Sexual Reproduction: Meiosis, Germ Cells, and Fertilization

21

Sex is not absolutely necessary. Single-celled organisms can reproduce by simple mitotic division, and many plants propagate vegetatively by forming multicellular offshoots that later detach from the parent. Likewise, in the animal kingdom, a solitary multicellular *Hydra* can produce offspring by budding (**Figure 21–1**), and sea anemones and marine worms can split into two half-organisms, each of which then regenerates its missing half. There are even some lizard species that consist only of females that reproduce without mating. Although such **asexual reproduction** is simple and direct, it gives rise to offspring that are genetically identical to their parent. **Sexual reproduction**, by contrast, mixes the genomes from two individuals to produce offspring that differ genetically from one another and from both parents. This mode of reproduction apparently has great advantages, as the vast majority of plants and animals have adopted it. Even many procaryotes and eucaryotes that normally reproduce asexually engage in occasional bouts of genetic exchange, thereby producing offspring with new combinations of genes. This chapter describes the cellular machinery of sexual reproduction. Before discussing in detail how the machinery works, however, we will briefly consider what sexual reproduction involves and what its benefits might be.

In This Chapter

OVERVIEW OF SEXUAL REPRODUCTION

Sexual reproduction occurs in **diploid** organisms, in which each cell contains two sets of chromosomes, one inherited from each parent. The specialized cells that carry out sexual reproduction, however, are **haploid**; that is, they each contain only one set of chromosomes. In the final step of sexual reproduction, a haploid cell of one individual fuses with a haploid cell of another, mixing the two genomes and restoring the diploid state. Sexual reproduction, therefore, requires a specialized type of cell division called *meiosis*, in which a diploid precursor cell gives rise to haploid progeny cells, rather than to diploid cells as occurs in ordinary mitotic cell division.

In sexually reproducing multicellular organisms, the haploid cells produced by meiosis develop into highly specialized **gametes**—*eggs* (or *ova*), *sperm* (or *spermatozoa*), pollen, or spores. In animals, females typically produce large and nonmotile eggs, whereas males typically produce small and motile sperm (**Figure 21–2**). At *fertilization*, a haploid sperm fuses with a haploid egg to form a diploid cell (a fertilized egg, or *zygote*), which contains a new combination of chromosomes. The zygote then develops into a new multicellular organism through repeated rounds of ordinary mitosis, followed by cell specialization, which includes the production of gametes (**Figure 21–3**A).

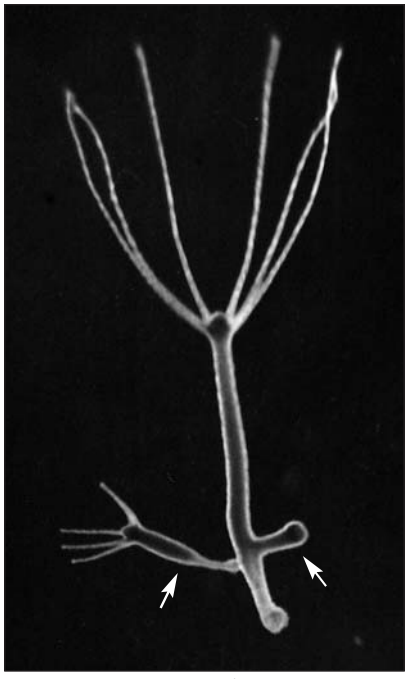


Figure 21–1 Photograph of a *Hydra* from which two new organisms are budding *(arrows).* The offspring, which are genetically identical to their parent, will eventually detach and live independently. (Courtesy of Amata Hornbruch.)

The Haploid Phase in Higher Eucaryotes Is Brief

In most organisms that reproduce sexually, diploid cells proliferate by mitotic cell division, and the haploid cells that form by meiosis do not proliferate. Some simple organisms, such as fission yeasts, are exceptional in that haploid cells

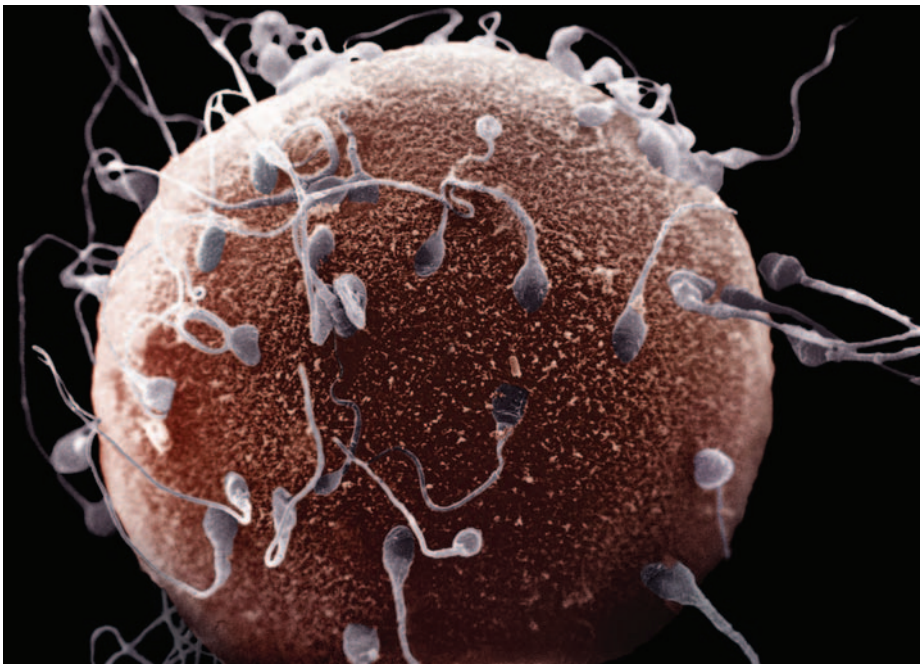


Figure 21–2 Scanning electron micrograph of an egg with many human sperm bound to its surface. Whereas the egg is immotile, the sperm are highly motile. Although many sperm are bound to the egg, only one will fertilize it, as we discuss later. (Courtesy of D. Phillips/ Science Photo Library.)

proliferate by mitotic cell division, and the diploid cells formed by the fusion of haploid cells proceed directly to meiosis to produce new haploid cells (Figure 21–3B). A less extreme exception occurs in plants, where both haploid and diploid cells proliferate. In all but the most primitive plants, such as mosses and ferns, however, the haploid phase is very brief and simple, while the diploid phase is extended into a long period of development and cell proliferation.

For almost all animals, including all vertebrates, only the diploid cells proliferate: the haploid gametes exist only briefly, do not divide at all, and are highly specialized for sexual fusion. In these organisms, it is useful to distinguish

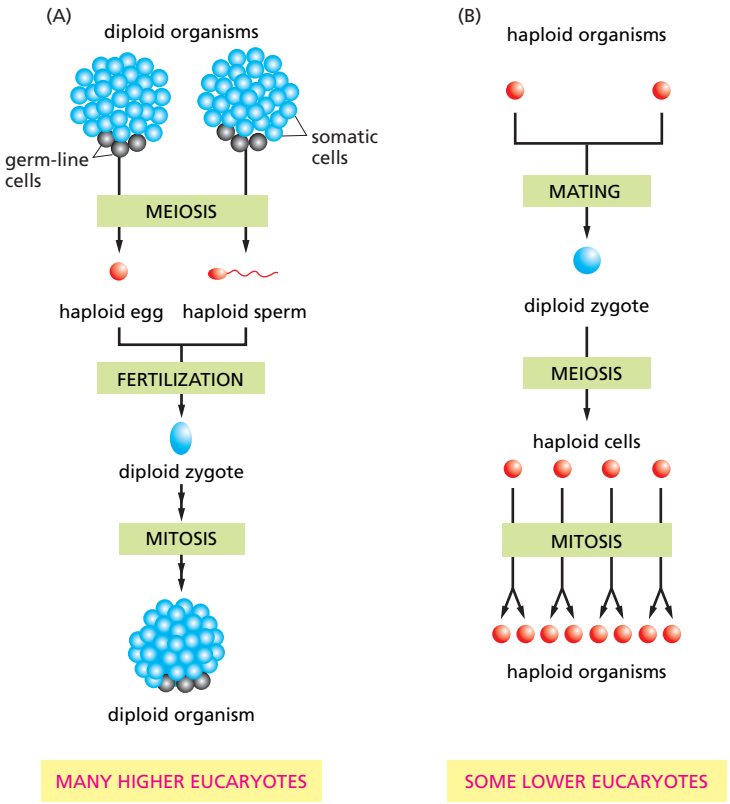


Figure 21–3 Haploid and diploid cells in the life cycles of some complex and simple eucaryotes. The haploid cells are shown in *red* and the diploid cells in *blue*. (A) Cells in most animals and plants usually proliferate in the diploid phase to form a multicellular organism; only the gametes (eggs and sperm in animals) are haploid, and they fuse at fertilization to form a diploid zygote, which develops into a new individual. The gametes develop from diploid germ-line cells *(gray)* in the gonads; all the rest of the cells in the organism are somatic cells. (B) In some simple eucaryotes such as fission yeast and the green alga *Chlamydomonas*, by contrast, the haploid cells proliferate, and the only diploid cell is the zygote, which exists transiently after mating.

between the cells of the **germ line** (or **germ cells**), which include gametes and their specified diploid precursor cells, and the **somatic cells**, which form the rest of the body and ultimately leave no progeny (see Figure 21–3A). In a sense, the somatic cells exist only to help the germ-line cells survive, develop, and transmit their DNA to the next generation.

Meiosis Creates Genetic Diversity

Sexually reproducing organisms inherit two full sets of chromosomes, one from each parent. Each set contains *autosomes,* which are common to all members of the species, and *sex chromosomes,* which are differently distributed according to the sex of the individual. Therefore, each diploid nucleus contains two closely similar versions of each autosome, plus a set of sex chromosomes appropriate to the sex of the individual. The two copies of each autosome, one from the mother and one from the father, are called **homologous chromosomes**, or **homologs**, and in most cells they maintain a separate existence as independent chromosomes. During meiosis, however, each chromosome must communicate with its unique homologous partner by physically pairing and undergoing genetic recombination. This communication is essential to enable the homologs to segregate accurately into different daughter cells during meiosis.

A crucial feature of meiosis is that it generates haploid cells that are genetically different from one another and from the two haploid cells that formed the organism in the first place. The genetic differences arise by two mechanisms. First, an individual gamete contains either the maternal or paternal version of each chromosome; because the choice of maternal or paternal occurs independently and randomly for each pair of homologs, the original maternal and paternal chromosomes are reshuffled into novel combinations in the haploid cells. Second, although the maternal and paternal versions of each chromosome have similar DNA sequences, they are not identical, and they undergo genetic recombination during meiosis—a process called *crossing-over* (discussed in Chapter 5) to produce novel hybrid versions of each chromosome; thus, each chromosome in a gamete contains a unique mixture of genetic information from both parents. We discuss these two mechanisms in more detail later (see Figure 21–13).

Sexual Reproduction Gives Organisms a Competitive Advantage

The machinery of sexual reproduction is elaborate, and the resources spent on it are large (**Figure 21–4**). What are its benefits, and why did it evolve? Sexually reproducing individuals produce varied offspring, whose varied genotypes are at least as likely to represent a change for the worse as a change for the better. Why, then, should they have a competitive advantage over individuals that breed true, by an asexual process? This question continues to perplex evolutionary biologists.

One advantage of sexual reproduction seems to be that the reshuffling of genes helps a species to survive in an unpredictably variable environment. If two parents produce many offspring with a wide variety of gene combinations, the chance that at least one of their progeny will have the combination of features necessary for survival in a changing environment is increased. Indeed, a population of budding yeast genetically engineered so that it cannot undergo meiotic genetic recombination and therefore cannot reproduce sexually adapts much less well over time to harsh environmental conditions than does the wild-type population, which can reproduce sexually.

Another advantage of sexual reproduction seems to be that it can help eliminate deleterious genes from a population: females generally mate with the fittest males, so that the least fit males leave no progeny and serve only as a sort of genetic trashcan. This stringent selection among males means that "good" genes are transmitted and "bad" genes are lost from the population more efficiently than they would otherwise be. As a result, members of the sexually reproducing population are expected to have much higher average fitness than members of an equivalent population that reproduces asexually.

Figure 21–4 A peacock displaying his elaborate tail. This extravagant plumage serves to attract females for the purpose of sexual reproduction. It has evolved because only the fittest and most handsome males leave progeny. (Courtesy of Cyril Laubscher.)

Whatever the benefits of sexual reproduction may be, it is striking that practically all complex present-day organisms have evolved largely through generations of sexual, rather than asexual, reproduction. Asexual organisms, although plentiful, seem mostly to have remained comparatively simple and primitive.

We now turn to the cellular mechanisms of sex, beginning with the events of meiosis. We then focus our discussion mainly on mammals. We first consider the diploid cells of the germ line that give rise to the gametes and how the sex of a mammal is determined. We then discuss the nature of the gametes themselves. Finally, we consider the process of fertilization, in which an egg and a sperm fuse to form a new diploid organism.

Summary

The sexual reproductive cycle involves an alternation of diploid and haploid states: diploid cells divide by meiosis to form haploid cells, and the haploid cells from two individuals fuse in pairs to form new diploid zygotes. In the process, genomes are mixed and recombined to produce individuals with novel genetic combinations. In most higher eucaryotes, diploid cells proliferate by mitosis, and only a small proportion of them (those of the germ line) undergo meiosis to produce haploid cells; the haploid cells develop into gametes, which are specialized for sexual reproduction, exist only briefly, and do not divide. Sexual reproduction is thought to be advantageous both because it produces individuals with novel genetic combinations, some of which can survive and procreate in an unpredictably variable environment, and because it provides an efficient way to eliminate harmful mutations from a population.

MEIOSIS

The realization that gametes are haploid came from an observation that also suggested that chromosomes carry genetic information. In 1883, it was discovered in a study of roundworms that the nucleus of an unfertilized egg and that of a sperm each contain two chromosomes, whereas the fertilized egg (zygote) contains four. This led to the chromosome theory of heredity, which explained the long-standing paradox that the maternal and paternal contributions to the character of the progeny seem to be equal, despite the enormous difference in size between the egg and sperm (see Figure 21–2).

The finding also implied that haploid germ cells arise from a special kind of cell division in which the number of chromosomes is precisely halved. This type of division, called **meiosis**—the Greek word for diminution or lessening—begins in animals in diploid germ-line cells in the ovaries or testes. It might seem as if meiosis could occur by a simple modification of mitosis, in which DNA synthesis (S phase) is omitted and a single cell division produces two haploid cells directly. Meiosis, however, is more complex than this and involves two cell divisions rather than one, but with only one round of DNA synthesis. It was not until the early 1930s, as a result of painstaking cytological and genetic studies, that the basic features of meiosis were established. More recently, genetic and molecular studies have begun to identify the various meiosis-specific proteins that cause meiotic chromosomes to behave differently from mitotic chromosomes and help mediate the crucial genetic recombination events that occur in meiosis. We will see that the recombination events are important not only for genetic mixing, but also for accurate chromosome segregation during meiosis.

Gametes Are Produced by Two Meiotic Cell Divisions

Meiosis uses much of the same molecular machinery and control systems that operate in ordinary mitosis. In this chapter, however, we focus on the special features of meiosis that distinguish it from mitosis. At the beginning of meiosis, as in mitosis, the chromosomes have replicated their DNA (in meiotic S phase), and the two copies are tightly bound together by *cohesin complexes* along their entire

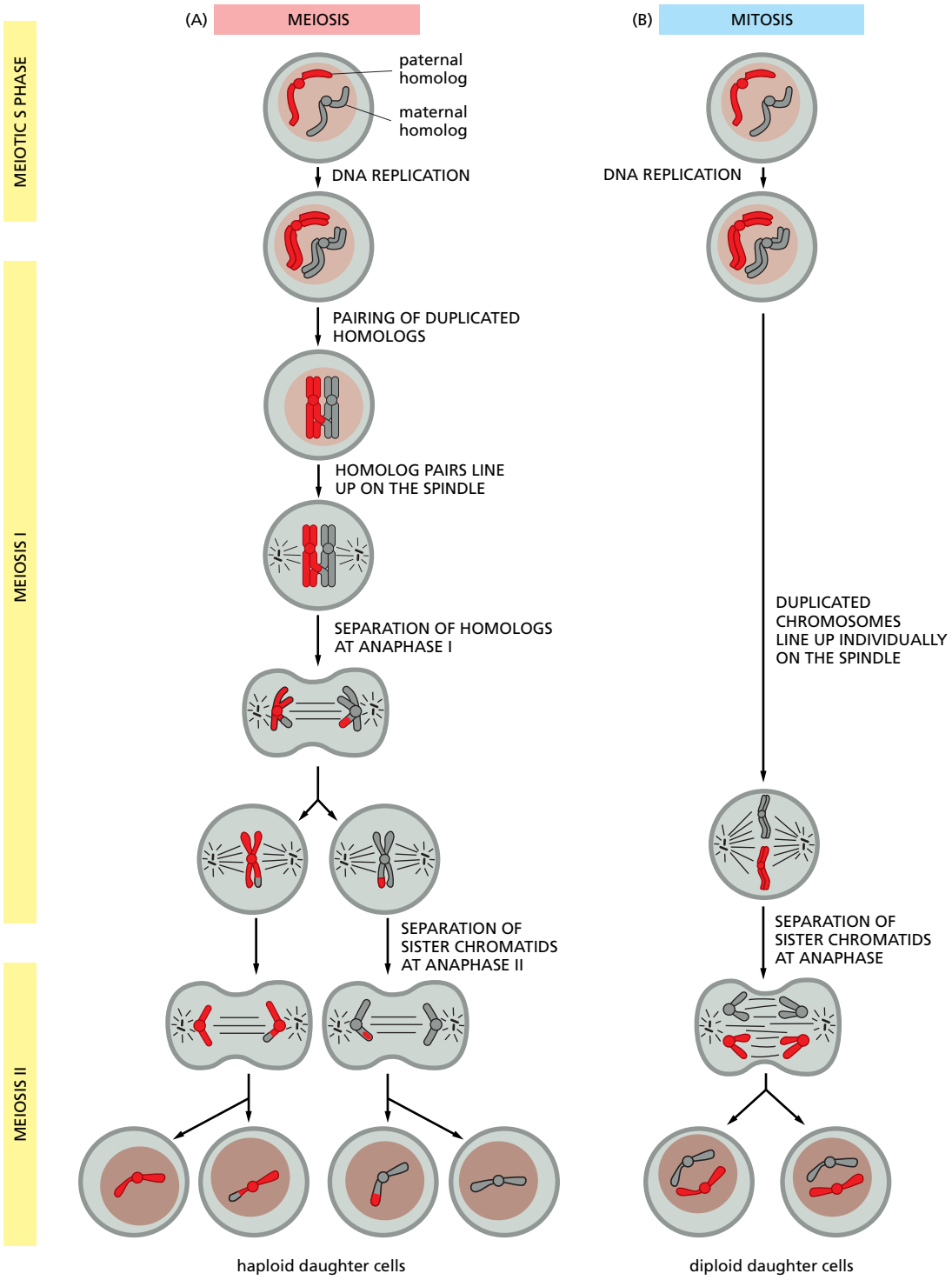


Figure 21–5 Comparison of meiosis and mitotic cell division. For clarity, only one pair of homologous chromosomes (homologs) is shown. (A) In meiosis, after DNA replication, two nuclear (and cell) divisions are required to produce the haploid gametes. The duplicated homologs, each consisting of tightly bound sister chromatids, pair up and are segregated into different daughter cells in meiosis I; the sister chromatids separate only in meiosis II. As indicated by the formation of chromosomes that are partly *red* and partly *gray*, homolog pairing in meiosis leads to genetic recombination (crossing-over) during meiosis I, as discussed later. Each diploid cell that enters meiosis therefore produces four genetically different haploid cells. <AGTG> (B) In mitosis, by contrast, homologs do not pair up, and the sister chromatids separate during the single division. Thus, each diploid cell that divides by mitosis produces two genetically identical diploid daughter cells.

length (see Figure 17–24) and are called **sister chromatids**. Unlike mitosis, however, meiosis has to produce gametes with half as many chromosomes as their diploid precursor cells. This is achieved by modifying the mitotic program so that a single round of DNA replication is followed by two successive rounds of chromosome segregation (**Figure 21–5**A). Recall that in mitosis (discussed in Chapter 17), the duplicated chromosomes line up in random order at the equator of the mitotic spindle, and the sister chromatids are pulled apart and segregated into the two daughter cells, so that each daughter inherits a complete diploid set of chromosomes and is genetically identical to the parent cell (Figure 21–5B). In division I of meiosis (**meiosis I**), by contrast, the duplicated paternal and maternal homologs (including the two replicated sex chromosomes) pair up along side each other and exchange genetic information through the process of genetic

recombination. They then line up at the equator of the meiotic spindle, after which the duplicated homologs rather than the sister chromatids are pulled apart and segregated into the two daughter cells. Only in division II of meiosis (**meiosis II**), which occurs without further DNA replication, are the sister chromatids pulled apart and segregated to produce haploid daughter cells. In this way, each diploid cell that enters meiosis produces four haploid cells, each of which inherits either the maternal or paternal copy of each chromosome, but not both (see Figure 21–5A).

Duplicated Homologs (and Sex Chromosomes) Pair During Early Prophase I

During mitosis in most organisms, homologous chromosomes behave independently of each other. During meiosis I, however, it is crucial that homologs recognize each other and associate physically in order for the maternal and paternal homologs to undergo genetic recombination and to segregate to different daughter cells at anaphase I. Special mechanisms mediate these intimate interactions between homologs.

The progressive juxtaposition of homologs occurs during a very prolonged meiotic prophase (prophase I), which can take hours in yeasts, days in mice, and weeks in higher plants. Like their mitotic counterparts, duplicated meiotic prophase chromosomes initially appear as long threadlike structures, in which the sister chromatids are so tightly glued together that they appear as one. It is during early prophase I that the homologs begin to associate along their length in a process called **pairing**, which, in some organisms at least, occurs initially through interactions between complementary DNA sequences (called *pairing sites*) in the two homologs; in most organisms, stable pairing requires genetic recombination between the homologs. As prophase I progresses, the homologs become more closely juxtaposed, forming a four-chromatid structure called a **bivalent** (**Figure 21–6**A). As we discuss later, genetic recombination begins during pairing in early prophase I, with the production of programmed double-strand breaks in chromatid DNA; some of these recombination events will later resolve into *crossovers*, where a fragment of a maternal chromatid is exchanged for a corresponding fragment of a homologous paternal chromatid (Figure 21–6B; also see Figure 5–64).

The pairing of homologs requires chromosome movements, but it is not known what drives these movements. The replicated chromosomes undergo major rearrangements within the nucleus during prophase I. The ends of the chromosomes (the *telomeres*) are tightly bound to the inner surface of the nuclear envelope. They are initially distributed diffusely there, but they then cluster transiently at one spot on the envelope and, later still, disperse again (**Figure 21–7**). Neither the mechanism nor the roles of these rearrangements are known, although they are thought to make prophase I faster and more efficient. One possibility is that they help prevent chromosome entanglements during prophase I. In fission yeast, telomere clustering is required for homolog pairing and crossing-over, but in some organisms it occurs after pairing is well underway.

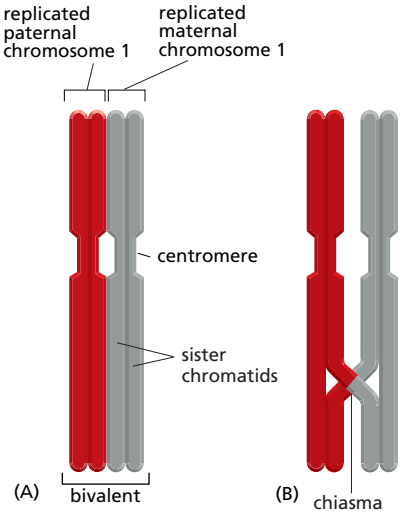


Figure 21–6 Homolog alignment and crossing-over. (A) The structure formed by two closely aligned duplicated homologs is called a *bivalent*. As in mitosis, the sister chromatids in each homolog are tightly connected along their entire lengths, as well as at their centromeres. At this stage, the homologs are usually joined together by a protein complex called the *synaptonemal complex* (not shown; see Figure 21–9). (B) A later-stage bivalent in which a single crossover event has occurred between non-sister chromatids. It is only when the synaptonemal complex disassembles and the paired homologs separate a little at the end of prophase I, as shown, that the crossover is seen microscopically as a thin connection between the homologs called a *chiasma*.

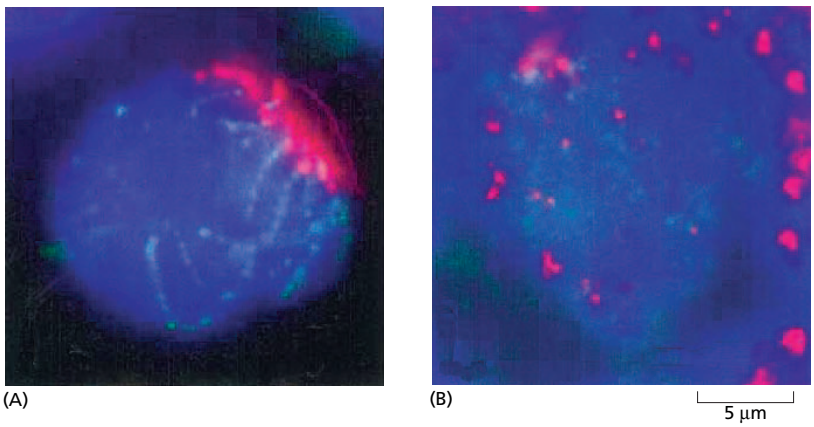


Figure 21–7 Rearrangements of telomeres during prophase in developing bovine eggs. The nucleus is stained *blue* and the telomeres are stained *red*. During prophase I, the telomeres are bound to the inner surface of the nuclear envelope. At first, they are dispersed around the nuclear envelope (not shown). Then they become clustered at one region of the envelope (A); finally, toward the end of prophase I, they disperse again (B). (From C. Pfeifer et al., *Dev. Biol.* 255:206–215, 2003. With permission from Elsevier.)

We have described the pairing of homologous autosomes during prophase I, but what happens to the sex chromosomes? This varies between different organisms. Female mammals have two X chromosomes, which pair and segregate like other homologs. But the males have one X and one Y chromosome. Although these chromosomes are not homologous, they too must pair and undergo crossing-over during prophase if they are to segregate normally at anaphase I. Pairing, crossing-over, and segregation are possible because of a small region of homology between the X and the Y at one or both ends of these chromosomes. The two chromosomes pair and crossover in this region during prophase I, ensuring that each sperm receives either one Y or one X chromosome and not both or neither. Thus, only two types of sperm are normally produced: those containing one Y chromosome, which will give rise to male embryos, and those containing one X chromosome, which will give rise to female embryos.

Homolog Pairing Culminates in the Formation of a Synaptonemal Complex

The paired homologs are brought into closer juxtaposition, with their structural axes (*axial cores*) about 400 nm apart, by a mechanism that depends in most species on the programmed double-strand DNA breaks that occur in sister chromatids. What pulls the axes together? One possibility is that the large protein machine, called a *recombination complex*, which assembles on a double-strand break in a chromatid, binds the matching DNA sequence in the nearby homolog and helps reel in this partner. This so-called *presynaptic alignment* of the homologs is followed by *synapsis,* in which the axial core of a homolog becomes tightly linked to the axial core of its partner by a closely packed array of *transverse filaments* to create a **synaptonemal complex**, which bridges the gap, now only 100 nm, between the homologs (**Figure 21–8**). Although crossing-over begins before the synaptonemal complex assembles, the final steps occur while the DNA is held in the complex (discussed in Chapter 5).

The morphological changes that occur during the pairing of meiotic chromosomes are the basis for dividing prophase I into five sequential stages—leptotene, zygotene, pachytene, diplotene, and diakinesis. As shown in **Figure 21–9**, prophase I starts with *leptotene,* when homologs condense and pair, and genetic recombination begins. At *zygotene,* the synaptonemal complex begins to assemble in local regions along the homologs; assembly initiates at sites where the homologs are closely associated and recombination events are occurring. At *pachytene,* the assembly process is complete, and the homologs are synapsed along their entire lengths. The pachytene stage can persist for days or longer, until desynapsis begins at *diplotene* with the disassembly of the synaptonemal complexes and the concomitant condensation and shortening of the chromosomes. It is only at this stage, after the complexes have disassembled, that the

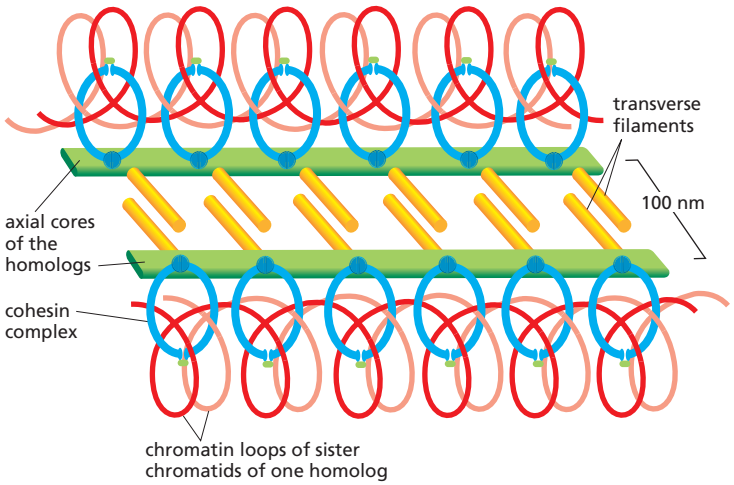


Figure 21–8 Simplified schematic drawing of a synaptonemal complex. Before the synaptonemal complex forms, recombination complexes assemble on double-strand DNA breaks on sister chromatids and help catalyze crossing-over between nonsister chromatid loops from opposite sides of the complex (not shown). (Modified from K. Nasmyth, *Annu. Rev. Genet.* 35:673–745, 2001. With permission from Annual Reviews.)

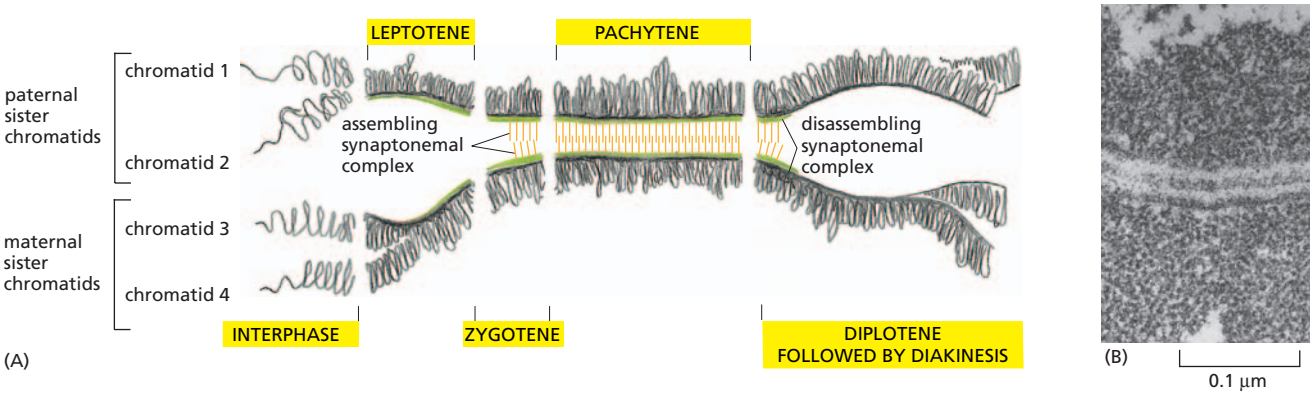


Figure 21–9 Homolog synapsis and desynapsis during the different stages of prophase I. (A) A single bivalent is shown schematically. At leptotene, the two sister chromatids coalesce, and their chromatid loops extend out together from a common axial core. The synaptonemal complex begins to assemble focally in early zygotene. Assembly continues through zygotene and is complete in pachytene. The complex disassembles in diplotene. (B) An electron micrograph of a synaptonemal complex from a meiotic cell at pachytene in a lily flower. (C and D) Immunofluorescence micrographs of prophase I cells of the fungus *Sordaria*. Partially synapsed bivalents at zygotene are shown in (C) and fully synapsed bivalents ore shown in (D). *Red arrowheads* in (C) point to regions where synapsis is still incomplete. (B, courtesy of Brian Wells; C and D, from A. Storlazzi et al., *Genes Dev.* 17:2675–2687, 2003. With permission from Cold Spring Harbor Laboratory Press.)

individual crossover events between nonsister chromatids can be seen as interhomolog connections called **chiasmata** (singular **chiasma**), which now play a crucial part in holding the compact homologs together (**Figure 21–10**). The homologs are now ready to begin the process of segregation. Prophase I ends with *diakinesis*—the stage of transition to metaphase I.

The proteins that form the transverse filaments that bridge between the axial cores of the homologs have been identified in several species, including yeasts, worms, flies, and mammals. They form homodimers that interact with each other across the 100 nm gap between the homologs, as illustrated in **Figure 21–11**. In most eucaryotes, these proteins are important for crossing-over, as mutants that lack them fail to form crossovers. The cohesin complexes that assemble on the DNA during S phase and bind the sister chromatids together during meiosis are major components of the axial core of each homolog (see Figure 21–8). Some of the cohesin subunits that operate in meiosis are the same as those that function in mitosis, whereas others are specific for meiosis. Both the crossovers and the cohesin complexes play crucial parts in segregating the homologs during meiotic division I, as we now discuss.

Homolog Segregation Depends on Meiosis-Specific, Kinetochore-Associated Proteins

One fundamental difference between meiosis I and mitosis (and meiosis II) is that in meiosis I homologs rather than sister chromatids separate and then segregate

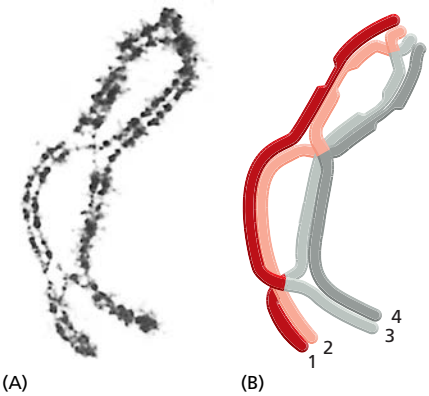


Figure 21–10 A bivalent with three chiasmata resulting from three crossover events. (A) Light micrograph of a grasshopper bivalent. (B) Drawing showing the arrangement of the crossovers in (A). Note that chromatid 1 has undergone an exchange with chromatid 3, and chromatid 2 has undergone exchanges with chromatids 3 and 4. Note also how the combination of the chiasmata and the tight attachment of the sister chromatid arms to each other (mediated by cohesin complexes) hold the two homologs together after the synaptonemal complex has disassembled; if either the chiasmata or the sister-chromatid adhesion failed to form, the homologs would come apart at this stage and not be segregated properly when the cell divides at the end of meiosis I. (A, courtesy of Bernard John.)

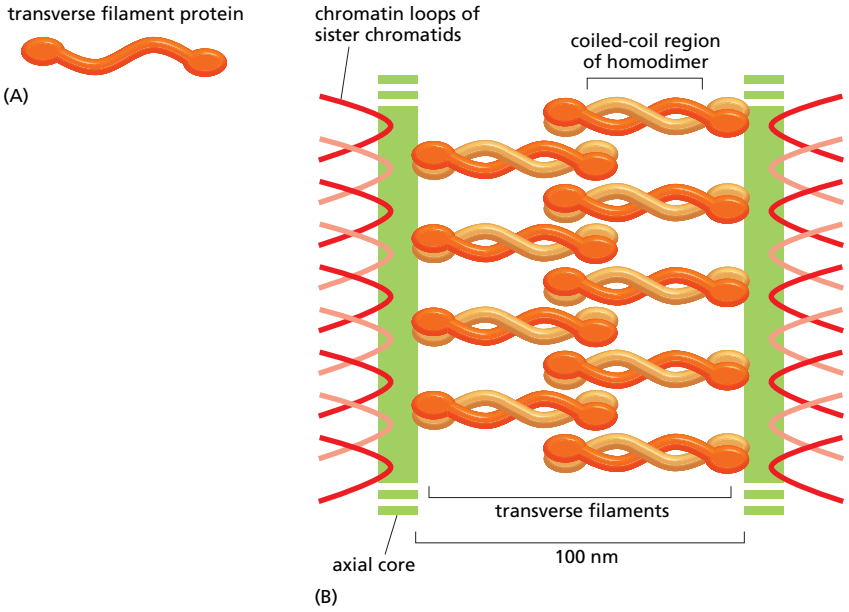


Figure 21–11 A molecular model of how transverse filaments may be formed by a single type of protein. (A) A diagram of the polypeptide chain showing the N- and C-terminal globular domains, connected by a coiled-coil region. (B) It is proposed that the protein forms homodimers, which then interact across the 100 nm gap separating the axial cores of the two homologs. (Adapted from S.L. Page and R.S. Hawley, *Science* 301:785–789, 2003. With permission from AAAS.)

into the two daughter cells (see Figure 21–5). This difference depends on three features of meiosis I that distinguish it from mitosis (**Figure 21–12**). First, the *kinetochores* (protein complexes associated with the centromeres, discussed in

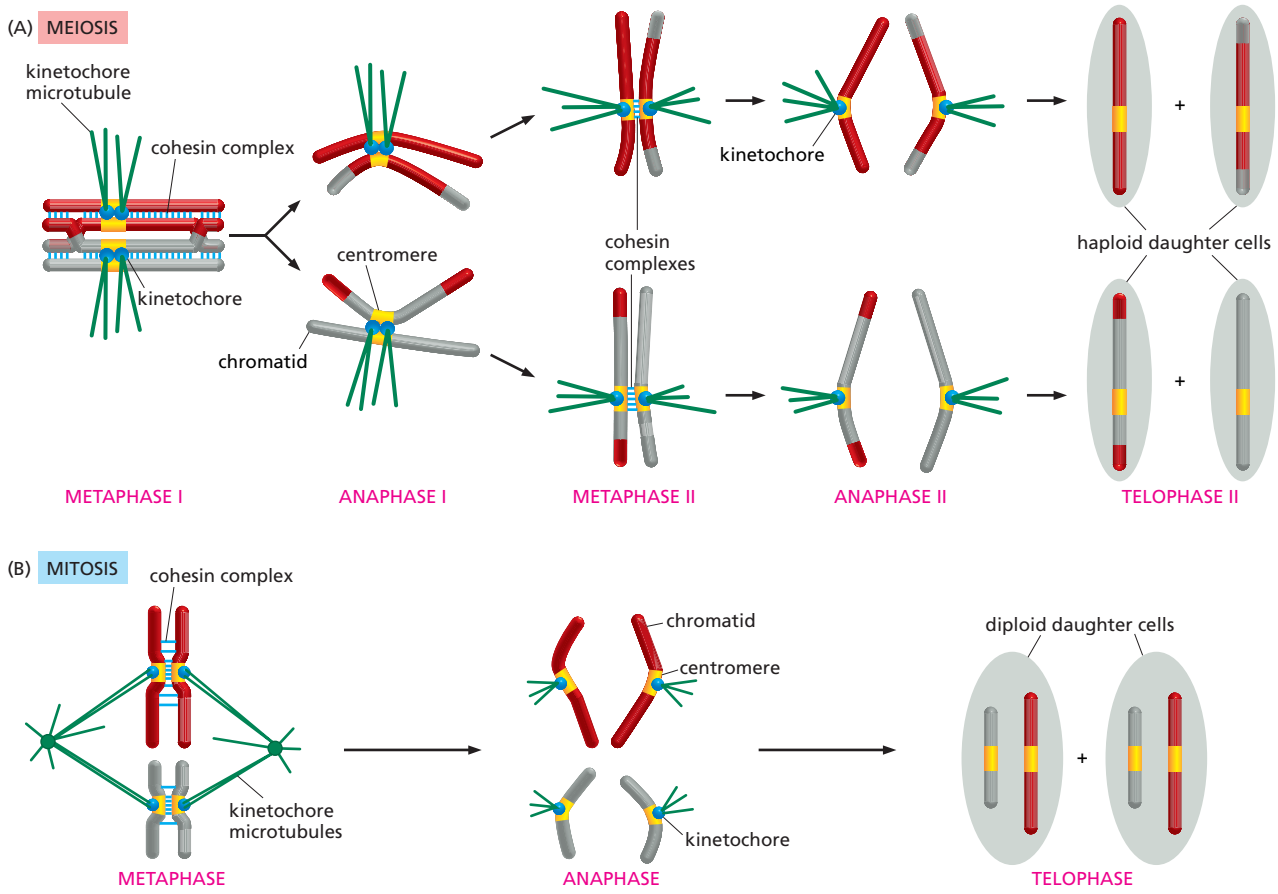


Figure 21–12 Comparison of chromosome behavior in meiosis I, meiosis II, and mitosis. Chromosomes behave similarly in mitosis and meiosis II, but they behave very differently in meiosis I. (A) In meiosis I, the two sister kinetochores are located side-by-side on each homolog at the sister centromeres and attach to microtubules emanating from the same spindle pole. The proteolytic destruction of the cohesin complexes along the sister chromatid arms unglues the arms and resolves the crossovers, allowing the duplicated homologs to separate at anaphase I, while the residual cohesin complexes at the centromeres keep the sisters together. The proteolytic destruction of the residual cohesin complexes at the centromeres allows the sister chromatids to separate at anaphase II. (B) In mitosis, by contrast, the two sister kinetochores attach to microtubules emanating from different spindle poles, and the two sister chromatids come apart at the start of anaphase and segregate into separate daughter cells (discussed in Chapter 17).

Chapters 4 and 17) on the two sister chromatids of a homolog attach to microtubules emanating from the same pole of the meiosis I spindle and thus segregate together into the same daughter cell at anaphase I; this contrasts with mitosis (and meiosis II), in which the kinetochores on the two sister chromatids of a chromosome attach to opposite poles of the spindle and therefore segregate into different daughter cells at anaphase. Second, a strong physical linkage is maintained between the homologs that resists the pulling forces of the meiosis I spindle until the bivalents are aligned at the equator of the spindle and the homologs separate at anaphase I. The chiasmata formed between nonsister chromatids and the cohesion between sister-chromatid arms cooperate in holding the homologs together (see Figure 21–10). Third, the arms of the sister chromatids separate at anaphase I, resolving the chiasmata and allowing the homologs to separate, but the sisters stay glued together in the region of their centromeres until anaphase II and therefore do not separate in anaphase I.

In micromanipulation experiments, meiosis I chromosomes transferred to a meiosis II spindle behave as they do in meiosis I, indicating that the specialized behavior of meiosis I chromosomes is determined by the chromosomes themselves rather than by the spindle or other cytoplasmic factors. Various meiosis-specific proteins associated with meiosis I chromosomes account for the special behavior, although they work together with non-meiosis-specific proteins that help mediate both mitosis and meiosis. Meiosis-specific protein complexes, for example, associate with the two kinetochores on each replicated homolog and help ensure that the two sister chromatids attach to microtubules emanating from a single spindle pole. Other proteins (called *shugoshins*) associated with kinetochores help ensure that sister kinetochores do not come apart at anaphase I when the proteolytic enzyme *separase* (discussed in Chapter 17) cleaves the cohesin complexes that tie the arms of sister chromatids together. One way that the shugoshins protect the cohesin complexes at centromeres is by recruiting a specific protein phosphatase to the centromeres; the phosphatase reverses the phosphorylation of the cohesin complexes that is required for separase to cleave them. Thus, whereas the chromatid arms come apart at anaphase I, the centromeres do not. The sisters separate only when separase cleaves the remaining cohesin complexes at the centromeres at anaphase II (see Figure 21–12A), when the shugoshins are gone.

Unlike meiosis I, meiosis II occurs rapidly and closely resembles a mitotic cell division, although it occurs without DNA replication. Prophase II is brief: the nuclear envelope breaks down as the new spindle forms, after which metaphase II, anaphase II, and telophase II usually follow in quick succession. After nuclear envelopes have formed around the four haploid nuclei produced at telophase II, cytokinesis occurs, and meiosis is complete.

Meiosis Frequently Goes Wrong

The sorting of chromosomes that takes place during meiosis is a remarkable feat of intracellular bookkeeping. In humans, each meiosis requires that the starting cell keep track of 92 chromatids (46 chromosomes, each of which has duplicated), distributing one complete set of each type of chromosome to each of the four haploid progeny cells. Not surprisingly, mistakes can occur in allocating the chromosomes during this elaborate process. Mistakes are especially common in human female meiosis, which arrests for years after diplotene: meiosis I is completed only at *ovulation*, and meiosis II only after the egg is fertilized. Indeed, such chromosome segregation errors during egg development are the commonest cause of both spontaneous abortion (miscarriage) and mental retardation in humans.

When homologs fail to separate properly—a phenomenon called **nondisjunction**—the result is that some of the haploid gametes produced lack a particular chromosome, while others have more than one copy of it. (Cells with an abnormal number of chromosomes are said to be *aneuploid*, whereas those with the correct number are said to be *euploid*.) Upon fertilization, aneuploid gametes form abnormal embryos, most of which die. Some survive, however. *Down syndrome* in humans, for example, which is the leading single cause of

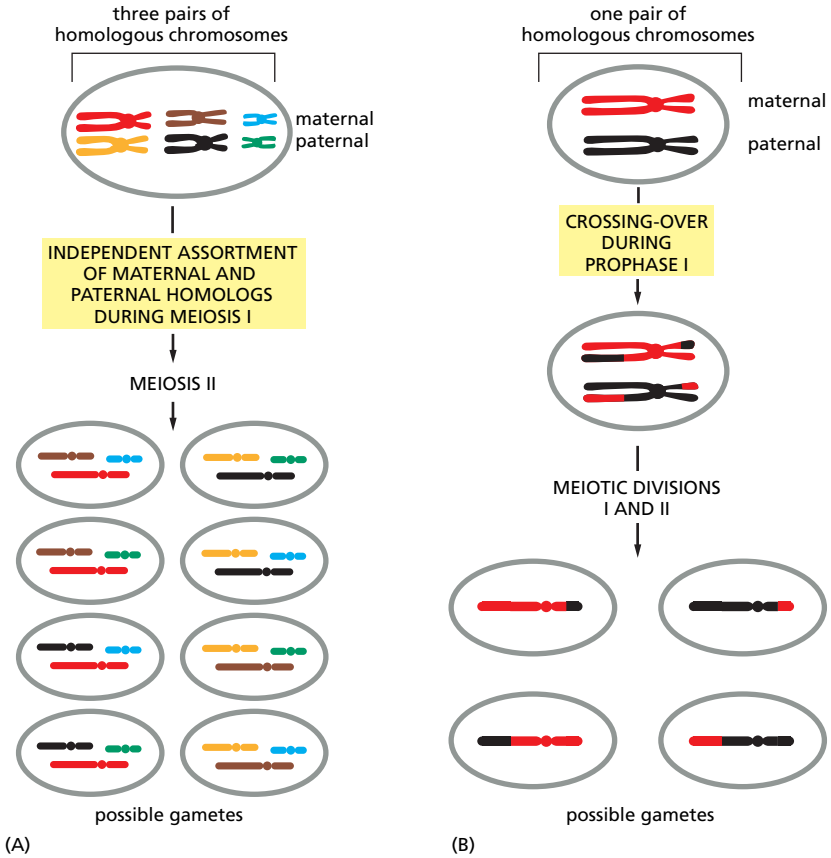


Figure 21–13 Two major contributions to the reassortment of genetic material that occurs in the production of gametes during meiosis. (A) The independent assortment of the maternal and paternal homologs during meiosis produces 2^n different haploid gametes for an organism with n chromosomes. Here $n = 3$, and there are 8 different possible gametes. (B) Crossing-over during prophase I exchanges DNA segments between homologous chromosomes and thereby re-assorts genes on individual chromosomes. Because of the many small differences in DNA sequence that always exist between any two homologs, both mechanisms increase the genetic variability of organisms that reproduce sexually.

mental retardation, is caused by an extra copy of chromosome 21, usually resulting from nondisjunction during meiosis I in the female ovary. Segregation errors during meiosis I increase greatly with advancing maternal age.

Despite its fallibility, almost all eucaryotes use meiosis, intermittently at least, to shuffle their genetic information before passing it on to the next generation. Crossing-over makes a major contribution to this genetic shuffling process, as we now discuss.

Crossing-Over Enhances Genetic Reassortment

Unless they are identical twins, which develop from a single zygote, no two offspring of the same parents are genetically the same. As we discussed earlier, this is because, long before the two gametes fuse at fertilization, two kinds of randomizing genetic reassortment have occurred in meiosis I, during the production of the gametes: the random distribution of maternal and paternal homologs, and crossing-over. The random distribution of maternal and paternal homologs (**Figure 21–13**A) could, in principle, produce 2^n genetically different gametes, where n is the haploid number of chromosomes. In humans, for example, each individual can produce at least $2^{23} = 8.4 \times 10^6$ genetically different gametes. But the actual number of variants is very much greater than this because of **chromosomal crossing-over** (or simply **crossing-over**), which is an outcome of homologous recombination (discussed in Chapter 5), in which DNA segments of homologous chromosomes are exchanged. In meiosis, when the exchange occurs between nonsister chromatids, it mixes the genetic constitution of each of the chromosomes (Figure 21–13B). On average, between two and three crossovers occur between each pair of human homologs (**Figure 21–14**).

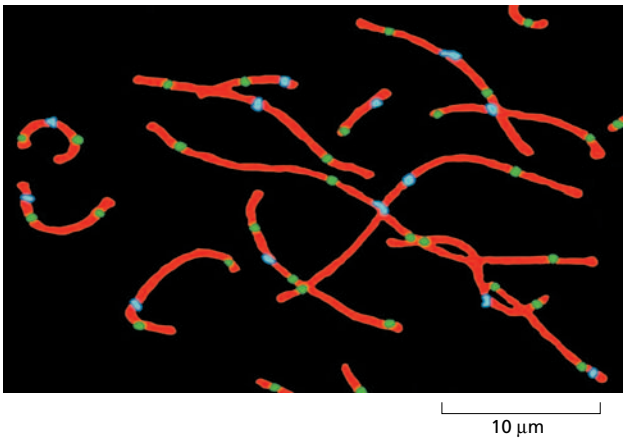


Figure 21–14 Crossovers between homologs in the human testis. In these immunofluorescence micrographs, antibodies have been used to stain the synaptonemal complexes (*red*), the centromeres (*blue*), and the sites of crossing-over (*green*). Note that all of the bivalents have at least 1 crossover and none have more than 3. (Modified from A. Lynn et al., *Science* 296:2222–2225, 2002. With permission from AAAS.)

The molecular details of crossing-over are discussed in Chapter 5 (see Figure 5–64). Briefly, a conserved meiosis-specific protein called *Spo11* initiates crossing-over by creating a double-strand break in the DNA of either a maternal or a paternal chromatid. A very large multienzyme *recombination complex*, containing double-strand DNA repair enzymes, assembles on the break and catalyzes homologous recombination. In most cases, these events do not result in a crossover. In some cases, however, homologous recombination leads to a *crossover,* where DNA segments are exchanged between two nonsister chromatids in a reciprocal fashion. As discussed earlier, after desynapsis, each crossover can be seen in the microscope as a chiasma (see Figure 21–10A). As illustrated in Figure 21–10B, each of the two sister chromatids of a homolog can form one or more crossovers with either of the two sister chromatids of its partner homolog.

Crossing-Over Is Highly Regulated

Crossing-over has two distinct functions in meiosis: it helps hold homologs together so that they are properly segregated to the two daughter cells produced by meiosis I, and it contributes to the genetic diversification of the gametes that are eventually produced. As might be expected, therefore, crossing-over is highly regulated: the number and location of double-strand breaks along each chromosome is controlled, as is the likelihood that a break will be converted into a crossover. Although the double-strand breaks that occur in meiosis I can be located almost anywhere along the chromosome (see Figure 21–14), they are not distributed uniformly: they cluster at "hot spots", where the chromatin is accessible, and occur only rarely in "cold spots", such as the heterochromatin regions around centromeres and telomeres.

At least two kinds of regulation influence the location and number of crossovers that form, neither of which is well understood. Both operate before the synaptonemal complex assembles. One ensures that at least one crossover forms between the members of each homolog pair, as is necessary for normal homolog segregation in meiosis I. In the other, called *crossover interference*, the presence of one crossover event inhibits another from forming close by, perhaps by locally depleting proteins required for converting a double-strand DNA break into a stable crossover.

Meiosis Is Regulated Differently in Male and Female Mammals

The basic mechanisms of meiosis have been conserved in evolution in all sexually reproducing eucaryotes. In all of these organisms, for example, most of meiosis is spent in prophase I, although the details of the timing of different stages vary among organisms (**Figure 21–15**). There are, however, some remarkable differences in the regulation of meiosis in different species and in different sexes of the same species. The difference between the two sexes is very striking in mammals.

In mammalian females, egg precursor cells *(oocytes)* begin meiosis in the fetal ovary but arrest after diplotene, after the synaptonemal complex has disassembled in meiosis I. They complete meiosis I only after the female has become sexually mature and the oocyte is released from the ovary during *ovulation*; moreover, the released oocyte completes meiosis II only if it is fertilized. Thus, there are special stop and start mechanisms during meiosis in female mammals. In humans, some oocytes remain arrested in meiosis I for 40 years or more, which is presumably at least part of the reason why nondisjunction increases dramatically in older women. In mammalian males, by contrast, meiosis only begins in sperm precursor cells *(spermatocytes)* in the testes at puberty and then goes on continuously, without the stop and start mechanisms that operate in female meiosis. It takes about 24 days for a human spermatocyte to complete meiosis.

There is also a big difference in the error rates of meiosis in mammalian females and males, and this is especially striking in humans. About 20% of human eggs are aneuploid, compared with 3–4% of human sperm, and, largely as a result of this, up to 25% of all human fetuses are aneuploid, and most of these result from nondisjunction in oocytes at meiosis I. Mammalian fertilization typically involves the ovulation of a small number of eggs at one end of the female reproductive tract and the entry of millions of sperm at the other. Given the relative scarcity of eggs, one might have expected that egg development would be subject to more stringent quality control than sperm development, but the opposite is the case. If meiosis goes wrong in male cells, a cell-cycle checkpoint mechanism (discussed in Chapter 17) is activated, which arrests meiosis and leads to cell death by apoptosis. Such checkpoint mechanisms apparently do not operate in female meiotic cells: if homolog segregation fails to occur normally, the cells continue through meiosis and produce aneuploid eggs. The male germ line, on the other hand, is thought to be the main source of another type of genetic error. Because many more mitotic cell divisions occur on the way to the production of a sperm, and each round of DNA replication is liable to error, the average number of new mutations contributed by fathers is larger than the number contributed by mothers.

The production of gametes involves more than just meiosis, and the other processes also differ for eggs and sperm. As we will see, by the end of meiosis, a mammalian egg is fully mature, whereas a sperm that has completed meiosis has only just begun its differentiation. Before discussing these gametes, however, we first consider how certain cells in the mammalian embryo initially become specified to develop into germ cells and how these cells then become committed to developing into either sperm or eggs, depending on the sex of the individual.

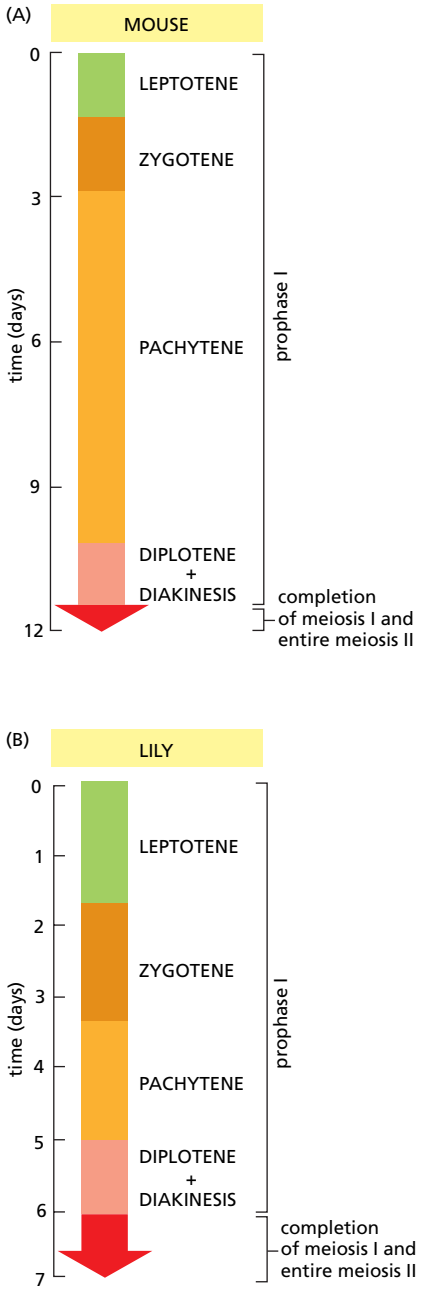


Figure 21–15 Comparison of times required for each of the stages of meiosis. (A) Approximate times for a male mammal (mouse). (B) Approximate times for the male tissue of a plant (lily). Times differ for male and female gametes (sperm and eggs, respectively) of the same species, as well as for the same gametes of different species. Meiosis in a human male, for example, lasts for 24 days, compared with 12 days in the mouse. In human females, it can last 40 years or more, because meiosis I arrests after diplotene. In all species, however, prophase I is always much longer than all the other meiotic stages combined.

Summary

Haploid gametes (eggs, sperm, pollen, and spores) are produced by meiosis, in which two successive cell divisions follow one round of DNA replication to give rise to four haploid cells from a single diploid cell. Meiosis is dominated by a prolonged prophase I, which can occupy 90% or more of the total meiotic period. At the start of prophase I, the chromosomes have replicated and consist of two tightly joined sister chromatids. Homologous chromosomes (homologs) then pair up side-by-side and become progressively more closely juxtaposed as prophase I proceeds. The tightly aligned homologs (bivalents) undergo genetic recombination, forming crossovers that can later be seen as chiasmata, which help hold each pair of homologs together during metaphase I. Both crossing-over and the independent segregation of the maternal and paternal copies of each chromosome during meiosis I have important roles in producing gametes that are genetically different from one another and from both parents. Meiosis-specific, kinetochore-associated proteins help ensure that both sister chromatids in a homolog attach to the same spindle pole; other kinetochore-associated proteins ensure that the homologs remain connected at their centromeres during anaphase I, so that homologs rather than sister chromatids are segregated in meiosis I. After the long meiosis I, meiosis II follows rapidly, without DNA replication, in a process that resembles mitosis, in that sister chromatids are pulled apart at anaphase.

PRIMORDIAL GERM CELLS AND SEX DETERMINATION IN MAMMALS

Sexual reproductive strategies vary enormously among different organisms. In the rest of this chapter, we focus mainly on the strategies used by mammals.

In all vertebrate embryos, certain cells are singled out early in development as progenitors of the gametes. These diploid **primordial germ cells** (**PGCs**) migrate to the developing gonads, which will form the *ovaries* in females and the *testes* in males. After a period of mitotic proliferation in the developing gonads, the PGCs undergo meiosis and differentiate into mature haploid gametes—either eggs or sperm. Later, the fusion of egg and sperm after mating initiates embryogenesis. The subsequent production of new PGCs in this new embryo begins the cycle again (see Figure 21–3A).

In this section, we consider how mammalian PGCs arise, how the sex of a mammal is determined, and how sex determination dictates whether the PGCs develop into sperm or eggs.

Signals from Neighbors Specify PGCs in Mammalian Embryos

In many animals, including many vertebrates, the unfertilized egg contains specific molecules localized in a particular region of the cytoplasm that determine which cells will become the germ cells. When the egg is fertilized and divides repeatedly to produce the cells of the early embryo, the cells that ultimately inherit these *germ cell determinants* become PGCs (**Figure 21–16**). Although the molecular nature and functions of the determinants are largely unknown, proteins of the *Vasa* family are a required component in all of these animals. Vasa proteins are structurally similar to ATP-dependent RNA helicases, but their precise function in germ cell determination remains a mystery.

By contrast, in other animals, including mammals, the egg cytoplasm does not contain localized germ cell determinants. Instead, signals from neighboring cells dictate which cells become PGCs. In mammals, all cells produced by the first few divisions of the fertilized egg are *totipotent*—that is, they have the potential to give rise to any of the cell types of the animal, including the germ cells and cells of extraembryonic tissues such as the placenta. Only later is a small group of cells induced by signals from neighbors to become PGCs. In mice, for instance, about 6 days after fertilization, signals (including bone morphogenic protein 4, BMP4) secreted by cells in tissue lying outside the embryo proper induce about 10 cells in the adjacent underlying embryo to become PGC precursors. These cells divide and mature to become PGCs, turning off the expression of a number of somatic cell genes and turning on the expression of genes involved in maintaining the special character of the germ cells.

Although different mechanisms specify PGCs in different animals, some of the mechanisms that control their proliferation and development have been

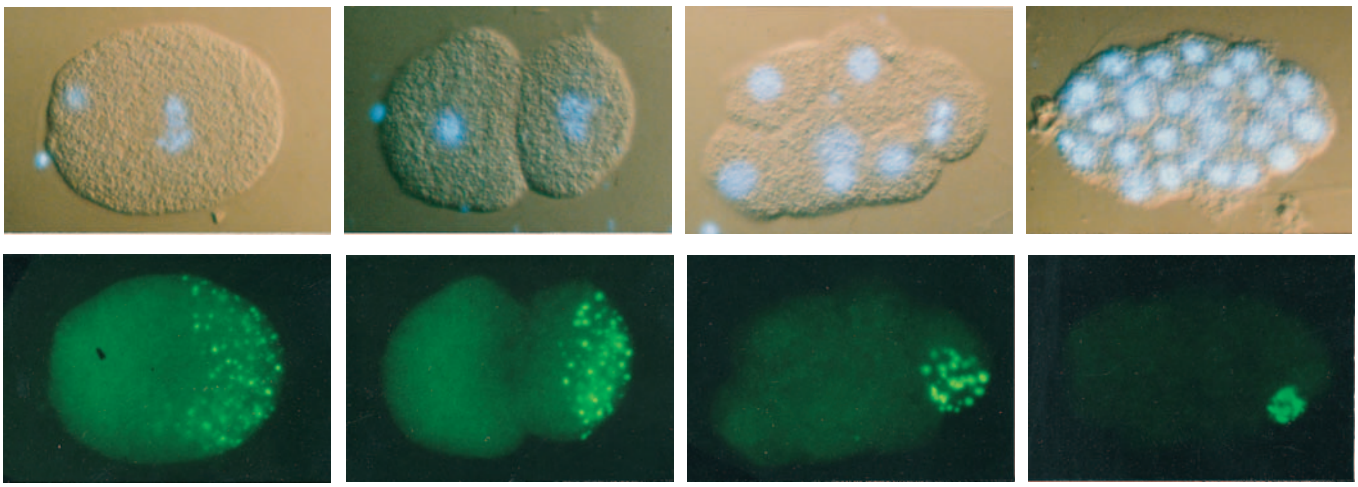


Figure 21–16 Segregation of germ cell determinants in the nematode *C. elegans*. The micrographs in the upper row show the pattern of cell divisions, with the cell nuclei stained *blue*; below, the same cells are stained with an antibody that labels (in *green*) small granules (called *P granules*) that function as germ cell determinants. The P granules are composed of RNA and protein molecules and are distributed randomly throughout the cytoplasm of the unfertilized egg (not shown). As shown in the far left-hand panels, after fertilization, the granules accumulate at one pole of the zygote. The granules are then segregated into one of the two daughter cells at each cell division. The single cell containing the P granules in the embryo shown in the far right-hand panels is the precursor of the germ line. (Courtesy of Susan Strome.)

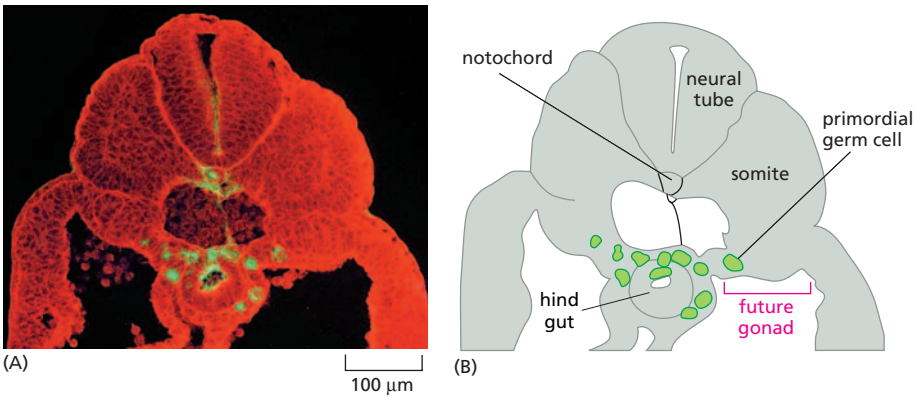


Figure 21–17 Migration of mammalian PGCs. (A) Fluorescence micrograph showing migrating PGCs in a cross section of an early mouse embryo. The PGCs are stained with a monoclonal antibody (in *green*) that specifically labels these cells at this stage of embryogenesis. The remaining cells in the embryo are stained with a lectin (in *red*) that binds to sialic acid, which is found on the surface of all cells. (B) Drawing corresponding to the micrograph shown in (A). (A, courtesy of Robert Anderson and Chris Wylie.)

conserved in evolution from worms to humans. For example, PGC development in all animals that have been studied depends on the suppression of somatic cell fates by gene repression, as well as on the inhibition of translation of specific mRNAs by *Nanos* RNA-binding proteins.

PGCs Migrate into the Developing Gonads

After mammalian PGCs develop, they proliferate and migrate to their final destination in the developing gonads (**Figure 21–17**). As they migrate through the embryo, various extracellular signal proteins produced by adjacent somatic cells signal them to survive, proliferate, and migrate. Among the secreted signal proteins that help attract PGCs into the developing gonad are *chemokines*, which bind to G-protein-coupled receptors (GPCRs) and guide the migration of various cell types, including PGCs and white blood cells (discussed in Chapter 23).

After the PGCs enter the developing gonad, which at this stage is called the *genital ridge,* they go through several more mitotic cell divisions, in the course of which they become specified to follow a pathway that will lead them to develop as either eggs or sperm.

When the PGCs first migrate into the embryonic gonad, however, they are not irreversibly committed to becoming gametes. When removed from the embryo and cultured in the presence of appropriate extracellular signal proteins, they convert into cells that can be maintained in culture indefinitely as a cell line that can produce any of the cell types of the body of the animal, although not the extraembryonic cells that go on to form structures such as the placenta; for this reason, they are said to be *pluripotent*, rather than totipotent. In these respects, these so-called *embryonic germ (EG) cells* resemble *embryonic stem (ES)* cells (discussed in Chapter 23). EG and ES cells are promising sources of various human cell types—both for drug testing and for the treatment of diseases such as heart attacks, strokes, and various neurodegenerative diseases, in which specific cell types die.

What determines whether the PGCs that migrate into the developing mammalian gonad develop into eggs or sperm? Surprisingly, it is not their own sex chromosome constitution but rather whether the genital ridge has begun to develop into an ovary or a testis, respectively. The sex chromosomes in the somatic cells of the genital ridge determine which type of gonad the ridge becomes. Although many genes influence the outcome, a single gene on the Y chromosome has an especially important role.

The *Sry* Gene Directs the Developing Mammalian Gonad to Become a Testis

Aristotle believed that the temperature of the male during sexual intercourse determined the offspring's sex: the higher the temperature, the greater the chance of producing a male. Had he been referring to lizards or alligators rather than to humans, he would have been closer to the truth, as in many egg-laying

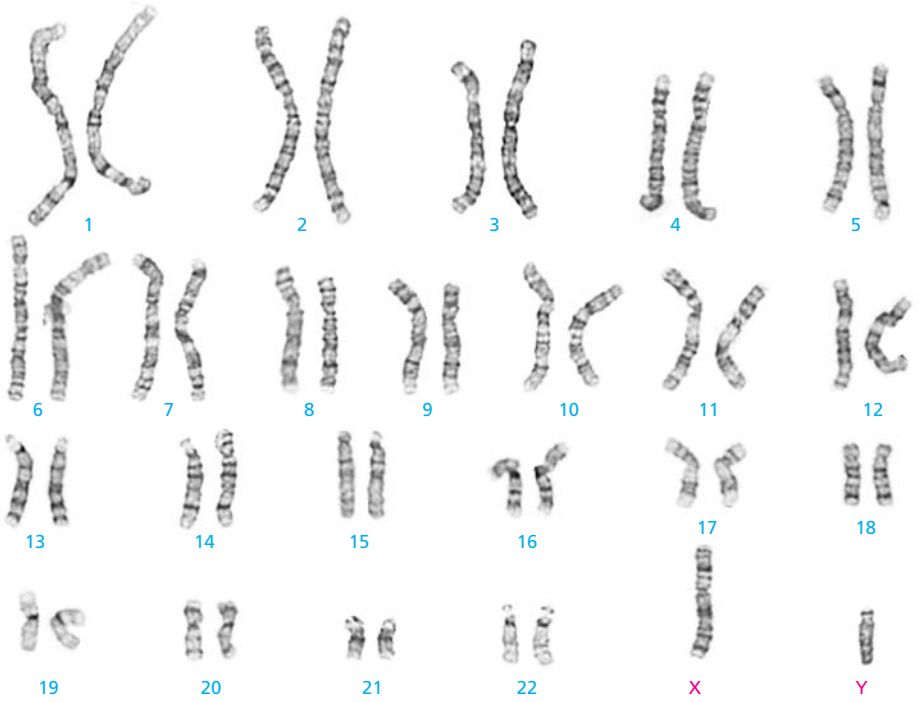


Figure 21–18 Chromosomes of a normal human male. The chromosomes have been labeled with Giemsa stain. See also Figures 4–10 and 4–11. Note the difference in size of the two sex chromosomes. Whereas the X chromosome contains more than 1000 genes, the Y chromosome contains only about 80. (Courtesy of Julie Robertson of the Wisconsin State Laboratory of Hygiene.)

reptiles the temperature of the incubating egg determines the sex of the offspring; in lizards and alligators, males develop at warm temperatures and females develop at cool temperatures. We now know, however, that sex chromosomes, rather than the temperature of the parents or embryo, determine the sex of a mammal.

Female mammals have two X chromosomes in all of their somatic cells, whereas males have one X and one Y. The presence or absence of the Y chromosome, which is the smallest human chromosome (**Figure 21–18**), determines the sex of the individual. Individuals with a Y chromosome develop as males no matter how many X chromosomes they have, whereas individuals without a Y chromosome develop as females, even if they have only one X chromosome. The sperm that fertilizes the egg determines the sex of the resulting zygote: eggs have a single X chromosome, whereas sperm can have either an X or a Y.

The Y chromosome influences the sex of the individual by directing the somatic cells of the genital ridge to develop into a testis instead of an ovary. Mammalian embryos are programmed to develop as females unless prevented from doing so by the testes, which direct the embryo to develop into a male. If the genital ridges are removed before they have started to develop into a testis or an ovary, a mammal develops into a female, regardless of the sex chromosomes it carries. This does not mean that signals are not required for the development of female-specific organs in mammals: the secreted signal protein Wnt4, for example, is required for normal mammalian ovary development.

The crucial gene on the Y chromosome that directs the genital ridge to develop into a testis instead of an ovary is called ***Sry***, for "sex-determining region of Y." Remarkably, when this gene is introduced into the genome of an XX mouse zygote, the transgenic embryo produced develops as a male, even though it lacks all of the other genes on the Y chromosome. Such *sex-reversed* mice, however, cannot produce sperm, because they lack the other genes on the Y chromosome that are required for sperm development. Similarly, XY humans with an inactivating mutation in *Sry* develop as females, even though they are otherwise genetically male.

Sry is expressed in a subpopulation of the somatic cells of the developing gonad, and it causes these cells to differentiate into **Sertoli cells**, which are the main type of supporting cells in the testis (see Figure 21–29). The Sertoli cells then direct sexual development along a male pathway by influencing other cells

in the genital ridge and elsewhere in the embryo in at least four ways:

1. They stimulate the newly arriving PGCs to develop along a pathway that produces sperm. They do so by inhibiting the cells from entering meiosis and developing along the pathway that produces eggs, as we discuss later.
2. They secrete *anti-Müllerian hormone,* which circulates in the blood and suppresses the development of the female reproductive tract by causing the Müllerian duct to regress (this duct otherwise gives rise to the oviduct, uterus, and upper part of the vagina).
3. They stimulate endothelial and smooth muscle cells in adjacent mesenchymal tissue to migrate into the developing gonad. These cells form critical elements within the testis that are required for normal sperm production, which begins when the organism reaches sexual maturity.
4. They help to induce other somatic cells in the developing gonad to become *Leydig cells,* which secrete the male sex hormone *testosterone* into the blood. The secretion of testosterone is responsible for inducing all male secondary sexual characteristics, including the structures of the male reproductive tract, such as the prostate and seminal vesicles, which develop from another duct called the Wolffian duct system. This duct system degenerates in the developing female because it requires testosterone to survive and develop. Testosterone secretion also helps masculinize the early developing brain, influencing sexual identity and sexual orientation, and thereby sexual behavior: female rats that are treated with testosterone around birth, for example, later display malelike sexual behavior.

The *Sry* gene encodes a gene regulatory protein (Sry) that binds to DNA and influences the transcription of other genes involved in Sertoli cell development. One crucial downstream gene encodes another gene regulatory protein related to Sry, which is called Sox9. The *Sox9* gene is not on the Y chromosome, but it is expressed in males in all vertebrates, unlike *Sry*, which is found only in mammals. If *Sox9* is expressed ectopically in the developing gonads of an XX mouse embryo, the embryo develops as a male, even if it lacks the *Sry* gene, suggesting that Sry normally acts by inducing the expression of *Sox9*. The Sox9 protein directly activates the transcription of at least some Sertoli-cell-specific genes, including the gene encoding anti-Müllerian hormone.

In the absence of either *Sry* or *Sox9*, the genital ridge of an XY embryo develops into an ovary instead of a testis. The supporting cells become *follicle cells* instead of Sertoli cells. Other somatic cells become *theca cells* (instead of Leydig cells), which, beginning at puberty, contribute to the production of the female sex hormone *estrogen*. The PGCs develop along a pathway that produces eggs rather than sperm (**Figure 21–19**), and the animal develops as a female.

How do Sertoli cells induce the PGCs that migrate into the developing gonad in males to follow the pathway leading to sperm production rather than to egg production? The mechanism depends on the small signal molecule *retinoic acid* (see Figure 15–13), which, in both sexes, is produced by cells in a transient tubular structure called the mesonephros that lies adjacent to the developing gonad. In the embryonic ovary, the retinoic acid induces the proliferating germ-line cells to enter meiosis and start down the pathway leading to egg production; the cells become arrested after diplotene of prophase I, where they remain until ovulation, beginning when the female reaches sexual maturity. In the embryonic testis, by contrast, Sertoli cells produce an enzyme that degrades retinoic acid, preventing the retinoic acid from the mesonephros from inducing the germ-line cells to enter meiosis and begin egg development. Only much later, when the male becomes sexually mature, do the germ-line cells in the testis begin producting sperm.

Many Aspects of Sexual Reproduction Vary Greatly between Animal Species

Although meiosis is highly conserved in all sexually reproducing eucaryotes, other aspects of sexual reproduction are extremely variable. We have seen that

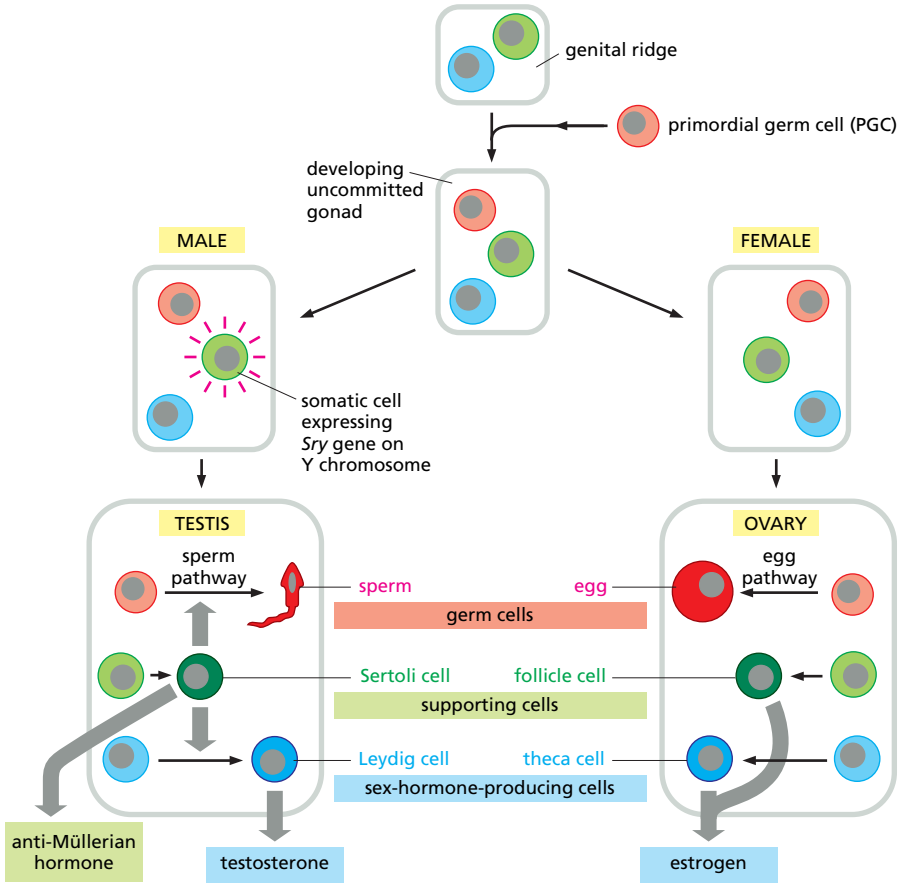


Figure 21–19 Influence of *Sry* on gonad development. The germ-line cells are shaded in *red,* and the somatic cells are shaded in *green* or *blue.* The change from light to darker color indicates that the cell has differentiated. The *Sry* gene acts in a subpopulation of somatic cells in the developing gonad to direct them to differentiate into Sertoli cells instead of follicle cells. The Sertoli cells then prevent the germ-line cells from developing along the egg pathway and help direct them down the sperm pathway of development, beginning at puberty. They also secrete anti-Müllerian hormone, which causes the Müllerian duct to regress, and they help to induce other somatic cells to differentiate into Leydig cells, which secrete testosterone (see Figure 21–29). In the absence of *Sry,* the germ-line cells commit to egg development, and the somatic cells develop into either follicle cells, which support egg development, or theca cells, which produce progesterone; the progesterone is converted to estrogen by the follicle cells. Whereas the testis begins secreting testosterone in the fetus, the ovary does not begin secreting estrogen until puberty.

an animal's sex can depend on either its chromosomes or the environment in which it develops. But even the genetic mechanisms of sex determination vary greatly. In *C. elegans* and *Drosophila*, for example, sex is determined by the ratio of X chromosomes to autosomes, rather than by the presence or absence of a Y chromosome, as in mammals. In *C. elegans*, sex determination depends mainly on transcriptional and translational controls on gene expression, whereas in *Drosophila* it depends on a cascade of regulated RNA splicing events, as discussed in Chapter 7. In *Drosophila*, moreover, the sex-specific character of each cell in the body is programmed individually by its own chromosomes, instead of being controlled mainly by hormones. It remains a mystery why some aspects of sexual reproduction have been conserved in evolution, while others have become so fundamentally different.

Summary

A small number of cells in the early mammalian embryo are signaled by their neighbors to become germ-line cells. The resulting primordial germ cells (PGCs) proliferate and migrate into the developing gonads. Here, the germ-line cells commit to develop into either eggs, if the gonad is becoming an ovary, or sperm, if the gonad is becoming a testis. A developing gonad will develop into an ovary unless its somatic cells contain a Y chromosome, in which case it develops into a testis. The Sry gene on the mammalian Y chromosome is crucial for testis development: it is expressed in a subpopulation of somatic cells in the developing gonad and directs them to differentiate into Sertoli cells, which then produce signal molecules that promote the development of male characteristics, and suppress the development of female characteristics. Mammalian embryos are programmed to follow a female pathway of development unless they are diverted to follow the male pathway by Sertoli cells.

EGGS

In one respect at least, an egg is the most remarkable of animal cells: once activated, it can give rise to a complete new individual within a matter of days or weeks. No other cell in a higher animal has this capacity. Activation is usually the consequence of *fertilization*—fusion of a sperm with the egg—but eggs can also be activated artificially by various nonspecific chemical or physical treatments. Indeed, some organisms, including a few vertebrates such as some lizards, normally reproduce from eggs that become activated in the absence of sperm—that is, **parthenogenetically**. Mammals are the only animals that cannot be produced parthenogenetically; because of *genomic imprinting* (discussed in Chapter 7), they require both maternal and paternal genetic contributions.

Although an egg can give rise to every cell type in the adult organism, it is itself a highly specialized cell, uniquely equipped for the single function of generating a new individual. The cytoplasm of an egg can even reprogram a somatic cell nucleus so that the nucleus can direct the development of a new individual, although the egg components responsible are mostly unknown. That is how the famous sheep Dolly was produced. The nucleus of an unfertilized sheep egg was removed with a glass pipette and replaced with the nucleus of an adult somatic cell. An electric shock was used to activate the egg, and the resulting embryo was implanted in the uterus of a surrogate mother. The resulting adult sheep had the genome of the donor somatic cell nucleus and was therefore a *clone* of the donor sheep.

The same approach, called *reproductive cloning*, has been used to produce clones of various mammals, including mice, rats, cats, dogs, goats, pigs, cattle, and horses (see Figure 21–38). In all cases, the efficiency is low: most of the clones die before birth and less than 5% of them develop to adulthood, probably because the transplanted somatic nucleus is not completely reprogrammed and so expresses many genes inappropriately.

In this section, we briefly consider some of the specialized features of an egg, before discussing how it undergoes its final preparations for fertilization.

Figure 21–20 The actual sizes of three eggs. The human egg is 0.1 mm in diameter.

An Egg Is Highly Specialized for Independent Development

The eggs of most animals are giant single cells. They contain stockpiles of all the materials needed for initial development of the embryo until the stage at which the new individual can begin feeding. Before the feeding stage, the giant cell cleaves into many smaller cells, but no net growth occurs. The mammalian embryo is an exception. It can start to grow early by taking up nutrients from the mother via the placenta. Thus, a mammalian egg, although still a large cell, is not as large as the egg of a frog or bird, for example. Eggs are typically spherical or ovoid, with a diameter of about 0.1 mm in humans and sea urchins (whose feeding larvae are tiny), 1–2 mm in frogs and fishes, and many centimeters in birds and reptiles (**Figure 21–20**). A typical somatic cell, by contrast, has a diameter of only about 10–30 μm (**Figure 21–21**).

The egg cytoplasm usually contains nutritional reserves in the form of **yolk**, which is rich in lipids, proteins, and polysaccharides and is often contained within discrete structures called *yolk granules*. In some species, a membrane encloses each yolk granule. In eggs that develop into large animals outside the mother's body, yolk can account for more than 95% of the volume of the cell. In mammals, whose embryos are largely nourished by their mothers via the placenta, there is little, if any, yolk.

The **egg coat** is another peculiarity of eggs. It is a specialized form of extracellular matrix consisting largely of glycoproteins—some secreted by the egg and some by surrounding cells. In many species, the major coat is a layer immediately surrounding the egg plasma membrane; in nonmammalian eggs, such as those of sea urchins or chickens, it is called the *vitelline layer*, whereas in mammalian eggs it is called the *zona pellucida* (**Figure 21–22**). This layer protects the egg from mechanical damage, and in many eggs it also acts as a species-specific barrier to sperm, admitting only those of the same or closely related species.

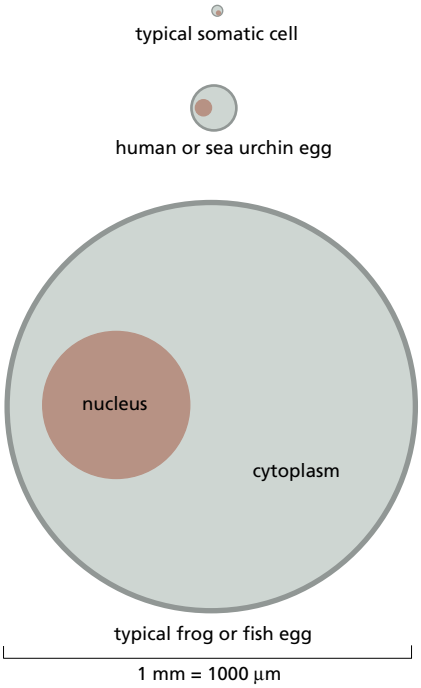


Figure 21–21 The relative sizes of various eggs, compared with that of a typical somatic cell.

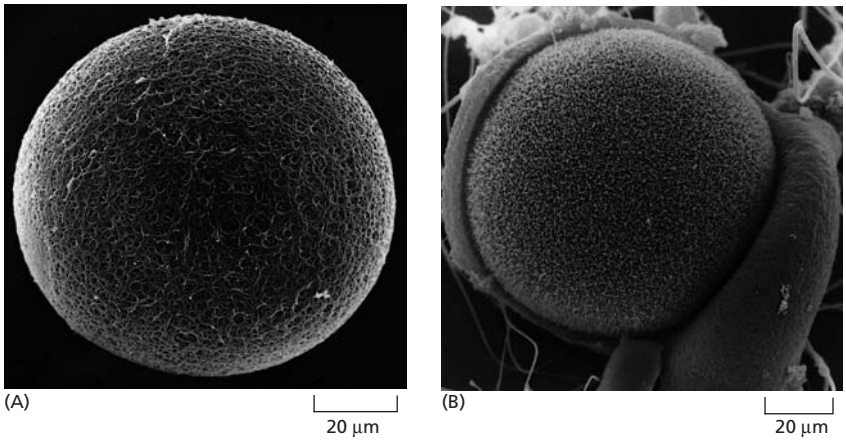


Figure 21–22 The zona pellucida. (A) Scanning electron micrograph of a hamster egg, showing the zona pellucida. (B) A scanning electron micrograph of a similar egg in which the zona (to which many sperm are attached) has been peeled back to reveal the underlying plasma membrane, which contains numerous microvilli. The zona is made entirely by the developing oocyte. (From D.M. Phillips, *J. Ultrastruct. Res.* 72:1–12, 1980. With permission from Elsevier.)

Many eggs (including those of mammals) contain specialized secretory vesicles just under the plasma membrane in the outer region, or *cortex*, of the egg cytoplasm. When a sperm activates the egg, these **cortical granules** release their contents by exocytosis; the contents of the granules alter the egg coat so as to help prevent more than one sperm from fusing with the egg.

Cortical granules are usually distributed evenly throughout the egg cortex. In many organisms, however, some egg cytoplasmic components have a strikingly asymmetrical distribution. Some of these localized components later serve as germ cell determinants (see Figure 21–16) or help establish the polarity of the embryo, as discussed in Chapter 22.

Eggs Develop in Stages

A developing egg, or **oocyte**, differentiates into a mature egg (or *ovum*) through a progressive series of changes. The timing of these changes is coordinated with the steps of meiosis, in which the germ cells go through their two final, highly specialized, divisions. As discussed earlier, oocytes arrest for a prolonged period in meiosis I while they grow in size and progressively differentiate; in many cases, after completing meiosis I, they arrest again in metaphase II while awaiting fertilization (although they can await fertilization at various other points, depending on the species).

While the details of oocyte development (**oogenesis**) vary from species to species, the general stages are similar, as outlined in **Figure 21–23**. Primordial germ cells migrate to the forming gonad to become *oogonia,* which proliferate by mitosis for a period before beginning meiosis I, at which point they are called *primary oocytes*; this usually occurs before birth in mammals. As discussed earlier, prior to the onset of meiosis I, the DNA replicates so that each chromosome consists of two sister chromatids; at the start of prophase I, the duplicated homologous chromosomes pair along their long axes, and crossing-over occurs between nonsister chromatids of these paired homologs (see Figure 21–10). After these events, the cell remains arrested after diplotene of prophase I for a period lasting from a few days to many years, depending on the species. During this long arrest period (or, in some cases, at the onset of sexual maturity), the primary oocytes synthesize a coat and cortical granules. The large oocytes of nonmammalian species also accumulate ribosomes, yolk, glycogen, lipid, and the mRNAs that will later direct the synthesis of proteins required for early embryonic growth and development. In many of these oocytes, we can observe the intensive biosynthetic activities in the structure of the chromosomes, which decondense and form lateral loops, taking on a characteristic "lampbrush" appearance, signifying that the genes in the loops are being busily transcribed (see Figures 4–54 and 4–55).

The next phase of oocyte development, *oocyte maturation*, usually does not occur until sexual maturity, when hormones stimulate the oocyte. Under these hormonal influences, the cell resumes its progress through meiosis I. The chromosomes recondense, the nuclear envelope breaks down, the meiotic

spindle assembles, and the replicated homologous chromosomes segregate at anaphase I into two sets, each containing half the original number of chromosomes. To end meiosis I, the cytoplasm divides asymmetrically to produce two cells that differ greatly in size: one is a small *polar body,* and the other is a large **secondary oocyte**, the precursor of the egg. At this stage, each chromosome is still composed of two sister chromatids held together at their centromeres. The sister chromatids do not separate until anaphase II, after which the cytoplasm of the large secondary oocyte again divides asymmetrically to produce the mature **egg** (or **ovum**) and a second small polar body, each with a haploid set of single chromosomes (see Figure 21–23). Because of these asymmetrical divisions of

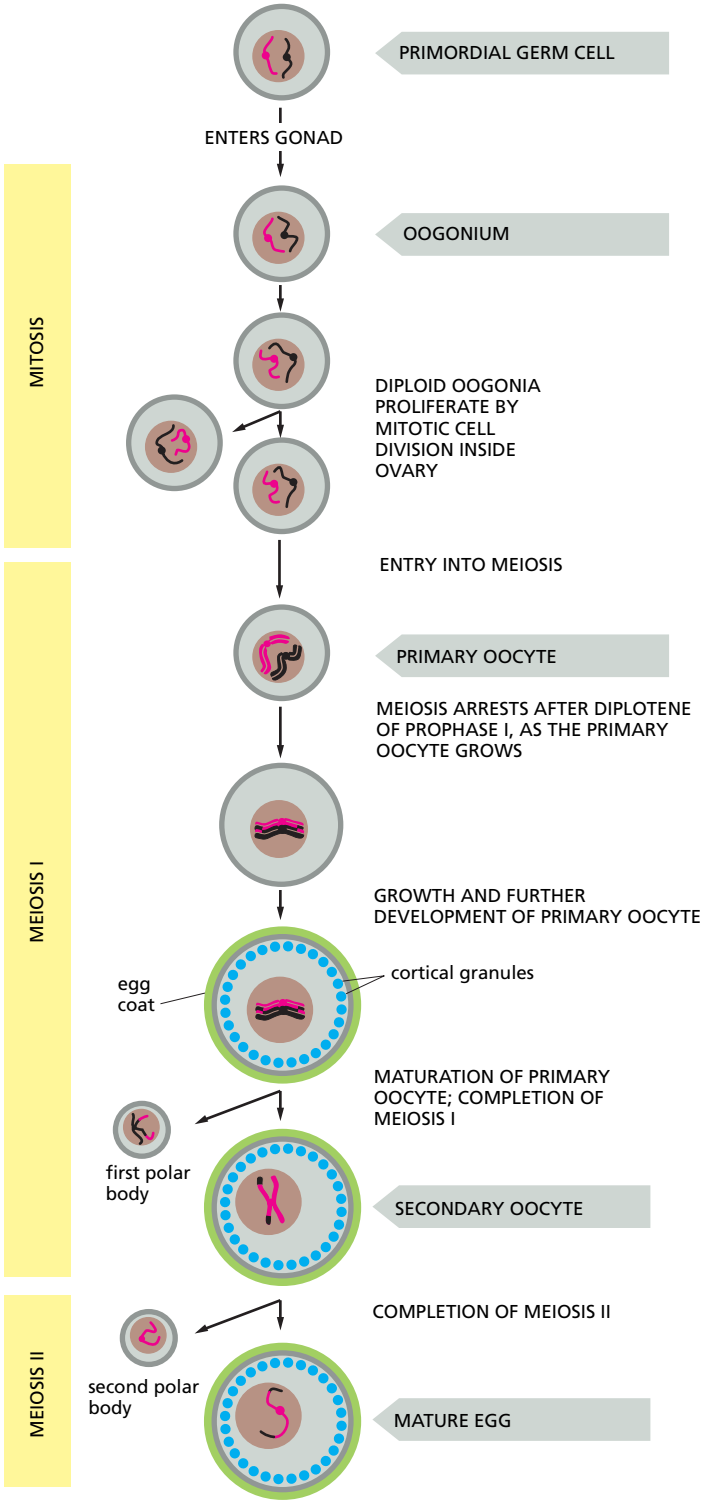


Figure 21–23 The stages of oogenesis. Oogonia develop from primordial germ cells (PGCs) that migrate into the developing gonad early in embryogenesis. For clarity, only one pair of homologous chromosomes is shown. After several mitotic divisions, oogonia begin meiosis and are now called primary oocytes. In mammals, primary oocytes are formed very early (between 3 and 8 months of gestation in the human embryo) and remain arrested after diplotene of prophase I until the female becomes sexually mature. At this point, a small number of primary oocytes periodically mature under the influence of hormones, completing meiosis I to produce secondary oocytes, which eventually undergo meiosis II to produce mature eggs (ova). The stage at which the egg or oocyte is released from the ovary and is fertilized varies from species to species. In most vertebrates, oocyte maturation is arrested at metaphase II, and the secondary oocyte completes meiosis II only after fertilization. All of the polar bodies eventually degenerate. In most animals, the developing oocyte is surrounded by specialized accessory cells that help to isolate and nourish it (not shown).

their cytoplasm, oocytes maintain their large size despite undergoing the two meiotic divisions. Both of the polar bodies are small, and they eventually degenerate.

In most vertebrates, oocyte maturation proceeds to metaphase of meiosis II, at which point they become arrested. At **ovulation**, the arrested secondary oocyte is released from the ovary, ready to be fertilized. If fertilization occurs, the block is lifted and the cell completes meiosis, becoming a mature egg. Because it is fertilized, it is also called a zygote.

Oocytes Use Special Mechanisms to Grow to Their Large Size

A somatic cell with a diameter of 10–20 μm typically takes about 24 hours to double its mass in preparation for cell division. At this rate of biosynthesis, such a cell would take a very long time to reach the thousand-fold greater mass of a mammalian egg with a diameter of 100 μm. It would take even longer to reach the million-fold greater mass of an insect egg with a diameter of 1000 μm. Yet, some insects live only a few days and manage to produce eggs with diameters even greater than 1000 μm. Eggs must have special mechanisms for achieving their large size.

One simple strategy for rapid growth is to have extra gene copies in the cell. Most of the growth of an oocyte occurs after DNA replication, during the prolonged arrest after diplotene in prophase I, when the diploid chromosome set is in duplicate (see Figure 21–23). In this way, it has twice as much DNA available for RNA synthesis as does an average somatic cell in the G_1 phase of the cell cycle. The oocytes of some species go even further to accumulate extra DNA: they produce many extra copies of certain genes. As discussed in Chapter 6, the somatic cells of most organisms contain 100 to 500 copies of the ribosomal RNA genes so as to produce enough ribosomes for protein synthesis. Eggs need even greater numbers of ribosomes to support the increased rate of protein synthesis required during early embryogenesis, and in the oocytes of many animals the ribosomal RNA genes are specifically amplified; some amphibian eggs, for example, contain 1 or 2 million copies of these genes.

Oocytes may also depend partly on the synthetic activities of other cells for their growth. Yolk, for example, is usually synthesized outside the ovary and imported into the oocyte. In birds, amphibians, and insects, yolk proteins are made by liver cells (or their equivalents), which secrete these proteins into the blood. Within the ovaries, oocytes use receptor-mediated endocytosis to take up the yolk proteins from the extracellular fluid (see Figure 13–46). Nutritive help can also come from neighboring accessory cells in the ovary. These can be of two types. In some invertebrates, some of the progeny of the oogonia become **nurse cells** instead of becoming oocytes. Cytoplasmic bridges connect these cells to the oocyte, allowing macromolecules to pass directly from the nurse cells into the oocyte cytoplasm (**Figure 21–24**). For the insect oocyte, the nurse cells manufacture many of the products—ribosomes, mRNA, protein, and so on—that vertebrate oocytes have to make for themselves.

The other accessory cells in the ovary that help to nourish developing oocytes are ordinary somatic cells called **follicle cells**, which surround each developing oocyte in both invertebrates and vertebrates. They are arranged as an epithelial layer around the oocyte (**Figure 21–25**, and see Figure 21–24), and they are connected to each other and to the oocyte by *gap junctions*, which permit the exchange of small molecules but not macromolecules (discussed in Chapter 19). Although follicle cells are unable to provide the oocyte with preformed macromolecules through these junctions, they can supply the smaller precursor molecules from which macromolecules are made. The critical importance of gap-junction communication has been elegantly demonstrated in the mouse ovary, where the gap-junction proteins *(connexins)* involved in connecting follicle cells to each other are different from those connecting follicle cells to the oocyte. If the genes that encode either of these proteins are disrupted in mice, both the follicle cells and oocytes fail to develop normally and the female mice are sterile. In many species, follicle cells secrete macromolecules that

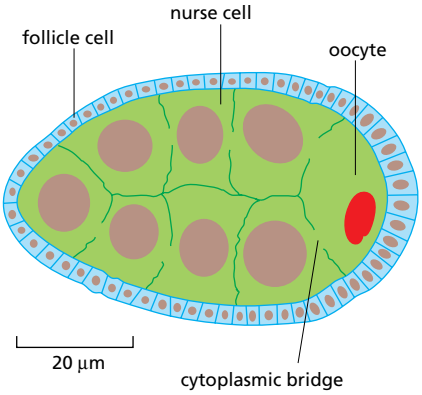
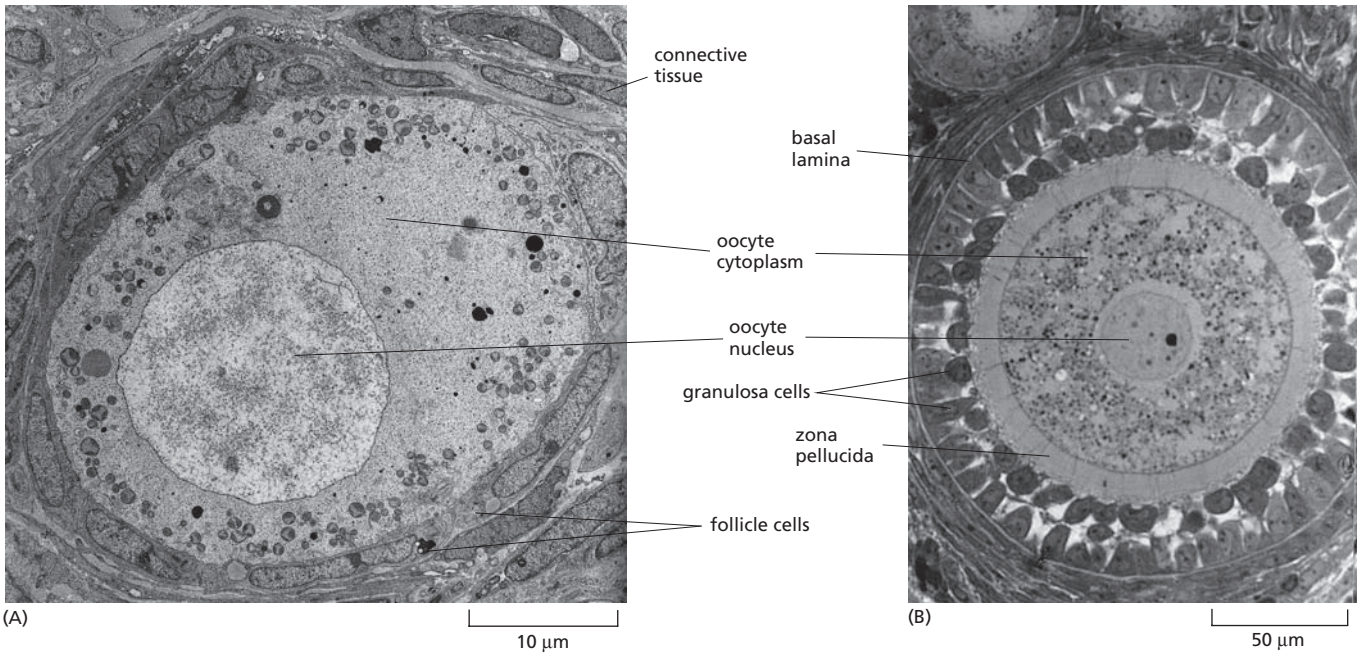


Figure 21–24 Nurse cells and follicle cells associated with a *Drosophila* oocyte. The nurse cells and the oocyte arise from a common oogonium, which gives rise to one oocyte and 15 nurse cells (only 7 of which are seen in this plane of section). These cells remain joined by cytoplasmic bridges, which result from incomplete cell division. Eventually, the nurse cells dump their cytoplasmic contents into the developing oocyte and then kill themselves. The follicle cells develop independently from mesodermal cells.



either contribute to the egg coat, are taken up by receptor-mediated endocytosis into the growing oocyte, or act on egg cell-surface receptors to control the spatial patterning and axial asymmetries of the egg (discussed in Chapter 22).

The communication between oocytes and their follicle cells occurs in both directions. The timing of developmental processes in the two sets of cells has to be coordinated, and it seems that this depends on signals from the oocyte to the follicle cells. Experiments in which young oocytes are combined with older follicle cells, or vice versa, show that an intrinsic developmental program in the oocyte normally controls the rate of follicle cell development.

Figure 21–25 Electron micrographs of developing primary oocytes in the rabbit ovary. (A) An early stage of primary oocyte development. Neither a zona pellucida nor cortical granules have developed, and a single layer of flattened follicle cells surrounds the oocyte. (B) A more mature primary oocyte, which is shown at a sixfold lower magnification because it is much larger than the oocyte in (A). This oocyte has acquired a thick zona pellucida and is surrounded by several layers of follicle cells (now called granulosa cells) and a basal lamina, which isolate the oocyte from the other cells in the ovary. The granulosa cells are connected to one another and to the oocyte by gap junctions. (From The Cellular Basis of Mammalian Reproduction [J. Van Blerkom and P. Motta eds.]. Baltimore–Munich: Urban & Schwarzenberg, 1979.)

Most Human Oocytes Die Without Maturing

Figure 21–26 outlines the stages in human oocyte development in the ovary. A single layer of follicle cells surrounds most of the primary oocytes in newborn girls. Such an oocyte, together with its surrounding follicle cells, is called a *primordial follicle* (see Figure 21–25A). Periodically, beginning sometime before birth, a small proportion of primordial follicles begin to grow to become *developing follicles*, in which multiple layers of follicle cells (now called *granulosa cells*)

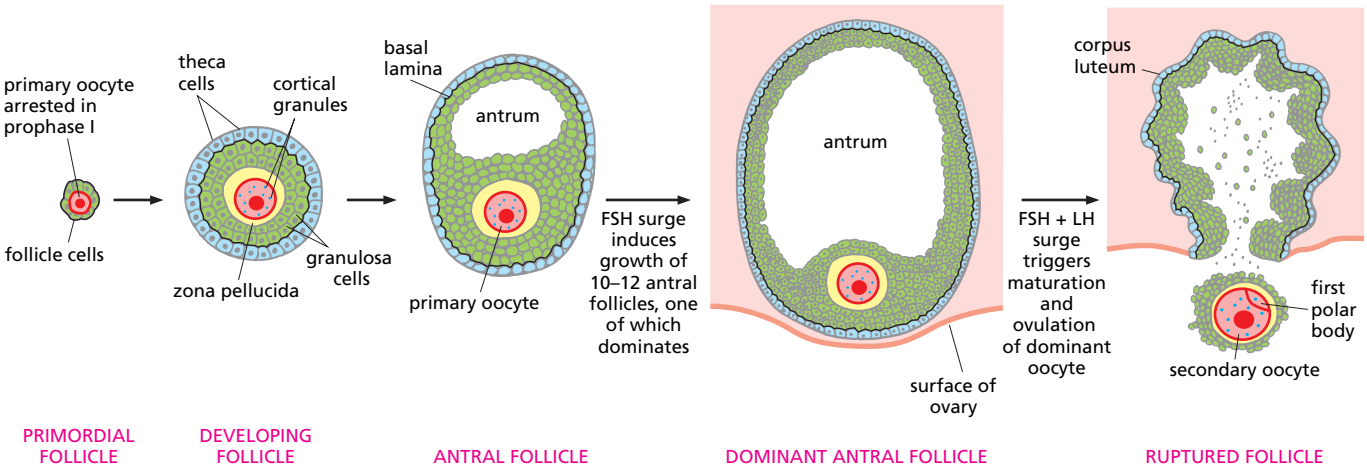


Figure 21–26 The stages in human oocyte development. Note that for most of its development the oocyte is surrounded by granulosa cells *(green),* which are separated from an outer layer of *theca cells (blue)* by an intervening basal lamina *(black).* After ovulation, the emptied follicle transforms into an endocrine structure, the *corpus luteum*, which secretes progesterone to help prepare the uterus for pregnancy. If fertilization does not occur, the corpus luteum regresses, and the lining of the uterus is sloughed off during menstruation.

surround the growing oocyte (see Figure 21–25B). It is not known what causes certain primordial follicles to begin growing. Some of these developing follicles go on to acquire a fluid-filled cavity, or *antrum,* to become *antral follicles.*

After puberty, about once each month, the pituitary secretes a surge of *follicle stimulating hormone (FSH)*, which accelerates the growth of about 10–12 antral follicles. One of these antral follicles becomes dominant, and, toward the middle of the menstral cycle, a surge in FSH and *luteinizing hormone (LH)* triggers *ovulation*: the dominant primary oocyte completes meiosis I, and the resulting secondary oocyte arrests at metaphase II; the follicle rapidly enlarges and ruptures at the surface of the ovary, releasing the secondary oocyte, still surrounded by a shell of granulosa cells embedded in a hyaluronan-rich gel-like matrix. The released oocyte is triggered to complete meiosis II only if a sperm fertilizes it within a day or so.

It remains a mystery why only a small proportion of the many antral follicles present in the ovaries at the time of the FSH surge each month are stimulated to accelerate their growth, and why only one of these matures and releases its oocyte, while the rest degenerate. Once the selected follicle has matured beyond a certain point, some feedback mechanism must operate to ensure that no other follicles complete maturation and ovulate during that cycle. Whatever the mechanism, the result is that, during the 40 or so years of a woman's reproductive life, only 400 to 500 oocytes will be released. All of the other million or so primary oocytes present at birth die without maturing. It is still a puzzle why so many oocytes are formed only to die in the ovaries.

Summary

Oocytes develop in stages from primordial germ cells (PGCs) that migrate into the developing gonad, where they become oogonia. After a period of mitotic proliferation, the oogonia begin meiosis I and are now called primary oocytes. Primary oocytes remain arrested after diplotene of prophase I for days to years, depending on the species. During this arrest period, they grow, synthesize a coat, and accumulate ribosomes, mRNAs, and proteins, often enlisting the help of other cells, including surrounding follicle cells. Bidirectional signaling between oocytes and their follicle cells is required for normal oocyte growth and development. In the process of hormonally induced oocyte maturation, primary oocytes complete meiosis I to form a small polar body and a large secondary oocyte, which proceeds to metaphase of meiosis II. In most vertebrates, the secondary oocyte arrests at metaphase II until stimulated by fertilization to complete meiosis and begin embryonic development.

SPERM

In most species, there are two radically different types of gametes. The egg is among the largest cells in an organism, whereas the **sperm** (**spermatozoon**, plural **spermatozoa**) is often the smallest. The egg and the sperm are optimized in opposite ways for the propagation of the genes they carry. The egg is nonmotile and aids the survival of the maternal genes by providing large stocks of raw materials for embryo growth and development, together with an effective protective wrapping. The sperm, by contrast, is optimized to propagate the paternal genes by exploiting this maternal investment: it is usually highly motile and streamlined for speed and efficiency in the task of fertilization. Competition between sperm is fierce, and the vast majority fail in their mission: of the billions of sperm released during a human male's reproductive life, only a few ever manage to fertilize an egg.

Sperm Are Highly Adapted for Delivering Their DNA to an Egg

Typical sperm are "stripped-down" cells, equipped with a strong flagellum to propel them through an aqueous medium but unencumbered by cytoplasmic

organelles such as ribosomes, endoplasmic reticulum, or Golgi apparatus, which are unnecessary for the task of delivering the DNA to the egg. Sperm, however, contain many mitochondria strategically placed where they can most efficiently power the flagellum. Sperm usually consist of two morphologically and functionally distinct regions enclosed by a single plasma membrane: the *tail,* which propels the sperm to the egg and helps it to burrow through the egg coat, and the *head,* which contains a highly condensed haploid nucleus (**Figure 21–27**). The DNA in the nucleus is extremely tightly packed, to minimize its volume for transport, and transcription is shut down. The chromosomes of many sperm lack the histones of somatic cells and are packaged instead with simple, highly positively charged proteins called *protamines*, as well as with sperm-specific histones.

In the head of most animal sperm, closely apposed to the anterior end of the nuclear envelope, is a specialized secretory vesicle called the **acrosomal vesicle**. This vesicle contains hydrolytic enzymes that are thought to help the sperm penetrate the egg's outer coat. When a sperm contacts the egg coat, the contents of the vesicle are released by exocytosis in the *acrosome reaction*. This reaction is required for the sperm to bind to the coat, burrow through it, and fuse with the egg.

The motile tail of a sperm is a long flagellum, whose central *axoneme* emanates from a basal body situated just behind the nucleus. As described in Chapter 16, the axoneme consists of two central singlet microtubules surrounded by nine evenly spaced microtubule doublets. The flagellum of some sperm (including those of mammals) differs from other flagella in that the 9 + 2 pattern of microtubules is surrounded by nine outer *dense fibers* (**Figure 21–28**). The dense fibers are stiff and noncontractile, and they are thought to restrict the flexibility of the flagellum and protect it from shear forces; defects in these fibers lead to abnormal sperm morphology and to infertility. The active bending of the flagellum is caused by the sliding of adjacent microtubule doublets past one another, driven by dynein motor proteins, which use the energy of ATP hydrolysis to slide the microtubules. The ATP is generated by a large number of highly specialized mitochondria that are concentrated in the anterior part of the sperm tail (called the *midpiece*), where the ATP is needed.

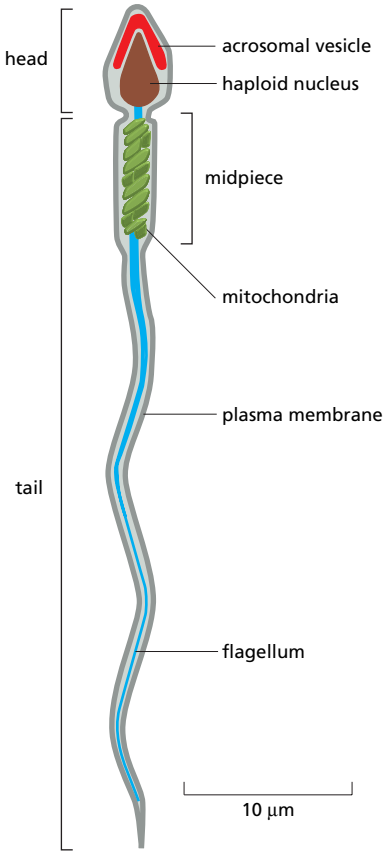


Figure 21–27 A human sperm. It is shown in longitudinal section.

Sperm Are Produced Continuously in the Mammalian Testis

In contrast to oocytes, which begin meiosis before birth and remain arrested after diplotene of prophase I until a human female reaches puberty, meiosis and sperm production (**spermatogenesis**) do not begin in the testes of human males until puberty. They then go on continuously in the epithelial lining of very long, tightly coiled tubes, called *seminiferous tubules*. Immature germ cells, called *spermatogonia* (singular, *spermatogonium*), are located around the outer edge of these tubes next to the basal lamina (**Figure 21–29**A). Most of these cells divide a limited number of times by mitosis before they stop proliferating and begin meiosis I, at which point they are now called *primary spermatocytes;* the primary spermatocytes give rise to *secondary spermatocytes,* which differentiate into *spermatids* and ultimately sperm (Figure 21–29B). A small proportion of the spermatogonia serve as stem cells, which divide slowly by mitosis throughout life, producing daughter cells that either remain stem cells or commit to maturation.

The stages of spermatogenesis and their relationship to meiosis are illustrated in **Figure 21–30**. During prophase I, the paired homologous chromosomes participate in crossing-over. The primary spermatocytes then complete

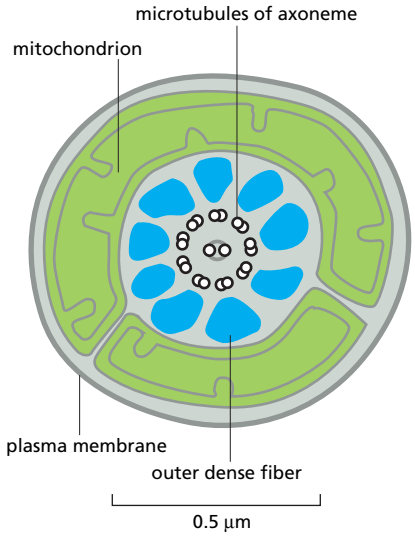
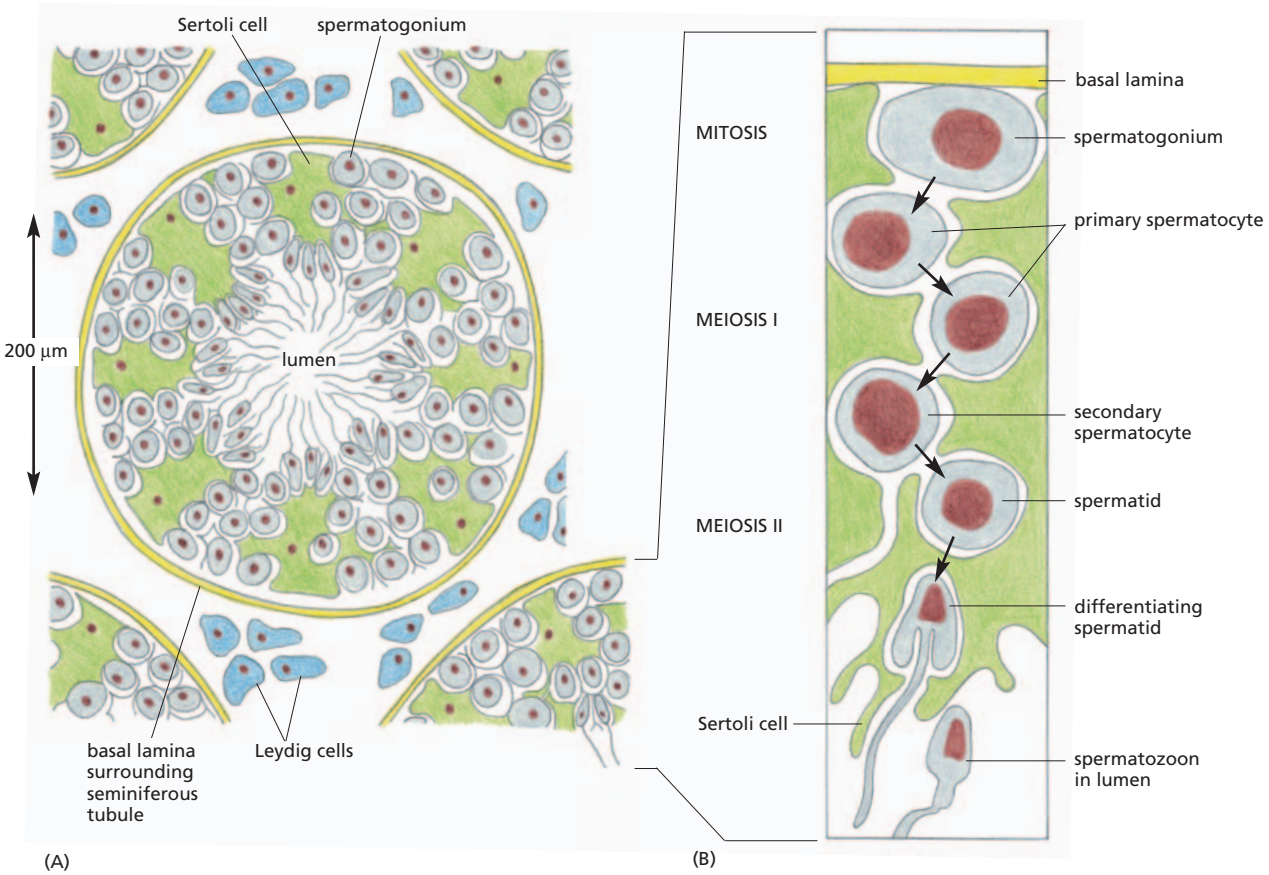


Figure 21–28 Drawing of the midpiece of a mammalian sperm as seen in cross section in an electron microscope. The core of the flagellum is composed of an axoneme surrounded by nine dense fibers. The axoneme consists of two singlet microtubules surrounded by nine microtubule doublets. The mitochondria (shown in *green*) are well placed for providing the ATP required for flagellar movement; they are distributed in an unusual spiral arrangement around the axoneme (see Figure 21–27).



meiosis I to produce two secondary spermatocytes, each containing 22 duplicated autosomal chromosomes and either a duplicated X or a duplicated Y chromosome. The two secondary spermatocytes derived from each primary spermatocyte proceed through meiosis II to produce four spermatids, each with a haploid number of single chromosomes. The haploid spermatids then undergo dramatic morphological changes as they differentiate into sperm, which escape into the lumen of the seminiferous tubule. The sperm subsequently pass into the *epididymis*, a coiled tube overlying the testis, where they are stored and undergo further maturation. The stored sperm are still not ready to fertilize an egg, however; as we discuss later, they undergo further maturation in the female genital tract—a process called *capacitation*.

Sperm Develop as a Syncytium

An intriguing feature of spermatogenesis is that once a spermatogonium begins to mature, its progeny no longer complete cytoplasmic division (cytokinesis) during mitosis and subsequent meiosis. Consequently, large clones of differentiating daughter cells that have descended from one maturing spermatogonium remain connected by cytoplasmic bridges, forming a syncytium (**Figure 21–31**). The cytoplasmic bridges persist until the very end of sperm differentiation, when individual sperm are released into the lumen of the seminiferous tubule. As a result, mature sperm are produced in synchronous batches in any given area of a seminiferous tubule. What is the function of this syncytial arrangement?

We saw earlier that oocytes grow and differentiate while containing the diploid set of chromosomes in duplicate. Sperm, by contrast, do not grow, and they undergo most of their differentiation after their nuclei have completed meiosis to become haploid. The presence of cytoplasmic bridges between them, however, means that each developing haploid sperm shares a common cytoplasm with its neighbors. In this way, it can be supplied with all the gene

Figure 21–29 Highly simplified drawings of a cross section of a seminiferous tubule in a mammalian testis. (A) All of the stages of spermatogenesis shown take place while the developing germline cells are in intimate association with Sertoli cells. Sertoli cells direct sexual differentiation along a male pathway. They are large cells, extending from the basal lamina to the lumen of the seminiferous tubule; they are required for the survival of the spermatogonia and are analogous to follicle cells in the ovary (see Figure 21–19). Spermatogenesis also depends on testosterone secreted by Leydig cells, located between the seminiferous tubules. (B) Spermatogonia divide by mitosis at the periphery of the seminiferous tubule. Some of these cells enter meiosis I to become primary spermatocytes; they then complete meiosis I to become secondary spermatocytes. The secondary spermatocytes then complete meiosis II to become spermatids, which differentiate into spermatozoa (sperm) and are released into the lumen of the tubule (see Figure 21–30). In man, it takes a spermatogonium about 24 days from the onset of meiosis to emergence as a spermatid and another 5 weeks for the spermatid to develop into a sperm.

products of a complete diploid genome. Developing sperm that carry a Y chromosome, for example, can be supplied with essential proteins encoded by genes on the X chromosome. Thus, the diploid genome directs sperm differentiation just as it directs egg differentiation.

Some of the genes that regulate spermatogenesis have been conserved in evolution from flies to humans. The *Daz* genes, for example, encode RNA-binding proteins and are located in a cluster on the human Y chromosome. The cluster is found to be deleted in a substantial proportion of infertile men, many of whom cannot make sperm. A *Drosophila* gene that is homologous to the human

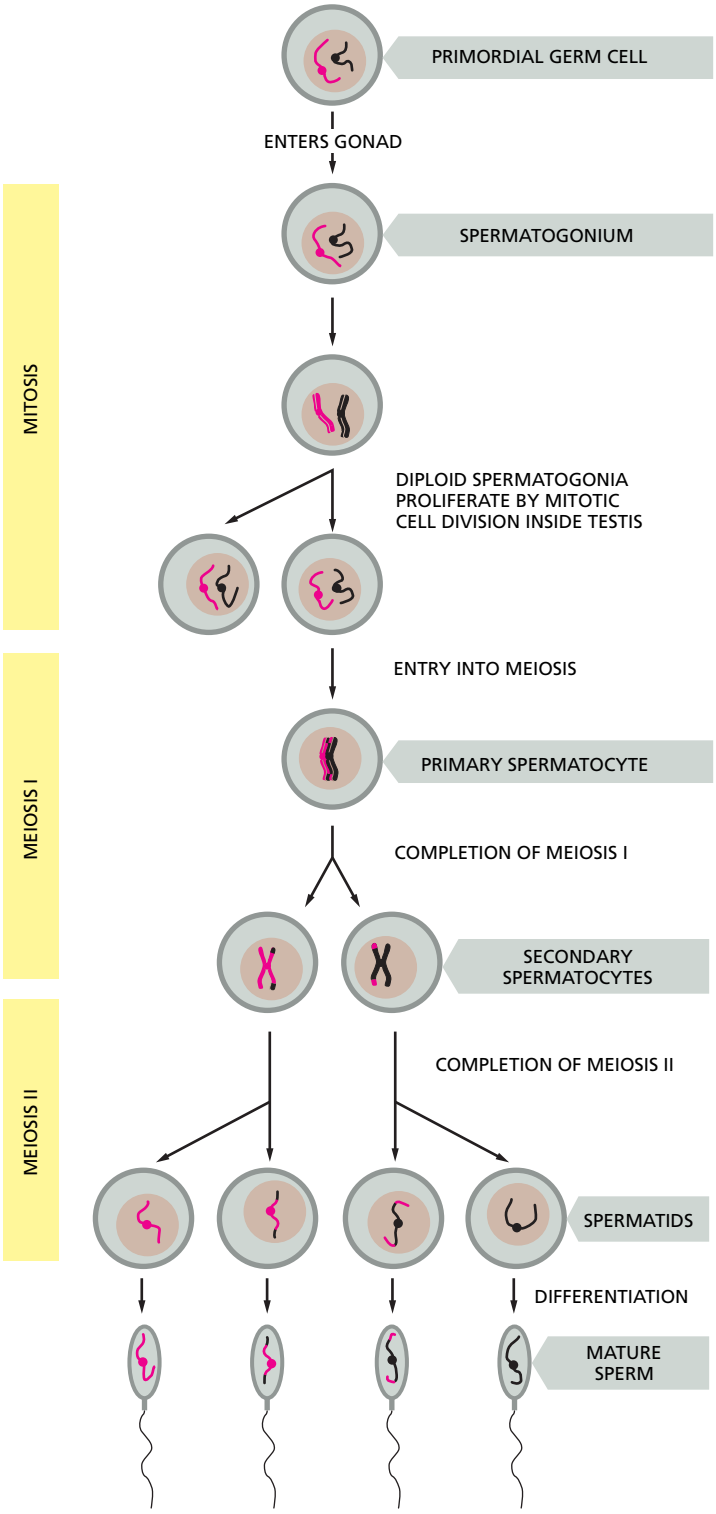


Figure 21–30 The stages of spermatogenesis. Spermatogonia develop from primordial germ cells (PGCs) that migrate into the developing gonad early in embryogenesis. When the animal becomes sexually mature, the spermatogonia begin to proliferate rapidly by mitosis. Some retain the capacity to divide indefinitely (as stem-cell spermatogonia). Others (maturing spermatogonia) undergo a limited number of mitotic division cycles before beginning meiosis to become spermatocytes, which eventually become haploid spermatids and then sperm. Spermatogenesis differs from oogenesis (see Figure 21–23) in several ways. (1) New cells enter meiosis continually from the time of puberty. (2) Each cell that begins meiosis gives rise to four mature gametes rather than one. (3) Mature sperm form by an elaborate process of cell differentiation that begins after meiosis is complete. (4) About twice as many cell divisions occur in the production of a sperm as in the production of an egg; in a mouse, for example, it is estimated that on average about 56 divisions occur from zygote to mature sperm, and about 27 divisions occur from zygote to mature egg.

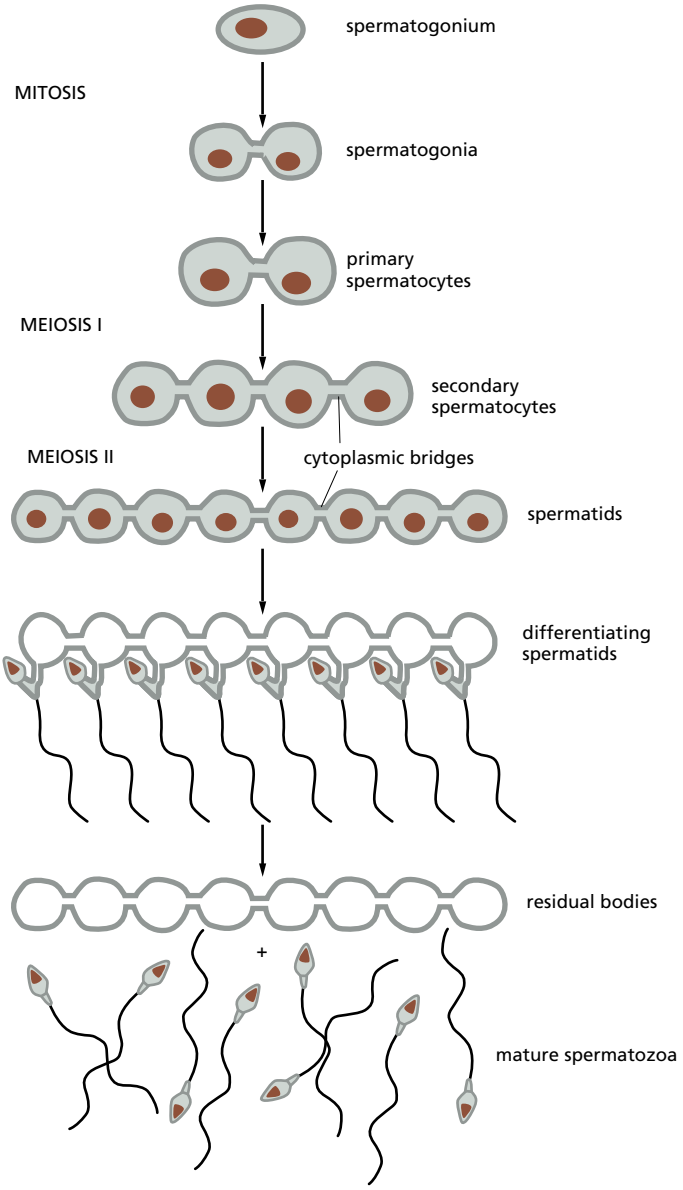


Figure 21–31 Cytoplasmic bridges in developing sperm cells and their precursors. The progeny of a single maturing spermatogonium remain connected to one another by cytoplasmic bridges throughout their differentiation into mature sperm. For the sake of simplicity, only two connected maturing spermatogonia are shown entering meiosis, eventually to form eight connected haploid spermatids. In fact, the number of connected cells that go through meiosis and differentiate synchronously is very much larger than shown here. Note that in the process of differentiating, most of the spermatid cytoplasm is discarded as *residual bodies*, which are phagocytosed by Sertoli cells.

Daz genes is similarly essential for spermatogenesis in the fly: Daz-deficient male flies are infertile because they cannot make sperm, but, remarkably, they can be cured by a human *Daz* transgene. RNA-binding proteins are especially important in spermatogenesis, because many of the genes expressed in the sperm lineage are regulated at the level of RNA translation.

Summary

A sperm is usually a small, compact cell, highly specialized for the task of fertilizing an egg. Whereas in women a large pool of oocytes is produced before birth, in men spermatogonia only begin to enter meiosis to produce spermatocytes (and sperm) after sexual maturation, and they continue to do so from then on. Each diploid primary spermatocyte gives rise to four mature haploid sperm. The process of sperm differentiation occurs after meiosis is complete, requiring five weeks in humans. Because the maturing spermatogonia and spermatocytes fail to complete cytokinesis, however, the progeny of a single maturing spermatogonium develop as a large syncytium. Thus, the products encoded by both parental chromosomes direct sperm differentiation, even though each sperm nucleus is haploid.

FERTILIZATION

Once released, egg and sperm alike are destined to die within minutes or hours unless they find each other and fuse in the process of **fertilization**. Through fertilization, the egg and sperm are saved: the egg is activated to begin its developmental program, and the haploid nuclei of the two gametes come together to form the diploid genome of a new organism. Fertilization was originally studied most intensively in marine invertebrates such as sea urchins and starfish, where fertilization occurs in seawater after the release of huge numbers of both sperm and eggs. Such external fertilization is easier to study than the internal fertilization of mammals, which normally occurs in the female reproductive tract after mating. In the late 1950s, however, it became possible to fertilize mammalian eggs *in vitro*, opening the way to an analysis of the cellular and molecular events in mammalian fertilization.

In this section, we focus on mammalian fertilization. We begin by considering the maturation of sperm that occurs during their passage through the female genital tract. We then discuss the binding of sperm to the egg coat (zona pellucida), which induces the *acrosome reaction*, required for the sperm to burrow through the zona and fuse with the egg. We next consider the binding of the sperm to the egg plasma membrane and its subsequent fusion with this membrane. After discussing how the fusion of a sperm activates the egg and how the haploid nuclei of the two gametes come together in the zygote to complete fertilization, we briefly consider the burgeoning field of assisted reproductive technology, which has revolutionized the treatment of human infertility and opened up new ways of manipulating the reproductive process.

Ejaculated Sperm Become Capacitated in the Female Genital Tract

Of the 300,000,000 or so human sperm ejaculated during coitus, only about 200 reach the site of fertilization in the *oviduct*. Once a sperm finds an egg, it must first migrate through the layers of granulosa cells that surround the egg and then bind to and cross the zona pellucida. Finally, it must bind to and fuse with the egg plasma membrane.

Ejaculated mammalian sperm are initially not competent to accomplish any of these tasks. They must first be modified by conditions in the female reproductive tract. Because it is required for sperm to acquire the capacity to fertilize an egg, the process is called **capacitation**. Capacitation takes about 5–6 hours in humans and is completed only when the sperm arrive in the oviduct. The sperm undergo extensive biochemical and functional changes, including changes in glycoproteins, lipids, and ion channels in the sperm plasma membrane and a large change in the resting potential of this membrane (the membrane potential moves to a more negative value so that the membrane becomes hyperpolarized). Capacitation is also associated with an increase in cytosolic pH, tyrosine phosphorylation of various sperm proteins, and the unmasking of cell-surface receptors that help bind the sperm to the zona pellucida. Capacitation alters two crucial aspects of sperm behavior: it greatly increases the motility of the flagellum, and it makes the sperm capable of undergoing the acrosome reaction.

Capacitation can occur *in vitro* in the appropriate culture medium and is usually a required part of *in vitro* fertilization. Three critical components are needed in the medium, all of which are normally in high concentration in the female genital tract—albumin, Ca^{2+}, and HCO_3^-. The albumin protein helps extract cholesterol from the plasma membrane, increasing the ability of this membrane to fuse with the acrosome membrane during the acrosome reaction. The Ca^{2+} and HCO_3^- enter the sperm and directly activate a soluble adenylyl cyclase enzyme in the cytosol to produce cyclic AMP (discussed in Chapter 15), which helps to initiate many of the changes associated with capacitation.

Capacitated Sperm Bind to the Zona Pellucida and Undergo an Acrosome Reaction

During ovulation, mammalian eggs are released from the ovary into the peritoneal cavity next to the entrance to the oviduct, into which they are rapidly swept. They are covered with several layers of granulosa cells embedded in an extracellular matrix that is rich in hyaluronic acid (discussed in Chapter 19). The granulosa cells may help the egg get picked up into the oviduct, and they may also secrete unidentified chemical signals that attract sperm to the egg.

On encountering an egg, a capacitated sperm first must penetrate the layers of granulosa cells, making use of a hyaluronidase enzyme on the surface of the sperm. It can then bind to the **zona pellucida** (see Figure 21–22). The zona usually acts as a barrier to fertilization across species, and removing it often eliminates this barrier. Human sperm, for example, will fertilize hamster eggs from which the zona has been removed with specific enzymes; not surprisingly, such hybrid zygotes fail to develop. Zona-free hamster eggs are sometimes used in infertility clinics to assess the fertilizing capacity of human sperm *in vitro* (**Figure 21–32**).

The zona pellucida of most mammalian eggs is composed mainly of three glycoproteins, all of which are produced exclusively by the growing oocyte. Two of them, ZP2 and ZP3, assemble into long filaments, while the other, ZP1, cross-links the filaments into a three-dimensional network. The ZP3 protein is crucial: female mice with a disrupted *Zp3* gene produce eggs that lack a zona and are infertile. *O*-linked oligosaccharides on ZP3 seem to be at least partly responsible for the species-specific binding of sperm to the zona. The binding of sperm to the zona is complex, however, and involves both ZP3-dependent and ZP3-independent mechanisms and a variety of proteins on the sperm surface.

The zona induces sperm to undergo the **acrosome reaction**, in which the contents of the acrosome are released by exocytosis (**Figure 21–33**). The acrosome reaction is required for normal fertilization, as it exposes various hydrolytic enzymes that are believed to help the sperm tunnel through the zona pellucida, and it alters the sperm surface so that it can bind to and fuse with the plasma membrane of the egg, as we discuss below. *In vitro*, purified ZP3 can trigger the acrosome reaction, possibly by activating a lectin-like receptor on the sperm surface, thought to be a transmembrane form of the enzyme galactosyltransferase. Receptor activation leads to an increase in Ca^{2+} in the sperm cytosol, which initiates the exocytosis.

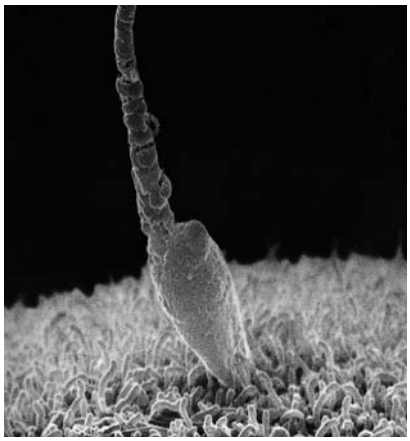


Figure 21–32 Scanning electron micrograph of a human sperm contacting a hamster egg. The zona pellucida of the egg has been removed, exposing the plasma membrane, which contains numerous microvilli. The ability of an individual's sperm to penetrate hamster eggs is used as an assay of male fertility; penetration of more than 10–25% of the eggs is considered to be normal. (Courtesy of David M. Phillips.)

The Mechanism of Sperm–Egg Fusion Is Still Unknown

After a sperm has undergone the acrosome reaction and penetrated the zona pellucida, it binds to the egg plasma membrane overlying the tips of microvilli on the egg surface (see Figure 21–32). The sperm binds initially by its tip and then by its side (see Figure 21–33). Neighboring microvilli on the egg surface rapidly elongate and cluster around the sperm to ensure that it is held firmly so that it can fuse with the egg. After fusion, all of the sperm contents are drawn into the egg, as the microvilli are resorbed.

The molecular mechanisms responsible for sperm–egg binding and fusion are largely unknown, although, after a number of false starts, two membrane proteins have been shown to be required for the fusion. One is a sperm-specific transmembrane protein of the immunoglobulin superfamily called *Izumo* (after a Japanese shrine dedicated to marriage). It becomes exposed on the surface of mouse and human sperm during the acrosome reaction. Anti-Izumo antibodies block the fusion, and Izumo-deficient mouse sperm fail to fuse with normal eggs, but it is still unknown how Izumo promotes sperm–egg fusion. The only protein on the egg surface demonstrated to be required for fusion with a sperm is the *CD9* protein, which is a member of the *tetraspanin* family, so-called because these proteins have four membrane-spanning segments. Normal sperm fail to fuse with CD9-deficient mouse eggs, indicating that sperm–egg fusion depends on CD9, but it is not known how. CD9 does not act alone on the egg surface to promote fusion: normal sperm also fail to fuse with eggs treated

with an enzyme that removes proteins attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor (discussed in Chapter 10), indicating that one or more GPI-linked proteins is also required for fusion, although the relevant protein or proteins have yet to be identified.

Sperm Fusion Activates the Egg by Increasing Ca^{2+} in the Cytosol

Fusion with a sperm activates the egg, causing the cortical granules to release their contents by exocytosis, a process called the *cortical reaction*. Meiosis, which was arrested in metaphase II, resumes, producing a second polar body, and a zygote, which begins to develop.

An increase in Ca^{2+} in the cytosol of the fertilized egg triggers all of these events. <AGGA> If the Ca^{2+} concentration in the cytosol of an unfertilized egg is increased artificially—either directly by an injection of Ca^{2+} or indirectly by the use of a Ca^{2+}-carrying ionophore (discussed in Chapter 11)—the eggs of all animals so far tested, including mammals, are activated. Conversely, preventing the increase in Ca^{2+} by injecting the Ca^{2+} chelator EGTA inhibits activation of the egg in response to fertilization.

When the sperm fuses with the egg plasma membrane in the normal way, it causes a local increase in cytosolic Ca^{2+}, which spreads through the cell in a wave (see Figure 15–40). The wave propagates by positive feedback: the increase in cytosolic Ca^{2+} causes Ca^{2+} channels to open, allowing still more Ca^{2+} to enter the cytosol. The initial wave of Ca^{2+} release is usually followed within a few minutes by Ca^{2+} oscillations (discussed in Chapter 15), which persist for several hours.

The fused sperm triggers the Ca^{2+} wave and oscillations by bringing a factor into the egg cytosol. An injection of an intact sperm, a sperm head, or a sperm extract into an egg does the same thing. All of these treatments increase the concentration of inositol 1,4,5-trisphosphate (IP_3), which releases Ca^{2+} from the endoplasmic reticulum and initiates the Ca^{2+} wave and oscillations (discussed in Chapter 15). A strong candidate for the critical factor that mammalian sperm introduce into the egg is a sperm-specific form of phospholipase C (PLCζ), which directly cleaves phosphoinositol 4,5-bisphosphate ($PI(4,5)P_2$) to produce IP_3 (and diacyglycerol) (see Figure 15–39).

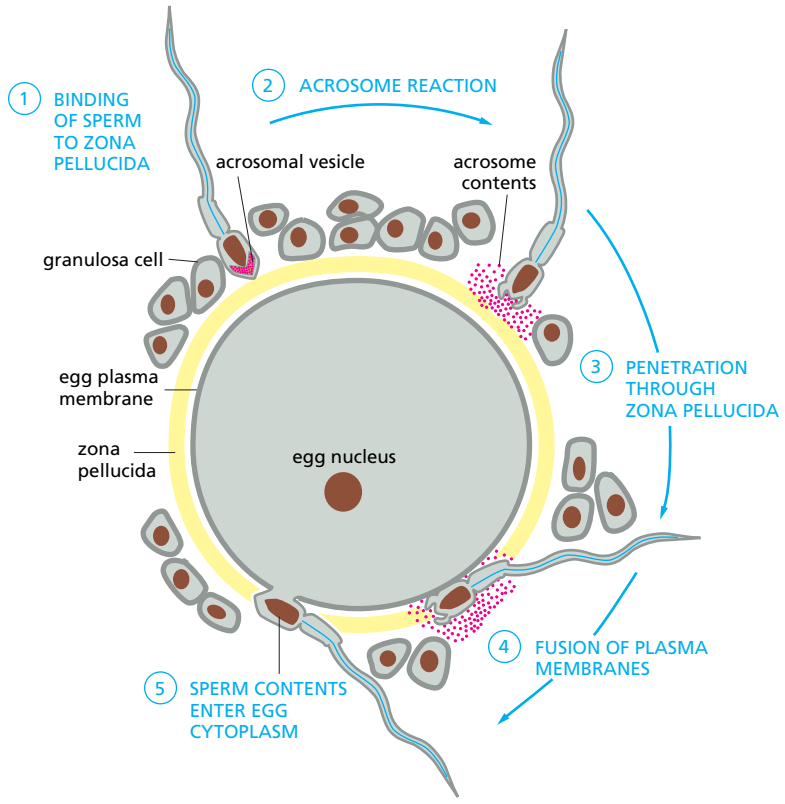


Figure 21–33 The acrosome reaction that occurs when a mammalian sperm fertilizes an egg. In mice, the zona pellucida is about 6 µm thick, and sperm tunnel through it at a rate of about 1 µm/min.

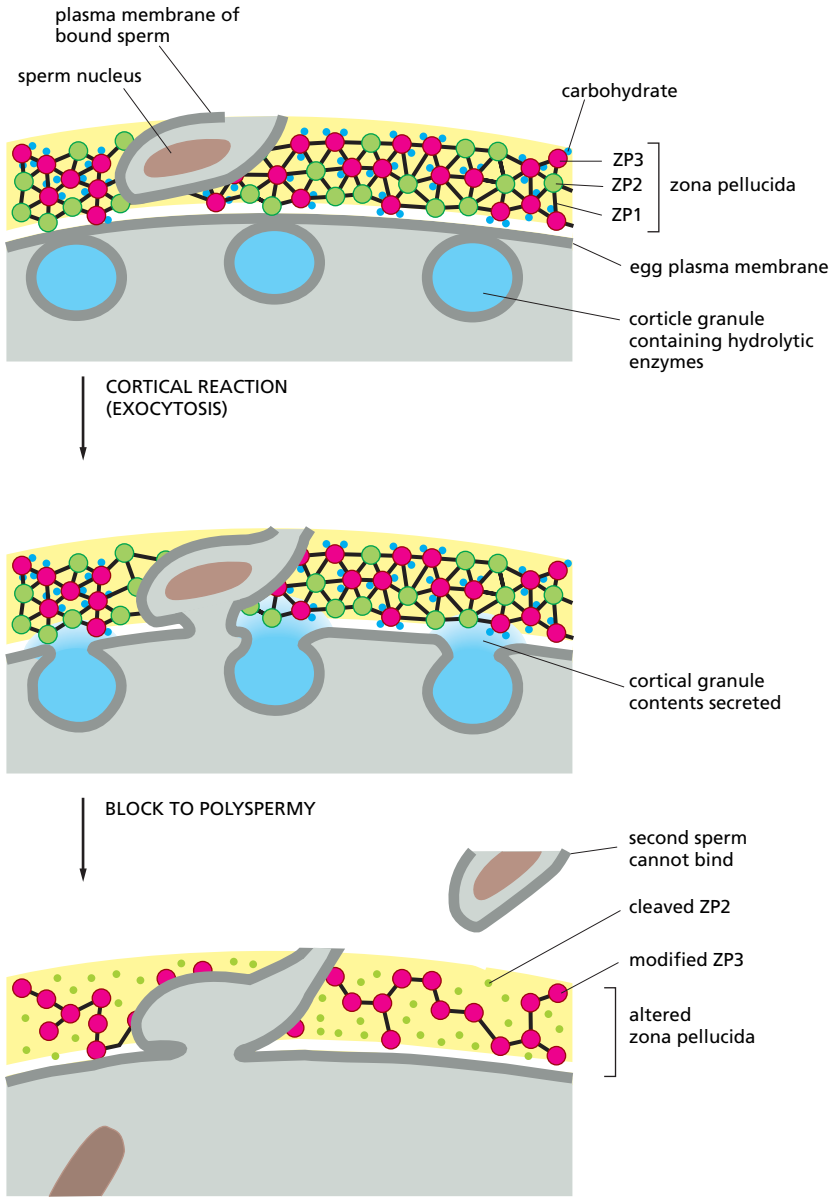


Figure 21–34 How the cortical reaction in a mouse egg is thought to prevent additional sperm from entering the egg. <TGAC> The released contents of the cortical granules inactivate ZP3 so it can no longer bind to the sperm plasma membrane. They also partly cleave ZP2, hardening the zona pellucida so that sperm cannot penetrate it. Together, these changes provide a block to polyspermy.

The Cortical Reaction Helps Ensure That Only One Sperm Fertilizes the Egg

Although many sperm can bind to an egg, normally only one fuses with the egg plasma membrane and injects its cytosol, nucleus, and other organelles into the egg cytoplasm. If more than one sperm fuses—a condition called *polyspermy*—extra or multipolar mitotic spindles are formed, resulting in faulty segregation of chromosomes during the first mitotic cell divisions; aneuploid cells are produced, and development usually stops.

Two mechanisms operate to ensure that only one sperm fertilizes the egg. First, a change in the egg plasma membrane caused by the fusion of the first sperm prevents other sperm from fusing. In sea urchin eggs, the change is a rapid depolarization of the egg membrane; in mammalian eggs, the mechanism is not known. The second block to polyspermy is provided by the egg **cortical reaction**, which releases various enzymes that change the structure of the zona pellucida so that sperm cannot bind to or penetrate it. Among the changes that occur in the mammalian zona is the inactivation of ZP3 so that it can no longer bind sperm or induce an acrosome reaction; in addition, ZP2 is cleaved, which somehow helps to make the zona impenetrable (**Figure 21–34**).

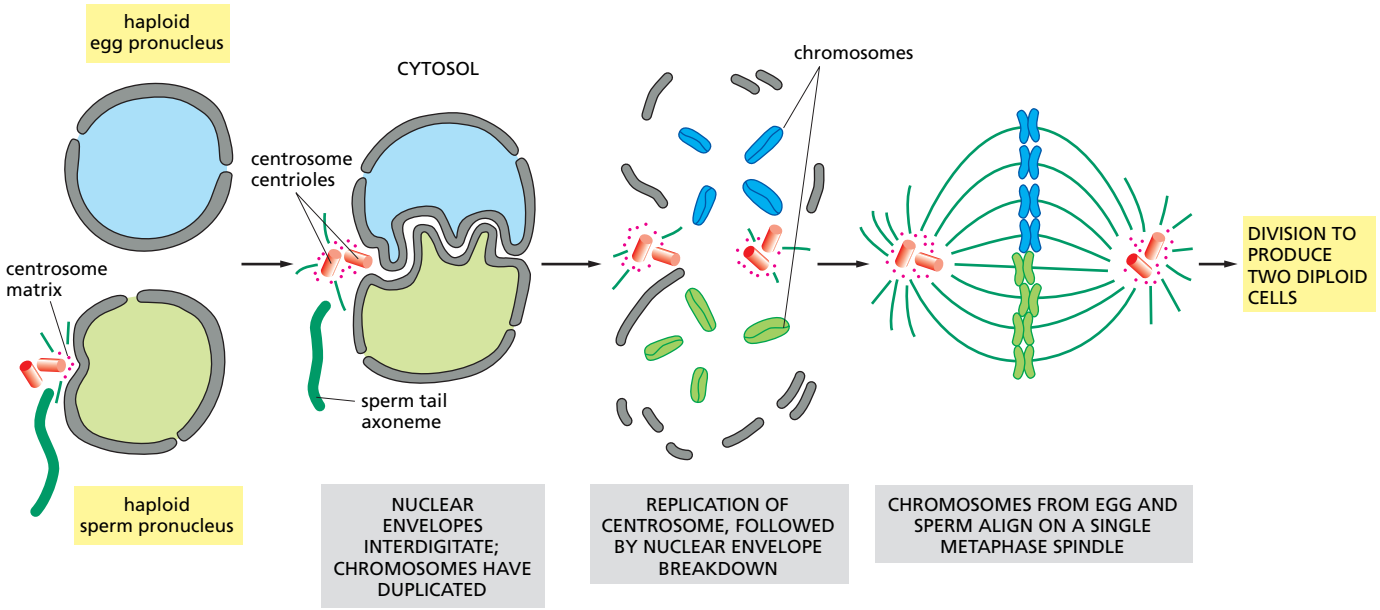


Figure 21–35 The coming together of the sperm and egg pronuclei after mammalian fertilization. The pronuclei migrate toward the center of the egg. When they come together, their nuclear envelopes interdigitate. The centrosome duplicates, the nuclear envelopes break down, and the chromosomes of both gametes are eventually integrated into a single mitotic spindle, which organizes the first cleavage division of the zygote. (Adapted from drawings and electron micrographs provided by Daniel Szöllösi.)

The Sperm Provides Centrioles as Well as Its Genome to the Zygote

Once fertilized, the egg is called a **zygote**. Fertilization is not complete, however, until the two haploid nuclei (called *pronuclei*)–one from the egg, the other from the sperm—have come together and combined their chromosomes into a single diploid nucleus. In fertilized mammalian eggs, the two pronuclei do not fuse directly as they do in many other species. They approach each other but remain distinct until after the membrane of each pronucleus has broken down in preparation for the zygote's first mitotic division (**Figure 21–35**).

In most animals, including humans, the sperm contributes more than its genome to the zygote. It also donates its centrioles—structures that are lacking in unfertilized human eggs. The sperm centrioles enter the egg along with the sperm nucleus and tail, and a centrosome forms around them. In humans, the centrosome duplicates, and the two resulting centrosomes then help organize the assembly of the first mitotic spindle in the zygote (**Figure 21–36**, and see Figure 21–35). This explains why polyspermy, in which several sperm contribute their centrioles to the egg, causes extra or multipolar mitotic spindles to form.

IVF and ICSI Have Revolutionized the Treatment of Human Infertility

About 10% of human couples have reduced fertility, such that the female partner fails to become pregnant after 12–18 months of unprotected sex. In roughly half of these cases it is the male that is the problem, and in half it is the female. Although there are numerous reasons for reduced fertility in both males and females, in the great majority of cases some form of assisted reproductive technology can solve the problem.

The first major breakthrough in the treatment of infertility occurred in 1978, with the birth of Louise Brown, the first child produced by ***in vitro* fertilization** (**IVF**). Before this success, there were heated debates about the ethics and safety of IVF—remarkably similar to the current ethical debates about the production and use of human embryonic stem (ES) cells. IVF is now a routine procedure and has produced more than a million children. To begin the process, the woman is usually pretreated with hormones to stimulate the simultaneous maturation of multiple oocytes. Just before their release by ovulation, the eggs are harvested from the ovary (using a long needle inserted through the vagina) and are fertilized in a culture dish with sperm from the man. After a few days in culture, 2 or

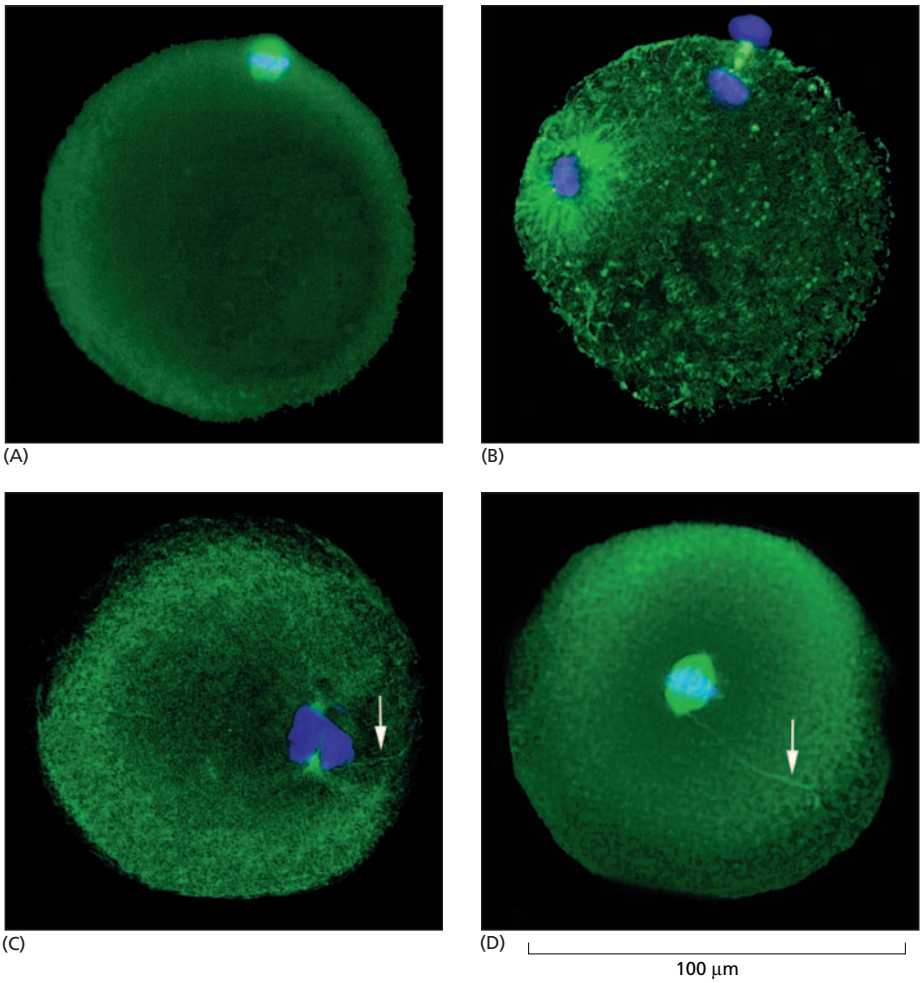


Figure 21–36 Immunofluorescence micrographs of human sperm and egg pronuclei coming together after *in vitro* fertilization. Spindle microtubules are stained in *green* with anti-tubulin antibodies, and DNA is labeled in *blue* with a DNA stain. (A) A meiotic spindle in a mature unfertilized secondary oocyte. (B) A fertilized egg extruding its second polar body about 5 hours after fusion with a sperm. The sperm head *(left)* has nucleated an array of microtubules. The egg and sperm pronuclei are still far apart. (C) The two pronuclei have come together. (D) By 16 hours after fusion with a sperm, the centrosome that entered the egg with the sperm has duplicated, and the daughter centrosomes have organized a bipolar mitotic spindle. The chromosomes of both pronuclei are aligned at the metaphase plate of the spindle. As indicated by the arrows in (C) and (D), the sperm tail is still associated with one of the centrosomes. (From C. Simerly et al., *Nat. Med.* 1:47–53, 1995. With permission from Macmillan Publishers Ltd.)

3 of the best-looking early embryos are transferred with a catheter into the woman's uterus; the remaining embryos are usually kept frozen in liquid nitrogen, for further implantations, if necessary. The main complication of IVF is multiple pregnancies, which occur in over 30% of cases, compared with about 2% in unassisted pregnancies.

The IVF procedure just described has enabled many previously infertile women to produce normal children. It does not, however, solve the problem for infertile men who usually produce too few or abnormal sperm. A second breakthrough, which occurred in 1992, provided the solution for most such men. In this modification of IVF, called **intracytoplasmic sperm injection** (**ICSI**), an egg is fertilized by injecting a single sperm into it (**Figure 21–37**). This strategy eliminates the need for large numbers of motile sperm and bypasses many of the hurdles that a sperm normally has to clear to fertilize an egg, including capacitation, swimming to the egg, undergoing an acrosomal reaction, burrowing through the zona pellucida, and fusing with the egg plasma membrane. ICSI has a success rate of better than 50% and has produced more than 100,000 children.

In addition to revolutionizing the treatment of infertility, IVF has opened up many new possibilities for manipulating the reproductive process. It has, for example, made it possible for parents carrying a defective gene to avoid passing the gene on to their children, by screening IVF embryos for the gene before implanting them into the uterus.

As discussed earlier, *in vitro* techniques for handling mammalian eggs have made it possible to produce clones of many types of mammals by transferring the nucleus of a somatic cell from the animal to be cloned into an unfertilized egg that has had its own nucleus removed or destroyed. It is not an easy procedure; the success rate is low, and it is still uncertain whether a human could be cloned in the same way. Moreover, there are serious ethical arguments about

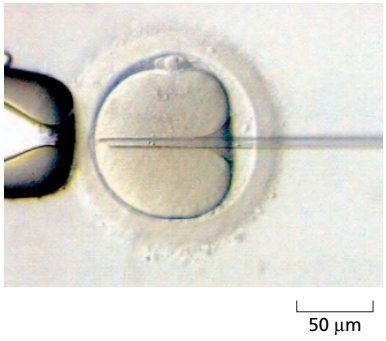


Figure 21–37 Intracytoplasmic sperm injection (ICSI). Light micrograph of a human secondary oocyte being held with a suction pipette (on the left) and injected with a single human sperm through a glass needle. The zona pellucida surrounds the egg and the polar body. (Courtesy of Reproductive Biology Associates, Atlanta, Georgia.)

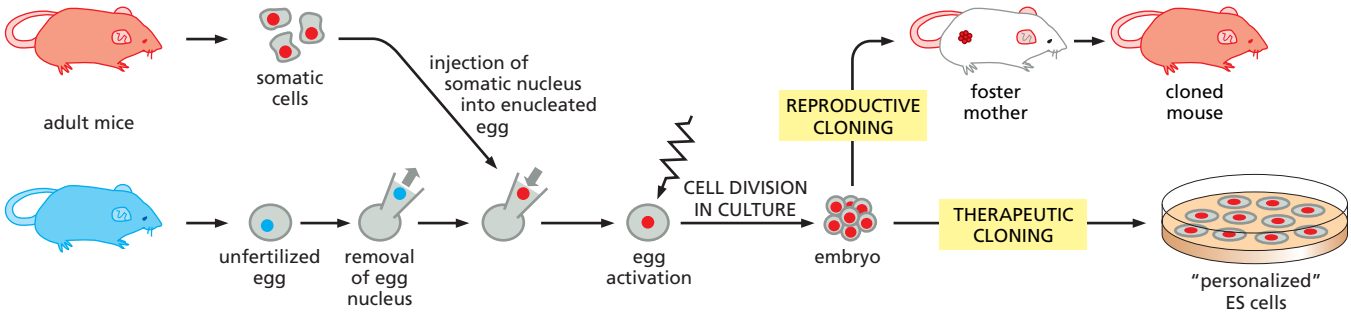


Figure 21–38 The difference between reproductive cloning and the preparation of "personalized" embryonic stem cells. In both cases, one produces a reconstructed embryo by removing (or destroying) the nucleus from an unfertilized egg and replacing it with the nucleus of a somatic cell from the animal to be cloned. The reconstructed egg is activated to develop by an electric shock. In *reproductive cloning*, the embryo that develops in culture is transplanted into the uterus of a foster mother and develops into a cloned animal. In the preparation of personalized embryonic stem (ES) cells (sometimes called *therapeutic cloning*), by contrast, the embryo is used to produce ES cells in culture, which can then be used to produce various specialized cell types for the treatment of the individual that provided the somatic nucleus; because the specialized cells produced by these ES cells are genetically the same as the donor of the somatic nucleus, they will not be immunologically rejected.

whether one should ever attempt to clone a human. There is general agreement, however, that it should not be attempted with existing technology, as the likelihood of producing an abnormal child is high; indeed, many countries and American states have made the attempt illegal.

Such *reproductive cloning*, however, should not be confused with *therapeutic cloning*, in which the early embryo produced *in vitro* from a such a reconstituted zygote is not implanted into a uterus to produce a new individual, but is instead used to make ES cells that are genetically the same as the donor of the somatic nucleus (**Figure 21–38**). Various types of specialized cells produced from such "personalized" ES cells could then be used to treat the donor, avoiding the problem of immunological rejection associated with using cells derived from genetically dissimilar ES cells. Clearly, societies will have to make some difficult decisions about how far they are willing to go in exploiting these new technologies to manipulate the reproductive process for the potential benefit of individuals. Alternatively, it might be possible in the future to produce personalized ES-like cells in ways that bypass these ethical dilemmas: in recent experiments, for example, genetic engineering was used to express in cultured mouse fibroblasts a number of gene regulatory proteins normally expressed in ES cells; when four such transgenes were expressed simultaneously, the fibroblasts behaved much like ES cells.

Fertilization marks the beginning of one of the most remarkable phenomena in all of biology—the process of embryogenesis, in which the zygote develops into a new individual. This is the subject of the next chapter.

Summary

Mammalian fertilization normally begins when a sperm, which has undergone capacitation in the female genital tract, binds to the zona pellucida surrounding an egg in the oviduct. This induces the sperm to undergo an acrosome reaction, releasing the contents of the acrosomal vesicle, which are thought to help the sperm to digest its way through the zona. The acrosome reaction is also required for the sperm to bind to and fuse with the egg plasma membrane. The fusion of the sperm with the egg induces a Ca^{2+} wave and oscillations in the egg cytosol, which activate the egg. The activation includes the egg cortical reaction, in which cortical granules release their contents, which alter the zona pellucida so that other sperm cannot bind to or penetrate it. The Ca^{2+} signal also triggers the development of the zygote, which begins after the two haploid pronuclei have come together and their chromosomes have aligned on a single mitotic spindle, which mediates the first mitotic division of the zygote. Many previously infertile couples can now reproduce thanks to IVF and ICSI.

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