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RESEARCH NEWS

SEPTEMBER 05, 2005

One Shot: Researchers Capture Pictures of Fusion Events That Enable Sperm to Penetrate Egg's Coating

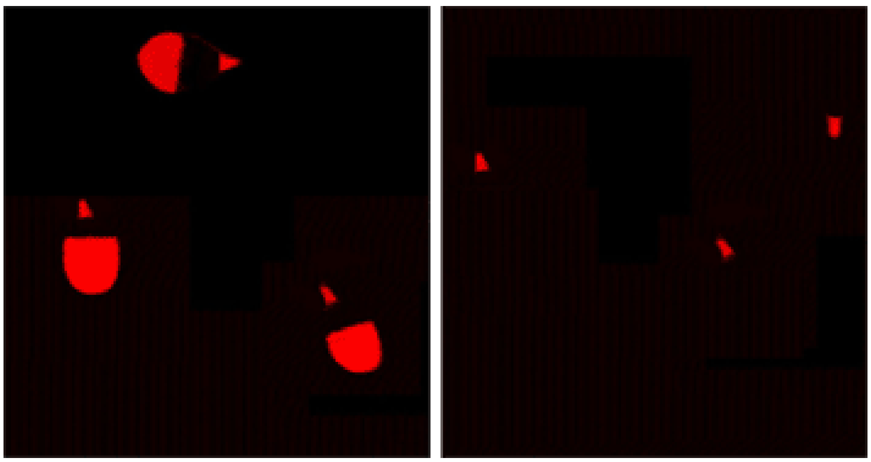

Image Title: Human sperm was stained red with an antibody that recognizes the SNARE protein, syntaxin. In the left panel, a botulin neurotoxin does not affect syntaxin in resting sperm because the protein is protected in cis-SNAREs complexes. At right, the neurotoxin is digesting syntaxin in sperm in which exocytosis has been stimulated by calcium. - Luis Mayorga and Gerardo De Blas

Researchers have capitalized on the unique properties of a sperm cell to follow cell membrane fusion as it occurs during fertilization, tracking the full cascade of events for the first time. The findings could reveal new ways to enhance or block fertilization, as well as how to control the secretion of neurotransmitters and hormones such as insulin.

Luis Mayorga, a Howard Hughes Medical Institute (HHMI) international research scholar, and colleagues at the National University of Cuyo School of Medicine in Mendoza, Argentina, took advantage of the cellular specialization that gives sperm one irreversible chance to fertilize an egg.

The group followed the sperm's secretion of the enzymes used to penetrate the protective outer coating that surrounds an egg. “Because the sperm has a single opportunity, this secretion has to be very well-regulated," said Mayorga. "If the sperm doesn't respond right on time, it won't get through the egg's coating." And since fertilization is one-way and all-or-nothing, so too is the fusion event that releases the sperm's enzymes. This tight control enabled Mayorga's laboratory to capture a molecular movie of fusion as it unfolded. Their findings are published in the September issue of the journal *Public Library of Science Biology*.

> "If the sperm doesn't respond right on time, it won't get through the egg's coating."
> **- Luis Segundo Mayorga**

Inside the sperm, the enzymes are contained in a small bag known as the acrosome. During fertilization, as the acrosome membrane meets the sperm's outer membrane, the two fuse together, and the enzymes are released outside the cell - much the same way a bubble rises to the surface of a soda and releases its gas into the air.

Mayorga, who has studied membrane fusion for more than 15 years, recognized an unexplored potential in this simple secretion event, called acrosomal exocytosis (AE). Preliminary experiments showed that AE uses the same basic fusion molecules as neuronal and endocrine cells. However, AE is much less complicated than fusion in these other cell types because it only happens once; other cells secrete the same substances again and again, requiring the fusion machinery to recycle multiple times.

The team evaluated the molecules that bring membranes together to fuse. These molecules, called SNAREs, work somewhat like Velcro to hold membranes in close enough proximity to merge. In sperm, the acrosome membrane containing the enzymes has one type of SNARE and the sperm's outer membrane has another.

In the beginning, the acrosome SNAREs are stuck to each other and the outer membrane SNAREs are stuck to each other in what are called *cis*-SNARE pairs. These must be broken apart so that acrosome SNAREs can pair with outer membrane SNAREs in *trans*-SNARE pairs. When a sperm encounters the egg surface, calcium is released into the sperm cell, which triggers molecules that break apart the *cis*-SNARE pairs.

The Argentine scientists found that immediately after this step, loose *trans*-SNARE pairs form between the acrosome membrane and the sperm outer membrane. Such loose SNARE formations have been hypothesized but never shown directly. Mayorga's group treated the *trans*-SNARE formations with toxins before the final stage of fusion and found that some, but not all, of the toxins inhibited the process. This indicated that the SNAREs were neither completely unpaired, which would make them most susceptible to toxins, nor were they clamped together in their final, tight configuration, which would make them resistant to toxins.

Then the researchers found that the final, tight *trans*-SNARE pairs form after more calcium is released from inside the acrosome. Once the acrosome membrane is locked to the outer membrane of the sperm by these tight pairs, a fusion factor causes the two membranes to fuse together, forming a pore that releases the enzymes outside the sperm where they can begin to digest the coating that surrounds the egg.

“In our experiments, it is very clear that those complexes have a loose form and are waiting for calcium to complete fusion,” said Mayorga. “We show step-by-step how membrane fusion is really occurring in acrosomal exocytosis.”

Many of the factors involved in AE will be important for manipulating fertilization—either to enhance it or block it, Mayorga said. Knowing the exact steps in the sperm's simple AE system could also help researchers working on the more complicated membrane fusion processes that are critical for proper cell divisions, infection of cells by bacteria and viruses, and secretion of hormones and neurotransmitters.

“The field is very actively searching for ways to regulate exocytosis—to regulate the insulin-producing beta cells of the pancreas to prevent diabetes or to get neurotransmitters released in the brain at the right time or concentration,” Mayorga pointed out. “Those are all examples of regulated exocytosis, and AE is a simple model they can use.”