ABSTRACT

Sea urchin embryos have long been used as ideal experimental materials. In this chapter, we describe research areas to which sea urchin embryos have contributed, and the potential fields for which they may serve as a model system. The most valuable feature of sea urchin embryos is the availability of a large amount of homogeneous material, which facilitates biochemical and molecular biological approaches. The ease of gamete handling enables detailed analysis of the mechanism of fertilization, and the transparency and synchrony of fertilized eggs facilitate investigations on cell division and the cell cycle. Sea urchin embryos are an ideal model system for signal transduction, because the number of constituent cells is small. The simple organization of the embryo simplifies the analysis of morphogenetic movements. Both primary and secondary mesenchyme cells are interesting populations for studying cell movement. Sea urchin embryos will continue to contribute to the analysis of various unsolved problems.

Key Words: Sea urchin embryo, Fertilization, Cell division, Cell cycle, Signal transduction, Specification, Gastrulation, Invagination, Morphogenesis, Cell movement.

HANDLING OF ANIMALS AND EMBRYOS

Sea urchins belong to the phylum Echinodermata (echinoderms), which consists of six classes (Crinoidea, Asteroidea, Concentricycloidea, Ophiuroidea, Echinoidea, and Holothuroidea). At present, nearly 900 species of sea urchins (class Echinoidea)—divided into nine orders—are known. Most live in intertidal zones or shallow seas, and their distributions extend from equatorial regions through the North and South Poles. Embryos of sea urchins are frequently used in biological and medical research including those of Strongylocentrotus purpuratus, Lytechinus variegatus, Lytechinus pictus, Arbacia punctulata (North America), Paracentrotus lividus (Mediterranean Sea), Psammechinus miliaris (North Sea), Hemicentrotus pulcherrimus, and Anthocidaris crassispina (Japan). The whole genome of S. purpuratus, a well known species in North America, has already been sequenced (http://sugp.caltech.edu/). In the United States, some species are available from commercial suppliers such as Susan Decker (Davie, FL) or Marinus Scientific (Long Beach, CA). Some marine laboratories also supply these materials in several countries. Adult sea urchins are reared in a small aquarium equipped with a simple filtering system and maintained without feeding for a couple of months.

During the breeding season, fertilizable oocytes are shed following intracoelomic injection of 0.5 M KCl. In some species, more than a million eggs are obtained from a single female. Usually, the testis is directly excised from adult males, and the leaked semen is diluted with seawater immediately before use. Fertilized eggs are obtained by simply adding the diluted sperm to an egg suspension.

For embryo culture, natural seawater should be filtered to remove debris and microorganisms. If fertilized eggs are deprived of the fertilization envelope for manipulations, it is preferable to add antibiotics (e.g., streptomycin or penicillin). Commercially supplied artificial seawater (e.g., Instant Ocean) is also available. At an appropriate density, fertilized eggs developed into pluteus larvae within 2–3 days without any special care. Detailed handling of animals and embryos has been described previously.1

FERTILIZATION

The finding of the acrosome reaction in starfish spermatozoa2 led to detailed analysis of fertilization processes, which was accompanied by improved electron microscopic and biochemical techniques. Many important concepts of fertilization (cortical reaction, activation, Ca²⁺ release, acid release, change in membrane potential, block to polyspermy, etc.) have come from numerous studies of sea urchin fertilization. The jelly coat of A. punctulata contains a chemoattractant for spermatozoa,3 although this has not yet been proved in other sea urchin species. When a sperm head attaches to the jelly coat (Figure 11-1A), the acrosomal vesicle breaks down and the acrosomal process forms. Proteolytic enzymes contained in the acrosomal vesicle digest the jelly coat and allow the spermatozoon to approach the egg. The first contact between sperm and egg is mediated by bindin on the acrosomal process and its receptor on microvilli extruded from the oocyte.4 The binding of bindin and its receptor shows species specificity, and leads to the membrane fusion of both gametes.
The influx of Ca\(^{2+}\) of intracellular signaling pathways for Ca\(^{2+}\) breakdown, the fertilization membrane is elevated (Figure 11–1B). This physical barrier is a late and permanent block to polyspermy. Even the number of microtubules that constitutes the mitotic spindle can be estimated from the intensity of birefringence.\(^7\)

By deforming fertilized eggs into various shapes, Rappaport has studied the spatial relationship between mitotic spindle and cleavage furrow.\(^8\) It is now widely accepted that differences in the density of the microtubules that reach the egg cortex determine the position of the cleavage furrow (i.e., the position of the contractile ring), which exerts the main force for cytokinesis (Figure 11–1C). The existence of the contractile ring was first reported in fertilized sea urchin eggs.\(^8\) To preserve this structure for electron microscopy, more than 500 combinations of buffers and fixatives were tested, but we can now detect microfilaments more easily using fluorescent phallacidins.

The fertilized eggs begin to cleave within 1.0–1.5 h after fertilization, and show radial and holoblastic cleavages. Up to the eight-cell stage, the cleavage pattern is typical (Figure 11–1D). In some species, blastomeres in the animal hemisphere are somewhat larger than those in the vegetal hemisphere. This is the first sign of the animal–vegetal axis. At the fourth cleavage, the animal blastomeres undergo meridional cleavage and give rise to eight blastomeres with the same volume (mesomeres). In the vegetal half, cleavage is horizontal and the cleavage plane shifts to the vegetal pole. As a result, four large blastomeres (macromeres) and small blastomeres (micromeres) are formed (Figure 11–1E). This formation of macromeres and micromeres is an excellent model for research of unequal cell division.

In most species, seven or eight rounds of synchronous cleavages occur at intervals of 0.5–1.0 h. The cell cycle then extends and the synchrony of division is lost. As is well known, the cell cycle is regulated by complexes of cyclin and Cdc(s) or Cdk(s). It is of note that cyclins were first found in sea urchin embryos.\(^10\) By the stage of hatching, the fertilized eggs usually undergo 10 cleavages and develop into spherical and hollow blastulas (Figure 11–1H). The time required for development to the hatching stage is 10–12 h in most species. The hatched blastula develops into a pluteus larva through a series of morphogenetic movements and organogenesis within 2–3 days (Figure 11–1I). It is of note that each part of the larval body comprises a monolayered epithelium.

**Figure 11–1.** Early development of sea urchin embryos. (A) Unfertilized egg. (B) Fusion of male and female pronuclei. (C) First cleavage. (D) Eight-cell stage. (E) Sixteen-cell stage. (F) Sixty-cell stage. The four tiers of blastomeres are named an\(_1\), an\(_2\), veg\(_1\), and veg\(_2\), along the animal–vegetal axis. (G) Late blastula stage. (H) Fate map drawn in the median plane of the swimming blastula. (I) Pluteus larva (48 h after fertilization in Hemicentrotus pulcherrimus). (E–G) Positions of Wnt8 and Nodal expressions are indicated in dark and light gray, respectively. (A–F) Frontal view. (G–I) Side view. Dotted lines in (G) indicate the boundaries of the veg\(_1\) and veg\(_2\) tiers. (H, I) Labels for SMCs (dark gray), foregut (reticulated), mid-gut (hatched), hind gut (dotted), and oral ectoderm (light gray) are common to Figure 11–2, which shows more details on stages between (H) and (I) of this figure. ap, animal pole; aoe, aboral ectoderm; cg, cortical granule; cr, contractile ring; fm, fertilization membrane; fp, female pronucleus; hl, hyaline layer; jc, jelly coat; ma, mitotic apparatus; mac, macromere; meso, mesomere; mic, micromere; mp, male pronucleus; oe, oral ectoderm; sp, spermatozoon; st, degenerating sperm tail; vm, vitelline membrane; vp, vegetal pole.

Following cell membrane fusion, the Na\(^+\)–K\(^+\) ion channels of the egg act to elevate the intracellular Na\(^+\) concentration and to depolarize the egg membrane. This electrical change is a rapid and transient block to polyspermy.\(^3\) The influx of Ca\(^{2+}\) at the fusion site triggers the breakdown of cortical granules (cortical reaction), which is a typical Ca\(^{2+}\)-induced Ca\(^{2+}\) release reaction. The exocytosis of cortical granules propagates from the sperm entry point toward the opposite site concentrically. With cortical granule breakdown, the fertilization membrane is elevated (Figure 11–1B). This physical barrier is a late and permanent block to polyspermy. Molecules contained in the cortical granules cover the egg surface and form the hyaline layer (Figure 11–1B). Sea urchin fertilization is providing a model system for the analysis of intracellular signaling pathways for Ca\(^{2+}\) release from internal supplies.

After entry into the egg, the sperm head decondenses and becomes a male pronucleus. Centrioles brought into the egg with the sperm nucleus act as a microtubule-organizing center. The female pronucleus is pulled toward the centrioles along a ray of microtubules from the growing sperm monaster\(^6\) and fuses with the male pronucleus. The transparency of the fertilized eggs enables us to observe these processes in detail.

**CELL DIVISION AND THE CELL CYCLE**

Fertilized sea urchin eggs have greatly contributed to the understanding of the mechanisms of cell division. This is due to the ease of handling and the transparency and synchrony of fertilized eggs. With differential interference contrast imaging or polarizing microscopy, dynamic changes in the mitotic apparatus—assembly and disassembly of microtubules—are observable (Figure 11–1C). The double gradient theory has explained the results obtained from classical deletion and recombination experiments on sea urchin embryos.\(^11\) Recent studies have revealed that micromeres and their descendants act as a signaling center for embryonic body patterning along the animal–vegetal axis. Briefly, β-catenin enters the nuclei of micromeres soon after their formation.\(^12\) Then, Wnt8