Mammalian Fertilization: Molecular Aspects of Gamete Adhesion, Exocytosis, and Fusion

Review

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General Introduction to Mammalian Fertilization

Fertilization is defined as the process of union of two germ cells, egg and sperm, whereby the somatic chromosome number is restored and the development of a new individual exhibiting characteristics of the species is initiated. If fertilization fails to take place, both egg and sperm degenerate relatively rapidly in the female reproductive tract, since the two highly differentiated cells cannot survive long on their own.

Among mammals, the process of union of germ cells includes several ordered steps (Gwatkin, 1977; Wassarman, 1987; Yanagimachi, 1994; Snell and White, 1996). It begins in the oviduct with binding of free-swimming sperm to the ovulated egg extracellular coat, the zona pellucida (ZP) (Figures 1 and Figure 2), and ends a short time later with fusion of egg and sperm plasma membranes to form a single "activated" cell, the zygote. Along the way, several recognizable events take place, including the sperm acrosome reaction (a form of cellular exocytosis), penetration of the egg ZP by sperm, and the egg cortical reaction and zona reaction. The latter results in alteration of the ZP such that free-swimming sperm are unable to bind to fertilized eggs. Each of these events in the fertilization pathway has been studied in some detail.

This review focuses on molecules currently thought to be involved in three steps of the mammalian fertilization process: (1) binding of sperm to eggs; (2) induction of the acrosome reaction by sperm; and (3) fusion of sperm and eggs. Much of the research discussed was carried out in mice. In recent years, considerable progress has been made toward delineating the molecular basis of each of these steps, especially in mice and other rodents. However, it is anticipated that some of the ideas presented may need to be modified as a result of ongoing research on a variety of mammals, including humans.

Specific Aspects of Mammalian Fertilization

The final steps of mammalian oogenesis and spermatogenesis prepare eggs and sperm, respectively, for fertilization. During ovulation, fully grown oocytes from antral (Graafian) follicles undergo "meiotic maturation," a process that transforms fully grown oocytes into unfertilized eggs prepared to interact with sperm (Wassarman and Albertini, 1994). Similarly, following deposition into and migration up the female reproductive tract, sperm undergo "capacitation," a process that enables sperm to bind to eggs and to undergo the acrosome reaction

(Darszon et al., 1996; Visconti and Kopf, 1998). Capacitation probably involves removal of inhibitory factors from sperm accompanied by membrane protein and lipid rearrangements and/or modifications. Apparently, some alterations are mediated, at least in part, by cAMP-dependent protein tyrosine phosphorylation, as well as by changes in pH and Ca^{2+} concentrations. Meiotic maturation of oocytes and capacitation of sperm propel gametes down a path that leads either to formation of a viable zygote or to degeneration of the cells.

Typically, very few ovulated eggs are found in oviducts of females (e.g., humans, ~1; mice, ~10). Similarly, relatively few sperm are found at the site of fertilization (~100–150) as compared to the number of sperm deposited into the female reproductive tract (~10^7). A very low percentage of ejaculated sperm ever make their way to the position of unfertilized eggs in the oviduct (~0.002%). Whether binding of sperm to eggs occurs due to a chance encounter of gametes in the oviduct or is promoted by a chemical gradient stimulus ("sperm chemotaxis"), as found with many nonmammalian species, remains to be resolved. In this context, it should be noted that there is good in vitro evidence for human sperm chemotaxis mediated by an egg follicular factor (Eisenbach and Tur-Kaspa, 1998).

It is well known that hybrids of certain mammalian species are viable; for example, mules derived from a cross between male donkeys and female horses. Does this mean that mammalian fertilization does not exhibit any species specificity? On the contrary, evidence from in vitro fertilization experiments strongly suggests that there are barriers to interspecies fertilization and that the egg ZP serves as a major barrier (Yanagimachi, 1994). The ZP can interfere with interspecies fertilization by failing to permit the initial binding of sperm to eggs, induction of the acrosome reaction, or penetration of bound sperm through the egg extracellular coat. Although the restrictions on binding are not absolute (e.g., mouse sperm bind to hamster eggs and hamster sperm bind to mouse eggs), they provide for a relatively high degree of species-specific fertilization in vitro (e.g., guinea pig and human sperm do not bind to mouse eggs). Notably, removal of the ZP from unfertilized eggs, thereby exposing egg plasma membrane directly to sperm, virtually eliminates the barrier to fertilization between species in vitro.

Molecular Aspects of Binding of Sperm to Eggs

A reductionist's view of the binding of sperm to eggs in mammals would have a molecule located on the sperm head ("egg-binding protein") recognizing and binding to a complementary molecule located on the egg ZP ("sperm receptor") in a species-specific manner. Such a situation is analogous to many other cellular adhesion events. Among these are binding of bacteria, animal viruses, and other pathogens to their cellular hosts, binding of pollen to the plant stigma, sexual agglutination in yeast, and binding of sperm to unfertilized eggs

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† This review is dedicated to the memory of the author's colleagues Eugenia Spanopoulou, Andrew Hodtsev, and Thomas Kreis.
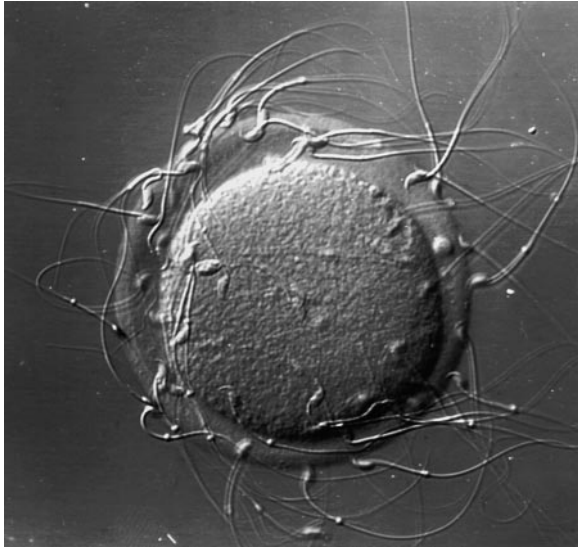
Figure 1. Light Photomicrograph (Nomarski DIC) of Mouse Sperm Bound to the ZP of an Unfertilized Mouse Egg In Vitro

Figure 3. Schematic Diagram of Some Molecular Features of a Mammalian Sperm Bound to the ZP of an Unfertilized Egg

Each sperm is bound to O-linked oligosaccharides of thousands of mZP3 molecules that are located periodically along ZP filaments. The filaments are composed of mZP2 and mZP3 and are cross-linked by mZP1. The active O-linked oligosaccharides of mZP3 that are recognized by sperm are colored pink.

(e.g., sea urchin, abalone, and *Xenopus*) during fertilization. In each case, complementary molecules that support highly specific cellular adhesion are found on the surfaces of participating cells. In certain cases, the cellular adhesion is thought to be carbohydrate mediated.

Egg Zona Pellucida Glycoproteins

The plasma membrane of all mammalian eggs is completely surrounded by a ZP. Nearly 20 years ago it was demonstrated that the mouse egg ZP ($\sim$6.2 μm thick containing $\sim$3.5 ng of protein) is composed of only three glycoproteins, called mZP1 ($\sim$200 kDa; dimer), mZP2
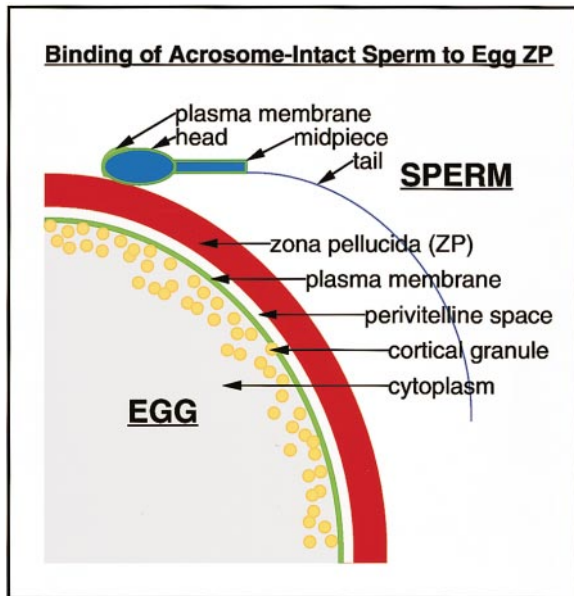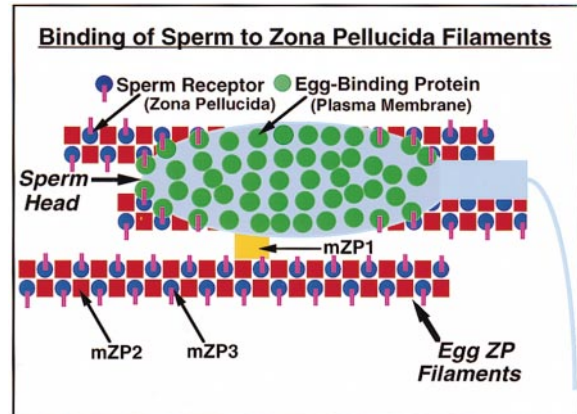

Figure 2. Schematic Diagram of Sperm–Egg Interaction in Mammals

Shown are morphological features of an acrosome-intact mammalian sperm bound to the ZP of an unfertilized egg by plasma membrane overlying the anterior region of the sperm head.

($\sim$120 kDa), and mZP3 ($\sim$83 kDa) (Bleil and Wassarman, 1980a; Wassarman, 1988). In mice, the three glycoproteins are synthesized exclusively by growing oocytes. Two of the glycoproteins, mZP2 and mZP3, interact with each other via noncovalent bonds to form long filaments bearing a 14–15 nm structural repeat that are interconnected by mZP1 (Figure 3). Each of these glycoproteins consists of a unique polypeptide that is heterogeneously glycosylated with both complex-type asparagine- (N-) linked and serine/threonine- (O-) linked oligosaccharides.

Targeted disruption of the *mZP3* gene by homologous recombination in embryonic stem (ES) cells has no effect on the phenotype of male mice, but it results in infertility in homozygous null females (Liu et al., 1996; Rankin et al., 1996). Ovaries from the homozygous null females (*mZP3*$^{-/-}$) contain growing oocytes that completely lack a ZP, while oocytes from heterozygous null females (*mZP3*$^{+/-}$) have a ZP that is about one-half the thickness ($\sim$2.7 μm) of the wild type (Wassarman et al., 1997). These results are consistent with the proposed structural role of mZP3, as well as current models for ZP structure (Wassarman and Mortillo, 1991; Wassarman et al., 1996).

Today, it is clear that the ZP of eggs from a wide variety of mammals, including humans, is composed of a small number of glycoproteins that are closely related (polypeptides $\sim$40%–90% similar) to mZP1–mZP3 (Wassarman, 1999). For example, the positions of the 13 cysteine residues, as well as all of the recognizable domains of mZP3 polypeptide (Figure 4), are conserved in human ZP3 polypeptide. Even the vitelline envelope surrounding eggs from fish, birds, and amphibians contains glycoproteins whose polypeptides resemble mZP1–mZP3. Thus, there is a significant evolutionary link between glycoproteins of the vitelline envelope of non-mammalian eggs and glycoproteins of the ZP of mammalian eggs. Apparently, these glycoproteins play essential structural roles in assembling the extracellular

LANDMARKS OF THE mZP3 POLYPEPTIDE

N-1

signal sequence (1-22)

homologous region
(70-240)

sperm combining-site
(329-334)

hinge region
(220-260)

furin cleavage site
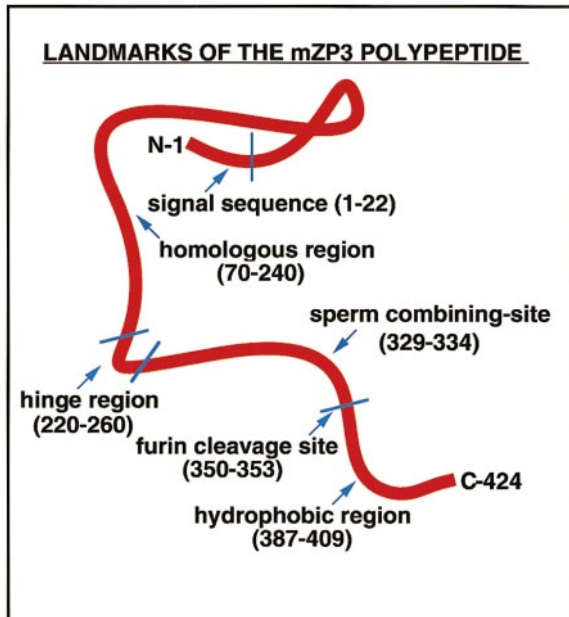(350-353)

C-424

hydrophobic region
(387-409)

Figure 4. Schematic Diagram of Some Molecular Landmarks of mZP3 Polypeptide

These features are common to all ZP3 polypeptides, from mouse to human ZP3.

coats during oogenesis. It will be of great interest in the future to compare the high-resolution structures of vitelline envelope and ZP glycoproteins.

mZP3 as a Sperm Receptor

Only acrosome-intact sperm bind to the ovulated mouse egg ZP (Florman and Storey, 1982; Bleil and Wassarman, 1983). Experimental evidence strongly supports the conclusion that, during binding of sperm to eggs, mZP3 serves as a receptor for sperm (Figure 3). For example, of the three glycoproteins that constitute the ZP, only purified mZP3 binds exclusively to heads of acrosome-intact sperm (i.e., to plasma membrane; $\sim 10^4$ molecules of mZP3 per sperm head) and thereby prevents sperm from binding to ovulated eggs in vitro (Bleil and Wassarman, 1980b, 1986; Wassarman, 1990; Mortillo and Wassarman, 1991; Wassarman and Litscher, 1995). Even at nanomolar concentrations, purified, unfertilized egg mZP3 is a very effective inhibitor of sperm binding in this competition assay. On the other hand, at similar concentrations, mZP3 from fertilized eggs or early embryos has no effect on binding of sperm to eggs in vitro. This is consistent with the failure of free-swimming sperm to bind to the ZP of fertilized eggs and preimplantation embryos. It can be concluded from these and other observations that, as a consequence of the zona reaction, mZP3 is altered such that free-swimming sperm can no longer recognize and bind to the glycoprotein (i.e., mZP3 is inactivated as a sperm receptor).

Sperm Binding to mZP3 Oligosaccharides

What is it that acrosome-intact sperm recognize and bind to on mZP3? The ability of mZP3 to act as a sperm receptor in vitro is not significantly affected by exposure of the glycoprotein to high temperatures, detergents, denaturants, or reducing agents, or by limited proteolysis of the glycoprotein. Even after extensive proteolytic digestion of mZP3, the small glycopeptides produced retain activity as a sperm receptor, although higher than normal concentrations ($\sim$50-fold) are required (Florman et al., 1984; Florman and Wassarman, 1985). These and other observations suggest that mZP3 polypeptide does not play a direct role in sperm receptor function.

On the other hand, there is considerable data to suggest that mZP3 oligosaccharides do play a direct role in sperm receptor function (Figure 3). For example, chemical or enzymatic removal of all mZP3 oligosaccharides (N- and O-linked) results in complete inactivation of the glycoprotein as a sperm receptor. Furthermore, O-linked oligosaccharides recovered from mZP3 by mild alkaline hydrolysis under reducing conditions (Florman and Wassarman, 1985; Bleil and Wassarman, 1988; Miller et al., 1992) and certain O-linked related oligosaccharides synthesized in the laboratory (Litscher et al., 1995; Johnston et al., 1998) inhibit binding of sperm to eggs in vitro at micromolar concentrations. Collectively, these and other observations suggest that species-specific binding of sperm to eggs in mammals is a carbohydrate-mediated event. On the other hand, the identity of the sugars on mZP3 recognized by sperm remains unresolved, especially in view of results with homozygous null mice (Thall et al., 1995; Asano et al., 1997; Lu and Shur, 1997).

Recent studies have utilized limited proteolysis (Rosiere and Wassarman, 1992; Litscher and Wassarman, 1996), exon swapping (Kinloch et al., 1995), and site-directed mutagenesis (Kinloch et al., 1995; Chen et al., 1998) to identify the location of essential O-linked oligosaccharides on mZP3 polypeptide. Results of such studies suggest that these oligosaccharides are located on just two of five serine residues, serine-332 and serine−334, in a region of polypeptide near the carboxyl terminus encoded by exon-7 of the *mZP3* gene (Figure 4). Interestingly, of the five serine residues, only these two are conserved from mouse to human ZP3. In this context, the numerous amino acid changes neighboring serine-332 and serine−334 that have occured during evolution may impose changes in the structure of O-linked oligosaccharides added to ZP3 and, thereby, affect species specificity of sperm–egg interaction (Wassarman and Litscher, 1995).

Egg-Binding Proteins on Sperm

There is an extensive literature that deals with the search for egg-binding proteins (EBPs) on sperm that complement sperm receptors on the egg ZP (Wassarman, 1995; Snell and White, 1996). During the past 20 years, as many as two dozen different sperm proteins/glycoproteins have been implicated in the binding of sperm to eggs. A variety of mammalian species, from mice to humans, and experimental methodologies (e.g., Western blotting, affinity chromatography, chemical cross-linking, and solid-phase assays) have been used to identify these sperm components, which include a number of enzymes and lectin-like proteins. In several instances the components have been cloned and sequenced (e.g., β-galactosyltransferase, Shur, 1999; sperm protein-56, Bookbinder et al., 1995; zonadhesin, Gao and Garbers, 1998; and sperm protein-17, Richardson et al., 1994), in one case a high-resolution three-dimensional structure has been determined (spermadhesin, Romero et al.,

Table 1. Proposed Candidate EBPs

Candidates	Comments	References
β-galactosyltransferase	Binds to GlcNAc residues on mZP3 specifically	Miller et al., 1992; Lu and Shur, 1997
Sperm protein-56	Binds to mZP3 oligosaccharides; contains sushi and unique domains	Cheng et al., 1994; Bookbinder et al., 1995
Zonadhesin	Binds to the egg zona pellucida; contains multiple types of domains	Hardy and Garbers, 1995; Gao and Garbers, 1998
Spermadhesins	Exhibit carbohydrate-binding activity; possess a CUB domain	Dostalova et al., 1995; Romero et al., 1997
Zona receptor kinase	Binds to mZP3; identical to c-mer tyrosine kinase	Burks et al., 1995; Bork, 1996; Tsai and Silver, 1996
Mannose-binding protein	α-D-mannosidase that binds to mannose residues on ZP	Cornwall et al., 1991
Galactose-binding protein	Related to asialoglycoprotein receptor	Abdullah and Kierzenbaum, 1989
Sperm protein-17	Sperm-specific autoantigen that binds to ZP	Richardson et al., 1994
Fertilization antigens	Bind to human ZP3; murine and human FA-1 and NZ-1	Zhu and Naz, 1997
Phospholipase A₂	Inhibitors of and antibodies against PLA₂ inhibit fertilization	Fry et al., 1992; Riffo and Parraga, 1996
Sperm agglutination antigen-1	Human sperm surface antigen	Diekman et al., 1997

1997), and, in at least one case, targeted mutagenesis of the gene has been carried out and homozygous null mice produced and characterized (β-galactosyltransferase, Lu and Shur 1997). A partial list of candidate EBPs, together with a brief description of each protein, is presented in Table 1.

Although a few candidate EBPs have been favored over others, even these remain controversial. For example, recently it was found that male mice which are homozygous null for β-galactosyltransferase ($gt^{-/-}$; long and short forms of the protein) are fertile (Lu and Shur, 1997). Although responses to mZP3 (e.g., induction of the acrosome reaction) are impaired in vitro, sperm from $gt^{-/-}$ mice bind to and fertilize eggs in vivo. These findings may at least minimize the role of β-galactosyltransferase in fertilization. Similarly, the realization that human zona receptor kinase (ZRK; Hu9), a protein tyrosine kinase, is identical to the proto-oncogene c-mer (Bork, 1996; Tsai and Silver, 1996) raises doubts about ZRK's proposed role in binding of sperm to eggs (Burks et al., 1995; Saling et al., 1996). Finally, the recent finding that AM67, an acrosomal matrix protein, and sp56 are orthologs (Foster et al., 1997) casts some doubt on a role for sp56 in binding of acrosome-intact sperm to the ZP (Bookbinder et al., 1995). These and other issues have contributed to the confusion that characterizes this area of research.

What accounts for the large number of sperm components identified as EBPs? The following four possibilities may be contributing factors to the confusing state of this area of research: (1) involvement of different sperm proteins as EBPs in different mammalian species; (2) participation of multiple sperm proteins as EBPs, acting either individually or as multiprotein complexes, in a particular mammalian species; (3) participation of multiple sperm proteins as EBPs, each with different affinities for the sperm receptor that may act in sequence, in a particular mammalian species; and (4) some of the in vitro assays used to assess EBP function may not mirror in vivo events. For example, in the latter context, the ability of an antibody directed against a sperm protein to inhibit binding of sperm to eggs does not necessarily mean that the antigen is an authentic EBP. To some degree, the use of sperm at different stages of capacitation, having an intact, partially reacted, or fully reacted acrosome, may also contribute to the diversity of proteins identified as EBPs. At this point, it is impossible to choose from among these and other possibilities. However, in the end, it is possible that a single class of sperm proteins may emerge as the bona fide EBP in many, if not all mammals.

Molecular Aspects of the Sperm Acrosome Reaction

The acrosome is a large secretory vesicle that overlies the nucleus in the apical region of the sperm head (Eddy and O'Brien, 1994; Yanagimachi, 1994). Acrosomal membrane just underlying the plasma membrane is referred to as "outer" acrosomal membrane, and that overlying the nucleus is referred to as "inner" acrosomal membrane. Morphologically, the acrosome reaction is seen as multiple fusions between outer acrosomal membrane and plasma membrane at the anterior region of sperm head, extensive formation of hybrid membrane vesicles, and exposure of inner acrosomal membrane and acrosomal contents (Cardullo and Florman, 1993) (Figure 5). Again, only acrosome-reacted sperm can penetrate the ZP and fuse with egg plasma membrane.

Until quite recently, the acrosomal serine protease, called acrosin, was considered essential for penetration of the ZP by sperm. However, sperm from mice that are homozygous nulls for acrosin ($Acr^{-/-}$) penetrate the ZP and fertilize eggs, suggesting that acrosin may not be essential for these steps (Baba et al., 1994). On the other hand, the absence of acrosin does cause a delay in penetration of the ZP by sperm that may be due to a delay in dispersal of acrosomal proteins during the acrosome reaction (Yamagata et al., 1998).

Acrosome Reaction Inducers

It is known that there are many different inducers of the acrosome reaction (e.g., progesterone, Roldan et al., 1994; Yanagimachi, 1994). However, it is now generally accepted that ZP3 is the natural agonist that initiates the acrosome reaction upon binding of acrosome-intact
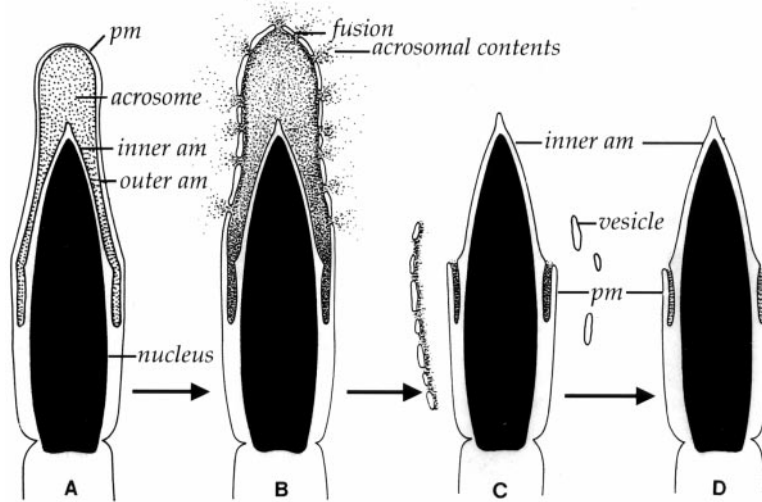
Figure 5. Schematic Diagram of Some Morphological Features of a Mammalian Sperm Undergoing and Completing the Acrosome Reaction

The course of the acrosome reaction is indicated by (A)–(D). An acrosome-intact sperm head is shown in (A). In (B), fusion between outer acrosomal membrane and plasma membrane is indicated. Hybrid membrane vesicles, composed of plasma and outer acrosomal membrane, are shown in (C) and (D). pm, plasma membrane; am, acrosomal membrane. This figure was adapted from Figure 16 in Yanagimachi (1994).

sperm to the ZP (Bleil and Wassarman, 1983; Ward and Kopf, 1993; Darszon et al., 1996; Florman et al., 1998) (Figure 6). Criteria now are available that permit one to distinguish between the so-called "spontaneous" acrosome reaction and the ZP3-induced acrosome reaction (e.g., sensitivity to pertussis toxin). While purified mZP3 and large mZP3 glycopeptides induce sperm to acrosome-react in vitro, small mZP3 glycopeptides and purified mZP3 O-linked oligosaccharides bind to sperm and inhibit their binding to eggs, but do not induce the acrosome reaction (Wassarman, 1988). In the latter context, it has been reported that cross-linking of small mZP3 glycopeptides bound to sperm can induce the sperm to acrosome-react (Leyton and Saling, 1989). These and other findings suggest that induction of the acrosome reaction by ZP3 will turn out to be dependent on multivalent interactions.

G Protein Signaling and Induction of the Acrosome Reaction

ZP3 stimulation of sperm activates G proteins, and activation of G_{i1} and G_{i2} accounts for the pertussis toxin
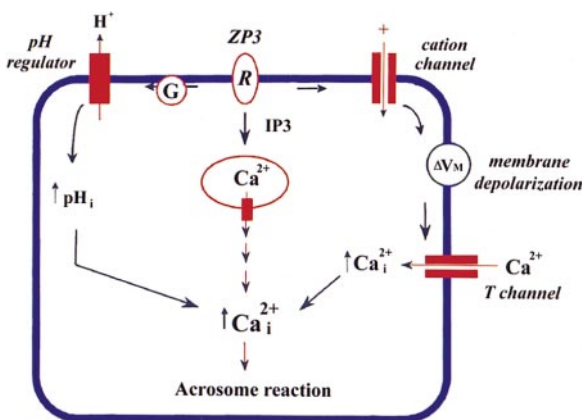

Figure 6. Schematic Diagram of the Acrosome Reaction in Mammals

Shown are some molecular events that can lead to induction and completion of the mammalian sperm acrosome reaction upon addition of ZP3 to sperm. This figure was adapted from one kindly provided by Dr. Harvey Florman, Tufts University School of Medicine, Boston, Massachusetts.

sensitivity of the acrosome reaction (Ward et al., 1994) (Figure 6). G protein activation by ZP3 has been demonstrated in sperm extracts (Ward et al., 1992), and participation of a second G protein, $G_{q/11}$, has been suggested (Walensky and Snyder, 1995). However, the receptors that activate sperm G proteins have remained elusive, as have the second messengers (e.g., cAMP) that are activated by G proteins during ZP3 stimulation of sperm. In this context, it has been reported that aggregation of β-galactosyltransferase on the sperm head, by either ZP3 or antibodies against the protein, leads to activation of a pertussis toxin-sensitive G protein complex and induction of the acrosome reaction (Gong et al., 1995).

Ion Channels and Induction of the Acrosome Reaction

ZP3 stimulation of sperm activates voltage-sensitive T type Ca^{2+} channels (Arnoult et al., 1996a, 1996b; Liévano et al., 1996) (Figure 6). Binding of ZP3 results in a depolarization of sperm membrane from ∼−60 mV to ∼−30 mV, values consistent with activation of T type channels, and is required for intracellular Ca^{2+} elevation and the acrosome reaction. It has been proposed that ZP3-induced opening of T type channels in sperm leads to a sustained release of Ca^{2+} from an internal store, perhaps via inositol-3,4,5-triphosphate (IP3) and IP3 receptors (Florman et al., 1998).

Intracellular Ca^{2+} and pH during the Acrosome Reaction

As in secretion by somatic cells, intracellular Ca^{2+} is necessary and sufficient to initiate the acrosome reaction (Figure 6). An elevated intracellular Ca^{2+} concentration is seen on progressing from resting uncapacitated sperm (50–100 nM), to capacitated sperm (125–175 nM), to ZP3- (agonist-) stimulated sperm (300–500 nM) (Florman, 1994). Similarly, ZP3-stimulated sperm exhibit a transiently elevated pH (from ∼6.6 to ∼6.8–7.0) that is sufficient to affect IP3 concentration and binding of IP3 to its receptor and, thereby, could lead to release of intracellular Ca^{2+} stores (Arnoult et al., 1996a; Florman et al., 1998). The pH increase may be regulated by an anion exchanger (e.g., a Na^+-dependent Cl^--HCO_3^- exchanger) and/or by an uncharacterized transport pathway. The alkalinization of sperm in response to ZP3 may also activate Ca^{2+}/calmodulin-dependent adenylyl
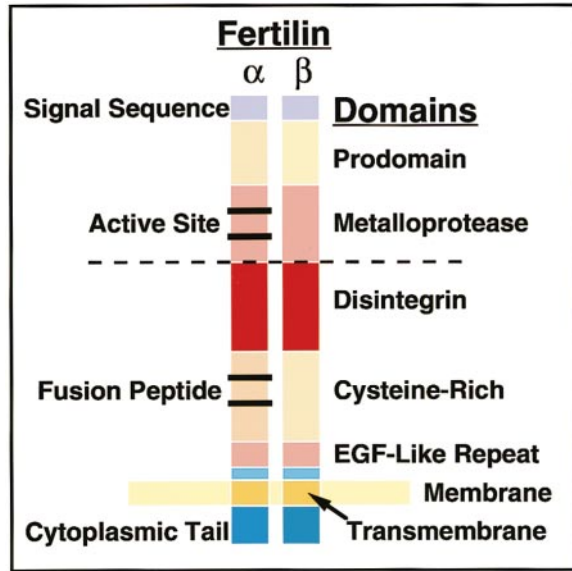
Figure 7. Schematic Diagram of Some Molecular Features of Fertilin-α and Fertilin-β Polypeptides

cyclase, protein phosphatase, protein kinases (A and C), tyrosine kinase, and various phospholipases (e.g., A_2, C, and D) (Darszon et al., 1996; Florman et al., 1998).

Molecular Aspects of Fusion of Sperm and Egg

Upon reaching the perivitelline space between egg plasma membrane and ZP (Figure 2), acrosome-reacted sperm can bind to and then fuse with egg plasma membrane. Fusion of sperm and egg occurs between the microvillous surface of ovulated eggs, which includes all but the region where the second metaphase plate and first polar body are located, and plasma membrane at the postacrosomal region of sperm (Yanagimachi, 1994). Apparently, this region of sperm membrane becomes capable of fusing with eggs only after the acrosome reaction has taken place; that is, completion of capacitation and the acrosome reaction are required to produce a fusible sperm.

Fertilin and Binding of Sperm to Egg
Plasma Membrane

One particular sperm protein, called fertilin, is currently thought to be responsible for binding of acrosome-reacted sperm to, and perhaps for fusion of bound sperm with, the egg plasma membrane (Blobel et al., 1992; Snell and White, 1996; Bigler et al., 1997; Myles and Primakoff, 1997; Evans et al., 1998). Fertilin (a.k.a. PH-30) is a heterodimer of α (guinea pig, ~60 kDa) and β (guinea pig, ~40 kDa) N-glycosylated subunits, both of which are members of the ADAM (contain a *d*isintegrin and a *m*etalloprotease domain; a.k.a. MDC, *m*etalloproteinase, *d*isintegrin, *c*ysteine-rich) family of transmembrane proteins (Wolfsberg and White, 1996; Black and White, 1998) (Figure 7). Both subunits are synthesized as precursors (~100 kDa) by spermatogenic cells and are proteolytically processed (removing pro- and metalloprotease domains) to the forms found on mature sperm. Peptides based on sequences at the disintegrin domain of fertilin-β, cyritestin (ADAM 3), and, perhaps,

fertilin-α, can prevent the binding of sperm to eggs from which the ZP has been removed in vitro (Myles et al., 1994; Almeida et al., 1995; Evans et al., 1995, 1997; Yuan et al., 1997). Therefore, it has been proposed that binding of acrosome-reacted sperm to egg plasma membrane is supported by interactions between fertilin's disintegrin domains and integrin (e.g., $\alpha_6\beta_1$) receptors (Hynes, 1992; Almeida et al., 1995; Graves, 1995) on unfertilized eggs.

Fertilin and Fusion of Sperm with Egg
Plasma Membrane

Fertilin-α possesses a moderately hydrophobic sequence, ~17–25 amino acids in length, in its cysteine-rich domain that may function as a "fusion peptide" following binding of sperm to egg plasma membrane (Blobel et al., 1992; Wolfsberg et al., 1995; Snell and White, 1996; Bigler et al., 1997) (Figure 7). This peptide can be modeled as an α helix having a strongly hydrophobic face (amphipathic helix), similar to the situation with viral fusion peptides. In this context, synthetic peptides corresponding to the fertilin-α hydrophobic sequence bind to membranes and induce fusion in vitro (Muga et al., 1994; Nidome et al., 1997), meltrin-α, an ADAM family member, apparently is involved in myoblast fusion (Yagami-Hiromasa et al., 1995), and a human testis-specific ADAM, proposed to have fusogenic activity, has been identified (Hooft van Huijsduijnen, 1998). It should be noted that several sperm surface proteins, other than fertilin-α, have been implicated in fusion of sperm and egg (Rochwerger et al., 1992; Allen and Green, 1995; Toshimori et al., 1998). Whether mammalian sperm–egg fusion follows a path similar to that proposed for influenza hemagglutinin mediated fusion (Hernandez et al., 1996) remains to be determined.

In conflict with the above proposal are results of experiments suggesting that some primates, including gorillas, orangutans, and humans, possess nonfunctional *fertilin*-α genes (Jury et al., 1997, 1998). Such studies indicate the presence of insertions, deletions, and in-frame termination codons in the single-copy gene encoding *fertilin*-α. Although the gene is expressed, it does not produce a functional protein. Here again, further experiments will be necessary in order to resolve this important issue.

Behavior of Sperm Lacking Fertilin-β

Recently, mice were produced that are homozygous nulls for *fertilin*-β (*fertilin*-β$^{-/-}$), and their phenotype was determined (Cho et al., 1998). The amount of fertilin-α in sperm was significantly reduced in mice lacking fertilin-β. Mutant females exhibited normal (i.e., wild type) fertility, whereas the fertility of males was severely impaired (~2% of wild type). Several differences were noted in the phenotype of sperm from *fertilin*-β$^{-/-}$ mice: (1) there was a significant decrease (~12% of wild type) in the ability of mutant sperm to bind to eggs lacking a ZP in vitro; (2) there was about a 50% reduction in the number of eggs fused with at least one mutant sperm in vitro; (3) binding of mutant sperm to the egg ZP was reduced to <0.1% in vitro; (4) the fertility of mutant sperm was reduced ~50-fold in vivo; and (5) although wild-type numbers of mutant sperm were found in the uterus after mating in vivo, very low numbers of sperm were found in the oviduct.

While the fertility of *fertilin*-$\beta^{-/-}$ males is greatly reduced compared to wild-type males, it is attributable to the paucity of mutant sperm that reach the oviduct ($<5\%$ of wild type) and to the inability of these sperm to bind to the ZP of ovulated eggs. The latter observation is surprising since fertilin-β is not a candidate EBP. Therefore, the dramatic reduction in fertility of *fertilin*-$\beta^{-/-}$ males is not only a consequence of the impaired ability of these sperm to bind to and fuse with egg plasma membrane. Furthermore, sperm from fertilin-$\beta^{-/-}$ males continue to fuse with egg plasma membrane in in vitro assays, albeit with reduced efficiency. There are several potential explanations for this behavior, including (1) mutant sperm enter the fusion pathway downstream of fertilin-β binding; (2) an additional fertilin-β-independent pathway to fusion exists (e.g., another ADAM protein, such as cyritestin, may be upregulated); and (3) fertilin-α and fertilin-β are not essential components of the gamete fusion pathway.

Final Comments

Mammalian fertilization is a complex process in which sperm and eggs participate in a series of ordered steps that culminate in formation of a zygote and development of a new individual of the species. Nearly all of these steps have their counterparts in somatic cells, and this has aided immensely in designing experiments on sperm, eggs, and fertilization. Although mammalian fertilization is a special case that involves some unique molecules, such as ZP glycoproteins, principles gained from somatic cell biology undoubtedly apply with respect to cellular adhesion (binding of sperm to the egg ZP and plasma membrane), exocytosis (acrosome and cortical reactions), and fusion of gametes. For example, the latter has been modeled after viral fusion events and the action of snake venoms.

During the past two decades a great deal of progress has been made toward establishing a "molecular pathway" for fertilization in mammals, from species-specific binding of capacitated, acrosome-intact sperm to the egg ZP, to induction of the acrosome reaction, to fusion of acrosome-reacted sperm with egg plasma membrane. While much of the progress has been made with mice and other rodents, it seems likely that homologs of molecules that participate in fertilization in these animals (e.g., sperm receptors and EBPs) also participate in fertilization in other mammals, including humans. Hopefully, the next decade of research will confirm and extend many of the ideas presented in this review and impact in a constructive manner on many aspects of human reproductive health.

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