface of other cells, histocompatibility antigens, which elicit an immune response during tissue and organ transplants, are present. Various **receptor molecules** are also found on the surfaces of cells. These molecules act as recognition sites that transfer specific chemical signals across the cell membrane into the cell.

Living organisms are categorized into two major groups depending on whether or not their cells contain a nucleus. The presence of a nucleus and other membranous organelles is the defining characteristic of **eukaryotic organisms**. The **nucleus** in eukaryotic cells is a membrane-bound structure that houses the genetic material, DNA, which is complexed with an array of acidic and basic proteins into thin fibers. During nondivisional phases of the cell cycle, the fibers are uncoiled and dispersed into chromatin (as mentioned above). During mitosis and meiosis, chromatin fibers coil and condense into chromosomes. Also present in the nucleus is the **nucleolus**, an amorphous component where ribosomal RNA (rRNA) is synthesized and where the initial stages of ribosomal assembly occur. The portions of DNA that encode rRNA are collectively referred to as the **nucleolus organizer region**, or the **NOR**.
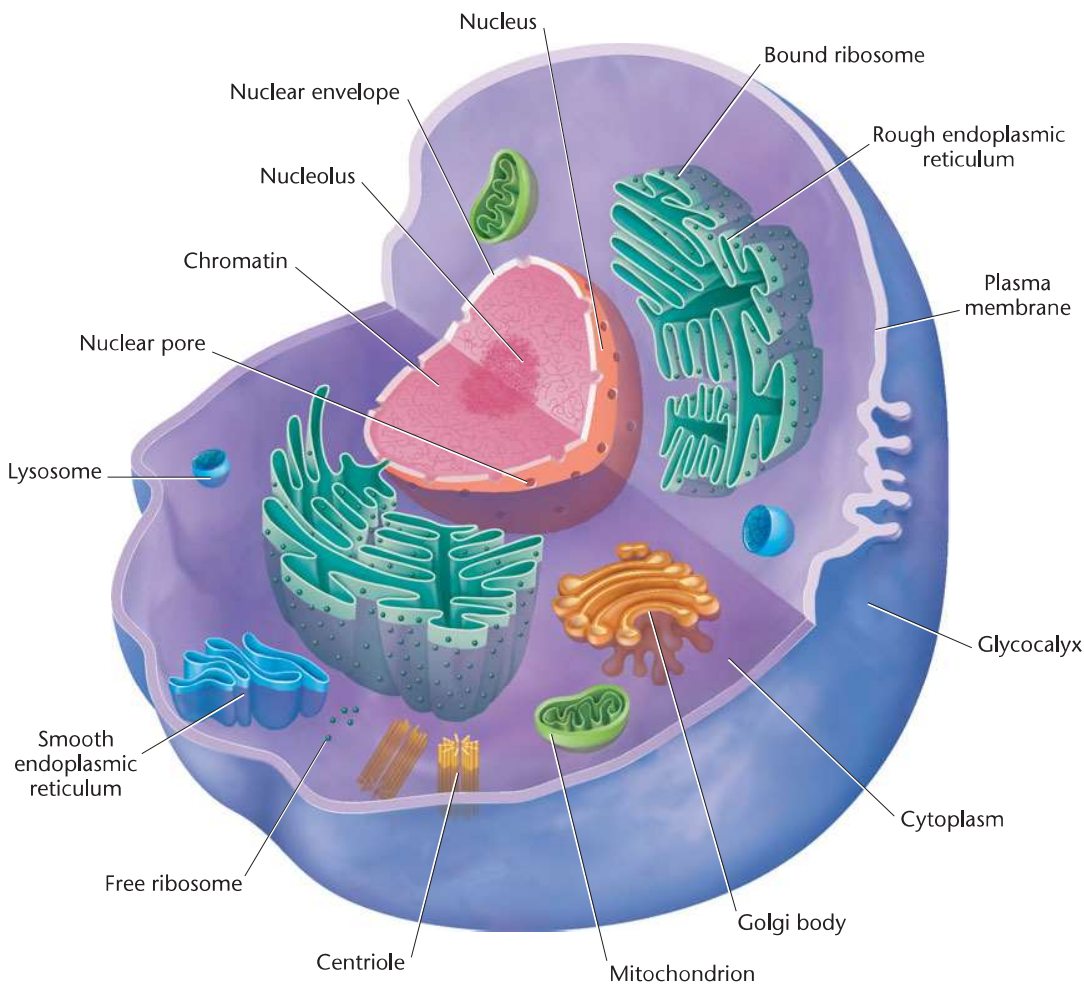
A generalized animal cell. The cellular components discussed in the text are emphasized here.

Prokaryotic organisms, of which there are two major groups, lack a nuclear envelope and membranous organelles. For the purpose of our brief discussion here, we will consider the *eubacteria,* the other group being the more ancient bacteria referred to as *archaea.* In eubacteria, such as *Escherichia coli,* the genetic material is present as a long, circular DNA molecule that is compacted into an unenclosed region called the **nucleoid**. Part of the DNA may be attached to the cell membrane, but in general the nucleoid extends through a large part of the cell. Although the DNA is compacted, it does not undergo the extensive coiling characteristic of the stages of mitosis, during which the chromosomes of eukaryotes become visible. Nor is the DNA associated as extensively with proteins as is eukaryotic DNA. Figure 2–2, which shows two bacteria forming by cell division, illustrates the nucleoid regions containing the bacterial chromosomes. Prokaryotic cells do not have a distinct nucleolus but do contain genes that specify rRNA molecules.

The remainder of the eukaryotic cell within the plasma membrane, excluding the nucleus, is referred to as **cytoplasm** and includes a variety of extranuclear cellular organelles. In the cytoplasm, a nonparticulate, colloidal material referred to as the **cytosol** surrounds and encompasses the cellular organelles. The cytoplasm also includes an extensive system of tubules and
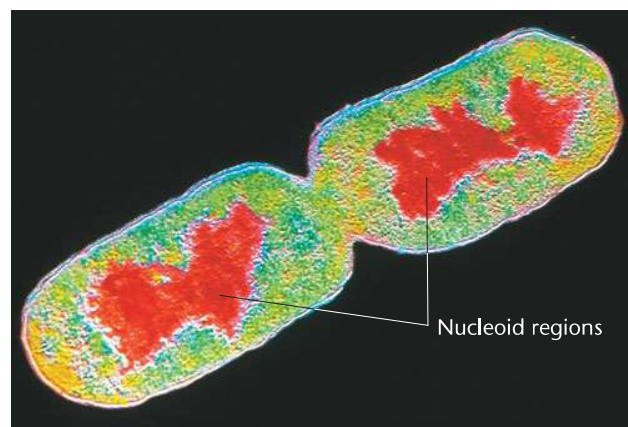
Color-enhanced electron micrograph of *E. coli* undergoing cell division. Particularly prominent are the two chromosomal areas (shown in red), called nucleoids, that have been partitioned into the daughter cells.

filaments, comprising the cytoskeleton, which provides a lattice of support structures within the cell. Consisting primarily of **microtubules**, which are made of the protein **tubulin**, and **microfilaments**, which derive from the protein **actin**, this structural framework maintains cell shape, facilitates cell mobility, and anchors the various organelles.

One organelle, the membranous **endoplasmic reticulum (ER)**, compartmentalizes the cytoplasm, greatly increasing the surface area available for biochemical synthesis. The ER appears smooth in places where it serves as the site for synthesizing fatty acids and phospholipids; in other places, it appears rough because it is studded with ribosomes. **Ribosomes** serve as sites where genetic information contained in messenger RNA (mRNA) is translated into proteins.

Three other cytoplasmic structures are very important in the eukaryotic cell's activities: mitochondria, chloroplasts, and centrioles. **Mitochondria** are found in most eukaryotes, including both animal and plant cells, and are the sites of the oxidative phases of cell respiration. These chemical reactions generate large amounts of the energy-rich molecule adenosine triphosphate (ATP). **Chloroplasts**, which are found in plants, algae, and some protozoans, are associated with photosynthesis, the major energy-trapping process on Earth. Both mitochondria and chloroplasts contain DNA in a form distinct from that found in the nucleus. They are able to duplicate themselves and transcribe and translate their own genetic information. It is interesting to note that the genetic machinery of mitochondria and chloroplasts closely resembles that of prokaryotic cells. This and other observations have led to the proposal that these organelles were once primitive free-living organisms that established symbiotic relationships with primitive eukaryotic cells. This theory concerning the evolutionary origin of these organelles is called the **endosymbiont hypothesis**.

Animal cells and some plant cells also contain a pair of complex structures called **centrioles**. These cytoplasmic bodies, located in a specialized region called the **centrosome**, are associated with the organization of spindle fibers that function in mitosis and meiosis. In some organisms, the centriole is derived from another structure, the basal body, which is associated with the formation of cilia and flagella (hair-like and whip-like structures for propelling cells or moving materials). Over the years, many reports have suggested that centrioles and basal bodies contain DNA, which could be involved in the replication of these structures. This idea is still being investigated.

The organization of **spindle fibers** by the centrioles occurs during the early phases of mitosis and meiosis. These fibers play an important role in the movement of chromosomes as they separate during cell division. They are composed of arrays of microtubules consisting of polymers of the protein tubulin.
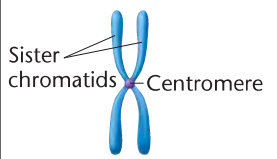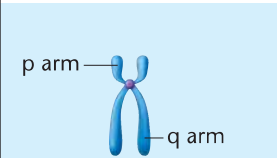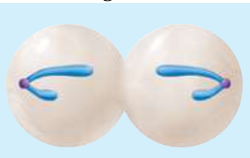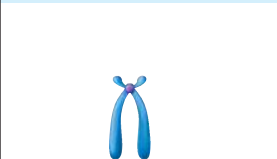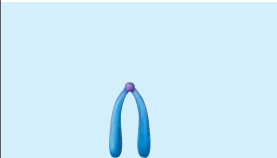
2.2
Chromosomes Exist in Homologous Pairs in Diploid Organisms

As we discuss the processes of mitosis and meiosis, it is important that you understand the concept of homologous chromosomes. Such an understanding will also be of critical importance in our future discussions of Mendelian genetics. Chromosomes are most easily visualized during mitosis. When they are examined carefully, distinctive lengths and shapes are apparent. Each chromosome contains a constricted region called the **centromere**, whose location establishes the general appearance of each chromosome. **Figure 2–3** shows chromosomes with centromere placements at different distances along their length. Extending from either side of the centromere are the arms of the chromosome. Depending on the position of the centromere, different arm ratios are produced. As Figure 2–3 illustrates, chromosomes are classified as **metacentric**, **submetacentric**, **acrocentric**, or **telocentric** on the basis of the centromere location. The shorter arm, by convention, is shown above the centromere and is called the **p arm** (p, for "petite"). The longer arm is shown below the centromere and is called the **q arm** (q because it is the next letter in the alphabet).

In the study of mitosis, several other observations are of particular relevance. First, all somatic cells derived from members of the same species contain an identical number of chromosomes. In most cases, this represents the **diploid number** (**2n**), whose meaning will become clearer below. When the lengths and centromere placements of all such chromosomes are examined, a second general feature is apparent. With the exception of sex chromosomes, they exist in pairs with regard to these two properties, and the members of each pair are called **homologous chromosomes**. So, for each chromosome exhibiting a specific length and centromere placement, another exists with identical features.

There are exceptions to this rule. Many bacteria and viruses have but one chromosome, and organisms such as yeasts and molds, and certain plants such as bryophytes (mosses), spend the predominant phase of their life cycle in the haploid stage. That is, they contain only one member of each homologous pair of chromosomes during most of their lives.

Figure 2–4 illustrates the physical appearance of different pairs of homologous chromosomes. There, the human mitotic chromosomes have been photographed, cut out of the print, and matched up, creating a display called a **karyotype**. As you can see, humans have a 2n number of 46 chromosomes, which on close examination exhibit a diversity of sizes and centromere placements. Note also that each of the 46 chromosomes in this karyotype is clearly a double

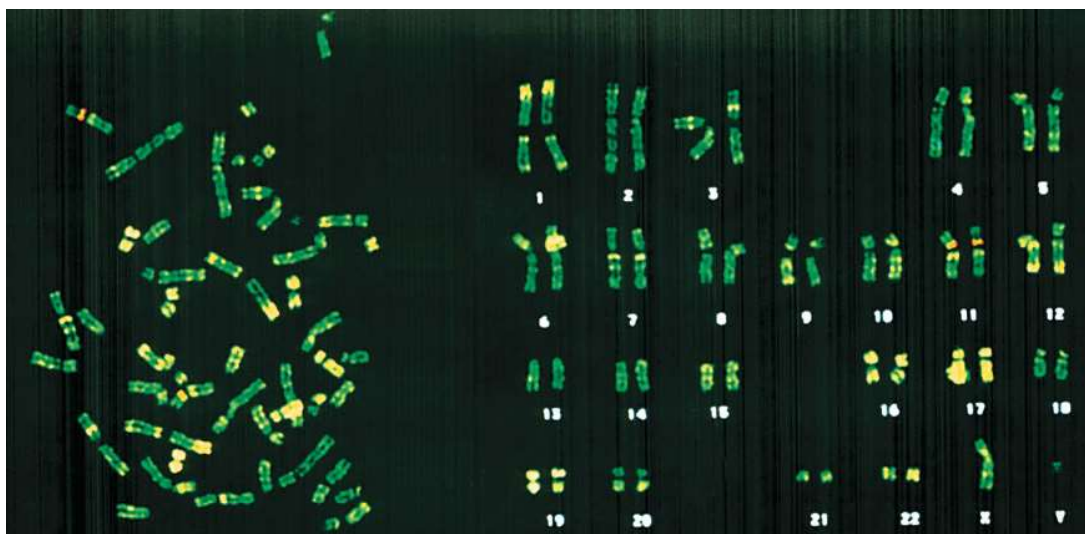| Centromere location	Designation	Metaphase shape	Anaphase shape
Middle	Metacentric	Sister chromatids — Centromere	←— Migration —→
Between middle and end	Submetacentric	p arm — / q arm	
Close to end	Acrocentric		
At end	Telocentric		

FIGURE 2–3   Centromere locations and the chromosome designations that are based on them. Note that the shape of the chromosome during anaphase is determined by the position of the centromere during metaphase.

The **haploid number** (*n*) of chromosomes is equal to one-half the diploid number. Collectively, the genetic information contained in a haploid set of chromosomes constitutes the **genome** of the species. This, of course, includes copies of all genes as well as a large amount of noncoding DNA. The examples listed in Table 2.1 demonstrate the wide range of *n* values found in plants and animals.

Homologous chromosomes have important genetic similarities. They contain identical gene sites along their lengths; each site is called a **locus** (pl. loci). Thus, they are identical in the traits that they influence and in their genetic potential. In sexually reproducing organisms, one member of each pair is derived from the maternal parent (through the ovum) and the other member is derived from the paternal parent (through the sperm). Therefore, each diploid organism contains two copies of each gene as a consequence of **biparental inheritance**, inheritance from two parents. As we shall see in the chapters on transmission genetics, the members of each pair of genes, while influencing the same characteristic or trait, need not be identical. In a population of members of the same species, many different alternative forms of the same gene, called **alleles**, can exist.

structure consisting of two parallel *sister chromatids* connected by a common centromere. Had these chromosomes been allowed to continue dividing, the sister chromatids, which are replicas of one another, would have separated into the two new cells as division continued.

FIGURE 2–4   A metaphase preparation of chromosomes derived from a dividing cell of a human male (left), and the karyotype derived from the metaphase preparation (right). All but the X and Y chromosomes are present in homologous pairs. Each chromosome is clearly a double structure consisting of a pair of sister chromatids joined by a common centromere.

TABLE 2.1

The Haploid Number of Chromosomes for a Variety of Organisms

Common Name	Scientific Name	Haploid Number
Black bread mold	*Aspergillus nidulans*	8
Broad bean	*Vicia faba*	6
Cat	*Felis domesticus*	19
Cattle	*Bos taurus*	30
Chicken	*Gallus domesticus*	39
Chimpanzee	*Pan troglodytes*	24
Corn	*Zea mays*	10
Cotton	*Gossypium hirsutum*	26
Dog	*Canis familiaris*	39
Evening primrose	*Oenothera biennis*	7
Frog	*Rana pipiens*	13
Fruit fly	*Drosophila melanogaster*	4
Garden onion	*Allium cepa*	8
Garden pea	*Pisum sativum*	7
Grasshopper	*Melanoplus differentialis*	12
Green alga	*Chlamydomonas reinhardtii*	18
Horse	*Equus caballus*	32
House fly	*Musca domestica*	6
House mouse	*Mus musculus*	20
Human	*Homo sapiens*	23
Jimson weed	*Datura stramonium*	12
Mosquito	*Culex pipiens*	3
Mustard plant	*Arabidopsis thaliana*	5
Pink bread mold	*Neurospora crassa*	7
Potato	*Solanum tuberosum*	24
Rhesus monkey	*Macaca mulatta*	21
Roundworm	*Caenorhabditis elegans*	6
Silkworm	*Bombyx mori*	28
Slime mold	*Dictyostelium discoideum*	7
Snapdragon	*Antirrhinum majus*	8
Tobacco	*Nicotiana tabacum*	24
Tomato	*Lycopersicon esculentum*	12
Water fly	*Nymphaea alba*	80
Wheat	*Triticum aestivum*	21
Yeast	*Saccharomyces cerevisiae*	16
Zebrafish	*Danio rerio*	25

The concepts of haploid number, diploid number, and homologous chromosomes are important for understanding the process of meiosis. During the formation of gametes or spores, meiosis converts the diploid number of chromosomes to the haploid number. As a result, haploid gametes or spores contain precisely one member of each homologous pair of chromosomes—that is, one complete haploid set. Following fusion of two gametes at fertilization, the diploid number is reestablished; that is, the zygote contains two complete haploid sets of chromosomes. The constancy of genetic material is thus maintained from generation to generation.

There is one important exception to the concept of homologous pairs of chromosomes. In many species, one pair, consisting of the **sex-determining chromosomes**, is often not homologous in size, centromere placement, arm ratio, or genetic content. For example, in humans, while females carry two homologous X chromosomes, males carry one Y chromosome in addition to one X chromosome (Figure 2–4). These X and Y chromosomes are not strictly homologous. The Y is considerably smaller and lacks most of the gene loci contained on the X. Nevertheless, they contain homologous regions and behave as homologs in meiosis so that gametes produced by males receive either one X or one Y chromosome.

2.3

Mitosis Partitions Chromosomes into Dividing Cells

The process of mitosis is critical to all eukaryotic organisms. In some single-celled organisms, such as protozoans and some fungi and algae, mitosis (as a part of cell division) provides the basis for asexual reproduction. Multicellular diploid organisms begin life as single-celled fertilized eggs called **zygotes**. The mitotic activity of the zygote and the subsequent daughter cells is the foundation for the development and growth of the organism. In adult organisms, mitotic activity is the basis for wound healing and other forms of cell replacement in certain tissues. For example, the epidermal cells of the skin and the intestinal lining of humans are continuously sloughed off and replaced. Cell division also results in the continuous production of reticulocytes that eventually shed their nuclei and replenish the supply of red blood cells in vertebrates. In abnormal situations, somatic cells may lose control of cell division, and form a tumor.

The genetic material is partitioned into daughter cells during nuclear division, or **karyokinesis**. This process is quite complex and requires great precision. The chromosomes must first be exactly replicated and then accurately partitioned. The end result is the production of two daughter nuclei, each with a chromosome composition identical to that of the parent cell.

Karyokinesis is followed by cytoplasmic division, or **cytokinesis**. This less complex process requires a mechanism that partitions the volume into two parts, then encloses each new cell in a distinct plasma membrane. As the cytoplasm is reconstituted, organelles either replicate themselves, arise from existing membrane structures, or are synthesized *de novo* (anew) in each cell.

Following cell division, the initial size of each new daughter cell is approximately one-half the size of the parent cell. However, the nucleus of each new cell is not appreciably

smaller than the nucleus of the original cell. Quantitative measurements of DNA confirm that there is an amount of genetic material in the daughter nuclei equivalent to that in the parent cell.

Interphase and the Cell Cycle

Many cells undergo a continuous alternation between division and nondivision. The events that occur from the completion of one division until the completion of the next division constitute the **cell cycle** (Figure 2–5). We will consider **interphase**, the initial stage of the cell cycle, as the interval between divisions. It was once thought that the biochemical activity during interphase was devoted solely to the cell's growth and its normal function. However, we now know that another biochemical step critical to the ensuing mitosis occurs during interphase: *the replication of the DNA of each chromosome.* This period, during which DNA is synthesized, occurs before the cell enters mitosis and is called the **S phase.** The initiation and completion of synthesis can be detected by monitoring the incorporation of radioactive precursors into DNA.

Investigations of this nature demonstrate two periods during interphase when no DNA synthesis occurs, one before and one after the S phase. These are designated **G1 (gap I)** and **G2 (gap II)**, respectively. During both of these intervals, as well as during S, intensive metabolic activity, cell growth, and cell differentiation are evident. By the end of G2, the volume of the cell has roughly doubled, DNA has been replicated, and mitosis (M) is initiated. Following mitosis, continuously dividing cells then repeat this cycle (G1, S, G2, M) over and over, as shown in Figure 2–5.
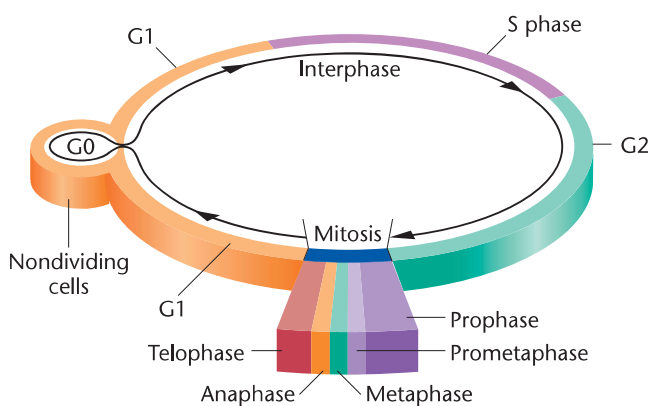
FIGURE 2–5   The stages comprising an arbitrary cell cycle. Following mitosis, cells enter the G1 stage of interphase, initiating a new cycle. Cells may become nondividing (G0) or continue through G1, where they become committed to begin DNA synthesis (S) and complete the cycle (G2 and mitosis). Following mitosis, two daughter cells are produced, and the cycle begins anew for both of them.

Interphase			Mitosis
G1	S	G2	M
5	7	3	1

Hours

Pro	Met	Ana	Tel
36	3	3	18

Minutes

FIGURE 2–6   The time spent in each interval of one complete cell cycle of a human cell in culture. Times vary according to cell types and conditions.
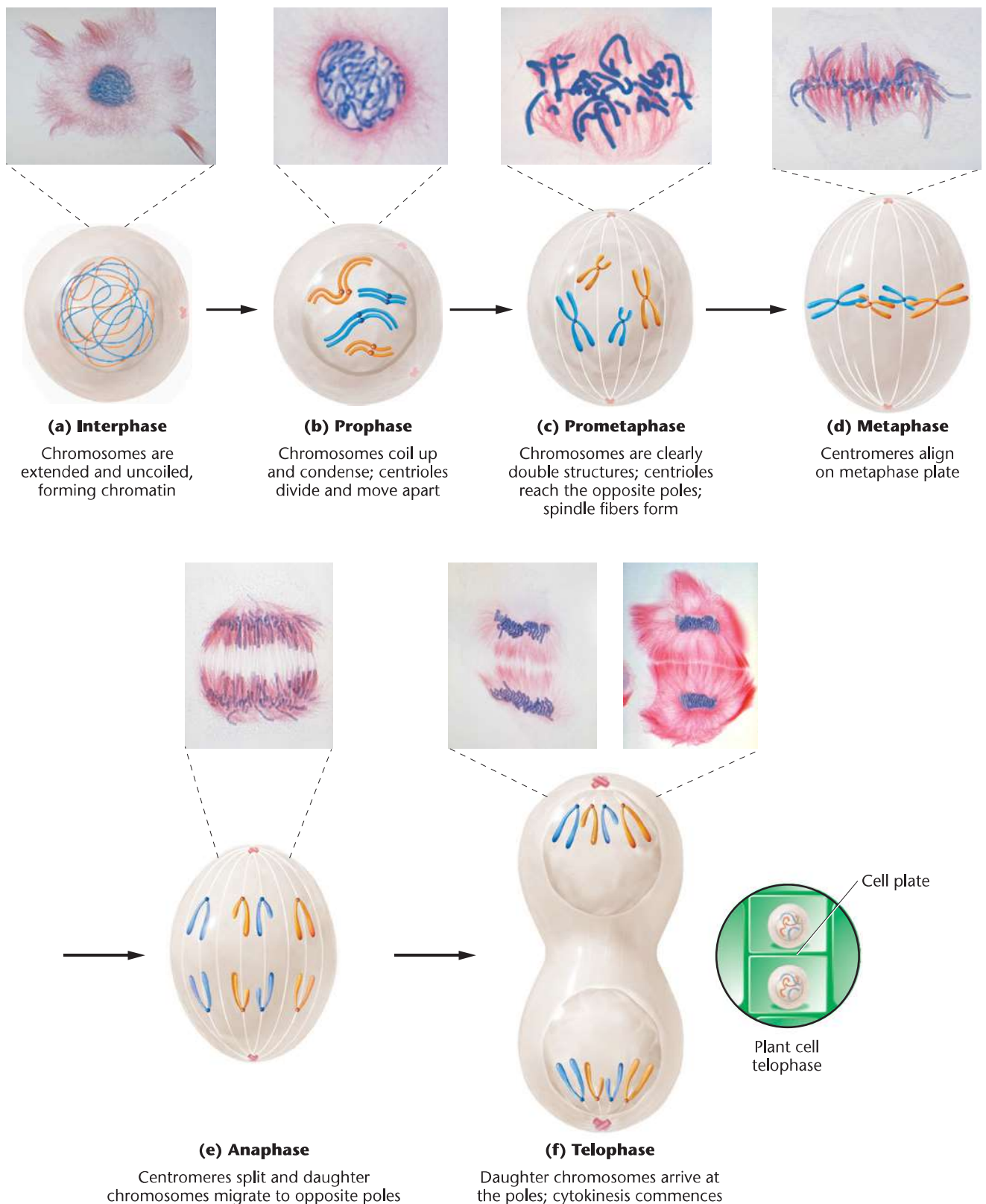
Much is known about the cell cycle based on *in vitro* (literally, "in glass") studies. When grown in culture, many cell types in different organisms traverse the complete cycle in about 16 hours. The actual process of mitosis occupies only a small part of the overall cycle, often less than an hour. The lengths of the S and G2 phases of interphase are fairly consistent in different cell types. Most variation is seen in the length of time spent in the G1 stage. Figure 2–6 shows the relative length of these intervals as well as the length of the stages of mitosis in a human cell in culture.

G1 is of great interest in the study of cell proliferation and its control. At a point during G1, all cells follow one of two paths. They either withdraw from the cycle, become quiescent, and enter the **G0 stage** (see Figure 2–5), or they become committed to proceed through G1, initiating DNA synthesis, and completing the cycle. Cells that enter G0 remain viable and metabolically active but are not proliferative. Cancer cells apparently avoid entering G0 or pass through it very quickly. Other cells enter G0 and never reenter the cell cycle. Still other cells in G0 can be stimulated to return to G1 and thereby reenter the cell cycle.

Cytologically, interphase is characterized by the absence of visible chromosomes. Instead, the nucleus is filled with chromatin fibers that are formed as the chromosomes uncoil and disperse after the previous mitosis [Figure 2–7(a)]. Once G1, S, and G2 are completed, mitosis is initiated. Mitosis is a dynamic period of vigorous and continual activity. For discussion purposes, the entire process is subdivided into discrete stages, and specific events are assigned to each one. These stages, in order of occurrence, are prophase, prometaphase, metaphase, anaphase, and telophase. They are diagrammed with corresponding photomicrographs in Figure 2–7.

Prophase

Often, over half of mitosis is spent in **prophase** [Figure 2–7(b)], a stage characterized by several significant occurrences. One of the early events in prophase of all animal cells is the

(a) Interphase

Chromosomes are extended and uncoiled, forming chromatin

(b) Prophase

Chromosomes coil up and condense; centrioles divide and move apart

(c) Prometaphase

Chromosomes are clearly double structures; centrioles reach the opposite poles; spindle fibers form

(d) Metaphase

Centromeres align on metaphase plate

(e) Anaphase

Centromeres split and daughter chromosomes migrate to opposite poles

(f) Telophase

Daughter chromosomes arrive at the poles; cytokinesis commences

Cell plate

Plant cell telophase

FIGURE 2–7   Drawings depicting mitosis in an animal cell with a diploid number of 4. The events occurring in each stage are described in the text. Of the two homologous pairs of chromosomes, one pair consists of longer, metacentric members and the other of shorter, submetacentric members. The maternal chromosome and the paternal chromosome of each pair are shown in different colors. In (f), a drawing of late telophase in a plant cell shows the formation of the cell plate and lack of centrioles. The cells shown in the light micrographs came from the flower of *Haemanthus,* a plant that has a diploid number of 8.

migration of two pairs of centrioles to opposite ends of the cell. These structures are found just outside the nuclear envelope in an area of differentiated cytoplasm called the centrosome (introduced in Section 2.1). It is believed that each pair of centrioles consists of one mature unit and a smaller, newly formed centriole.

The centrioles migrate to establish poles at opposite ends of the cell. After migrating, the centrioles are responsible for organizing cytoplasmic microtubules into the spindle fibers that run between these poles, creating an axis along which chromosomal separation occurs. Interestingly, the cells of most plants (there are a few exceptions), fungi, and certain algae seem to lack centrioles. Spindle fibers are nevertheless apparent during mitosis. Therefore, centrioles are not universally responsible for the organization of spindle fibers.

As the centrioles migrate, the nuclear envelope begins to break down and gradually disappears. In a similar fashion, the nucleolus disintegrates within the nucleus. While these events are taking place, the diffuse chromatin fibers have begun to condense, until distinct threadlike structures, the chromosomes, become visible. It becomes apparent near the end of prophase that each chromosome is actually a double structure split longitudinally except at a single point of constriction, the centromere. The two parts of each chromosome are called **sister chromatids** because the DNA contained in each of them is genetically identical, having formed from a single replicative event. Sister chromatids are held together by a multi-subunit protein complex called **cohesin**. This molecular complex is originally formed between them during the S phase of the cell cycle when the DNA of each chromosome is replicated. Thus, even though we cannot see chromatids in interphase because the chromatin is uncoiled and dispersed in the nucleus, the chromosomes are already double structures, which becomes apparent in late prophase. In humans, with a diploid number of 46, a cytological preparation of late prophase reveals 46 chromosomes randomly distributed in the area formerly occupied by the nucleus.

Prometaphase and Metaphase

The distinguishing event of the two ensuing stages is the migration of every chromosome, led by its centromeric region, to the equatorial plane. The equatorial plane, also referred to as the *metaphase plate,* is the midline region of the cell, a plane that lies perpendicular to the axis established by the spindle fibers. In some descriptions, the term **prometaphase** refers to the period of chromosome movement [**Figure 2–7**(c)], and the term **metaphase** is applied strictly to the chromosome configuration following migration.

Migration is made possible by the binding of spindle fibers to the chromosome's **kinetochore**, an assembly of multilayered plates of proteins associated with the centromere. This structure forms on opposite sides of each paired
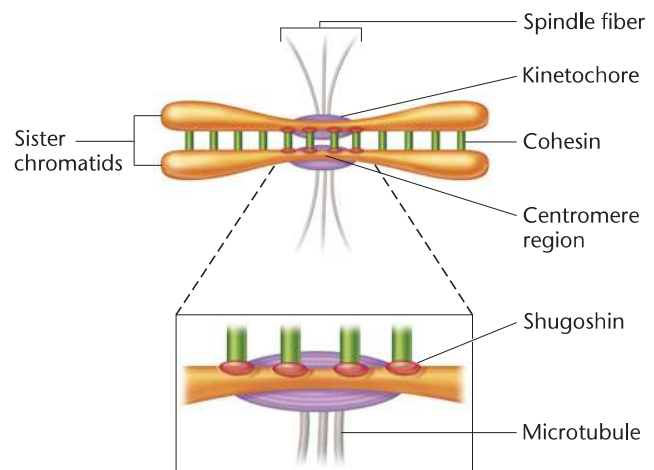
FIGURE 2–8   The depiction of the alignment, pairing, and disjunction of sister chromatids during mitosis, involving the molecular complexes cohesin and shugoshin and the enzyme separase.

centromere, in intimate association with the two sister chromatids. Once properly attached to the spindle fibers, cohesin is degraded by an enzyme, appropriately named *separase,* and the sister chromatid arms disjoin, except at the centromere region. A unique protein family called **shugoshin** (from the Japanese meaning guardian spirit) protects cohesin from being degraded by separase at the centromeric regions. The involvement of the cohesin and shugoshin complexes with a pair of sister chromatids during mitosis is depicted in **Figure 2–8**.

We know a great deal about spindle fibers. They consist of microtubules, which themselves consist of molecular subunits of the protein tubulin (we noted earlier that tubulin-derived microtubules also make up part of the cytoskeleton). Microtubules seem to originate and "grow" out of the two centrosome regions (which contain the centrioles) at opposite poles of the cell. They are dynamic structures that lengthen and shorten as a result of the addition or loss of polarized tubulin subunits. The microtubules most directly responsible for chromosome migration make contact with, and adhere to, kinetochores as they grow from the centrosome region. They are referred to as **kinetochore microtubules** and have one end near the centrosome region (at one of the poles of the cell) and the other end anchored to the kinetochore. The number of microtubules that bind to the kinetochore varies greatly between organisms. Yeast (*Saccharomyces*) has only a single microtubule bound to each plate-like structure of the kinetochore. Mitotic cells of mammals, at the other extreme, reveal 30 to 40 microtubules bound to each portion of the kinetochore.

At the completion of metaphase, each centromere is aligned at the metaphase plate with the chromosome arms

extending outward in a random array. This configuration is shown in Figure 2–7(d).

Anaphase

Events critical to chromosome distribution during mitosis occur during **anaphase**, the shortest stage of mitosis. During this phase, sister chromatids of each chromosome, held together only at their centromere regions, *disjoin* (separate) from one another—an event described as **disjunction**—and are pulled to opposite ends of the cell. For complete disjunction to occur: (1), shugoshin must be degraded, reversing its protective role; (2), the cohesin complex holding the centromere region of each sister chromosome is then cleaved by separase; and (3) sister chromatids of each chromosome are pulled toward the opposite poles of the cell (Figure 2–8). As these events proceed, each migrating chromatid is now referred to as a **daughter chromosome**.

Movement of daughter chromosomes to the opposite poles of the cell is dependent on the centromere–spindle fiber attachment. Recent investigations reveal that chromosome migration results from the activity of a series of specific molecules called motor proteins found at several locations within the dividing cell. These proteins, described as **molecular motors**, use the energy generated by the hydrolysis of ATP. Their effect on the activity of microtubules serves ultimately to shorten the spindle fibers, drawing the chromosomes to opposite ends of the cell. The centromeres of each chromosome *appear* to lead the way during migration, with the chromosome arms trailing behind. Several models have been proposed to account for the shortening of spindle fibers. They share in common the selective removal of tubulin subunits at the ends of the spindle fibers. The removal process is accomplished by the molecular motor proteins described above.

The location of the centromere determines the shape of the chromosome during separation, as you saw in Figure 2–3. The steps that occur during anaphase are critical in providing each subsequent daughter cell with an identical set of chromosomes. In human cells, there would now be 46 chromosomes at each pole, one from each original sister pair. Figure 2–7(e) shows anaphase prior to its completion.

Telophase

Telophase is the final stage of mitosis and is depicted in Figure 2–7(f). At its beginning, two complete sets of chromosomes are present, one set at each pole. The most significant event of this stage is cytokinesis, the division or partitioning of the cytoplasm. Cytokinesis is essential if two new cells are to be produced from one cell. The mechanism of cytokinesis differs greatly in plant and animal cells, but the end result is the same: two new cells are produced. In plant cells, a **cell plate** is synthesized and laid down across the region of the metaphase plate. Animal cells, however, undergo a constriction of the cytoplasm, much as a loop of string might be tightened around the middle of a balloon.

It is not surprising that the process of cytokinesis varies in different organisms. Plant cells, which are more regularly shaped and structurally rigid, require a mechanism for depositing new cell wall material around the plasma membrane. The cell plate laid down during telophase becomes a structure called the **middle lamella**. Subsequently, the primary and secondary layers of the cell wall are deposited between the cell membrane and middle lamella in each of the resulting daughter cells. In animals, complete constriction of the cell membrane produces the **cell furrow** characteristic of newly divided cells.

Other events necessary for the transition from mitosis to interphase are initiated during late telophase. They generally constitute a reversal of events that occurred during prophase. In each new cell, the chromosomes begin to uncoil and become diffuse chromatin once again, while the nuclear envelope reforms around them, the spindle fibers disappear, and the nucleolus gradually reforms and becomes visible in the nucleus during early interphase. At the completion of telophase, the cell enters interphase.

Cell-Cycle Regulation and Checkpoints

The cell cycle, culminating in mitosis, is fundamentally the same in all eukaryotic organisms. This similarity in many diverse organisms suggests that the cell cycle is governed by a genetically regulated program that has been conserved throughout evolution. Because disruption of this regulation may underlie the uncontrolled cell division characterizing malignancy, interest in how genes regulate the cell cycle is particularly strong.

A mammoth research effort over the past 15 years has paid high dividends, and we now have knowledge of many genes involved in the control of the cell cycle. This work was recognized by the awarding of the 2001 Nobel Prize in Medicine or Physiology to Lee Hartwell, Paul Nurse, and Tim Hunt. As with other studies of genetic control over essential biological processes, investigation has focused on the discovery of mutations that interrupt the cell cycle and on the effects of those mutations. As we shall return to this subject in Chapter 19 during our consideration of cancer, what follows is a very brief overview.

Many mutations are now known that exert an effect at one or another stage of the cell cycle. First discovered in yeast, but now evident in all organisms, including humans, such mutations were originally designated as *cell division cycle (cdc)* **mutations**. The normal products of many of the mutated genes are enzymes called **kinases** that can add phosphates to other proteins. They serve as "master control" molecules functioning in conjunction with proteins called **cyclins**. Cyclins bind to these kinases (creating *cyclin-dependent kinases*),

activating them at appropriate times during the cell cycle. Activated kinases then phosphorylate other target proteins that regulate the progress of the cell cycle. The study of *cdc* mutations has established that the cell cycle contains at least three major *checkpoints,* where the processes culminating in normal mitosis are monitored, or "checked," by these master control molecules before the next stage of the cycle commences.

Checkpoints are named according to where in the cell cycle monitoring occurs (Figure 2–5). The first of three, the **G1/S checkpoint**, monitors the size the cell has achieved since its previous mitosis and also evaluates the condition of the DNA. If the cell has not reached an adequate size or if the DNA has been damaged, further progress through the cycle is arrested until these conditions are "corrected." If both conditions are "normal" at G1/S, then the cell is allowed to proceed from G1 to the S phase of the cycle. The second checkpoint is the **G2/M checkpoint**, where DNA is monitored prior to the start of mitosis. If DNA replication is incomplete or any DNA damage is detected and has not been repaired, the cell cycle is arrested. The final checkpoint occurs during mitosis and is called the **M checkpoint** (sometimes referred to as the *Spindle Assembly checkpoint*). Here, both the successful formation of the spindle fiber system and the attachment of spindle fibers to the kinetochores associated with the centromeres are monitored. If spindle fibers are not properly formed or if attachment is inadequate, mitosis is arrested.

The importance of cell-cycle control and these checkpoints can be demonstrated by considering what happens when this regulatory system is impaired. Let's assume, for example, that the DNA of a cell has incurred damage leading to one or more mutations impairing cell-cycle control. If allowed to proceed through the cell cycle as one of the population of dividing cells, this genetically altered cell would divide

uncontrollably—precisely the definition of a cancerous cell. If instead the cell cycle is arrested at one of the checkpoints, the cell may effectively be removed from the population of dividing cells, preventing its potential malignancy.

Meiosis Reduces the Chromosome Number from Diploid to Haploid in Germ Cells and Spores

The process of meiosis, unlike mitosis, reduces the amount of genetic material by one-half. Whereas in diploids mitosis produces daughter cells with a full diploid complement, meiosis produces gametes or spores with only one haploid set of chromosomes. During sexual reproduction, gametes then combine through fertilization to reconstitute the diploid complement found in parental cells. **Figure 2–9** compares the two processes by following two pairs of homologous chromosomes.

The events of meiosis must be highly specific since by definition, haploid gametes or spores contain precisely one member of each homologous pair of chromosomes. If successfully completed, meiosis ensures genetic continuity from generation to generation.

The process of sexual reproduction also ensures genetic variety among members of a species. As you study meiosis, you will see that this process results in gametes that each contain unique combinations of maternally and paternally derived chromosomes in their haploid complement. With such a tremendous genetic variation among the gametes, a huge number of maternal-paternal chromosome combinations are possible at fertilization. Furthermore, you will see that the meiotic event referred to as **crossing over** results in genetic exchange between members of each homologous pair of chromosomes. This process creates intact chromosomes that are mosaics of the maternal and paternal homologs from which they arise, further enhancing the potential genetic variation in gametes and the offspring derived from them. Sexual reproduction therefore reshuffles the genetic material, producing offspring that often differ greatly from either parent. Thus, meiosis is the major source of genetic recombination within species.
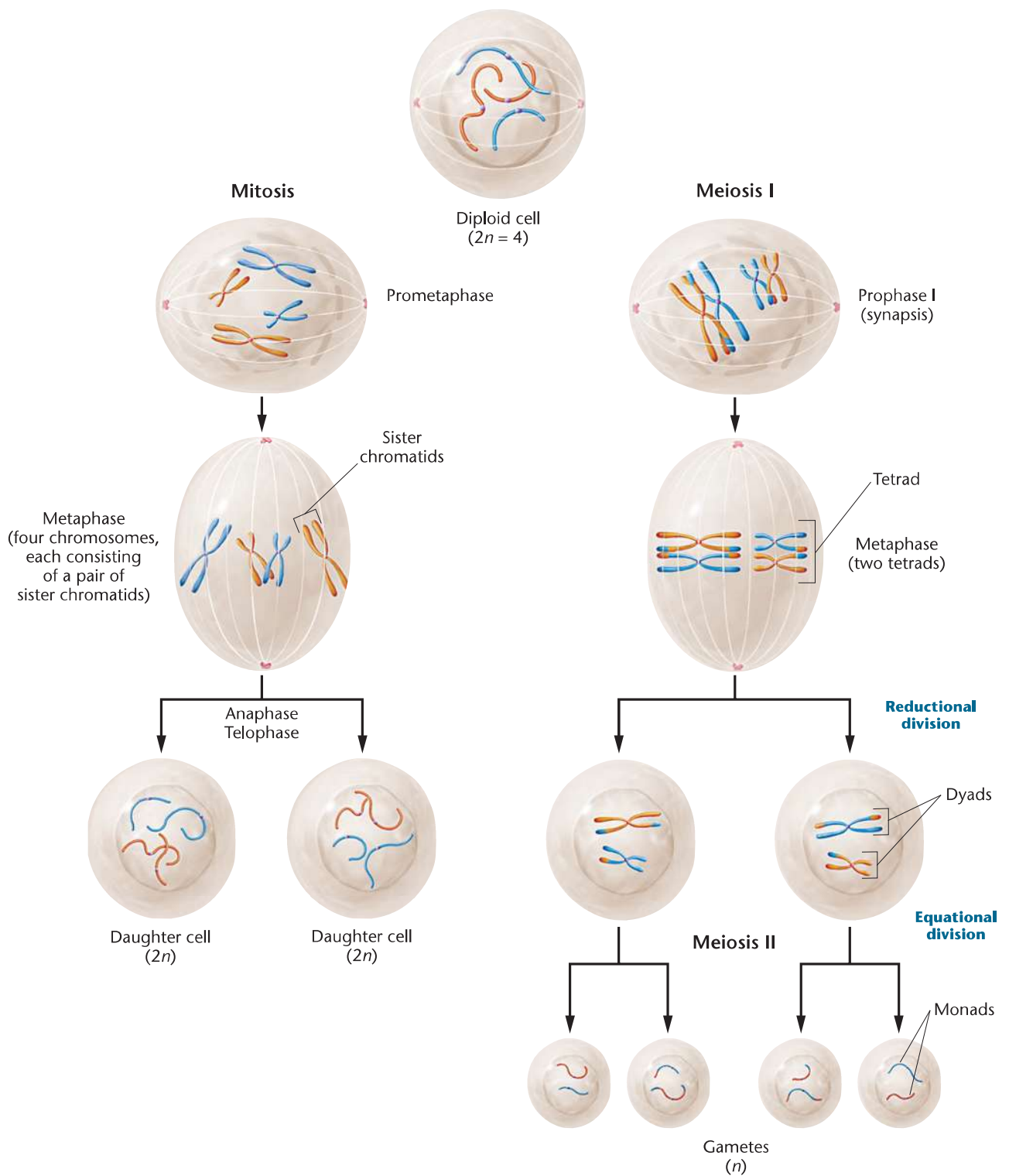
An Overview of Meiosis

In the preceding discussion, we established what might be considered the goal of meiosis: the reduction to the haploid complement of chromosomes. Before considering the phases of this process systematically, we will briefly summarize how diploid cells give rise to haploid gametes or spores. You should refer to the right-hand side of Figure 2–9 during the following discussion.

NOW SOLVE THIS

2–1 With the initial appearance of the feature we call "Now Solve This," a short introduction is in order. The feature occurs several times in this and all ensuing chapters, each time providing a problem related to the discussion just presented. A "Hint" is then offered that may help you solve the problem. Here is the first problem:

(a) If an organism has a diploid number of 16, how many chromatids are visible at the end of mitotic prophase?

(b) How many chromosomes are moving to each pole during anaphase of mitosis?

■ HINT: *This problem involves an understanding of what happens to each pair of homologous chromosomes during mitosis. The key to its solution is to understand that throughout mitosis, the members of each homologous pair do not pair up, but instead behave independently.*

FIGURE 2–9 Overview of the major events and outcomes of mitosis and meiosis. As in Figure 2–7, two pairs of homologous chromosomes are followed.

We have established that in mitosis each paternally and maternally derived member of any given homologous pair of chromosomes behaves autonomously during division. By contrast, early in meiosis, homologous chromosomes form pairs; that is, they *synapse* (or undergo) **synapsis.** Each

synapsed structure, initially called a **bivalent**, eventually gives rise to a **tetrad** consisting of four chromatids. The presence of four chromatids demonstrates that both homologs (making up the bivalent) have, in fact, duplicated. Therefore, to achieve haploidy, two divisions are necessary. The first

division occurs in meiosis I and is described as a **reductional division** (because the number of centromeres, each representing one chromosome, is *reduced* by one-half). Components of each tetrad—representing the two homologs—separate, yielding two **dyads**. Each dyad is composed of two sister chromatids joined at a common centromere. The second division occurs during meiosis II and is described as an **equational division** (because the number of centromeres remains *equal*). Here each dyad splits into two **monads** of one chromosome each. Thus, the two divisions potentially produce four haploid cells.

The First Meiotic Division: Prophase I

We turn now to a detailed account of meiosis. Like mitosis, meiosis is a continuous process. We assign names to its stages and substages only to facilitate discussion. From a genetic standpoint, three events characterize the initial stage, **prophase I** (**Figure 2–10**). First, as in mitosis, chromatin present in interphase thickens and coils into visible chromosomes. And, as in mitosis, each chromosome is a double structure, held together by the molecular complex called *cohesin*. Second, unlike mitosis, members of each homologous pair of chromosomes pair up, undergoing synapsis. Third, crossing over occurs between chromatids of synapsed homologs. Because of the complexity of these genetic events, this stage of meiosis is divided into five substages: leptonema, zygonema, pachynema, diplonema,* and diakinesis. As we discuss these substages, be aware that, even though it is not immediately apparent in the earliest phases of meiosis, the DNA of chromosomes has been replicated during the prior interphase.

Leptonema During the **leptotene stage**, the interphase chromatin material begins to condense, and the chromosomes, though still extended, become visible. Along each chromosome are **chromomeres**, localized condensations that resemble beads on a string. Evidence suggests that a process called **homology search**, which precedes and is essential to the initial pairing of homologs, begins during leptonema.

Zygonema The chromosomes continue to shorten and thicken during the **zygotene stage**. During the process of homology search, homologous chromosomes undergo initial alignment with one another. This so-called *rough pairing* is complete by the end of zygonema. In yeast, homologs are separated by about 300 nm, and near the end of zygonema, structures called *lateral elements* are visible between paired homologs. As meiosis proceeds, the overall length of the lateral elements along the chromosome increases, and a more extensive ultrastructural component called the

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*These are the noun forms of these substages. The adjective forms (leptotene, zygotene, pachytene, and diplotene) are also used in the text.
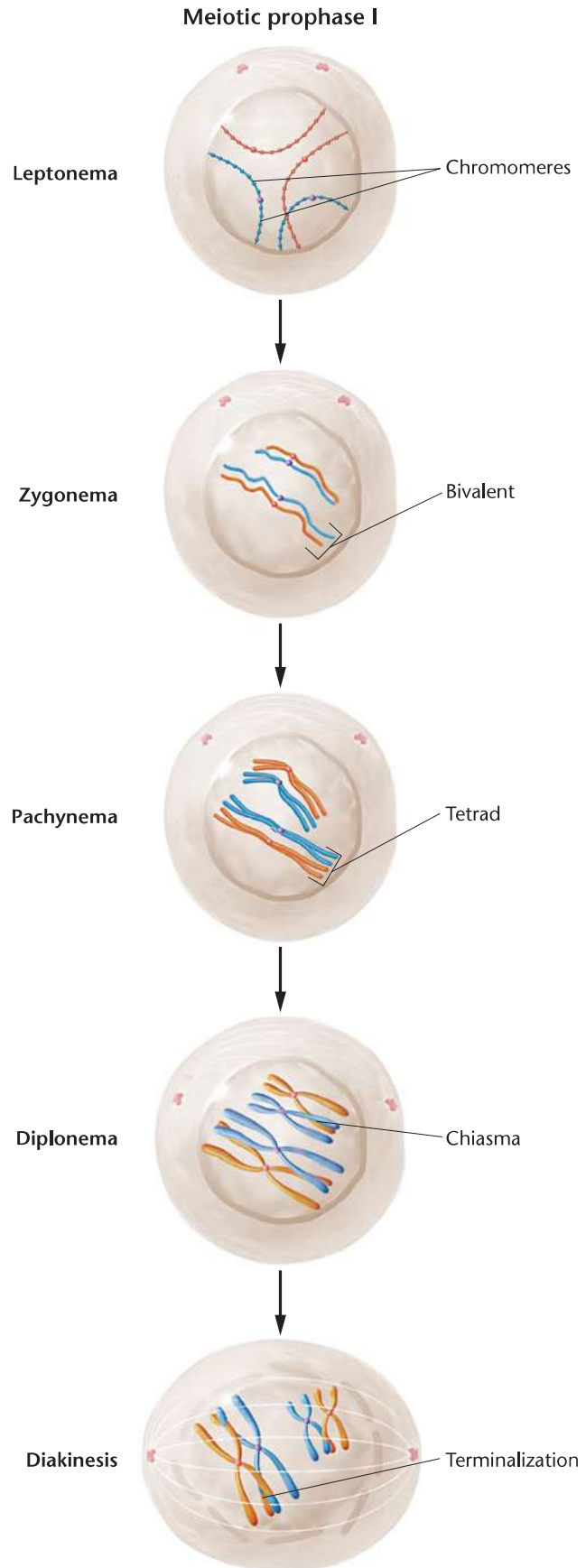
Meiotic prophase I

FIGURE 2–10   The substages of meiotic prophase I for the chromosomes depicted in Figure 2–9.

synaptonemal complex begins to form between the homologs. This complex is believed to be the vehicle responsible for the pairing of homologs. In some diploid organisms, this synapsis occurs in a zipper-like fashion, beginning at the ends of chromosomes attached to the nuclear envelope.

It is upon completion of zygonema that the paired homologs are referred to as bivalents. Although both members of each bivalent have already replicated their DNA, it is not yet visually apparent that each member is a double structure. The number of bivalents in each species is equal to the haploid (*n*) number.

Pachynema In the transition from the zygotene to the **pachytene stage**, the chromosomes continue to coil and shorten, and further development of the synaptonemal complex occurs between the two members of each bivalent. This leads to synapsis, a more intimate pairing. Compared to the rough-pairing characteristic of zygonema, homologs are now separated by only 100 nm.

During pachynema, each homolog is now evident as a double structure, providing visual evidence of the earlier replication of the DNA of each chromosome. Thus, each bivalent contains four member chromatids. As in mitosis, replicates are called *sister chromatids*, whereas chromatids from maternal and paternal members of a homologous pair are called *nonsister chromatids*. The four-membered structure, also referred to as a tetrad, contains two pairs of sister chromatids.

Diplonema During the ensuing **diplotene stage**, it is even more apparent that each tetrad consists of two pairs of sister chromatids. Within each tetrad, each pair of sister chromatids begins to separate. However, one or more areas remain in contact where chromatids are intertwined. Each such area, called a **chiasma** (pl. **chiasmata**), is thought to represent a point where nonsister chromatids have undergone genetic exchange through the process referred to above as *crossing over*. Although the physical exchange between chromosome areas occurred during the previous pachytene stage, the result of crossing over is visible only when the duplicated chromosomes begin to separate. Crossing over is an important source of genetic variability, and as indicated earlier, new combinations of genetic material are formed during this process.

Diakinesis The final stage of prophase I is **diakinesis**. The chromosomes pull farther apart, but nonsister chromatids remain loosely associated at the chiasmata. As separation proceeds, the chiasmata move toward the ends of the tetrad. This process of **terminalization** begins in late diplonema and is completed during diakinesis. During this final substage, the nucleolus and nuclear envelope break down,

and the two centromeres of each tetrad attach to the recently formed spindle fibers. By the completion of prophase I, the centromeres of each tetrad structure are present on the metaphase plate of the cell.

Metaphase, Anaphase, and Telophase I

The remainder of the meiotic process is depicted in **Figure 2–11**. After meiotic prophase I, stages similar to those of mitosis occur. In the first division, **metaphase I**, the chromosomes have maximally shortened and thickened. The terminal chiasmata of each tetrad are visible and appear to be the major factor holding the nonsister chromatids together. Each tetrad interacts with spindle fibers, facilitating its movement to the metaphase plate. The alignment of each tetrad prior to the first anaphase is random: Half of the tetrad (one of the dyads) will subsequently be pulled to one or the other pole, and the other half moves to the opposite pole.

During the stages of meiosis I, a single centromeric region holds each pair of sister chromatids together. It appears as a single unit, and a kinetechore forms around each one. As in our discussion of mitosis (see Figure 2–8), cohesin plays the major role in keeping sister chromatids together. At **anaphase I**, cohesin is degraded between sister chromatids, except at the centromere region, which, as in mitosis, is protected by a shugoshin complex. Then, one-half of each tetrad (a dyad) is pulled toward each pole of the dividing cell. This separation process is the physical basis of disjunction, the separation of homologous chromosomes from one another. Occasionally, errors in meiosis occur and separation is not achieved. The term **nondisjunction** describes such an error. At the completion of the normal anaphase I, a series of dyads equal to the haploid number is present at each pole.

If crossing over had not occurred in the first meiotic prophase, each dyad at each pole would consist solely of either paternal or maternal chromatids. However, the exchanges produced by crossing over create mosaic chromatids of paternal and maternal origin.

In many organisms, **telophase I** reveals a nuclear membrane forming around the dyads. In this case, the nucleus next enters into a short interphase period. If interphase occurs, the chromosomes do not replicate because they already consist of two chromatids. In other organisms, the cells go directly from anaphase I to meiosis II. In general, meiotic telophase is much shorter than the corresponding stage in mitosis.

The Second Meiotic Division

A second division, referred to as **meiosis II**, is essential if each gamete or spore is to receive only one chromatid from each original tetrad. The stages characterizing meiosis II are shown on the right side of Figure 2–11. During **prophase II**, each dyad is composed of one pair of sister chromatids attached

Metaphase I   Anaphase I   Telophase I
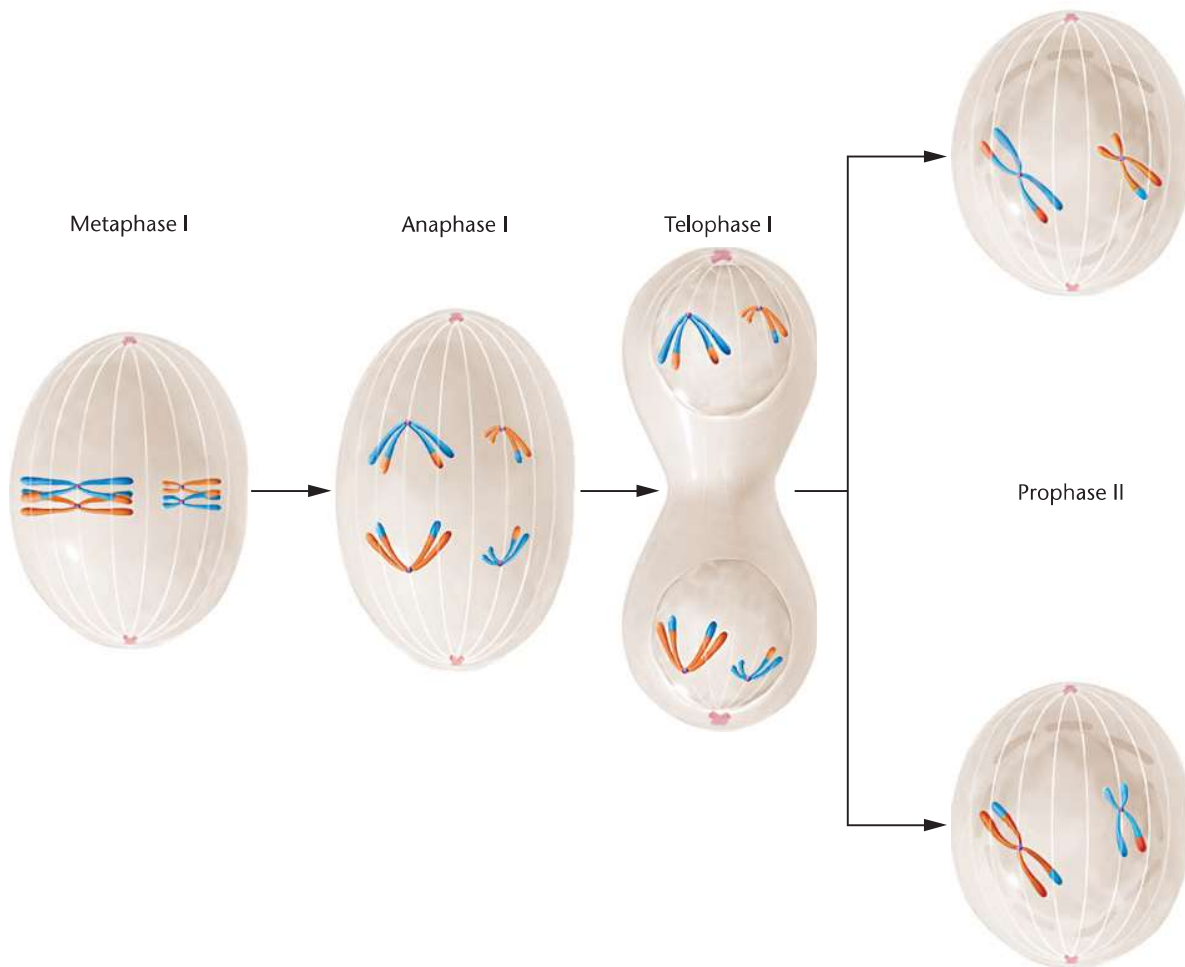
Prophase II

The major events in meiosis in an animal cell with a diploid number of 4, beginning with metaphase I. Note that the combination of chromosomes in the cells following telophase II is dependent on the random orientation of each tetrad and dyad when they align on the equatorial plate during metaphase I and metaphase II. Several other combinations, which are not shown, can also be produced. The events depicted here are described in the text.

by the common centromeric region. During **metaphase II**, the centromeres are positioned on the equatorial plate. When the shugoshin complex is degraded, the centromeres separate, **anaphase II** is initiated, and the sister chromatids of each dyad are pulled to opposite poles. Because the number of dyads is equal to the haploid number, **telophase II** reveals one member of each pair of homologous chromosomes present at each pole. Each chromosome is now a monad. Following cytokinesis in telophase II, four haploid gametes may result from a single meiotic event. At the conclusion of meiosis II, not only has the haploid state been achieved, but if crossing over has occurred, each monad contains a combination of maternal and paternal genetic information. As a result, the offspring produced by any gamete will receive a mixture of genetic information originally present in his or her grandparents.

NOW SOLVE THIS

2–2 An organism has a diploid number of 16 in a primary oocyte. (a) How many tetrads are present in the first meiotic prophase? (b) How many dyads are present in the second meiotic prophase? (c) How many monads migrate to each pole during the second meiotic anaphase?

■ HINT: *This problem involves an understanding of what happens to the maternal and paternal members of each pair of homologous chromosomes during meiosis. The key to its solution is to understand that maternal and paternal homologs synapse during meiosis. Once each chromatid has duplicated, creating a tetrad in the early phases of meiosis, each original pair behaves as a unit and leads to two dyads during anaphase I.*

Metaphase II   Anaphase II   Telophase II   Haploid gametes
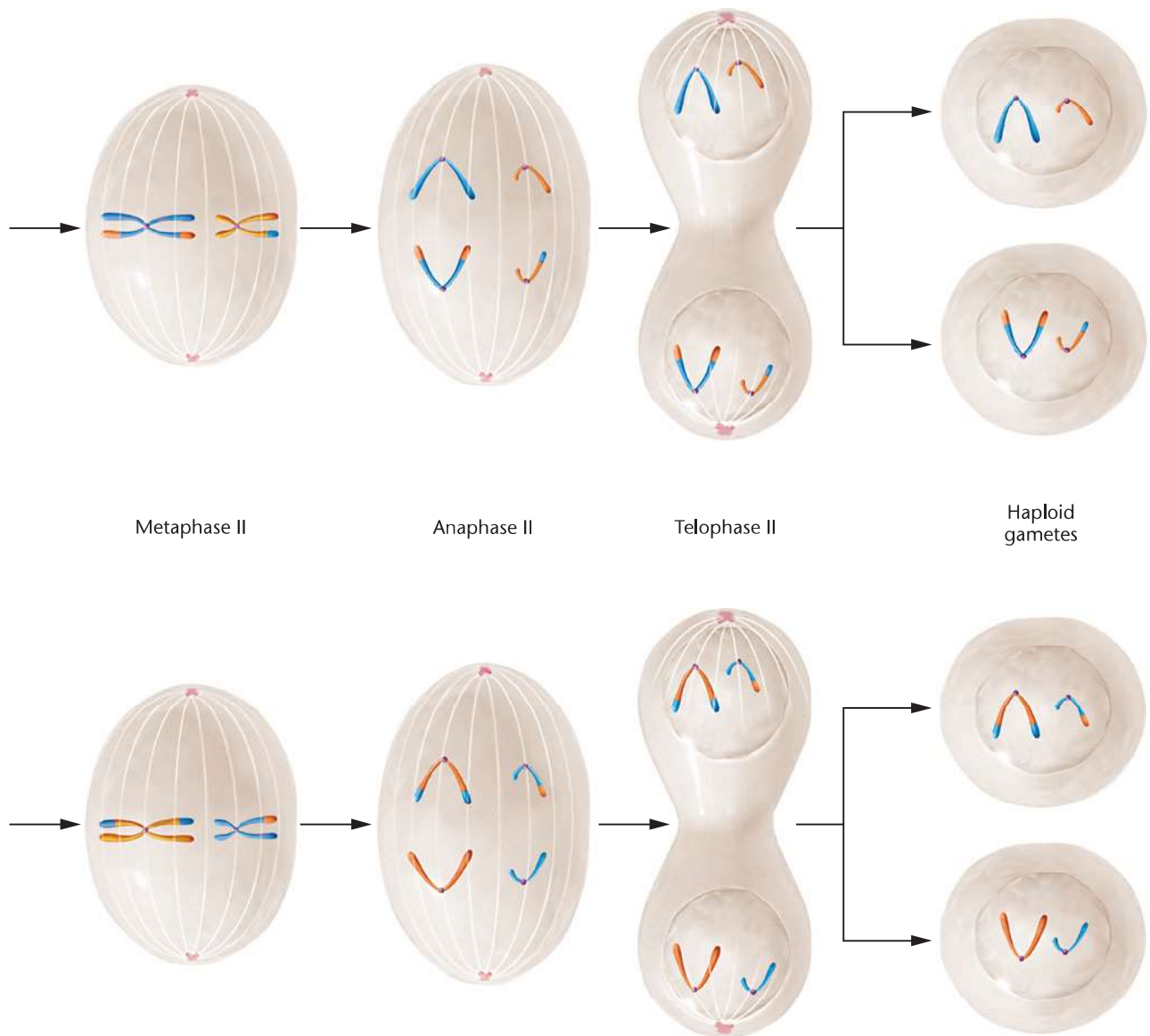
FIGURE 2–11 (*Continued*)

Meiosis thus significantly increases the level of genetic variation in each ensuing generation.

2.5
The Development of Gametes Varies in Spermatogenesis Compared to Oogenesis

Although events that occur during the meiotic divisions are similar in all cells participating in gametogenesis in most animal species, there are certain differences between the production of a male gamete (spermatogenesis) and a female gamete (oogenesis). **Figure 2–12** summarizes these processes.

Spermatogenesis takes place in the testes, the male reproductive organs. The process begins with the enlargement of an undifferentiated diploid germ cell called a **spermatogonium**. This cell grows to become a **primary spermatocyte**, which undergoes the first meiotic division. The products of this division, called **secondary spermatocytes**, contain a haploid number of dyads. The secondary spermatocytes then undergo meiosis II, and each of these cells produces two haploid **spermatids**. Spermatids go through a series of developmental changes, **spermiogenesis**, to become highly specialized, motile **spermatozoa**, or **sperm**. All sperm cells produced during spermatogenesis contain the haploid number of chromosomes and equal amounts of cytoplasm.
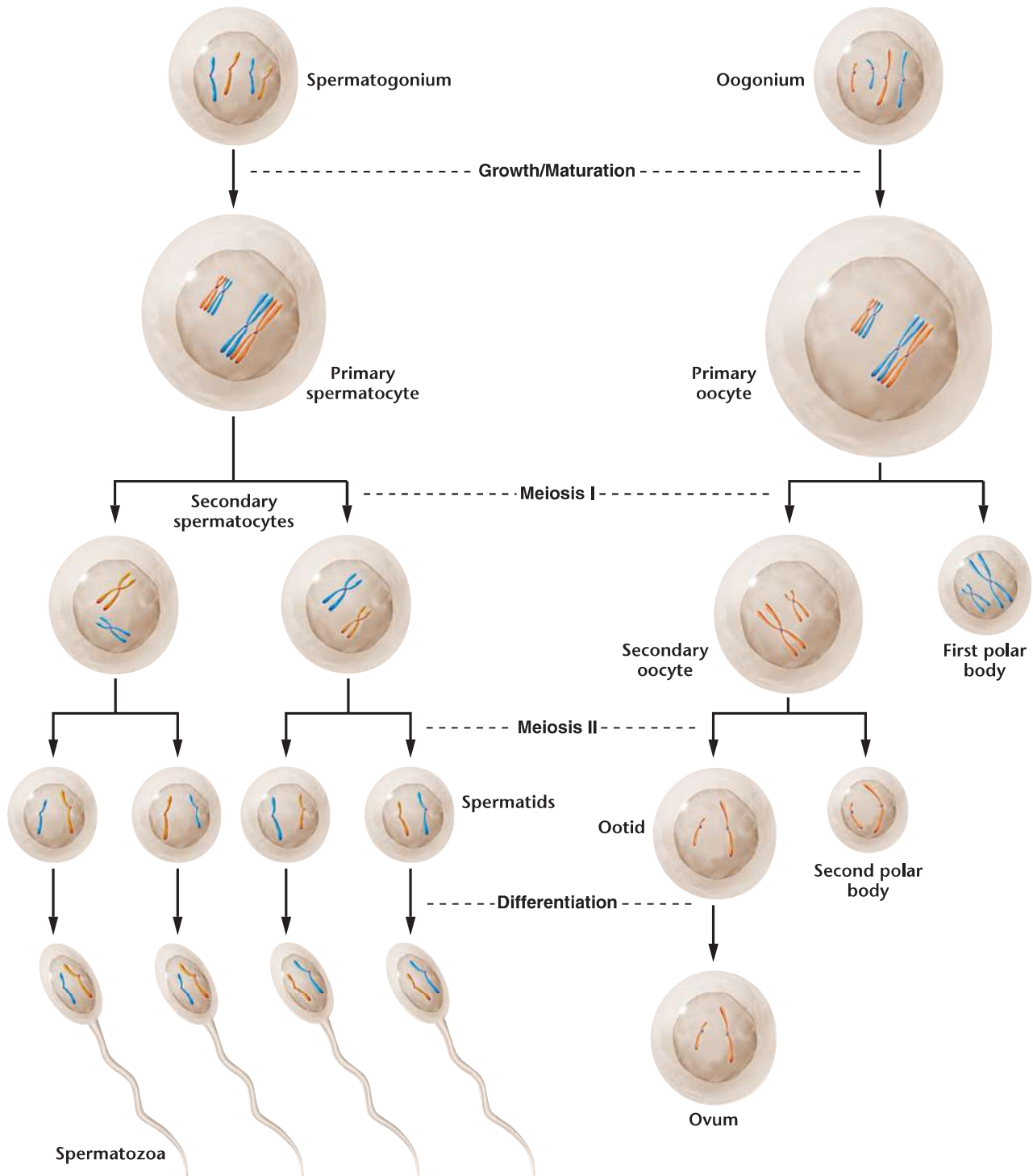
FIGURE 2–12   Spermatogenesis and oogenesis in animal cells.

Spermatogenesis may be continuous or may occur periodically in mature male animals; its onset is determined by the species' reproductive cycles. Animals that reproduce year-round produce sperm continuously, whereas those whose breeding period is confined to a particular season produce sperm only during that time.

In animal **oogenesis**, the formation of **ova** (sing. **ovum**), or eggs, occurs in the ovaries, the female reproductive organs. The daughter cells resulting from the two meiotic divisions of this process receive equal amounts of genetic material, but they do *not* receive equal amounts of cytoplasm. Instead, during each division, almost all the cytoplasm of

the **primary oocyte**, itself derived from the **oogonium**, is concentrated in one of the two daughter cells. The concentration of cytoplasm is necessary because a major function of the mature ovum is to nourish the developing embryo following fertilization.

During anaphase I in oogenesis, the tetrads of the primary oocyte separate, and the dyads move toward opposite poles. During telophase I, the dyads at one pole are pinched off with very little surrounding cytoplasm to form the **first polar body**. The first polar body may or may not divide again to produce two small haploid cells. The other daughter cell produced by this first meiotic division contains most of the cytoplasm and is called the **secondary oocyte**. The mature ovum will be produced from the secondary oocyte during the second meiotic division. During this division, the cytoplasm of the secondary oocyte again divides unequally, producing an **ootid** and a **second polar body**. The ootid then differentiates into the mature ovum.

Unlike the divisions of spermatogenesis, the two meiotic divisions of oogenesis may not be continuous. In some animal species, the second division may directly follow the first. In others, including humans, the first division of all oocytes begins in the embryonic ovary but arrests in prophase I. Many years later, meiosis resumes in each oocyte just prior to its ovulation. The second division is completed only after fertilization.

NOW SOLVE THIS

2–3 Examine Figure 2–12, which shows oogenesis in animal cells. Will the genotype of the second polar body (derived from meiosis II) always be identical to that of the ootid? Why or why not?

■ HINT: *This problem involves an understanding of meiosis during oogenesis. The key to its solution is to take into account that crossing over occurred between each pair of homologs during meiosis I.*

2.6
Meiosis Is Critical to Sexual Reproduction in All Diploid Organisms

The process of meiosis is critical to the successful sexual reproduction of all diploid organisms. It is the mechanism by which the diploid amount of genetic information is reduced to the haploid amount. In animals, meiosis leads to the formation of gametes, whereas in plants haploid spores are produced, which in turn lead to the formation of haploid gametes.

Each diploid organism stores its genetic information in the form of homologous pairs of chromosomes. Each pair consists of one member derived from the maternal parent and one from the paternal parent. Following meiosis, haploid cells potentially contain either the paternal or the maternal representative of every homologous pair of chromosomes. However, the process of crossing over, which occurs in the first meiotic prophase, further reshuffles the alleles between the maternal and paternal members of each homologous pair, which then segregate and assort independently into gametes. These events result in the great amount of genetic variation present in gametes.

It is important to touch briefly on the significant role that meiosis plays in the life cycles of fungi and plants. In many fungi, the predominant stage of the life cycle consists of haploid vegetative cells. They arise through meiosis and proliferate by mitotic cell division. In multicellular plants, the life cycle alternates between the diploid **sporophyte stage** and the haploid **gametophyte stage** (**Figure 2–13**). While one or the other predominates in different plant groups during this "alternation of generations," the processes of meiosis and fertilization constitute the "bridges" between the sporophyte and gametophyte stages. Therefore, meiosis is an essential component of the life cycle of plants.

2.7
Electron Microscopy Has Revealed the Physical Structure of Mitotic and Meiotic Chromosomes

Thus far in this chapter, we have focused on mitotic and meiotic chromosomes, emphasizing their behavior during cell division and gamete formation. An interesting question is why chromosomes are invisible during interphase but visible during the various stages of mitosis and meiosis. Studies using electron microscopy clearly show why this is the case.

Recall that, during interphase, only dispersed chromatin fibers are present in the nucleus [**Figure 2–14(a)**]. Once mitosis begins, however, the fibers coil and fold, condensing into typical mitotic chromosomes [**Figure 2–14(b)**]. If the fibers comprising a mitotic chromosome are loosened, the areas of greatest spreading reveal individual fibers similar to those seen in interphase chromatin [**Figure 2–14(c)**]. Very few fiber ends seem to be present, and in some cases, none can be seen. Instead, individual fibers always seem to loop back into the interior. Such fibers are obviously twisted and coiled around one another, forming the regular pattern of folding in the mitotic chromosome. Starting in late telophase of mitosis and continuing during G1 of interphase, chromosomes unwind to form the long fibers characteristic of chromatin, which consist of DNA and associated proteins, particularly proteins called *histones*. It is in this physical
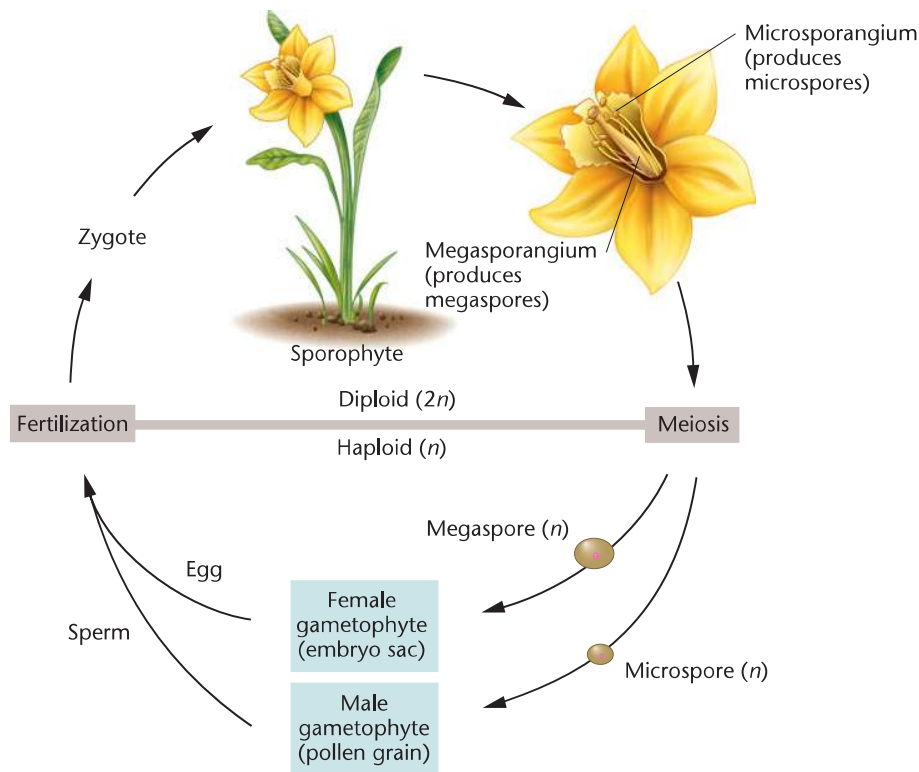
FIGURE 2–13   Alternation of generations between the diploid sporophyte (2n) and the haploid gametophyte (n) in a multicellular plant. The processes of meiosis and fertilization bridge the two phases of the life cycle. In angiosperms (flowering plants), like the one shown here, the sporophyte stage is the predominant phase.
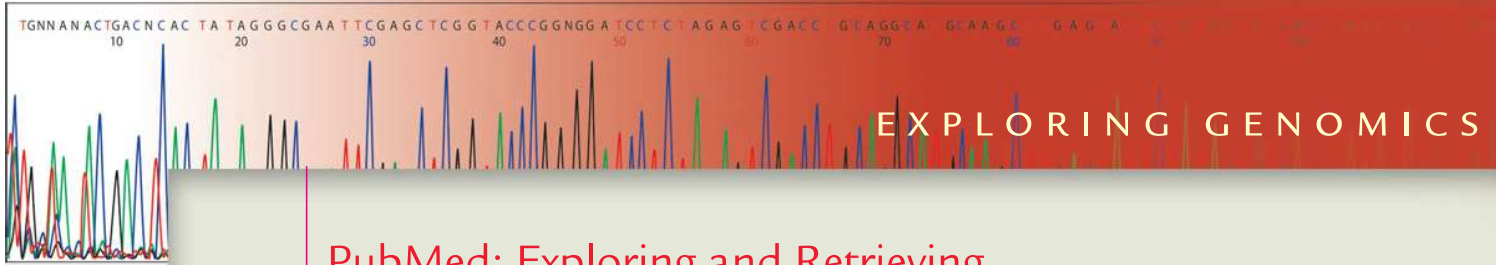
arrangement that DNA can most efficiently function during transcription and replication.

Electron microscopic observations of metaphase chromosomes in varying degrees of coiling led Ernest DuPraw to postulate the **folded-fiber model**, shown in Figure 2–14(c). During metaphase, each chromosome consists of two sister chromatids joined at the centromeric region. Each arm of the chromatid appears to be a single fiber wound much like a skein of yarn. The fiber is composed of tightly coiled double-stranded DNA and protein. An orderly coiling–twisting–condensing process appears to facilitate the transition of the interphase chromatin into the more condensed mitotic chromosomes. Geneticists believe that during the transition from interphase to prophase, a 5000-fold compaction occurs in the length of DNA within the chromatin fiber! This process must be extremely precise given the highly ordered and consistent appearance of mitotic chromosomes in all eukaryotes. Note particularly in the micrographs the clear distinction between the sister chromatids constituting each chromosome. They are joined only by the common centromere that they share prior to anaphase.

(a)   (b)   (c)
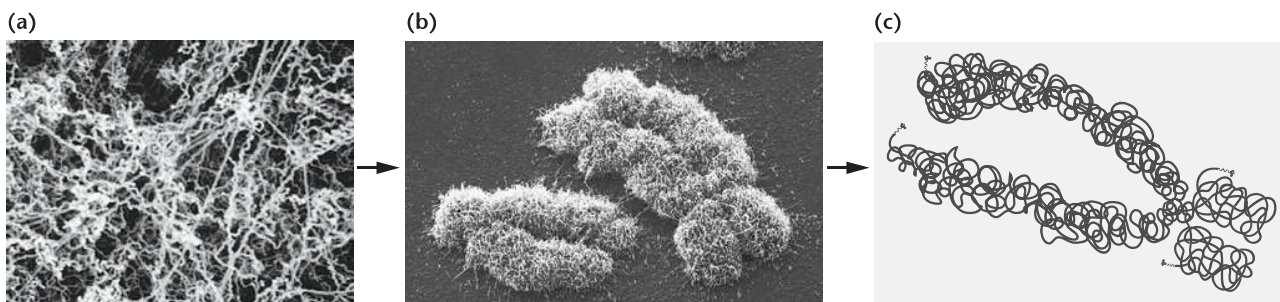
FIGURE 2–14   Comparison of (a) the chromatin fibers characteristic of the interphase nucleus with (b) metaphase chromosomes that are derived from chromatin during mitosis. Part (c) diagrams a mitotic chromosome, showing how chromatin is condensed to produce it. Part (a) is a transmission electron micrograph and part (b) is a scanning electron micrograph.

TGNN A N ACTGACN C AC TA TAGGGCGAA T TCGAGC TCGG T ACCCGGNGG A TCC TC T AGAG T CGACC GC AGGC A GC A AG C   GAG A
   10   20   30   40   50   60   70   80

PubMed: Exploring and Retrieving Biomedical Literature

MG *Study Area: Exploring Genomics*

In this era of rapidly expanding information on genomics and the biomedical sciences, scientists must be conversant in the use of multiple on-line databases. These resources provide access to DNA and protein sequences, genomic data, chromosome maps, microarray gene-expression networks, and molecular structures, as well as to the bioinformatics tools necessary for data manipulation. Perhaps the most central database resource is PubMed, an online tool for conducting literature searches and accessing biomedical publications.

PubMed is an Internet-based search system developed by the National Center of Biotechnology Information (NCBI) at the National Library of Medicine. Using PubMed, one can access over 15 million articles in over 4600 biomedical journals. The full text of many of the journals can be obtained electronically through college or university libraries, and some journals (such as *Proceedings of the National Academy of Sciences USA; Genome Biology;* and *Science*) provide free public access to articles within certain time frames.

In this exercise, we will explore PubMed to answer questions about relationships between tubulin, human cancers, and cancer therapies, as well as the genetics of spermatogenesis.

■ Exercise I – Tubulin, Cancer, and Mitosis

In this chapter we were introduced to tubulin and the dynamic behavior of microtubules during the cell cycle. Cancer cells are characterized by continuous and uncontrolled mitotic divisions.

Is it possible that tubulin and microtubules contribute to the development of cancer? Could these important structures be targets for cancer therapies?

1. To begin your search for the answers, access the PubMed site at **www.ncbi.nlm.nih.gov/entrez/query. fcgi?DB=pubmed.**

2. In the SEARCH box, type "tubulin cancer" and then select the "Go" button to perform the search.

3. Select several research papers and read the abstracts.

   To answer the question about tubulin's association with cancer, you may want to limit your search to fewer papers, perhaps those that are review articles. To do this:

1. Select the "Limits" tab near the top of the page.

2. Scroll down the page and select "Review" in the "Type of Article" list.

3. Select "Go" to perform the search.

   Explore some of the articles, as abstracts or as full text, available in your library or by free public access. Prepare a brief report or verbally share your experiences with your class. Describe two of the most important things you learned during your exploration and identify the information sources you encountered during the search.

■ Exercise II – Human Disorders of Spermatogenesis

Using the methods described in Exercise I, identify some human disorders associated with defective spermatogenesis. Which human genes are involved in spermatogenesis? How do defects in these genes result in fertility disorders? Prepare a brief written or verbal report on what you have learned and what sources you used to acquire your information.

C A S E   S T U D Y | Timing is everything

A man in his early 20s received chemotherapy and radiotherapy as treatment every 60 days for Hodgkin's disease. After unsuccessful attempts to have children, he had his sperm examined at a fertility clinic, upon which multiple chromosomal irregularities were discovered. When examined within 5 days of a treatment, extra chromosomes were often present or one or more chromosomes were completely absent. However, such irregularities were not observed 38 days and later after a treatment.

1. How might a geneticist explain the time-related differences in chromosomal irregularities?
2. Do you think that exposure to chemotherapy and radiotherapy of a spermatogonium would cause more problems than exposure to a secondary spermatocyte?
3. What is the obvious advice that the man received regarding fertility while he remained under treatment?

Summary Points

1. The structure of cells is elaborate and complex, with most components involved directly or indirectly with genetic processes.
2. In diploid organisms, chromosomes exist in homologous pairs, where each member is identical in size, centromere placement, and gene loci. One member of each pair is derived from the maternal parent, and the other from the paternal parent.
3. Mitosis and meiosis are mechanisms by which cells distribute the genetic information contained in their chromosomes to progeny cells in a precise, orderly fashion.
4. Mitosis, which is but one part of the cell cycle, is subdivided into discrete stages that initially depict the condensation of chromatin into the diploid number of chromosomes. Each chromosome first appears as a double structure, consisting of a pair of identical sister chromatids joined at a common centromere. As mitosis proceeds, centromeres split and sister chromatids are pulled apart by spindle fibers and directed toward opposite poles of the cell. Cytoplasmic division then occurs, creating two new cells with the identical genetic information contained in the progenitor cell.
5. Meiosis converts a diploid cell into haploid gametes or spores, making sexual reproduction possible. As a result of chromosome duplication, two subsequent meiotic divisions are required to achieve haploidy, whereby each haploid cell receives one member of each homologous pair of chromosomes.
6. There is a major difference between meiosis in males and in females. Spermatogenesis partitions the cytoplasmic volume equally and produces four haploid sperm cells. Oogenesis, on the other hand, collects the bulk of cytoplasm in one egg cell and reduces the other haploid products to polar bodies. The extra cytoplasm in the egg contributes to zygote development following fertilization.
7. Meiosis results in extensive genetic variation by virtue of the exchange of chromosome segments during crossing over between maternal and paternal chromatids and by virtue of the random separation of maternal and paternal chromatids into gametes. In addition, meiosis plays an important role in the life cycles of fungi and plants, serving as the bridge between alternating generations.
8. Mitotic chromosomes are produced as a result of the coiling and condensation of chromatin fibers of interphase into the characteristic form of chromatids.

INSIGHTS AND SOLUTIONS

This appearance of "Insights and Solutions" begins a feature that will have great value to you as a student. From this point on, "Insights and Solutions" precedes the "Problems and Discussion Questions" at each chapter's end to provide sample problems and solutions that demonstrate approaches you will find useful in genetic analysis. The insights you gain by working through the sample problems will improve your ability to solve the ensuing problems in each chapter.

1. In an organism with a diploid number of $2n = 6$, how many individual chromosomal structures will align on the metaphase plate during (a) mitosis, (b) meiosis I, and (c) meiosis II? Describe each configuration.

Solution: (a) Remember that in mitosis, homologous chromosomes do not synapse, so there will be six double structures, each consisting of a pair of sister chromatids. In other words, the number of structures is equivalent to the diploid number.

(b) In meiosis I, the homologs have synapsed, reducing the number of structures to three. Each is called a tetrad and consists of two pairs of sister chromatids.

(c) In meiosis II, the same number of structures exist (three), but in this case they are called dyads. Each dyad is a pair of sister chromatids. When crossing over has occurred, each chromatid may contain parts of one of its nonsister chromatids, obtained during exchange in prophase I.
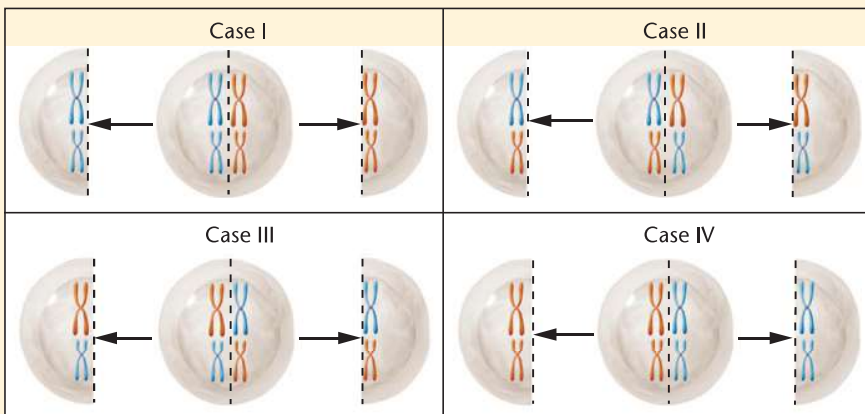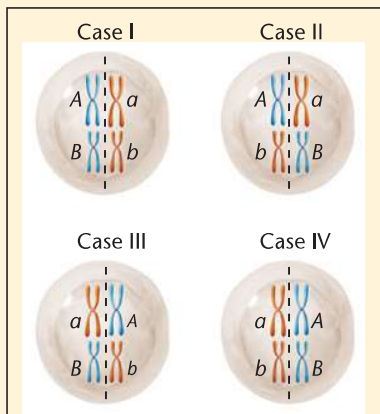

Solution for #2

2. Disregarding crossing over, draw all possible alignment configurations that can occur during metaphase for the chromosomes shown in Figure 2–11.

   Solution: As shown in the following diagram, four configurations are possible when $n = 2$.

3. For the chromosomes in the previous problem, assume that each of the larger chromosomes has a different allele for a given gene, A OR a, as shown. Also assume that each of the smaller chromosomes has a different allele for a second gene, B OR b. Calculate the probability of generating each possible combination of these alleles (AB, Ab, aB, ab) following meiosis I.

   Solution: As shown in the accompanying diagram:

Case I	AB and ab
Case II	Ab and aB
Case III	aB and Ab
Case IV	ab and AB

   Solution for #3

Total: $AB = 2 \; (p = 1/4)$

$Ab = 2 \; (p = 1/4)$

$aB = 2 \; (p = 1/4)$

$ab = 2 \; (p = 1/4)$

4. How many different chromosome configurations can occur following meiosis I if three different pairs of chromosomes are present ($n = 3$)?

   Solution: If $n = 3$, then eight different configurations would be possible. The formula 2^n, where n equals the haploid number, represents the number of potential alignment patterns. As we will see in the next chapter, these patterns are produced according to the Mendelian postulate of *segregation,* and they serve as the physical basis of another Mendelian postulate called *independent assortment.*

5. Describe the composition of a meiotic tetrad during prophase I, assuming no crossover event has occurred. What impact would a single crossover event have on this structure?

   Solution: Such a tetrad contains four chromatids, existing as two pairs. Members of each pair are sister chromatids. They are held together by a common centromere. Members of one pair are maternally derived, whereas members of the other are paternally derived. Maternal and paternal members are called nonsister chromatids. A single crossover event has the effect of exchanging a portion of a maternal and a paternal chromatid, leading to a chiasma, where the two involved chromatids overlap physically in the tetrad. The process of exchange is referred to as crossing over.

Problems and Discussion Questions

HOW DO WE KNOW?

1. In this chapter, we focused on how chromosomes are distributed during cell division, both in dividing somatic cells (mitosis) and in gamete- and spore-forming cells (meiosis). At the same time, we found many opportunities to consider the methods and reasoning by which much of this information was acquired. From the explanations given in the chapter,
   (a) How do we know that chromosomes exist in homologous pairs?
   (b) How do we know that DNA replication occurs during interphase, not early in mitosis?
   (c) How do we know that mitotic chromosomes are derived from chromatin?

2. What role do the following cellular components play in the storage, expression, or transmission of genetic information: (a) chromatin, (b) nucleolus, (c) ribosome, (d) mitochondrion, (e) centriole, (f) centromere?

3. Discuss the concepts of homologous chromosomes, diploidy, and haploidy. What characteristics do two homologous chromosomes share?

4. If two chromosomes of a species are the same length and have similar centromere placements and yet are not homologous, what is different about them?

5. Describe the events that characterize each stage of mitosis.

6. What designations are assigned to chromosomes on the basis of their centromere placement, and where is the centromere located in each case?

7. Contrast telophase in plant and animal mitosis.

8. Describe the phases of the cell cycle and the events that characterize each phase.

9. Contrast the end results of meiosis with those of mitosis.

10. Define and discuss these terms: (a) synapsis, (b) bivalents, (c) chiasmata, (d) crossing over, (e) chromomeres, (f) sister chromatids, (g) tetrads, (h) dyads, (i) monads.

11. Contrast the genetic content and the origin of sister versus non-sister chromatids during their earliest appearance in prophase I of meiosis. How might the genetic content of these change by the time tetrads have aligned at the equatorial plate during metaphase I?

12. Given the end results of the two types of division, why is it necessary for homologs to pair during meiosis and not desirable for them to pair during mitosis?

13. Contrast spermatogenesis and oogenesis. What is the significance of the formation of polar bodies?

14. Explain why meiosis leads to significant genetic variation while mitosis does not.

15. A diploid cell contains three pairs of homologous chromosomes designated C1 and C2, M1 and M2, and S1 and S2. No crossing over occurs. What combinations of chromosomes are possible in (a) daughter cells following mitosis? (b) cells undergoing the first meiotic metaphase? (c) haploid cells following both divisions of meiosis?

16. Considering the preceding problem, predict the number of different haploid cells that could be produced by meiosis if a fourth chromosome pair (W1 and W2) were added.

17. During oogenesis in an animal species with a haploid number of 6, one dyad undergoes nondisjunction during meiosis II. Following the second meiotic division, this dyad ends up intact in the ovum. How many chromosomes are present in (a) the mature ovum and (b) the second polar body? (c) Following fertilization by a normal sperm, what chromosome condition is created?

18. What is the probability that, in an organism with a haploid number of 10, a sperm will be formed that contains all 10 chromosomes whose centromeres were derived from maternal homologs?

19. During the first meiotic prophase, (a) when does crossing over occur; (b) when does synapsis occur; (c) during which stage are the chromosomes least condensed; and (d) when are chiasmata first visible?

20. Describe the role of meiosis in the life cycle of a vascular plant.

21. Contrast the chromatin fiber with the mitotic chromosome. How are the two structures related?

22. Describe the "folded-fiber" model of the mitotic chromosome.

23. You are given a metaphase chromosome preparation (a slide) from an unknown organism that contains 12 chromosomes. Two that are clearly smaller than the rest appear identical in length and centromere placement. Describe all that you can about these chromosomes.

24. If one follows 50 primary oocytes in an animal through their various stages of oogenesis, how many secondary oocytes would be formed? How many first polar bodies would be formed? How many ootids would be formed? If one follows 50 primary spermatocytes in an animal through their various stages of spermatogenesis, how many secondary spermatocytes would be formed? How many spermatids would be formed?

25. The nuclear DNA content of a single sperm cell in *Drosophila melanogaster* is approximately 0.18 picogram. What would be the expected nuclear DNA content of a primary spermatocyte in *Drosophila*? What would be the expected nuclear DNA content of a somatic cell (non-sex cell) in the G1 phase? What would be the expected nuclear DNA content of a somatic cell at metaphase?

Extra-Spicy Problems

As part of the "Problems and Discussion Questions" section in this and each subsequent chapter, we shall present a number of "Extra-Spicy" genetics problems. We have chosen to set these apart in order to identify problems that are particularly challenging. You may be asked to examine and assess actual data, to design genetics experiments, or to engage in cooperative learning. Like genetic varieties of peppers, some of these experiences are just spicy and some are very hot. We hope that you will enjoy the challenges that they pose.

For Questions 26–31, consider a diploid cell that contains three pairs of chromosomes designated AA, BB, and CC. Each pair contains a maternal and a paternal member (e.g., A^m and A^p). Using these designations, demonstrate your understanding of mitosis and meiosis by drawing chromatid combinations as requested. Be sure to indicate when chromatids are paired as a result of replication and/or synapsis. You may wish to use a large piece of brown manila wrapping paper or a cut-up paper grocery bag for this project and to work in partnership with another student. We recommend cooperative learning as an efficacious way to develop the skills you will need for solving the problems presented throughout this text.

26. In mitosis, what chromatid combination(s) will be present during metaphase? What combination(s) will be present at each pole at the completion of anaphase?

27. During meiosis I, assuming no crossing over, what chromatid combination(s) will be present at the completion of prophase? Draw all possible alignments of chromatids as migration begins during early anaphase.

28. Are there any possible combinations present during prophase of meiosis II other than those that you drew in Problem 27? If so, draw them.

29. Draw all possible combinations of chromatids during the early phases of anaphase in meiosis II.

30. Assume that during meiosis I none of the *C* chromosomes disjoin at metaphase, but they separate into dyads (instead of monads) during meiosis II. How would this change the alignments that you constructed during the anaphase stages in meiosis I and II? Draw them.

31. Assume that each gamete resulting from Problem 30 fuses, in fertilization, with a normal haploid gamete. What combinations will result? What percentage of zygotes will be diploid,

containing one paternal and one maternal member of each chromosome pair?

32. A species of cereal rye (*Secale cereale*) has a chromosome number of 14, while a species of Canadian wild rye (*Elymus canadensis*) has a chromosome number of 28. Sterile hybrids can be produced by crossing *Secale* with *Elymus*.

    (a) What would be the expected chromosome number in the somatic cells of the hybrids?

    (b) Given that none of the chromosomes pair at meiosis I in the sterile hybrid (Hang and Franckowlak, 1984), speculate on the anaphase I separation patterns of these chromosomes.

33. An interesting procedure has been applied for assessing the chromosomal balance of potential secondary oocytes for use in human *in vitro* fertilization. Using fluorescence *in situ* hybridization (FISH), Kuliev and Verlinsky (2004) were able to identify individual chromosomes in first polar bodies and thereby infer the chromosomal makeup of "sister" oocytes.

    (a) Assume that when examining a first polar body you saw that it had one copy (dyad) of each chromosome but two dyads of chromosome 21. What would you expect to be the chromosomal 21 complement in the secondary oocyte? What consequences are likely in the resulting zygote, if the secondary oocyte was fertilized?

    (b) Assume that you were examining a first polar body and noted that it had one copy (dyad) of each chromosome except chromosome 21. Chromosome 21 was completely absent. What would you expect to be the chromosome 21 complement (only with respect to chromosome 21) in the secondary oocyte? What consequences are likely in the resulting zygote if the secondary oocyte was fertilized?

    (c) Kuliev and Verlinsky state that there was a relatively high number of separation errors at meiosis I. In these cases the centromere underwent a premature division, occurring at meiosis I rather than meiosis II. Regarding chromosome 21, what would you expect to be the chromosome 21 complement in the secondary oocyte in which you saw a single chromatid (monad) for chromosome 21 in the first polar body? If this secondary oocyte was involved in fertilization, what would be the expected consequences?