THE SECRET LIFE OF SPERM

Far from being mere DNA delivery boys, it's now becoming clear that sperm also ship a complex cargo of RNA and proteins that may be crucial for an embryo's early development. **Claire Ainsworth** reports.

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"What's in sperm?" demands Tim Karr. It's an unusual start to a lab visit, and I confess that I know little more than the sketchy textbook picture. I'm not alone, says Karr, and that is the whole problem. "How can we not know?" he asks. "It probably explains why we don't understand sex."

Karr, who works at the University of Bath, UK, is convinced that a more detailed knowledge of the molecular biology of sperm will help answer some of biology's most fundamental questions. This is what prompted him to spend the past three years dissecting fruitfly testes and developing methods to study the protein content—or proteome—of their sperm.

Sperm are amenable to detailed proteomic analysis because they contain no more than a few hundred proteins. But this apparent simplicity is deceptive. Karr's team is one of several to have found that there is far more to sperm than we thought. In addition to the DNA instructions that spell out a male's contribution to a new life, these sleek, whip-powered cells have in the past few years been shown to carry other pieces of cellular machinery, such as RNA and proteins. This discovery is changing our understanding of fertility, development and the evolution of sex. "It really challenges some basic ideas," says Stephen Krawetz, a biologist at Wayne State University School of Medicine in Detroit, Michigan.

Because sperm have to swim far and fast, biologists have come to view them like racing cars: streamlined and stripped down of all unnecessary bits and pieces. Generally speaking, the DNA in animal sperm is tightly packed inside a sleek head structure that contains little of the cytoplasm that fills most other cells. Behind the head is the midpiece, containing more than 50 power units called mitochondria that drive the lashing motion of the attached tail.

Egged on

Given the shortage of cytoplasm, and the lack of any detectable protein synthesis in mature sperm heads, biologists had long assumed that sperm contribute little to an embryo bar the father's genes. In contrast, the egg is replete with molecules such as proteins and RNAs that nourish and direct the development of the embryo. "The idea was that the egg was supplying everything and Dad was just tagging along with his DNA," says Krawetz.

Recent discoveries have revealed the error of this view. Studies now suggest that defects in sperm can disrupt embryo development even if the genes carried by the cells are perfectly normal¹. And there are hints that faulty sperm could be the cause of a significant number of miscarriages, says David Miller, a reproductive biologist at the University of Leeds, UK. "So we know sperm is important."

But what does a sperm deliver? One popular misconception is that only the head enters the egg, while the tail is discarded. But in most

species, the entire cell enters the egg — midpiece, tail and all². And in many mammals, midpiece and tail structures persist in the embryo for several cell divisions³. This results in a large number of proteins and other molecules being delivered to the egg. In mammals other than rodents, these include a piece of cellular machinery called the centrosome, which coordinates the molecular ropes cells use to haul chromosomes around during cell division⁴.

Until 2002, this was thought to be an isolated example. But then a team headed by Anthony Lai at the University of Wales in Cardiff discovered that sperm also deliver a molecule called PLC ζ that triggers the waves of calcium ions that activate a fertilized egg⁵. And a bigger surprise came when Krawetz and Miller studied sperm from 10 fertile men and found that they contained some 3,000 different kinds of messenger RNA⁶. Some of them coded for proteins needed for early embryo development; others were previously unknown, and had no equivalents in the egg.

Male delivery

This suggested that sperm could deliver RNAs that help direct an embryo's early development. Some biologists were sceptical, arguing that the RNAs were simply non-functional leftovers from the process of sperm development. But Krawetz, Miller and others have since gathered more evidence, and last year showed that a specific package of RNAs are indeed transferred from sperm to egg⁷. Earlier this year, Krawetz and his colleagues found that these include micro-RNAs, which don't code for proteins but are known to play a role in controlling gene activity⁸.

It remains unclear what the transferred RNA does. The fact that cloning works, and the creation last year of a mouse by combining the nuclei of two eggs⁹, both suggest it is not absolutely essential for embryo development. But these processes are grossly inefficient and often result in birth defects or abnormal gene activity, which hints that the paternal RNA may be important.

Miller suggests that messenger RNAs help protect paternal genes that are needed soon after fertilization from being shut down as sperm mature. Normally, most of a sperm's DNA is tightly wrapped up and gagged by proteins called protamines. The RNAs could stick to the genes that code for them and stop this, he argues. Another possibility is that paternal RNAs, particularly micro-RNAs, might be involved in controlling imprinting — the differential activation of genes according to whether they are inherited from the mother or the father. Certainly, the idea that the RNAs have no function is becoming a minority view. "Sperm are so sleek and have such powerful methods for eliminating everything that causes drag that I don't believe they are vestigial," says Gerald Schatten, a reproductive biologist at the University of Pittsburgh, Pennsylvania.

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As well as DNA, sperm deliver at least one molecule that helps to activate a fertilized egg.

The new view of sperm as carriers of molecules crucial for early embryo development has thought-provoking implications for reproductive medicine. Comparing the RNA profiles of fertile and infertile men might reveal causes of unexplained infertility, says Miller.

Such studies may also raise questions about the wisdom of an *in vitro* fertilization technique called intracytoplasmic sperm injection, or ICSI, used to help men whose sperm do not fertilize their partner's eggs. ICSI involves injecting faulty or immature sperm — which might lack the normal complement of RNAs — directly into eggs. So far, there are no clear signs of problems among children conceived by ICSI, although long-term follow-up is needed to confirm the safety of the technique.

Now the action in sperm biology is moving from RNA to proteins. Last year, Christopher Barratt, a reproductive biologist at the University of Birmingham, UK, published the first proteomic study of male infertility. His aim was not to produce a complete proteome for sperm; instead, his team looked for differences in the protein profiles in the sperm of an infertile and a fertile man. The researchers found at least 20 proteins present in significantly different quantities¹⁰, giving them a starting point to study cases of unexplained infertility and suggesting targets for new contraceptives.

Origins of life

The coming months could see the first publications from groups, including Karr's, that are conducting more comprehensive proteomic studies. Being able to compare the structure and content of the proteomes of sperm from different species should help researchers understand the evolution and origin of sperm. In particular, having a comprehensive cata-

logue of proteins to compare between different species may reveal how natural selection is operating on them, says Steve Dorus, a postdoc in Karr's lab. "It should give us some pretty powerful information about what our ancestors' core sperm attributes were."

While Karr's group works on fruitfly sperm, Victor Vacquier, a reproductive biologist at the Scripps Institution of Oceanography in La Jolla, California, is producing a catalogue of the proteins found in the outer membrane of sperm of the purple sea urchin (*Strongylocentrotus purpuratus*). His particular interest lies in understanding how sperm and egg interact and recognize each other. Surprisingly, scientists know comparatively little about the molecules that interact when a sperm comes into contact with an egg's surface. But they do know that, in some species, such proteins can evolve extraordinarily rapidly¹¹. Vacquier believes that these fast-evolving proteins may help explain why some animal populations become reproductively isolated, leading to the formation of new species.

The origin of species, the evolution of sex, the mysteries of infertility — these are some of the biggest and most intriguing questions in biology. Long dismissed as mere delivery boys, it seems that sperm are about to be put on the promotion fast track.

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