

chapter 4

Fertilization



Figure 4.1 Fertilization in the rat. This scanning electron micrograph shows an egg from which the egg envelope, or zona pellucida, has been removed to make the fertilizing sperm better visible. The sperm's head has been engulfed by the egg; the midpiece and tail are still outside. The egg surface is covered with fingerlike projections called microvilli, which enlarge the egg surface and provide a reservoir of plasma membrane to be used during cleavage.

4.1	Interactions before Sperm-Egg Adhesion	76
	Some Sperm Must Undergo Capacitation before They Can Fertilize Eggs	77
	Many Sperm Are Attracted to Eggs by Chemical Signals	77
4.2	Fertilization in Sea Urchins	78
	Sea Urchin Sperm Undergo the Acrosome Reaction before They Adhere to the Vitelline Envelope	79
	Method 4.1 Immunostaining	80
	Sea Urchin Sperm Adhere to Eggs with an Acrosomal Protein Called Bindin	81
	Gamete Fusion Leads to the Formation of a Fertilization Cone	82
4.3	Fertilization in Mammals	83
	Mouse Sperm Undergo the Acrosome Reaction after They Adhere to the Zona Pellucida	83
	A Bioassay Is a Powerful Strategy to Reveal a Biologically Active Component	85
	Mouse Sperm Adhere to a Specific Zona Pellucida Protein	85
	Antibodies to Sperm-Egg Adhesion Proteins Can Act as Contraceptives	87
4.4	Egg Activation	88
	Egg Activation May Be Triggered by Different Signaling Mechanisms	88
	A Temporary Rise in Ca^{2+} Concentration Is Followed by Activation of Protein Kinase C	89
	Egg Activation Triggers the Completion of Meiosis and the Fusion of the Gametes' Haploid Genomes	90
	Activation Accelerates the Egg's Metabolism in Preparation for Cleavage	91
4.5	Blocks to Polyspermy	91
	The Fertilization Potential Serves as a Fast Block to Polyspermy	92
	The Cortical Reaction Causes a Slow Block to Polyspermy	93
4.6	The Principle of Overlapping Mechanisms	93
4.7	Parthenogenesis	95
		75

MOST higher organisms reproduce sexually by forming two types of haploid gametes, eggs and sperm. Both are highly specialized and short-lived unless they unite to form a newly developing organism, through a process called fertilization (Fig. 4.1). Fertilization begins with the approach of the sperm to the egg and ends with the formation of the diploid zygote. The entire process can be broken down into four major steps:

- The sperm's approach to the egg
- The sperm's penetration of egg envelopes with or without adhesion to the vitelline envelope
- Plasma membrane contact and fusion
- Egg activation

Many mechanisms have evolved in animals to ensure that gametes meet at the appropriate time and place and under the right conditions to achieve fertilization. Thus, fertilization follows a series of well-choreographed interactions between males and females, and between sperm and eggs. Parental mating behavior brings eggs and sperm close together before the sperm are released and begin to swim with their flagella. In some organisms, chemical attractants produced by the egg itself or by ovarian cells guide the sperm in their final approach to the egg.

Sperm that encounter an egg face a series of obstacles because the envelopes that protect the egg are also formidable barriers to sperm entry. Several solutions to this dilemma have evolved. Some of the hardest egg coats, such as the calciferous shells of bird eggs, are laid down only after sperm have been admitted to the egg. The eggs of other animals, including fish and insects, have tiny holes called *micro-pyles* in their shells through which sperm can enter. In most species, however, the sperm must penetrate one or more egg envelopes before the plasma membranes of the two gametes can fuse (Fig. 4.2). Of particular importance is the *vitelline envelope*, a coat of proteins laid down by the oocyte before ovulation. In mammals, the vitelline envelope is a thick, transpar-

ent layer of glycoproteins called the *zona pellucida*.

In many species, a critical step in fertilization is sperm-egg adhesion, the binding of sperm to the vitelline envelope. The underlying molecular mechanisms are species-specific: Sperm adhere more stably to eggs from the same species than to eggs from another species. This species-specificity is particularly important for aquatic organisms, whose eggs may be exposed to sperm from many other species.

After adhesion to an egg, sperm penetrate the vitelline envelope using enzymes produced in the *acrosome*. When the first sperm finally reaches the egg cell proper, the plasma membranes of the two gametes fuse, forming a cytoplasmic bridge between them. The bridge widens and allows the sperm head to enter the egg. The two haploid nuclei derived from the egg and the sperm unite so that both sets of chromosomes are passed on together during cleavage divisions.

Plasma membrane contact or gamete fusion triggers a chain of events collectively called *egg activation*. These events include resumption of the cell cycle in eggs that have been arrested since oocyte maturation. In many species, activation also entails measures to protect the fertilized egg against the entry of additional sperm, which would disrupt mitoses.

Much of our knowledge about fertilization stems from observations and experiments in vitro, which are easily carried out with animals that shed their gametes into water. Therefore sea urchins, marine worms, fish, and amphibians have traditionally been the organisms chosen for research on fertilization. More recent studies have focused on mammalian fertilization, with the goal of developing better infertility treatments and new methods of contraception. Since research on fertilization in mammals is now beginning to rival the depth and sophistication of work on aquatic organisms, this chapter will focus on fertilization in both sea urchins and mice.

4.1 Interactions before Sperm-Egg Adhesion

Parental mating behavior ensures that eggs and sperm are delivered close to each other. Further success of the fertilization process now depends on interactions between the gametes themselves. In many species, eggs or associated cells release chemical attractants to which sperm respond by swimming actively toward them. In

mammals and other animals as well, sperm must undergo a biochemical conditioning process before they are capable of fertilizing an egg. The processes of chemical attraction and sperm conditioning may in fact be related. At least in mammals, there are indications that only those sperm that are at the peak of their fertilizing capability respond to a chemoattractant released from the ovary at ovulation.

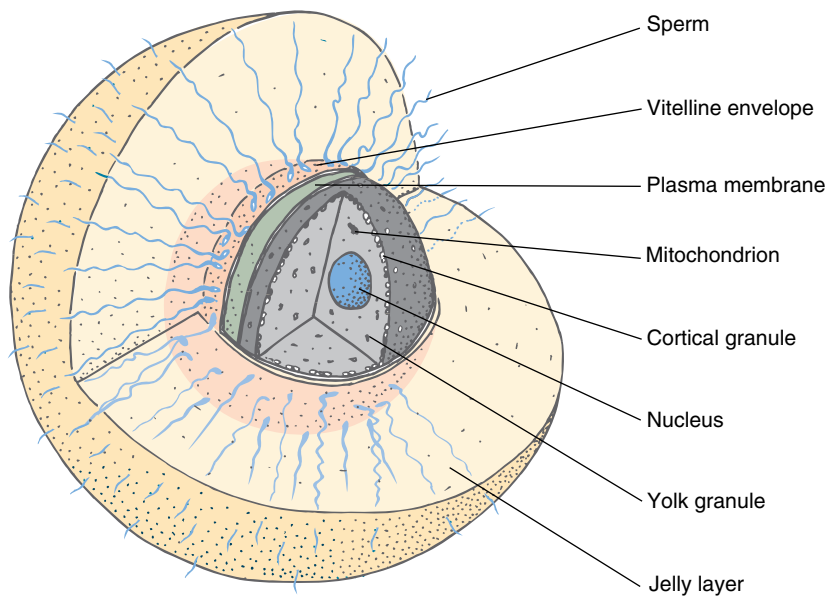


Figure 4.2 Sea urchin egg, drawn to show the egg coats that a sperm must penetrate before reaching the egg cell proper. The sperm first encounters a thick jelly layer, which it penetrates with digestive enzymes from its acrosome. Next the sperm binds to receptors at the level of the vitelline envelope and then pierces the vitelline envelope. Now the plasma membranes of sperm and egg make contact and fuse. Just beneath the egg plasma membrane are thousands of membranous vesicles called cortical granules. They will undergo exocytosis after sperm-egg fusion and shed their contents into the perivitelline space between egg and vitelline envelope, thus modifying the vitelline envelope so that no additional sperm can fertilize the egg. The diameter of the egg cell is about 75 μm .

SOME SPERM MUST UNDERGO CAPACITATION BEFORE THEY CAN FERTILIZE EGGS

Early attempts to fertilize mammalian eggs *in vitro* were unsuccessful because experimenters used freshly ejaculated sperm, which are unable to penetrate the *zona pellucida*. Sperm normally acquire this capability in the course of a few hours inside the female genital tract, through a process called *capacitation* (C. R. Austin, 1952). Capacitation also takes place *in vitro* when sperm are incubated with fluid from the uterus or oviduct. Prior to capacitation, mammalian sperm are in a state of low activity, apparently saving their energy and responsiveness until they are most likely to encounter an egg. A capacitated sperm is metabolically more active and beats its flagellum rapidly (Gwatkin, 1977). Similar behavior has also been observed in the sperm of some invertebrates (Wikramanayake et al., 1992).

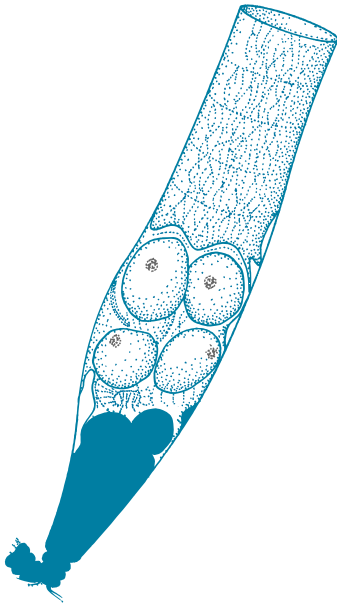
The molecular mechanisms of capacitation are still under investigation. The sperm plasma membrane undergoes changes in lipid composition and other alterations that will facilitate fertilization (Yanagimachi, 1994). Other capacitation events may be reorganizations in the *cytoskeleton* at the sperm tip or the removal of "coating factors" that mask egg-binding sites at the sperm surface.

MANY SPERM ARE ATTRACTED TO EGGS BY CHEMICAL SIGNALS

Although parental mating behavior brings egg and sperm together, most sperm complete their journey to the egg by swimming. In mammals, sperm is ejaculated into the vagina or directly into the uterus. From here, the sperm must travel to the site of fertilization in the upper oviduct. Muscular actions of the uterus and oviducts contribute significantly to the movement of the sperm. Nevertheless, of the vast number of sperm (100 million to 500 million in humans) deposited in the vagina, only a few hundred reach the fertilization site. This extreme reduction in number is very intriguing and does not seem to be fully explained as a means of selecting against abnormal sperm.

Sperm movement is guided by chemical attractants produced by the egg or by ovarian cells, at least in some species. Oriented movement in response to an external chemical signal is called *chemotaxis*. Chemotactic behavior in sperm has been observed in various groups of animals, such as hydrozoans, molluscs, echinoderms, urochordates, and mammals, including humans (R. Miller, 1985; Eisenbach and Ralt, 1992). In the hydrozoan *Campanularia*, eggs develop in an ovary-like structure called a *gonangium*, which has a funnel-shaped opening through which sperm must enter to reach the eggs. Richard Miller (1966) showed that the funnel produces an attractant that activates sperm and causes them to turn toward the funnel (Fig. 4.3). This attractant is species-specific; thus, funnel extract from *C. calceofera* attracts sperm from the same species but not from a related species, *C. flexuosa*. In another hydrozoan, *Orthopyxis caliculata*, the attractant is released by the eggs instead of the gonangium (R. Miller, 1978). Its release is timed precisely: The sperm of this hydrozoan are attracted by eggs that have completed their second meiotic division, not by less mature eggs or oocytes.

Several sperm attractants have been isolated from sea urchins. One, called *resact*, is found in the jelly layer surrounding the egg of *Arbacia punctulata* (G. E. Ward et al., 1985). Resact is a small peptide consisting of 14 amino acids. At very low concentrations, resact attracts sperm in a species-specific way. However, when sperm are pretreated with high concentrations of resact they lose their chemotactic ability. This result suggests that the sperm surface may have specific receptors that become saturated at a certain resact concentration. In fact, such receptors have been identified in the plasma membrane of *Arbacia* sperm (Bentley et al., 1986). How these



receptors allow sperm to find the direction of increasing chemoattractant concentration—that is, the way to the attractant's source, is still unresolved.

Preliminary evidence for sperm chemoattractants in mammals came from reports that mammalian sperm accumulate in the oviduct *below* the fertilization site and remain relatively motionless for hours until ovulation has occurred. Then the sperm become motile again, and a small number of them find their way to the fertilization site. These observations were corroborated by *in vitro* experiments indicating that human sperm respond chemotactically to a substance present in the follicular fluid released during ovulation (Ralt et al., 1991). However, the chemical nature of the putative attractant is not yet known, nor is it clear whether the signal is released by the egg itself or by other ovarian cells. Curiously, only a fraction of all sperm appear to be receptive to the attractant at any particular time (Cohen-Dayag et al., 1994). Thus, the putative chemoattractant in follicular fluid may selectively attract sperm that are at the peak of their ability to fertilize (Eisenbach and Tur-Kaspa, 1999). According to this hypothesis, the egg encounters only the selected sperm upon ovulation; the immature and overmature sperm are excluded from the competition.

4.2 Fertilization in Sea Urchins

Sea urchins have been classical animals for studies on fertilization (Epel, 1977). One advantage of these organisms

is the copious amount of gametes they produce. During each breeding season, a sea urchin female spawns 400 million eggs, while a male releases 100 billion sperm. Producing so many gametes seems necessary because they quickly disperse in the ocean. A second advantage is that sea urchin fertilization occurs naturally in seawater and can therefore be easily studied *in vitro*. Early investigators may also have liked sea urchins because they provided a reason for them to spend their summers at marine stations, where they would meet biologists from other laboratories to trade new ideas and laboratory tricks.

The process of sea urchin fertilization can be broken down into five steps as shown in Figure 4.4. The sperm approaches by *chemotaxis* (step 1) and encounters the egg's protective coats. Upon contact with the egg jelly, the acrosome at the tip of the sperm releases its contents by exocytosis, in a process known as the *acrosome reaction* (step 2). Acrosomal enzymes digest a hole into the egg jelly while a slender projection called the *acrosomal process* rapidly forms at the tip of the sperm. Next the acrosomal process encounters the *vitelline envelope*, to which it adheres species-specifically, a step called *sperm-egg adhesion* (step 3). A hole lysed into the vitelline envelope finally gives the sperm access to the egg cell proper. The plasma membranes of egg and sperm touch, a step referred to as *plasma membrane contact* (step 4). It is followed quickly by *gamete fusion* (step 5), the event that most narrowly defines fertilization: The plasma

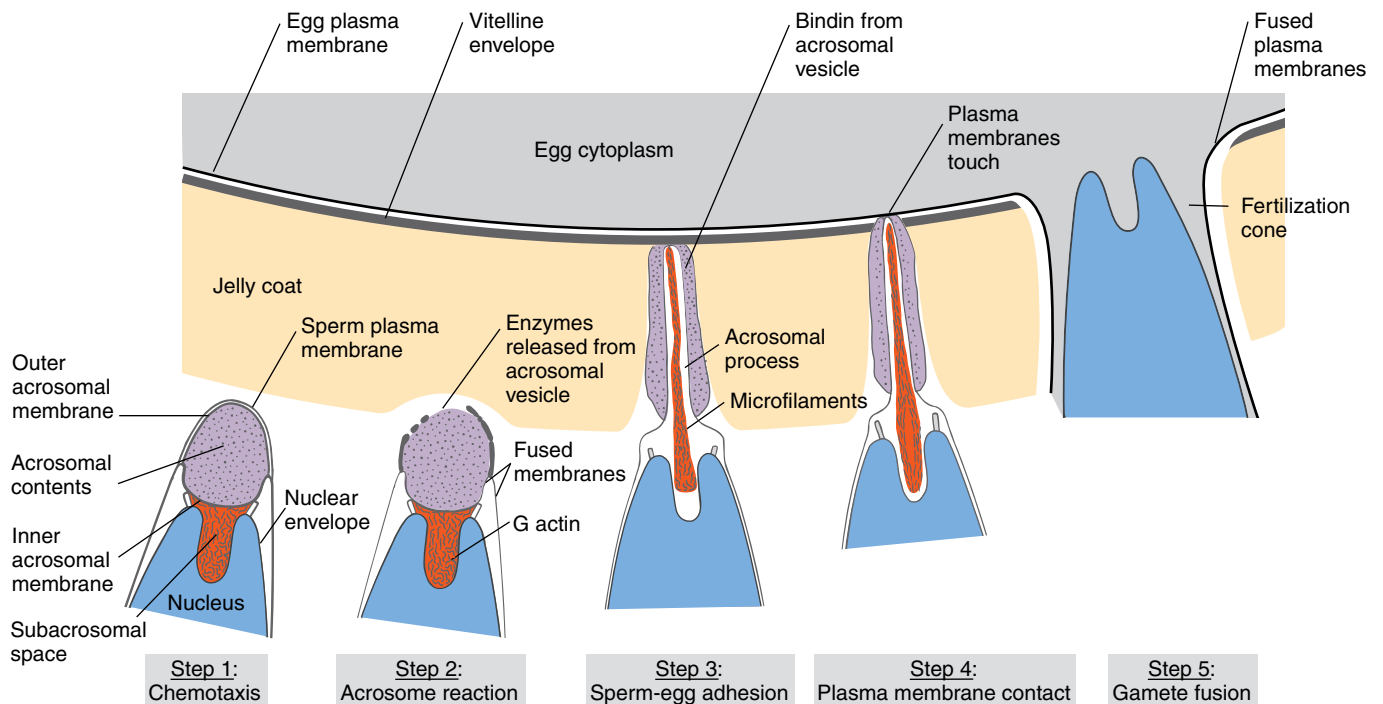


Figure 4.4 Overall process of sea urchin fertilization broken down into five steps. **Step 1:** Chemotactic approach of the sperm. The entire sperm is surrounded by a plasma membrane. The acrosome at the tip of the sperm is filled with contents including lytic enzymes and bindin. Its membrane can be regionally subdivided into the outer acrosomal membrane and the inner acrosomal membrane. The subacrosomal space between acrosome and nucleus is filled with globular actin (G actin). **Step 2:** Acrosome reaction. Upon contact with the egg's jelly coat, the acrosome undergoes exocytosis. The outer acrosomal membrane and the adjacent part of sperm plasma membrane vesiculate; the remainder of sperm plasma membrane and the inner acrosomal membrane fuse along the line indicated. Lytic enzymes released from the acrosome dissolve the jelly coat. The acrosomal process forms rapidly as globular actin from the subacrosomal space self-assembles into microfilaments (see step 3). The acrosomal process is covered with a membrane that originally served as the inner acrosomal membrane. **Step 3:** Sperm-egg adhesion. Bindin from the acrosome now sticking to the acrosomal process adheres species-specifically to the vitelline envelope. **Step 4:** Plasma membrane contact. After lysis of the vitelline envelope, the plasma membranes of sperm and egg touch, an event that may trigger the cascade of events collectively known as egg activation. **Step 5:** Gamete fusion. The plasma membranes of egg and sperm fuse, thus forming one membrane covering both gametes. The egg forms a cone of cytoplasm, called the fertilization cone, which actively engulfs the sperm nucleus. Note that the jelly layer is shown thinner, and the sperm larger, than proportional to the egg size.

membranes of egg and sperm fuse with each other to form a plasma membrane that surrounds both cells. Plasma membrane contact or gamete fusion trigger a cascade of events in the egg collectively called *egg activation*, which jumpstarts the egg on its way into embryonic development. One of the first visible signs of egg activation is the formation of the *fertilization cone*, a mound of cytoplasm in which the egg engulfs the sperm nucleus.

SEA URCHIN SPERM UNDERGO THE ACROSOME REACTION BEFORE THEY ADHERE TO THE VITELLINE ENVELOPE

In sea urchins, the *acrosome reaction* occurs when the sperm contacts certain components in the egg's jelly coat. The critical egg jelly components are generally glycoproteins (Ward and Kopf, 1993; S. H. Keller and Vacquier, 1994) although in *Strongylocentrotus purpuratus* it seems

to be a fucose sulfate polymer without any associated peptide that triggers the acrosome reaction (Vacquier and Moy, 1997). The critical egg jelly component seems to bind to a receptor in the sperm plasma membrane, which releases intracellular signals, including a transient rise in calcium ion (Ca^{2+}) concentration. Interestingly, the same signals are also involved in coordinating the responses of the egg to fertilization (see Section 4.4).

The acrosome is the membranous organelle at the tip of the sperm that contains enzymes and other components used by the sperm to adhere to the egg and to lyse its way through the egg's protective coats. The portion of the acrosomal membrane that faces the sperm plasma membrane is known as the *outer acrosomal membrane*. The remainder, called the *inner acrosomal membrane*, faces the *subacrosomal space* located between the acrosome and the sperm nucleus (Fig. 4.4). The subacrosomal space contains globular actin, which will

method 4.1

Immunostaining

The *immunostaining* procedure relies on blood serum proteins known as *immunoglobulins*, or *antibodies*, which are produced by B lymphocytes of vertebrates. Each clone of lymphocytes makes one type of antibody, directed against a specific *epitope* (binding site) on an antigen (a foreign molecule or cell). A given antigen may have several different epitopes and may therefore react with different antibodies. *Polyclonal antibodies* are prepared from blood serum of immunized mammals, into which an antigenic preparation has been injected. These antibodies are produced by multiple lymphocyte clones and directed against several epitopes of the antigen. In contrast, *monoclonal antibodies* are the products of single lymphocyte clones and directed against a single epitope (see Method 11.1).

Scientists use immunostaining to locate antigens at their normal sites of occurrence. If polyclonal antibodies are used, the antigen of interest is injected into a laboratory mammal, often a rabbit. Blood is taken from the rabbit at intervals, and the serum is tested for the presence of antibodies against the antigen. *Preimmune serum* prepared from the same rabbit before the first antigen injection is kept as a control. When the antibody concentration in the rabbit's blood is sufficiently high, a larger blood sample is taken, and the immunoglobulin fraction (the serum proteins containing the antibodies of interest) is prepared. This preparation is the *primary antibody*, which binds selectively to the antigen of interest.

One way of making the primary antibody visible is to conjugate it to a fluorescent molecule or some other label that can be detected under the microscope. Most researchers use a *secondary antibody*, prepared in a different mammalian species and directed against the primary antibody as an antigen. (Secondary antibodies avoid potentially detrimental effects of conjugated labels on the specificity of the primary antibody. Because one primary antibody molecule is bound by several secondary antibodies the latter also amplify the signal. Commercially available secondary antibodies are made in a goat or a swine and are directed against antigenic sites found in all rabbit immunoglobulins.) The secondary antibody is conjugated to a fluorescent dye, such as *fluorescein*, or to a stable enzyme, for example peroxidase, which generates a dark precipitate from two colorless reagents, diaminobenzidine and hydrogen peroxide.

For immunostaining, the primary and secondary antibodies are added in sequence to the antigen (Fig. 4.m1). The specimen containing the antigen is preserved

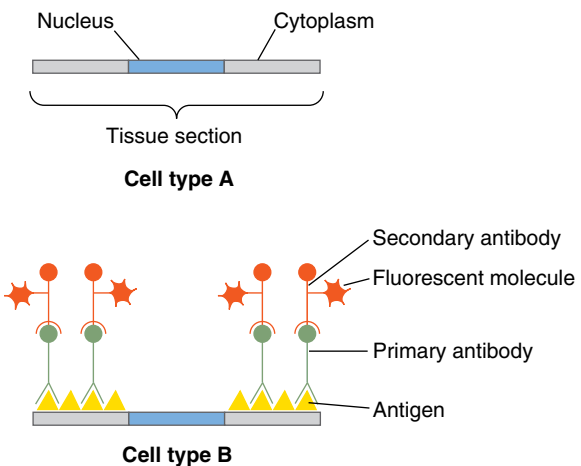


Figure 4.m1 Immunostaining is used to reveal the position of a known antigen in sections of fixed tissue. In this hypothetical example, the question is whether the antigen of interest is present in cell type A or B, and whether the antigen is located in the nucleus or in the cytoplasm. Primary antibody directed against the antigen (triangles) is allowed to interact with the sectioned tissue. Secondary antibody directed against the primary antibody is covalently linked with a fluorescent molecule or some other conjugate. The distribution of the conjugate over the section reveals that the antigen is present here in the cytoplasm of cell type B.

with a fixative that leaves the antigenic sites exposed and fairly undisturbed so that they will bind to the primary antibody. Unless the specimen is transparent or can be cleared, it must be embedded and sectioned for microscopic viewing. On each section, a drop of primary antibody is pipetted and allowed to bind to the antigen. Unbound primary antibody is washed off before the secondary antibody is added and allowed to bind to the primary antibody. Unbound secondary antibody is washed off, so that only specifically bound antibody remains on the section. Depending on the conjugate attached to the secondary antibody, further reagents are added to make the conjugate visible.

When viewed under the microscope, the fluorescent stain or visible precipitate shows where the secondary antibody is located. Assuming that secondary antibody is bound *only* to primary antibody, and that primary antibody is bound *only* to the antigen of interest, the conjugate reveals where the antigen of interest is located in the sectioned specimen. These assumptions need to be confirmed by appropriate controls using specimens without antigen or with immunoglobulin prepared from preimmune serum in lieu of primary antibody.

rapidly self-assemble into microfilaments once the acrosome reaction gets started.

The two major results of the acrosome reaction in sea urchin sperm are the *exocytosis* of the acrosomal contents and the formation of the *acrosomal process*. For exocytosis, the outer acrosomal membrane and the overlying portion of the sperm plasma membrane break up into small vesicles, thus releasing the acrosomal contents (Fig. 4.4). Acrosomal enzymes digest, or lyse, a hole through the egg jelly so that the sperm gains access to the vitelline envelope. Simultaneously, a long extension called the *acrosomal process* forms at the tip of the sperm. It is covered by what was originally the inner acrosomal membrane and has now become part of the sperm plasma membrane. The rapid extension of the acrosomal process is driven by the *self-assembly* of actin stored in the subacrosomal space (see Figs. 2.11 and 4.4).

Sea urchin sperm that have penetrated the egg jelly encounter the egg's vitelline envelope. If egg and sperm are from the same species, they adhere to each other stably. This step of *sperm-egg adhesion* is relatively *species-specific*; if sperm and egg are from different species they will adhere less stably or not at all. (Heterospecific fertilizations between closely related sea urchin species have been achieved in the laboratory, but they require about a million times higher sperm concentration than homospecific fertilizations.) The biological importance of this species-specificity is easy to see: Any gamete fertilized by a gamete from another species will not give rise to fertile offspring and therefore will not live on. It is also thought that the molecules mediating sperm-egg adhesion play critical roles in the evolution of new species (Vacquier, 1998).

Although other steps of fertilization, from mating behavior to gamete fusion, are also more or less species-specific, sperm-egg adhesion is particularly critical in animals that shed their gametes in the open water, where the chance of encountering gametes from other species is relatively high.

SEA URCHIN SPERM ADHERE TO EGGS WITH AN ACROSOMAL PROTEIN CALLED BINDIN

A sea urchin spermatozoon adheres to the egg by its acrosomal process. Investigators therefore searched for any molecules that would occur on the outside of the acrosomal process and stick to the vitelline envelope of eggs from the same species but not—or less so—to the vitelline envelope of eggs from other species.

The critical acrosomal component was first prepared from the sperm of the sea urchin *Strongylocentrotus purpuratus* (Vacquier and Moy, 1977; Vacquier, 1980). The sperm were suspended in a solution that dissolved the plasma membrane and acrosomal membrane but preserved the acrosomal contents as a more or less intact granule. The granules were separated from other sperm

components and analyzed by gel electrophoresis. The researchers observed one major band, a protein with an apparent molecular weight of 30,500, which they termed *bindin*. Two experiments indicated that bindin is the adhesive material by which sea urchin sperm adhere to eggs.

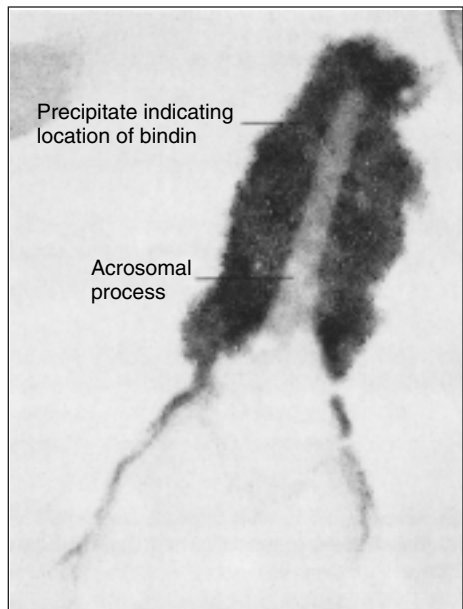
To test whether bindin was coating the acrosomal process as expected, Moy and Vacquier (1979) used a very versatile procedure known as *immunostaining* (Method 4.1). It revealed a thick coat of bindin covering the acrosomal process of acrosome-reacted sperm (Fig. 4.5). Control sperm, which had not undergone the acrosome reaction, were not stained by the procedure. In newly fertilized eggs, bindin was found at the site where acrosomal processes adhered. Thus bindin was present exactly when and where it should be as a mediator of sperm-egg adhesion.

To test whether bindin was adhering to eggs in a species-specific manner, Glabe and Lennarz (1979) designed a *competitive cell aggregation experiment*. They mixed equal numbers of dejellied *Strongylocentrotus purpuratus* and *Arbacia punctulata* eggs with bindin from either species in culture dishes. (Bindin is poorly soluble in water without detergent and forms particles in seawater.) The dishes were kept on a rotary shaker for a few minutes and then inspected under the microscope. Each bindin preparation agglutinated mostly eggs from its own species (Fig. 4.6). In order to test whether the bindin particles were directly holding the eggs together, the researchers labeled bindin with a fluorescent dye, fluorescein. Agglutinated eggs showed fluorescent particles precisely at the spots where they were stuck together. Thus bindins adhere specifically to eggs from the same species. Bindins from different sea urchin species are similar but not identical in size and amino acid composition, in accord with the species-specificity of their function (Glabe and Clark, 1991).

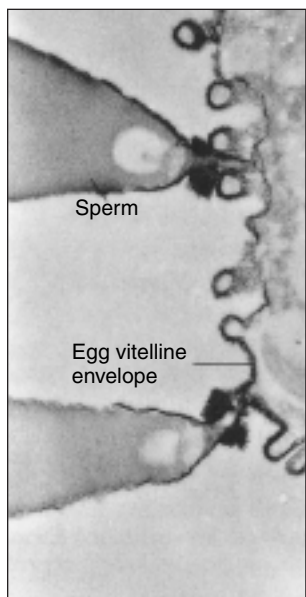
Questions

1. Why were the contents of the acrosomal vesicle a logical place to start the search for a bindinlike component?
2. If bindin from species A would agglutinate eggs from species A and S equally well, what should have been the relative frequencies of A-A, A-S, and S-S egg associations?

The experiments described above show that bindin is present exactly when and where a sperm adhesive is expected to act, and that bindin can account for the species-specificity of sperm-egg adhesion. Following sperm-egg adhesion, sperm penetrate the vitelline envelope with lytic enzymes stored in the acrosome (Colwin and Colwin, 1960). The sperm plasma membrane then comes in contact with the egg plasma membrane. This *plasma membrane contact* may trigger a series of events collectively called *egg activation*, which we will discuss in Section 4.4.



(a)



(b)

Figure 4.5 Localization of bindin on the acrosomal process of sea urchin sperm after the acrosomal reaction (a) and after binding to the egg at the level of the vitelline envelope (b). The bindin location was revealed by immunostaining thin sections for viewing under the electron microscope. Primary rabbit antibodies were prepared against sea urchin bindin, and secondary swine antibodies against rabbit IgG were conjugated with peroxidase. When furnished with the appropriate substrates, the enzyme produced an electron-dense precipitate, which revealed the location of bindin.

The specific receptor(s) for bindin on the egg surface still need to be identified. It is not clear whether a single receptor molecule is sufficient to dock the sperm, trigger plasma membrane fusion, and initiate egg activation. Any candidate molecule should meet the following

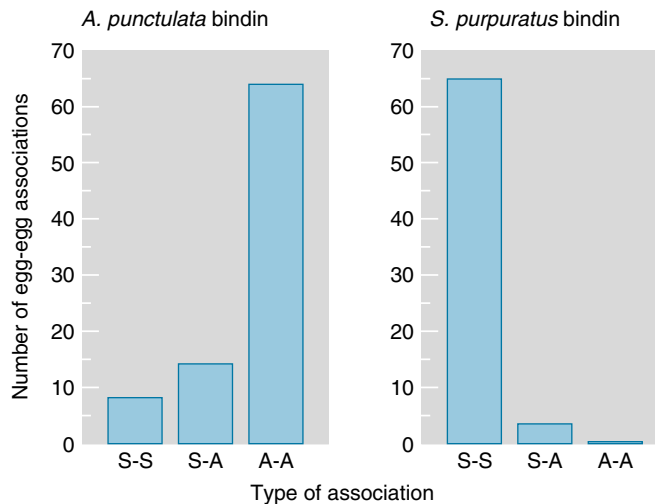


Figure 4.6 Species-specific agglutination of sea urchin eggs by bindin. A suspension containing equal numbers of eggs of *Strongylocentrotus purpuratus* (S) and *Arbacia punctulata* (A) was mixed with small amounts of bindin particles from one of the species and gently shaken for 2 to 5 min. The resulting egg-egg associations were scored on the basis of different egg pigmentations as S-S, A-S, or A-A. Each bindin agglutinated mostly eggs from its own species.

criteria: It is expected to be linked to the egg plasma membrane or to the vitelline envelope. It should also bind to sperm and its bindin species-specifically. Moreover, it should inhibit fertilization by competing with eggs for binding sites on sperm. Finally, antibodies against the candidate molecule should block fertilization.

GAMETE FUSION LEADS TO THE FORMATION OF A FERTILIZATION CONE

After plasma membrane contact has occurred, egg and sperm proceed with *gamete fusion*, the event that lies at the heart of fertilization. In sea urchins, gamete fusion always occurs at the tip of the acrosomal process and often involves an egg microvillus (Fig. 4.7). In some species, the part of the egg membrane that fuses with the sperm is restricted to the area near the egg nucleus, which contains special glycoproteins in the plasma membrane (Freeman and Miller, 1982; Freeman, 1996).

At the site of gamete fusion, the fertilized egg forms a protrusion called the *fertilization cone*. The cone engulfs the sperm as it sinks into the egg (Fig. 4.8). This process involves a movement of egg cytoplasm into the region surrounding the sperm nucleus (Longo, 1989). The fertilization cone often grows for several minutes after gamete fusion and then regresses. The formation of the cone is associated with the polymerization of cortical actin into microfilaments; *cytochalasin*, a drug that interferes with microfilament assembly, inhibits cone formation. It is thought that the fertilization cone and microfilaments facilitate sperm entry, but the mechanisms involved are still unclear.

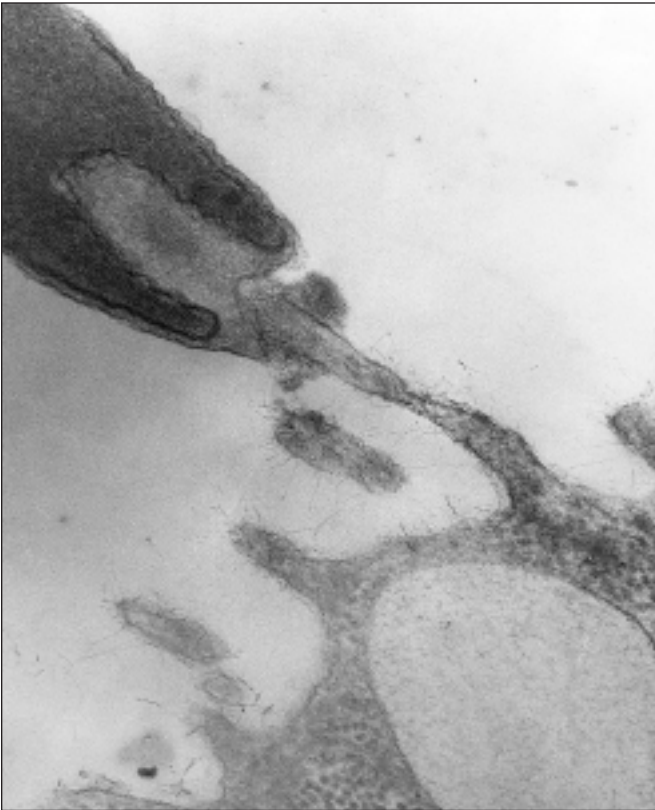


Figure 4.7 Gamete fusion during sea urchin fertilization. The electron micrograph shows a cytoplasmic connection between the acrosomal process of the sperm (top left) and a microvillus from the egg.

4.3 Fertilization in Mammals

Another group of animals frequently used for studies on fertilization are various nonhuman mammals (Yanagimachi, 1994). Mice and other laboratory mammals are often used as models for humans, who may benefit greatly from fertilization research for the purposes of overcoming infertility or preventing unwanted pregnancies. Investigators of mammalian fertilization have to contend with the fact that eggs are relatively difficult to obtain. Also, for in vitro studies, the fluids of the female reproductive tract must be mimicked by appropriately formulated media. In addition, for any observations on in vitro fertilized eggs beyond the blastocyst stage the embryos have to be implanted into the uterus of a female. However, researchers working with mice have the advantage of being able to harness the power of genetics, and this advantage will become more valuable as research in this field evolves toward an analysis of the key molecules involved (Allen and Green, 1997; Snell and White, 1996).

MOUSE SPERM UNDERGO THE ACROSOME REACTION AFTER THEY ADHERE TO THE ZONA PELLUCIDA

The first obstacle encountered by a mammalian sperm approaching a newly ovulated egg is the layer of *granulosa* cells embedded in a loose extracellular matrix (see Figs. 3.21 and 3.22). A sperm plasma membrane protein known as PH20 has an enzymatic activity that digests the major matrix component, hyaluronic acid. This action, along with active burrowing movements, allows the sperm to make its way to the zona pellucida (Lin et al., 1994).

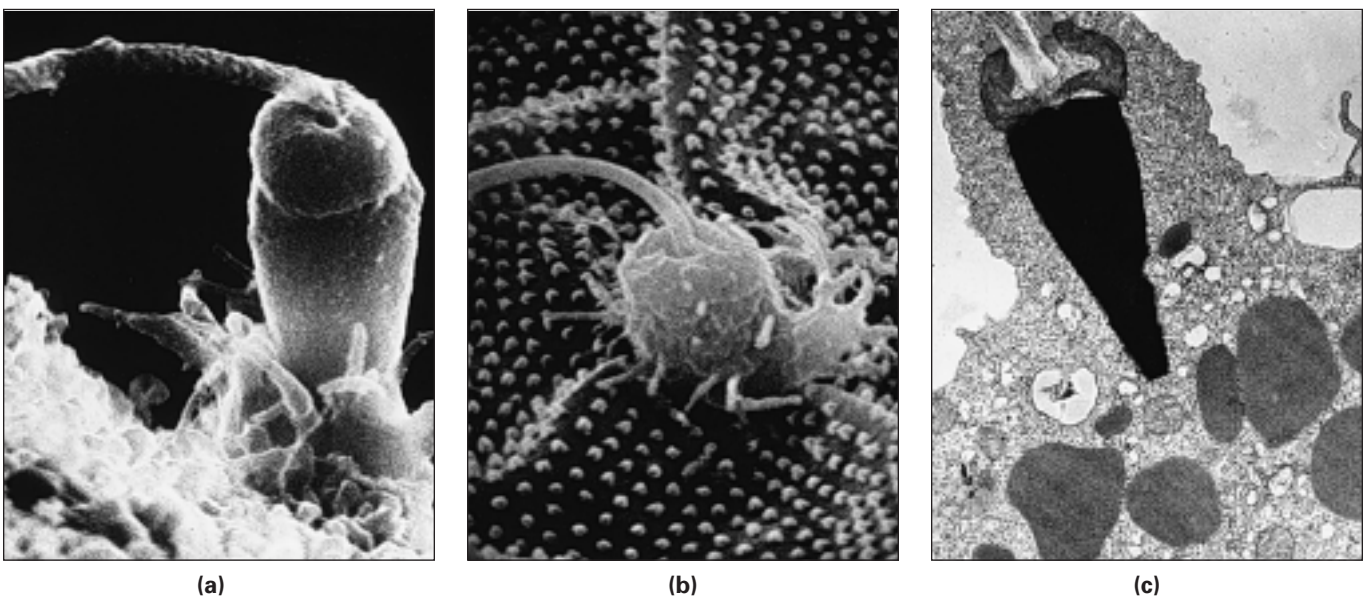


Figure 4.8 Sperm incorporation into sea urchin egg. (a, b) Scanning electron micrographs showing the internalization of the sperm head into the egg. (c) Transmission electron micrograph showing the sperm head engulfed by a cytoplasmic mound known as the fertilization cone.

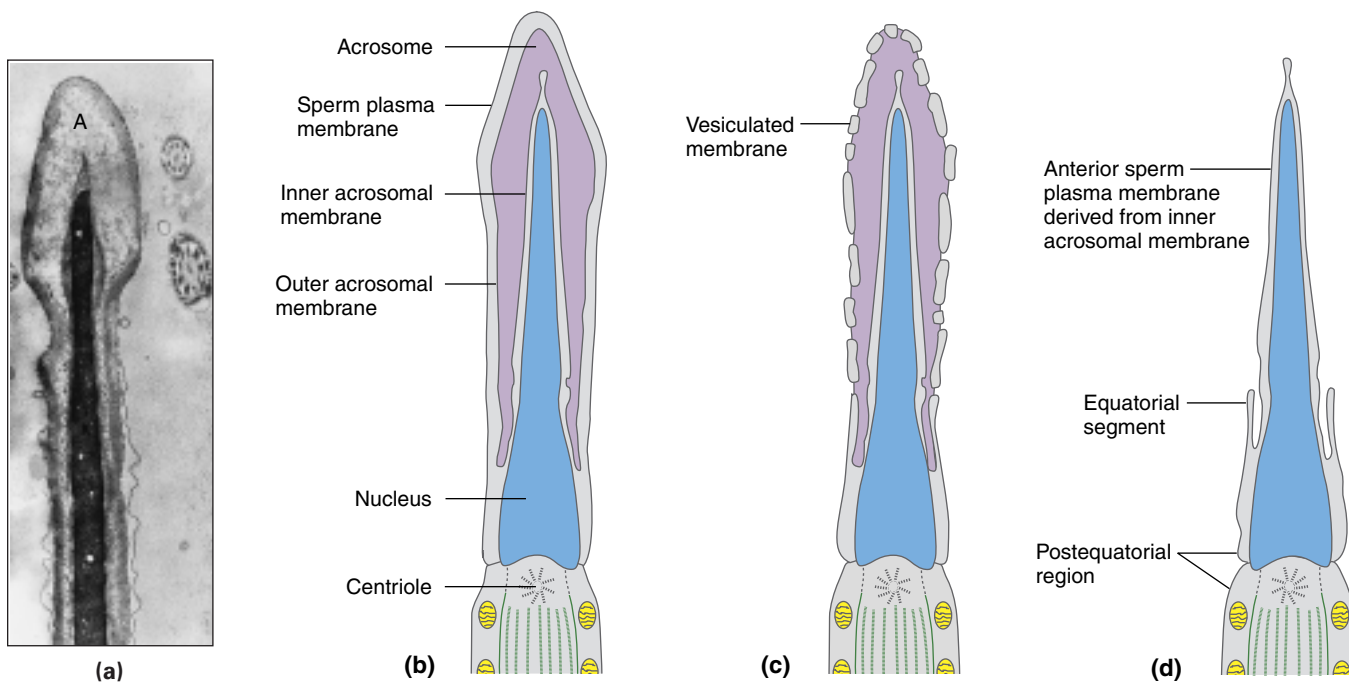


Figure 4.9 Acrosome reaction in mammals. **(a)** Transmission electron micrograph of a hamster sperm before the acrosome reaction. A = acrosome. **(b)** Drawing of sperm head before acrosome reaction, with sperm plasma membrane and acrosomal membrane intact. **(c)** During the acrosome reaction, the sperm plasma membrane and the outer acrosomal membrane fuse at many places, forming numerous vesicles. **(d)** After the acrosome reaction, the sperm plasma membrane consists of three regions: the anterior region derived from part of the inner acrosomal membrane, an equatorial segment, which has not vesiculated, and the postequatorial region.

The timing of the acrosome reaction may vary among mammalian species, and it is not always known with certainty due to the technical problems associated with studying mammalian fertilization *in vivo*. In rabbits, the acrosome reaction seems to be triggered when the sperm burrows through the *granulosa cells*. In mice, all available evidence indicates that the acrosome reaction is elicited when the sperm adheres to the *zona pellucida*, the mammalian equivalent of the vitelline envelope of sea urchins.

The acrosome in most mammals forms an extended cap over the anterior portion of the nucleus (Fig. 4.9). The *outer acrosomal membrane* lies underneath the plasma membrane while the *inner acrosomal membrane* lies close to the nuclear envelope. During the acrosome reaction, the outer acrosomal membrane and the sperm plasma membrane fuse at many points, breaking up the two membranes into many small vesicles. These vesicles remain connected to one another by a matrixlike component in the acrosomal contents until eventually the matrix is dissolved and the vesicles dissociate. The fusion of the outer acrosomal and plasma membranes, however extensive, stops short of the posterior edge of the acrosome, leaving a collarlike fold of membrane, the *equatorial segment*, to encircle the sperm head. As a result, the sperm plasma membrane now consists of three distinct regions: an anterior region derived from the inner acrosomal membrane, the equatorial segment, and

the postequatorial region (Fig. 4.9d). In contrast to sea urchins, mammalian sperm also have less subacrosomal material and do not form an acrosomal process.

The sperm of mice and many other mammals undergo the acrosome reaction after adhering to the *zona pellucida* with the side of the anterior region of their heads (Fig. 4.10). Completion of the acrosome reaction depends on an increase in intracellular Ca^{2+} concentration (Shirikawa and Miyazaki, 1999), as is the case for sea urchins (see Section 4.2). Having undergone the acrosome reaction, the fertilizing mammalian sperm forms a hole—or widens a preexisting open area—in the *zona pellucida* by an as yet unknown mechanism. Thereafter, the sperm jettisons the acrosomal matrix with the membranous vesicles covering its head, and wriggles its way into the perivitelline space between the *zona* and the egg plasma membrane.

Once inside the perivitelline space, the sperm contacts the egg plasma membrane with the equatorial or the postequatorial region of their plasma membrane. Although both of these regions are exposed in acrosome-intact sperm, the acrosome reaction is nevertheless required for gamete fusion: Eggs from which the *zona pellucida* has been removed experimentally fuse only with acrosome-reacted sperm, not with acrosome-intact sperm. These observations suggest that the acrosome reaction modifies proteins located in the equatorial or postequatorial region, or that these regions somehow

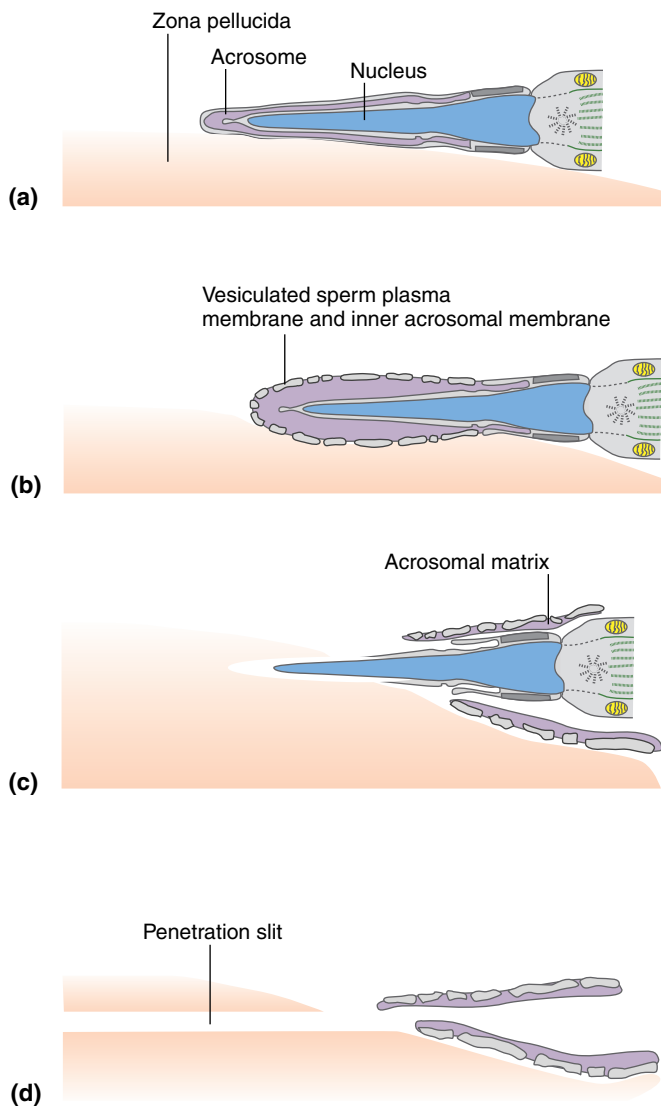


Figure 4.10 Mammalian sperm (a) adhering to the zona pellucida, (b, c) undergoing the acrosome reaction, and (c, d) breaching the zona pellucida on its way to the egg cell proper. The sperm overcomes the forces of adhesion between its own plasma membrane and the zona by leaving behind the shell of membranous vesicles and acrosomal matrix generated during the acrosome reaction.

acquire new molecules from elsewhere as part of the acrosome reaction.

The nature of the molecules involved in the contact and fusion of mammalian egg and sperm, and the mechanisms of their deployment, are currently under active investigation (Allen and Green, 1997). Particular interest has focused on a family of plasma membrane proteins with extracellular domains that have prometalloprotease activity and function in cell adhesion. A member of this family, known as PH30 or *fertilin*, accumulates in the plasma membrane of mammalian sperm. Mutant mice lacking fertilin have sperm that are deficient in migration from the uterus into the oviduct, in adhesion to the

egg zona pellucida, in plasma membrane contact, and in sperm-egg fusion (Cho et al., 1998). A similar sperm protein is also required for fertilization in *Xenopus* (Shilling et al., 1997, 1998).

A BIOASSAY IS A POWERFUL STRATEGY TO REVEAL A BIOLOGICALLY ACTIVE COMPONENT

Sperm-egg adhesion in mice, as in sea urchins, is mediated by specific molecules on the surfaces of egg and sperm. In sea urchins, *bindin* has been established as the adhesive molecule on the part of the sperm, whereas the bindin receptor(s) of the egg are still being investigated as discussed earlier. The converse is the current situation in mammals: While the adhesive molecules on the side of the sperm is still a matter of debate, their counterpart on the zona pellucida is well characterized, at least in mice.

The sperm-binding component in mouse zona was identified by means of a *bioassay*—that is, a strategy used to screen various cellular fractions or molecules for their ability to elicit a biological response that is clearly defined and readily scored. Such bioassays are generally very powerful, as the following example will demonstrate. In this instance, the investigators sought to identify the mouse zona pellucida component(s) to which mouse sperm naturally adhere.

To establish such a bioassay, Bleil and Wassarman (1980) fertilized mouse eggs under conditions in which the sperm were in limited supply (Fig. 4.11). Before fertilization, the sperm were preincubated in medium containing zona pellucida glycoproteins. Unfertilized eggs were then added to the cultures to allow sperm-egg adhesion. After gently pipetting the eggs to remove loosely “attached” sperm, the sperm firmly “bound” to each egg were counted. The average number of sperm bound after preincubation in pure medium was normalized to 100%. This percentage decreased dramatically when the sperm were preincubated with zona pellucida glycoproteins from unfertilized eggs.

The bioassay outlined above was designed to test zona glycoproteins and their components for the ability to compete with intact eggs for sperm adhesion. The most effective competitor(s) in this assay would thus be identified as the most likely candidate(s) for being the zona component to which sperm naturally adhere. Other bioassays to be discussed in Sections 8.3 and 9.5 have been designed to identify cytoplasmic components that are segregated unevenly during cleavage and impart distinct patterns of gene expression on blastomeres.

MOUSE SPERM ADHERE TO A SPECIFIC ZONA PELLUCIDA PROTEIN

The zona pellucida is synthesized by the growing oocyte as a thick but porous structure. Its major constituents are three glycoproteins (designated ZP1, ZP2, and ZP3), which assemble into long, interconnected filaments

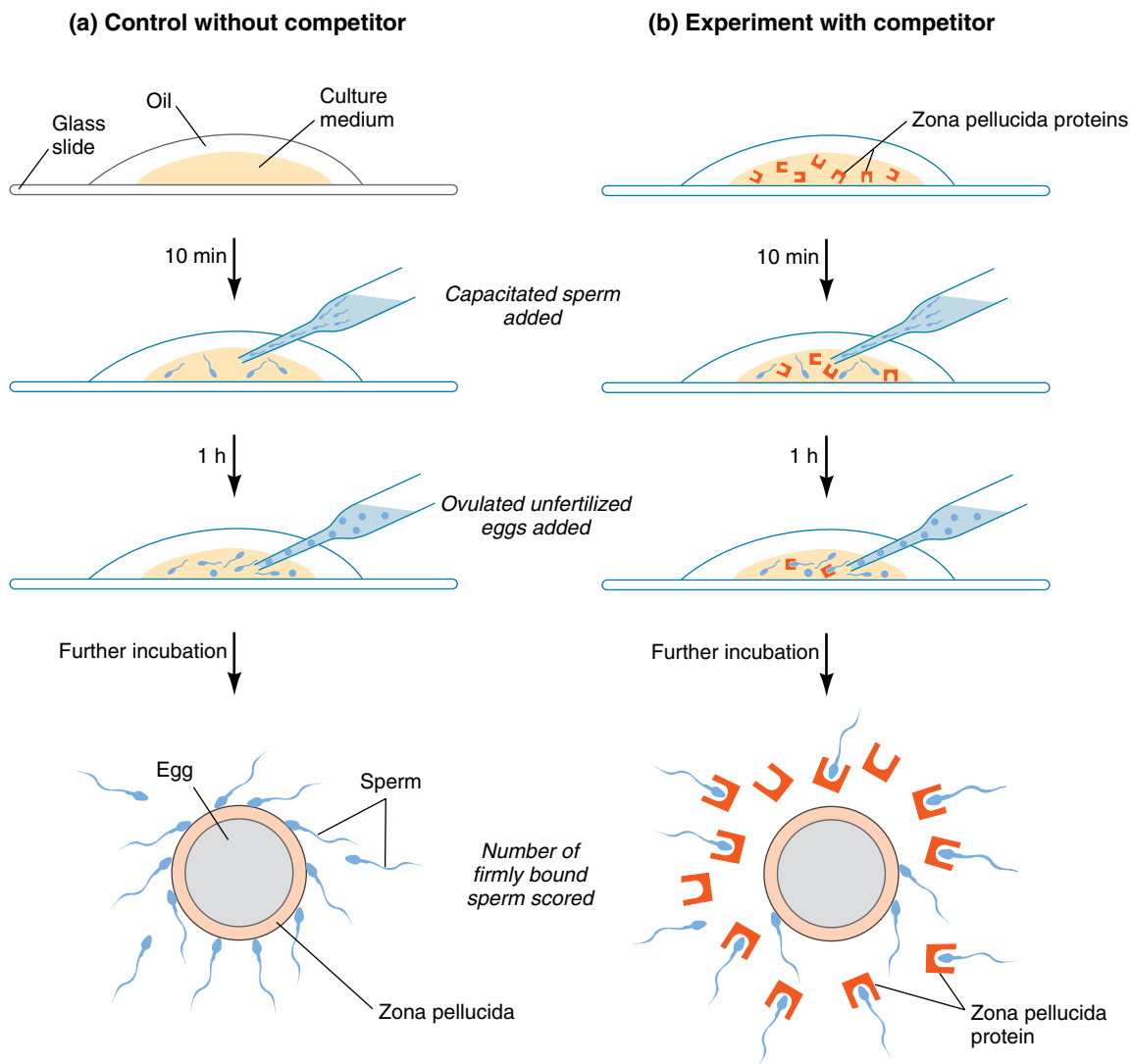


Figure 4.11 Bioassay to identify the mouse zona pellucida component to which the sperm adheres. **(a)** Control experiment without competitor. A drop of culture medium is kept on a glass slide under oil to prevent desiccation. Capacitated sperm are added to the drop from a glass micropipette. After 1 h of incubation, unfertilized ovulated eggs are added. After further incubation, the eggs are washed to remove loosely attached sperm. The average number of firmly bound sperm per egg is scored. **(b)** Same experiment except that zona proteins, or moieties thereof, are added to the culture medium. If the added components bind to sperm in solution, they effectively compete with the eggs for binding sites on the sperm, thus reducing the number of sperm bound to the eggs. For results see Figure 4.12.

(Wassarman, 1987, 1990). The zonae of other mammals, including humans, consist of similar glycoproteins.

In order to identify the zona protein, and the exact moiety thereof, to which mouse sperm bind, Bleil and Wassarman (1980) used the bioassay described above (Fig. 4.11). As part of establishing this bioassay, they had already shown that total zona proteins from unfertilized eggs inhibit the binding of intact unfertilized eggs to capacitated sperm. In contrast, the same glycoproteins from 2-cell embryos did not have this inhibitory effect. Apparently, zona glycoproteins from unfertilized eggs were competing with intact

unfertilized eggs for binding sites on the sperm. In 2-cell embryos, the zona glycoproteins seemed to be modified so that sperm could no longer bind to them.

Once this bioassay was established, each zona glycoprotein was purified and tested individually in the same way. Only ZP3 inhibited sperm-egg adhesion. The other zona proteins did not compete with unfertilized eggs for binding sites on the sperm (Fig. 4.12). Evidently, ZP3 is the only zona glycoprotein that binds to mouse sperm during the initial sperm-egg adhesion.

Molecular analysis of ZP3 showed that its backbone is a single polypeptide of about 400 amino acids (Wassarman, 1990). Extending from the polypeptide are many *oligosac-*

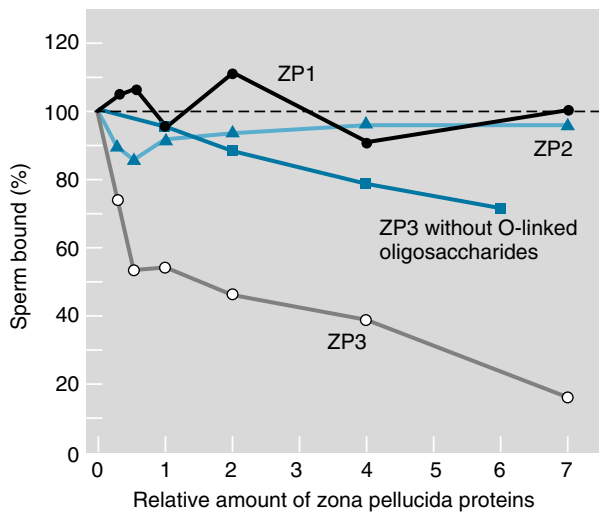


Figure 4.12 Competition of mouse ZP3 with intact eggs for sperm binding sites. Sperm were preincubated with purified proteins (ZP1, ZP2, ZP3) from mouse zonae pellucidae before unfertilized test eggs were added (see Fig. 4.11). The eggs were scored later for the number of firmly bound sperm. The hatched line (100% level) represents the average number of sperm bound per egg in control experiments without zona pellucida components added to the culture medium. Sperm preincubated with ZP1 or ZP2 bound to the test eggs at near-control levels, but sperm preincubated with ZP3 showed a reduced ability to bind. The inhibitory action of ZP3 depended largely on its O-linked oligosaccharides. (Apparent fertilization rates greater than 100% are an artifact of the method used for data processing.)

charides—that is, short chains of connected sugars. Some of these are *O-linked oligosaccharides*, which means that they are linked to an oxygen atom on either one of two amino acids: serine or threonine. To reveal which of the ZP3 components function in sperm adhesion, ZP3 was pre-treated with various agents to destroy specific parts of the molecule. The pretreated ZP3 was then tested again as a competitor in the bioassay. Removal of the oligosaccharides destroyed the competitive function of ZP3, whereas cutting the polypeptide enzymatically into short pieces did not have this effect. Specifically, removing the O-linked oligosaccharides with a mild alkali left ZP3 an unfit competitor (Florman and Wassarman, 1985). Conversely, the O-linked oligosaccharides alone competed as well as intact ZP3. The exact nature of the moiety within the O-linked oligosaccharide that is critical for sperm adhesion still needs to be established. It also unclear whether the ZP3 polypeptide plays a direct role in adhesion or if it only determines the “presentation” of the O-linked oligosaccharides (Wassarman and Litscher, 1995).

Questions

1. In the bioassay described above, why was it important that the sperm be present in limited supply rather than in excess?

2. In the course of preparing zona proteins from eggs and 2-cell embryos, it became apparent that zona pellucida from embryos is much harder to dissolve in various aqueous media. What does this difference in solubility suggest?

Additional lines of evidence confirmed that the ZP3 glycoprotein is the critical molecule for sperm-egg adhesion in mice (Bleil and Wassarman, 1986). First, purified and radiolabeled ZP3 from *unfertilized eggs* binds only to the head of the sperm, not to its midpiece or tail. This is consistent with the observation that sperm bind by the head to the egg zona pellucida. Second, purified ZP3 from the *embryonic* zona pellucida does not bind to sperm, confirming the earlier observation that zona pellucida proteins from 2-cell embryos are inactive in the competitive binding assay.

The mouse sperm membrane protein(s) that bind specifically to ZP3 are still a matter of debate (Snell and White, 1996). Perhaps the leading candidate so far is an enzyme, *N-acetylglucosamine galactosyltransferase (GalTase)*, which is located at the tip of acrosome-intact sperm (Shur, 1989; D. J. Miller et al., 1992; Gong et al., 1995). The biochemical function of GalTase is to transfer galactose to an oligosaccharide chain ending with *N-acetylglucosamine*. However, since galactose is not present in the oviduct, the enzyme is not able to complete its function and so remains stuck to the terminal *N-acetylglucosamines* of the O-linked oligosaccharides of ZP3. There is also evidence that other sperm proteins adhere to ZP3 (Bleil and Wassarman, 1990; Leyton et al., 1992, 1995), and it seems possible that two or more adhesion molecules could act synergistically in sperm-egg adhesion.

ANTIBODIES TO SPERM-EGG ADHESION PROTEINS CAN ACT AS CONTRACEPTIVES

Research on fertilization has included the development of medical applications designed either to help infertile couples to have children or to help fertile couples to avoid unwanted children. Past research into sperm capacitation has greatly improved the success of in vitro fertilization; knowledge of the hormonal control of ovulation has provided the basis for the development of the most commonly used oral contraceptive today.

A recently developed method for *intracytoplasmic sperm injection (ICSI)* inserts a single sperm directly into the cytoplasm of a mature oocyte. It has already helped men with very low sperm counts or high proportions of defective sperm to have children. This method avoids the rigorous sperm selection process that occurs during normal conception. The question whether the condition that has left the father infertile is heritable, and if so, how his child may be affected, is usually left unanswered. So far, it appears that ICSI does not significantly change the risk of spontaneous abortion, stillbirth, or

malformation at birth (Bonduelle et al., 1999). However, many more ICSI children must be compared to normally conceived controls before any changes in the frequency of rare diseases become statistically detectable.

On the other hand, the overall growth of the human population on Earth is making effective contraceptive methods the only sustainable way of avoiding both large-scale famines and environmental degradation. Contraceptive vaccines may become viable alternatives to traditional contraceptives where the latter are impractical or deemed undesirable (Burks et al., 1995; Talwar and Raghupathy, 1994). Since sperm-egg adhesion and plasma membrane contact depend on a few specific molecules, it may be possible to block these processes using antibodies that do not interfere with other vital functions. Such antibodies might be elicited by injecting suitable antigens, such as the zona proteins or sperm proteins that mediate sperm adhesion.

Immunization with zona proteins from hamster makes female mice infertile, but after the antibody concentration decreases, the mice are capable of bearing normal young again (Gwatkin, 1977). Similarly, female mice immunized with mouse ZP3 produce antibodies that coat the zonae pellucidae of their developing oocytes. These mice are also infertile as long as the antibody concentration remains high (Millar et al., 1989). Unfortunately, the injected mice also developed ovarian diseases.

Sperm proteins are another target for contraceptive antibodies. Clinical data indicate that human infertility may be caused by anti-sperm antibodies in either the male or the female partner. (Normally, such an autoimmune reaction in males is prevented by the *blood-testis barrier* described in Section 3.4.) In guinea pigs immunized with a guinea pig sperm protein, the vaccination was fully contraceptive in both males and females, and the effects were reversible (Primakoff et al., 1988). None of the existing procedures is effective and safe for human application yet, but the results obtained so far are encouraging enough to continue related investigations (Feng et al., 1999).

4.4 Egg Activation

Fertilization triggers *egg activation*, a series of events that stimulates the quiescent egg to reenter the cell cycle and begin development (Table 4.1; Fig. 4.13; Epel, 1997). The activation cascade entails intracellular signaling mechanisms that coordinate the numerous steps involved. The signals include in particular *calcium ion* (Ca^{2+}) *transients*—that is, short rises or oscillations in Ca^{2+} concentration, and the activation of protein kinase C. Activation also accelerates the egg's metabolism including DNA replication and protein synthesis in preparation for fast-paced cleavage divisions. Another important part of egg activation in many species is the prevention of multiple fertilizations (see Section 4.5.)

table 4.1 Sequence of Events in Sea Urchin Fertilization

Event	Time after Plasma Membrane Contact ^a
Fertilization potential appears (fast block to polyspermy)	Before 3 s
Plasma membrane fusion	Before 15 s ^b
Inositol trisphosphate and diacylglycerol produced	Before 15 s ^b
Intracellular calcium release	40–120 s
Cortical reaction (slow block to polyspermy)	40–100 s
Sperm entry	1–2 min ^b
Activation of NAD kinase	1–2 min
Increase in O_2 consumption	1–3 min
Na^+/H^+ exchange, increase in pH	1–5 min
Sperm chromatin decondensation	2–10 min ^c
Sperm nucleus migration to egg center	2–10 min ^c
Activation of protein synthesis	After 5 min
Fusion of pronuclei	20 min ^d
Initiation of DNA synthesis	20–40 min
First mitotic prophase	60–80 min
First cleavage	85–95 min

^a The times listed are estimates based on data from *Lytechinus pictus* kept at 16–18°C (Whitaker and Steinhardt, 1985), except as noted.

^b Laurinda A. Jaffe (personal communication).

^c Based on data from *Clypeaster japonicus* (Hamaguchi and Hiramoto, 1980).

^d Based on data from *Strongylocentrotus purpuratus* (Epel, 1977).

EGG ACTIVATION MAY BE TRIGGERED BY DIFFERENT SIGNALING MECHANISMS

How does fertilization trigger egg activation? There are two likely scenarios (Whitaker and Swann, 1993). First, egg activation could be triggered by *gamete fusion*, when sperm introduce the activating component into the egg. Second, egg activation could be triggered earlier by *plasma membrane contact*, when the sperm activates a receptor on the egg surface.

According to the first scenario, a component introduced with the sperm cytoplasm into the egg cytoplasm, or with the sperm plasma membrane into the egg plasma membrane, triggers egg activation. In support of this hypothesis, egg activation responses were found to be triggered by cytoplasmic proteins isolated from sperm and injected into eggs of a marine worm (Stricker, 1997), a sea urchin (Galione et al., 1997), and a mouse (Sette et al., 1997). The activation responses so triggered always included Ca^{2+} oscillations, apparently released from Ca^{2+} stores in the *endoplasmic reticulum*.

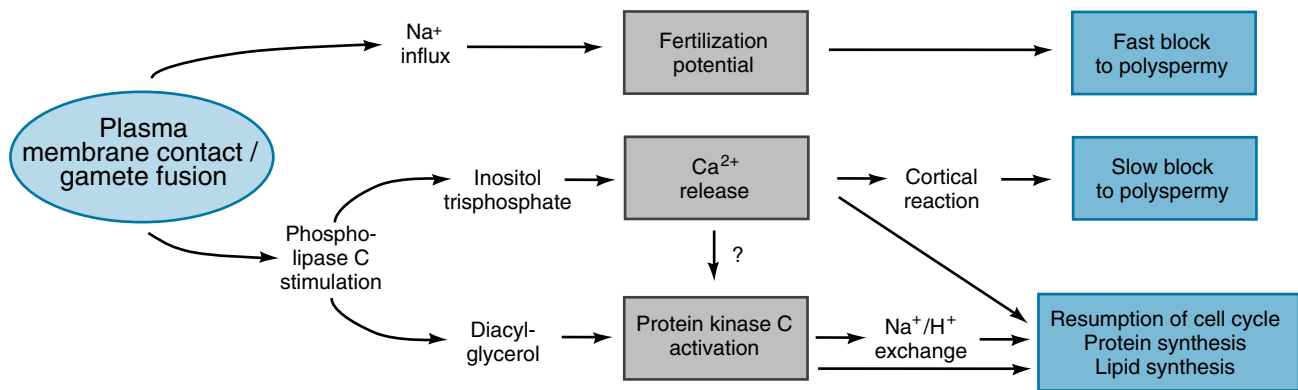


Figure 4.13 Flow chart of key steps in sea urchin egg activation. See also Table 4.1.

According to the second scenario, contact with sperm plasma membrane activates a sperm receptor on the egg surface, located presumably in the egg plasma membrane. (Note that these hypothetical sperm receptors in the egg plasma membrane are thought to be different from molecules like ZP3, which mediate sperm adhesion to the zona pellucida or other vitelline envelope.) The search for receptors that may function in egg activation have been guided by the observation that the activation process requires four well-known intracellular messengers: *inositol trisphosphate* (IP_3), *diacylglycerol* (DAG), *protein kinase C* (PKC), and *calcium ion* (Ca^{2+}). A key enzyme in the release of these messengers is the enzyme *phospholipase C* (PLC), as diagrammed in Figure 2.27. PLC hydrolyses phosphatidylinositol bisphosphate (PIP_2) into IP_3 , which releases Ca^{2+} from cytoplasmic storage sites, and DAG, which activates PKC.

IP_3 and a receptor to which it binds have been found in newly fertilized eggs of many species (Parys et al., 1994). Two modes of generating IP_3 involving different types of PLC have been tested. First, *G proteins*, which may be activated by the hypothetical sperm receptor in the egg plasma membrane, activate *phospholipase C- β* (PLC- β), which cleaves membrane-bound PIP_2 . Second, a *receptor tyrosine kinase*, or RTK (see Fig. 2.28), which may be stimulated by sperm-egg contact, or a cytoplasmic tyrosine kinase (TK) introduced by the sperm, activates *phospholipase C- γ* (PLC- γ), which also cleaves PIP_2 and causes the same downstream events as PLC- β .

Several experiments indicate that both the G protein/PLC- β and the TK/PLC- γ pathways may be involved. For example, stimulation of G proteins by receptors not normally present in eggs is *sufficient* to trigger activation of frog and mouse eggs (Kline et al., 1988; G. D. Moore et al., 1993). The G protein/PLC- β pathway is also *necessary* for hamster egg activation (Miyazaki, 1988). In starfish eggs, activation of PLC- γ is necessary for egg activation, but the G protein/PLC- β pathway works as well (Shilling et al., 1994; Carroll et al., 1997). In *Xenopus* eggs, stimulation of an RTK that activates PLC- γ is *sufficient* to trigger complete activa-

tion (Yim et al., 1994). However, neither PLC- β nor PLC- γ is *necessary* for activating *Xenopus* eggs, suggesting that yet another pathway for making IP_3 is functioning in these eggs (Runft et al., 1999).

Taken together, the available data indicate that Ca^{2+} transients are a universal link in the chain of egg activation signals, and that IP_3 is commonly used to release Ca^{2+} from intracellular stores. The mechanisms for generating IP_3 differ among organisms and may be initiated by plasma membrane contact and/or gamete fusion. This diversity again demonstrates that critical biological processes such as egg activation are often supported by two or more molecular mechanisms, which may have evolved to act alternatively or synergistically, depending upon the species.

A TEMPORARY RISE IN Ca^{2+} CONCENTRATION IS FOLLOWED BY ACTIVATION OF PROTEIN KINASE C

Ca^{2+} transients are naturally triggered by fertilization and have been found to be a regular feature of egg activation in all species studied so far (Stricker, 1999). In large eggs, the increased concentration of free Ca^{2+} spreads like a wave, beginning at the site of sperm entry (Fig. 4.14). The wavelike propagation seems to follow the release of Ca^{2+} from intracellular stores in response to either IP_3 or Ca^{2+} itself. The released Ca^{2+} , along with the DAG generated simultaneously with IP_3 , activates PKC (see Fig. 2.27). Both Ca^{2+} and PKC are key regulators of the egg activation process (see Fig. 4.13).

To test whether Ca^{2+} transients are *sufficient* for later activation events to occur, investigators have added Ca^{2+} to unfertilized eggs. This was done by microinjecting Ca^{2+} into unfertilized eggs (Hamaguchi and Hiramoto, 1981), or by keeping unfertilized eggs in a Ca^{2+} -containing medium and making them permeable to Ca^{2+} with drugs called *ionophores* (Steinhardt and Epel, 1974). Either treatment triggers several downstream activation steps, showing that Ca^{2+} is sufficient for these steps to occur.

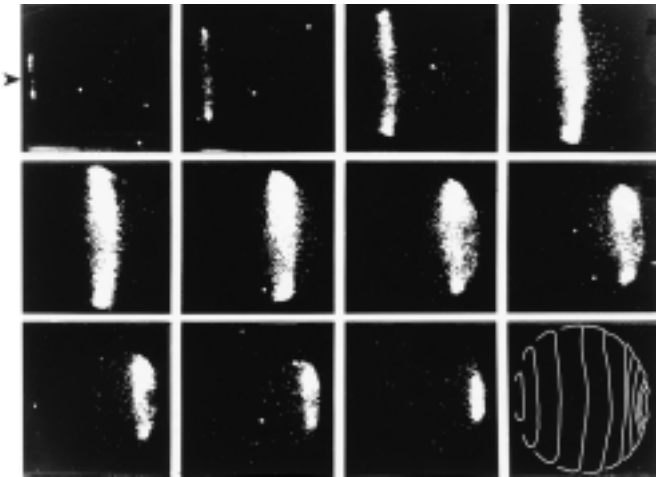


Figure 4.14 Ca^{2+} wave in a fertilized fish egg (*Oryzias latipes*). The egg has been injected with aequorin, a protein that emits light when it binds to Ca^{2+} ions. The photographs were taken at 10-s intervals to show the wave of Ca^{2+} that begins near the sperm entry point (arrowhead at the top left panel) and passes across the egg. The bottom right panel outlines the leading edges of the light front seen in the other 11 panels.

To test whether Ca^{2+} transients are *necessary* for all subsequent activation events to occur, researchers have removed Ca^{2+} from sea urchin eggs before fertilization. This was achieved by injecting eggs with molecules that bind up Ca^{2+} (Zucker and Steinhart, 1978). The procedure rendered eggs metabolically inactive. However, mammalian eggs did undergo several activation steps even in the absence of a Ca^{2+} transient when PKC was stimulated by other means, and they failed to complete meiosis when PKC was inhibited (Gallicano et al., 1993, 1997a). It appears that groups of animals may differ in their dependence on PKC in their egg activation cascades (Gallicano et al., 1997b).

EGG ACTIVATION TRIGGERS THE COMPLETION OF MEIOSIS AND THE FUSION OF THE GAMETES' HAPLOID GENOMES

A very important aspect of egg activation is the resumption of the cell cycle, which occurs in three major steps. First, in most species, fertilization releases the egg from its second meiotic block, allowing the formation of a haploid egg nucleus. Second, the haploid egg nucleus and its male counterpart, the sperm nucleus, interact to form the diploid genome of the embryo. Third, egg activation initiates the rapid mitotic cycles that characterize embryonic cleavage.

The unfertilized eggs of amphibians and most mammals are arrested during the second metaphase of meiosis. The arrest is caused by a sustained high level of *M-phase promoting factor* (MPF). The high MPF level in turn depends on a high concentration of *mos* protein,

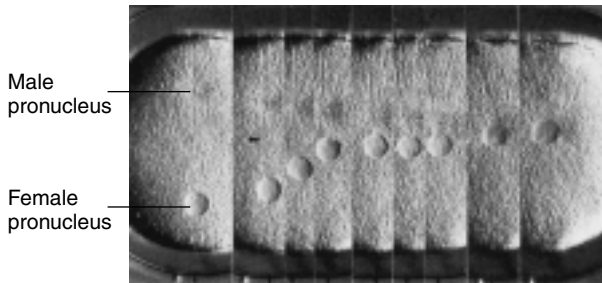


Figure 4.15 Fusion of pronuclei in the fertilized egg of the sea urchin *Clypeaster japonicus*. This series of photographs, which covers a time span of 800 s, shows the male pronucleus surrounded by an aster of microtubules. The pronuclei move toward each other and fuse.

and presumably another protein, during oocyte maturation (see Fig. 3.20). This situation changes when Ca^{2+} is released as part of the egg activation process. Ca^{2+} activates *calmodulin*, a Ca^{2+} -dependent protein, which in turn activates a calmodulin-dependent kinase, designated *CaM K_{II}*. The active CaM K_{II} promotes two effects, which seem to occur independently of each other (Lorca et al., 1993). First, the high level of MPF that has kept the egg arrested in metaphase II is reduced so that the cell cycle can continue. Second, the *mos* protein, the *cytostatic factor* that has maintained the MPF level high in the unfertilized egg, is degraded. In addition, the release of Ca^{2+} inhibits *MAPK*, a step that is necessary and sufficient for initiating DNA synthesis (Carroll et al., 2000).

Completion of meiosis renders a haploid egg nucleus, the *female pronucleus*. Its male counterpart derives from the sperm nucleus, the envelope of which disintegrates into small vesicles, thus exposing the sperm chromatin to the egg cytoplasm (G. R. Green and E. L. Poccia, 1985). The sperm nucleus then swells as its chromatin decondenses, and its envelope reconstitutes. At this time, the former sperm nucleus is called the *male pronucleus*. Meanwhile, the centrosome introduced by the sperm has organized an aster of microtubules, the *sperm aster* (Fig. 4.15). As soon as sperm aster microtubules contact the female pronucleus, *motor proteins* begin to move the pronuclei toward each other.

The fusion of a male and a female gamete concludes the process of fertilization by forming a single cell, the zygote. Both gametes contribute corresponding sets of chromosomes to the zygote. Nearly all of the zygote cytoplasm and its organelles are, of course, derived from the egg. However, the *centrosome* in most species, including humans, is lost during oogenesis and is restored to the zygote from the centriole pair located at the base of the sperm's flagellum. Upon fertilization, this centriole pair recruits egg components to rebuild a centrosome, which replicates and organizes the first mitotic spindle in the zygote (Schatten, 1994; Simerly 1995). The flagellum and the mitochondria of the sperm enter the egg but usually disintegrate, although in some species

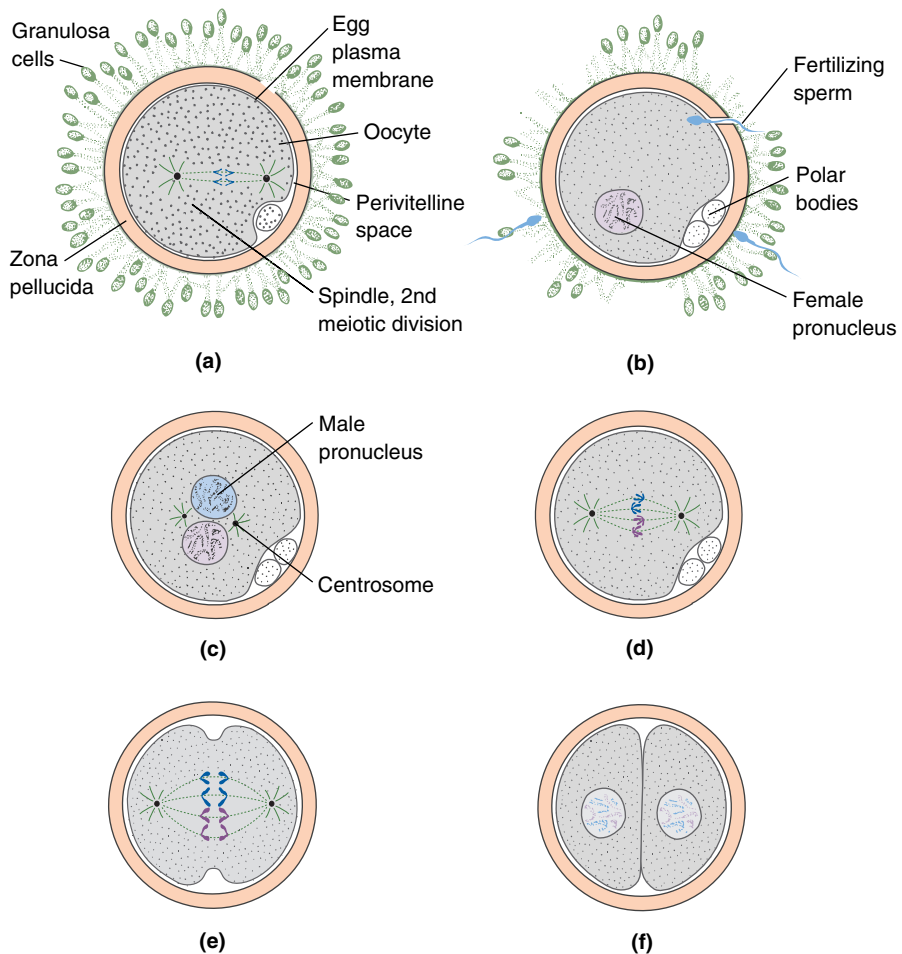


Figure 4.16 Fertilization of the human egg. (a) Ovulated egg arrested in metaphase II. (b) One sperm has entered the egg, which has completed meiosis II and formed a female pronucleus. The second polar body has been given off. (c) The sperm nucleus decondenses and forms the male pronucleus. (d) The nuclear envelopes of both pronuclei disintegrate, and their chromosomes are incorporated into a common mitotic spindle. (e) Anaphase of first embryonic cleavage. (f) Embryo at the 2-cell stage.

the sperm mitochondria are conserved during early cleavage (Giles et al., 1980; Zouros et al., 1992; Sutovsky et al., 1996). The plasma membrane of the zygote consists mostly of the egg membrane, with a small contribution by the sperm plasma membrane.

The process by which a diploid nucleus forms from the male and female pronuclei varies among groups of animals. In sea urchins and their relatives, the envelopes of the pronuclei fuse in a process called *syngamy* and form a common nuclear envelope around the maternal and paternal chromosomes. However, other animals, including most mammals, skip this step. Instead, the male and female pronuclei retain separate nuclear envelopes until just prior to mitosis. Then, both nuclear envelopes break up, and the maternal and paternal chromosomes align within the same mitotic spindle (Fig. 4.16). Diploid nuclei therefore do not originate until after the first mitosis in these animals.

ACTIVATION ACCELERATES THE EGG'S METABOLISM IN PREPARATION FOR CLEAVAGE

While the unfertilized egg is generally a sleepy cell, fertilization is the wake-up call to a stage of fast-paced action: *cleavage*. Cleavage entails the rapid replication of DNA, including the synthesis of its building blocks, the nucleotides. In addition, histones and other chromosomal proteins need to be synthesized in quantity. The same holds for cyclins, cyclin-dependent kinases, and other proteins involved in regulating the cell cycle. In most species, the mRNAs for the synthesis of early embryonic proteins have been stored during oogenesis. Thus, egg activation entails a massive recruitment of stored maternal mRNA into polysomes for rapid translation. Besides proteins, the embryo needs to synthesize large amounts of plasma membrane to cover the additional cell surfaces generated by cleavage. A key step in accelerating membrane formation is the activation of an enzyme, NAD⁺ kinase, which phosphorylates NAD⁺ to NADP⁺, a coenzyme in lipid biosynthesis (Epel et al., 1981).

The start signal for many of these metabolic steps is a surge of intracellular Ca²⁺ concentration as discussed earlier. An additional signal, at least in sea urchin eggs, is a sudden *rise in intracellular pH*, which is caused by plasma membrane proteins that pump hydrogen ions (H⁺) out of the

egg by exchanging them for sodium ions (Na⁺) from the surrounding medium (see Fig. 4.13). Procedures that prevent this increase in pH inhibit DNA and protein synthesis, whereas artificially raising the pH of unfertilized eggs boosts protein synthesis (Winkler, 1988).

4.5 Blocks to Polyspermy

Eggs of many species have thousands of adhesion sites on the vitelline envelope, presumably to ensure that fertilization can occur wherever a lone sperm may land on the egg. However, the excess of adhesion sites is potentially troublesome: it could lead to fertilization of a single egg by more than one sperm. Such a condition, known as *polyspermy*, would be disastrous in many species. The fertilization of a sea urchin egg by two sperm, for example, results in a zygote with three

haploid sets of chromosomes. Also, the two centrosomes introduced by the two sperm set up a mitotic spindle with four poles. The ensuing cleavage produces blastomeres with irregular numbers of chromosomes (see Fig. 15.3). Such embryos usually die early (Boveri, 1907).

Multiple mechanisms have evolved to prevent either polyspermy itself or its consequences. Many species, including some insects, most salamanders, reptiles, and birds, are naturally polyspermic, but extra sperm are somehow inactivated in the eggs of these animals. In other species, polyspermy itself is prevented, typically by two independent mechanisms. One mechanism, known as the *fast block to polyspermy*, is rapid but temporary. The other mechanism, called the *slow block to polyspermy*, takes time to get under way but is permanent.

THE FERTILIZATION POTENTIAL SERVES AS A FAST BLOCK TO POLYSPERMY

When sea urchin eggs are fertilized in the laboratory with excess sperm, they rarely become polyspermic. As soon as one sperm contacts the egg, conditions change to prevent the plasma membrane from fusing with additional sperm. Some inhibitory signal must spread around the entire egg surface from the site of first plasma membrane contact. This mechanism is effective even when sperm concentrations are high enough for many sperm-egg collisions per second. Given an egg diameter of 0.1 mm, diffusion of a chemical signal would be much too slow for a response time of less than 1 s. Only an electrical signal can travel fast enough.

By inserting an electrode into an egg and placing another electrode on the outside, one can measure an electric potential across the plasma membrane. This is known as the *resting potential*, which is generated—as it is in neurons—by concentration differences in potassium (K^+) and sodium (Na^+) ions inside and outside the egg. In unfertilized sea urchin eggs, the resting potential is about -75 mV (L.A. Jaffe, 1976; Nuccitelli and Grey, 1984). Upon contact with a sperm, the membrane potential changes temporarily to a *fertilization potential* of near $+20$ mV (see Fig. 4.13; Fig. 4.17). The membrane potential remains positive for about 1 min and then gradually returns to the resting potential.

The fertilization potential is caused by increased membrane permeability to certain ions, a change that represents the first step in the egg activation cascade. The nature of the ion channels that generate the fertilization potential varies among animal groups (L. A. Jaffe and Gould, 1985; Elinson, 1986). In sea urchin eggs, the fertilization potential is caused by an increase in the membrane's permeability to sodium ions (Na^+), temporarily allowing these positively charged ions to enter the egg. In frog eggs, the fertilization potential results from an increase in the membrane's permeability to chloride ions (Cl^-), allowing these negatively charged ions to leave the egg.

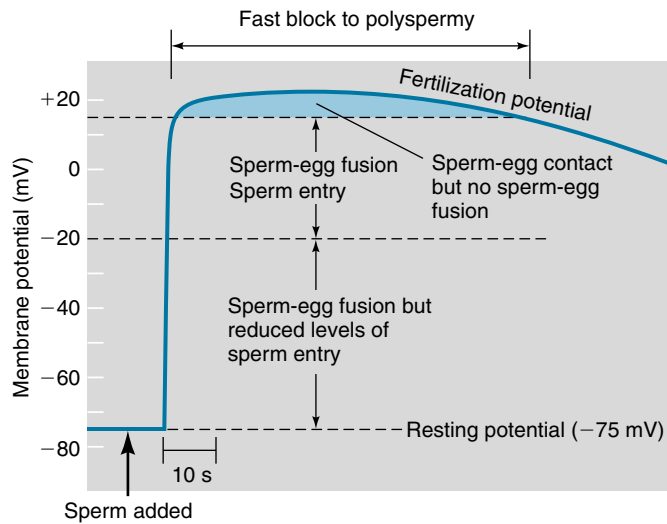


Figure 4.17 Resting potential and fertilization potential in the egg of the sea urchin *Strongylocentrotus purpuratus*. The resting potential is negative inside the cytoplasm (-75 mV). The first sperm-egg contact triggers a fertilization potential, which rises to near $+20$ mV for about 1 min. Whereas the resting potential permits membrane fusion, sperm entry requires a shift to a membrane potential between -20 and $+17$ mV. During this shift, which takes about 1 s (scale bar represents 10 s), a fused sperm begins to enter. A potential above $+17$ mV allows the first sperm to complete its entry, but any additional sperm make only membrane contact and do not proceed with sperm-egg fusion; thus the fertilization potential acts as a fast block to polyspermy. This block lasts for about 1 min, after which the membrane potential returns to intermediate values that allow all fertilization events to occur.

The fertilization potential serves as a fast block to polyspermy in sea urchin eggs. This was shown by using electric current to maintain, or “clamp,” the membrane potential of sea urchin eggs at different levels and then exposing these eggs to sperm (L. A. Jaffe, 1976). At a constant membrane potential of $+20$ mV or more, sperm adhered, but membrane fusion did not occur. The molecular basis for the observed voltage dependence of membrane fusion is still under investigation.

In addition to controlling membrane fusion, the membrane potential also affects fertilization cone formation and sperm entry, at least in sea urchins (Lynn et al., 1988). If the egg's membrane potential is held between -70 and -20 mV, fertilization cones begin to form. However, the cones do not develop fully, and sperm entry usually fails (Fig. 4.17). At membrane potentials between -20 and $+17$ mV, membrane contact is followed by membrane fusion, full fertilization cone formation, and sperm entry. At membrane potentials above $+17$ mV, sperm contact elicits no further response from the egg.

Evidently, a sperm can initiate fertilization at -70 mV, since this is the resting potential, but for sperm entry to proceed, the membrane potential must shift to a more positive level. Only during the fleeting moment

(about 1 s) while the membrane potential rises from -70 to $+17$ mV is a sperm admitted to the egg. Once membrane fusion has occurred, entry of the *first* sperm can continue at $+20$ mV, but additional sperm cannot enter because they fail to fuse with the egg. However, the electrical block to polyspermy is only temporary, because the intermediate potential that allows fertilization is restored after about 1 min.

THE CORTICAL REACTION CAUSES A SLOW BLOCK TO POLYSPERMY

Another key event in egg activation is the *cortical reaction*, also known as the *zona reaction* in mammals (see Fig. 4.13). The cortical reaction is the exocytosis of *cortical granules*, which are membrane-bound vesicles derived from the Golgi apparatus and deployed beneath the egg plasma membrane during oocyte maturation (Laidlaw and Wessel, 1994). An unfertilized mouse egg has about 4000 cortical granules, while a sea urchin egg has 15,000 or so. During egg activation, the cortical granules undergo *exocytosis*, releasing their contents into the *perivitelline space* between the plasma membrane and the vitelline envelope (Fig. 4.18). In sea urchins, the cortical reaction is followed by the regulated exocytosis of additional vesicles that leads to the sequential construction of an extracellular layer of materials surrounding the fertilized egg (Matese et al., 1997).

The cortical reaction begins soon after sperm-egg contact and is completed in one or two minutes, depending on egg size. In mammals, the cortical reaction seems to be triggered by protein kinase C (PKC), which in turn is activated by diacylglycerol, and possibly, Ca^{2+} (see Fig. 2.27; Gallicano et al., 1993; Olds et al., 1995). In sea urchins, the cortical reaction appears to be triggered by a more direct effect of Ca^{2+} . Both a calcium wave and cortical granule exocytosis sweep across the egg in a wave initiated at the point of sperm entry, with exocytosis following calcium release after about 6 s (Matese and Clay, 1998). The exocytosis of cortical granules—similar to the exocytosis of the acrosomal vesicle—involves interactions between so-called SNARE proteins located on the cytoplasmic faces of the exocytosing vesicles and their target plasma membranes (Rothman, 1994; Conner et al., 1997).

In sea urchin eggs, the components released during the cortical reaction have three major effects (Fig. 4.18). First, proteases cleave the proteins that tether the vitelline envelope to the plasma membrane. At the same time, the cortical granules shed complex polysaccharides known as *glycosaminoglycans*, which attract water into the perivitelline space. The resulting gelatinous layer, known as the *hyaline layer*, lifts the vitelline envelope off the egg plasma membrane. Second, *peroxidases* harden the vitelline envelope by cross-linking adjacent proteins. The hardened envelope is then called the *fertilization envelope*. Third, enzymes modify sperm receptors and other components of the vitelline envelope so that sperm no longer adhere to the egg surface.

The cortical reaction causes a slow block to polyspermy. Its protective action may be required because the egg underneath the fertilization envelope is still fertilizable: If such an egg is stripped of its fertilization envelope and hyaline layer, then additional sperm will enter. However, the fertilization envelope permanently inhibits the penetration of more sperm and even causes previously adhering sperm to fall off (Fig. 4.19).

The *zona reaction* in mammalian eggs is similar to the cortical reaction in sea urchin eggs and has very similar results. Certain enzymes released from the cortical granules cross-link the zona proteins, thus making the zona impermeable to sperm. Other enzymes modify the ZP3 glycoprotein, so that ZP3 no longer binds to sperm or elicits the acrosome reaction.

4.6 The Principle of Overlapping Mechanisms

Many steps in development rely on two or more mechanisms that complement or reinforce each other. We call this the *principle of overlapping mechanisms*. The eminent embryologist Hans Spemann (1938) referred to it as the “principle of double assurance” or the “synergetic principle of development.” The degree of overlap between different mechanisms that support the same biological process differs from case to case. In this chapter, we have come across overlapping mechanisms for sperm-egg adhesion, for the penetration of the zona pellucida, for triggering egg activation, and for preventing polyspermy. With regard to the last case, we saw that many animal eggs have two blocks to polyspermy: a fast block relying on an electric membrane potential, and a slow block relying on the formation of the fertilization envelope. The latter event in turn relies on three mechanisms—namely, the cleavage of the tethering proteins and formation of the hyaline layer, together lifting the vitelline envelope off the egg plasma membrane; the hardening of the vitelline envelope, which makes it impermeable to additional sperm; and the release of sperm-binding molecules from the vitelline envelope.

The fast block to polyspermy holds up about as long as it takes for the slow block to become effective (see Table 4.1). This is an example of minimal overlap between different mechanisms that serve the same biological function. In contrast, the three inhibitory mechanisms that constitute the slow block appear to be redundant. In theory, there should be no need for the vitelline envelope to harden or to lift off the plasma membrane when the sperm-binding molecules have been modified or shed. There are two possible explanations for the fact that these seemingly redundant mechanisms still exist. First, each mechanism might serve as a fail-safe mechanism to back up the other two. If each mechanism by itself eliminated only 9 out of 10 extra sperm, then the three mechanisms together would eliminate 999 of 1000 extra sperm. This would be adequate

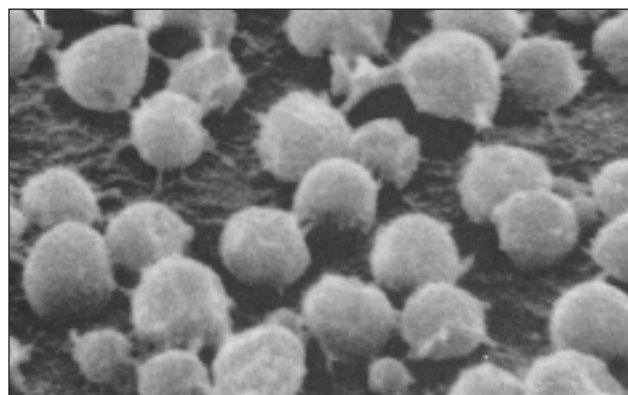
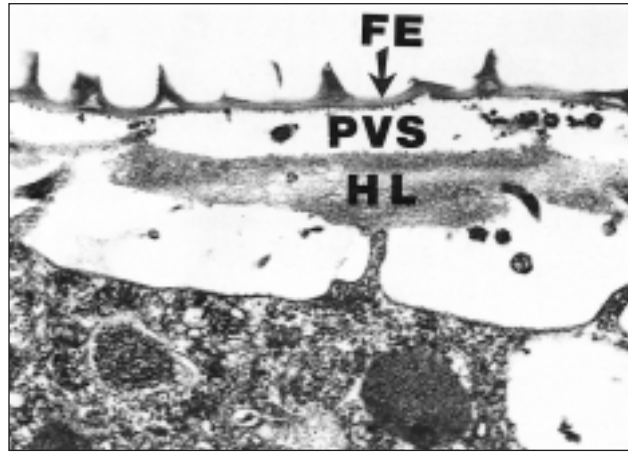
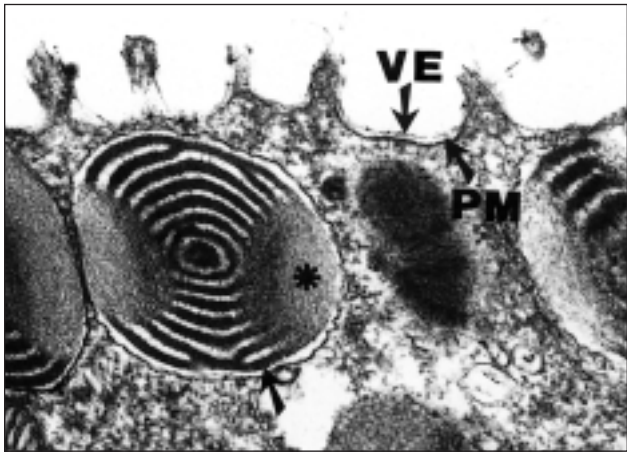
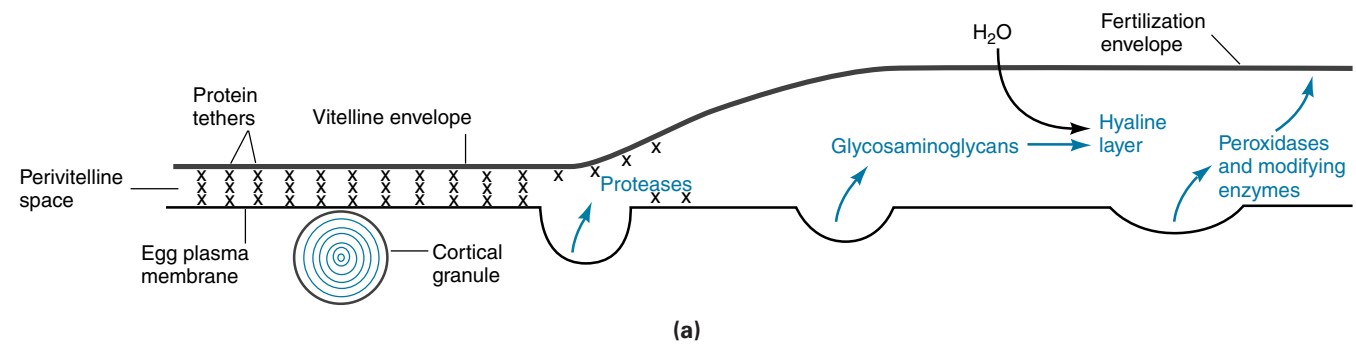


Figure 4.18 Cortical reaction in the sea urchin egg. **(a)** As part of egg activation, the cortical granules beneath the plasma membrane undergo exocytosis, releasing into the perivitelline space several components that support three major processes. First, proteases cleave the proteins that tether the vitelline envelope to the plasma membrane. At the same time, glycosaminoglycans also released from the cortical granules attract water into the perivitelline space and form a hyaline layer that lifts the vitelline envelope off the egg plasma membrane. Second, peroxidases harden the vitelline envelope by cross-linking adjacent proteins. The hardened envelope is then called the fertilization envelope. Third, other enzymes modify vitelline envelope components so that sperm no longer adhere to the egg surface. **(b)** Transmission electron micrograph showing the cortex of an unfertilized sea urchin egg (*Strongylocentrotus purpuratus*). The vitelline envelope (VE) is closely applied to the plasma membrane (PM) and follows the contours of the microvilli. The cortical granules contain lamellar and amorphous (asterisk) components. **(c)** Ten minutes after fertilization, the fertilization envelope (FE) is separated from the egg by a hyaline layer (HL), which builds up in the perivitelline space (PVS). **(d)** Scanning electron micrograph showing the inner aspect of the plasma membrane of an unfertilized egg. The cortical granules (round bodies) are still intact.

protection even for an egg that is exposed to an excess of sperm. Another explanation for the persistence of seemingly redundant mechanisms for one biological function is that each mechanism may have one or more additional functions. For instance, the hardened fertilization envelope also provides mechanical protection, and the hyaline layer also forms a firm coat around the blastula

cells, which prevents these cells from leaving the embryo. Thus, what seem to be redundant mechanisms for one function may actually be part of a larger network of mechanisms serving several functions.

Overlapping mechanisms may be crucial for the organism, but they can also be a challenge to researchers. If a given biological function is supported by multiple

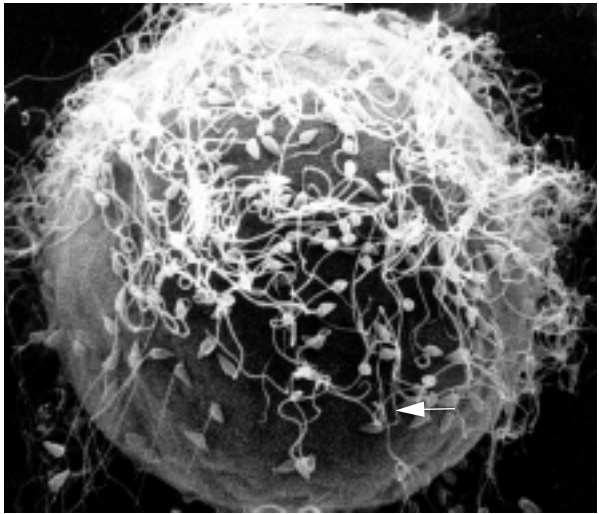
mechanisms, it is very difficult to analyze any one of them. One way of testing whether a certain mechanism is *necessary* for a given function is to block the mechanism (by mutation, drugs, or other means) and to

observe whether the function fails. This test may not reveal a necessary mechanism among a group of overlapping mechanisms, because each blocked mechanism is backed up, at least to an extent, by others that are still working. In this situation, investigators have to interfere with several mechanisms simultaneously before the biological function of interest fails and yields to analysis.

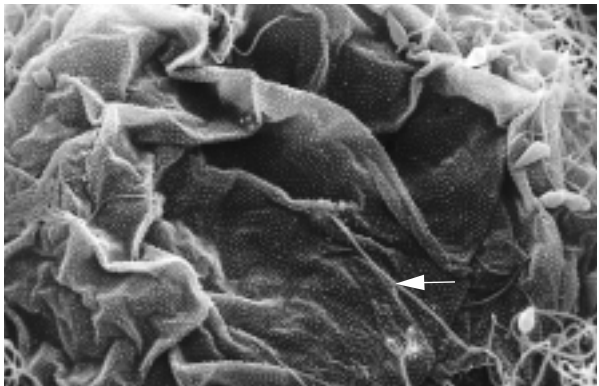
4.7 Parthenogenesis

Some animals can reproduce by *parthenogenesis* (Gk. *parthenos*, "virgin"; *genesis*, "origin"), which is the development of viable offspring from unfertilized eggs. In aphids and other insects, parthenogenesis alternates with sexual reproduction, a reproductive strategy known as *facultative parthenogenesis*. Females reproduce parthenogenetically during the summer when food is abundant. At the end of the season, a generation of males and females develops and reproduces sexually. The fertilized eggs from this generation overwinter and develop into females during the next spring. In other insects, including the honeybee, parthenogenesis is coupled with sex determination. Fertilized eggs give rise to diploid females, while unfertilized eggs develop into haploid males called *drones*. Some species, mostly invertebrates but also some lizards, reproduce by *obligatory parthenogenesis*. Their populations consist entirely of females.

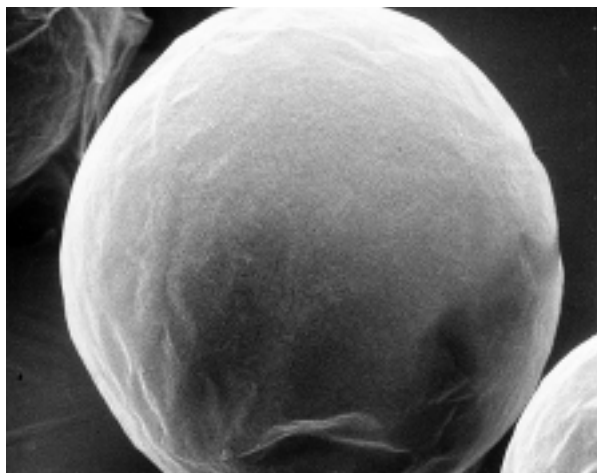
Parthenogenetic species are faced with three problems. First, they need to compensate for the reduction in number of chromosomes that normally occurs during meiosis. They can do this through several mechanisms, such as skipping one meiotic division or fusing two haploid nuclei after meiosis. The second problem in parthenogenetic species is egg activation. The silver salamander, *Ambystoma platineum*, solves this problem by enlisting the help of males from another species. There are no *A. platineum* males, but the females mate with males of a closely related salamander, *A. jeffersonianum*. The sperm from these males only activate the eggs of *A. platineum* and do not contribute their genome to the offspring (Uzzell, 1964). Other parthenogenetic species use different stimuli to activate their eggs. In parasitic wasps, unfertilized eggs are activated by friction or distortion when they pass through the narrow ovipositor (Went and Krause, 1973). The third problem



(a)



(b)



(c)

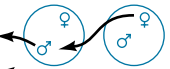
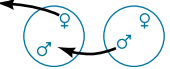
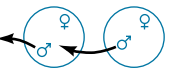
Figure 4.19 Removal of excess sperm after the cortical reaction in the egg of the sea urchin *Strongylocentrotus purpuratus*. (a) Scanning electron micrograph of an egg 15 s after the addition of sperm. (b) Thirty seconds after the addition of sperm. The arrow marks the tail of the fertilizing sperm. The progress of the cortical reaction is indicated by the zone of sperm detachment surrounding the fertilizing sperm. The wrinkles in the fertilization envelope are an artifact of fixation. (c) Three minutes after the addition of sperm. The fertilization envelope has hardened and no longer wrinkles during fixation. All sperm have detached.

in parthenogenesis is the lack of a centrosome, which is normally introduced by the sperm and serves to organize the first mitotic spindle. However, a centrosome may also be contributed by the egg, or formed from smaller components in the egg cytoplasm, or replaced functionally by other microtubule organizing centers.

In the laboratory, a variety of physical and chemical treatments have been used to activate eggs. One of the most commonly used methods is simply to prick the eggs with a needle, preferably one that has been dipped in blood or other tissues. This treatment may trigger an early event in the activation cascade, such as the Ca^{2+} transient, which then elicits the subsequent events. Impurities on the pricking needle may also serve as nucleating centers for spindle formation. For development to advanced stages, the diploid chromosome number must usually be restored. However, adults have been obtained, with varying success, through artificial activation of unfertilized eggs from sea urchins, starfish, silk moths, fishes, and frogs (Beatty, 1967).

Natural parthenogenesis is unknown in mammals. Unfertilized mouse eggs activated in a series of experiments did not develop beyond day 11, halfway through their gestation. By transplanting pronuclei between fertilized mouse eggs, one can construct zygotes with two female pronuclei and no male pronucleus (Table 4.2). Such zygotes develop into bimaternal embryos, which cease development at about the same time as parthenogenetic

table 4.2 Pronuclear Transplantation Experiments with Fertilized Mouse Eggs

Class of Reconstructed Zygotes	Operation	Number of Successful Transplants	Number of Progeny Surviving
Bimaternal		339	0
Bipaternal		328	0
Control		348	18

Source: McGrath and Solter (1984) and Gilbert (1991). Used by permission.

embryos. Zygotes with two male pronuclei and no female pronucleus develop into bipaternal embryos; they also die midway through gestation but show different defects from the bimaternal embryos. Control zygotes with one male and one female pronucleus, generated by the same transplantation technique, can develop normally (McGrath and Solter, 1984). These results indicate that in mammals both a female pronucleus and a male pronucleus are needed for embryonic development. Certain mammalian genes can be activated only in the female pronucleus, whereas other genes must be introduced through the male germ line in order to be expressed (Surani et al., 1986; see also Section 16.6).

SUMMARY

Fertilization is the union of two haploid gametes—one egg and one sperm—to form a diploid zygote from which a new individual develops. In addition to properly timed parental mating behavior, some animals use chemical attractants and capacitation mechanisms to ensure that the egg and the sperm are ready for fertilization when they encounter each other. In most species, sperm penetrate several egg coats before reaching the egg plasma membrane. Of particular importance is the vitelline envelope—in mammals called the zona pellucida—which is a coat of proteins laid down by the oocyte before ovulation. The interaction of the sperm with the vitelline envelope or another egg coat triggers the acrosome reaction, in which the sperm releases the contents of its acrosome. Enzymes from the acrosome help the sperm to lyse its way to the egg cell proper. Sperm adhere to the egg surface in a species-specific way, by means of matching molecules on the sperm head and the vitelline envelope of an egg from the same species.

The contact or fusion of the egg and sperm plasma membranes triggers a cascade of egg responses collectively called egg activation. It includes sperm entry, transient rises in calcium ion (Ca^{2+}) concentration and pH, acceleration of many metabolic processes, and resumption of the cell cycle. In many species, egg activation also entails changes at the egg surface that prevent polyspermy. The eggs of such species typically use a fast but temporary electrical block before a slower, permanent block sets in. The slow block results from the cortical reaction, in which cortical granules located beneath the egg plasma membrane release their contents by exocytosis. The cortical reaction alters the vitelline envelope in several ways that make it impermeable to sperm.

Some animals can reproduce by parthenogenesis, the development of viable offspring from unfertilized eggs. These species use several mechanisms to avoid or compensate for the reduction in number of chromosomes that occurs during meiosis and to trigger egg activation.