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Worm Sperm Points to Fertility Deficits

A study of sperm formation in the worm *Caenorhabditis elegans* may point toward a major cause of infertility in humans. The proteins that package sperm DNA into a compact molecular assemblage known as chromatin are similar in worms and humans, despite the many differences between the two organisms.

The identification of many of these proteins, reported in an advance online publication on August 30, 2006, in *Nature*, provides important targets for the study of infertility and contraception in humans, said Barbara Meyer, a Howard Hughes Medical Institute investigator at the University of California, Berkeley, who was the leader of the team that conducted the research.

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— **Barbara J. Meyer**

Nearly one in six human couples suffers from infertility, and defects in a male's sperm are partly or wholly responsible in about half of these cases. The sperm cells of infertile males often contain DNA that is fragmented or not as tightly packed as normal sperm. Meyer and her colleagues decided to investigate the formation of sperm in *C. elegans* to learn more about the molecular mechanisms that may cause such cases of infertility in humans. Sperm look very different between a worm and a human, she said, but the actual processes that allow the packing and tight structure of DNA in the sperm head are very similar.

The study began when Diana Chu, a postdoctoral fellow in Meyer's lab who is now an assistant professor at San Francisco State University, decided to take advantage of a collaboration that Meyer had established with John Yates at the Scripps Research Institute in La Jolla, California. Yates had developed a sophisticated technology that combines liquid chromatography, mass spectrometry, and bioinformatics to identify the hundreds of proteins involved in complex cellular mechanisms from small samples of biological

material. Building on earlier work done in Meyer's lab on cell division, Chu decided to use the technology to study spermatogenesis. We decided that looking at all the proteins associated with sperm formation wouldn't be so complicated, she said.

Chu began by isolating cells from *C. elegans* that were in the process of forming sperm. Yates's technology produced a list of 1,099 proteins found in association with the chromatin in those cells—far too many to begin analyzing the functions of each protein separately. Chu therefore used two experimental strategies to shorten the list. She focused on the proteins that were most well represented in the chromatin from sperm-forming cells. She applied Yates's technology to cells that were forming eggs and found 812 proteins associated with the chromatin in those cells. Since she wanted to focus on proteins that were unique to sperm formation, she subtracted the proteins found in egg-producing cells from those found in sperm. "The idea was to reduce the molecular complexity by removing shared proteins that are not sperm specific," Chu said.

Chu was left with 132 proteins enriched in cells that make sperm. She and her colleagues at Berkeley then began knocking down the production of the proteins one by one using a technique called RNA interference, in which short strands of the nucleic acid RNA are introduced into a cell to block the function of the gene containing the instructions for a particular protein. For 50 of the 132 proteins, blocking the function of the corresponding gene resulted in sterile worms that either failed to make embryos or made defective embryos that died.

Chu and her colleagues then compared the proteins they had identified in *C. elegans* to proteins known to occur in humans and mice. Many were quite similar and appeared to be involved in such processes as cell division and the compaction of DNA in chromatin. Some of these proteins had been knocked out previously by others in mice by removing the gene responsible for the protein, and the resulting mice strains were often infertile. In her lab at San Francisco State University, Chu is now looking at the biological nitty gritty of how these proteins work at the molecular level.

Meyer said it's remarkable that so much can be learned about sperm formation in mammals from the study of a millimeter-long worm. Policymakers might think that it's important just to look at humans and mammals, she noted. But the truth is that these lower organisms are invaluable because so many of the basic biological pathways are conserved. You can do studies in these simpler organisms like knocking out large numbers of genes quickly and inexpensively that you can't do with more complex organisms. It takes looking at a humble organism like the worm to discover large sets of proteins and to make sense of their functions.

The next step, said Meyer, is what Chu is doing—exploring through scientific collaborations the function of these proteins in *C. elegans* and ultimately in mammals to find the genes responsible for cases of infertility in humans. The people who work with mice and humans need to take the list we've generated

and start looking for candidate proteins responsible for infertility, said Meyer. Many of the genes that we have identified map to the parts of chromosomes that human genetics researchers have identified as important for fertility. This paper sets the stage for the technologies and intellectual approaches that are going to be very important for doing this research in mammals, including humans.

New approaches to male contraception may be another important application of their research, said Meyer. What is severely lacking in the study of contraception is the development of methods that could work in males, she said. So to design drugs that would interfere with spermatogenesis without causing deleterious side effects in males, we have to understand enough about the proteins and genes that are specific to sperm. These are the kinds of studies that will identify those targets.