The events of meiosis appear to be coordinated through cytoplasmic connections between the dividing cells. Whereas the daughter cells formed by mitosis routinely separate from each other, the products of the meiotic cell divisions remain coupled to each other by **cytoplasmic bridges**. These bridges are seen during the formation of sperm and eggs throughout the animal kingdom (Pepling and Spradling 1998).

WEBSITE 19.3 **Proteins involved in meiosis.** The phenomenon of homologous pairing and crossing-over is being analyzed in several organisms and may involve DNA repair enzymes.

WEBSITE 19.4 **Human meiosis.** Nondisjunction, the failure of chromosomes to sort properly during meiosis, is not uncommon in humans. Its frequency increases with maternal age.

Sidelights & Speculations

Big Decisions: Mitosis or Meiosis? Sperm or Egg?

In many species, the germ cells migrating into the gonad are bipotential and can differentiate into either sperm or eggs, depending on their gonadal environment. When the ovaries of salamanders are experimentally transformed into testes, the resident germ cells cease their oogenic differentiation and begin developing as sperm (Burns 1930; Humphrey 1931). Similarly, in the housefly and mouse, the gonad is able to direct the differentiation of the germ cells (McLaren 1983; Inoue and Hiroyoshi 1986). Thus, in most organisms, the sex of the gonad and of its germ cells is the same.

But what about hermaphroditic animals, in which the change from sperm production to egg production is a naturally occurring physiological event? How is the same animal capable of producing sperm during one part of its life and oocytes during another part? Using *Caenorhabditis elegans*, Kimble and her colleagues identified two "decisions" that presumptive germ cells have to make. The first is whether to enter meiosis or to remain a mitotically dividing stem cell. The second is whether to become an egg or a sperm.

Recent evidence shows that these decisions are intimately linked. The mitotic/meiotic decision is controlled by a single nondividing cell at the end of each gonad, the **distal tip cell**. The germ cell precursors near this cell divide mitotically, forming the pool of germ cells; but as these cells get farther away from the distal tip cell, they enter meiosis. If the distal tip cell is destroyed by a focused laser beam, all the germ cells enter meiosis, and if the distal tip cell is placed in a different location in the gonad, germ line stem cells are generated near its new position (Figure 19.17; Kimble 1981; Kimble and White 1981). The distal tip cell extends long filaments that touch the distal germ cells. The extensions contain in their cell membranes the Lag-2 protein, a *C. elegans* homologue of Delta (Henderson et al. 1994; Tax et al. 1994; Hall et al. 1999). The Lag-2 protein maintains the germ cells in mitosis and inhibits their meiotic differentiation.

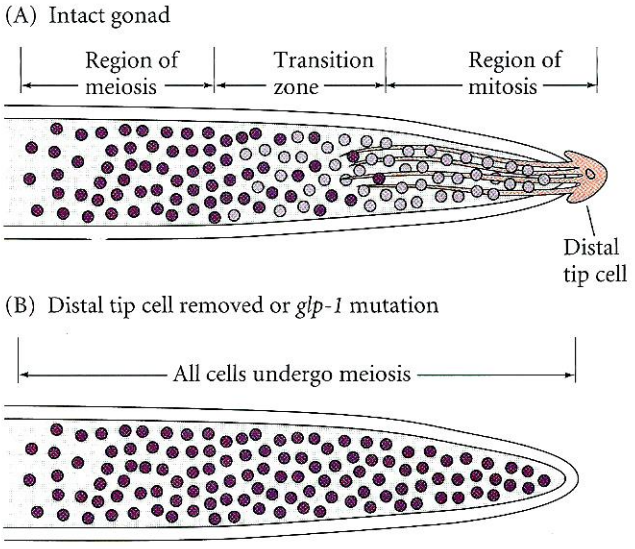

Figure 19.17
Regulation of the mitosis-or-meiosis decision by the distal tip cell of the *C. elegans* ovotestis. (A) Intact gonad early in development with regions of mitosis (light-colored cells) and meiosis. The membrane of the distal tip cell's extensions contains the *C. elegans* homologue of the Delta protein, while the PGCs contain the *C. elegans* homologue of Notch. (B) Gonad after laser ablation of the distal tip cell or mutation of the *glp-1* gene. All germ cells enter meiosis.

Austin and Kimble (1987) isolated a mutation that mimics the phenotype obtained when the distal tip cell is removed. It is not surprising that this mutation involves the gene encoding GLP-1, the *C. elegans* homologue of Notch—the receptor for Delta. All the germ cell precursors of nematodes homozygous for the recessive mutation of *glp-1* initiate meiosis, leaving no mitotic population. Instead of the 1500 germ cells usually found in the fourth larval stage of hermaphroditic development, these mutants produce only 5 to 8 sperm cells. When genetic chimeras are made in which wild-type germ cell precursors are found within a mutant larva, the wild-type cells are able to respond to the distal tip cells and undergo mitosis. However, when mutant germ cell precursors are found within wild-type larvae, they all enter meiosis. Thus, the *glp-1* gene appears to be responsible for enabling the germ cells to respond to the distal tip cell's signal.*

*The *glp-1* gene appears to be involved in a number of inductive interactions in *C. elegans*. You will no doubt recall that GLP-1 protein is also needed by the AB blastomere for it to receive inductive signals from the EMS blastomere to form pharyngeal muscles (see Chapter 8).

After the germ cells begin their meiotic divisions, they still must become either sperm or ova. Generally, in each hermaphrodite gonad (called an ovotestis), the most proximal germ cells produce sperm, while the most distal (near the tip) become eggs (Hirsh et al. 1976). This means that the germ cells entering meiosis early become sperm, while those entering meiosis later become eggs. The genetics of this switch are currently being analyzed. The laboratories of Hodgkin (1985) and Kimble (Kimble et al. 1986) have isolated several genes needed for germ cell pathway selection, but the switch appears to involve the activity or inactivity of *fem-3* mRNA. Figure 19.18 presents a scheme for how these genes might function. During early development, the *fem* genes, especially *fem-3*, are critical for the specification of sperm cells. Loss-of-function mutations of these genes convert XX nematodes into females (i.e., spermless hermaphrodites). As long as the FEM proteins are made in the germ cells, sperm are produced. FEM protein is thought to activate the *fog* genes (whose loss-of-function mutations cause the feminization of the germ line and eliminate spermatogenesis). The *fog* gene products activate the genes involved in transforming the germ cell into sperm and also inhibit those genes that would otherwise direct the germ cells to initiate oogenesis.

Oogenesis can begin only when *fem* activity is suppressed. This suppression appears to act at the level of RNA translation. The 3′ untranslated region (3′ UTR) of the *fem-3* mRNA contains a sequence that binds a repressor protein during normal development. If this region is mutated such that the repressor cannot bind, the *fem-3* mRNA remains translatable, and oogenesis never occurs. The result is a hermaphrodite body that produces only sperm (Ahringer and Kimble 1991; Ahringer et al. 1992). The *trans*-acting repressor of the *fem-3* message is a combination of the Nanos and Pumilio proteins (the same combination that represses *hunchback* message translation in *Drosophila*). The up-regulation of Pumilio expression may be critical in regulating the germ line switch from spermatogenesis to oogenesis, since Nanos is made constitutively. Nanos appears to be necessary in *C. elegans* (as it is in *Drosophila*) for the survival of all germ line cells (Kraemer et al. 1999).

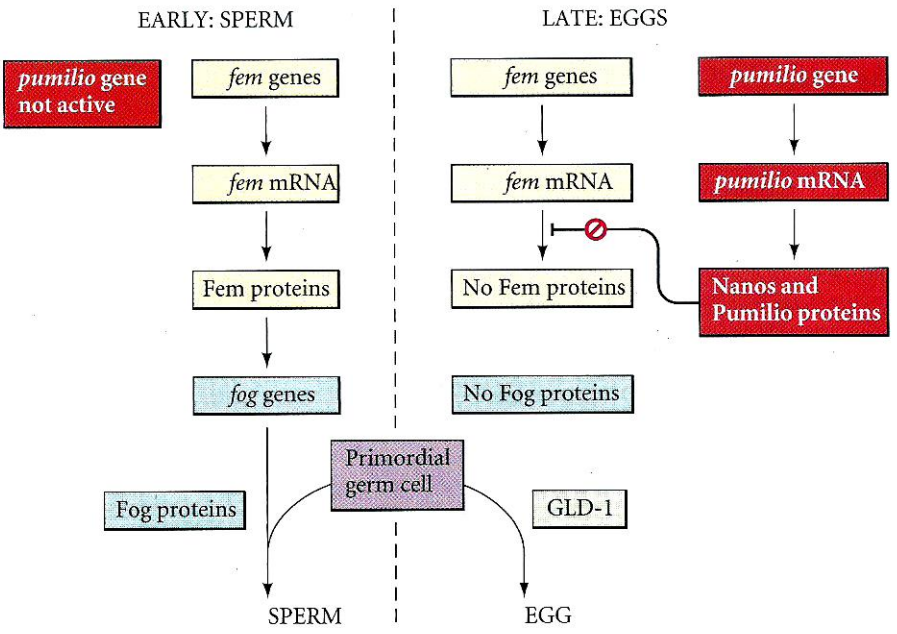

Figure 19.18
Model of sex determination switch in the germ line of *C. elegans* hermaphrodites. Sex determination in somatic tissues shows a hierarchy of negative regulation. In the early larva, Pumilio is not synthesized, and the *fem* mRNA is able to be translated. The FEM proteins activate the *fog* genes, whose proteins cause the germ cells to undergo spermatogenesis. Later in development, *pumilio* is activated, and combines with Nanos to make a repressor of *fem* translation. Without FEM, the GLD-1 protein can function to make certain the germ cell undergoes oogenesis.

WEBSITE 19.5 **Germ line sex determination in *C. elegans*.** The establishment of whether a germ cell is to become a sperm or an egg involves multiple levels of inhibition. Translational regulation is seen in several of these steps.

Spermatogenesis

Forming the haploid spermatid

While the reductive divisions of meiosis are conserved in every eukaryotic kingdom of life, the regulation of meiosis in mammals differs dramatically between males and females. The differences between **oogenesis**, the production of eggs, and **spermatogenesis**, the production of sperm, are outlined in Table 19.1.

Spermatogenesis is the production of sperm from the primordial germ cells. Once the vertebrate PGCs arrive at the genital ridge of a male embryo, they become incorporated into the sex cords. They remain there until maturity, at which time the sex cords hollow out to form the seminiferous tubules, and the epithelium of the tubules differentiates into the Sertoli cells. The initiation of spermatogenesis during puberty is probably regulated by the synthesis of BMP8b by the spermatogenic germ cells, the **spermatogonia**. When BMP8b reaches a critical concentration, the germ cells begin to differentiate. The differentiating cells produce high levels of BMP8b, which can then further stimulate their differentiation. Mice lacking BMP8b do not initiate spermatogenesis at puberty (Zhao et al. 1996).

The spermatogenic germ cells are bound to the Sertoli cells by N-cadherin molecules on both cell surfaces and by galactosyltransferase molecules on the spermatogenic cells

TABLE 19.1 Sexual dimorphism in mammalian meioses

Female oogenesis	Male spermatogenesis
Meiosis initiated once in a finite population of cells	Meiosis initiated continuously in a mitotically dividing stem cell population
One gamete produced per meiosis	Four gametes produced per meiosis
Completion of meiosis delayed for months or years	Meiosis completed in days or weeks
Meiosis arrested at first meiotic prophase and reinitiated in a smaller population of cells	Meiosis and differentiation proceed continuously without cell cycle arrest
Differentiation of gamete occurs while diploid, in first meiotic prophase	Differentiation of gamete occurs while haploid, after meiosis ends
All chromosomes exhibit equivalent transcription and recombination during meiotic prophase	Sex chromosomes excluded from recombination and transcription during first meiotic prophase

Source: Handel and Eppig 1998.

that bind a carbohydrate receptor on the Sertoli cells (Newton et al. 1993; Pratt et al. 1993). The Sertoli cells nourish and protect the developing sperm cells, and spermatogenesis—the developmental pathway from germ cell to mature sperm—occurs in the recesses between the Sertoli cells (Figure 19.19). The processes by which the PGCs generate sperm have been studied in detail in several organisms, but we will focus here on spermatogenesis in mammals.

After reaching the gonad, the PGCs divide to form **type A_1 spermatogonia**. These cells are smaller than the PGCs and are characterized by an ovoid nucleus that contains chromatin associated with the nuclear membrane. Type A_1 spermatogonia are found adjacent to the outer basement membrane of the sex cords. They are stem cells, and at maturity, they are thought to divide to make another type A_1 spermatogonium as well as a second, paler type of cell, the type A_2 spermatogonium. Thus, each type A_1 spermatogonium is a stem cell capable of regenerating itself as well as producing a new cell type. The A_2 spermatogonia divide to produce the A_3 spermatogonia, which then beget the type A_4 spermatogonia. It is possible that each of the type A spermatogonia are stem cells, capable of self-renewal.

The A_4 spermatogonium has three options: it can form another A_4 spermatogonium (self-renewal); it can undergo cell death (apoptosis); or it can differentiate into the first committed stem cell type, the **intermediate spermatogonium**. Intermediate spermatogonia are committed to becoming spermatozoa, and they divide mitotically once to form **type B spermatogonia**. These cells are the precursors of the spermatocytes and are the last cells of the line that undergo mitosis. They divide once to generate the **primary spermatocytes**—the cells that enter meiosis.

The transition between spermatogonia and spermatocytes appears to be mediated by glial cell line-derived neurotrophic

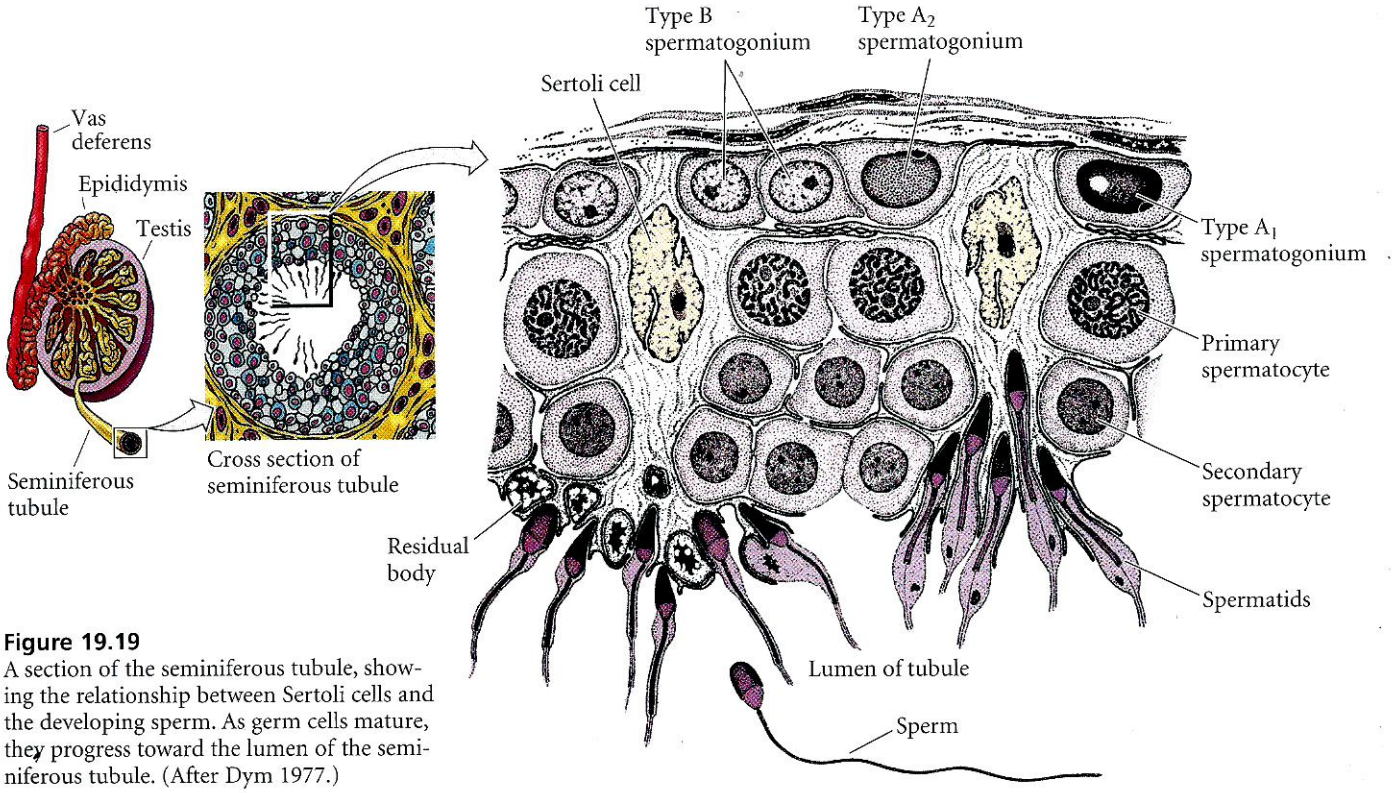

Figure 19.19
A section of the seminiferous tubule, showing the relationship between Sertoli cells and the developing sperm. As germ cells mature, they progress toward the lumen of the seminiferous tubule. (After Dym 1977.)

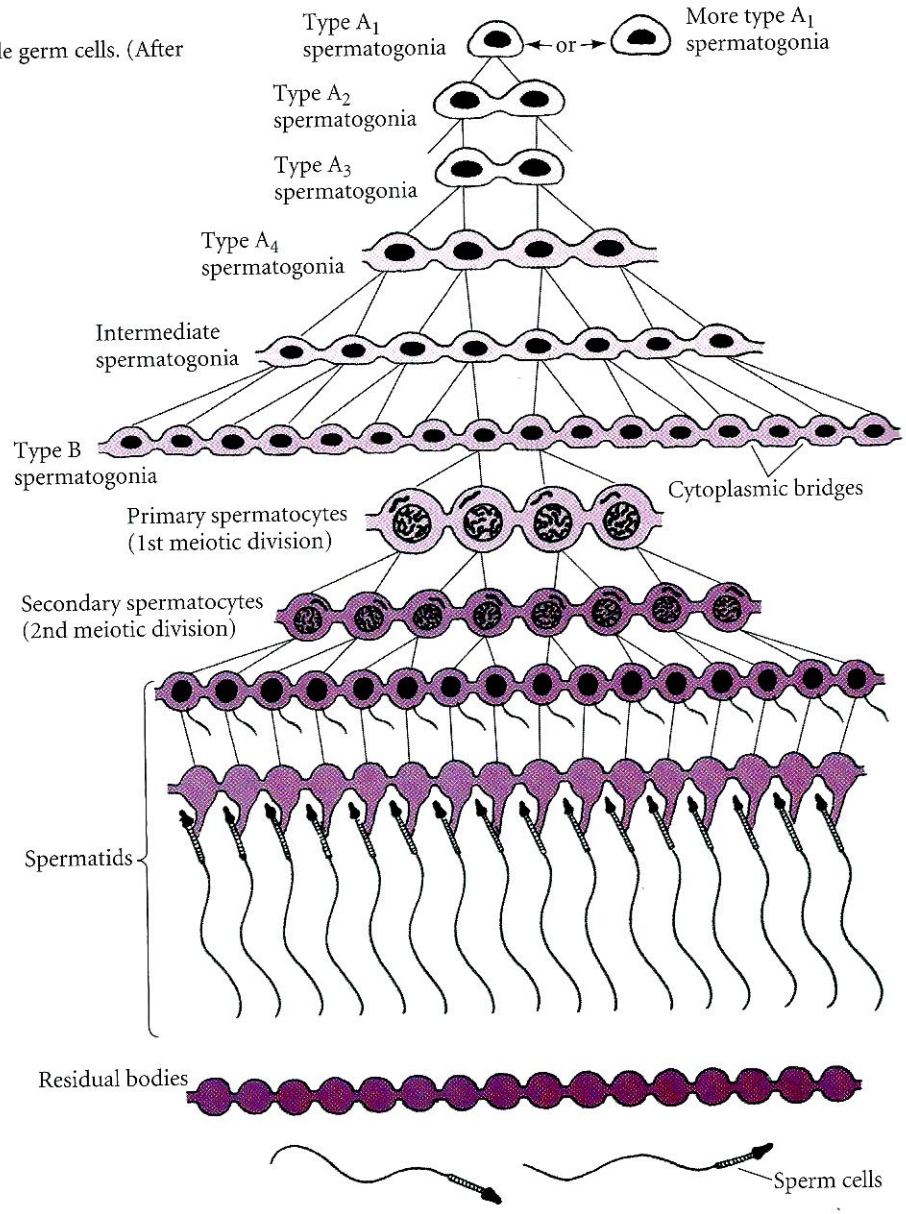

Figure 19.20
The formation of syncytial clones of human male germ cells. (After Bloom and Fawcett 1975.)

factor (GDNF), which is secreted by the Sertoli cells. GDNF levels determine whether the dividing spermatogonia remain spermatogonia or enter the pathway to become spematocytes. Low levels of GDNF favor the differentiation of the spermatogonia, while high levels favor self-renewal of the stem cells (Meng et al. 2000). Since GDNF is upregulated by follicle-stimulating hormone (FSH), GDNF may serve as a link between the Sertoli cells and the endocrine system, and it provides a mechanism for FSH to instruct the testes to produce more sperm (Tadokoro et al. 2002).

Looking at Figure 19.20, we find that during the spermatogonial divisions, cytokinesis is not complete. Rather, the cells form a syncytium whereby each cell communicates with the others via cytoplasmic bridges about 1 μm in diameter (Dym and Fawcett 1971). The successive divisions produce clones of interconnected cells, and because ions and molecules readily pass through these cytoplasmic bridges, each cohort matures synchronously. During this time, the spermatocyte nucleus often transcribes genes whose products will be used later to form the axoneme and acrosome.

Each primary spermatocyte undergoes the first meiotic division to yield a pair of **secondary spermatocytes**, which complete the second division of meiosis. The haploid cells thus formed are called **spermatids**, and they are still connected to one another through their cytoplasmic bridges. The spermatids that are connected in this manner have haploid nuclei, but are functionally diploid, since a gene product made in one cell can readily diffuse into the cytoplasm of its neighbors (Braun et al. 1989).

During the divisions from type A_1 spermatogonium to spermatid, the cells move farther and farther away from the basement membrane of the seminiferous tubule and closer to its lumen (see Figure 19.19). Thus, each type of cell can be found in a particular layer of the tubule. The spermatids are located at the border of the lumen, and here they lose their cytoplasmic connections and differentiate into spermatozoa. In humans, the progression from spermatogonial stem cell to mature spermatozoa takes 65 days (Dym 1994).

WEBSITE 19.6 **Gonial syncytia: Bridges to the future.** The products of meiotic divisions are connected by cytoplasmic bridges. The functions of these connections may differ between those cells producing sperm and those producing eggs.

Spermiogenesis: The differentiation of the sperm

The mammalian haploid spermatid is a round, unflagellated cell that looks nothing like the mature vertebrate sperm. The next step in sperm maturation, then, is **spermiogenesis** (or **spermateliosis**), the differentiation of the sperm cell. For fertilization to occur, the sperm has to meet and bind with an egg, and spermiogenesis prepares the sperm for these functions of motility and interaction. The process of mammalian sperm differentiation was shown in Figure 7.2. The first step is

the construction of the acrosomal vesicle from the Golgi apparatus. The acrosome forms a cap that covers the sperm nucleus. As the acrosomal cap is formed, the nucleus rotates so that the cap will be facing the basement membrane of the seminiferous tubule. This rotation is necessary because the flagellum, which is beginning to form from the centriole on the other side of the nucleus, will extend into the lumen. During the last stage of spermiogenesis, the nucleus flattens and condenses, the remaining cytoplasm (the residual body or "cytoplasmic droplet") is jettisoned, and the mitochondria form a ring around the base of the flagellum.

One of the major changes in the nucleus is the replacement of the histones by protamines. Transcription of the genes for protamines is seen in the early haploid spermatids, although translation is delayed for several days (Peschon et al. 1987). Protamines are relatively small proteins that are over 60% arginine. During spermiogenesis, the nucleosomes dissociate, and the histones of the haploid nucleus are eventually replaced by protamines. This causes the complete shutdown of transcription in the nucleus and facilitates its assuming an almost crystalline structure. The resulting sperm then enter the lumen of the tubule.

In the mouse, development from stem cell to spermatozoon takes 34.5 days. The spermatogonial stages last 8 days, meiosis lasts 13 days, and spermiogenesis takes up another 13.5 days. In humans, sperm development takes nearly twice as long. Because type A_1 spermatogonia are stem cells, spermatogenesis can occur continuously. Each day, some 100 million sperm are made in each human testicle, and each ejaculation releases 200 million sperm. Unused sperm are either resorbed or passed out of the body in urine. During his lifetime, a human male can produce 10^{12} to 10^{13} sperm (Reijo et al. 1995).

WEBSITE 19.7 **Gene expression during spermatogenesis.** Transcription occurs both from the diploid spermatocyte nucleus and from the haploid spermatid nucleus. Posttranscriptional control is also important in regulating sperm gene expression.

WEBSITE 19.8 **The Nebenkern.** Sperm mitochondria are often highly modified to fit the streamlined cell. The mitochondria of flies fuse together to form a structure called the Nebenkern; this fusion is controlled by the *fuzzy onions* gene.

Oogenesis

Oogenic meiosis

Oogenesis—the differentiation of the ovum—differs from spermatogenesis in several ways. Whereas the gamete formed by spermatogenesis is essentially a motile nucleus, the gamete formed by oogenesis contains all the materials needed to initiate and maintain metabolism and development. Therefore, in addition to forming a haploid nucleus, oogenesis also builds up a store of cytoplasmic enzymes, mRNAs, organelles, and metabolic substrates. While the sperm becomes differentiated for motility, the egg develops a remarkably complex cytoplasm.

The mechanisms of oogenesis vary among species more than those of spermatogenesis. This variation should not be surprising, since patterns of reproduction vary so greatly among species. In some species, such as sea urchins and frogs, the female routinely produces hundreds or thousands of eggs at a time, whereas in other species, such as humans and most mammals, only a few eggs are produced during the lifetime of an individual. In those species that produce thousands of ova, the germ cells, called **oogonia**, are self-renewing stem cells that endure for the lifetime of the organism. In those species that produce fewer eggs, the oogonia divide to form a limited number of egg precursor cells. In the human embryo, the thousand or so oogonia divide rapidly from the second to the seventh month of gestation to form roughly 7 million germ cells (Figure 19.21). After the seventh month of embryonic development, however, the number of germ cells drops precipitously. Most oogonia die during this period, while the remaining oogonia enter the first meiotic division (Pinkerton et al. 1961). These latter cells, called **primary oocytes**, progress through the first meiotic prophase until the diplotene stage, at which point they are maintained until the female matures. With the onset of purberty, groups of oocytes periodically resume meiosis. Thus, in the human female, the first part of meiosis begins in the embryo, and the signal to resume meiosis is not given until roughly 12 years later. In fact, some oocytes are maintained in meiotic

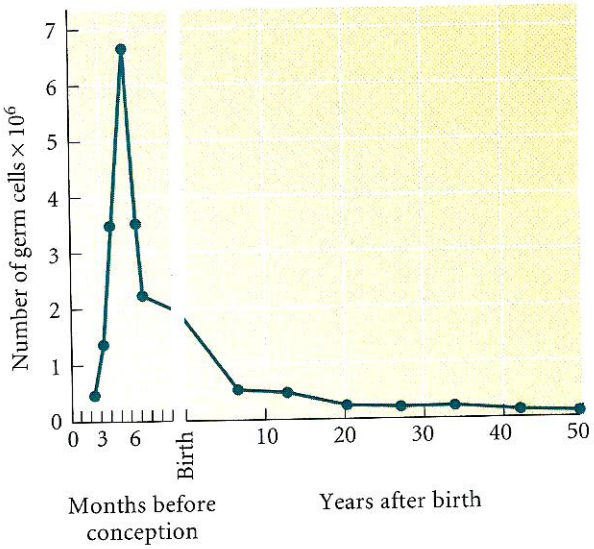

Figure 19.21
Changes in the number of germ cells in the human ovary over the life span. (After Baker 1970.)

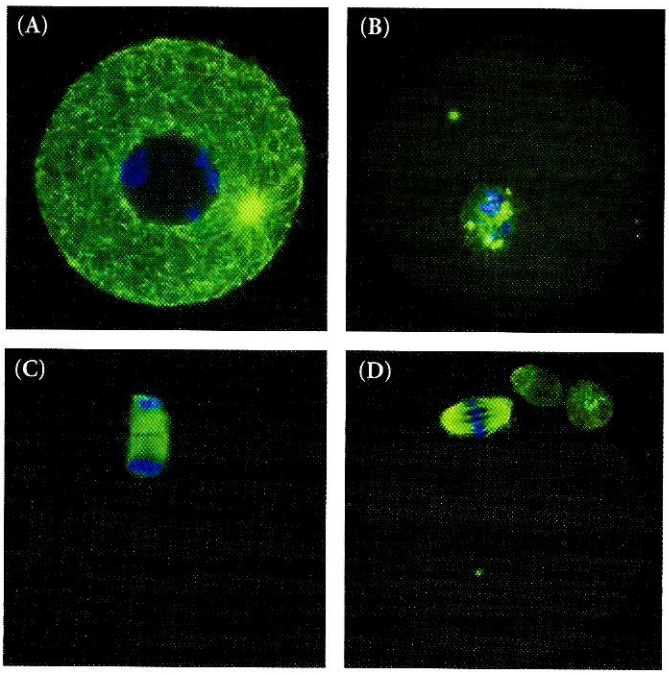

Figure 19.22
Meiosis in the mouse oocyte. The tubulin of the microtubules is stained green; the DNA is stained blue. (A) Mouse oocyte in meiotic prophase. The large haploid nucleus (the germinal vesicle) is still intact. (B) The nuclear envelope of the germinal vesicle breaks down as metaphase begins. (C) Meiotic anaphase I, wherein the spindle migrates to the periphery of the egg and releases a small polar body. (D) Meiotic metaphase II wherein the second polar body is given off (the first polar body has also divided). (From De Vos 2002; photographs courtesy of L. De Vos.)

prophase for nearly 50 years. As Figure 19.21 indicates, primary oocytes continue to die even after birth. Of the millions of primary oocytes present at birth, only about 400 mature during a woman's lifetime.

Oogenic meiosis also differs from spermatogenic meiosis in its placement of the metaphase plate. When the primary oocyte divides, its nucleus, called the **germinal vesicle**, breaks down, and the metaphase spindle migrates to the periphery of the cell. At telophase, one of the two daughter cells contains hardly any cytoplasm, whereas the other cell retains nearly the entire volume of cellular constituents (Figure 19.22). The smaller cell is called the **first polar body**, and the larger cell is referred to as the **secondary oocyte**. During the second division of meiosis, a similar unequal cytokinesis takes place. Most of the cytoplasm is retained by the mature egg (ovum), and a second polar body receives little more than a haploid nucleus. Thus, oogenic meiosis conserves the volume of oocyte cytoplasm in a single cell rather than splitting it equally among four progeny.

In a few species of animals, meiosis is greatly modified such that the resulting gamete is diploid and need not be fertilized to develop. Such animals are said to be **parthenogenetic** (Greek, "virgin birth"). In the fly *Drosophila mangabeirai*, one of the polar bodies acts as a sperm and "fertilizes" the oocyte after the second meiotic division. In other insects (such as *Moraba virgo*) and in the lizard *Cnemidophorus uniparens*, the oogonia double their chromosome number before meiosis, so that the halving of the chromosomes restores the diploid number. The germ cells of the grasshopper *Pycnoscelus surinamensis* dispense with meiosis altogether, forming diploid ova by two mitotic divisions (Swanson et al. 1981). All of these species consist entirely of females. In other species, haploid parthenogenesis is widely used not only as a means of reproduction, but also as a mechanism of sex determination. In the Hymenoptera (bees, wasps, and ants), unfertilized eggs develop into males, whereas fertilized eggs, being diploid, develop into females. The haploid males are able to produce sperm by abandoning the first meiotic division, thereby forming two sperm cells through second meiosis.

Maturation of the oocyte in amphibians

The egg is responsible for initiating and directing development, and in some species (as we saw above), fertilization is not even necessary. The accumulated material in the oocyte cytoplasm includes energy sources and energy-producing organelles (the yolk and mitochondria); the enzymes and precursors for DNA, RNA, and protein syntheses; stored messenger RNAs; structural proteins; and morphogenetic regulatory factors that control early embryogenesis. A partial catalogue of the materials stored in the oocyte cytoplasm is shown in Table 19.2, while a partial list of stored mRNAs is shown in Table 5.2. Most of this accumulation takes place during meiotic prophase I, which is often subdivided into two phases, **previtellogenesis** (Greek, "before yolk formation") and **vitellogenesis**.

The eggs of fish and amphibians are derived from an oogonial stem cell population that can generate a new cohort of oocytes each year. In the frog *Rana pipiens*, oogenesis takes

TABLE 19.2 Cellular components stored in the mature oocyte of *Xenopus laevis*

Component	Approximate excess over amount in larval cells
Mitochondria	100,000
RNA polymerases	60,000–100,000
DNA polymerases	100,000
Ribosomes	200,000
tRNA	10,000
Histones	15,000
Deoxyribonucleoside triphosphates	2,500

Source: After Laskey 1979.

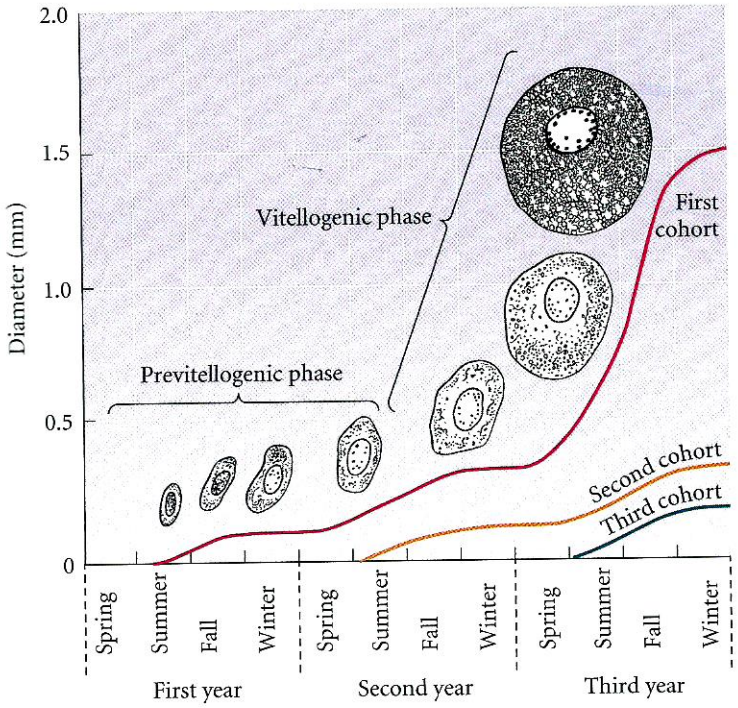

Figure 19.23
Growth of oocytes in the frog. During the first 3 years of life, three cohorts of oocytes are produced. The drawings follow the growth of the first-generation oocytes. (After Grant 1953.)

3 years. During the first 2 years, the oocyte increases its size very gradually. During the third year, however, the rapid accumulation of yolk in the oocyte causes the egg to swell to its characteristically large size (Figure 19.23). Eggs mature in yearly batches, with the first cohort maturing shortly after metamorphosis; the next group matures a year later.

Vitellogenesis occurs when the oocyte reaches the diplotene stage of meiotic prophase. Yolk is not a single substance, but a mixture of materials used for embryonic nutrition. The major yolk component in frog eggs is a 470-kDa protein called **vitellogenin**. It is not made in the frog oocyte (as are the major yolk proteins of organisms such as annelids and crayfish), but is synthesized in the liver and carried by the bloodstream to the ovary (Flickinger and Rounds 1956; Danilchik and Gerhart 1987).

As the yolk is being deposited, the organelles also become arranged asymmetrically. The cortical granules begin to form from the Golgi apparatus; they are originally scattered randomly through the oocyte cytoplasm, but later migrate to the periphery of the cell. The mitochondria replicate at this time, dividing to form a "mitochondrial cloud." These millions of mitochondria will be apportioned to the different blastomeres during cleavage. (In *Xenopus*, new mitochondria will not be formed until after gastrulation is initiated.) As vitellogenesis nears an end, the oocyte cytoplasm becomes stratified. The cortical granules, mitochondria, and pigment granules are located at the periphery of the oocyte, within the actin-rich cortex. Within the inner cytoplasm, distinct gradients emerge. While the yolk platelets become more heavily concentrated at the vegetal pole of the oocyte, the glycogen granules, ribosomes, lipid vesicles, and endoplasmic reticulum are found toward the animal pole. Even specific mRNAs stored in the cytoplasm become localized to certain regions of the oocyte.

WEBSITE 19.9 **Hormonal control of yolk production.** Vitellogenesis in amphibians is mediated primarily by estrogen. Estrogen instructs the liver to express and secrete vitellogenin, and this protein is absorbed from the blood by the young oocyte.

WEBSITE 19.10 **Transporting the Vg1 mRNA.** The Vera protein specifically binds to the 3′ UTR of the *Vg1* message. Vera may link *Vg1* mRNA to a set of endoplasmic reticulum vesicles that are translocated to the vegetal cortex.

WEBSITE 19.11 **Establishment of egg polarity.** In several species, the developing oocyte is a flagellated cell whose flagellum marks the future animal pole of the egg. This flagellum is lost during oogenesis.

Completion of amphibian meiosis: Progesterone and fertilization

Amphibian primary oocytes can remain in the diplotene stage of meiotic prophase for years. This state resembles the G_2 phase of the mitotic cell division cycle (see Chapter 8). Resumption of meiosis in the amphibian oocyte requires progesterone. This hormone is secreted by the follicle cells in response to gonadotropic hormones secreted by the pituitary gland. Within 6 hours of progesterone stimulation, **germinal vesicle breakdown (GVBD)** occurs, the microvilli retract, the nucleoli disintegrate, and the chromosomes contract and migrate to the animal pole to begin division. Soon afterward, the first meiotic division occurs, and the mature ovum is released from the ovary by a process called **ovulation**. The ovulated egg is in second meiotic metaphase when it is released (Figure 19.24).

How does progesterone enable the egg to break its dormancy and resume meiosis? To understand the mechanisms by which this activation is accomplished, it is necessary to briefly review the model for early blastomere division (see Chapter 8). Entry into the mitotic (M) phase of the cell cycle (in both meiosis and mitosis) is regulated by **mitosis-promoting factor**, or **MPF** (originally called "maturation-promoting factor" after its meiotic function). MPF contains two subunits, **cyclin B** and the **p34** protein. The p34 protein is a cyclin-dependent-kinase—its activity is dependent upon the presence

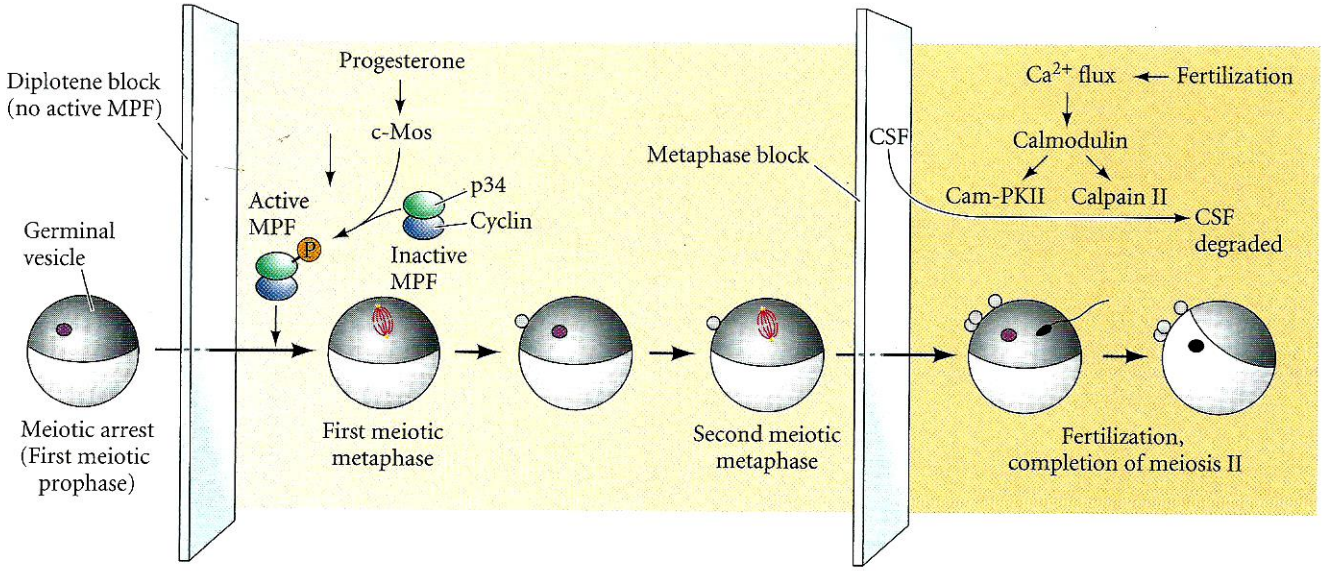

Figure 19.24
Schematic representation of *Xenopus* oocyte maturation, showing the regulation of meiotic cell division by progesterone and fertilization. Oocyte maturation is arrested at the diplotene stage of first meiotic prophase by the lack of active MPF. Progesterone activates the production of the c-mos protein. This protein initiates a cascade of phosphorylation that eventually phosphorylates the p34 subunit of MPF, allowing the MPF to become active. The MPF drives the cell cycle through the first meiotic division, but further division is blocked by CSF, a compound containing c-mos and cdk2. Upon fertilization, calcium ions released into the cytoplasm are bound by calmodulin and are used to activate two enzymes, calmodulin-dependent protein kinase II and calpain II, which inactivate and degrade CSF. Second meiosis is completed, and the two haploid pronuclei can fuse. At this time, cyclin B is resynthesized, allowing the first cell cycle of cleavage to begin.

of cyclin. Since all the components of MPF are present in the amphibian oocyte, it is generally thought that progesterone somehow converts a pre-MPF complex into active MPF.

The mediator of the progesterone signal is the **c-mos** protein. Progesterone reinitiates meiosis by causing the egg to polyadenylate the maternal *c-mos* mRNA that has been stored in its cytoplasm (Sagata et al. 1988; Sheets et al. 1995; Mendez et al. 2000). This message is translated into a 39-kDa phosphoprotein. This c-mos protein is detectable only during oocyte maturation and is destroyed quickly upon fertilization. Yet during its brief lifetime, it plays a major role in releasing the egg from its dormancy. The c-mos protein activates a phosphorylation cascade that phosphorylates and activates the p34 subunit of MPF (Ferrell and Machleder 1998; Ferrell 1999). The active MPF allows the germinal vesicle to break down and the chromosomes to divide. If the translation of *c-mos* is inhibited by injecting *c-mos* antisense mRNA into the oocyte, germinal vesicle breakdown and the resumption of oocyte maturation do not occur.

However, oocyte maturation then encounter a second block. MPF can take the chromosomes only through the first meiotic division and the prophase of the second meiotic division. The oocyte is arrested again in the metaphase of the second meiotic division. This metaphase block is caused by the combined actions of c-mos and another protein, cyclin-dependent kinase 2 (cdk2; Gabrielli et al. 1993). These two proteins are subunits that together form **cytostatic factor** (**CSF**), which is found in mature frog eggs, and which can block cell cycles in metaphase (Matsui 1974). It is thought that CSF prevents the degradation of cyclin.

The metaphase block is broken by fertilization. Evidence suggests that the calcium ion flux attending fertilization enables the calcium-binding protein **calmodulin** to become active. Calmodulin, in turn, can activate two enzymes that inactivate CSF: calmodulin-dependent protein kinase II, which inactivates the cdk2 kinase, and calpain II, a calcium-dependent protease that degrades c-mos (Watanabe et al. 1989; Lorca et al. 1993). This action promotes cell division in two ways. First, without CSF, cyclin can be degraded, and the meiotic division can be completed. Second, calcium-dependent protein kinase II also allows the centrosome to duplicate, thus forming the poles of the meiotic spindle (Matsumoto and Maller 2002). The coordination of fertilization and meiosis appears to be intimately coordinated through the release and binding of calcium ions.

Gene transcription in oocytes

In most animals (insects being a major exception), the growing oocyte is active in transcribing genes whose products are necessary for cell metabolism, for oocyte-specific processes, or for early development before the zygote-derived nuclei begin to function. In mice, for instance, the growing diplotene oocyte is actively transcribing the genes for zona pellucida

Meroistic oogenesis in insects

There are several types of oogenesis in insects, but most studies have focused on those insects, such as *Drosophila* and moths, that undergo **meroistic oogenesis**, in which cytoplasmic connections remain between the cells produced by the oogonium. In *Drosophila*, each oogonium divides four times to produce a clone of 16 cells connected to one another by **ring canals**. The production of these interconnected cells, called **cystocytes**, involves a highly ordered array of cell divisions (Figure 19.28). Only those two cells having four interconnections are capable of developing into oocytes, and of those two, only one becomes the egg. The other begins meiosis but does not complete it. Thus, only one of the 16 cystocytes can become an ovum. All the other cells become **nurse cells**. As it turns out, the cell destined to become the oocyte is that cell residing at the most posterior tip of the egg chamber, or **ovariole**, that encloses the 16-cell clone. However, since the nurse cells are connected to the oocyte by cytoplasmic bridges, the entire complex can be seen as one egg-producing unit.

The oocytes of meroistic insects do not pass through a transcriptionally active stage, nor do they have lampbrush chromosomes. Rather, RNA synthesis is largely confined to the nurse cells, and the RNA made by those cells is actively transported into the oocyte cytoplasm (see Figure 9.13A). Oogenesis takes place in only 12 days, so the nurse cells are very metabolically active during this time. They are aided in their transcriptional efficiency by becoming polytene. Instead of having two copies of each chromosome, they replicate their chromosomes until they have produced 512 copies. The 15 nurse cells pass ribosomal and messenger RNAs as well as

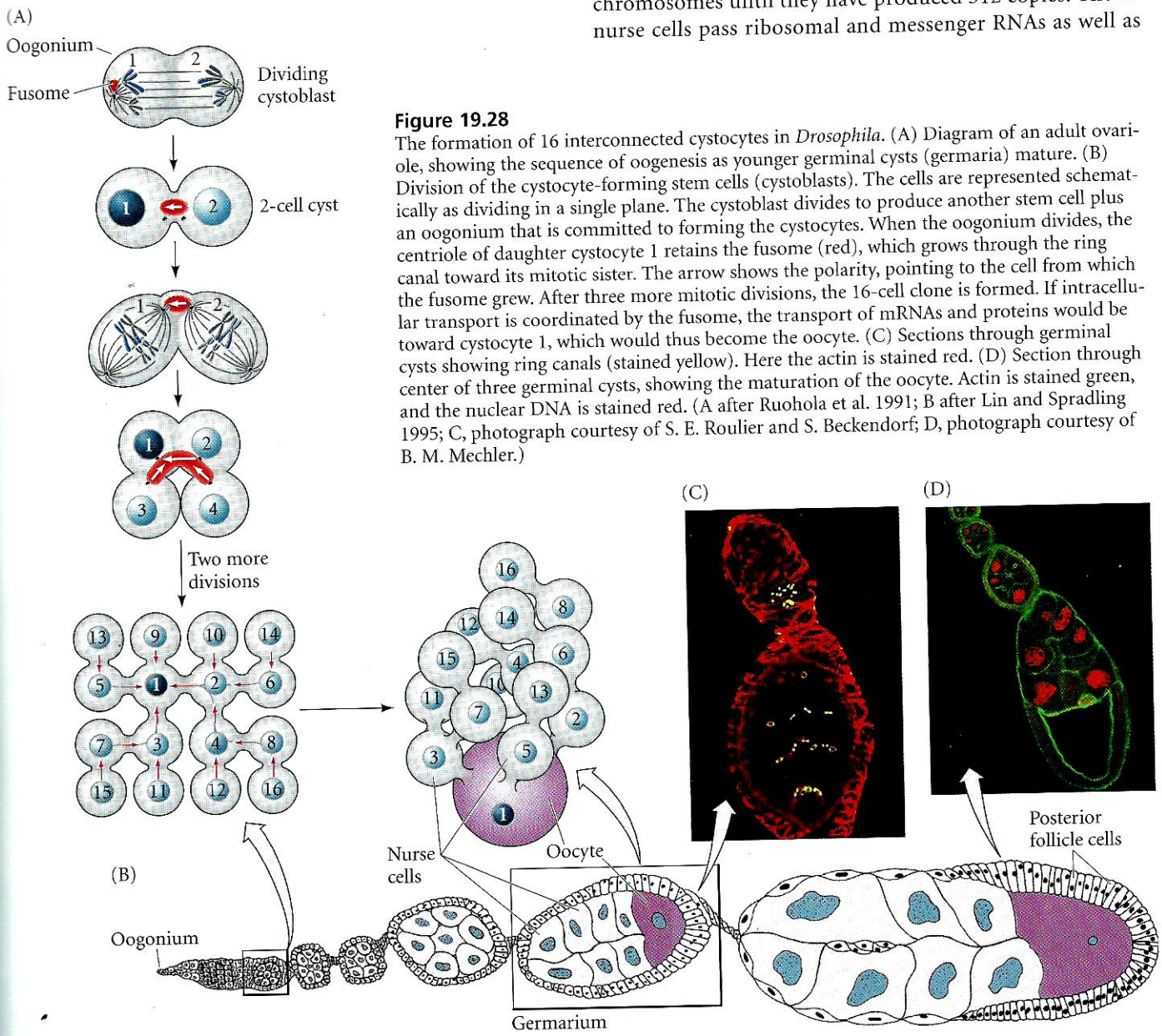

Figure 19.28
The formation of 16 interconnected cystocytes in *Drosophila*. (A) Diagram of an adult ovariole, showing the sequence of oogenesis as younger germinal cysts (germaria) mature. (B) Division of the cystocyte-forming stem cells (cystoblasts). The cells are represented schematically as dividing in a single plane. The cystoblast divides to produce another stem cell plus an oogonium that is committed to forming the cystocytes. When the oogonium divides, the centriole of daughter cystocyte 1 retains the fusome (red), which grows through the ring canal toward its mitotic sister. The arrow shows the polarity, pointing to the cell from which the fusome grew. After three more mitotic divisions, the 16-cell clone is formed. If intracellular transport is coordinated by the fusome, the transport of mRNAs and proteins would be toward cystocyte 1, which would thus become the oocyte. (C) Sections through germinal cysts showing ring canals (stained yellow). Here the actin is stained red. (D) Section through center of three germinal cysts, showing the maturation of the oocyte. Actin is stained green, and the nuclear DNA is stained red. (A after Ruohola et al. 1991; B after Lin and Spradling 1995; C, photograph courtesy of S. E. Roulier and S. Beckendorf; D, photograph courtesy of B. M. Mechler.)

proteins into the oocyte cytoplasm, and entire ribosomes may be transported as well. The mRNAs do not associate with polysomes, which suggests that they are not immediately active in protein synthesis (Paglia et al. 1976; Telfer et al. 1981).

The meroistic ovary confronts us with some interesting problems. If all the cystocytes are connected so that proteins and RNAs shuttle freely among them, why should they have different developmental fates? Why should one cell become the oocyte while the others become "RNA-synthesizing factories," sending mRNAs, ribosomes, and even centrioles into the oocyte? Why is the flow of protein and RNA in one direction only?

As the cystocytes divide, a large, spectrin-rich structure called the **fusome** forms and spans the ring canals between the cells (Figure 19.28D). It is constructed asymmetrically, as it always grows from the spindle pole that remains in one of the cells after the first division (Lin and Spradling 1995; de Cuevas and Spradling 1998). The cell that retains the greater part of the fusome during the first division becomes the oocyte. It is not yet known if the fusome contains oogenic determinants, or if it directs the traffic of materials into this particular cell.

Once the patterns of transport are established, the cytoskeleton becomes actively involved in transporting mRNAs from the nurse cells into the oocyte cytoplasm (Cooley and Theurkauf 1994). An array of microtubules that extends through the ring canals is critical for oocyte determination. In the nurse cells, the Exuperantia protein binds *bicoid* message to the microtubules and transports it to the anterior of the oocyte (Cha et al. 2001; see Chapter 8). If the microtubular array is disrupted (either chemically or by mutations such as *bicaudal-D* or *egalitarian*), the nurse cells gene products are transmitted in all directions, and all 16 cells differentiate into nurse cells (Gutzeit 1986; Theurkauf et al. 1992, 1993; Spradling 1993). The Bicaudal-D and Egalitarian proteins are probably core components of a dynein motor system that transports mRNAs and proteins throughout the oocyte (Bullock and Ish-Horowicz 2001). It is possible that some compounds transported from the nurse cells into the oocyte become associated with transport proteins, such as dynein and kinesin, which would enable them to travel along the tracks of microtubules extending through the ring canals (Theurkauf et al. 1992; Sun and Wyman 1993). The *oskar* message, for instance, is linked to kinesin through the Barentsz protein, and kinesin can transport the *oskar* message to the posterior of the oocyte (van Eeden et al. 2001).

Actin may become important for maintaining the polarity of transport during later stages of oogenesis. Mutations that prevent actin microfilaments from lining the ring canals prevent the transport of mRNAs from the nurse cells to the oocyte, and disruption of the actin microfilaments randomizes the distribution of mRNA (Cooley et al. 1992; Watson et al. 1993). Thus, the cytoskeleton controls the movement of organelles and RNAs between nurse cells and oocyte such that developmental cues are exchanged only in the appropriate direction.

WEBSITE 19.13 ***Drosophila* spermatogenesis.** *Drosophila* sperm are derived from stem cells that maintain a long-term capacity to divide. The renewal of stem cells is specified by the STAT pathway, which responds to a specific support cell signal.

Maturation of the mammalian oocyte

Ovulation in mammals follows one of two patterns, depending on the species. One type of ovulation is stimulated by the act of copulation. Physical stimulation of the cervix triggers the release of gonadotropins from the pituitary. These gonadotropins signal the egg to resume meiosis and initiate the events that will expel it from the ovary. This mechanism ensures that most copulations will result in fertilized ova, and animals that utilize this method of ovulation—such as rabbits and minks—have a reputation for procreative success.

Most mammals, however, have a periodic ovulation pattern, in which the female ovulates only at specific times of the year. This ovulatory period is called **estrus** (or its English equivalent, "heat"). In these animals, environmental cues, most notably the amount and type of light during the day, stimulate the hypothalamus to release gonadotropin-releasing hormone. This factor stimulates the pituitary to release the gonadotropins—follicle-stimulating hormone (FSH) and luteinizing hormone (LH)—that cause the ovarian follicle cells to proliferate and secrete estrogen. The estrogen enters certain neurons and evokes the pattern of mating behavior characteristic of the species. The gonadotropins also stimulate follicular growth and initiate ovulation. Thus, mating behavior and ovulation occur close together.

Humans have a variation on the theme of periodic ovulation. Although human females have cyclical ovulation (averaging about once every 29.5 days) and no definitive yearly estrus, most of human reproductive physiology is shared with other primates. The characteristic primate periodicity in maturing and releasing ova is called the **menstrual cycle** because it entails the periodic shedding of blood and endothelial tissue from the uterus at monthly intervals.* The menstrual cycle represents the integration of three very different cycles:

*The periodic shedding of the uterine lining is a controversial topic. Some scientists speculate that menstruation is an active process, with adaptive significance in evolution. Profet (1993) proposed that menstruation is a crucial immunological adaptation, protecting the uterus against infections contracted from semen or other environmental agents. Strassmann (1996) suggested that the cyclicity of the endometrium is an energy-saving adaptation that is important in times of poor nutrition. Vaginal bleeding would be a side effect of this adaptive process. Finn (1998) claimed that menstruation has no adaptive value and is necessitated by the immunological crises that are a consequence of bringing two genetically dissimilar organisms together in the uterus. Martin (1992) pointed out that it might even be wrong to think of there being a single function of menstruation, and that its roles might change during a woman's life cycle.

1. The ovarian cycle, the function of which is to mature and release an oocyte.
2. The uterine cycle, the function of which is to provide the appropriate environment for the developing blastocyst.
3. The cervical cycle, the function of which is to allow sperm to enter the female reproductive tract only at the appropriate time.

These three functions are integrated through the hormones of the pituitary, hypothalamus, and ovary.

> VADE MECUM[2] **Oogenesis in mammals.** The development of the mammalian ovum and its remarkable growth during the primary oocyte stage are the subject of photographs and QuickTime movies of histological sections through a mammalian ovary.
> **[Click on Gametogenesis]**

The majority of the oocytes within the adult human ovary are maintained in the diplotene stage of the first meiotic prophase (often referred to as the **dictyate state**). Each oocyte is enveloped by a primordial follicle consisting of a single layer of epithelial granulosa cells and a less organized layer of mesenchymal thecal cells (Figure 19.29). Periodically, a group of primordial follicles enters a stage of follicular growth. During this time, the oocyte undergoes a 500-fold increase in volume (corresponding to an increase in oocyte diameter from 10 μm in a primordial follicle to 80 μm in a fully developed follicle).

Concomitant with oocyte growth is an increase in the number of granulosa cells, which form concentric layers around the oocyte. This proliferation of granulosa cells is mediated by a paracrine factor, GDF9, a member of the TGF-β family (Dong et al. 1996). Throughout this growth period, the oocyte remains in the dictyate stage. The fully grown follicle thus contains a large oocyte surrounded by several layers of granulosa cells. The innermost of these cells will stay with the ovulated egg, forming the **cumulus**, which surrounds the egg in the oviduct. In addition, during the growth of the follicle, an **antrum** (cavity) forms, which becomes filled with a complex mixture of proteins, hormones, and other molecules. Just as the maturing oocyte synthesizes paracrine factors that allow the follicle cells to proliferate, the follicle cells secrete growth and differentiation factors (TGF-β2, VEGF, leptin, FGF2) that allow the oocyte to grow and bring blood vessels into the follicular region (Antczak et al. 1997).

At any given time, a small group of follicles is maturing. However, after progressing to a certain stage, most oocytes and their follicles die. To survive, the follicle must be exposed to a wave of gonadotropic hormone release, "catch the wave" at the right time, and ride it until it peaks. Thus, for oocyte maturation to occur, the follicle needs to be at a certain stage of development when the waves of gonadotropin arise.

The first day of vaginal bleeding is considered to be day 1 of the menstrual cycle (Figure 19.30). This bleeding represents the sloughing off of endometrial tissue and blood vessels that

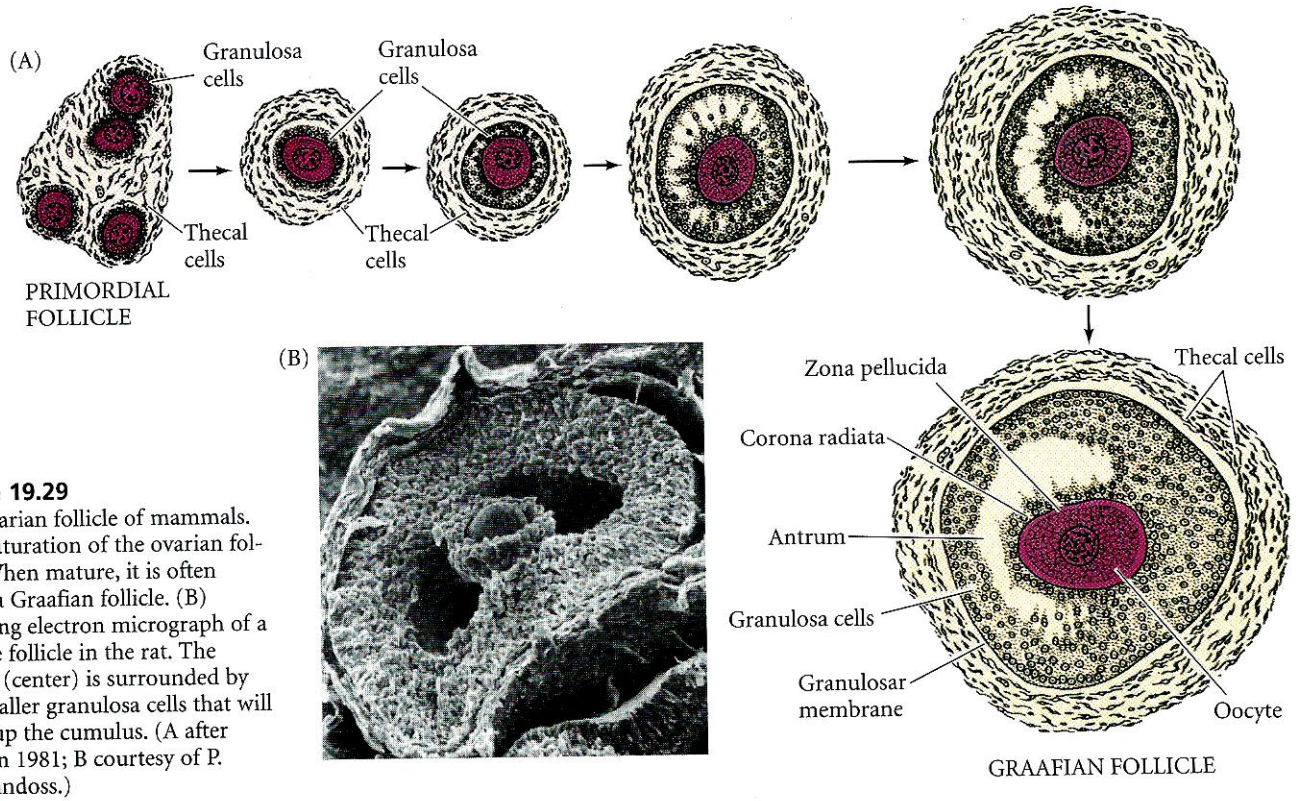

Figure 19.29
The ovarian follicle of mammals. (A) Maturation of the ovarian follicle. When mature, it is often called a Graafian follicle. (B) Scanning electron micrograph of a mature follicle in the rat. The oocyte (center) is surrounded by the smaller granulosa cells that will make up the cumulus. (A after Carlson 1981; B courtesy of P. Bagavandoss.)

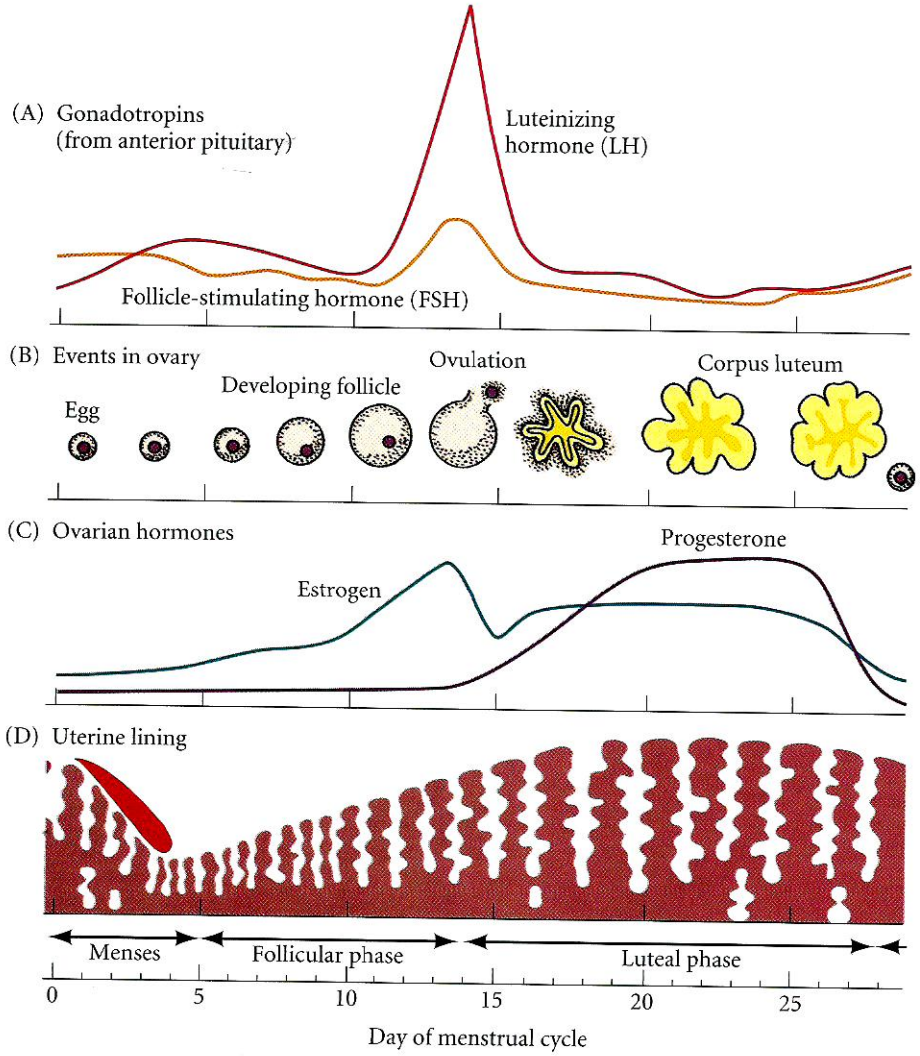

Figure 19.30
The human menstrual cycle. The coordination of (B) ovarian and (D) uterine cycles is controlled by (A) the pituitary and (C) the ovarian hormones. During the follicular phase, the egg matures within the follicle, and the uterine lining is prepared to receive a blastocyst. The mature egg is released around day 14. If a blastocyst does not implant in the uterus, the uterine wall begins to break down, leading to menstruation.

would have aided the implantation of the blastocyst. In the first part of the cycle (called the **proliferative** or **follicular phase**), the pituitary starts secreting increasingly large amounts of FSH. Any maturing follicles that have reached a certain stage of development respond to this hormone with further growth and cellular proliferation. FSH also induces the formation of LH receptors on the granulosa cells. Shortly after this period of initial follicle growth, the pituitary begins secreting LH. In response to LH, the dictyate meiotic block is broken. The nuclear membranes of competent oocytes break down, and the chromosomes assemble to undergo the first meiotic division. One set of chromosomes is kept inside the oocyte, and the other ends up in the small polar body. Both are encased by the zona pellucida, which has been synthesized by the growing oocyte. It is at this stage that the egg will be ovulated.

The two gonadotropins, acting together, cause the follicle cells to produce increasing amounts of estrogen, which has at least five major activities in regulating the further progression of the menstrual cycle:

1. It causes the uterine endometrium to begin its proliferation and to become enriched with blood vessels.
2. It causes the cervical mucus to thin, thereby permitting sperm to enter the inner portions of the reproductive tract.
3. It causes an increase in the number of FSH receptors on the granulosa cells of the mature follicles while causing the pituitary to lower its FSH production. It also stimulates the granulosa cells to secrete the peptide hormone inhibin, which also suppresses pituitary FSH secretion.
4. At low concentrations, it inhibits LH production, but at high concentrations, it stimulates it.
5. At very high concentrations and over long durations, estrogen interacts with the hypothalamus, causing it to secrete gonadotropin-releasing hormone.

As estrogen levels increase as a result of follicular production, FSH levels decline. LH levels, however, continue to rise as more estrogen is secreted. As estrogen continues to be made (days 7–10), the granulosa cells continue to grow.

Starting on day 10, estrogen secretion rises sharply. This rise is followed at midcycle by an enormous surge of LH and a smaller burst of FSH. Experiments with female monkeys have shown that exposure of the hypothalamus to greater than 200 pg of estrogen per milliliter of blood for more than 50 hours results in hypothalamic secretion of gonadotropin-releasing hormone. This factor causes the subsequent release of FSH and LH from the pituitary. Within 10–12 hours after the gonadotropin peak, the egg is ovulated (Figure 19.31; Garcia et al. 1981).

Although the detailed mechanism of ovulation is not yet known, the physical expulsion of the mature oocyte from the follicle appears to be the result of an LH-induced increase in collagenase, plasminogen activator, and prostaglandins within the follicle (Lemaire et al. 1973). Prostaglandins may cause localized contractions in the smooth muscles of the ovary and may also increase the flow of water from the ovarian capillaries, increasing fluid pressure in the antrum (Diaz-Infante et al. 1974; Koos and Clark 1982). If ovarian prostaglandin synthesis is inhibited, ovulation does not take place. In addition, collagenase and the plasminogen activator protease loosen and digest the extracellular matrix of the follicle (Beers et al. 1975; Downs and Longo 1983). The mRNA for plasminogen activator has been dormant in the oocyte cytoplasm. LH causes this message to be polyadenylated and translated into this powerful protease (Huarte et al. 1987). The result of LH, then, is increased follicular pressure coupled with the degradation of the follicle wall. A hole is digested through which the ovum can burst.

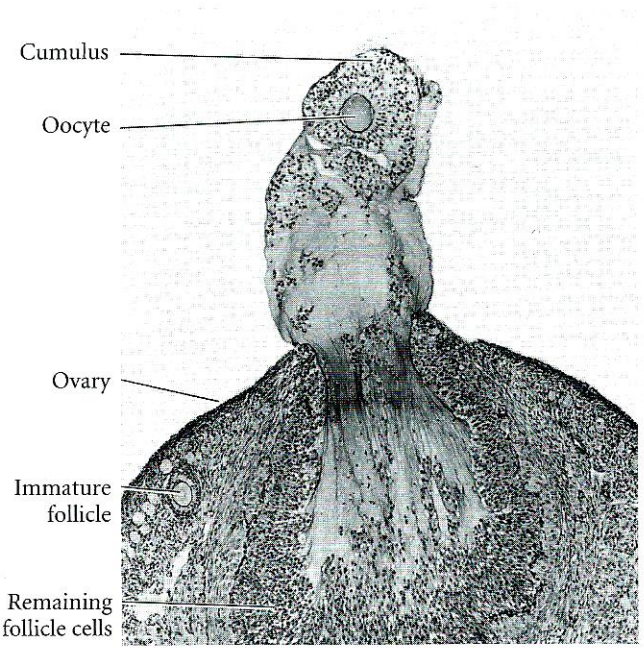

Figure 19.31
Ovulation in the rabbit. The ovary of a living, anesthetized rabbit was exposed and observed. When the follicle started to ovulate, the ovary was removed, fixed, and stained. (Photograph courtesy of R. J. Blandau.)

Following ovulation, the **luteal phase** of the menstrual cycle begins. The remaining cells of the ruptured follicle, under the continued influence of LH, become the **corpus luteum**. (They are able to respond to this LH because the surge in FSH stimulates them to develop even more LH receptors.) The corpus luteum secretes some estrogen, but its predominant secretion is **progesterone**. This steroid hormone circulates to the uterus, where it completes the job of preparing the uterine tissue for blastocyst implantation, stimulating the growth of the uterine wall and its blood vessels. Blocking the progesterone receptor with the synthetic steroid **mifepristone** (RU486) stops the uterine wall from thickening and prevents the implantation of a blastocyst* (Couzinet et al. 1986; Greb et al. 1999).

Progesterone also inhibits the production of FSH, thereby preventing the maturation of any more follicles and ova. (For this reason, a combination of estrogen and progesterone has been used in birth control pills. The growth and maturation of new ova are prevented as long as FSH is inhibited.)

If the ovum is not fertilized, the corpus luteum degenerates, progesterone secretion ceases, and the uterine wall is sloughed off. With the decline in serum progesterone levels, the pituitary secretes FSH again, and the cycle is repeated. However, if fertilization occurs, the trophoblast secretes a new hormone, **luteotropin**, which causes the corpus luteum to remain active and serum progesterone levels to remain high. Thus, the menstrual cycle enables the periodic maturation and ovulation of human eggs and allows the uterus to periodically develop into an organ capable of nurturing a developing organism for 9 months.

WEBSITE 19.14 **The reinitiation of mammalian meiosis.** The hormone-mediated disruption of communication between the oocyte and its surrounding follicle cells may critical in the resumption of meiosis in female mammals.

We are now back where we began: the stage is set for fertilization to take place. The egg and the sperm will both die if they do not meet. As F. R. Lillie recognized in 1919, "The elements that unite are single cells, each on the point of death; but by their union a rejuvenated individual is formed, which constitutes a link in the eternal process of Life."

*This is why RU486 is used for postconception birth control. RU486 is thought to compete for the progesterone receptor inside the nucleus. RU486 can bind to the progesterone site in the receptor, and the receptor-RU486 complex appears to form heterodimers with the normal progesterone-carrying progesterone receptor. When this RU486-progesterone complex binds to progesterone-responsive enhancer elements on the DNA, transcription from these sites is inhibited (Vegeto et al. 1992; Spitz and Bardin 1993).