16 The Saga of the Germ Line

And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

T.S. ELLIOT (1942)

When the spermatozoon enters the egg, it enters a cell system which has already achieved a certain degree of organization.

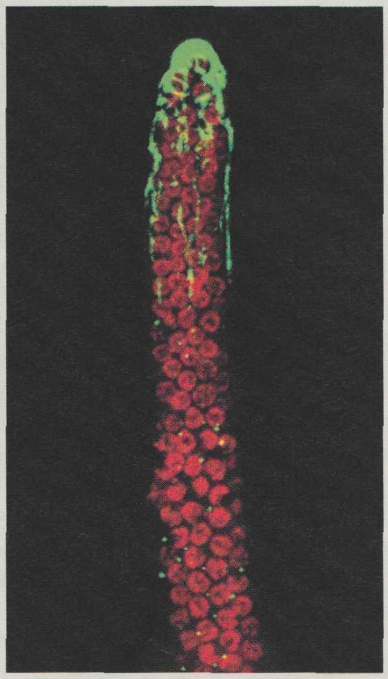
ERNST HADORN (1955)

WE ARE ABOUT TO COME FULL CIRCLE. We began our analysis of animal development by discussing fertilization, and we will finish our studies of individual development by investigating **gametogenesis**, the processes by which the sperm and the egg are formed. In addition to forming its own body, an individual animal must set aside cells that will provide the material and instructions for initiating bodies in the *next* generation. Germ cells provide the continuity of life between generations, and the mitotic ancestors of our own germ cells once resided in the gonads of reptiles, amphibians, fish, and invertebrates.

In many animals, including insects, roundworms, and vertebrates, there is a clear and early separation of germ cells from somatic cell types. In several other animal phyla (and throughout the entire plant kingdom), this division is not as well established. In these animal species (which include cnidarians, flatworms, and tunicates), somatic cells readily form new organisms. The zooids, buds, and polyps of many invertebrate phyla testify to the ability of somatic cells to give rise to new individuals (Liu and Berrill 1948; Buss 1987).

In those organisms in which there is an established germ line that separates from the somatic cells early in development, the germ cells often do not arise within the gonad itself. Rather, the gamete progenitor cells—the **primordial germ cells (PGCs)**—arise elsewhere and migrate into the developing gonads. The first step in gametogenesis, then, involves forming the PGCs and getting them into the genital ridge as the gonad is forming. Therefore, our discussion of gametogenesis will include:

- Formation of the germ plasm and the determination of the primordial germ cells
- Migration of the PGCs into the developing gonads
- The process of meiosis and the modifications of meiosis for forming sperm and eggs
- Differentiation of the sperm and egg cells (gametogenesis)
- Hormonal control of gamete maturation and ovulation



Germ Plasm and the Determination of the Primordial Germ Cells

All sexually reproducing animals arise from the fusion of gametes—sperm and eggs. All gametes arise from primordial germ cells. In most laboratory model organisms (including frogs, nematodes, and flies), the primordial germ cells are specified autonomously by cytoplasmic determinants in the egg that are parceled

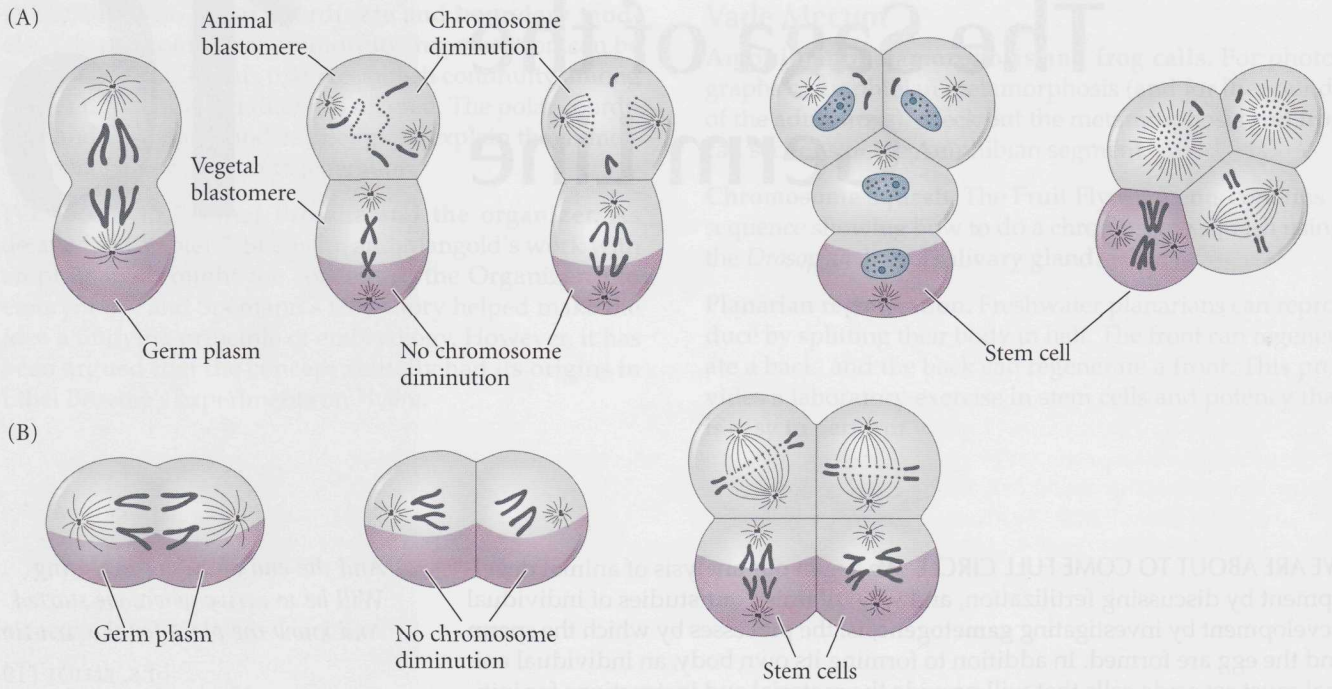


FIGURE 16.1 Distribution of germ plasm during cleavage of normal and centrifuged zygotes of *Parascaris*. (A) In normal cleavage, the germ plasm is localized in the vegetalmost blastomere, as shown by the lack of chromosomal diminution in that particular cell. Thus, at the 4-cell stage, the embryo has a single stem cell for its gametes. (B) When centrifugation is used to displace the first cleavage by 90 degrees, both of the resulting cells have vegetal germ plasm, and neither cell undergoes chromosome diminution. After the second cleavage, both of these two cells give rise to germinal stem cells. (After Waddington 1966.)

out to specific cells during cleavage. However, evidence suggests that in the majority of species (including salamanders and mammals), the germ cells are specified by interactions among neighboring cells (Extravour and Akam 2003). In those species in which determination of the primordial germ cells is brought about by the autonomous localization of specific proteins and mRNAs, these cytoplasmic components are collectively referred to as the **germ plasm**.

Germ cell determination in nematodes

BOVERI'S EXPERIMENTS ON *PARASCARIS* Theodor Boveri (1862–1915) was the first person to observe an organism's chromosomes throughout its development. In so doing, he discovered a fascinating feature in the development of the roundworm *Parascaris aequorum* (formerly known as *Ascaris megalocephala*). This nematode worm has only two chromosomes per haploid cell, thereby allowing detailed observations of its individual chromosomes. The cleavage plane of the first embryonic division is unusual in that it is equatorial, separating the animal half from the vegetal half of the zygote (Figure 16.1A). More bizarre, however, is the behavior of the chromosomes in the subsequent division of these first two blastomeres. The chromosomes in the animal blastomere fragment into dozens of pieces just before this cell divides. This phenomenon is called **chromosome diminution**, because only a portion of the original chromosome survives. Numerous genes are lost when the chromosomes fragment, and these genes are not included in the newly formed nuclei (Tobler et al. 1972; Müller et al. 1996).

Meanwhile, in the vegetal blastomere, the chromosomes remain normal. During second cleavage, the animal cell splits meridionally while the vegetal cell again divides equatorially. Both vegetally derived cells have normal chromosomes. However, the chromosomes of the more animally located of these two vegetal blastomeres fragment before the third cleavage. Thus, at the 4-cell stage, only one cell—the most vegetal—contains a full set of genes. At successive cleavages, nuclei with diminished chromosomes are given off from this vegetalmost line until the 16-cell stage, when there are only two cells with undiminished chromosomes. One of these two blastomeres gives rise to the germ cells; the other eventually undergoes chromosome diminution and forms more somatic cells. The chromosomes are kept intact only in those cells destined to form the germ line. If this were not the case, the genetic information would degenerate from one generation to the next. The cells that have undergone chromosome diminution generate the somatic cells.

Boveri has been called the last of the great observers of embryology and the first of the great experimenters. Not content with observing the retention of the full chromo-

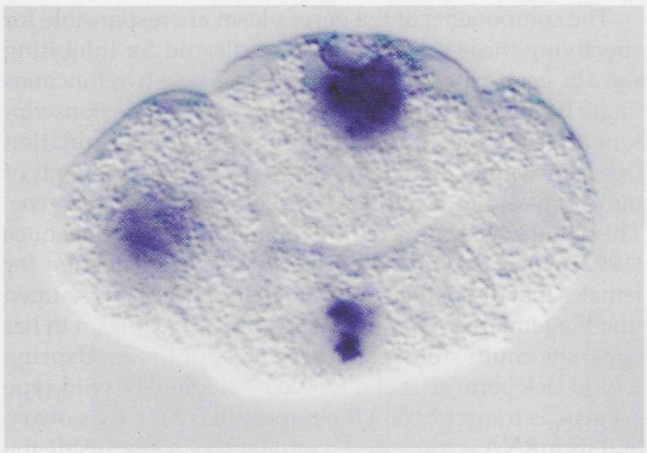


FIGURE 16.2 Inhibition of transcription in germ cell precursors of *Caenorhabditis elegans*. The photograph shows in situ hybridization to β-galactosidase mRNA expressed under control of the *pes-10* promoter. The *pes-10* gene is one of the earliest genes expressed in *C. elegans*. The P-blastomere that gives rise to the germ cells (far right) does not transcribe the gene. (From Seydoux and Fire 1994, courtesy of G. Seydoux.)

some complement by the germ cell precursors, he set out to test whether a specific region of cytoplasm protects the nuclei within it from diminution. If so, any nucleus happening to reside in this region should remain undiminished. In 1910, Boveri tested this hypothesis by centrifuging *Parascaris* eggs shortly before their first cleavage. This treatment shifted the orientation of the mitotic spindle. When the spindle forms perpendicular to its normal orientation, both resulting blastomeres contain some of the vegetal cytoplasm (Figure 16.1B). Boveri found that after the first division, neither nucleus underwent chromosomal diminution. However, the next division was equatorial along the animal-vegetal axis. Here the resulting animal blastomeres both underwent diminution, whereas the two vegetal cells did not. Boveri concluded that the vegetal cytoplasm contains a factor (or factors) that protects nuclei from chromosomal diminution and determines germ cells.

C. ELEGANS In the nematode *Caenorhabditis elegans*, the germline precursor cell is the P4 blastomere. The **P-granules** that enter this cell are critical for instructing it to become the germline precursor (see Figure 5.45). The P-granule protein repertoire includes several transcriptional inhibitors and RNA-binding proteins, including homologues of *Drosophila* Vasa, Piwi, and Nanos, whose functions we will discuss below (Kawasaki et al. 1998; Seydoux and Strome 1999; Subramanian and Seydoux 1999). In addition, as discussed in Chapter 5, the *C. elegans* germ plasm contains the PIE-1 protein, which prevents the phosphorylation of RNA polymerase II, thereby preventing transcription in the germ cell lineage (Ghosh and Seydoux 2008). This is critical for preventing the germ line from differentiating into somatic cells, and germ cell differentiation cannot commence until the disappearance of PIE-1 in later embryonic stages. Until that time, the germline nuclei are silenced (Figure 16.2). It is possible that these germ plasm proteins can aggregate into P-granules only in the P1–P4 cells. The MEX-5 and PAR-1 proteins inhibit P-granule stability in the remaining somatic cells (Brangwynne et al. 2009).

See WEBSITE 16.1
Germline sex determination in *C. elegans*

See WEBSITE 16.2
Mechanisms of chromosome diminution

Germ cell determination in insects

In *Drosophila*, PGCs form as a group of **pole cells** at the posterior pole of the cellularizing blastoderm. These nuclei migrate into the posterior region at the ninth nuclear division and become surrounded by the **pole plasm**, a complex collection of mitochondria, fibrils, and **polar granules** (Figure 16.3; Mahowald 1971a,b; Schubiger and Wood 1977). If the pole cell nuclei are prevented from reaching the pole plasm, no germ cells will be made (Mahowald et al. 1979). The germ cells are responsible for forming the **germline stem cells**, each of which divides asymmetrically to produce another stem cell and a differentiated daughter cell called a **cystoblast**. Cystoblasts undergo four mitotic divisions with incomplete cytokinesis to form a cluster of 16 cells interconnected by cytoplasmic bridges called

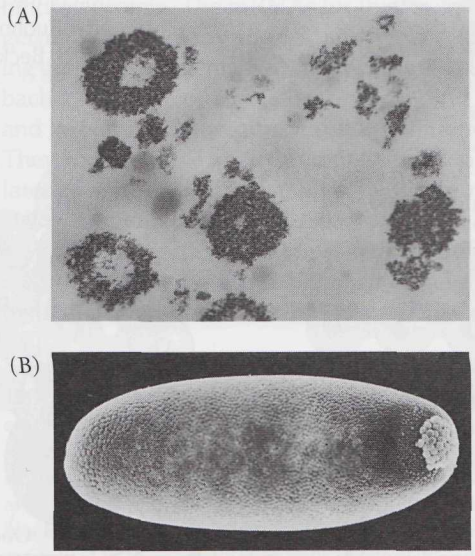


FIGURE 16.3 Pole plasm of *Drosophila*. (A) Electron micrograph of polar granules from particulate fraction of *Drosophila* pole cells. (B) Scanning electron micrograph of a *Drosophila* embryo just prior to completion of cleavage. The pole cells can be seen at the right of the photograph. (Photographs courtesy of A. P. Mahowald.)

ring canals. 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 becomes an ovum; the remaining 15 cells become **nurse cells** (Figure 16.4).

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**, enclosing the 16-cell clone. However, since the nurse cells are connected to the oocyte by the ring canals, the entire complex can be seen as one egg-producing unit. The nurse cells produce numerous RNAs and proteins that ultimately are transported into the oocyte through the ring canals.

The components of the germ plasm are responsible for specifying these cells to be germ cells and for inhibiting somatic gene expression in these cells. These two functions might be interrelated, since the inhibition of gene transcription appears to be essential for germ cell determination (see Santos and Lehmann 2004). One of the components of the pole plasm is the mRNA of the ***germ cell-less*** (***gcl***) gene. This gene was discovered by Jongens and his colleagues (1992) when they mutated *Drosophila* and screened for females that did not have "grandoffspring." They assumed that if a female did not place functional pole plasm in her eggs, she could still have offspring—but those offspring would lack germ cells and would be sterile. The wild-type *gcl* gene is transcribed in the nurse cells of the fly's ovary, and its mRNA is transported into the egg. Once inside the egg, it is transported to the posteriormost portion and

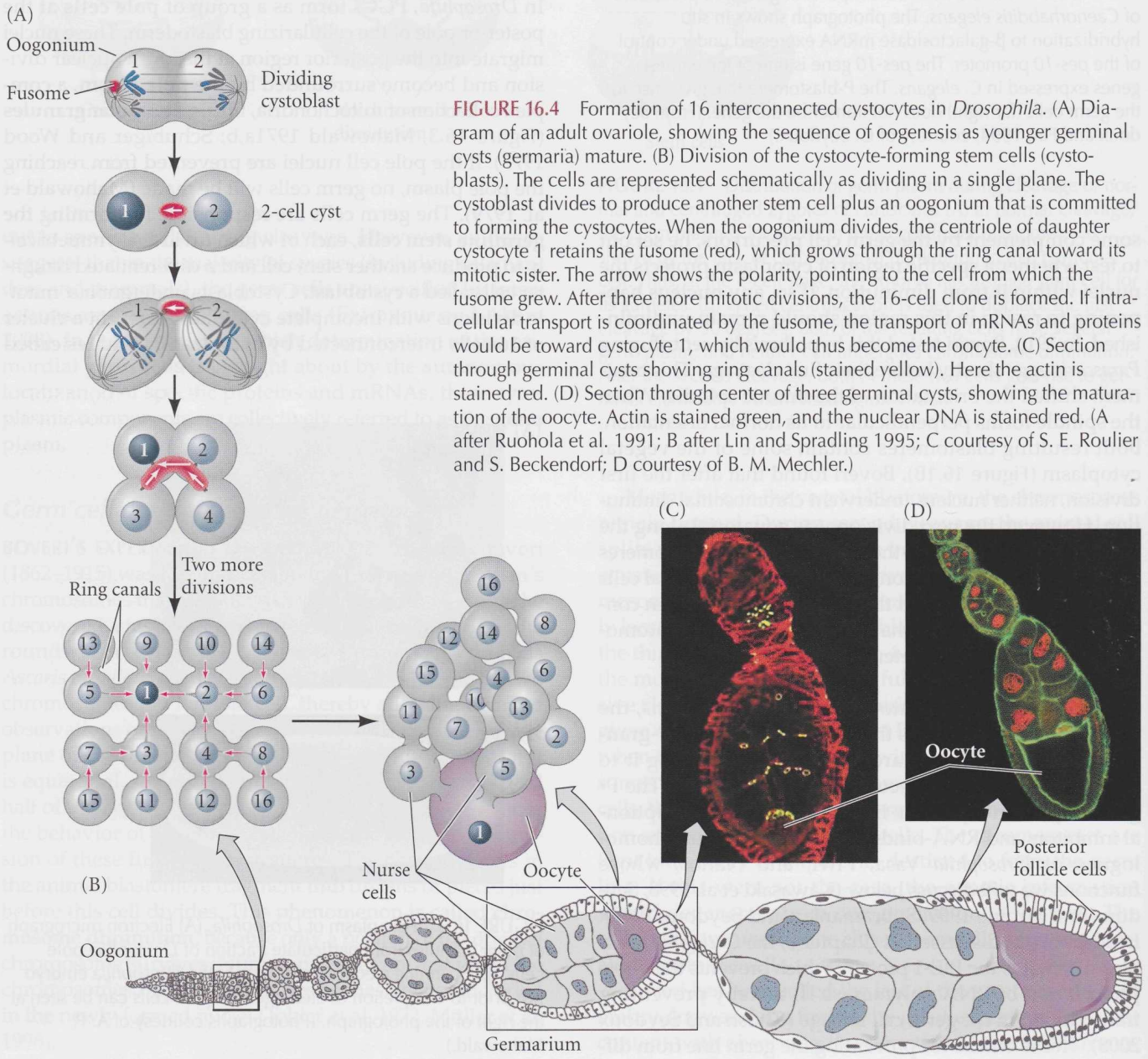


FIGURE 16.4 Formation of 16 interconnected cystocytes in *Drosophila*. (A) Diagram of an adult ovariole, showing the sequence of oogenesis as younger germinal cysts (germaria) mature. (B) Division of the cystocyte-forming stem cells (cystoblasts). The cells are represented schematically as dividing in 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) Section 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 courtesy of S. E. Roulier and S. Beckendorf; D courtesy of B. M. Mechler.)

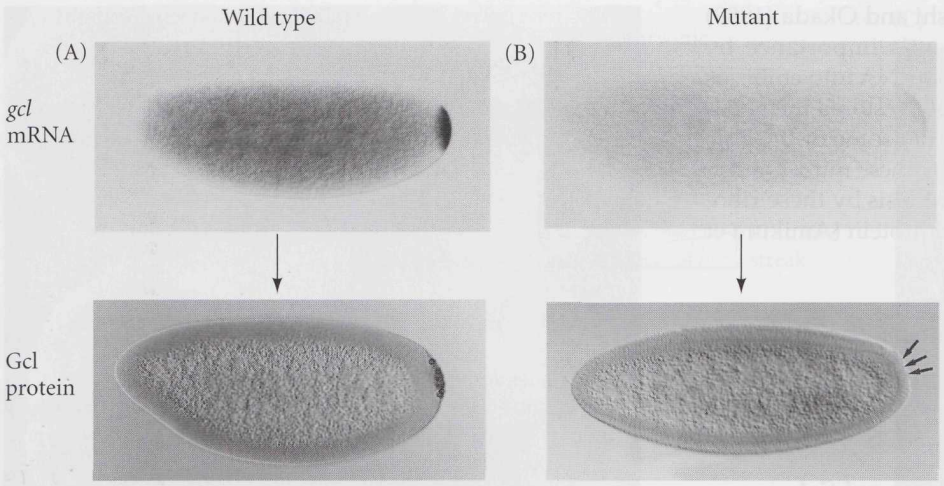


FIGURE 16.5 Localization of *germ cell-less* gene products in the posterior of the *Drosophila* egg and embryo. The *gcl* mRNA can be seen in the posterior pole of early-cleavage embryos produced by wild-type females (A), but not in embryos produced by *gcl*-deficient mutant females (B). The protein encoded by the *gcl* gene can be detected in the germ cells at the cellular blastoderm stage of embryos produced by wild-type females, but not in embryos from mutant females (arrows). (From Jongens et al. 1992, courtesy of T. A. Jongens.)

resides in what will become the pole plasm. This message is translated into protein during the early stages of cleavage (Figure 16.5A). The *gcl*-encoded protein appears to enter the nucleus, and it is essential for pole cell production. Flies with mutations of this gene lack germ cells (Figure 16.5B). The *gcl* gene encodes a nuclear envelope protein that prevents gene transcription and is critical for specifying the pole cells (Leatherman et al. 2002). Homologues of the *gcl* gene have been found in the germ cells of mice and humans, and the mouse *Gcl* gene also represses transcription. Human males with mutant *GCL* genes have defective spermatozoa and are often sterile (Nili et al. 2001; Kleiman et al. 2003).

A second protein found in *Drosophila* pole plasm (and one that becomes localized in the polar granules) is the **polar granule component** (**Pgc**). *Pgc* mRNA is part of the original pole plasm and becomes translated there. The Pgc protein inhibits transcription, and it does so (like PIE-1 in *C. elegans*) by preventing the phosphorylation of RNA polymerase II (Martinho et al. 2004; Hanyu-Nakamura et al. 2008; see Figure 2.7). Without this phosphorylation, the RNA polymerase cannot transcribe any genes. If the maternal *Pgc* gene is mutated, the germ cells begin expressing the genes characteristic of their neighboring somatic cells.

A third set of pole plasm components are the **posterior group determinants**. The Oskar protein (see Chapter 6) appears to be the critical member of this group, since expression of *oskar* mRNA in ectopic sites will cause the nuclei in those areas to form germ cells. The genes that restrict Oskar to the posterior pole are also necessary for germ cell formation (Ephrussi and Lehmann 1992; Newmark et al. 1997; Riechmann et al. 2002). Moreover, Oskar appears to be the limiting step of germ cell formation, since adding more *oskar* message to the oocyte causes more germ cells to form. Oskar functions by localizing the proteins and RNAs necessary for germ cell formation (such as *germ cell-less*) to the posterior pole (Ephrussi and Lehmann 1992; Snee and Macdonald 2004).

One of the mRNAs localized by Oskar is the *Nanos* message, whose product is essential for posterior segment formation and germ cell specification. Pole cells lacking Nanos do not migrate into the gonads and fail to become gametes. While Gcl and Pgc appear to be critical in regulating transcription, Nanos appears to be essential for inhibiting the translation of certain messages. In embryos lacking Nanos, the germline cells usually die; but if inhibited from dying, these germline cells can become somatic cells (see Chapter 6). Nanos thus prevents the pole cells from activating the pathway that would lead to the formation of somatic cells (Hayashi et al. 2004).

Another of the posterior mRNAs encodes **Vasa**, an RNA-binding protein. The mRNAs for this protein are seen in the germ plasm of many species, and Vasa is critical for initiating germ cell differentiation and meiosis (Ghabrial and Schüpbach 1999). Two other nucleic acid-binding proteins, **Piwi*** and its relative **Aubergine**, are also found in the pole plasm. They, too, have the ability to repress transcription. Piwi will later become critical in establishing the germ cell as a stem cell in the gonad (Cox et al. 1998; Megosh et al. 2006).

There are numerous components of the pole plasm that we know little about (see Santos and Lehmann 2004). For instance, mitochondrial ribosomes are seen transiently in

*Piwi appears to be required for stem cell maintenance and proliferation throughout the eukaryotic kingdoms. In addition to being present in germ stem cells, *Piwi* genes have also been found expressed in the totipotent stem cells of planaria and regenerating annelids. Inhibiting *Piwi* gene expression in the adult flatworm blocks the worm's regeneration (Reddien 2004). *Piwi* is also expressed in the somatic stem cells of jellyfish and is upregulated immediately before transdifferentiation. The continuous low expression of Piwi in differentiated cells of jellyfish may underlie their ability to remodel their bodies so profoundly (Seipel et al. 2004). *Piwi* may even be responsible for stem cell maintenance across kingdoms: two *Piwi* genes in *Arabidopsis* are crucial for maintaining meristem proliferation at the root and shoot of the plant (Bohmert et al. 1998; Moussian et al. 1998).

the *Drosophila* pole plasm. Kobayashi and Okada (1989) demonstrated ribosomal component's importance by showing that injecting mitochondrial RNA into embryos formed from ultraviolet-irradiated eggs restored their ability to form pole cells. It is possible that some of the pole plasm mRNAs are being translated by these mitochondrial ribosomes. Inhibiting protein synthesis by these ribosomes impairs production of the Gcl protein (Amikura et al. 2005).

VADE MECUM
Germ cells in the *Drosophila* embryo

See WEBSITE 16.3
The insect germ plasm

Germ cell determination in frogs and fish

FROGS Cytoplasmic localization of germ cell determinants has also been observed in vertebrate embryos. Bounoure (1934) showed that the vegetal region of fertilized frog eggs contains material with staining properties similar to those of *Drosophila* pole plasm. He was able to trace this cortical cytoplasm into the few cells in the presumptive endoderm that would normally migrate into the genital ridge. By transplanting genetically marked cells from one embryo into another of a differently marked strain, Blackler (1962) showed that these cells are the primordial germ cell precursors.

The germ plasm of amphibians consists of germinal granules and a matrix around them. It contains many of the same RNAs and proteins (including the large and small mitochondrial ribosomal RNAs) as the pole plasm of *Drosophila*, and they appear to repress transcription and translation (Kloc et al. 2002). The early movements of amphibian germ plasm have been analyzed in detail by Savage and Danilchik (1993), who labeled the germ plasm with a fluorescent dye. They found that the germ plasm of unfertilized eggs consists of tiny "islands" that appear to be tethered to the yolk mass near the vegetal cortex. These islands move with the vegetal yolk mass during the cortical rotation just after fertilization. After this rotation, the islands are released from the yolk mass and begin fusing together and migrating to the vegetal pole. Their aggregation depends on microtubules, and their movement to the vegetal pole depends on a kinesin-like protein that may act as the motor for germ plasm movement (Robb et al. 1996; Quaas and Wylie 2002). Savage and Danilchik (1993) found that UV light prevents vegetal surface contractions and inhibits the migration of germ plasm to the vegetal pole. Furthermore, the *Xenopus* homologues of *Nanos* and *Vasa* messages are specifically localized to the vegetal region (Figure 16.6; Forristall et al. 1995; Ikenishi et al. 1996; Zhou and King 1996).

ZEBRAFISH In zebrafish, the germ plasm forms a dense structure characterized by polar granules, mitochondria,

FIGURE 16.6 Germ plasm at the vegetal pole of frog embryos. In situ hybridization to the mRNA for *Xcat2* (the *Xenopus* homologue of *Nanos*) localizes the message in the vegetal cortex of first-cleavage (upper) and fourth-cleavage (lower) embryos. (After Kloc et al. 1998, courtesy of L. Etkin.)

and concentrated mRNAs. Two of these mRNAs are *Vasa* and *Nanos*. These messages are maternally supplied, and they appear to be associated with the cleavage furrows of the early dividing egg (Yoon et al. 1997). *Vasa* mRNA and other components of the germ plasm form a compact structure that is inherited by only one of the two daughter cells at each division. Thus, at late cleavage (around 1000 cells), only four cells have the germ plasm. However, after this stage, the germ plasm is distributed evenly at cell division, creating four clusters of primordial germ cells (see Figure 16.12).

Germ cell determination in mammals

In insects, frogs, nematodes, and flies, the germ cells are determined by material in the egg cytoplasm. However, in mammals, there is no obvious germ plasm. Rather, germ cells are *induced* in the embryo (Wakahara 1996; Hayashi et al. 2007).

In mice, the germ cells form at the posterior region of the epiblast, at the junction of the extraembryonic ectoderm, epiblast, primitive streak, and allantois (Figure 16.7A,B). This is called the posterior proximal epiblast because it is close (proximal) to the extraembryonic ectoderm, and it will be at the posterior of the embryo. Thus, the cells that become the PGCs in mice are not intrinsically different from the other cells of the epiblast and contain no specific germ plasm. Rather, the posterior epiblast cells are induced by the extraembryonic tissue. Wnts from the visceral endoderm are probably responsible for giving the posterior proximal epiblast cells the competence to

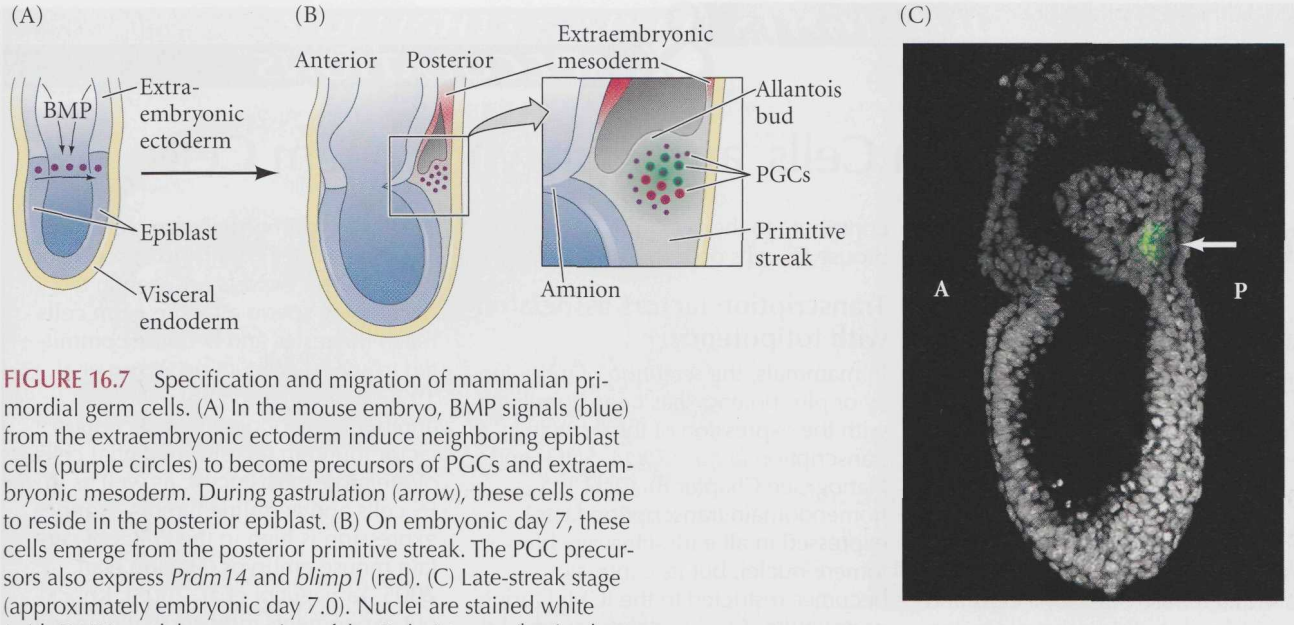


FIGURE 16.7 Specification and migration of mammalian primordial germ cells. (A) In the mouse embryo, BMP signals (blue) from the extraembryonic ectoderm induce neighboring epiblast cells (purple circles) to become precursors of PGCs and extraembryonic mesoderm. During gastrulation (arrow), these cells come to reside in the posterior epiblast. (B) On embryonic day 7, these cells emerge from the posterior primitive streak. The PGC precursors also express *Prdm14* and *blimp1* (red). (C) Late-streak stage (approximately embryonic day 7.0). Nuclei are stained white (with DAPI), and the expression of the *Prdm14* gene (having been fused to the mRNA of a green fluorescent protein) is seen by the green fluorescence. Arrowhead shows expression in the PGC in the extraembryonic mesoderm. A, anterior; P, posterior. (A,B after Hogan 2002; C from Yamaji et al. 2008, courtesy of M. Saitou.)

respond to BMP signals provided by the extraembryonic ectoderm.* This happens during gastrulation (at about day 6.5 in mice), before the germ layers are established (Figure 16.7C; Pfister et al. 2007; Yamaji et al. 2008; Ohinata et al. 2009).

The BMPs induce the expression of *blimp1* and *Prdm14* in a small cluster of cells (about six cells in the 6.5-day mouse embryo). Blimp1 is a transcriptional regulator that represses somatic-type gene expression, while activating those genes (such as *Sox2* and *Nanog*) associated with pluripotency. Blimp1 also activates the germline determinant *Nanos3*, which protects the germ cells against apoptosis during their migration (Tsuda et al. 2003). Prdm14 helps establish pluripotency by also activating Sox2, and it is critical for the chromatin modifications that will later silence the genome of the germ cells (Yamaji et al. 2008). Cells that express *blimp1* and *Prdm14* are restricted to the germ cell fate (Saitou et al. 2002; Ohinata et al. 2005).

The requirement for germ cell induction was shown by transplanting clumps of tissue from the distal portions of the epiblast to the proximal posterior portion of the epiblast. These cells then gave rise to PGCs (Tam and Zhou 1996). Moreover, cultured epiblast cells exposed to Wnt signals and BMP4 gave rise to PGCs. When such PGCs from cultured male epiblast cells were transferred into testicular tubules, they produced viable sperm that could fertilize mouse eggs (Ohinata et al. 2009).

*This induction can occur only in the posteriormost region of the epiblast; BMP antagonists prevent it from occurring in the trunk and anterior.

The inert genome hypothesis

As indicated above, one of the critical events in specifying germ cells appears to be the global repression of gene expression According to this hypothesis, the cells become germ cells because they are forbidden to become any other type of cell (Nieuwkoop and Sutasurya 1981; Wylie 1999; Cinalli et al. 2008). This suppression of transcription is seen in the germ cells of several species, including mammals, flies, frogs, and nematodes (see Figure 16.2). In mice, the germ cells undergo extensive chromatin modification (Seki et al. 2007), causing them to become transcriptionally inert at embryonic day 8.5 (as they begin migrating). Many of the components in the germ plasm (such as Gcl, Pgc, Piwi, and Nanos in *Drosophila* and PIE-1 in *C. elegans*) act by inhibiting either transcription or translation (Leatherman et al. 2002; de las Heras et al. 2009). Many such proteins are found throughout the animal kingdom. It is interesting that when animal germ cells are separated from the somatic cells—whether in chicks, mice, or flies—the germ cells are often specified *outside* the developing body proper. Perhaps this exile into an extraembryonic "enclave" insulates the primordial germ cells from paracrine signaling taking place within the somatic cells of the growing embryo (Dickson 1994). Once the repression of somatic gene expression is accomplished, the germ cells can return to the embryo and travel to the gonads. This germ cell migration will be our next topic.

SIDELIGHTS & SPECULATIONS

Pluripotency, Germ Cells, and Embryonic Stem Cells

Primordial germ cells and embryonic stem cells are both characterized by their ability to generate any cell type in the embryo. Embryonic stem (ES) cells are derived from the inner cell masses of mammalian blastocysts and are believed to be the functional equivalent of the inner cell mass (ICM) blastomeres (see Chapter 8). One of the best pieces of evidence for this equivalence is that when ES cells are injected into the ICMs of mouse blastocysts, they behave like mouse blastocyst cells and contribute cells to the embryo. One of the interesting species-specific differences between human and mouse ES cells is that human ES cells appear to contribute to the trophoblast, whereas mouse ES cells do not (Xu et al. 2002).

Transcription factors associated with totipotency

In mammals, the retention of totipotency or pluripotency has been correlated with the expression of three nuclear transcription factors: Oct4, Stat3, and Nanog (see Chapter 8). Oct4 is a homeodomain transcription factor expressed in all early-cleavage blastomere nuclei, but its expression becomes restricted to the ICM. During gastrulation, Oct4 becomes expressed solely in those posterior epiblast cells thought to give rise to the primordial germ cells. After that, Oct4 is seen only in the primordial germ cells and, later, in oocytes (Figure 16.8; see also Figure 8.18). Oct4 is not seen in the developing sperm after the germ cells reach the testes and become committed to sperm production (Yeom et al. 1996; Pesce et al. 1998). Nanog is another homeodomain transcription factor found in the pluripotential cells of the mouse blastocyst, as well as in ES cells and germline tumors. Nanog expression is high in the PGCs of certain mouse embryos (Hatano et al. 2005; Yamaguchi et al. 2005). Knockout experiments indicate that Nanog is critical in maintaining the pluripotency of stem cells, and overexpression experiments demonstrate that elevated

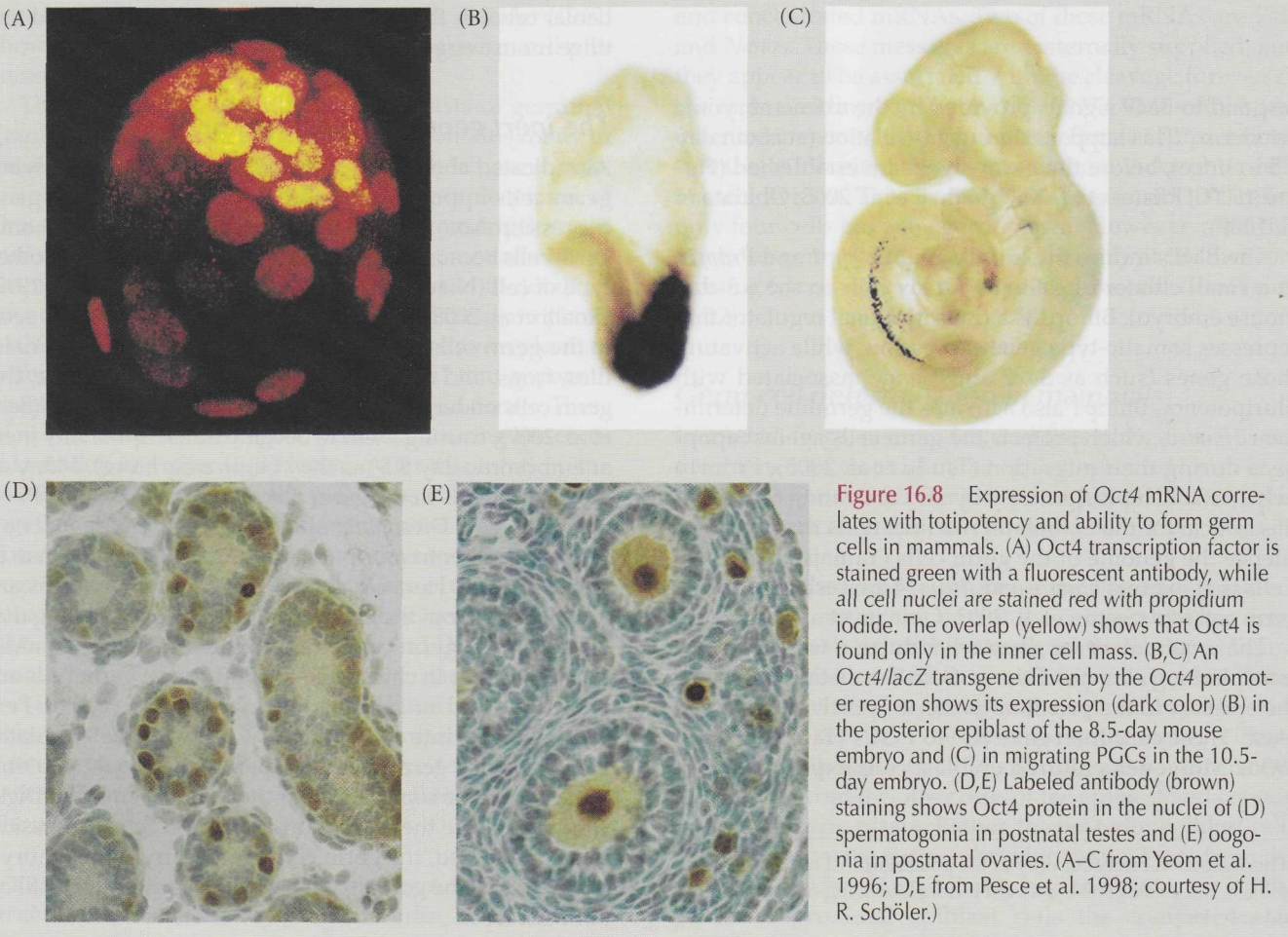


Figure 16.8 Expression of *Oct4* mRNA correlates with totipotency and ability to form germ cells in mammals. (A) Oct4 transcription factor is stained green with a fluorescent antibody, while all cell nuclei are stained red with propidium iodide. The overlap (yellow) shows that Oct4 is found only in the inner cell mass. (B,C) An *Oct4/lacZ* transgene driven by the *Oct4* promoter region shows its expression (dark color) (B) in the posterior epiblast of the 8.5-day mouse embryo and (C) in migrating PGCs in the 10.5-day embryo. (D,E) Labeled antibody (brown) staining shows Oct4 protein in the nuclei of (D) spermatogonia in postnatal testes and (E) oogonia in postnatal ovaries. (A–C from Yeom et al. 1996; D,E from Pesce et al. 1998; courtesy of H. R. Schöler.)

SIDELIGHTS & SPECULATIONS (Continued)

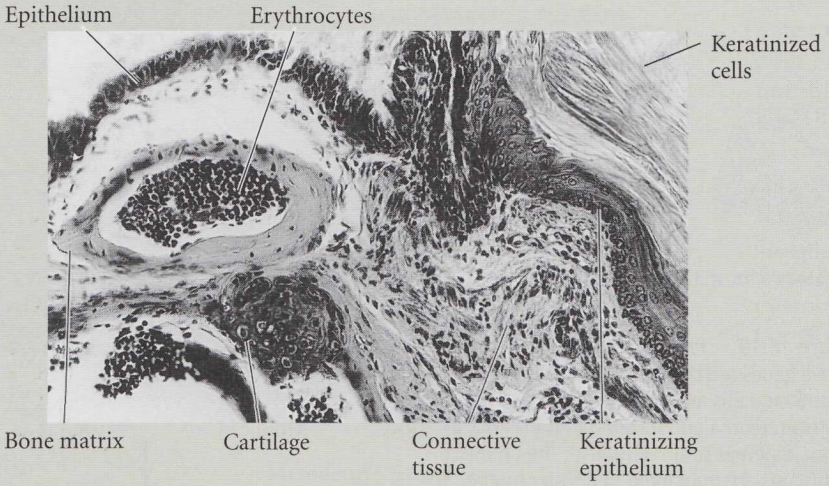


Figure 16.9 Photomicrograph of a section through a mouse teratocarcinoma, showing numerous differentiated cell types. (From Gardner 1982; photograph by C. Graham, courtesy of R. L. Gardner.)

Nanog negates the need for Stat3 and by itself maintains Oct4 transcription in ES cells (Chambers et al. 2003; Mitsui et al. 2003).

The same pluripotency and transcription factor expression pattern is seen not only in the PGCs but in two derivatives of the PGCs: cultured embryonic germ cells, and tumorous germ cells called *teratocarcinomas*.

Embryonic germ cells

When PGCs are first placed into culture, they resemble ES cells. Stem cell factor increases the proliferation of migrating mouse primordial germ cells in culture, and this proliferation can be further increased by adding another growth factor, leukemia inhibition factor (LIF). However, the life span of these PGCs is short, and the cells soon die. But if an additional mitotic regulator—basic fibroblast growth factor, Fgf2—is added, a remarkable change takes place. The cells continue to proliferate, producing pluripotent embryonic stem cells with characteristics resembling those of the inner cell mass (Matsui et al. 1992; Resnick et al. 1992; Rohwedel et al. 1996). These PGC-derived cells are called **embryonic germ (EG) cells**, and they have the potential to differentiate into all the cell types of the body.

In 1998, researchers in John Gerhart's laboratory cultured human EG cells (Shamblott et al. 1998). These cells were able to generate differentiated cells from all three primary germ layers, so they are presumably pluripotent. Such cells could be used medically to create neural or hematopoietic stem cells, which might be used to regenerate damaged neural or blood tissues. EG cells are often considered ES cells, and the distinction of their origin is ignored.

Embryonal carcinoma cells

What happens if a PGC became malignant? In one type of tumor, the germ cells become embryonic stem cells, much like the Fgf2-treated PGCs in the experiment above. This type of tumor is called a **teratocarcinoma**. Whether spontaneous or experimentally produced, teratocarcinomas contain an undifferentiated stem cell population that has biochemical and developmental properties remarkably similar to those of the inner cell mass (Graham 1977; see Parson 2004). Moreover, these stem cells not only divide but can also differentiate into a wide variety of tissues, including gut and respiratory epithelia, muscle, nerve, cartilage, and bone (Figure 16.9). These undifferentiated pluripotential stem cells are called **embryonal carcinoma (EC) cells**. Once differentiated, these cells no longer divide, and are therefore no longer malignant. Such tumors can give rise to most of the tissue types in the body (Stevens and Little 1954; Kleinsmith and Pierce 1964; Kahan and Ephrussi 1970). Thus, the teratocarcinoma stem cells mimic early mammalian development, but the tumor they form is characterized by random, haphazard development.

In 1981, Stewart and Mintz formed a mouse from cells derived in part from a teratocarcinoma stem cell. Stem cells that had arisen in a teratocarcinoma of an agouti (yellow-tipped) strain of mice were cultured for several cell generations and were seen to maintain the characteristic chromosome complement of the parental mouse. Individual stem cells descended from the tumor were injected into the blastocysts of black-furred mice. The blastocysts were then transferred to the uterus of a foster mother, and live mice were born. Some of these mice had coats of two colors, indicating that the tumor cell had integrated itself into the embryo. This, in itself, is a remarkable demonstration that the tissue context is critical for the phenotype of a cell—a malignant cell was made nonmalignant.

But the story does not end here. When these chimeric mice were mated to mice carrying alleles recessive to those of the original tumor cell, the alleles of the tumor cell were expressed in many of the offspring. This means that the originally malignant tumor cell had produced many, if not all, types of normal somatic cells, and had even produced normal, functional germ cells! When such mice (being heterozygous for tumor cell genes) were mated with each other, the resulting litter contained mice that were homozygous for a large number of genes from the tumor cell (Figure 16.10). Thus, germ cell tumors can retain their pluripotency.

Germ cells and stem cells: Possible interactions

One idea emerging from this study is that some descendants of the pluripotent cells (such as the teratocarcinoma or ES cells) form PGCs that can undergo meiosis to form sperm or eggs. Indeed, there is evidence that ES cells

(Continued on next page)

SIDELIGHTS & SPECULATIONS (Continued)

can develop into oogonia that enter meiosis and recruit adjacent cells into follicle-like structures (Hübner et al. 2003). There is also parallel evidence that mouse ES cells can be made to differentiate into spermatocytes that can become functional sperm when transplanted into testes (Toyooka et al. 2003). These studies need to be extensively confirmed and extended to humans if they are to provide a new way of curing infertility.

It is even possible that ES, EG, and EC cells have a common origin in the presumptive PGC cells. Zwaka and Thomson (2005) hypothesize that the ES cells are actually the equivalent of PGCs and not of the inner cell mass. Not every inner cell mass blastomere can become an ES cell, and Zwaka and Thomson suggest that perhaps the successful stem cells are those that have been positioned next to trophoblast at the future posterior proximal region of the embryo. In other words, the blastomeres that become the ES cells might actually be the presumptive PGCs. While this idea remains hypothetical, it would relate these four pluripotent cell types.

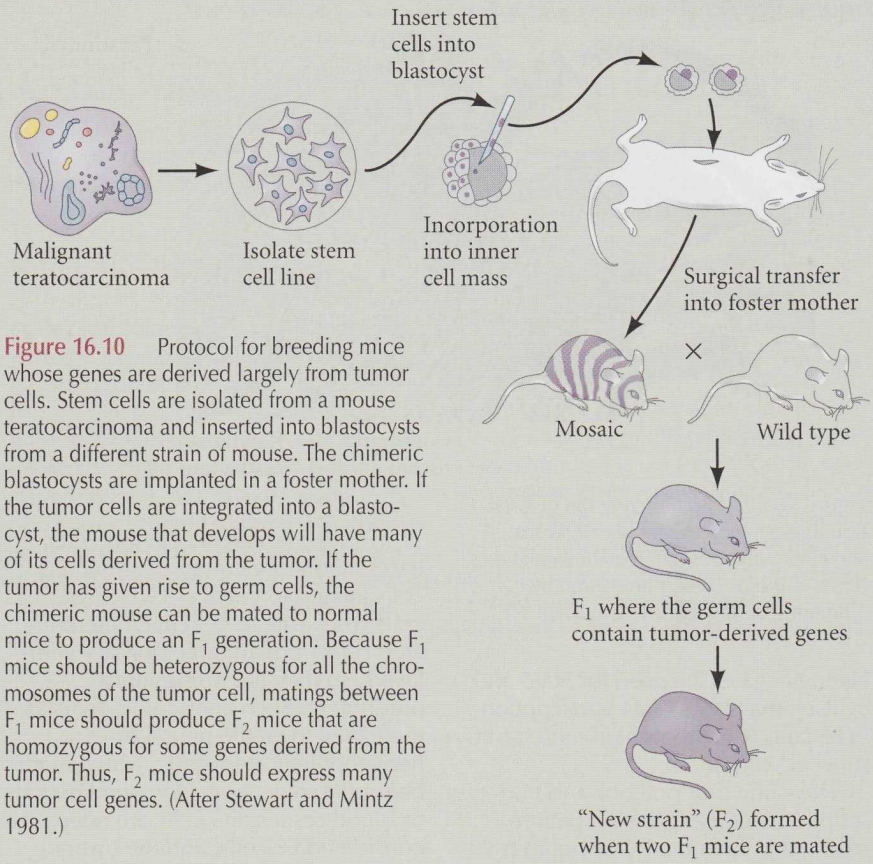


Figure 16.10 Protocol for breeding mice whose genes are derived largely from tumor cells. Stem cells are isolated from a mouse teratocarcinoma and inserted into blastocysts from a different strain of mouse. The chimeric blastocysts are implanted in a foster mother. If the tumor cells are integrated into a blastocyst, the mouse that develops will have many of its cells derived from the tumor. If the tumor has given rise to germ cells, the chimeric mouse can be mated to normal mice to produce an F_1 generation. Because F_1 mice should be heterozygous for all the chromosomes of the tumor cell, matings between F_1 mice should produce F_2 mice that are homozygous for some genes derived from the tumor. Thus, F_2 mice should express many tumor cell genes. (After Stewart and Mintz 1981.)

Germ Cell Migration

Germ cell migration in Drosophila

During *Drosophila* embryogenesis, the primordial germ cells move from the posterior pole to the gonads. The first step in this migration is a passive one, wherein the 30–40 pole cells are displaced into the posterior midgut by the movements of gastrulation (Figure 16.11A,B). The germ cells are actively prevented from migrating during this stage (Jaglarz and Howard 1994; Li et al. 2003). In the second step, the gut endoderm triggers the germ cells to actively migrate by diapedesis (i.e., squeezing amoebically) through the blind end of the posterior midgut (Kunwar et al. 2003). The germ cells migrate from the endoderm into the visceral mesoderm. In the third step, the PGCs split into two groups, each of which will become associated with a developing gonad primordium.

In the fourth step, the germ cells migrate to the gonads, which are derived from the lateral mesoderm of parasegments 10–12 (Warrior 1994; Jaglarz and Howard 1995; Broihier et al. 1998). This step involves both attraction and repulsion. The products of the *wunen* genes appear to be responsible for directing the migration of the primordial germ cells from the endoderm into the mesoderm and their division into two streams (Figure 16.11C–E). This protein is expressed in the endoderm immediately before PGC migration and in many other tissues that the germ cells avoid, and it appears to be repelling the germ cells. In loss-of-function mutants of this gene, the PGCs wander randomly (Zhang et al. 1997; Hanyu-Nakamura et al. 2004; Sano et al. 2005).

HMG-CoA reductase, the product of the *columbus* gene, appears to be critical for attracting the *Drosophila* PGCs to the gonads (Van Doren et al. 1998). This protein is made in the mesodermal cells of the gonads and probably acts as part of a biosynthetic pathway required to produce lipids that either modulate the activity of a germ cell attractant or act directly to attract PGCs (Ricardo and Lehmann 2009). In loss-of-function mutants of this gene, the PGCs wander randomly from the endoderm, and if the *columbus* gene is expressed in other tissues (such as the nerve cord), those tissues will attract the PGCs. In the last step, the gonad coalesces around the germ cells, allowing the germ cells to

SIDELIGHTS & SPECULATIONS (Continued)

can develop into oogonia that enter meiosis and recruit adjacent cells into follicle-like structures (Hübner et al. 2003). There is also parallel evidence that mouse ES cells can be made to differentiate into spermatocytes that can become functional sperm when transplanted into testes (Toyooka et al. 2003). These studies need to be extensively confirmed and extended to humans if they are to provide a new way of curing infertility.

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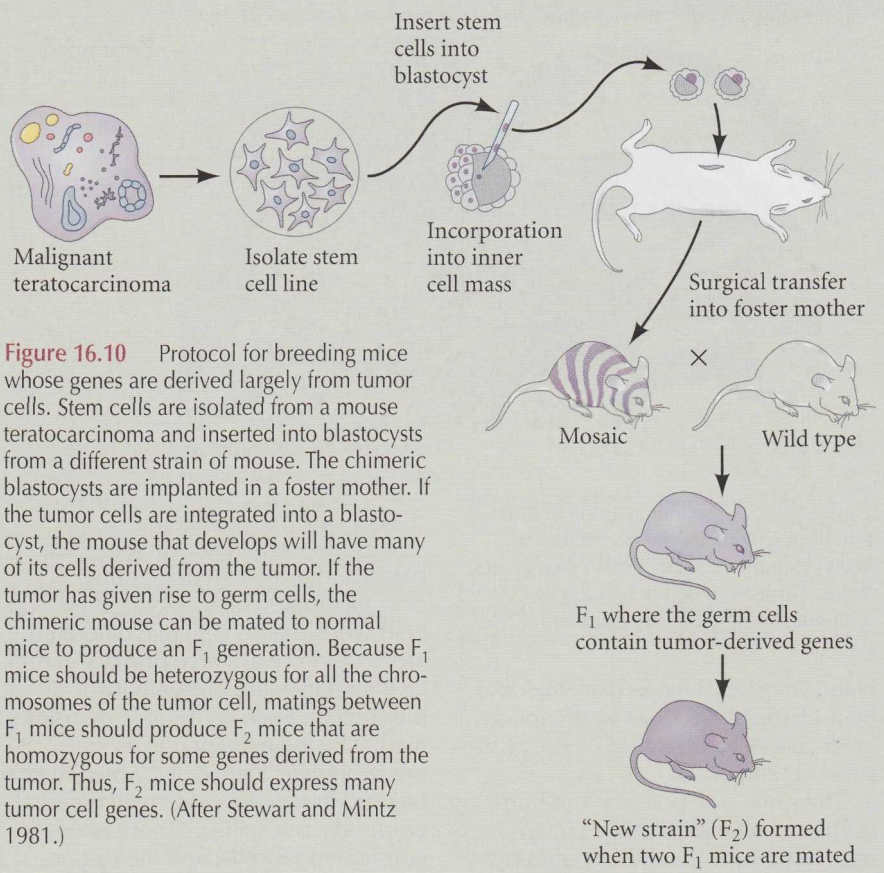

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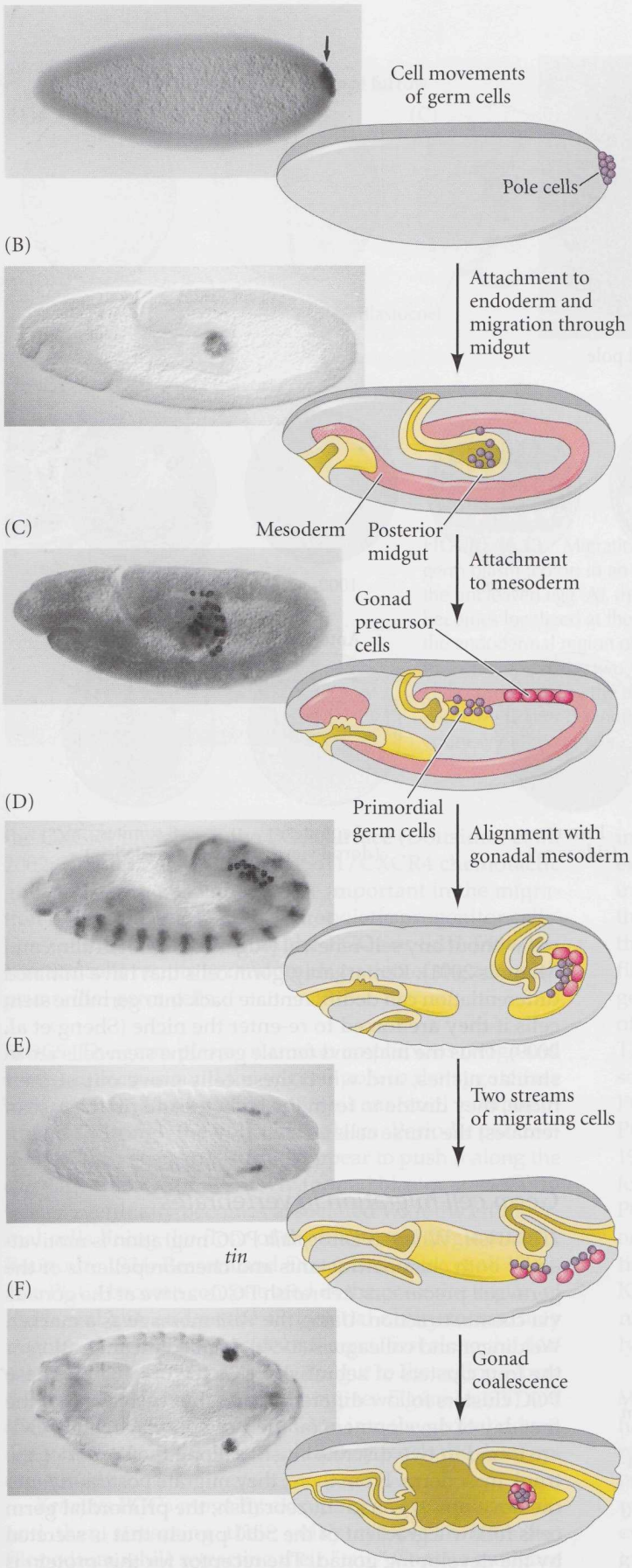


divide and mature into gametes (Figure 16.11F). This step requires E-cadherin (Jenkins et al. 2003).

Neither the gonads nor the germ cells differentiate until metamorphosis. During the larval stages, both the PGCs and the somatic gonadal cells divide, but they remain relatively undifferentiated. At the larval-pupal transition, gonadal morphogenesis occurs (Godt and Laski 1995; King 1970). During this transition, those PGCs in the anterior region of the gonad become the germline stem cells (Asaoka and Lin 2004), which divide asymmetrically to produce both another stem cell and a cystoblast. The cystoblasts eventually develop into an egg chamber (King 1970; Zhu and Xie 2003; see Chapter 6).

We are just beginning to understand how the germline stem cells retain their stem cell properties in the gonad (Gilboa and Lehmann 2004). As mentioned in Part Opener III, stem cells must be in a "niche" that supports their proliferation and inhibits their differentiation. Daughter cells that travel outside this niche begin differentiating. In ovaries, the germline stem cells are attached to the stromal cap, where they are maintained by the BMP4-like factor Decapentaplegic (Dpp). Dpp protein represses the gene encoding a transcription factor (Bag-of-marbles) that initiates oogenesis; Dpp cannot reach cells that leave the stromal cap. Without Dpp to repress the *bag-of marbles* gene, the germline cell begins the developmental cascade that produces the 15 nurse cells and the single oocyte (Chen and McKearin 2003; Decotto and Spradling 2005).

The stem cells of the male germ line are connected to "hub" cells that create a stem cell microenvironment by secreting BMP signals as well as the Unpaired protein. Unpaired activates the JAK-STAT pathway in the germline stem cells (see Figure 3.23). If the JAK-STAT signaling pathway is disrupted, the germline stem cells differentiate into spermatogo-

FIGURE 16.11 Migration of germ cells in the *Drosophila* embryo. The left column shows the germ plasm as stained by antibodies to Vasa, a protein component of the germ plasm (D has been counterstained with antibodies to Engrailed protein to show the segmentation; E and F are dorsal views). The right column diagrams the movements of the germ cells. (A) Germ cells originate from the pole plasm at the posterior end of the egg. (B) Passive movements carry the PGCs into the posterior midgut. (C) PGCs move through the endoderm and into the caudal visceral mesoderm by diapedesis. The *wunen* gene product expressed in the endoderm expels the PGCs, while the product of the *columbus* gene expressed in the caudal mesoderm attracts them. (D–F) Movements of the mesoderm bring the PGCs into the region of parasegments 10–12, where the mesoderm coalesces around them to form the gonads. (Photographs from Warrior et al. 1994, courtesy of R. Warrior; diagrams after Howard 1998.)

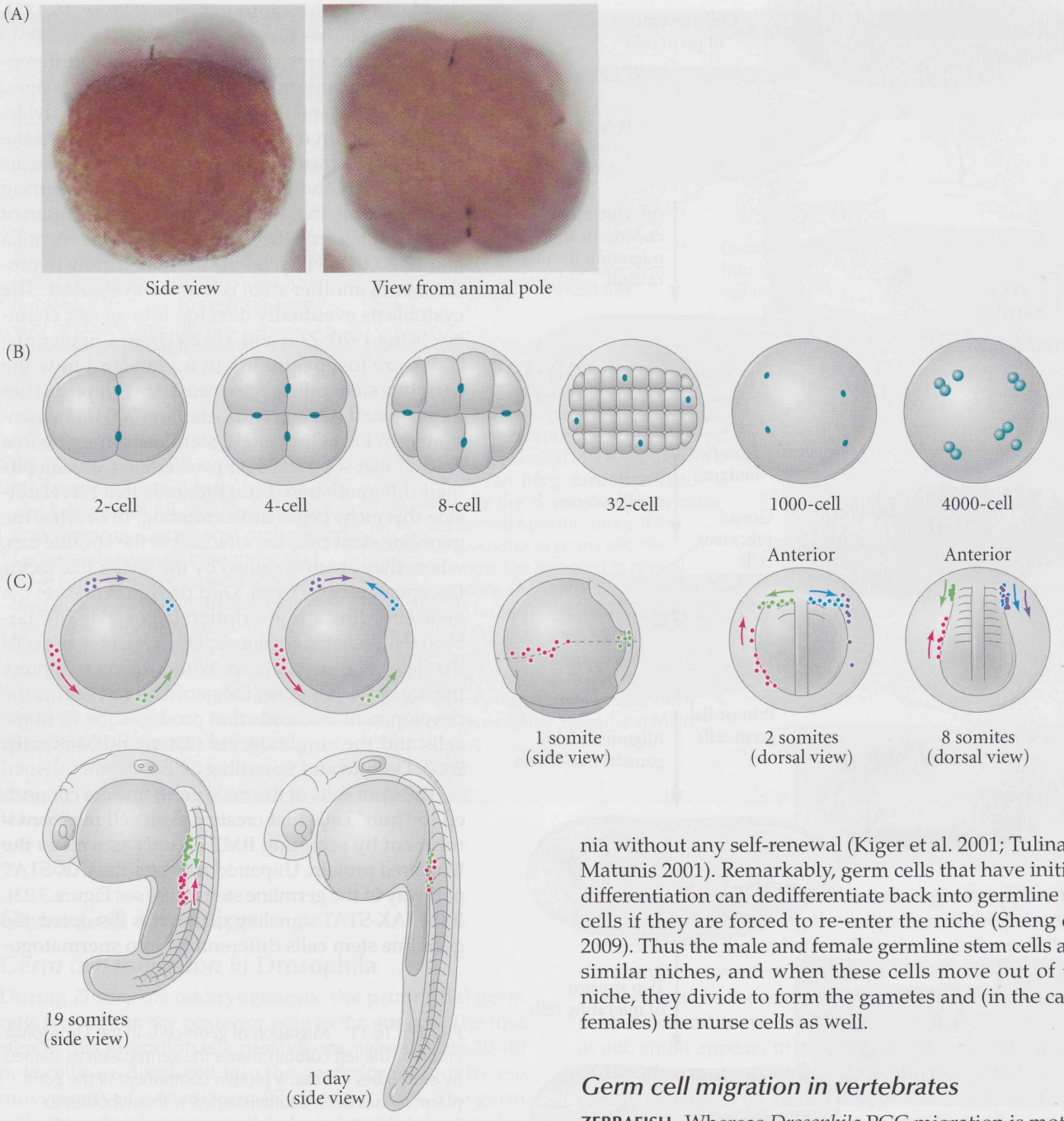


FIGURE 16.12 Specification and migration of germ cells in zebrafish. (A) In situ hybridization of *Vasa* mRNA, showing the accumulation of these messages and germ plasm along the cleavage planes during the first two divisions of the *Danio rerio* embryo. (B,C) Germ plasm movement during zebrafish cleavage (B) and further development (C). The earlier embryos are viewed from the animal pole; other view orientations are noted. The germ plasm remains in four clusters (colored dots). Movement of the clusters is probably caused by both attractive and repulsive chemical interactions. The route to the gonad is directed by the Sdf1 chemoattractant. (A courtesy of N. Hopkins; B,C after Yoon 1997.)

nia without any self-renewal (Kiger et al. 2001; Tulina and Matunis 2001). Remarkably, germ cells that have initiated differentiation can dedifferentiate back into germline stem cells if they are forced to re-enter the niche (Sheng et al. 2009). Thus the male and female germline stem cells are in similar niches, and when these cells move out of their niche, they divide to form the gametes and (in the case of females) the nurse cells as well.

Germ cell migration in vertebrates

ZEBRAFISH Whereas *Drosophila* PGC migration is motivated by both chemoattractants and chemorepellents of the germ cell precursors, zebrafish PGCs arrive at the gonads via chemoattraction. Using the *Vasa* message as a marker, Weidinger and colleagues (1999) detailed the migration of the four clusters of zebrafish PGCs (Figure 16.12). These PGC clusters follow different routes, but by the end of the first day of development (at the 1-somite stage), the PGCs are found in two discrete clusters along the border of the trunk mesoderm. From there, they migrate posteriorly into the developing gonad. In zebrafish, the primordial germ cells follow a gradient of the Sdf1 protein that is secreted by the developing gonad. The receptor for this protein is

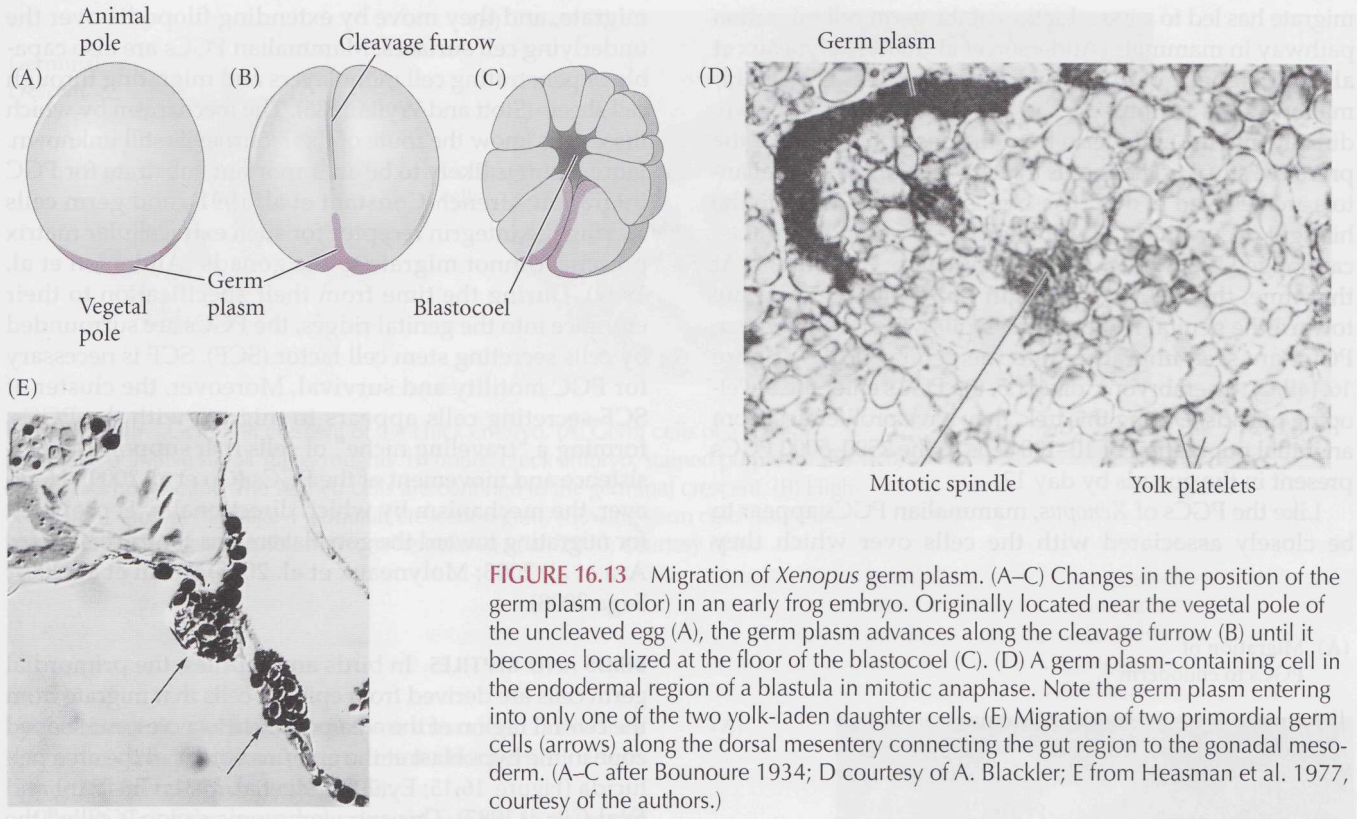


FIGURE 16.13 Migration of *Xenopus* germ plasm. (A–C) Changes in the position of the germ plasm (color) in an early frog embryo. Originally located near the vegetal pole of the uncleaved egg (A), the germ plasm advances along the cleavage furrow (B) until it becomes localized at the floor of the blastocoel (C). (D) A germ plasm-containing cell in the endodermal region of a blastula in mitotic anaphase. Note the germ plasm entering into only one of the two yolk-laden daughter cells. (E) Migration of two primordial germ cells (arrows) along the dorsal mesentery connecting the gut region to the gonadal mesoderm. (A–C after Bounoure 1934; D courtesy of A. Blackler; E from Heasman et al. 1977, courtesy of the authors.)

the CXCR4 protein on the PGC surface (Doitsidou et al. 2002; Knaut et al. 2003). This Sdf1/CXCR4 chemotactic guidance system is known to be important in the migration of lymphocytes and hematopoietic progenitor cells. Loss of either CXCR4 from the PGCs or Sdf1 from the somatic cells results in random migration of the zebrafish primordial germ cells.

FROGS The germ plasm of anuran amphibians (frogs and toads) collects around the vegetal pole in the zygote (see Figure 16.6). During cleavage, this material is brought upward through the yolky cytoplasm. Periodic contractions of the vegetal cell surface appear to push it along the cleavage furrows of the newly formed blastomeres. Germ plasm eventually becomes associated with the endodermal cells lining the floor of the blastocoel (Figure 16.13; Bounoure 1934; Ressom and Dixon 1988; Kloc et al. 1993). The PGCs become concentrated in the posterior region of the larval gut, and as the abdominal cavity forms, they migrate along the dorsal side of the gut, first along the dorsal mesentery (which connects the gut to the region where the mesodermal organs are forming; see Figure 16.13E) and then along the abdominal wall and into the genital ridges. They migrate up this tissue until they reach the developing gonads.

Xenopus PGCs move by extruding a single filopodium and then streaming their yolky cytoplasm into that filopodium while retracting their "tail." Contact guidance in this migration seems likely, as both the PGCs and the extracellular matrix over which they migrate are oriented in the direction of the migration (Wylie et al. 1979). Furthermore, PGC adhesion and migration can be inhibited if the mesentery is treated with antibodies against *Xenopus* fibronectin (Heasman et al. 1981). Thus, the pathway for germ cell migration in these frogs appears to be composed of an oriented fibronectin-containing extracellular matrix. The fibrils over which the PGCs travel lose this polarity soon after migration has ended. As they migrate, *Xenopus* PGCs divide about three times, so that approximately 30 PGCs will colonize the gonads (Whitington and Dixon 1975; Wylie and Heasman 1993). These cells will divide to form the germ cells. The mechanism by which the *Xenopus* PGCs are directed to the gonad involves a CXCR4 protein on the PGC responding to a Sdf1 ligand along the migration path (Nishiumi et al. 2005; Takeuchi et al. 2010). Knocking out the the CXCR4 mRNA with morpholinos results in fewer PGCs reaching the gonads, and ectopically expressing Sdf1 will misdirect the PGCs into other areas.

MAMMALS Based on differential staining of fixed tissue, it had long been thought that the mouse germ cell precursors migrated from the posterior epiblast into the extraembryonic mesoderm and then back again into the embryo by way of the allantois (see Chiquoine 1954; Mintz 1957). However, the ability to label mouse primordial germ cells with green fluorescent protein and to watch these living cells

migrate has led to a reevaluation of the germ cell migration pathway in mammals (Anderson et al. 2000; Molyneaux et al. 2001; Tanaka et al. 2005). First, it appears that mammalian PGCs forming in the posterior epiblast migrate directly into the endoderm from the posterior region of the primitive streak. (Those cells that are seen to enter the allantois are believed to die.) These cells find themselves in the hindgut (Figure 16.14A). Although they move actively, they cannot get out of the gut until about embryonic day 9. At that time, the PGCs exit the gut but do not yet migrate toward the genital ridges. By the following day, however, PGCs are seen migrating into the genital ridges (Figure 16.14B,C). By embryonic day 11.5, the PGCs enter the developing gonads. During this trek, they have proliferated from an initial population of 10–100 cells to the 2500–5000 PGCs present in the gonads by day 12.

Like the PGCs of *Xenopus*, mammalian PGCs appear to be closely associated with the cells over which they migrate, and they move by extending filopodia over the underlying cell surfaces. Mammalian PGCs are also capable of penetrating cell monolayers and migrating through cell sheets (Stott and Wylie 1986). The mechanism by which these cells know the route of their journey is still unknown. Fibronectin is likely to be an important substrate for PGC migration (ffrench-Constant et al. 1991), and germ cells lacking the integrin receptor for such extracellular matrix proteins cannot migrate to the gonads (Anderson et al. 1999). During the time from their specification to their entrance into the genital ridges, the PGCs are surrounded by cells secreting stem cell factor (SCF). SCF is necessary for PGC motility and survival. Moreover, the cluster of SCF-secreting cells appears to migrate with the PGCs, forming a "traveling niche" of cells that support the persistence and movement of the PGCs (Gu et al. 2009). However, the mechanism by which directionality is provided for migrating toward the gonads remains controversial (see Ara et al. 2003; Molyneaux et al. 2003; Farini et al. 2007; Saga 2008).

BIRDS AND REPTILES In birds and reptiles, the primordial germ cells are derived from epiblast cells that migrate from the central region of the area pellucida to a crescent-shaped zone in the hypoblast at the anterior border of the area pellucida (Figure 16.15; Eyal-Giladi et al. 1981; Ginsburg and Eyal-Giladi 1987). This extraembryonic region is called the **germinal crescent**, and the PGCs multiply there.

Unlike those of amphibians and mammals, the PGCs of birds and reptiles migrate to the gonads primarily by means of the bloodstream (Figure 16.16). When blood vessels form in the germinal crescent (anterior to the future head region), the PGCs enter those vessels and are carried

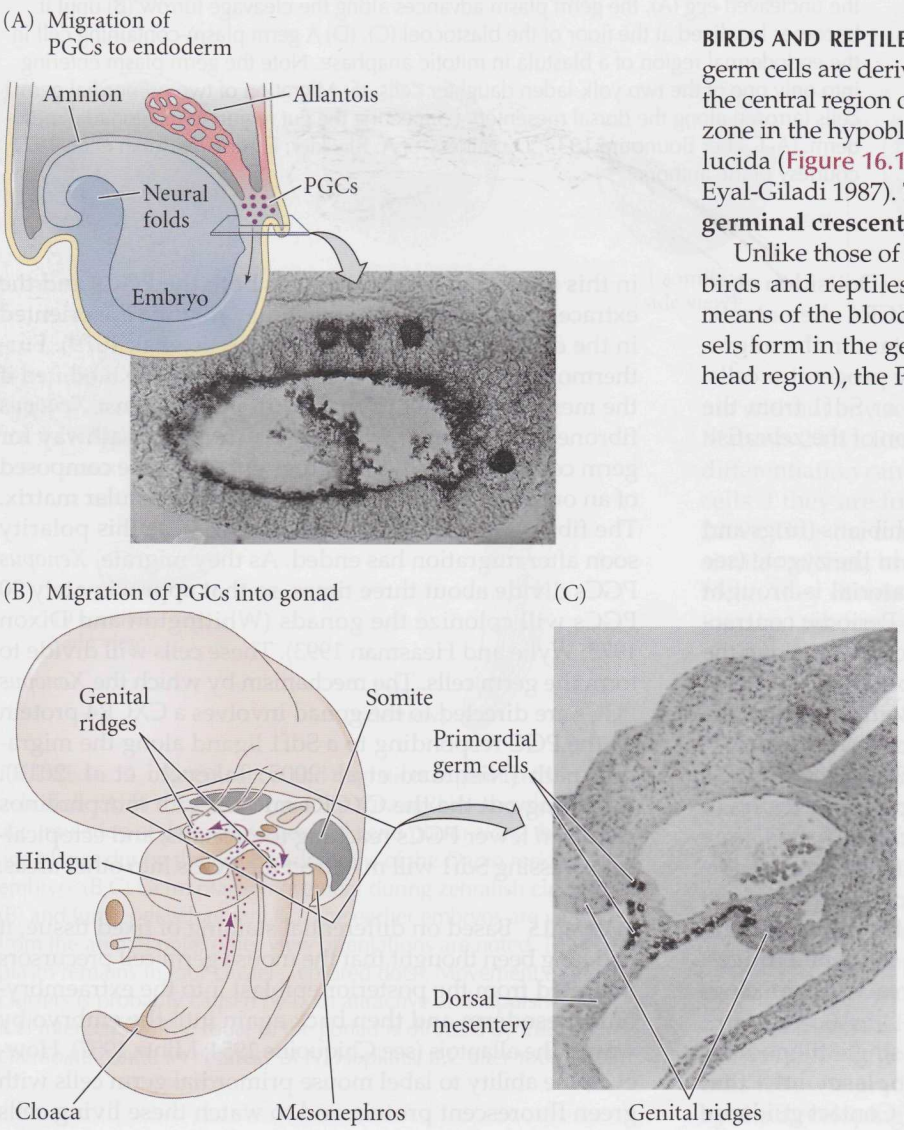


FIGURE 16.14 Primordial germ cell migration in the mouse. (A) On day 8, the PGCs established in the posterior epiblast (see Figure 16.7) migrate into the definitive endoderm of the embryo. The photo shows four large PGCs (stained for alkaline phosphatase) in the hindgut of a mouse embryo. (B) The PGCs migrate through the gut and, dorsally, into the genital ridges. (C) Alkaline phosphatase-staining cells are seen entering the genital ridges around embryonic day 11. (A from Heath 1978; C from Mintz 1957, courtesy of the author.)

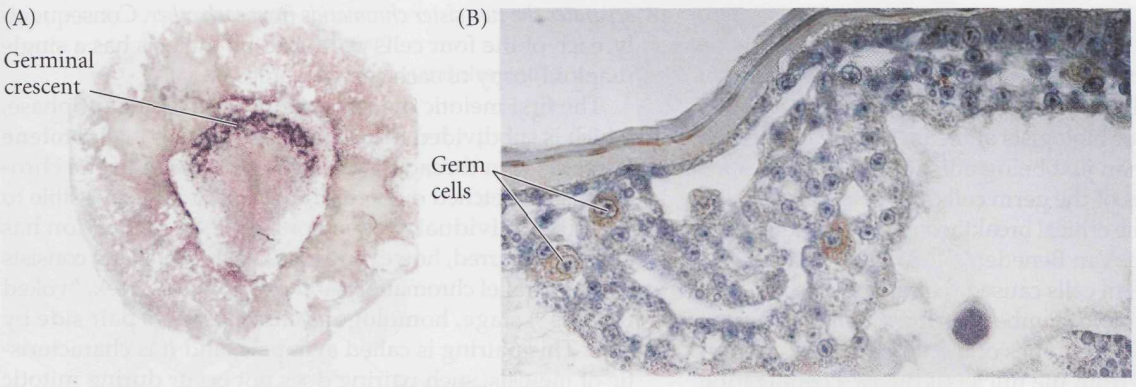


FIGURE 16.15 Germinal crescent of the chick embryo. (A) Germ cells of a stage 4 (definitive primitive streak stage, roughly 18 hours) chick embryo, stained purple for the chick Vasa homologue. The stained cells are confined to the germinal crescent. (B) Higher magnification of the stage 4 germinal crescent region, showing germ cells (stained brown) in the thickened epiblast. (From Tsunekawa et al. 2000, courtesy of N. Tsunekawa.)

by the circulation to the intermediate mesoderm. Here they leave the circulation and migrate into the genital ridges (Swift 1914; Nakamura et al. 2007).

The PGCs of the germinal crescent appear to enter the blood vessels by diapedesis, a type of amoeboid movement common to lymphocytes and macrophages that enables cells to squeeze between the endothelial cells of small blood vessels. In some as-yet-undiscovered way, the PGCs are instructed to exit the blood vessels and enter the gonads (Pasteels 1953; Dubois 1969; Nakamura et al. 2007). Evidence for chemotaxis comes from studies in which circulating chick PGCs were isolated from the blood and cultured between gonadal rudiments and other embryonic tissues (Kuwana et al. 1986). During a 3-hour incubation, *the PGCs migrated specifically into the gonadal rudiments.*

The molecules that chick PGCs use for chemotaxis may be the same Sdf1/CXCR4 chemotactic system seen in zebrafish. Like mammals, chicks only use chemotaxis during the latter stages of migration. Thus, after they leave the blood vessels, chick PGCs appear to utilize Sdf1 gradients to reach the gonads (Stebler et al. 2004). Indeed, if Sdf1-secreting cells are transplanted into late-stage chick embryos, the PGCs will be attracted to them.

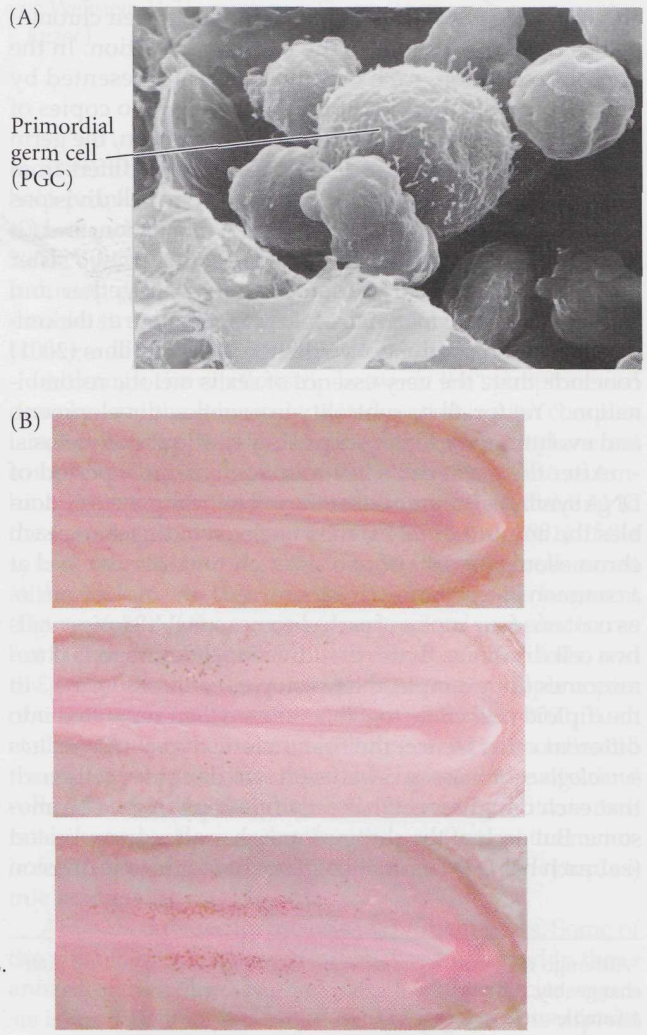


FIGURE 16.16 Migration of primordial germ cells in the chick embryo. (A) Scanning electron micrograph of a chick PGC in a capillary of a gastrulating embryo. Note the larger size of the PGC, as well as the microvilli on its surface. (B) After leaving the blood vessels, PGCs migrate into the intermediate mesodermal region that forms the gonad. These whole mounts show chick PGCs (stained with antibodies against the Vasa protein) in the posterior region of stage 14, 15, and 17 embryos. (A from Kuwana 1993, courtesy of T. Kuwana; B from Nakamura et al. 2007, courtesy of T. Takahiro.)

Meiosis

Meiosis is perhaps the most revolutionary invention of eukaryotes. It is difficult now to appreciate how startling this concept was for biologists at the end of the nineteenth century. Rather than just being a list of Greek names for the different stages of the germ cell cycle, the discovery of meiosis signaled the critical breakthrough for the investigation of inheritance. Van Beneden's 1883 observations that the divisions of germ cells caused the resulting gametes to contain half the diploid number of chromosomes "demonstrated that the chromosomes of the offspring are derived in equal numbers from the nuclei of the two conjugating germ-cells and hence equally from the two parents" (Wilson 1924). All subsequent theories of heredity, including the Sutton-Boveri model that united Mendelism with cell biology, are based on meiosis as the mechanism for sexual reproduction and the transmission of genes from one generation to the next. So let's return to the primordial germ cells that have migrated to the gonads.

Once in the gonad, the PGCs continue to divide mitotically, often producing millions of potential gamete precursors. The germ cells of both male and female gonads are then faced with the necessity of reducing their chromosomes from the diploid to the haploid condition. In the haploid condition, each chromosome is represented by only one copy, whereas diploid cells have two copies of each chromosome. To accomplish this reduction, the germ cells undergo **meiosis** (see Figure 1.5). Meiosis differs from mitosis in that (1) meiotic cells undergo two cell divisions without an intervening period of DNA replication, and (2) homologous chromosomes (each consisting of two sister chromatids joined at a kinetochore) pair together and recombine genetic material. Meiosis is therefore at the center of sexual reproduction. Villeneuve and Hillers (2001) conclude that "the very essence of sex is meiotic recombination." Yet for all its centrality in genetics, development, and evolution, we know surprisingly little about meiosis.

After the germ cell's last mitotic division, a period of DNA synthesis occurs, so that the cell initiating meiosis doubles the amount of DNA in its nucleus. In this state, each chromosome consists of two sister **chromatids** attached at a common kinetochore.* (In other words, the diploid nucleus contains four copies of each chromosome.) Meiosis entails two cell divisions. In the first division, homologous chromosomes (for example, the two copies of chromosome 3 in the diploid cell) come together and are then separated into different cells. Hence, the first meiotic division *splits two homologous chromosomes* between two daughter cells such that each daughter cell has only one copy of the chromosome. But each of the chromosomes has already replicated (i.e., each has two chromatids). The second meiotic division *separates the two sister chromatids from each other*. Consequently, each of the four cells produced by meiosis has a single (haploid) copy of each chromosome.

The first meiotic division begins with a long prophase, which is subdivided into five stages. During the **leptotene** (Greek, "thin thread") stage, the chromatin of the chromatids is stretched out very thinly, and it is not possible to identify individual chromosomes. DNA replication has already occurred, however, and each chromosome consists of two parallel chromatids. At the **zygotene** (Greek, "yoked threads") stage, homologous chromosomes pair side by side. This pairing is called **synapsis**, and it is characteristic of meiosis; such pairing does not occur during mitotic divisions. Although the mechanism whereby each chromosome recognizes its homologue is not known (see Barzel and Kupiec 2008), synapsis seems to require the presence of the nuclear membrane and the formation of a proteinaceous ribbon called the **synaptonemal complex**. This complex is a ladder-like structure with a central element and two lateral bars (von Wettstein 1984; Schmekel and Daneholt 1995). The chromatin becomes associated with the two lateral bars, and the chromosomes are thus joined together (Figure 16.17A,B).

Examinations of meiotic cell nuclei with the electron microscope (Moses 1968; Moens 1969) suggest that paired chromosomes are bound to the nuclear membrane, and Comings (1968) has suggested that the nuclear envelope helps bring together the homologous chromosomes. The configuration formed by the four chromatids and the synaptonemal complex is referred to as a **tetrad** or a **bivalent**.

During the next stage of meiotic prophase, **pachytene** (Greek, "thick thread"), the chromatids thicken and shorten. Individual chromatids can now be distinguished under the light microscope, and crossing over may occur. **Crossing over** represents an exchange of genetic material whereby genes from one chromatid are exchanged with homologous genes from another. Crossing over may continue into the next stage, **diplotene** (Greek, "double threads"). Here, the synaptonemal complex breaks down, and the two homologous chromosomes start to separate. Usually, however, they remain attached at various points called **chiasmata**, which are thought to represent regions where crossing over is occurring (Figure 16.17C). The diplotene stage is characterized by a high level of gene transcription. In some species, the chromosomes of both male and female germ cells take on the "lampbrush" appearance characteristic of chromosomes that are actively making RNA (see below).

During the next stage, **diakinesis** (Greek, "moving apart"), the kinetochores move away from each other, and the chromosomes remain joined only at the tips of the chromatids. This last stage of meiotic prophase ends with the breakdown of the nuclear membrane and the migration of the chromosomes to the **metaphase plate**. Anaphase of meiosis I does not commence until the chromosomes are properly aligned on the mitotic spindle fibers. This alignment is accomplished by proteins that prevent cyclin B

*Although the terms *centromere* and *kinetochore* are often used interchangeably, the *kinetochore* is the complex protein structure that assembles on a sequence of DNA known as the *centromere*.

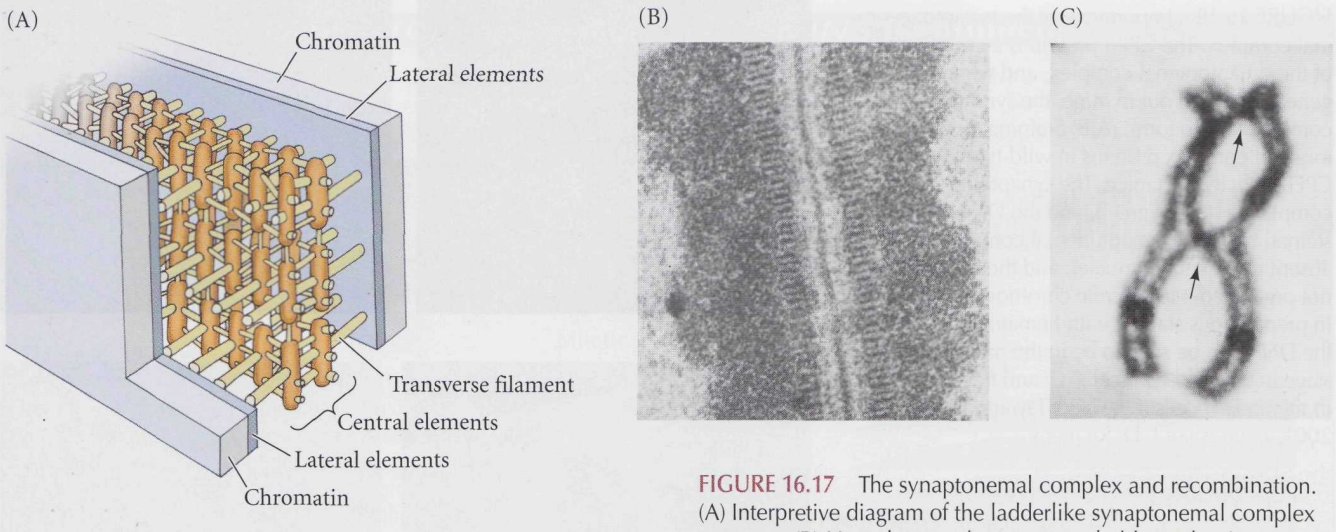


FIGURE 16.17 The synaptonemal complex and recombination. (A) Interpretive diagram of the ladderlike synaptonemal complex structure. (B) Homologous chromosomes held together in a synaptonemal complex during the zygotene phase of the first meiotic prophase in a *Neottiella* (mushroom) oocyte. (C) Chiasmata in diplotene bivalent chromosomes of salamander oocytes. Kinetochores are visible as darkly stained circles; the arrows point to the two chiasmata. (A after Schmekel and Daneholt 1995; B from von Wettstein 1971, courtesy of D. von Wettstein; C courtesy of J. Kezer.)

from being degraded until after all the chromosomes are securely fastened to microtubules. If these proteins are deficient, aneuploidies such as Down syndrome can occur (Homer et al. 2005; Steuerwald et al. 2005).

During anaphase I, the homologous chromosomes are separated from each other in an independent fashion. This stage leads to telophase I, during which two daughter cells are formed, each cell containing one partner of each homologous chromosome pair. After a brief **interkinesis**, the second division of meiosis takes place. During this division, the kinetochore of each chromosome divides during anaphase so that each of the new cells gets one of the two chromatids, the final result being the creation of four haploid cells. Note that meiosis has also reassorted the chromosomes into new groupings. First, each of the four haploid cells has a different assortment of chromosomes. Humans have 23 different chromosome pairs; thus 2^{23} (nearly 10 million) different haploid cells can be formed from the genome of a single person. In addition, the crossing-over that occurs during the pachytene and diplotene stages of prophase I further increases genetic diversity and makes the number of potential different gametes incalculably large.

See WEBSITE 16.4
Human meiosis

Meiosis is the core of sexual reproduction, and it seems to have arisen in the ancestor of fungi, plants, and animals. Indeed, nearly all the genes and proteins used in yeast (fungus) meiosis also function in mammalian meiosis. This observation has allowed the identification of a "core meiotic recombination complex" used by plants, fungi, and animals. This meiotic recombination complex is built on rings of the **cohesin proteins**, which encircle the sister chromatids. The rings of cohesin resist the pulling forces of the spindle microtubules and thereby keep the sister chromatids attached together (Haering et al. 2008; Brar et al. 2009). This complex recruits another set of proteins that help promote pairing between the homologous chromosomes and allow recombination to occur (Pelttari et al. 2001; Villeneuve and Hillers 2001). These recombination-inducing proteins are involved in making and repairing double-stranded DNA breaks. The cohesin proteins will be degraded in the second meiotic division.

Although the relationship between the synaptonemal complex and the cohesin-recruited recombination complex is not clear, it appears in mammals that the synaptonemal complex stabilizes the associations initiated by the recombination complex, giving a morphological scaffolding to the tenuous protein connections (Pelttari et al. 2001). If the synaptonemal complex fails to form, the germ cells arrest at the pachytene stage and their chromosomes fragment (Figure 16.18). If the murine synaptonemal complex forms but lacks certain proteins, chiasmata formation fails, and the germ cells are often aneuploid (having multiple copies of one or more chromosomes) (Tay and Richter 2001; Yuan et al. 2002). 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**.

Animals can modify meiosis in different ways. Some of the most interesting modifications have occurred in those animal species that have no males. In these species, meiosis is modified such that the resulting gamete is diploid

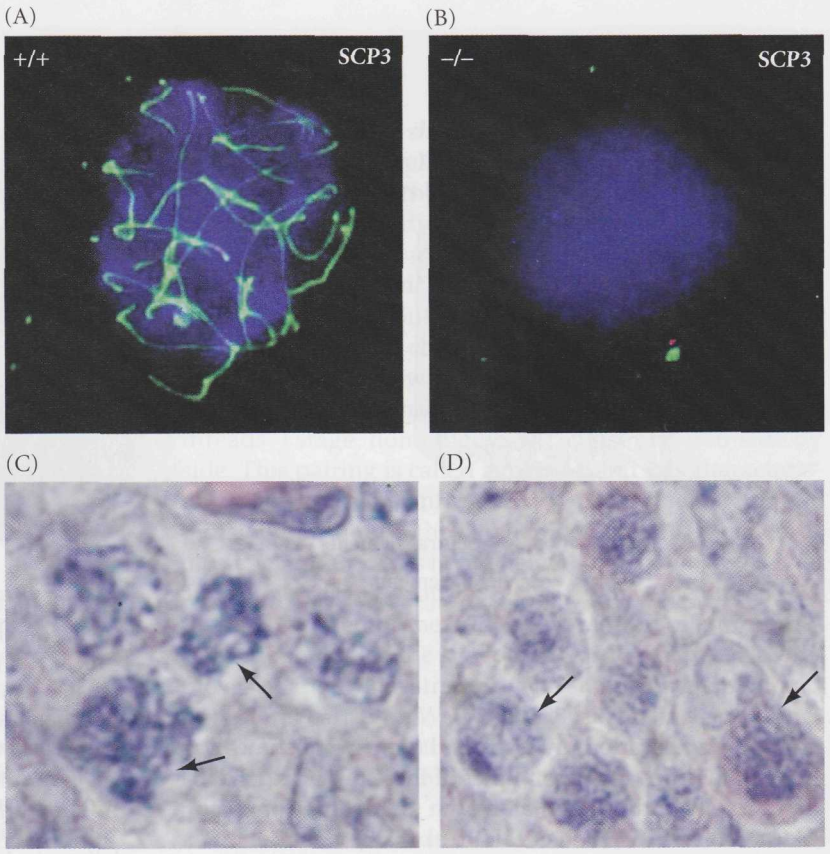


FIGURE 16.18 Importance of the synaptonemal complex. The CPEB protein is a constituent of the synaptonemal complex, and when this gene is knocked out in mice, the synaptonemal complex fails to form. (A,B) Staining for synaptonemal complex proteins in wild-type (A) and CPEB-deficient (B) mice. The synaptonemal complex is stained green, and the DNA is stained blue. The synaptonemal complex is absent in the mutant nuclei, and their DNA is not organized into discrete chromosomes. (C,D) In preparations stained with hematoxylin-eosin, the DNA can be seen to be in the pachytene stage in the wild-type cells (C) and fragmented in the mutant cells (D). (From Tay and Richter 2001, courtesy of J. D. Richter.)

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 (a meiotic cell having very little cytoplasm) acts as a sperm and "fertilizes" the oocyte after the second meiotic division. In some other insects and in the lizard *Cnemidophorus uniparens*, the oogonia further 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 haploid eggs develop into males, whereas fertilized eggs are diploid and 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.

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 that of its germ cells is the same.

But what about hermaphroditic animals, where 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.

There is evidence that these decisions are intimately linked. The mitosis/meiosis decision in *C. elegans* is controlled by a single nondividing cell—the **distal tip cell**—located at the end of each gonad. The germ cell precursors near this cell divide mitotical-

SIDELIGHTS & SPECULATIONS (Continued)

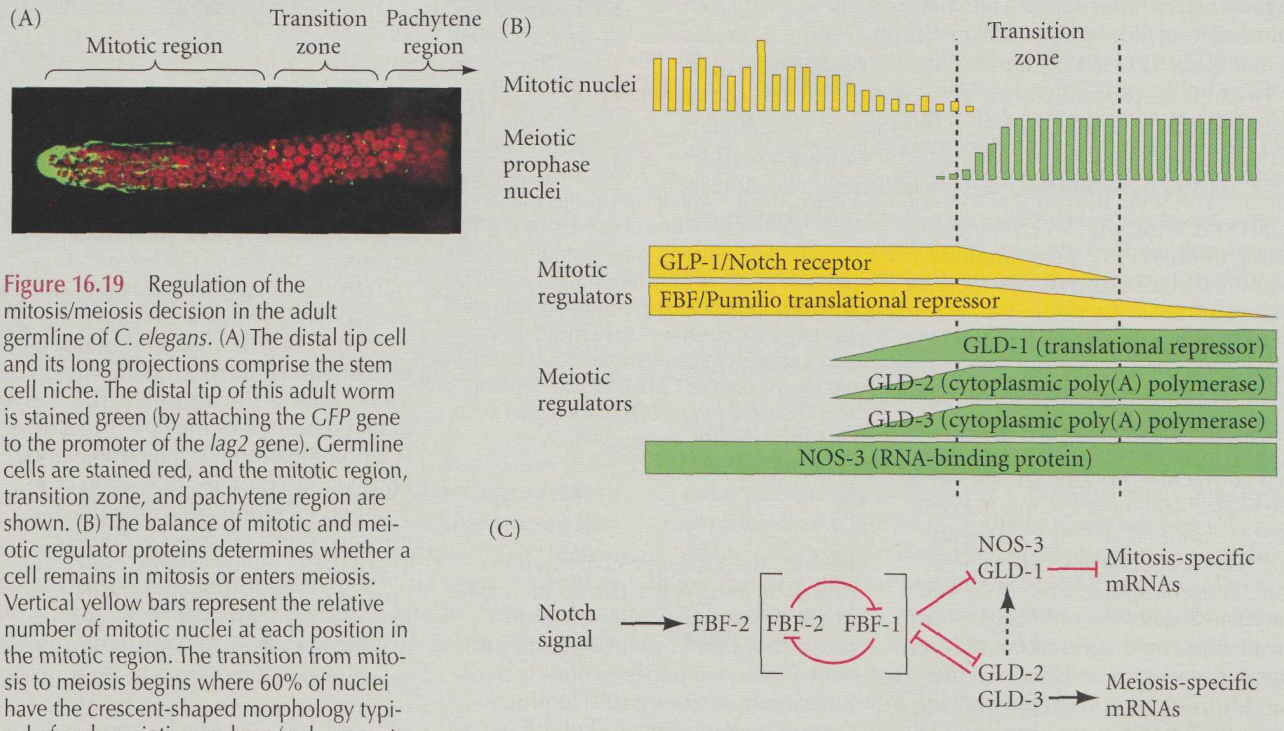


Figure 16.19 Regulation of the mitosis/meiosis decision in the adult germline of *C. elegans*. (A) The distal tip cell and its long projections comprise the stem cell niche. The distal tip of this adult worm is stained green (by attaching the *GFP* gene to the promoter of the *lag2* gene). Germline cells are stained red, and the mitotic region, transition zone, and pachytene region are shown. (B) The balance of mitotic and meiotic regulator proteins determines whether a cell remains in mitosis or enters meiosis. Vertical yellow bars represent the relative number of mitotic nuclei at each position in the mitotic region. The transition from mitosis to meiosis begins where 60% of nuclei have the crescent-shaped morphology typical of early meiotic prophase (red crescents in A). Green bars represent the percentage of nuclei in meiotic prophase at a given position along the distal-proximal axis. Levels of GLP-1 and FBF mitotic regulators are high throughout the mitotic region and decrease dramatically as the germ cells enter meiosis (horizontal yellow bands). Conversely, levels of most meiotic regulators (horizontal green bands) gradually increase in the proximal part of the mitotic region, reaching high levels as germ cells enter meiosis. (One exception is NOS-3, which is distributed uniformly throughout the germ line.) (C) Simplified summary of a network controlling the mitosis/meiosis decision. Notch signaling activates FBF-2. FBF-1 and FBF-2 are very similar proteins whose negative feedback loop may specify the size of the mitotic region, as it negatively regulates levels of GLD-1 and GLD-3, inhibiting meiosis in the distal region of the germ line. (After Kimble and Crittenden 2005; A courtesy of S. Crittenden and J. Kimble.)

ly, 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, germline stem cells are generated near its new position (Kimble 1981; Kimble and White 1981). The distal tip cell extends long filaments that touch the distal germ cells (Figure 16.19A). 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). LAG-2 maintains the germ cells in mitosis and inhibits their meiotic differentiation.

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 5–8 sperm cells. When genetic chimeras are made in which wild-type germ cell precursors are found in 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 in 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.*

As is usual in development, the binary decision entails both a push and a pull (Figure 16.19B). The decision to enter meiosis must be amplified by a decision to end mitosis. This appears to be accomplished by the FBF (*fem-3* mRNA-binding factor) proteins, similar to the *Drosophila* Pumilio RNA-binding protein mentioned in Chapter 6. Notch appears to activate FBFs, which are translational repressors of the GLD (germline development) proteins. GLD-1 (in combination with a Nanos protein) suppresses the translation of mitosis-specific mes-

*The *glp-1* gene appears to be involved in a number of inductive interactions in *C. elegans*. You may recall that GLP-1 is also needed by the AB blastomere for it to receive inductive signals from the EMS blastomere to form pharyngeal muscles (see Chapter 5).

(Continued on next page)

SIDELIGHTS & SPECULATIONS (Continued)

sages. This includes suppressing the translation of *glp-1* mRNA (Eckmann et al. 2002, 2004; Marin and Evans 2003; Kimble and Crittenden 2005). FBF also represses the translation of GLD-2 and GLD-3, two proteins necessary for polyadenylating meiosis-specific mRNAs, allowing them to be translated. Thus, the Notch signal, acting through FBF, simultaneously promotes mitosis and blocks meiosis (Figure 16.19C).

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, and those entering meiosis later become eggs. (It also means that, unlike the situation in vertebrates, the germ cells form in the gonads.) 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 16.20 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 *C. elegans* 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 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.

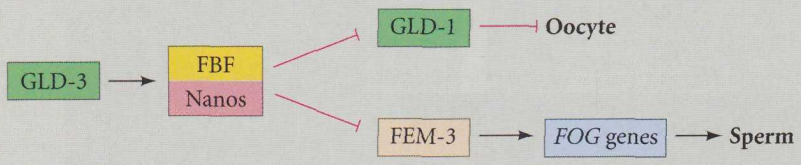


Figure 16.20 Model of sex determination switch in the germ line of *C. elegans* hermaphrodites. FBF and Nanos simultaneously promote oogenesis (by blocking an inhibitor) and inhibit spermatogenesis (by blocking an activator). Expression of the *fbf* and *nanos* genes appears to be regulated by a GLD-3 protein. As long as GLD-3 is made, sperm are produced. After GLD-3 production stops, the germ cells become oocytes. (After Eckmann et al. 2004.)

Oogenesis can begin only when FEM activity is suppressed. This suppression appears to act at the level of RNA translation. The 3′ untranslated region of *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 takes place. 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 FBF with the Nanos and Pumilio proteins (the same combination that represses *hunchback* message translation in *Drosophila*).

As in the meiosis/mitosis decision, there are pushes and pulls in the sex-determining pathways. The same Nanos and FBF signal that inhibits the sperm-producing *fem-3* message also inhibits the oocyte-inhibiting *gld-1* message. Thus, the Nanos/FBF signal simultaneously blocks sperm production (by inhibiting an activator) while promoting oocyte production (by inhibiting the inhibitor). The use of the same proteins in both the mitosis/meiosis decision and the sperm/egg decision has allowed Eckmann and colleagues (2004) to speculate that these two pathways evolved from a single original pathway whose function was to regulate the balance between cell growth and cell differentiation.

Gamete Maturation

The regulation of meiosis can differ dramatically between males and females. The egg is usually a nonmotile cell that has conserved its cytoplasm and has stored the ribosomes, mitochondria, and mRNAs needed to initiate development. The sperm is usually a smaller, motile cell that has eliminated most of its cytoplasm to become a nucleus attached to a propulsion system. As we will soon see, there are often large differences between **oogenesis**, the production of eggs, and **spermatogenesis**, the production of sperm. Thus, gametogenesis is more than making the nucleus haploid. The formation of the sperm involves constructing the flagellum and the acrosome. Constructing the egg involves building the organelles involved in fertilization, synthesizing and positioning the mRNAs and proteins used in early development, and accumulating energy sources and energy-producing organelles (ribosomes, yolk, and mitochondria) in the cytoplasm. A partial catalogue of the materials stored in the oocyte cytoplasm of a frog is shown in Table 16.1; a partial list of stored mRNAs found in several organisms was shown in Table 2.2.

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

TABLE 16.1 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.

humans and most other mammals, only a few eggs are produced during an individual's lifetime. In those species that produce thousands of ova each breeding season, the female PGCs produce **oogonia**, 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.

Maturation of the oocytes in frogs

The eggs of sea urchins, 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 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 16.21). Eggs mature in yearly batches, with the first cohort maturing shortly after metamorphosis; the next group matures a year later.

VITELLOGENESIS **Vitellogenesis**—the accumulation of yolk proteins—occurs when the oocyte reaches the diplotene stage of meiotic prophase. Yolk is not a single substance, but a mixture of materials 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).

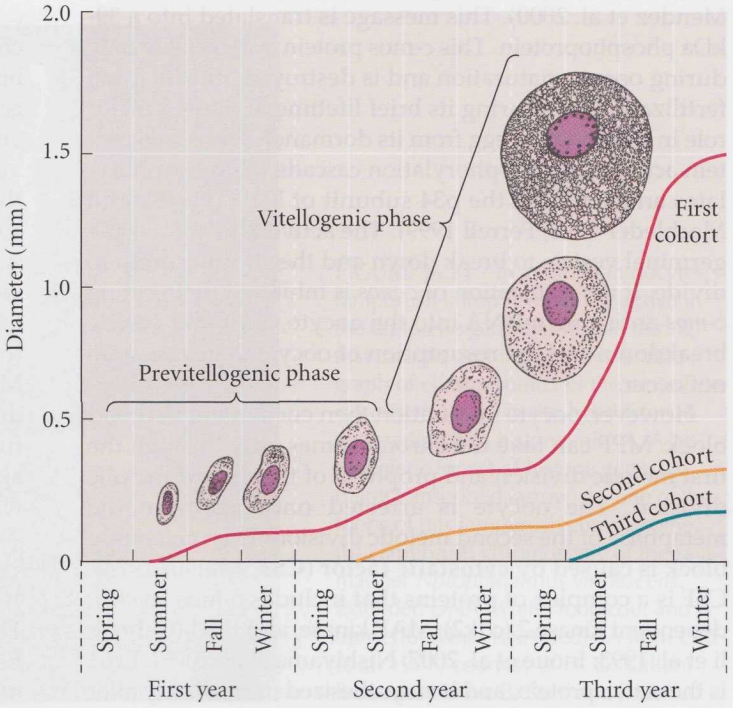


FIGURE 16.21 Growth of oocytes in the frog. During the first 3 years of life, three cohorts of oocytes are produced. The drawings follow the growth of the first-generation oocytes. (After Grant 1953.)

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. Resumption of meiosis in the amphibian oocyte is thought to require 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 16.22).

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 5). 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 on the presence 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.

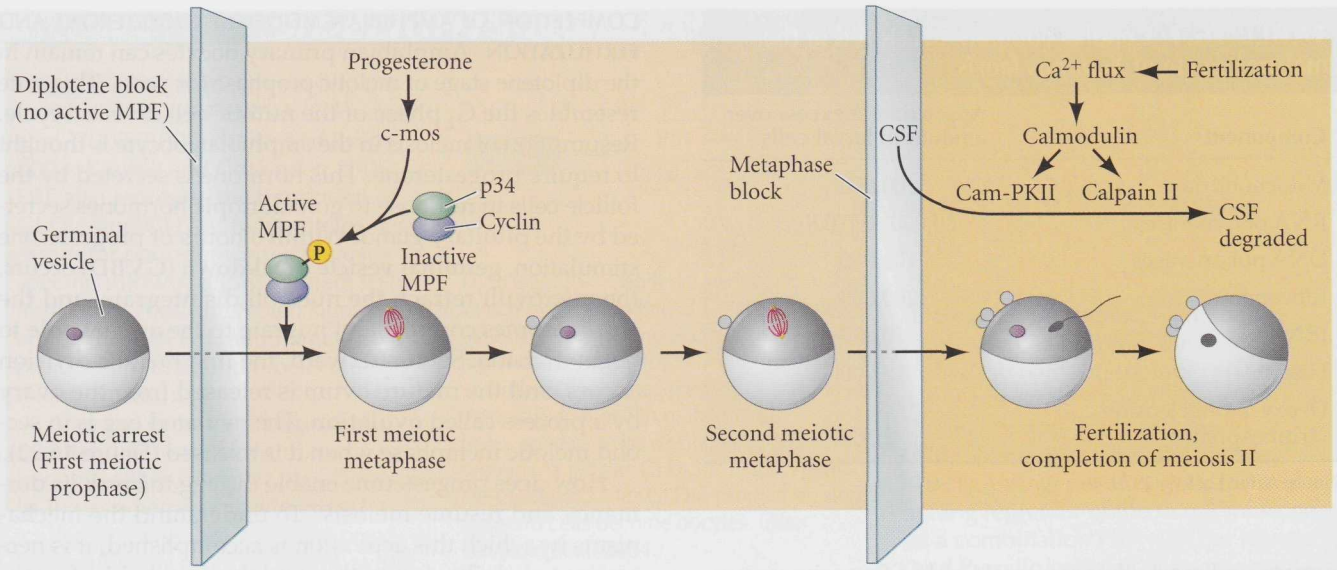


FIGURE 16.22 Schematic representation of *Xenopus* oocyte maturation, showing the regulation of meiotic cell division by progesterone and fertilization. Oocyte maturation is arrested at the diplotene stage of first meiotic prophase by the lack of active MPF. Progesterone activates the production of the 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, cyclin-dependent kinase 2, and Erp1. CSF inhibits the anaphase-promoting complex from degrading cyclin. 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.

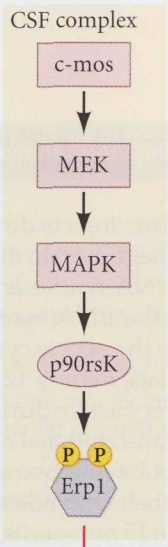
The mediator of the progesterone signal is the **c-mos** protein. Progesterone reinitiates meiosis by causing the egg to polyadenylate the maternal *c-mos* mRNA that has been stored in its cytoplasm (Sagata et al. 1988; Sheets et al. 1995; Mendez et al. 2000). This message is translated into a 39-kDa 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 encounters a second block. MPF can take the chromosomes only through the first meiotic division and prophase of the second meiotic division. The oocyte is arrested once again in the metaphase of the second meiotic division. This metaphase block is caused by **cytostatic factor** (**CSF**; Matsui 1974). CSF is a complex of proteins that includes c-mos, cyclin-dependent kinase 2 (cdk2), MAP kinase, and Erp1 (Gabrielli et al. 1993; Inoue et al. 2007; Nishiyama et al. 2007). Erp1 is the active protein, and it is synthesized immediately after the first meiotic division. The proteins of the CSF complex interact, eventually activating Erp1 by phosphorylating it. Phosphorylated Erp1 blocks the degradation of cyclin by the anaphase-promoting complex (Figure 16.23).

This metaphase block is broken by fertilization. The calcium ion flux attending fertilization activates the calcium-binding protein **calmodulin**, and calmodulin, in turn, can activate two enzymes that inactivate CSF. These enzymes are calmodulin-dependent protein kinase II, which inactivates cdk2, 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). In 1911, Frank Lillie wrote, "The nature of the inhibition that causes the need for fertilization is a most fundamental problem." The solution to that problem appears to be oocyte-derived CSF and the sperm-induced wave of calcium ions.

Gene transcription in amphibian oocytes

The amphibian oocyte has certain periods of very active RNA synthesis. During the diplotene stage, certain chromosomes stretch out large loops of DNA, causing them to



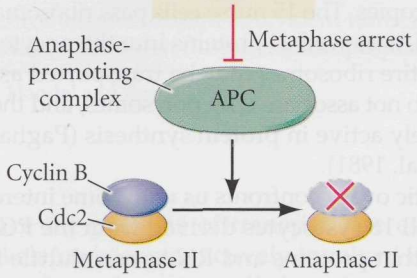
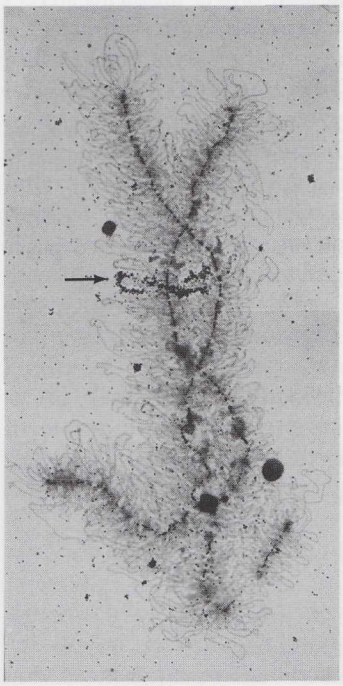


FIGURE 16.23 The main pathway leading to metaphase arrest in the second meiotic division. The CSF protein complex consists of c-mos, three transducer kinases, and the effector protein Erp1. Activation of c-mos activates the kinases, which eventually phosphorylate Erp1. Phophorylated Erp1 binds to and inhibits the anaphase-promoting complex, thus blocking the degradation of cyclin B that would allow the cell to enter anaphase. (After Inoue et al. 2007.)

resemble a lampbrush (which was a handy instrument for cleaning test tubes in the days before microfuges). In situ hybridization reveals these **lampbrush chromosomes** to be sites of RNA synthesis. Oocyte chromosomes can be incubated with a radioactive RNA probe and autoradiography used to visualize the precise locations where genes are being transcribed (Figure 16.24A). Electron micrographs of gene transcripts from lampbrush chromosomes also enable one to see chains of mRNA coming off each gene as it is transcribed (Figure 16.24B; also see Hill and MacGregor 1980).

In addition to mRNA synthesis, ribosomal RNA and transfer RNA are also transcribed during oogenesis. Figure 16.25A shows the pattern of rRNA and tRNA synthesis during *Xenopus* oogenesis. Transcription appears to begin in early (stage I, 25–40 μm) oocytes, during the diplotene stage of meiosis. At this time, all the rRNAs and tRNAs needed for protein synthesis until the mid-blastula stage are made, and all the maternal mRNAs needed for early development are transcribed. This stage lasts for months in *Xenopus*. The rate of rRNA production is prodigious. The *Xenopus* oocyte genome has over 1800 genes encoding 18S and 28S rRNA (the two large RNAs that form the ribosomes), and these genes are selectively amplified such that there are over 500,000 genes making rRNA in the oocyte (Figure 16.25B; Brown and Dawid 1968). When the mature (stage VI) oocyte reaches a certain size, its chromosomes condense, and the rRNA genes are no longer tran-

(A)



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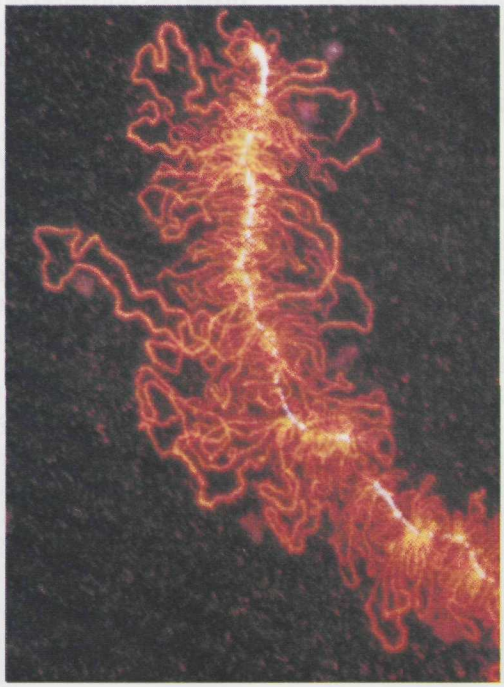


FIGURE 16.24 In amphibian oocytes, lampbrush chromosomes are active in the diplotene germinal vesicle during first meiotic prophase. (A) Autoradiograph of chromosome I of the newt *Triturus cristatus* after in situ hybridization with radioactive histone mRNA. A histone gene (or set of histone genes) is being transcribed (arrow) on one of the loops of this lampbrush chromosome. (B) Lampbrush chromosome of the salamander *Notophthalmus viridescens*. Extended DNA (white) loops out and is transcribed into RNA (red). (A from Old et al. 1977, courtesy of H. G. Callan; B courtesy of M. B. Roth and J. Gall.)

scribed. This "mature oocyte" condition can also last for months. Upon hormonal stimulation, the oocyte completes its first meiotic division and is ovulated. The mRNAs stored by the oocyte now join with the ribosomes to initiate protein synthesis. Within hours, the second meiotic division has begun, and the egg is fertilized in second meiotic metaphase. The embryo's genes do not begin active transcription until the mid-blastula transition (Newport and Kirschner 1982).

As we saw in Chapter 2, the oocytes of several species make two classes of mRNAs—those for immediate use in the oocyte, and those that are stored for use during early development. In frogs, the translation of stored oocyte messages (maternal mRNAs) is initiated by progesterone as the egg is about to be ovulated. One of the results of the MPF activity induced by progesterone may be the phosphorylation of proteins on the 3' UTR of stored oocyte mRNAs. The phosphorylation of these factors is associated with the lengthening of the polyA tails of the stored messages and their subsequent translation (Paris et al. 1991).

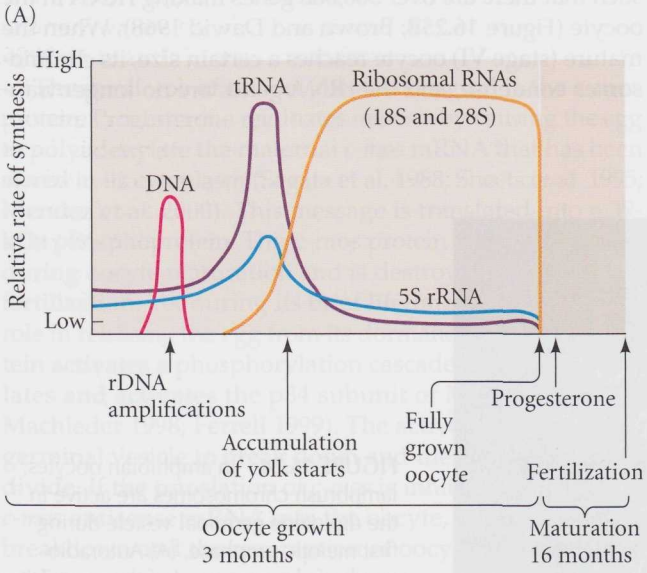
Meroistic oogenesis in insects

There are several types of oogenesis in insects, but most studies have focused on those insects (including *Drosophila* and moths) that undergo **meroistic oogenesis**, in which cytoplasmic connections remain between the cells produced by the oogonium.

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 6.7). Oogenesis takes place in only 12 days, so the nurse cells are metabolically very active during this time. Nurse cells 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 proteins into the oocyte cytoplasm, and entire ribosomes may be transported as well. The mRNAs do not associate with polysomes, and 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 16 cystocytes derived from the PGC are connected so that proteins and RNAs can shuttle freely among them, how do 15 cystocytes become RNA-producing nurse cells while one cell is fated to become 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 (see Figure 16.4A). 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.



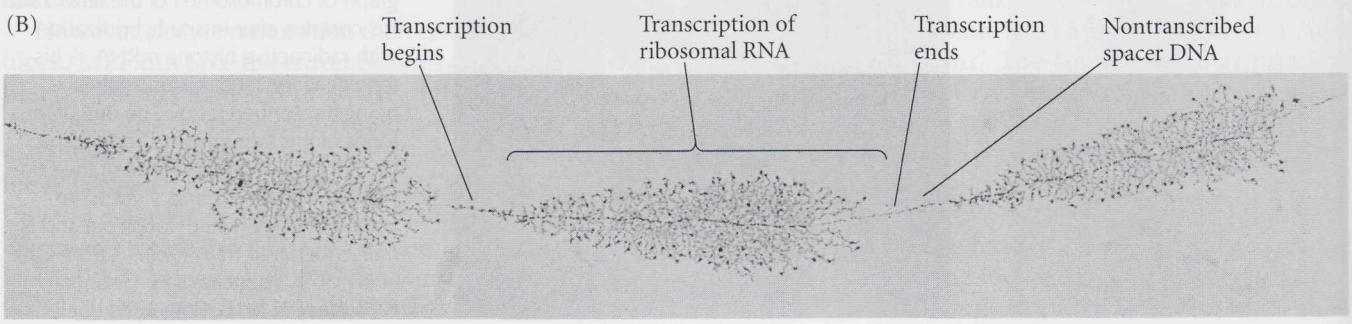


FIGURE 16.25 Ribosomal and transfer RNA production in *Xenopus* oocytes. (A) Relative rates of DNA, tRNA, and rRNA synthesis in amphibian oogenesis during the last 3 months before ovulation. (B) Transcription of the large RNA precursor of the 28S, 18S, and 5.8S ribosomal RNAs. These units are tandemly linked together, with some 450 per haploid genome. (A after Gurdon 1976; B courtesy of O. L. Miller, Jr.)

TABLE 16.2 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.

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 (see Figure 16.4C) 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 6). If the microtubular array is disrupted (either chemically or by mutations such as *bicaudal-D* or *egalitarian*), the nurse cell 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; see Figure 6.7).

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.

Gametogenesis in Mammals

As outlined in Table 16.2, there are profound differences between spermatogenesis and oogenesis in mammals. One of the fundamental differences concerns the timing of meiosis onset. In females, meiosis begins in the embryonic gonads; in males, meiosis is not initiated until puberty. This critical difference in timing is due to retinoic acid (RA) produced by the mesonephric kidneys (Figure 16.26). This RA stimulates the germ cells to undergo a new round of DNA replication and initiate meiosis (Baltus et al. 2006; Bowles et al. 2006; Lin et al. 2008). In males, however, the embryonic testes secrete the RA-degrading enzyme Cyp26b1. This prevents RA from promoting meiosis. Later, Nanos2 will be expressed in the male germ cells, and this will also prevent meiosis and ensure that the cells follow the pathway to become sperm (Koubova et al. 2006; Suzuki and Saga 2008).

VADE MECUM Gametogenesis in mammals

Spermatogenesis

Once mammalian PGCs arrive at the genital ridge of a male embryo, they are called **gonocytes** and become incorporated into the sex cords (Culty 2009). They remain there until maturity, at which time the sex cords hollow out to form the **seminiferous tubules**. The epithelium of the tubules differentiates into the **Sertoli cells** that will nourish and protect the developing sperm cells. The gonocytes differentiate into a population of stem cells that have recently been named the **undifferentiated type A spermatogonia** (Yoshida et al. 2007). These cells can reestablish spermatogenesis when transferred into mice whose sperm production was eliminated by toxic chemicals. They appear to reside in stem cell niches created by the junction of Sertoli cells, interstitial (testosterone-producing) cells, and blood vessels.

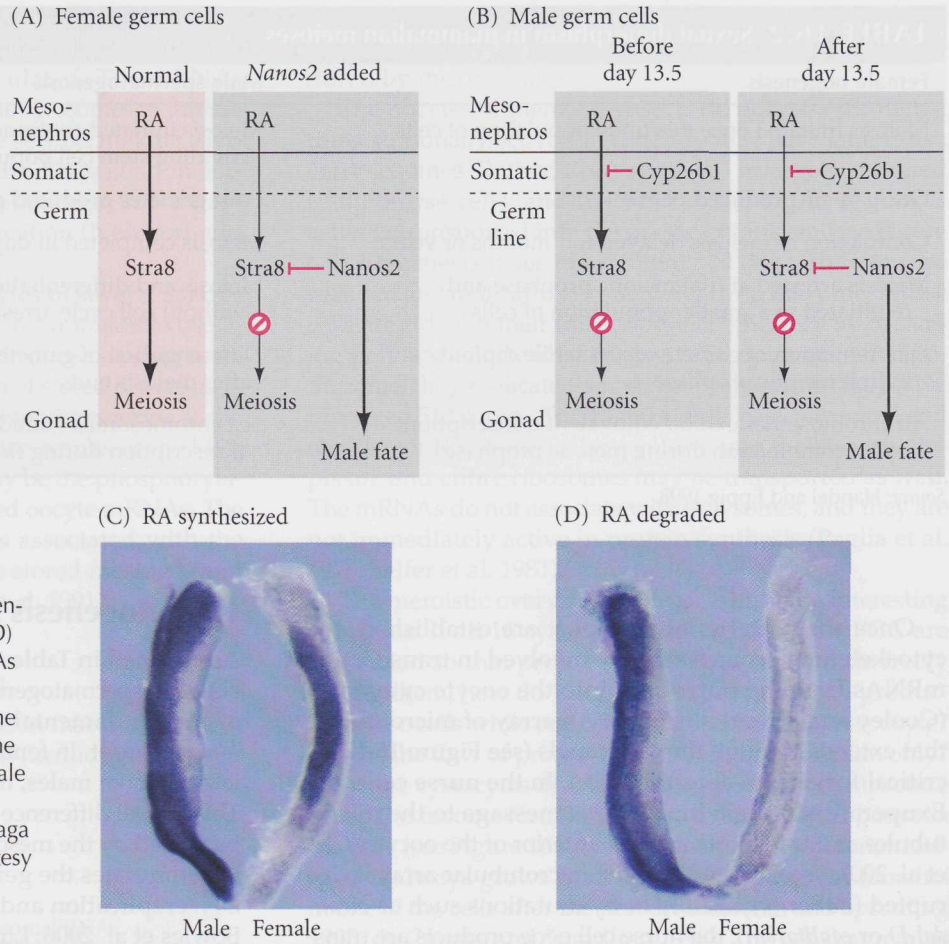


FIGURE 16.26 Retinoic acid (RA) determines the timing of meiosis and sexual differentiation of mammalian germ cells. (A) In female mouse embryos, RA secreted from the mesonephros reaches the gonad and triggers meiotic initiation via the induction of Stra8 transcription factor in female germ cells (beige). However, if activated *Nanos2* genes are added to female germ cells, they suppress Stra8 expression, leading the germ cells into a male pathway (gray). (B) In embryonic testes, Cyp26b1 blocks RA signaling, thereby preventing male germ cells from initiating meiosis until embryonic day 13.5 (left panel). After embryonic day 13.5, when Cyp26b1 expression is decreased, Nanos2 is expressed and prevents meiotic initiation by blocking Stra8 expression. This induces male-type differentiation in the germ cells (right panel). (C,D) Day 12 mouse embryos stained for mRNAs encoding the RA-synthesizing enzyme Aldh1a2 (C) and the RA-degrading enzyme Cyp26b1 (D). The RA-synthesizing enzyme is seen in the mesonephros of both the male and female; the RA-degrading enzyme is seen only in the male gonad. (A,B from Saga 2008; C,D from Bowles et al. 2006, courtesy of P. Koopman.)

The decision to proliferate or differentiate may involve interactions between the Wnt and BMP pathways. Wnt signaling appears to promote proliferation of stem cells, and the spermatogonia appear to have receptors for both Wnts and BMPs (Golestaneh et al. 2009). The initiation of spermatogenesis during puberty is probably regulated by the synthesis of BMPs 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 the surfaces of both cell types, and by galactosyltransferase molecules on the spermatogenic cells that bind a carbohydrate receptor on the Sertoli cells (Newton et al. 1993; Pratt et al. 1993). Spermatogenesis—the developmental pathway from germ cell to mature sperm—occurs in the recesses between the Sertoli cells (Figure 16.27).

FORMING THE HAPLOID SPERMATID The undifferentiated type A_1 spermatogonia (sometimes called the dense type A spermatogonia) are found adjacent to the outer basal lamina of the sex cords. They are stem cells, and upon reaching maturity are thought to divide to make another type A_1 spermatogonium as well as a second, paler type of cell, the type A_2 spermatogonia. The A_2 spermatogonia divide to produce type A_3 spermatogonia, which then beget type A_4 spermatogonia. The A_4 spermatogonia are thought to differentiate into the first committed stem cell type, the **intermediate spermatogonia**. Intermediate spermatogonia are committed to becoming spermatozoa, and they divide mitotically once to form **type B spermatogonia** (see Figure 16.27). 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 the opposing influences of glial cell line-derived neurotrophic factor (GDNF) and stem cell factor (SCF), both of which are secreted by the Sertoli cells. GDNF levels determine whether the dividing spermatogonia remain spermatogonia or enter the pathway to become spermatocytes. Low levels of GDNF favor the differentiation of the spermatogonia, whereas high levels favor self-renewal of the stem cells (Meng et al. 2000).

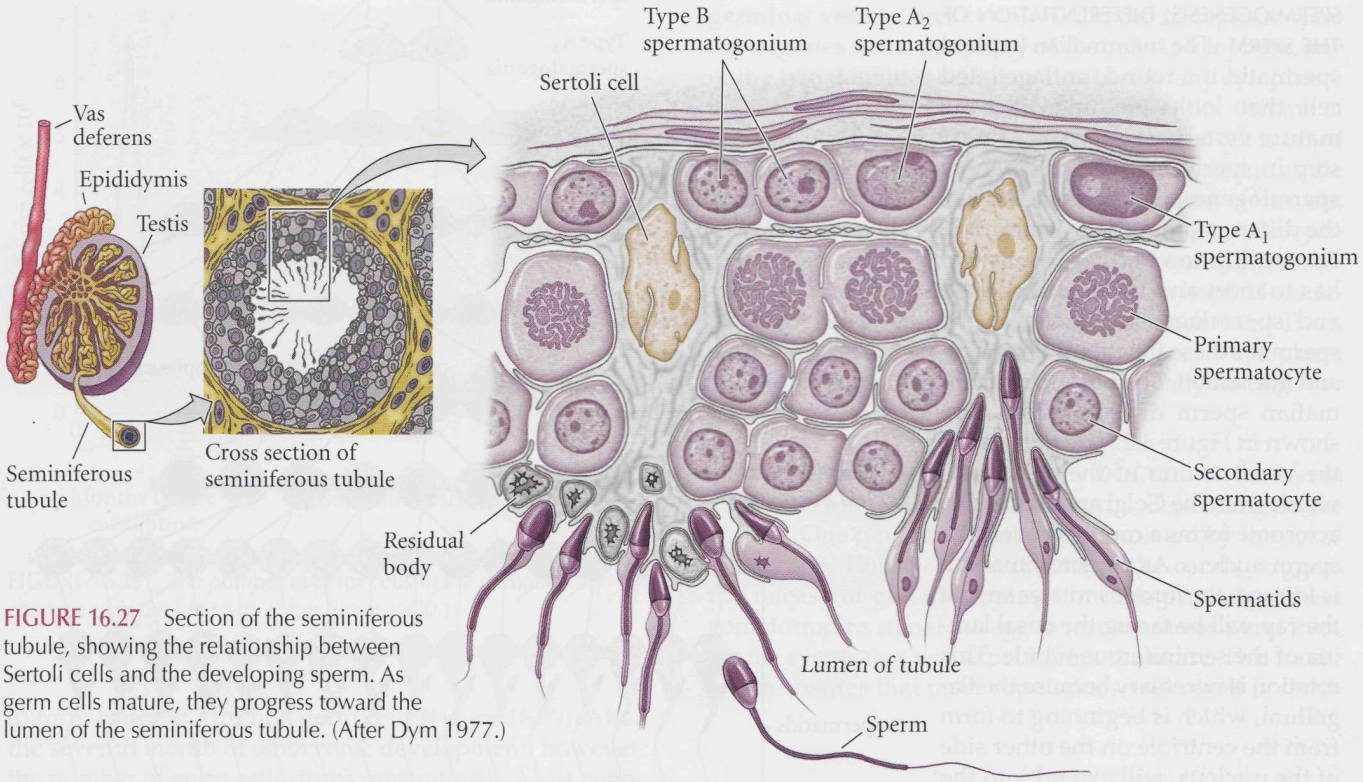


FIGURE 16.27 Section of the seminiferous tubule, showing the relationship between Sertoli cells and the developing sperm. As germ cells mature, they progress toward the lumen of the seminiferous tubule. (After Dym 1977.)

SCF promotes the transition to spermatogenesis (Rossi et al. 2000). Since both GDNF and SCF are upregulated by follicle-stimulating hormone (FSH), these two factors may serve as a link between the Sertoli cells and the endocrine system, and they provide a mechanism for FSH to instruct the testes to produce more sperm (Tadokoro et al. 2002). Keeping the stem cells in equilibrium—producing neither too many undifferentiated cells nor too many differentiated cells—is not easy. Mice with the *luxoid* mutation are sterile because they lack a transcription factor that regulates this division. All their spermatogonia become sperm at once, leaving the testes devoid of stem cells (Buaas et al. 2004; Costoya et al. 2004).

Looking at Figure 16.28, we find that during the spermatogonial divisions, cytokinesis is not complete. Rather, the cells form a syncytium in which 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 spermatogonia to spermatids, the cells move farther and farther away from the basal lamina of the seminiferous tubule and closer to its lumen (see Figure 16.27; Siu and Cheng 2004). 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).

The processes of spermatogenesis require a very specialized network of gene expression (Sassone-Corsi 2002). Not only are histones substantially remodeled and replaced by sperm-specific variants (see below), but even the basal RNA polymerase II transcription factors are exchanged for sperm-specific variants. The TFIID complex, which contains the TATA-binding protein and 14 TAFs, functions in the recognition of RNA polymerase. One of these TAFs, TAF4b, is a sperm-specific TAF required for mouse spermatogenesis (Falender et al. 2005). Without this factor, the spermatogonial stem cells fail to make Ret (the receptor for GDNF) or the luxoid transcription factor, and spermatogenesis fails to occur.

SPERMIOGENESIS: 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 4.2. 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 basal lamina 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.

During spermiogenesis, the histones of the spermatogonia are often replaced by histone variants, and widespread nucleosome dissociation takes place. This remodeling of nucleosomes might also be the point at which the PGC pattern of methylation is removed and the male genome-specific pattern of methylation is established on the sperm DNA (see Wilkins 2005). As spermiogenesis ends, the histones of the haploid nucleus are eventually replaced by protamines.* This replacement results in the complete shutdown of transcription in the nucleus and facilitates the nucleus assuming an almost crystalline structure (Govin et al. 2004). The resulting sperm then enter the lumen of the seminiferous tubule.

See WEBSITE 16.5 The Nebenkern

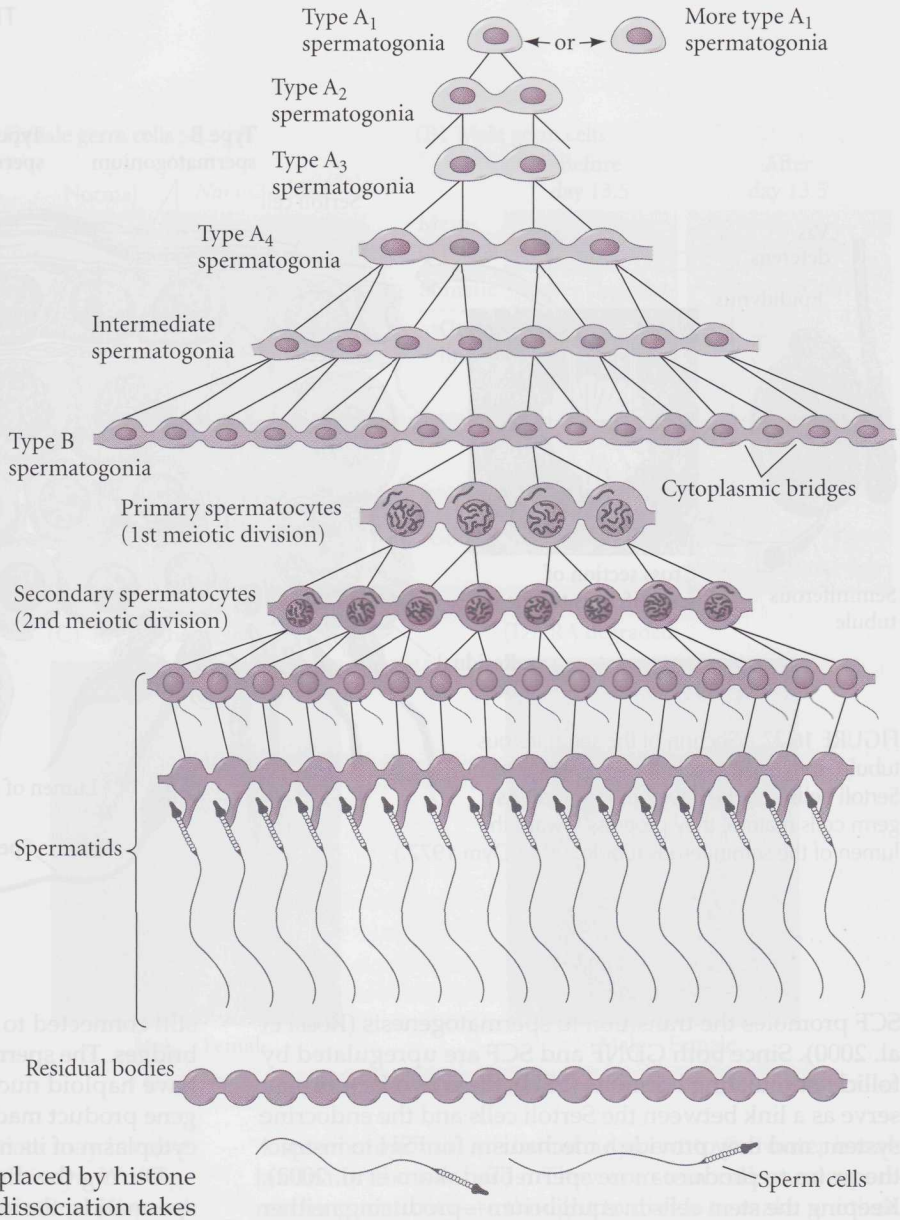


FIGURE 16.28 Formation of syncytial clones of human male germ cells. (After Bloom and Fawcett 1975.)

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 another 13.5 days. Human sperm development takes nearly twice as long. Because the original type A 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).

Oogenesis

In the human embryo, the thousand or so oogonia divide rapidly from the second to the seventh month of gestation

*Protamines are relatively small proteins that are over 60% arginine. Transcription of the genes for protamines is seen in the early haploid spermatids, although translation is delayed for several days (Peschon et al. 1987). The replacement, however, is not complete, and "activating" nucleosomes, having trimethylated H3K4, cluster around developmentally significant loci, including Hox gene promoters, certain microRNAs, and imprinted loci that are paternally expressed (Hammoud et al. 2009).

FIGURE 16.29 *The number of germ cells in the human ovary changes over the life span.* (After Baker 1970.)

to form roughly 7 million germ cells (Figure 16.29). 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 puberty, 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 prophase for nearly 50 years. As Figure 16.29 illustrates, primary oocytes continue to die. Of the millions of primary oocytes present at her birth, only about 400 mature during a woman's lifetime.*

See WEBSITE 16.6 Synthesizing oocyte ribosomes

OOGENIC MEIOSIS Oogenic meiosis 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 16.30). 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 (the ovum), and a second polar body receives little more than a haploid nucleus. (The first polar body usually does not divide.) Thus, oogenic meiosis conserves the volume of oocyte cytoplasm in a single cell rather than splitting it equally among four progeny.

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 fertil-

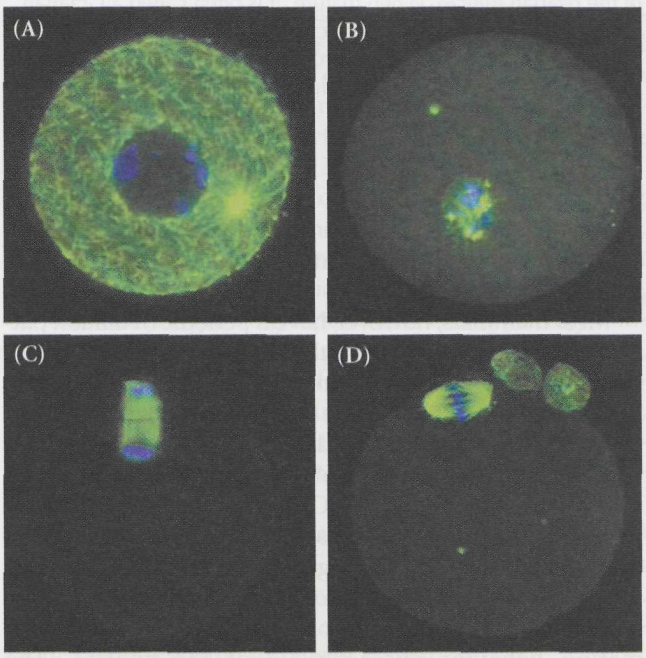


FIGURE 16.30 Meiosis in the mouse oocyte. The tubulin of the microtubules is stained green; the DNA is stained blue. (A) Mouse oocyte in meiotic prophase. The large haploid nucleus (the germinal vesicle) is still intact. (B) The nuclear envelope of the germinal vesicle breaks down as metaphase begins. (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, courtesy of L. De Vos.)

*It has long been thought that the number of oocytes in a female mammal (including humans) is established during embryogenesis and never increases (Zuckerman 1951). Radioactive labeling of oocyte nuclei also supported the view that the number of oocytes is fixed during embryonic life (see Telfer 2004). Recently, however, Zou and colleagues (2008) have claimed to find female germline stem cells in the ovaries of adult mice. Such a finding would mean that the loss of female fertility in mammals might not be due solely to the aging of oocytes but also to the depletion of the PGC stem cells (Oktem and Oktay 2009). Thus, the ovary may have some regenerative abilities that remain unexplored.

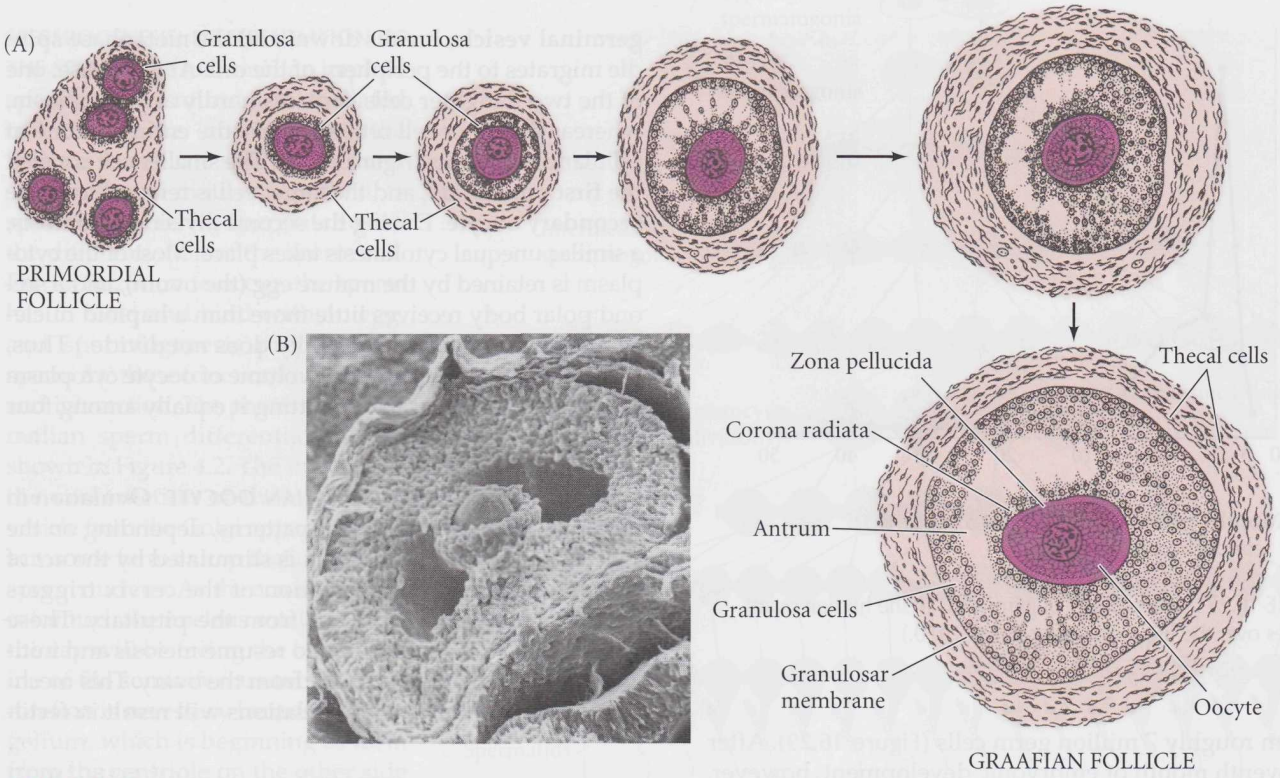


FIGURE 16.31 The ovarian follicle of mammals. (A) Maturation of the ovarian follicle. When mature, it is often called a Graafian follicle. (B) Scanning electron micrograph of a mature follicle in the rat. The oocyte (center) is surrounded by the smaller granulosa cells that will make up the cumulus. (A after Carlson 1981, B courtesy of P. Bagavandoss.)

ized 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** (**GRH**). GRH 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. 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:

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.

*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) and Howes (2010) have argued that menstruation is a crucial immunological adaptation, protecting the uterus against infections contracted from semen or other environmental agents. Strassmann (1996) suggested that cyclicity of the endometrium is an energy-saving adaptation in times of poor nutrition, and that vaginal bleeding would be a side effect of this adaptive process. Finn (1998) claimed that menstruation has no adaptive value but 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 be wrong to think of there being a single function of menstruation—its role might change during a woman's life cycle.

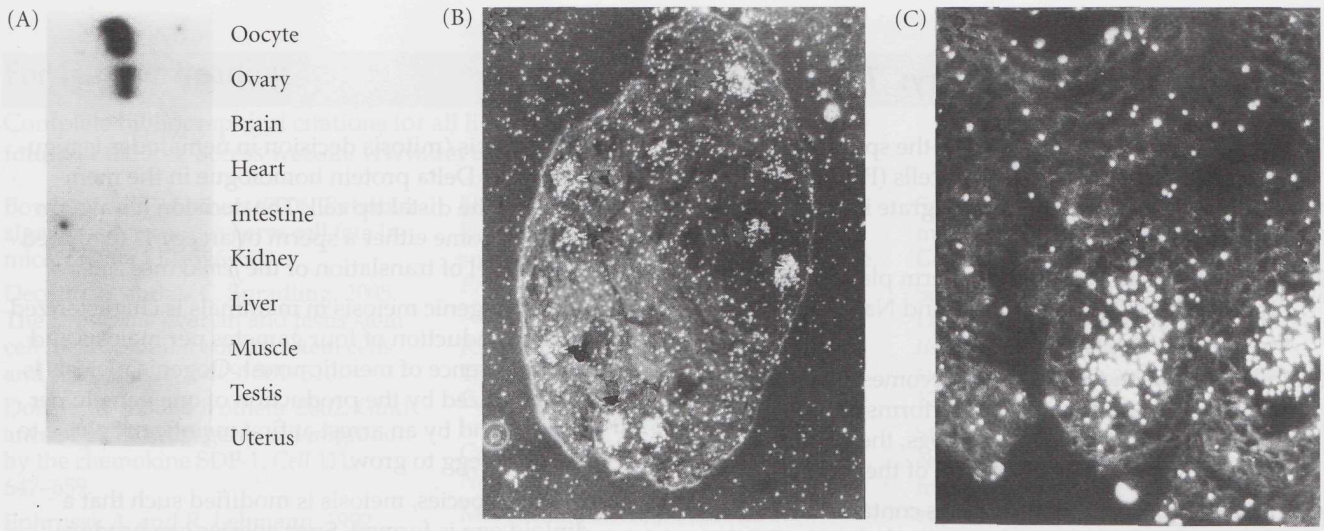


FIGURE 16.32 Expression of the *ZP3* gene in the developing mouse oocyte. (A) Northern blot of *ZP3* mRNA accumulation in the tissues of a 13-day mouse embryo. A radioactive probe to the *ZP3* message found it expressed only in the ovary, and specifically in the oocytes. (B) When the luciferinase reporter gene is placed onto the *ZP3* promoter and inserted into the mouse genome, *luciferinase* message is seen only in the developing oocytes of the ovary. (C) Higher magnification of a section of (B), showing two of the ovarian follicles containing maturing oocytes. (A from Roller et al. 1989; B,C from Lira et al. 1990; photographs courtesy of P. Wassarman.)

The majority of the oocytes in 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 16.31). 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).

See WEBSITE 16.7
Hormones and mammalian egg maturation

See WEBSITE 16.8
The reinitiation of mammalian meiosis

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 and 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). The oocytes are maintained in the dictyate stage by the ovarian follicle cells. The release from this dictyate stage and the reinitiation of meiosis are driven by lutenizing hormone from the pituitary. The pituitary hormone is received by the granulosa cells of the ovary, and the granulosa cells send a paracrine or juxtacrine signal that induces activation of mitosis-promoting factor (MPF) in the oocyte (Eppig et al. 2004; Mehlmann et al. 2004).

Meanwhile, the growing oocyte is actively transcribing genes whose products are necessary for cell metabolism, for oocyte-specific processes, and 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 proteins ZP1, ZP2, and ZP3 (see Figure 4.31). Moreover, these genes are transcribed only in the oocyte and not in any other cell type, as the proteins essential for fertilization are being synthesized (Figure 16.32; Roller et al. 1989; Lira et al. 1990; Epifano et al. 1995).

The fertilizable mammalian oocyte is arrested in second meiotic metaphase by MPF. As in amphibian oocytes, a cytostatic factor stabilizes the cyclin B-Cdc2 MPF dimers. Shoji and colleagues (2006) showed that loss of the cytostatic factor occurred upon egg activation.

Coda

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."

Snapshot Summary: *The Saga of the Germ Line*

1. The precursors of the gametes—the sperm and eggs—are the primordial germ cells (PGCs). They form outside the gonads and migrate into the gonads during development.
2. In many species, a distinctive germ plasm exists. It often contains the Oskar, Vasa, and Nanos proteins or the mRNAs encoding them.
3. In *Drosophila*, the germ plasm becomes localized in the posterior of the embryo and forms pole cells, the precursors of the gametes. In frogs, the germ plasm originates in the vegetal portion of the oocyte.
4. The germ plasm in many species contains inhibitors of transcription and translation, such that the PGCs derived from them are thought to be both translationally and transcriptionally silent.
5. In amphibians, the germ cells migrate on fibronectin matrices from the posterior larval gut to the gonads. In mammals, a similar migration is seen, and fibronectin pathways may also be used. Stem cell factor (SCF) is critical in this migration, and the germ cells proliferate as they travel.
6. In birds, the germ plasm is first seen in the germinal crescent. The germ cells migrate through the blood, then leave the blood vessels and migrate into the genital ridges.
7. In zebrafish, the germ cell determinants enter specific cells that are attracted to the gonad by a gradient of chemoattractants such as the Sdf1 protein.
8. Germ cell migration in *Drosophila* occurs in several steps involving transepithelial migration, repulsion from the endoderm, and attraction to the gonads.
9. Once the germ cells reach the gonads, they may initiate meiosis. The timing and details of this process depend on the species and sex of the organism. In humans and mice, germ cells entering ovaries initiate meiosis while in the embryo; germ cells entering testes do not initiate meiosis until puberty.
10. Before meiosis, the DNA is replicated and the resulting sister chromatids remain bound at the kinetochore. Homologous chromosomes are connected through the synaptonemal complex.
11. The first division of meiosis separates the homologous chromosomes. The second division of meiosis splits the kinetochore and separates the chromatids.
12. The meiosis/mitosis decision in nematodes is regulated by a Delta protein homologue in the membrane of the distal tip cell. The decision for a germ cell to become either a sperm or an egg is regulated at the level of translation of the *fem-3* message.
13. Spermatogenic meiosis in mammals is characterized by the production of four gametes per meiosis and by the absence of meiotic arrest. Oogenic meiosis is characterized by the production of one gamete per meiosis and by an arrest at first meiotic prophase to allow the egg to grow.
14. In some species, meiosis is modified such that a diploid egg is formed. Such species can produce a new generation parthenogenetically, without fertilization.
15. The egg not only synthesizes numerous compounds, but also absorbs material produced by other cells. Moreover, it localizes many proteins and messages to specific regions of the cytoplasm, often tethering them to the cytoskeleton.
16. The *Xenopus* oocyte transcribes actively from lampbrush chromosomes during the first meiotic prophase.
17. In *Drosophila*, nurse cells make mRNAs that enter the developing oocyte. Which of the cells derived from the primordial germ cell becomes the oocyte and which become nurse cells is determined by the fusome and the pattern of divisions.
18. In mammals, retinoic acid from the mesonephros initiates germ cell meiosis in the ovaries. In the testes, however, retinoic acid is degraded and meiosis is blocked until puberty.
19. In male mammals, the PGCs generate stem cells that last for the life of the organism. PGCs do not become stem cells in female mammals (although in many other animal groups, PGCs do become germ stem cells in the ovaries).
20. In female mammals, germ cells initiate meiosis and are retained in the first meiotic prophase (dictyate stage) until ovulation. In this stage, they synthesize mRNAs and proteins that will be used for gamete recognition and early development of the fertilized egg.

For Further Reading

Complete bibliographical citations for all literature cited in this chapter can be found at the free-access website www.devbio.com

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Go Online

WEBSITE 16.1 Germline 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.

WEBSITE 16.2 Mechanisms of chromosome diminution. The somatic cells do not lose DNA randomly. Rather, specific regions of DNA are lost during chromosome diminution.

WEBSITE 16.3 The insect germ plasm. The insect germinal cytoplasm was discovered as early as 1911, when Hegner found that removing the posterior pole cytoplasm of beetle eggs caused sterility in the resulting adults.

WEBSITE 16.4 Human meiosis. Nondisjunction—the failure of chromosomes to sort properly during meiosis—is not uncommon in humans. Its frequency increases with maternal age.

WEBSITE 16.5 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.

WEBSITE 16.6 Synthesizing oocyte ribosomes. Ribosomes are almost a "differentiated product" of the oocyte, and the *Xenopus* oocyte contains 20,000 times as many ribosomes as somatic cells do. Gene repetition and gene amplification are both used to transcribe these enormous amounts of rRNA.

WEBSITE 16.7 Hormones and mammalian egg maturation. To survive, the follicle and its oocyte have to "catch the wave" of gonadotropic hormone release. The hormones of the menstrual cycle synchronize egg maturation with the anatomical changes of the uterus and cervix.

WEBSITE 16.8 The reinitiation of mammalian meiosis. The hormone-mediated disruption of communication between the oocyte and its surrounding follicle cells may be critical in the resumption of meiosis in female mammals.

Vade Mecum

Germ cells in the *Drosophila* embryo. In the Fruit Fly segment, a view of gametogenesis follows the primordial germ cells of the living *Drosophila* embryo from their formation as pole cells through gastrulation as they move from the posterior end of the embryo into the region of the developing gonad.

Gametogenesis in mammals. Stained sections of testis and ovary illustrate the process of gametogenesis, the streamlining of developing sperm, and the remarkable growth of the egg as it stores nutrients for its long journey. You can see this in movies and labeled photographs that take you at each step deeper into the mammalian gonad.