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clusively by men. The conclusions, whether with respect to mutations or civilization, are likely to be inaccurate.

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REVIEW

Penetration, Adhesion, and Fusion in Mammalian Sperm-Egg Interaction

Paul Primakoff[1] and Diana G. Myles[2]

Fertilization is the sum of the cellular mechanisms that pass the genome from one generation to the next and initiate development of a new organism. A typical, ovulated mammalian egg is enclosed by two layers: an outer layer of ~5000 cumulus cells and an inner, thick extracellular matrix, the zona pellucida. To reach the egg plasma membrane, sperm must penetrate both layers in steps requiring sperm motility, sperm surface enzymes, and probably sperm-secreted enzymes. Sperm also bind transiently to the egg zona pellucida and the egg plasma membrane and then fuse. Signaling in the sperm is induced by sperm adhesion to the zona pellucida, and signaling in the egg by gamete fusion. The gamete molecules and molecular interactions with essential roles in these events are gradually being discovered.

In mammals, fertilization is completed by the direct interaction of sperm and egg, a process mediated primarily by gamete surface proteins. Therefore, an essential task in the study of sperm-egg interaction is an exploration of the capabilities of a distinct set of surface proteins, some gamete specific and others more widely expressed. On gametes, these proteins act in a sequential pattern to orchestrate the close approach and ultimate fusion of the two cells.

Sperm penetration of the cumulus. To penetrate the substantial cumulus cell barrier surrounding ovulated eggs of most mammalian species, sperm use hyperactivated motility (*1*) and a glycosylphosphatidylinositol (GPI)-anchored surface hyaluronidase, named PH-20 (Fig. 1A) (*2*). The motility and surface hyaluronidase are necessary, and perhaps sufficient, to digest a path through the extracellular matrix of the cumulus cells; no proteases have yet been implicated in this process.

Sperm interaction with the zona pellucida. The egg's zona pellucida is a cell type–specific extracellular matrix or coat composed of three glycoproteins termed ZP1, ZP2, and ZP3. Sperm that reach and bind to the zona pellucida receive a signal to acrosome react, i.e., release by exocytosis the contents of their large secretory granule, the acrosome (Fig. 1B).

The currently favored model is that sperm bind to O-linked carbohydrate on ZP3. Sperm preincubation with ZP3 strongly inhibits sperm binding to the zona, whereas preincubation with ZP1 or ZP2 has no effect (*3*). Other studies show that sperm binding can be blocked by O-linked oligosaccharides of ZP3, present on Ser^{332} and Ser^{334} near the ZP3 COOH-terminus (*4*, *5*). Thus, sperm adhesion to the zona is a carbohydrate-mediated event. A requirement for

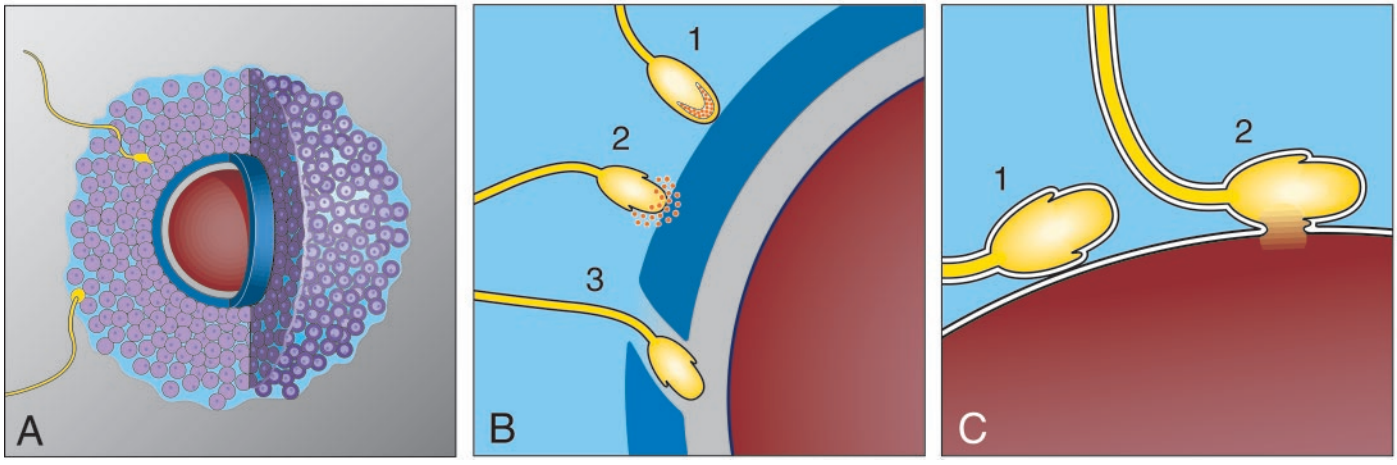

Fig. 1. (**A**) Sperm penetration of cumulus cells (purple) to reach zona (navy blue). (**B**) Egg depicted with cumulus cells removed; sperm 1 binds to the zona pellucida (navy blue); sperm 2 undergoes exocytosis, releasing acrosomal contents (orange-red); sperm 3 penetrates the zona pellucida and begins entry into perivitelline space (gray). (**C**) Sperm 1 binds to the egg plasma membrane by the side of its head, in a central region (equatorial region); sperm 2 fuses with the egg plasma membrane.

ZP3 in sperm-zona binding has not been confirmed by gene-knockout studies because ZP3-null eggs do not make a zona pellucida (Table 1). An approach to overcome this difficulty is to "rescue" zona formation with a human ZP3 transgene. ZP3-null female mice carrying a human ZP3 transgene make a zona of normal appearance. The females are fertile, and mouse sperm, but not human sperm, bind to the hybrid zonae. Possible interpretations of this experiment are that either ZP3 is not the protein to which sperm bind or ZP3 is the sperm-binding protein, but human ZP3 receives "mouselike" glycosylation in the mouse ovary (*6*). Deeper understanding of the function of ZP3 O-linked carbohydrate in sperm binding has been hampered by the absence of structural information about this carbohydrate (*7*).

A major effort has been made to define the sperm surface protein(s) that binds to ZP3 and enables acrosome-intact sperm to bind to the zona. Many (~15) candidates have been proposed, but none has found wide acceptance (*8*, *9*). The methods used so far to establish that a candidate has a required function in sperm adhesion to the zona have not been definitive. An attempt to confirm sperm-zona adhesion activity by gene knockout has been reported for only one sperm protein that putatively binds ZP3, a sperm surface enzyme, galactosyl transferase (GalT). Compared with wild-type sperm, GalT-null sperm show substantially reduced binding of soluble ZP3 and no ZP3-induced acrosome reaction. These results suggest that GalT is an essential ZP3 binding protein, functioning in ZP3-induced signaling. GalT-null male mice are fertile, which may reflect the ability of knockout sperm to acrosome react spontaneously in vivo in a situation where their normal, triggered path to acrosome react is blocked. GalT is not required for sperm adhesion to the zona, because GalT-null sperm bind to the zona at higher levels than do wild-type sperm (*10*).

Use of direct biochemical approaches to purify sperm proteins with high affinity for the zona (or ZP3) have identified p47 (*11*), sp56, and zonadhesin. Additional sequence and localization studies indicate that sp56 is present in the acrosomal contents (*12*) and zonadhesin is also present in the acrosomal contents and/or acrosomal membrane (*13*). Thus, neither sp56 nor zonadhesin has an appropriate cell surface localization to participate in acrosome-intact sperm binding to the zona. After the acrosome reaction, these soluble zona binding proteins might have a sperm-to-zona adhesive function before the acrosomal matrix disperses and/or an antiadhesive function, promoting sperm penetration of the zona, after the matrix is solubilized.

The predicament of not knowing the sperm surface protein(s) that enables acrosome-intact sperm to bind to the zona should change as new ideas and approaches become available. For example, mice have been obtained with gene knockouts for the sperm surface proteins fertilin β (*14*) or cyritestin (*15*, *16*) or the spermatogenesis-specific chaperone calmegin (*17*). The knockout males are infertile and produce sperm that cannot bind to the zona (Table 1). [Male mice with a knockout for angiotensin-converting enzyme show a related phenotype, but the defect in sperm-zona binding is quite mild (*18*).] The calmegin-null sperm have been shown to lack fertilin β (*19*). Fertilin β and cyritestin are members of the ADAM family (A Disintegrin And Metalloprotease) and were initially studied to define their putative role in gamete fusion (see below). Both fertilin β and cyritestin knockout sperm, through an unknown mechanism, lose not only the deleted gene product but other membrane proteins as well (*16*). Because these knockout sperm cannot bind to the zona, analysis of their phenotype should offer another resource to understand the adhesion process.

Sperm acrosome reaction and penetration of the zona pellucida. ZP3-induced exocytosis of the acrosomal contents proceeds through two sperm signaling pathways. In the first, ZP3 binding to GalT and other potential receptors results in activation of a heterotrimeric GTP-binding protein and phospholipase C (PLC), thus elevating the concentration of cytoplasmic calcium. In the second pathway, ZP3 binding to the same receptor(s) stimulates a transient influx of calcium through T-type channels. In a later phase of the signaling, these initial ZP3-induced events produce additional calcium entry through Trp family calcium channels, resulting in a sustained increase in cytoplasmic calcium concentration that triggers exocytosis (*20*, *21*).

During or after the acrosome reaction, the fertilizing sperm detaches from the zona pellucida. It penetrates through the thick zona, cutting a penetration slit that is just as wide as the sperm head (Fig. 1B). Motility, proteases (*1*), and glycosidases (*22*) are apparently involved in this penetration. The proteases could be sperm surface, membrane-anchored proteases (*23*) or soluble proteases from the acrosomal contents (*24*). Investigation of this problem could be advanced by a proteomics approach to the acrosomal contents that would reveal which proteins are present and perhaps suggest functions for them.

Sperm-egg plasma membrane binding and fusion. Sperm, having penetrated the zona, bind to and fuse with the egg plasma membrane (Fig. 1C). In the search for sperm surface proteins that function in this process, most attention has recently been given to the sperm members of the ADAM family, specifically fertilin and cyritestin. A major part of the ADAMs' appeal is that they have an adhesion module, the disintegrin domain, leading directly to the idea that eggs will have an appropriate plasma membrane adhesion partner, i.e., an integrin (*25*).

Peptides representing the active site of the disintegrin domain from either fertilin β (ADAM 2) or cyritestin (ADAM 3) inhibit sperm plasma membrane binding and fusion (*26*). Furthermore, the fertilin β peptide binds to the integrin α6β1 on the egg surface, and GoH3, a monoclonal antibody to α6, blocks sperm adhesion and fusion with zona-free eggs (*27*, *28*). Sperm cyritestin (ADAM 3) may also bind to egg α6β1. These findings are the foundation of a model in which fertilin β and/or cyritestin on sperm and α6β1 on eggs are adhesion partners that bind the gametes together in a way that leads to fusion (*29*–*31*).

This model is contradicted by gene-knockout data on these proteins. Fertilin β–null sperm fuse at ~50%, and cyritestin-null sperm at 100%, of the wild-type rate. Sperm from the double knockout (lacking fertilin β and cyritestin) also fuse at ~50% of the wild-type rate

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Table 1. Gamete surface proteins and zona pellucida proteins with reported gene knockout.

Gamete protein	KO phenotype: Major features
Sperm protein	
Galactosyl transferase	Fertile males; ZP3-induced acrosome reaction is defective; increase in sperm binding to zona (*10*)
Fertilin β	Infertile males; small effect on sperm-egg fusion; defective in binding to zona and migrating into oviduct (*14*)
Cyritestin	Infertile males; no effect on sperm-egg fusion; defective in binding to zona (*15*, *16*)
Angiotensin-converting enzyme	Infertile males; mild defect in sperm binding to zona; defective in migrating into oviduct (*18*)
Catsper	Infertile males; defective calcium channel, defective motility (*42*)
Egg protein	
ZP1, ZP2	Infertile females; structurally defective zona (*43*, *44*)
ZP3	Infertile females; no zona made (*45*, *46*)
α6 integrin	Neonatal lethal; no effect on sperm adhesion/fusion with egg plasma membrane (*32*)
CD9	Infertile females; eggs defective in plasma membrane fusion with sperm (*34*–*36*)

(*16*). These findings show that fertilin β and cyritestin are not individually or together required for gamete membrane fusion (*14*–*16*). In addition, eggs carrying a deletion of the gene for the α6 integrin subunit can bind to and fuse normally with sperm (*32*). Thus, none of the specific proteins acting in the current ADAM-integrin model for adhesion/fusion are required for sperm-egg fusion, and other molecules must exist on the surface of gametes that can act in sperm-egg fusion. These could be other members of the ADAM and integrin families or entirely different proteins.

Research on other egg surface proteins has pointed in two new directions. Egg surface proteins with a GPI anchor have been implicated because PI-PLC treatment releases these proteins from the surface and blocks gamete fusion. Two egg GPI-anchored proteins have been detected, with relative molecular masses of ~70 and ~35 to 45 kD, but have not yet been identified (*33*). More compelling evidence establishes an essential role for egg surface CD9. Female mice carrying a gene knockout for CD9 are infertile; they produce eggs that mature normally, but are defective in sperm-egg fusion (*34*–*36*). CD9, a member of the tetraspanin family, spans the plasma membrane four times, having two extracellular loops (one small, one large) and short cytoplasmic NH_2-terminal and COOH-terminal tails. One defined role of tetraspanins is to organize functional, multimolecular complexes on the surface of the cell expressing the tetraspanin. In other cases, tetraspanins may (also) bind a soluble ligand or a ligand on an adhering cell (*37*, *38*). Recent evidence suggests that CD9 on eggs may act in cis by interacting with other egg surface molecules (*39*). In addition, CD9-knockout oocytes injected with wild-type CD9 mRNA show a high level of rescue of their fusion ability. However, if the injected CD9 mRNA carries a subtle mutation in the CD9 large extracellular loop (residues 173 to 175, Ser-Phe-Gln→Ala-Ala-Ala), no fusion ability is restored to injected CD9 knockout oocytes. These data suggest that Ser-Phe-Gln is an active site in CD9 that associates with and regulates the egg fusion machinery (*39*).

Sperm-egg fusion stimulates the first signaling pathway(s) in development. The initial events in this pathway, preceding an essential rise in intracellular Ca^{2+} concentration, remain unknown (*40*).

Conclusions. Mammalian fertilization has been inherently difficult to study because of the temperamental nature of in vitro fertilization assays and the small amount of eggs obtainable. Nonetheless, current and emerging strategies—e.g., gene knockout (Table 1), signal peptide traps (*41*), and structural analysis of sperm protein–egg protein complexes—will provide deeper understanding of this fundamental biological process. This increased understanding is needed to generate clinical advances for treatment of infertility and novel contraceptive strategies.

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REVIEW

Deciphering the Cross-Talk of Implantation: Advances and Challenges

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Implantation involves a series of steps leading to an effective reciprocal signaling between the blastocyst and the uterus. Except for a restricted period when ovarian hormones induce a uterine receptive phase, the uterus is an unfavorable environment for blastocyst implantation. Because species-specific variations in implantation strategies exist, these differences preclude the formulation of a unifying theme for the molecular basis of this event. However, an increased understanding of mammalian implantation has been gained through the use of the mouse model. This review summarizes recognized signaling cascades and new research in mammalian implantation, based primarily on available genetic and molecular evidence from implantation studies in the mouse. Although the identification of new molecules associated with implantation in various species provides valuable insight, important questions remain regarding the common molecular mechanisms that govern this process. Understanding the mechanisms of implantation promises to help alleviate infertility, enhance fetal health, and improve contraceptive design.

The success of any species depends on its reproductive efficiency. For sexual reproduction, an egg and sperm must overcome many obstacles to fuse and co-mingle their genetic material at fertilization. The zygote develops into a blastocyst with two cell lineages (the inner cell mass and the trophectoderm), migrates within the reproductive tract, and ultimately implants into a transiently permissive host tissue, the uterus. However, the molecular basis of the road map connecting the blastocyst with the endometrium across species is diverse (*1*) and not fully understood. Recent advances have identified numerous molecules involved in implantation (*1*–*4*), yet new discoveries have not yielded a unifying scheme for the mechanisms of implantation.

Uterine Preparation and Blastocyst Competency for Implantation

Uterine receptivity is defined as a restricted period when a uterus supports blastocyst attachment (*5*). Although progesterone and estrogen play major roles in a species-specific