DEVELOPMENTAL BIOLOGY

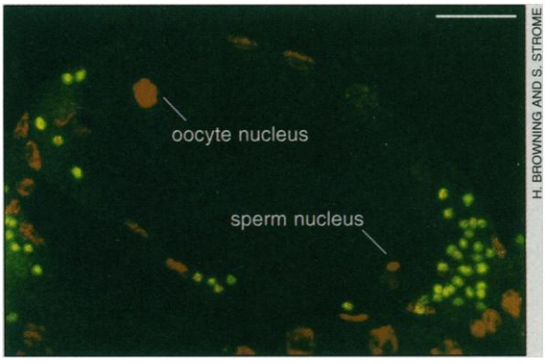
Sperm Protein Makes Its Mark Upon the Worm Embryo

In creatures from worms to people, it takes two sexes to reproduce, but it's often the female who gets stuck with the real work of childbearing. This division of labor is even mirrored in sperm and eggs. The unfertilized eggs of fruit flies, for example, already contain the molecular signals needed to direct one of the first events in embryonic growth, the creation of distinct body segments. The paternal contribution to early development, in contrast, seems paltry. Sperm carries nuclear material and organelles called centrosomes—organizing sites for cell division—that come into play later on, but no biochemical factors that guide early embryogenesis have been traced back to the father.

In the January issue of the journal *Development*, however, molecular biologist Heidi Browning of the University of Colorado and developmental geneticist Susan Strome of Indiana University report that SPE-11, a protein produced only in the sperm of the nematode *Caenorhabditis elegans*, may play a crucial role during the first few minutes after the embryo is fertilized. When fertilized by sperm lacking the protein, a *C. elegans* egg yields embryos that fail to complete fundamental developmental tasks and never grow into multicelled embryos—a fate that can be reversed by engineering the egg to express SPE-11 on its own. The scientists suggest that the protein may be part of the sperm-delivered "activation signal" that helps coordinate the crucial intermingling of genetic material from the sperm and the egg.

Developmental researchers call the results a major step forward. "This is the first example of an unambiguous paternal effect by a substance that's brought into the egg," says Sam Ward, a developmental biologist at the University of Arizona who collaborated with Strome in early work on the gene that produces SPE-11. Barbara Wakimoto, a developmental geneticist at the University of Washington, adds that the findings should reveal what sperm does beyond shuttling DNA: "Not very much is known about the regulation of events after the sperm gets into the egg, and that's what these paternal effects are going to be telling us." That tale may take a while to complete, however: Browning and Strome note that they haven't determined exactly how SPE-11 acts in the *C. elegans* oocyte.

The mystery of SPE-11 began almost accidentally, as part of a 1987 search in Ward's lab, then at the Carnegie Institute of Washington, for mutations in the *spe* genes. These genes direct *C. elegans* spermatogenesis—the creation of sperm cells by the species' two sexual types, males and self-fertilizing hermaphrodites. Most of the mutations found by Ward, Strome, and graduate students David Hill and Diane Shakes prevent sperm production or the entry of sperm into the egg, called an oocyte. But one—in the gene called *spe-11*—had a different effect. "The mutant sperm entered the egg, but the embryo didn't get very far in development. That was an unusual defect," says Strome. Oocytes fertilized by these sperm became short-lived single-cell embryos, incapable, among other things, of forming a complete protective eggshell or later cleaving into daughter cells through mitosis.



Daddy's home. In this *C. elegans* embryo, the protein SPE-11 *(green)* has dispersed from its location around the sperm nucleus just after sperm entry. The protein appears to play a crucial role in early embryo development. (Scale bar = 10 micrometers)

While researchers had found two other such "paternal-effect embryonic-lethal" mutations, both in fruit flies, Strome explains that these gene products seem to control the behavior of male chromosomes after fertilization and have not yet been found to interact directly with factors in the fly egg. The product of the *spe-11* gene, in contrast, seemed to affect the embryo as a whole, male and female contributions alike.

It still wasn't certain, however, whether the *spe-11* product created these effects by somehow acting as a sperm shepherd, equipping the sperm to fertilize oocytes properly, or by influencing the newly fertilized embryo more directly. To answer that question, Browning, then a doctoral student in Strome's lab, started cloning and sequencing the gene in 1990. In the current paper, she and Strome report that it encodes a novel protein, SPE-11. The protein is carried in the sperm's perinuclear region, a band of granular material surrounding the nucleus, and disappears into the oocyte cytoplasm shortly after sperm entry (see photo).

After identifying the protein, the researchers learned that it did appear to have a direct effect on the embryo. If the protein acted only on the sperm, then genetically engineering an oocyte to produce it would not reverse the effects of *spe-11*-less mutant sperm. But when oocytes expressing the *spe-11* gene were fertilized by the mutant sperm, they developed into healthy embryos. This experiment provided the crucial evidence, says Strome, that SPE-11 "need not be supplied by sperm. It can instead be supplied by the oocyte. Therefore it probably does function in the one-cell embryo."

The specifics of that function remain unknown. But because mutant *spe-11* embryos show defects in the earliest events after fertilization, "our best guess is that SPE-11 is involved in oocyte activation," a cascade of events that alters the metabolism of the egg and initiates embryonic development, says Browning. Researchers in Strome's laboratory are looking for substances that bind to SPE-11, hoping to uncover how the protein may fit into the biochemistry of activation.

If SPE-11 is an activation factor, says Wakimoto, it may provide biologists' first clues to how the embryo synchronizes the flurry of changes that take place after sperm entry. Among other things, the sperm nucleus must unpack its chromosomes, migrate to the center of the egg, and merge there with the egg's own nucleus—all in a matter of minutes. "Having a sperm product fire up those events is a neat way of making sure that the maternal and paternal contributions are coordinated—that the programs get started at the same time," Wakimoto says.

The discovery of cousins of the *spe-11* gene in two other *Caenorhabditis* species, meanwhile, is allowing Strome's lab to identify the most highly conserved segments of the gene, which they will then use in a search for homologs outside the nematode family. That could lead to "important new insights," says Frank Longo, a cell and developmental biologist at the University of Iowa. "Proteins are found in a perinuclear position in the sperm of other species, including mammals, but it's not known what they are involved with. Now we have an opening into this whole area."

–Wade Roush

Additional Reading
Michael Whitaker and Karl Swann, "Lighting the fuse at fertilization," *Development* **117** (1), pp. 1–12 (1993).