Feb 4, 2005
Topic: Fertilization

Outline
Gametes are specialized cells
  Sperm
  Egg
Species specific gamete recognition/binding
Prevention of polyspermy
  Fast block
  Slow block
Activation of egg metabolism

Reading
Ch. 7
Understand Fig 7.17 and 7.27

Important concepts
- Cell types are specialized for specific functions. Gametes are examples.
- Cells can respond to external signals using transcriptional and post-transcriptional mechanisms. Prevention of polyspermy requires response within seconds.
- Responses to a signaling event may be localized to a region or compartment of the cell.
- Not all signaling molecules are proteins.
- Eggs are quiescent, and the fertilized egg must activate metabolic processes (DNA synthesis, translation, etc.)

Question to class:
1. What are the goals of fertilization? Why are these goals important to the early development of the organism?
2. What are the specialized functions of the egg during fertilization and formation of the zygote? How is the egg structurally specialized to accomplish these functions?
3. What are the functions of the sperm during fertilization? How is the sperm structurally specialized to accomplish these functions?
4. What is meant by egg activation? Give 3 examples of cellular events that occur during egg activation. Why are each of these events important?

Responsible for
#1 Last names A – D
#2 Last names E – K
#3 Last names L – P
#4 Last names R – Z
Student answers to this question will structure discussion in class
Topics I expect to cover in class:

Fertilization requires:
- Recognition: It’s important that an egg fuse with a sperm of the same species
- Prevention of polyspermy
- Combining maternal and paternal genomes
- Activating egg metabolism

A. Gametes are specialized cells
   • Be able to relate gamete structure to function.

A1. **SPERM** (sea urchin or mammalian) See figure 7.2 in text

1. Functions are
   - Travel relatively long distances
   - Recognize and penetrate egg
   - Transfer haploid genome to ovum to form a zygote

2. Schematic of sperm (students should be able to label)

![Schematic of sperm](image)

- **Head:**
  - haploid nucleus
  - acrosomal vesicle
  - (a derivative of the golgi that contains proteolytic enzymes)

- **Midpiece:** mitochondria

- **Tail:** flagellum

Sea urchin sperm initiates final maturation (motility) upon release into seawater.

3. Relate structure to function
   - Flagellum
   - Mitochondria
     - (Most cytoplasm eliminated)
   - Acrosomal vesicle
   - Haploid nucleus
A2. Egg (see Figure 7.4 in text)

1. Egg schematic. Students should be able to localize the following:
   Jelly layer (sea urchin) OR layer of cumulus cells (mammals)
   Extracellular matrix: Vitelline membrane (sea urchin) OR zona pellucida (mammals)
   Nucleus arrested in meiosis (see Fig 7.5)
   Cytoplasmic contents must support early development of embryo
      Proteins/ yolk
      mRNAs
      tRNAs and ribosomes
      Morphogenic factors (regulatory molecules that will direct development)
   Cortical granules
      Contain proteolytic enzymes

B. Gamete recognition and binding

B1. Specificity of interaction is a big problem for sea urchin, because sea urchins release their gametes into the ocean. The sperm chemotax towards proteins in the jelly coat.

See pages 194-196 of the textbook for information regarding capacitation and sperm chemotaxis in mammals.

B2. Sea Urchin

Acrosomal reaction (Fig 7.8 and 7.10 of text) exocytosis
   Induced by polysaccharide in jelly coat
   Species specificity

Reaction includes:
• Fusion of the acrosomal vesicle with the sperm plasma membrane and release of vesicle contents:
   Contact with egg jelly coat induces a rapid influx of calcium ions into the sperm head. This induces exocytosis. Exocytosis of the acrosomal vesicle can be induced by increasing the calcium concentration in the environment.
   Proteolytic enzymes in the vesicle digest the jelly coat.

Extension of the acrosomal process: polymerization of Gactin to form actin filaments.
Fig 7.8 and 7.10

Binding to vitelline membrane is species specific.
  Sperm protein is bindin
  Vitelline membrane protein is a glycoprotein receptor. (A receptor is a protein that
  interacts with an extracellular signaling protein and initiates cellular response to that signal.)

B3. Mammals
Mammalian eggs don’t have jelly coats. Instead, they are surrounded by a layer of cumulus
cells. Sperm must navigate this maze of surrounding cells to reach the egg.

Zona pellucida is an extracellular protein matrix secreted by the egg.
In some respects, the zona pellucida is analogous to the sea urchin vitelline membrane.

C. Prevention of polyspermy
(Why is this important? See Fig 7.21)

Fast block to polyspermy
  Present in sea urchin, but not mammals
Slow block to polyspermy
  Present in both sea urchin and mammals

Sea Urchin
C1. Fast block
  The fast block is transient, lasting only a minute.

The egg resting potential is negative with respect to surrounding sea water (-70mV). [There
are more positive ions (mostly sodium) in sea water than in the egg cytoplasm.]

Within 1-3 seconds after binding of the first sperm, a small influx of sodium ions is
permitted. This causes the resting potential of the egg to rise to +20 mV. The sperm cannot
fuse with the membrane one this positive resting potential has been achieved.

Experimental evidence:
  Decrease sodium concentration in the sea water and induce polyspermy
  Artificially manipulate the electical potential of the cell and inhibit fertilization (by
  increasing the membrane potential) or allow polyspermy (by maintaining a negative
  membrane potential).
C2. Slow block to polyspermy

Sperm binding leads to the release of intracellular stores of calcium, which in turn causes the exocytosis of the cortical granules. See Fig 7.25

The ~15,000 cortical granules exocytose their contents, which include

- **Proteases** (mammals and sea urchin): dissolve the vitelline envelope
- Mucopolysaccarides: produce an osmotic gradient, causing water to rush into the space between the plasma membrane and the vitelline membrane
- Peroxidase: crosslinks proteins in the envelope
- Hyalin protein: surrounds egg and supports the forming embryo

The egg cell surface proteins that initiate the intracellular calcium ions are not known. However, some of the intracellular signaling proteins have been defined. IP3 plays a central role. (Figure 7.28)

C. Activation of egg metabolism
(Fig 7.27, 7.28, 7.29)

The egg is metabolically quiescent. Upon fertilization, the maternal genome completes meiosis and the haploid nucleus fuses with the paternal pronucleus to create a diploid genome.
The egg contains maternal mRNAs that must be translated to initiate and guide early developmental processes.
Egg metabolism of sugars must also be initiated, to create the energy needed for processes such as mitosis and protein synthesis.
Lipid metabolism and synthesis is needed to create or modify membranes during cell division and development.

C1. Calcium mediated
C2. Increase in intracellular pH