chapter 19 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. ELIOT (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 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. 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, such as 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 species (which include cnidarians, flatworms, and tunicates), somatic cells can readily become germ cells even in adult 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 do not arise within the gonad itself. Rather, their precursors—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. Our discussion of gametogenesis will include:

1. The formation of the germ plasm and the determination of the PGCs
2. The migration of the PGCs into the developing gonads
3. The process of meiosis and the modifications of meiosis for forming sperm and eggs
4. The differentiation of the sperm and egg
5. The hormonal control of gamete maturation and ovulation

Germ Plasm and the Determination of the Primordial Germ Cells

All sexually reproducing organisms arise from the fusion of gametes—sperm and eggs. All gametes arise from the primordial germ cells. In many instances (including frogs, nematodes, and flies), the primordial germ cells are specified autonomously by cytoplasmic determinants in the egg that are then parceled out to specific cells during cleavage. In other instances (such as salamanders and mammals), the germ cells are specified by interactions among neighboring cells. In those species wherein the determination of the primordial germ cells is brought about by the autonomous lo-

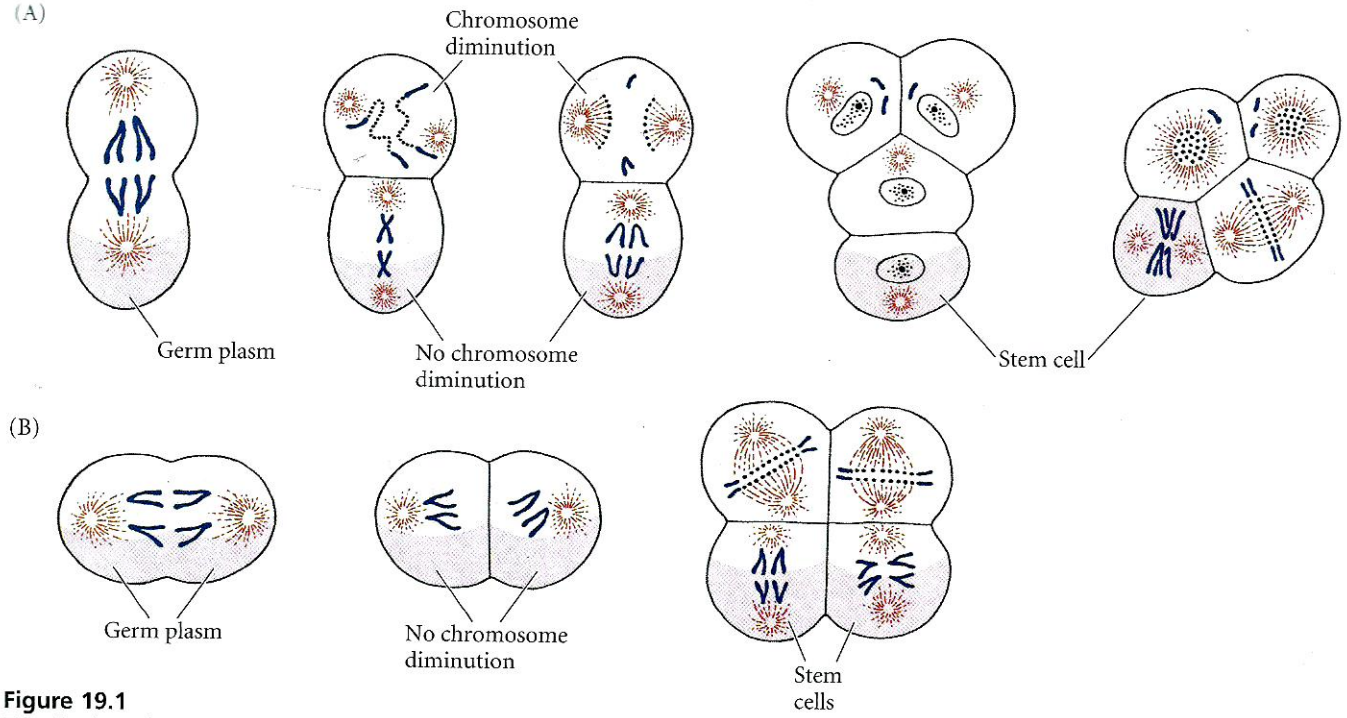


Figure 19.1
Distribution of germ plasm during cleavage of (A) normal and (B) centrifuged zygotes of *Parascaris*. (A) The germ plasm is normally conserved in the most vegetal blastomere, as shown by the lack of chromosomal diminution in that particular cell. Thus, at the 4-cell stage, the embryo has one stem cell for its gametes. (B) When the first cleavage is displaced 90 degrees by centrifugation, both resulting cells have vegetal germ plasm, and neither cell undergoes chromosome diminution. After the second cleavage, these two cells give rise to germinal stem cells. (After Waddington 1966.)

calization of specific proteins and mRNAs, these cytoplasmic components are collectively referred to as the **germ plasm**.

Germ cell determination in nematodes

Theodor Boveri (1862–1915; see Figure 4.2) 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 *Ascaris megalocephala*). This nematode has only two chromosomes per haploid cell, allowing for detailed observations of the 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 19.1A). More bizarre, however, is the behavior of the chromosomes in the subsequent division of these first two blastomeres. The ends of 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 chromosome 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. Boveri (1910) 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 should contain some of the vegetal cytoplasm (see

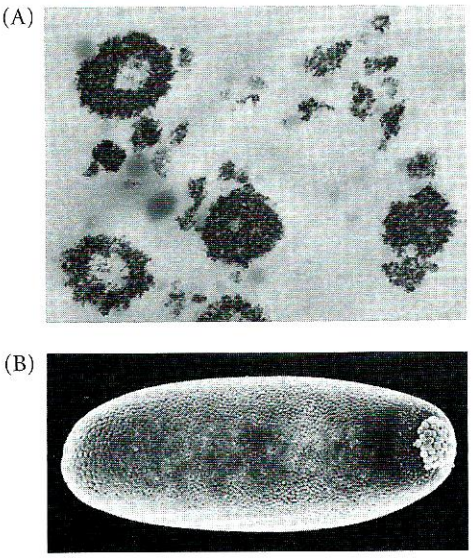


Figure 19.2
The pole plasm of *Drosophila*. (A) Electron micrograph of polar granules from particulate fraction of *Drosophila* pole cells. (B) Scanning electron micrograph of a *Drosophila* embryo just prior to completion of cleavage. The pole cells can be seen at the right of this picture. (Photographs courtesy of A. P. Mahowald.)

Figure 19.1B). Indeed, 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.

In the nematode *C. elegans*, the germ line precursor cell is the P4 blastomere. The **P-granules** enter this cell, and they appear critical for instructing it to become the germ line precursor (see Figure 8.43). The components of the P-granules include several transcriptional inhibitors and RNA-binding proteins, including homologues of the *Drosophila* Vasa and Nanos proteins, whose functions we will discuss below (Kawasaki et al. 1998; Seydoux and Strome 1999; Subramanian and Seydoux 1999).

WEBSITE 19.1 **Mechanisms of chromosome diminution.** The somatic cells do not lose DNA randomly. Rather, specific regions of DNA are lost during chromosome diminution.

Germ cell determination in insects

In *Drosophila*, PGCs form as a group of cells (**pole cells**) at the posterior pole of the cellularizing blastoderm. These nuclei migrate into the posterior region at the ninth nuclear division, and they become surrounded by the **pole plasm**, a complex collection of mitochondria, fibrils, and **polar granules** (Figure 19.2; 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).

Nature has provided confirmation of the importance of both the pole plasm and its polar granules. 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 who 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 be sterile (since they would lack germ cells). 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 resides within what will become the pole plasm (Figure 19.3A). This message is translated into protein during the early stages of cleavage (Figure 19.3C). 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 19.3B,D).

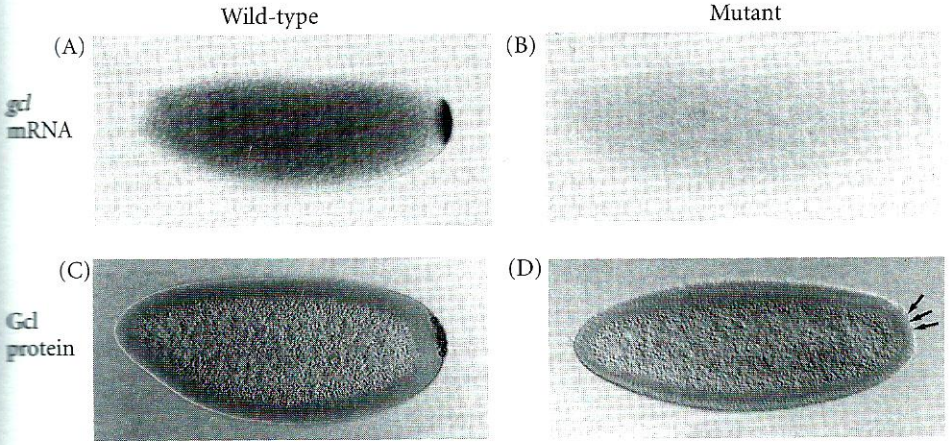


Figure 19.3
Localization of *germ cell-less* gene products in the posterior of the egg and embryo. (A, B) The *gcl* mRNA can be seen in the posterior pole of early-cleavage embryos produced by wild-type females (A), but not in embryos produced by *gcl*-deficient mutant females (B). (C, D) The protein encoded by the *gcl* gene can be detected in the germ cells at the cellular blastoderm stage of embryos produced by wild-type females (C), but not in embryos from mutant females (D). (From Jongens et al. 1992; photographs courtesy of T. A. Jongens.)

VADE MECUM[2] **Germ cells in the *Drosophila* embryo.** This segment 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. **[Click on Fruit Fly]**

A second set of pole plasm components are the posterior determinants mentioned in Chapter 9. **Oskar** appears to be the critical protein 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 the formation of more germ cells (Ephrussi and Lehmann 1992). Oskar functions by causing the localization of the proteins and RNAs necessary for germ cell formation. One of these RNAs is the *nanos* message, whose product is essential for posterior segment formation. **Nanos** is also essential for germ cell formation. Pole cells lacking Nanos do not migrate into the gonads and fail to become gametes. Nanos appears to be important in preventing mitosis and transcription during germ cell development (Kobayashi et al. 1996; Deshpande et al. 1999). Another one of these RNAs encodes **Vasa**, an RNA-binding protein. The mRNAs for this protein are seen in the germ plasm of many species.

A third germ plasm component, **mitochondrial ribosomal RNA (mtrRNA)**, was a big surprise. Kobayashi and Okada (1989) showed that the injection of mtrRNA into embryos formed from ultraviolet-irradiated eggs restores the ability of these embryos to form pole cells. Moreover, in normal fly eggs, the small and large mtrRNAs are located outside the mitochondria solely in the pole plasm of cleavage-stage embryos, where they appear as components of the polar granules (Kobayashi et al. 1993; Amikura et al. 1996; Kashikawa et al. 1999). While mtrRNA is involved in directing the formation of the pole cells, it does not enter them. The Tudor protein localizes to the germ plasm, and it appears to be critical for the export of these mtrRNAs from the mitochondria and into the cytoplasm (Amikura et al. 2001).

A fourth component of *Drosophila* pole plasm (and one that becomes localized in the polar granules) is a nontranslatable RNA called **polar granule component (PGC)**. While its exact function remains unknown, the pole cells of transgenic female flies making antisense RNA against PGC fail to migrate to the gonads (Nakamura et al. 1996).

WEBSITE 19.2 **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.

Germ cell determination in amphibians

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 (Figure 19.4). 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 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 the germ plasm in amphibians 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 germ plasm islands move with the vegetal yolk mass during the cortical rotation just after fertilization. After the 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

Figure 19.4
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; photograph courtesy of L. Etkin.)

(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 (Forristall et al. 1995; Ikenishi et al. 1996; Zhou and King 1996). So, like the *Drosophila* pole plasm, the cytoplasm from the vegetal region of the *Xenopus* zygote contains the determinants for germ cell formation. Moreover, several of the components are the same in the two species.

The inert genome hypothesis

The components of the germ plasm have not all been catalogued. Indeed, in the birds and mammals, such a list has hardly even been started. Moreover, we still do not know the functions of the proteins (such as Vasa and Nanos) and nontranslated RNAs found in the germ plasm. One hypothesis (Nieuwkoop and Sutasurya 1981; Wylie 1999) is that the components of the germ plasm inhibit both transcription and translation, thereby preventing the cells containing it from differentiating into anything else. According this hypothesis, the cells become germ cells because they are forbidden to become any other type of cell. This suppression of transcription is seen in the germ cells of several species, including flies, frogs, and nematodes (Figure 19.5). Many of the proteins in the germ plasm (such as Gcl) act by inhibiting either transcription or translation (Leatherman et al. 2002).

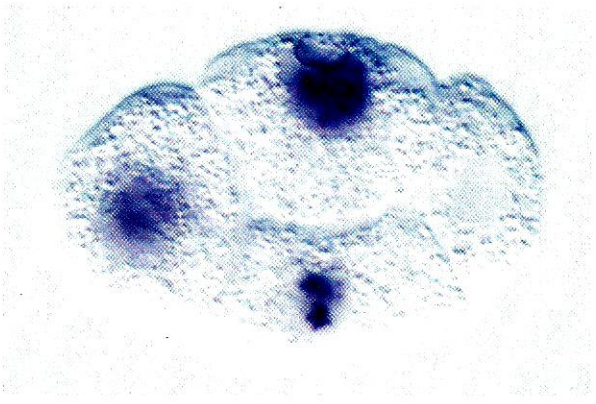


Figure 19.5
Inhibition of transcription in germ cell precursors of *C. elegans*. The photograph shows in situ hybridization to β-galactosidase mRNA expressed under control of the *pes-10* promoter. The *pes-10* gene is one of the earliest genes expressed in *C. elegans*. The P-blastomere that gives rise to the germ cells does not transcribe the gene. (From Seydoux and Fire 1994; photograph courtesy of G. Seydoux.)

Germ Cell Migration

Germ cell migration in amphibians

The germ plasm of anuran amphibians (frogs and toads) collects around the vegetal pole in the zygote. 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 19.4; see also 19.6A–E; 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) and then along the abdominal wall and into the genital ridges. They migrate up this tissue until they reach the developing gonads (Figure 19.6F). *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 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, and approximately 30 PGCs colonize the gonads (Whitington and Dixon 1975; Wylie and Heasman 1993). These cells will divide to form the germ cells.

The primordial germ cells of urodele amphibians (salamanders) have an apparently different origin, which has been traced by reciprocal transplantation experiments to the regions of the mesoderm that involute through the ventrolateral lips of the blastopore. Moreover, there does not seem to be any particular localized "germ plasm" in salamander eggs. Rather, the interaction of the dorsal endoderm cells and animal hemisphere cells creates the conditions needed to form germ cells in the areas that involute through the ventrolateral lips (Sutasurya and Nieuwkoop 1974; Wakahara 1996). So in salamanders, the PGCs are formed by induction within the mesodermal region and presumably follow a different path into the gonads.

*This statement does not necessarily hold true for all anurans. In the frog *Rana pipiens*, the germ cells follow a similar route, but may be passive travelers along the mesentery rather than actively motile cells (Subtelny and Penkala 1984). The migration of fish PGCs follows a similar route, and there may be species differences as to whether the PGCs are active or passive travelers (Braat et al. 2000).

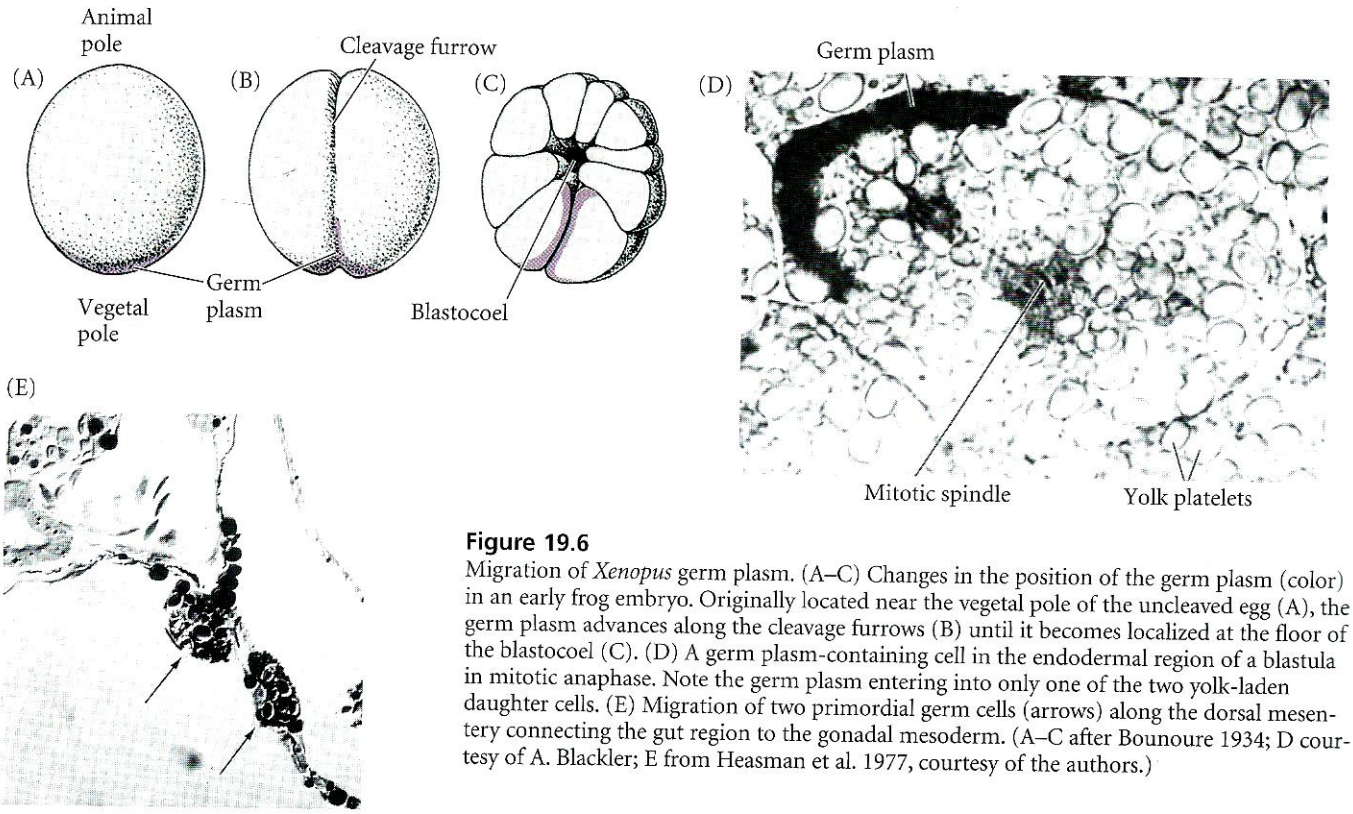


Figure 19.6
Migration of *Xenopus* germ plasm. (A–C) Changes in the position of the germ plasm (color) in an early frog embryo. Originally located near the vegetal pole of the uncleaved egg (A), the germ plasm advances along the cleavage furrows (B) until it becomes localized at the floor of the blastocoel (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.)

Germ cell formation and migration in mammals

There is no obvious germ plasm in mammals, and mammalian germ cells are not morphologically distinct during early development. Rather, germ cells are induced in the embryo. 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 19.7A). At day 6.5 of embryonic development, BMP4 and BMP8b from the extraembryonic ectoderm give certain cells in this area the ability to produce germ cells (Lawson et al. 1999; Ying et al. 2000). The cluster of cells capable of generating PGCs express *fragilis*, a gene encoding a particular transmembrane protein. However, these *fragilis*-expressing cells can form both PGCs and some somatic cells. In the center of this cluster of cells is a small group of cells that also expresses *stella*.* These cells are restricted to the germ cell fate (Saitou et al. 2002).

Based on differential staining of fixed tissue, it had long been thought that the mouse germ cell precursors migrated from the epiblast into the extraembryonic mesoderm and then back again into the embryo by way of the allantois (see Chiquoine 1954; Mintz 1957; and earlier editions of this textbook). However, the ability to label mouse primordial germ cells with green fluorescent protein and to watch these living cells migrate has caused a reevaluation of the germ cell migration pathway in mammals (Anderson et al. 2000; Molyneaux et al. 2001). First, it appears that mammalian PGCs migrate directly into the endoderm from the posterior region of the primitive streak. (The cells that enter the allantois are believed to die.) These *stella*-expressing cells find themselves in the hindgut. 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 19.7E). 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 (Figure 19.8).

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. These cells are also capable of penetrating cell monolayers and migrating through cell sheets (Stott and Wylie 1986). The mechanism by which the PGCs know the route of this journey is still unknown. Fibronectin is likely to be an im-

*It is not known what the Stella protein does. The sequence of this protein gives few clues other than that it contains some highly basic regions (and therefore might be able to associate with nucleic acids) and that it has a splicing factor motif in its carboxy terminus. It may therefore be involved in chromosome organization or RNA processing.

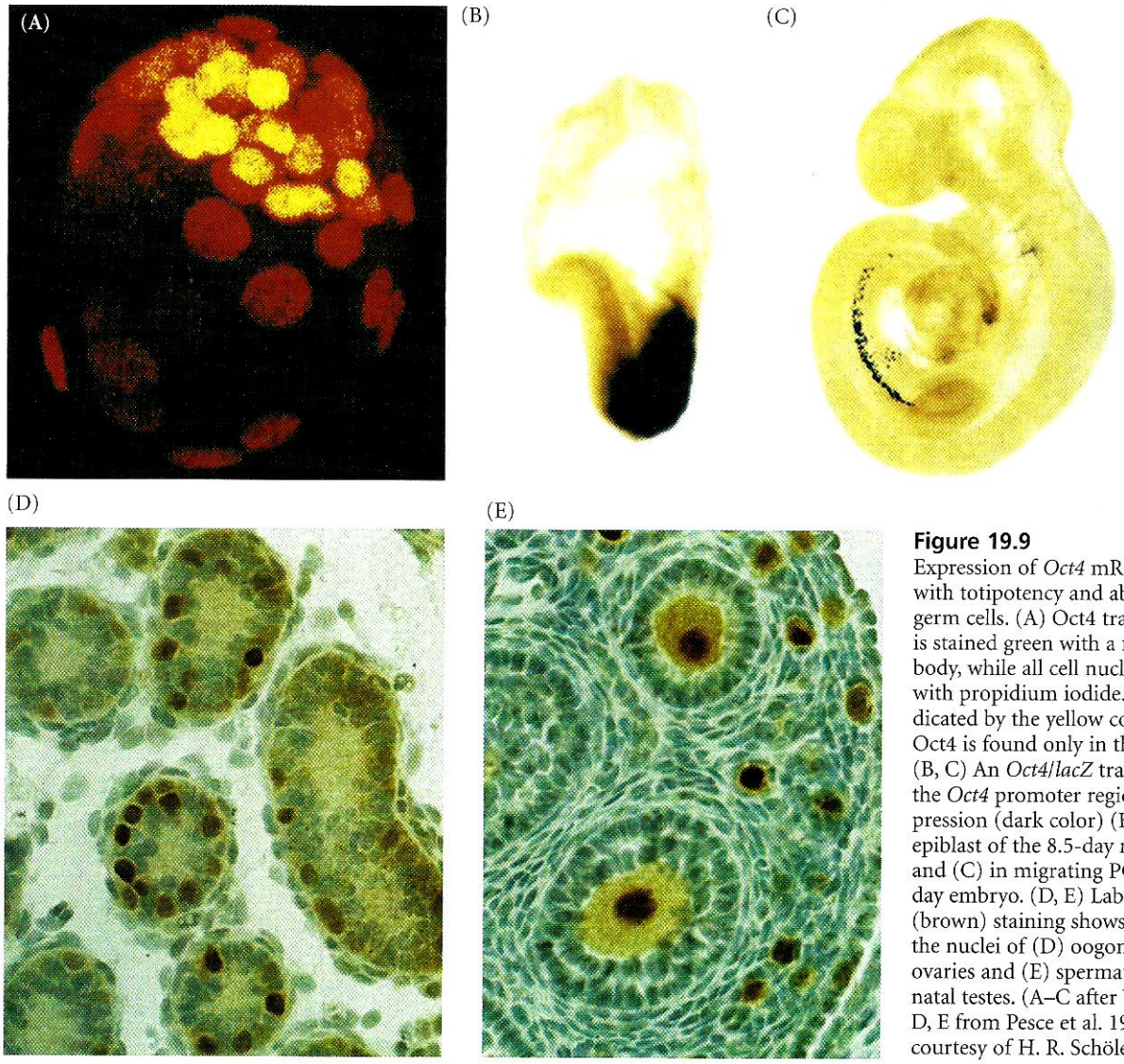


Figure 19.9
Expression of *Oct4* mRNA correlates with totipotency and ability to form germ cells. (A) Oct4 transcription factor is stained green with a fluorescent antibody, while all cell nuclei are stained red with propidium iodide. The overlap (indicated by the yellow color) shows that Oct4 is found 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) oogonia in postnatal ovaries and (E) spermatogonia in postnatal testes. (A–C after Yeom et al. 1996; D, E from Pesce et al. 1998; photographs courtesy of H. R. Schöler.)

portant substrate for PGC migration (ffrench-Constant et al. 1991), and germ cells that lack the integrin receptor for such extracellular matrix proteins cannot migrate to the gonads (Anderson et al. 1999). Directionality may be provided by a gradient of soluble protein.* In vitro evidence suggests that the genital ridges of 10.5-day mouse embryos secrete a diffusible TGF-β1-like protein that is capable of attracting mouse PGCs (Godin et al. 1990; Godin and Wylie 1991). Whether the genital ridge is able to provide such cues in vivo remains to be tested.

Although no germ plasm has been found in mammals, the retention of totipotency has been correlated with the expression of a nuclear transcription factor, Oct4. This factor is expressed in all of the early-cleavage blastomere nuclei, but its expression becomes restricted to the inner cell mass. During gastrulation, it becomes expressed solely in those posterior epiblast cells thought to give rise to the primordial germ cells. After that, this protein is seen only in the primordial germ cells, and later in oocytes (Figure 19.9; Yeom et al. 1996; Pesce et al. 1998). (Oct4 is not seen in the developing sperm after the germ cells reach the testes and become committed to sperm production.)

The proliferation of the PGCs appears to be promoted by stem cell factor, the same growth factor needed for the proliferation of neural crest-derived melanoblasts and hematopoietic stem cells (see Chapter 6). Stem cell factor is produced by the cells lining the migration pathway and remains bound to their cell membranes. It appears that the presentation of this protein on cell membranes is important for its activity. Mice

*In zebrafish, the primordial germ cells appear to be following a gradient of the SDF-1 protein, which is known to be a chemoattractant for mammalian lymphocytes (Doitsidou et al. 2002).

homozygous for mutations in the genes for either stem cell factor or its receptor (c-Kit) are deficient in germ cells (as well as melanocytes and blood cells) (see Dolci et al. 1991; Matsui et al. 1991). The addition of stem cell factor to PGCs taken from 11-day mouse embryos stimulates their proliferation for about 24 hours and appears to prevent programmed cell death that would otherwise occur (Godin et al. 1991; Pesce et al. 1993).

Sidelights & Speculations

EG Cells, ES Cells, and Teratocarcinomas

Embryonic germ (EG) 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, John Gearhart's laboratory (Shamblott et al. 1998) cultured human EG cells. These cells were able to generate differentiated cells from all three primary germ layers, and they are presumably totipotent. Such cells could be used medically to create neural or hematopoietic stem cells, which might be used to regenerate damaged neural or blood tissues (see Chapter 4). EG cells are often considered embryonic stem (ES) cells, and the distinction of their origin is ignored.

Embryonic stem (ES) cells

Embryonic stem (ES) cells were described in Chapter 4; these cells are derived from the inner cell mass. ES cells and EG cells can be transfected with recombinant genes and inserted into blastocysts to create transgenic mice. Such a mammalian germ cell or stem cell contains within it all the information needed for subsequent development.

What would happen if such a cell became malignant? In one type of tumor, the germ cells become embryonic stem cells, like the FGF2-treated PGCs in the experiment above. This type of tumor is called a **teratocarcinoma**. Whether spontaneous or experimentally produced, a teratocarcinoma contains an undifferentiated stem cell population that has biochemical and developmental properties remarkably similar to those of the inner cell mass (Graham 1977). 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 19.10). 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. Thus, the teratocarcinoma stem cells mimic early mammalian development, but the tumor they form is characterized by random, haphazard development.

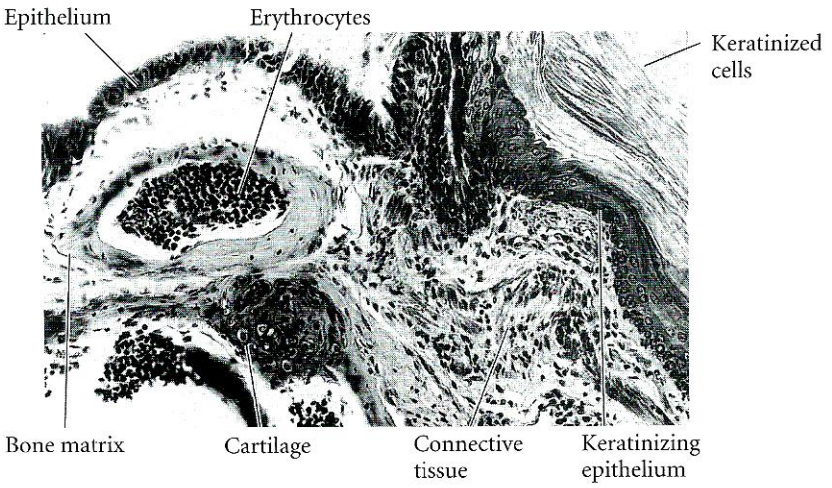


Figure 19.10
Photomicrograph of a section through a teratocarcinoma, showing numerous differentiated cell types. (From Gardner 1982; photograph by C. Graham, courtesy of R. L. Gardner.)

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 resultant litter contained mice that were homozygous for a large number of genes from the tumor cell (Figure 19.11).

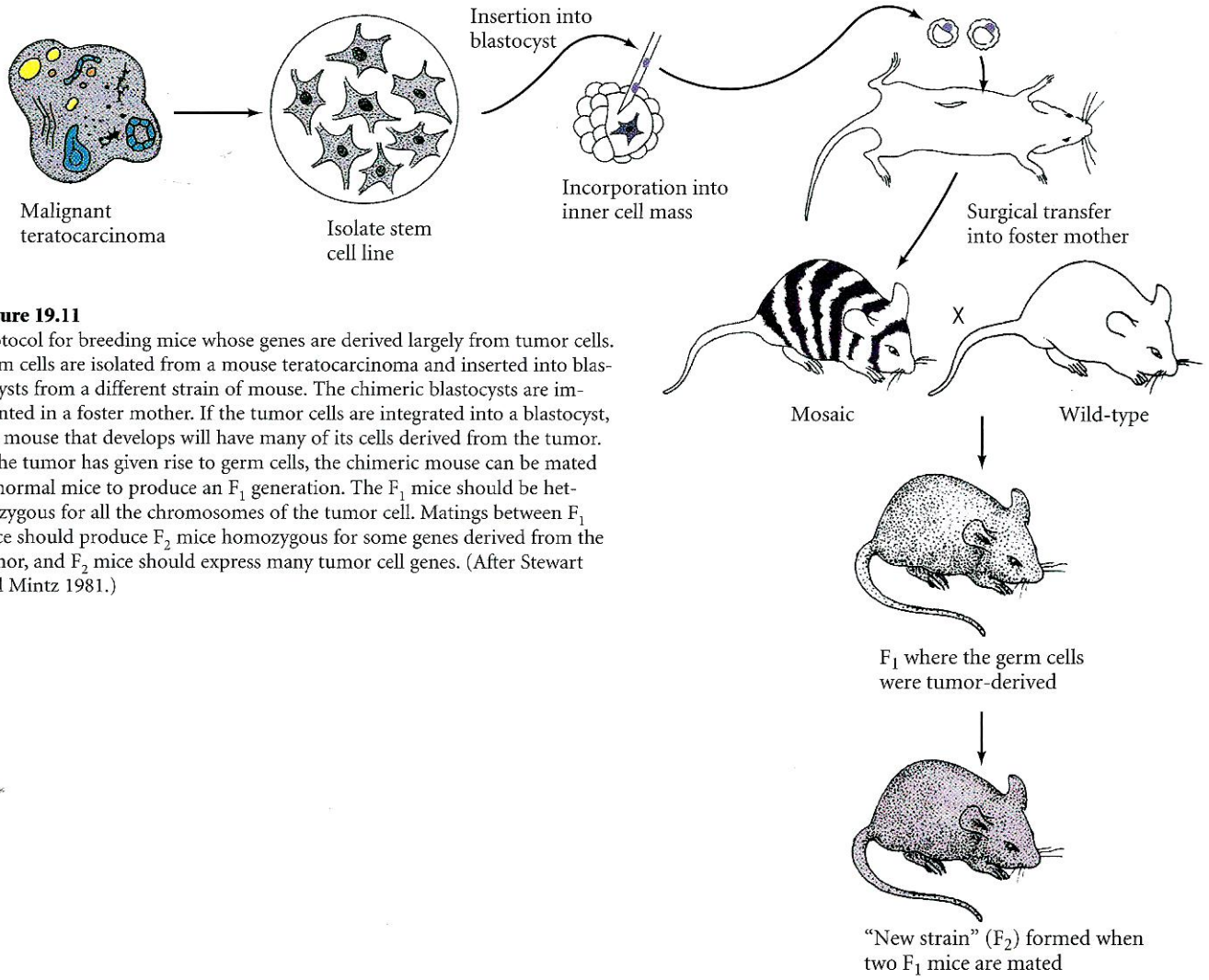


Figure 19.11
Protocol for breeding mice whose genes are derived largely from tumor cells. Stem cells are isolated from a mouse teratocarcinoma and inserted into blastocysts from a different strain of mouse. The chimeric blastocysts are implanted in a foster mother. If the tumor cells are integrated into 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. The F_1 mice should be heterozygous for all the chromosomes of the tumor cell. Matings between F_1 mice should produce F_2 mice homozygous for some genes derived from the tumor, and F_2 mice should express many tumor cell genes. (After Stewart and Mintz 1981.)

Germ cell migration in 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 19.12; 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 19.13). When blood vessels form in the germinal crescent, the PGCs enter those vessels and are carried by the circulation to the region where the hindgut is forming. Here they leave the circulation, become associated with the mesentery, and migrate into the genital ridges (Swift 1914; Mayer 1964; Kuwana 1993; Tsunekawa et al. 2000).

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). Evidence for chemotaxis comes from studies (Kuwana et al. 1986) in which circulating chick PGCs were isolated from the blood and cultured between gonadal rudiments and other embryonic tissues. The PGCs migrated specifically into the gonadal rudiments during a 3-hour incubation.

Germ cell migration in Drosophila

During *Drosophila* embryogenesis, the primordial germ cells move from the posterior pole to the gonads in a manner similar to that of mammalian germ cells (Figure 19.14). 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. In the second step, the gut endoderm

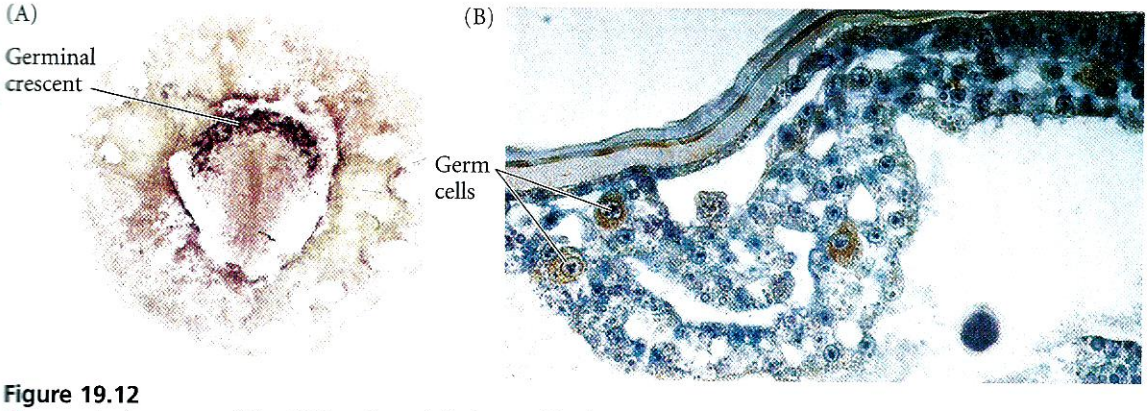


Figure 19.12
The germinal crescent of the chick embryo. (A) Germ cells of a definitive primitive streak-stage (stage 4, roughly 18-hour) chick embryo, stained (purple) for the chick Vasa homologue protein. The stained cells are confined to the germinal crescent. (B) Higher magnification of the stage 4 germinal crescent region, showing germ cells (stained brown) in the thickened epiblast. (Anterior is to the right.) (From Tsunekawa et al. 2000; photographs courtesy of N. Tsunekawa.)

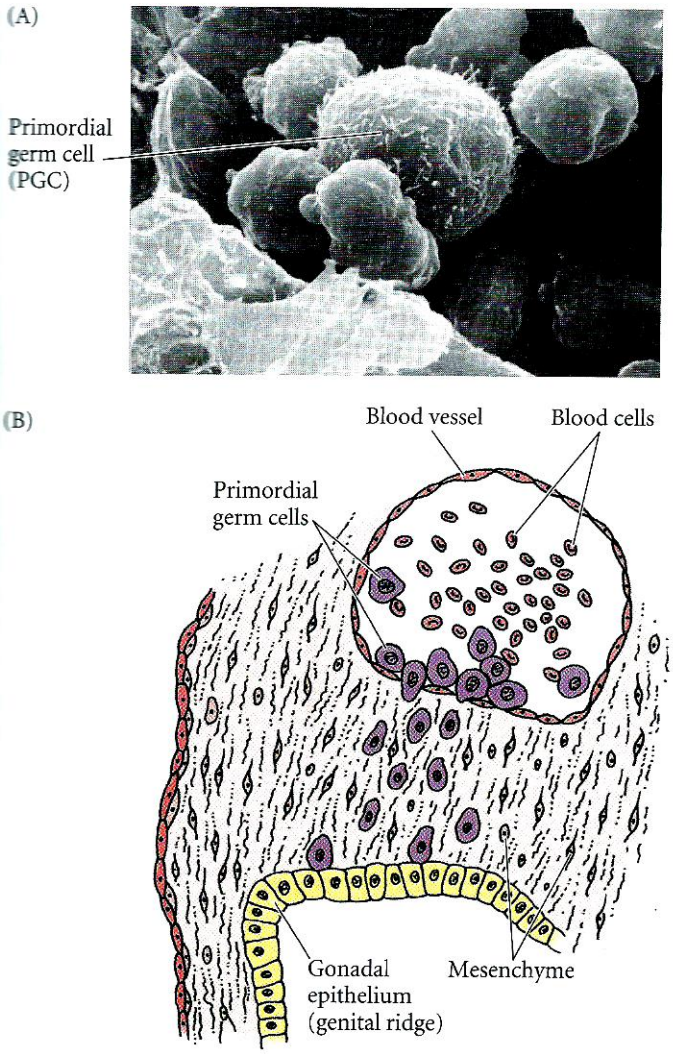
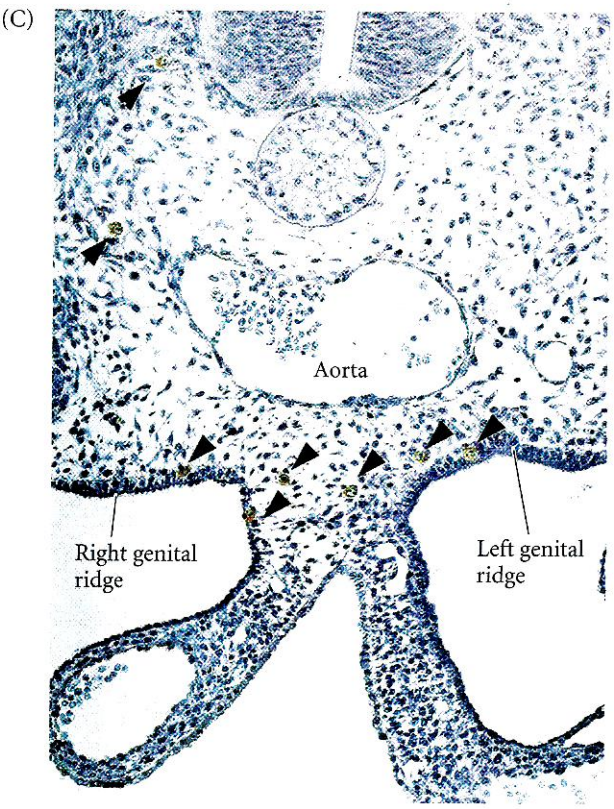


Figure 19.13
Migration of primordial germ cells in the chick embryo. (A) Scanning electron micrograph of a chick PGC in a capillary of a gastrulating embryo. The PGC can be identified by its large size and by the microvilli on its surface. (B) Diagram of transverse section near the prospective gonadal region of a chick embryo. Several PGCs within a blood vessel cluster next to the gonadal epithelium. One PGC is crossing through the blood vessel endothelium, and another PGC is already located adjacent to the gonadal epithelium. (C) Having passed through the endothelium of the dorsal aorta, chick germ cells (arrowheads) migrate toward the genital ridges of the embryo. (A from Kuwana 1993, courtesy of T. Kuwana; B after Romanoff 1960; C from Tsunekawa et al. 2000, courtesy of N. Tsunekawa.)



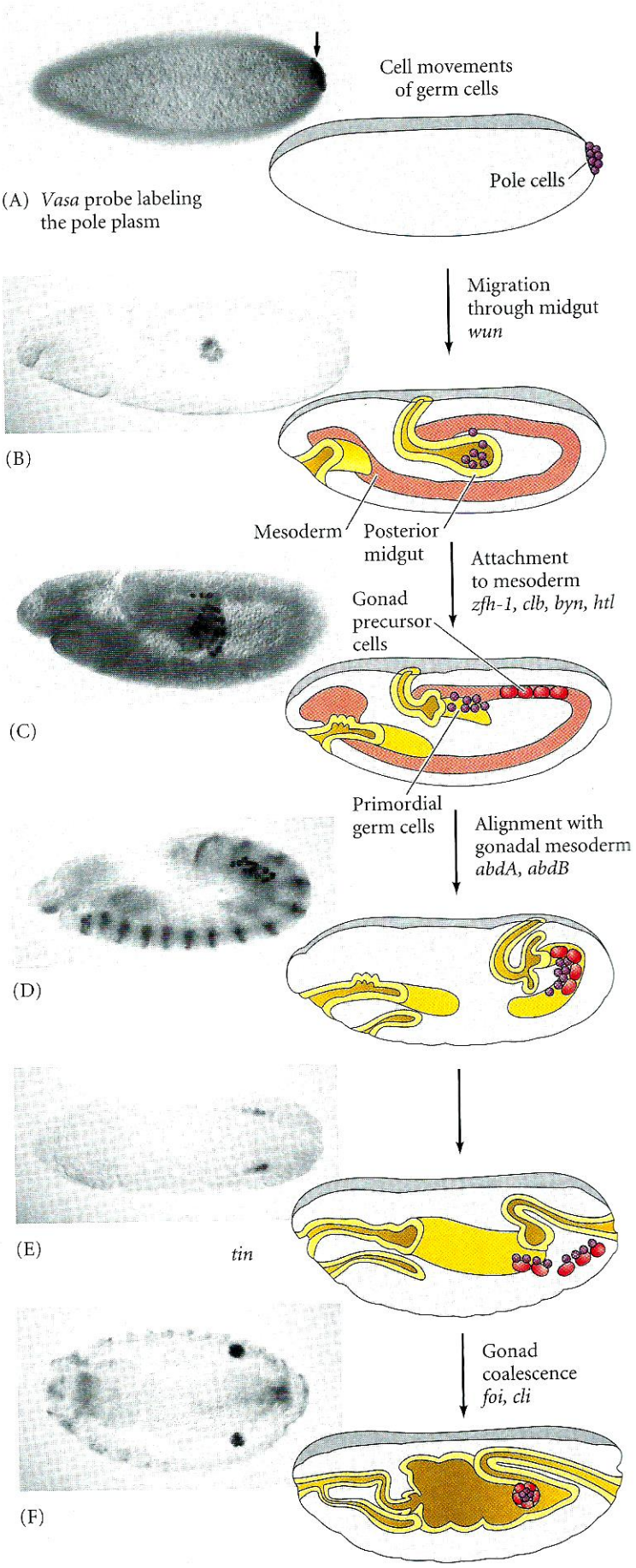


Figure 19.14
Migration of germ cells in the *Drosophila* embryo. The left column shows the germ plasm as stained by antibodies to Vasa, a protein component of the germ plasm (D has been counterstained with antibodies to Engrailed protein to show the segmentation, and E and F are dorsal views.) The right column diagrams the movements of the germ cells. (A) The 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) The PGCs move through the endoderm and into the caudal visceral mesoderm by diapedesis. The *wunen* (*wun*) gene product expressed in the endoderm expels the PGCs, while the product of the *columbus* (*clb*) gene expressed in the caudal mesoderm attracts them. (D–F) The movements of the mesoderm bring the PGCs into the region of the tenth through twelfth segments, where the mesoderm coalesces around them to form the gonads. (Photographs from Warrior et al. 1994, courtesy of R. Warrior; diagrams after Howard 1998.)

triggers active diapedesis in the PGCs, which travel through the blind end of the posterior midgut, migrating 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 PGCs 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 product of the *wunen* gene appears to be responsible for directing the migration of the PGCs from the endoderm into the mesoderm. This protein is expressed in the endoderm immediately before PGC migration, and it repels the PGCs. In loss-of-function mutants of this gene, the PGCs wander randomly (Zhang et al. 1997). Two proteins appear to be critical for the attracting the *Drosophila* PGCs to the gonads. One is the product of the *columbus* gene, the other is Hedgehog (Moore et al. 1998; Van Doren et al. 1998; Deshpande et al. 2001). These proteins are made in the mesodermal cells of the gonads. In loss-of-function mutants of either gene, the PGCs wander randomly from the endoderm, and if either 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 divide and mature into gametes.

Meiosis

Once in the gonad, the primordial germ cells continue to divide mitotically, 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