Orchestrating the Sperm-Egg Summit

Even before sperm meets egg, a host of chemical factors and cellular events act in concert to produce the two cells that will ultimately combine during fertilization. When sperm and egg finally meet, specific receptor molecules control their interaction. And after fertilization, a chemical signal from the early embryo to the mother allows her body to recognize the pregnancy. Researchers described these and other events in reproduction during a special symposium on reproductive biology at the recent AAAS meeting in Boston.

Interferon-Like Factor Signals Pregnancy

In humans, the mother's body recognizes a pregnancy at about 1 week after fertilization, when the very early embryo implants itself into the uterine wall and secretes human chorionic gonadotrophin. In sheep, cows, and pigs, the chemical signal that a pregnancy exists is a protein made by the trophoblast cells that surround the early embryo and later form the placenta. Michael Roberts of the University of Missouri in Columbia reports that oTP-1 (ovine trophoblast protein–1) in sheep appears to be a member of the interferon family of proteins.

"It is important in pregnancy that the conceptus be recognized, otherwise the embryo would be lost," says Roberts. "In sheep, cows, and pigs, the most likely protein for triggering maternal recognition is an interferon-like molecule." Interferons, which are produced by virus-infected T lymphocytes, are known primarily for their antiviral activity. The discovery that such a protein helps to sustain pregnancy before the embryo implants into the uterine wall is a new and totally unexpected finding.

In the pregnant ewe, oTP-1 has two kinds of biological effects. First, it seems to change the pattern of prostaglandin synthesis in endometrial cells that line the uterus. As a result, the ovary continues to produce progesterone, the steroid hormone that is necessary to maintain the lining of the uterus during pregnancy. Second, oTP-1 may protect the early embryo from immunological destruction by the mother, a role that is still not well understood.

Roberts and Kazuhiko Imakawa, also of the University of Missouri, have cloned and sequenced the gene for oTP-1. The protein has a molecular weight of more than 19,000 daltons and is initially synthesized as a larger precursor. "This protein shows considerable sequence homology between interferons of the α family, specifically the α_{II} family," says Roberts.

Approximately one-half of the amino acids in oTP-1 are the same as the α-interferon from humans, rats, cows, mice, and pigs. In addition, the regions of the protein that are held in a three-dimensional conformation by disulfide bridges are completely conserved. Like α-interferon, oTP-1 inhibits the replication of several kinds of DNA viruses in vitro and also inhibits proliferation of cultured cells.

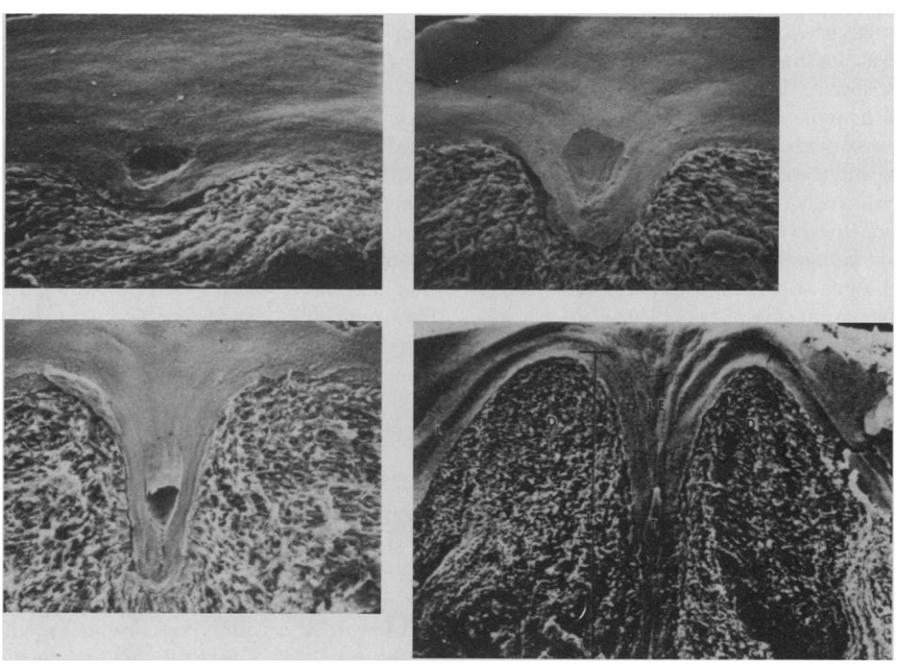
Roberts and Imakawa have studied the sheep trophoblast protein extensively and find that it is synthesized from day 12 to day 22 of pregnancy. According to Roberts the 12- to 16-day embryo produces most of the oTP-1, and it is the major product of the sheep trophoblast cells during this very early developmental stage. "Trophoblast cells seem to be preprogrammed to make oTP-1," says Imakawa. "Its messenger RNA is long-lived compared to the mRNA for other interferons."

Later in pregnancy, after the synthesis of oTP-1 has been shut off and the sheep embryo has implanted into the uterine wall, the fully formed placenta produces what appears to be true α-interferon.

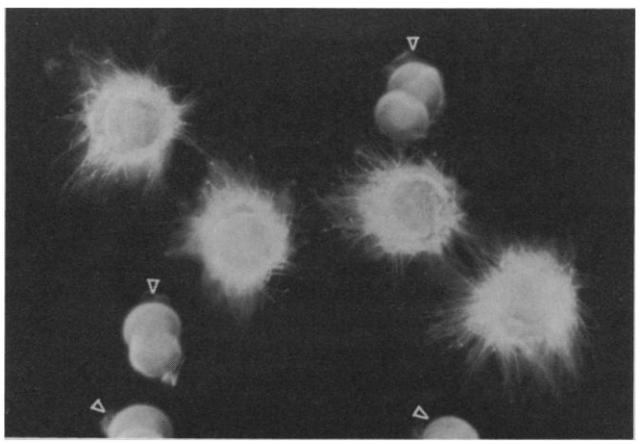
Sperm Receptor Gene Sequenced

During reproduction in mammals, one sperm fertilizes an egg. But before that occurs, many sperm bind to specific receptor molecules in the zona pellucida, the thick coat that surrounds the unfertilized egg. Paul Wassarman of the Roche Institute of Molecular Biology in Nutley, New Jersey, and his colleagues have now cloned and sequenced the gene for ZP3 (zona pellucida 3), the sperm receptor. The researchers also report that sugar residues in ZP3 are the actual binding sites for sperm.

"I think everyone now agrees that ZP3 is the sperm receptor in the mouse, and they are identifying similar molecules in other mammals," says Wassarman. To date, no one has published results on a human sperm



Embryo implantation. *After fertilization, the rat embryo implants rapidly into the uterine wall and forms an implantation chamber (upper left to lower right). The embryo of a sheep implants much more slowly than a rat embryo, and secretes a factor that allows the ewe's body to recognize the pregnancy.* [*Photo courtesy of A. Enders*]



Sperm-egg binding

Many mouse sperm bind to the zona pellucida of a single unfertilized egg, with the sperm tails giving the egg a fuzzy appearance. Sperm do not bind to fertilized eggs that have divided to produce two cells (arrowheads). [Photo courtesy of P. Wassarman]

receptor. But in the mouse, the interaction between sperm and ZP3 molecules is extensive. "We think that each sperm binds to tens of thousands of receptors and interacts with each ZP3 molecule at multiple sites," says Wassarman.

Wassarman, Ross Kinloch, and Richard Roller, also of Roche, find that the ZP3 gene is expressed only in oocytes during a very brief period of time. "ZP3 synthesis begins when the oocyte starts to increase in volume, which occurs at the time of sexual maturity in the mouse." says Wassarman. "By the time the oocyte becomes an egg, ZP3 production falls to very low levels." The protein is synthesized as a large precursor molecule that is processed in stages before sugar residues are added.

In mice oocytes, ZP3 has three major functions (*Science*, 30 January 1987, p. 553). Its first role is to act as a binding site for sperm. "The ZP3 receptor allows sperm of the same species to bind to the egg," says Wassarman. "It confers species specificity to the interaction." Sperm from other species are actually capable of fertilizing the egg, an abnormal event that occurs if the zona pellucida containing ZP3 is removed. But with the zona and ZP3 intact, this cross-species interaction cannot take place.

Last year, Wassarman and Harvey Florman, now of the University of Wisconsin in Madison, showed that the precise binding site for sperm on ZP3 is a carbohydrate region. "The important part of this molecule is an O-linked oligosaccharide, α-galactose," says Wassarman, meaning that α-galactose sugar residues are attached to the protein part of ZP3 at two kinds of amino acid residues, serine or threonine. More recently, Wassarman and Jeffrey Bliel, also of Roche, discovered that removal of the α-galactose residues from ZP3 prevents sperm binding in vitro, evidence that strongly supports their contention that the carbohydrate part of ZP3, rather than the protein, is the active binding site.

The second function of ZP3 is to stimulate membrane fusion in the acrosome, a structure located within the head of a sperm that contains enzymes that break down the zona pellucida. "After recognition and binding, sperm have to undergo the acrosome reaction, which makes the plasma membrane over the head of the sperm able to fuse with the outer acrosomal membrane," says Wassarman. "That enables the sperm to penetrate through the zona pellucida and fuse with the plasma membrane of the egg." Sperm-egg fusion then results in fertilization and the formation of a zygote, which carries the full number of chromosomes for that particular species.

The third role of ZP3 is structural. "ZP3 interacts with ZP2 [another glycoprotein] and forms the backbone of the filaments in the zona pellucida," says Wassarman. The two glycoproteins alternate to make the long filaments that are interconnected by a third glycoprotein, ZP1.

After fertilization, the ZP3 molecule becomes inactive. "ZP3 is still there, but it is modified so that it is no longer recognized by sperm," says Wassarman. He proposes that enzymes from the oocyte cortical granules, which lie just under the egg plasma membrane and are released at fertilization, somehow alter the carbohydrate residues on ZP3 so that no more sperm can bind.

New Functions for a Testes Growth Factor

A growth factor found only in the testes stimulates cell proliferation and the synthesis of proteins necessary for continual sperm production. "We now have a growth factor that is unique to the testes," says Anthony Bellvé of the Columbia University College of Physicians and Surgeons in New York. "Spermiogenesis growth factor (SGF) has effects that differ from those of any other hormones known to regulate cell division and differentiation in the testes."

The new data about SGF have opened up a whole new field of investigation about what controls gonadal growth and function, says Bellvé. "During spermatogenesis, you start off with one stem cell progenitor that ultimately produces about 500 to 600 mature sperm," he says. A number of pituitary hormones—follicle-stimulating hormone, luteinizing hormone, growth hormone, and prolactin—influence sperm maturation. They enter the blood and stimulate the production of various proteins at different stages of development. In contrast, the release and action of SGF appears to be confined to the testes.

SGF is a protein factor made by Sertoli cells, important structural elements in the testes that also provide pyruvate and lactate as an energy source to developing sperm. Researchers have known since 1980 that SGF stimulates cell proliferation in the testes of a developing mammal, and Bellvé's new results point to its role in the adult.

During postnatal development in the mouse, SGF turns on the division of three cell types in the testes—Sertoli cells, muscle-like peritubular cells, and Leydig cells, which lie in the spaces outside the seminiferous tubules and make testosterone. Also during development, the growth factor increases blood vessel production in the testes.

After the mouse is 10 days old, Sertoli cells stop dividing, but binding sites for SGF persist. This led Bellvé, Wenxin Zheng, and Theresa Butwell, also of Columbia, to determine what role the growth factor has in the adult animal. "In adult mice, SGF induces the production of six to eight as yet unidentified proteins," says Bellvé. "In addition, the growth factor dramatically increases the synthesis and secretion of a known protein, sulfated glycoprotein-2 (SGP2) in cultures of transformed Sertoli cells."

Although the precise function of SGP2 is still not clear, the protein is made and secreted by Sertoli cells and it becomes part of the sperm cell membrane, according to Michael Griswold of Washington State University in Pullman. He and Michael Collard, also of Washington State, have cloned and sequenced SGP2 and a related glycoprotein, SGP1. "The two molecules either deliver certain lipids to sperm cells or they alter lipids that are already in the plasma membrane of sperm cells," said Griswold in an interview with *Science*.

More information is needed to determine how SGF and the proteins it regulates, including SGP2, influence the continual production of sperm. ■

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