Fertilization: Intercellular Communication & Signal Transduction

Introduction

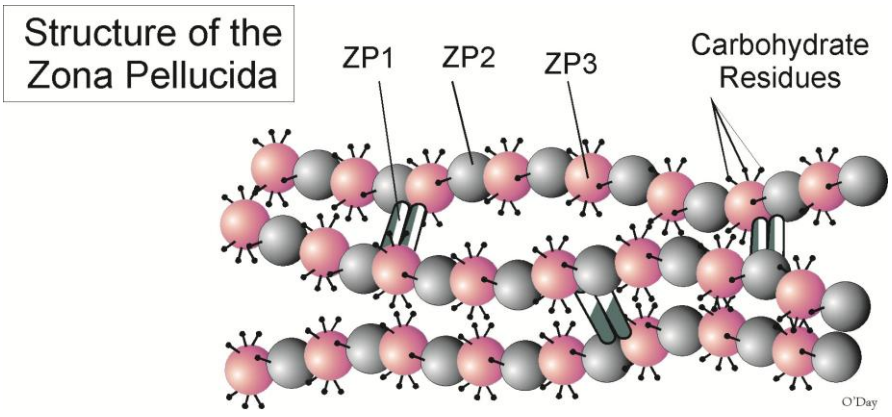
The sperm and the egg communicate with each other during the process of fertilization to ensure that everything goes according to plan. First the male and female gametes have to ensure that they are compatible, then a series of interactions follows that leads to each event occurring at the proper place and time for the formation of the zygote. The first line of defense of the egg is the coats, especially the zona pellucida, that surround it. These keep foreign sperm out and prepare human sperm for fertilization.

Zona Pellucida (ZP)

- Protein coat surrounding the egg
- Species-specific barrier to sperm binding and penetration; keeps sperm of foreign species out
- Not 100% effective; some cross-species fertilization can occur (e.g., horse & donkey = mule; some species of monkeys can cross fertilize)
- Remove the ZP and other species sperm can fertilize and egg (e.g., Hamster Test for Male Fertility; human sperm can fertilize the zona-less hamster egg)

The Zona Pellucida is made up of three major glycoproteins:

- ZP3 mediates Sperm-Specific Egg Binding
- ZP2 mediates subsequent sperm binding
- ZP1 cross-links ZP2 and ZP3 as protein meshwork; not essential for fertilization but is important for structural integrity of zona pellucida; mice that lack ZP1 produce embryos that hatch prematurely causing developmental problems (Rankin et al, 1999. Development 126: 3847-3855).

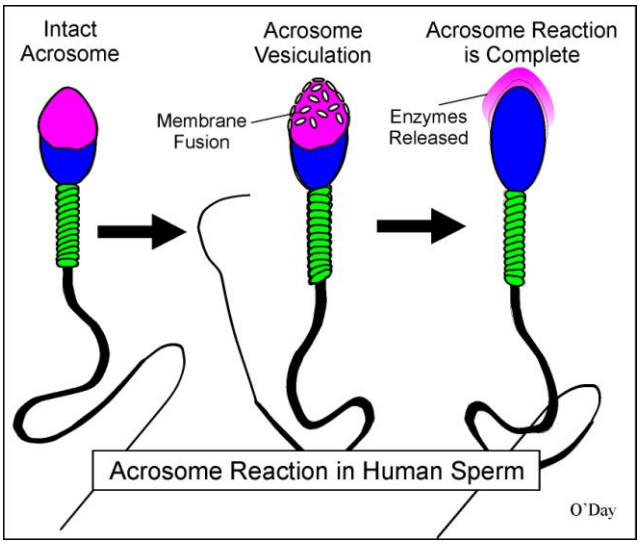


The above diagram demonstrates how ZP1, ZP2 and ZP3 interact to form the zona pellucida. Learn this structure now because we'll re-examine it when we discuss the process of sperm-egg binding and, later, the block to polyspermy in mammals. This model is derived from research on mice and may vary slightly in humans (after Wassarman, 1990. Develop. Biol. 108: 1-17). However, the relevance of the mouse model in understanding human fertilization was recently shown when mutant mice which lacked ZP3 were given the human ZP3 gene in place of their own ZP3 gene (Rankin et al, 1998. Development 125: 2415-2424). The genes encoding mouse and human ZP3 are over 2/3 identical. The mutant mice which lacked ZP3 couldn't

bind the sperm to the zona pellucida and thus did not get fertilized. When their missing ZP3 gene was replaced with a human ZP3 gene, the mice made the human ZP3 and it was incorporated into their zona pellucida. With the human ZP3 in their zonas the eggs could be fertilized by mouse sperm revealing that human and mouse ZP3 can mediate the same critical events.

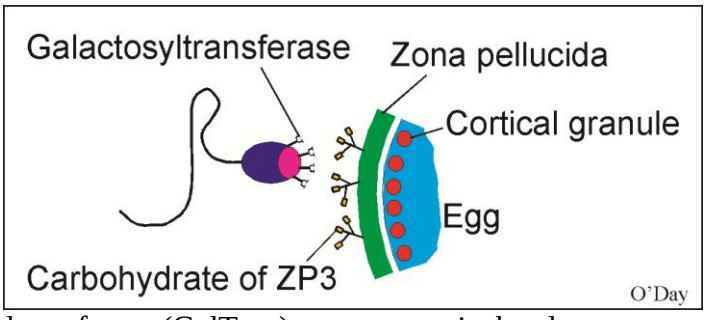
The Acrosome Reaction: Morphological events

When it contacts the egg cell layers, the sperm has an intact acrosome. In normal fertilization, stimulation of the sperm by agents from the cumulus oophorus and especially the corona radiata followed by binding to the zona pellucida, leads to the acrosome reaction prior to egg binding. The following sequence of events shows the morphological events of the acrosome reaction.



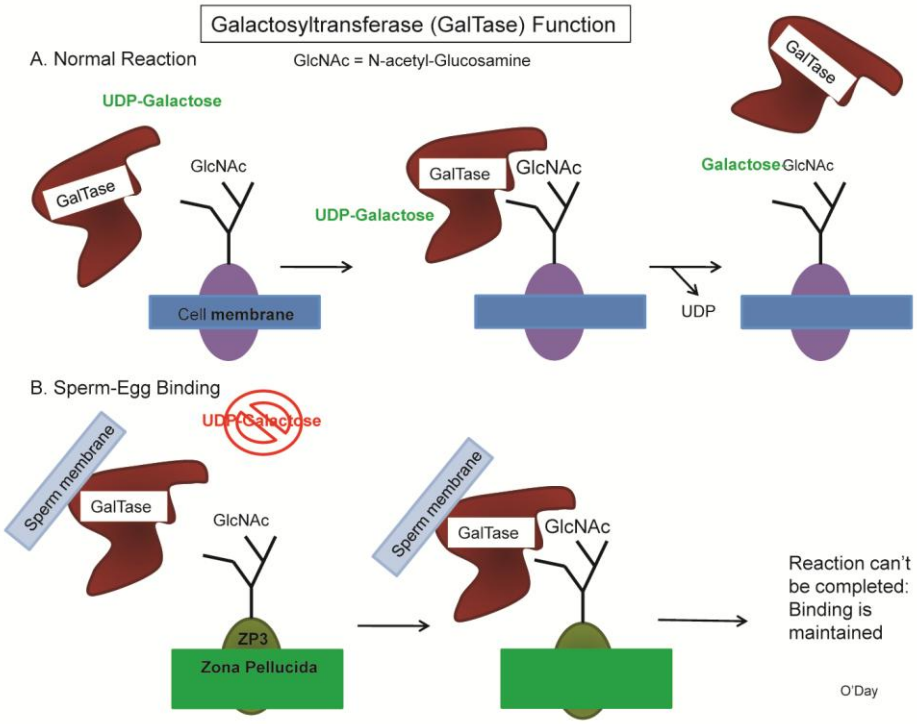
The Acrosome Reaction: Galactosyltransferase binding

The mechanism of mammalian sperm-egg binding is under intense scrutiny. The problem is that the data from various labs is often contradictory. The possibility likely exists that there are various fall-back mechanisms in play so that if a mutation in one pathway affects sperm-egg binding then the sperm and egg use other binding mechanisms to ensure fertilization occurs. In vitro the cumulus and zona radiata cells are removed. Here we will look at only one of the classic mechanisms of sperm-egg binding that leads to the acrosome reaction: galactosyltransferase binding that occurs at the zona pellucida.



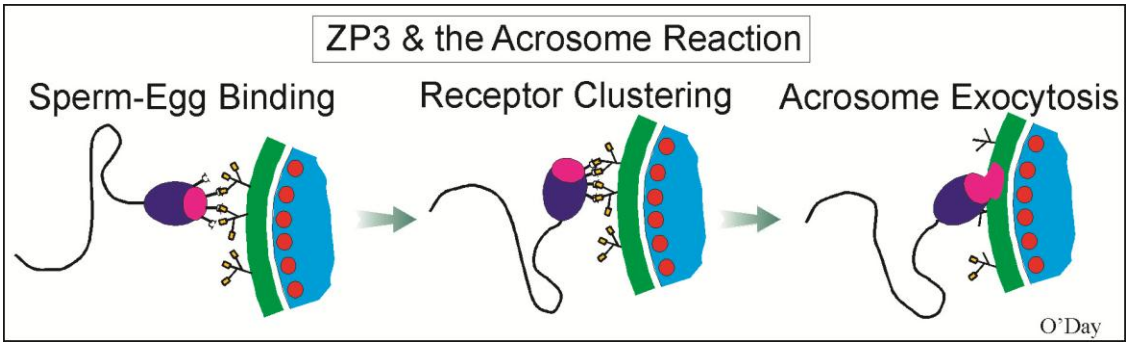
- Each sperm has galactosyltransferase (GalTase) enzymes on its head
- Galactosyltransferase is an enzyme that transfers a sugar group from one molecule to another
- Egg has ZP3 in ZP
- At binding, the cortical granules are intact
- Acrosome is also intact

At least part of the events involved in sperm binding to the egg and the resulting acrosome reaction are due to an enzyme reaction. Unlike normal enzyme reactions in which the enzyme binds its substrate and produces products, this enzyme reaction cannot go to completion. Since it can't complete the reaction the enzyme and the substrate remain attached to each other.



- Each sperm binds to sugar (N-acetylglucosamine, GlcNAc) residues in ZP3 via galactosyltransferase (GalTase) enzymes in the sperm cell membrane. Thus the GlcNAc is the substrate for the GalTase enzyme.
- The binding doesn't result in sugar transfer because a major component (actually UDP-galactose) needed for the enzyme reaction is missing; so the sperm remain attached via the sugar-enzyme binding because the enzyme reaction cannot go to completion .

ZP3 and the Acrosome Reaction

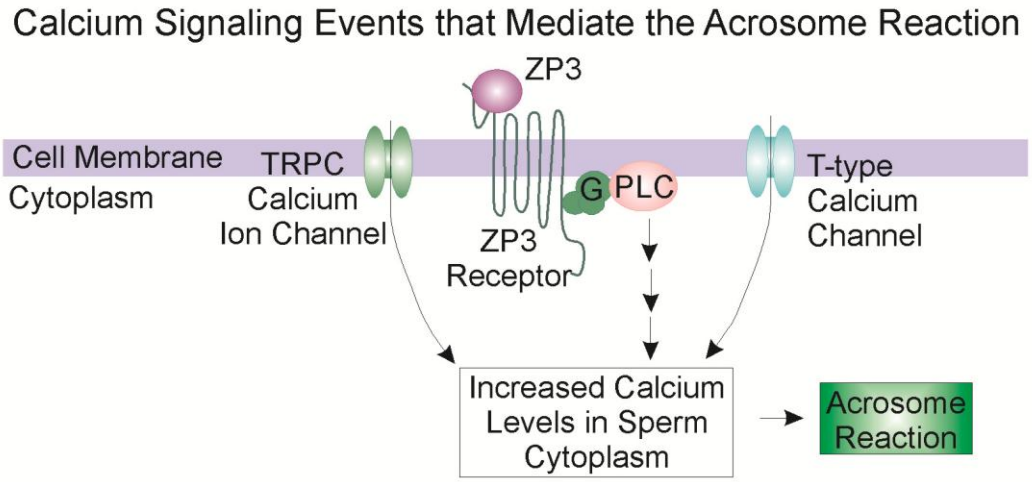


- As more GalTase enzymes bind more GlcNAc substrates the receptors on the sperm head cluster together
- This clustering alters the sperm cell membrane causing calcium levels to increase in the sperm cytoplasm
- Calcium levels further increase because GalTase/GlcNAc binding has initiated signal transduction events.
- The increase of intracellular calcium mediates the fusion of the acrosomal and sperm cell membranes
- This allows the contents of the acrosome to flow out
- The released Acrosomal enzymes now begin to digest a path for the sperm through the zona pellucida

The role of GalTase in sperm-binding to ZP3 in the zona pellucida and acting as a subsequent mediator of the acrosome reaction was proven using knockout mice. The sperm of mutant mice that lack the GalTase gene (GalTase-null mice) do not undergo the acrosome reaction and show extremely poor zona penetration.

Calcium & the Acrosome Reaction

Calcium is known to trigger many biomembrane fusion events especially those involving exocytosis (e.g., transmitter release from synaptic vesicles in nerve cells). Calcium plays a part in the exocytosis of the acrosome during fertilization as evidenced by the following and other results. When calcium ionophores (chemicals that cause channels to form in membranes allowing calcium to flow through) are added to sperm they induce the acrosome reaction.



G = G protein; PLC = Phospholipase C; TRPC = Transient Receptor Potential Cation; T-type: is a voltage dependent calcium channel

Continued research has revealed that the increased calcium levels that drive the acrosome reaction come from various sources as shown in the following diagram. Binding of ZP3 to the ZP3 receptor in the sperm membrane activates G protein signaling that in turn activates phospholipase C ultimately causing intracellular levels of calcium to increase. TRPC calcium ion channels and T-type calcium channels in the sperm cell membrane allow calcium to influx from outside the cell. Together the increase calcium levels drive the membrane fusion events of the acrosome reaction.

Sperm Binding to the Zona & the Egg Cell Membrane

In addition to GalTase and acrosin, there are other candidate proteins for sperm-zona binding. Once through the zona, the sperm then contacts the egg cell membrane. Binding of the sperm cell membrane to the egg cell membrane is a critical step that occurs prior to sperm-egg fusion and also initiates the cortical reaction (discussed below). Of several proteins that mediate these membrane interactions, fertilin b appears to be important, at least in mice. Mouse mutants that lack fertilin b have a markedly reduced fertility.

Block to Polyspermy

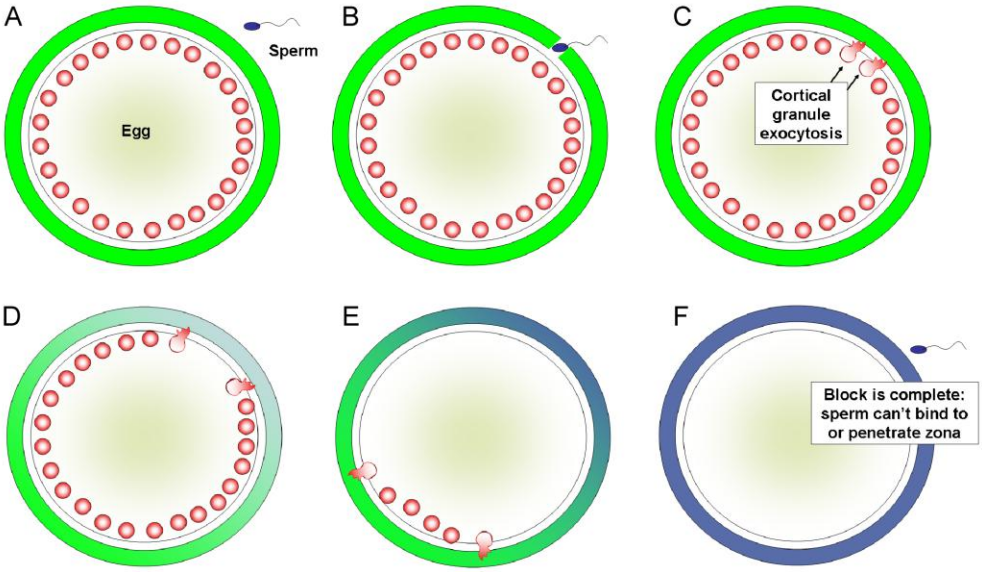
- Polyspermy is the penetration of the egg by more than one sperm
- Polyspermy leads to abnormal development because the chromosomal # is altered
- Normally the low number of sperm cells in the fallopian tubes in mammals reduces chances of polyspermy
- Special blocks to polyspermy exist:
  1. Fast Block: electrical change in egg membrane;
  2. Slow Block: modification of zona pellucida

The Cortical Reaction

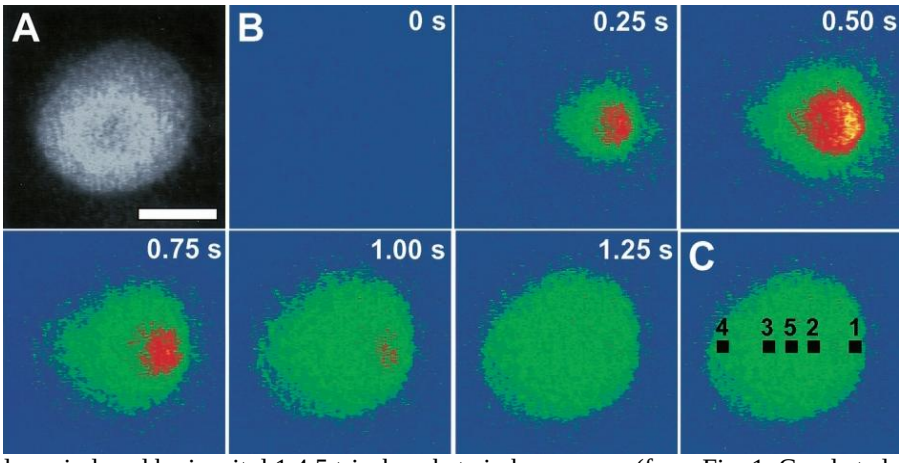
Calcium not only plays a role in the acrosome reaction, it also mediates the subsequent event of cortical granule exocytosis in the egg. Again much of the following is gleaned from studies on the mouse and other mammalian species.

This is the sequence of events:

- Sperm-egg binding releases calcium ions into cytoplasm
- Ca^{2+} induces local exocytosis of cortical granules
- Granules release to stimulate adjacent cortical granules to undergo exocytosis
- Wave of exocytosis occurs around egg in 3 dimensions from original site of sperm entry



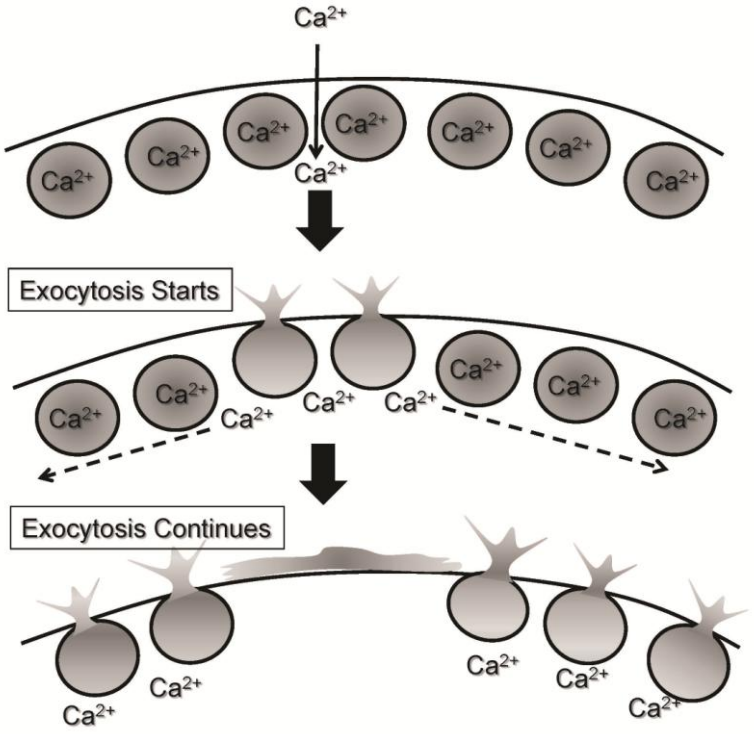
A. Sperm approaches egg. B. Sperm penetrates Zona (green). C. Contact with egg stimulates local exocytosis of cortical granules. D. Adjacent cortical granules in 3 dimensions exocytose their contents which begin to change the zona (now seen as blue). E. Continued exocytosis and Zona modification continues until all cortical granules are gone (D) and the block to polyspermy is complete.



Wave of calcium release induced by inositol 1,4,5 trisphosphate in human egg (from Fig. 1, Goud et al, 2002. Human Mol. Reprod. 8: 912-918)

If this is not clear to you, think of a meteorite hitting the earth. The shock waves and fallout emanating from the point of contact would travel in three dimensions over the surface of the globe. The acrosome reaction occurs in a similar pattern with the sperm initiating a wave of cortical granule exocytosis that spreads out

from the point of initiation until the ZP3 of the whole zona pellucida has been altered. Here's a diagram that summarizes the initiating events.



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