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Why Did the Sperm Cross the Cumulus? To Get to the Oocyte. Functions of the Sperm Surface Proteins PH-20 and Fertilin in Arriving at, and Fusing with, the Egg[1]

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ABSTRACT

The sperm surface has an active role in the events of fertilization. The definition of the sperm surface in both its composition and domain organization begins during spermatogenesis and continues until the moment of sperm-egg fusion. Alterations of the surface proceed as a result of internal programming and environmental cues from both the male and female reproductive tracts, including interactions with the egg itself. We have investigated the sperm surface to understand its domain organization and the ongoing changes in this organization as well as the role of specific surface proteins in fertilization. Much of our research has concentrated on two surface proteins: PH-20 and fertilin. PH-20 is a single-chain protein, anchored in the membrane via a glycosyl phosphatidylinositol (GPI) anchor. The N-terminal domain of the molecule has a hyaluronidase activity. The hyaluronidase activity of PH-20 on the sperm plasma membrane enables sperm to penetrate the layer of cumulus cells surrounding the oocyte. PH-20 has a second function, unrelated to its hyaluronidase activity, in the binding of acrosome-reacted sperm to the zona pellucida (secondary sperm-zona binding). The fertilin molecule is an α,β heterodimer whose two subunits are closely related transmembrane proteins. Fertilin β has a disintegrin domain that has high sequence homology with the snake disintegrins, a known class of soluble integrin ligands. The binding site of the β disintegrin domain functions to bind sperm to the egg plasma membrane via a mechanism that leads to sperm-egg fusion. The precursor of fertilin α, made in the testis, has an active metalloprotease site that could function in spermatogenesis. This metalloprotease domain is removed by proteolytic processing in the testis. Mature fertilin α on sperm also has a hydrophobic, putative "fusion peptide" that may promote the process of lipid bilayer fusion between sperm and egg plasma membranes. Fertilin α and β are the first identified members of a new gene family of transmembrane proteins, the ADAM family, so called because they contain A Disintegrin And Metalloprotease domain. Many distinct ADAMs have now been found in diverse tissues and species (*Drosophila* to human) and are proposed to have a variety of functions in development and the adult. In addition to fertilin, other ADAMs are also present on the sperm plasma membrane and may participate with fertilin in sperm-egg fusion.

INTRODUCTION

The final function of the sperm cell is to fuse with the egg plasma membrane. At the moment of fusion it ceases to be a sperm and becomes part of a newly formed cell, the zygote. The fusion between sperm and egg plasma membranes is the culmination of a sperm journey that relies on both sperm motility and the interaction between the sperm surface and the various environments that it encounters during its progression towards the egg. In this article, we discuss the role of the mammalian sperm surface in these events, particularly focusing on two surface proteins that we have studied in our laboratory. The first protein is PH-20, a single-chain protein anchored in the cell membrane by a glycosyl phosphatidylinositol (GPI) anchor. PH-20 is a bifunctional protein with a role in cumulus penetration and binding of acrosome-reacted sperm to the zona pellucida. The other protein, fertilin (originally termed PH-30), is a heterodimeric protein with two subunits (α and β) that both have a transmembrane region. Fertilin functions in sperm-egg membrane fusion. Both of these proteins were originally identified by functional screens of a monoclonal antibody library using in vitro fertilization assays [1, 2]. Their localization on the sperm surface and biological activities were subsequently studied with the goal of enhancing our understanding of the cellular and molecular basis of fertilization.

ORGANIZATION OF THE SPERM SURFACE

In the time between the entry of sperm into the female reproductive tract and the fusion of a single sperm with the egg plasma membrane, a series of functions involving the sperm surface occur (Fig. 1). Some of these events may involve multiple surface molecules, and in some instances a single molecule might be involved in more than one surface function. A remarkable aspect of the sperm surface is that this series of changing functions occurs in the absence of new membrane protein synthesis. Once the sperm leaves the testis, modifications to the molecular makeup of the sperm membrane must occur by other processes [3].

Although not well understood, one of the possible mechanisms for regulating sperm function in the absence of new protein synthesis may depend on the organization of sperm membrane proteins into specific plasma membrane domains. Most, if not all, of the mammalian sperm plasma membrane proteins are localized in these domains [4–6]. During the progression of sperm through functional changes in both the male and female reproductive tracts, specific proteins are able to move from one domain to another [7–12], and these migrations possibly affect their activity (Table 1). For example, such regulation by localization has been observed in *C. elegans* mutants, in which the proper localization of a surface receptor is required for signaling activity [13].

There are several different mechanisms by which changes in protein localization could bring about changes in protein function. By changing patterns of localization, the proteins also may change their surface density and level of expression. For example, PH-20 moving first from a whole-cell pattern to a posterior head pattern increases its surface density approximately 2-fold [8]. At the time of the

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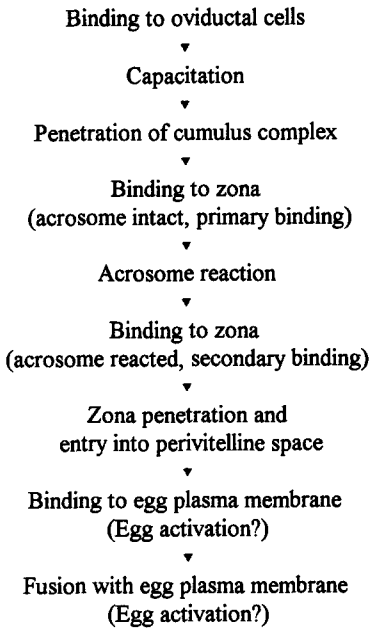


FIG. 1. Sperm in the female reproductive tract.

acrosome reaction, the level of surface expression increases an additional ~2.5-fold as the population of PH-20 originally on the posterior head plasma membrane joins a second population of PH-20 that preexists on the inner acrosomal membrane (and therefore is not on the surface) before the acrosome reaction [11, 14].

Changes in patterns of localization also serve to change the membrane environment. One result of this modification is that the relocalized protein is brought into contact with a different set of other membrane proteins that could either activate or inhibit its function. Changes in localization also mean that the protein in its new domain will have an altered lipid environment because plasma membrane lipids of sperm also appear to sort preferentially into these separate domains [15]. Potentially related to change in position in terms of interactions with either new lipids or other proteins are the observed changes in protein diffusion [9, 16–18], both in the rate of diffusion (diffusion coefficient) and percentage of recovery (Table 2).

One important finding in recent work is that the domain localization of sperm plasma membrane proteins is to some degree species-specific. For example, PH-20 on the plasma membrane of acrosome-intact sperm is on the posterior head in guinea pig sperm [11], the anterior or whole head in mouse, monkey, and human sperm [19, 20] and, remarkably, on the tail of non-capacitated rat sperm [12]. Rat PH-20 (originally termed 2B1 antigen) migrates to the sperm head during capacitation, so that it may be able to function in a fashion similar to PH-20 in other species [12] (R. Jones and L. Hall, personal communication). Likewise, fertilin is on the posterior head (and not the equatorial segment) in guinea pig sperm [2] and is exclusively on the equatorial segment in mouse sperm (unpublished results). In both species, fertilin is localized in a region that is large enough to participate in membrane fusion.

In addition to changes in localization, proteolytic processing occurs after insertion of sperm membrane proteins into the plasma membrane and probably affects protein function. Proteolytic cleavage has been observed for both PH-20 [21] and fertilin [8, 22]. Proteolytic processing of fertilin is discussed in more detail below.

APPROACH OF THE SPERM TO THE EGG PLASMA MEMBRANE

Role of Sperm Surface PH-20 during Cumulus Cell Penetration

In most mammalian species, sperm approaching the oocyte encounter a substantial layer of cumulus cells embedded in an extracellular matrix rich in hyaluronic acid. This cumulus cell complex, typically comprising about 3000 cumulus cells, forms a barrier that sperm must penetrate before they can achieve close approach to the egg. Sperm acrosome-reacted before reaching the cumulus remain stuck on the outer edge of the cumulus complex, apparently bound to the cumulus cells [23]. In species in which the approach has been studied in some detail, including hamsters [24] and guinea pigs (unpublished results), only acrosome-intact sperm are observed penetrating the cumulus complex. This finding argues against a model in which enzymes released from the acrosome function to disperse the extracellular matrix so that sperm can penetrate between the cumulus cells. Our recent work indicates that PH-20 is

TABLE 1. Examples of changes in position of membrane proteins in plasma membrane domains of mammalian sperm.*

Protein	When changed	Domain change	Reference
PH-20	Epididymal passage	Whole cell to posterior head	[7]
Fertilin (PH-30)	Epididymal passage	Whole head to posterior head	[8]
AH-50	Epididymal passage	Whole head to anterior head	[8]
CE9	Epididymal passage	Posterior tail to anterior tail	[9]
Surface galactosyl transferase	Epididymal passage	Anterior head over acrosome to more restricted acrosomal "cap"	[10]
PT-1	Capacitation	Posterior tail to whole tail	[11]
2B1 (rat PH-20)	Capacitation	Tail to anterior head	[12]
PH-20	Acrosome reaction	Posterior head to inner acrosomal membrane	[11]

* This is not meant to be an exhaustive list. Other examples exist, but we have chosen proteins that are relevant to other studies discussed in this paper or examples that are associated with our laboratory.

TABLE 2. Diffusion characteristics of some sperm surface proteins.

Protein	Surface domain	D (diffusion coefficient) × 10^9 cm^2/sec	R (% recovery)	Reference
PH-20	Whole head, testicular sperm	0.019	72	[16]
PH-20	Posterior head, acrosome intact epididymal sperm	0.18	73	[17]
PH-20	IAM, acrosome reacted epididymal sperm	1.7	78	[17]
PT-1	Posterior tail, epididymal sperm	2.5	> 90	[18]
CE9	Posterior tail, testicular sperm	1.1	100	[9]

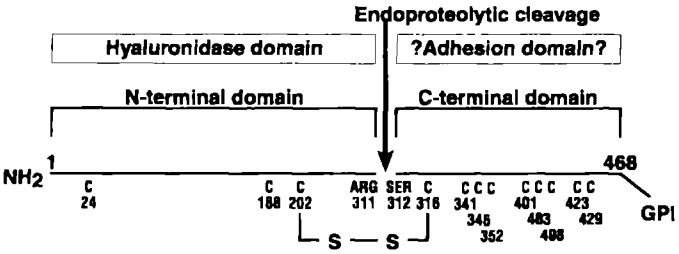


FIG. 2. Structural organization of the PH-20 protein. The endoproteolytic cleavage occurs at the time of the acrosome reaction, and the two fragments remain associated by one or more disulfide bridges. One hypothetical cysteine bridge is shown and the other cysteine residues are identified (C). The protein is associated with the plasma membrane by a GPI anchor.

on the plasma membrane of sperm from all species tested (guinea pig, mouse, monkey, and human) and that hyaluronidase activity of plasma membrane PH-20 is required for sperm to pass through the cumulus layer. For example, in the mouse system, we developed an assay to observe sperm penetration through the cumulus to the zona of cumulus-intact eggs. Antibodies directed against mouse PH-20 that inhibit its hyaluronidase activity prevent sperm penetration through the cumulus; antibodies that recognize mouse PH-20 but do not affect hyaluronidase activity have no effect on cumulus penetration [19].

Sperm Proteins and Zona Binding

Once sperm reach the zona pellucida, they bind to it. In some species (mouse for example), only acrosome-intact sperm have been observed to initiate the binding to the zona (primary binding). In the case of the mouse, binding of acrosome-reacted sperm to the zona (secondary binding) apparently occurs in vitro only when sperm have acrosome-reacted on the zona [23]. Primary and secondary binding are thought to involve two different zona proteins: ZP3 (primary binding) and ZP2 (secondary binding) [25, 26]. In other species (guinea pig for example), sperm in vitro can initiate binding to the zona pellucida when they are acrosome-intact ([27] and Schroer, Yudin, Myles and Overstreet, unpublished results) or acrosome-reacted [27, 28]. The binding of acrosome-intact sperm to the zona has been studied mostly in mice, and several sperm proteins may be involved [29]. Our studies have focused on secondary binding, more easily studied in guinea pigs, in which sperm in vitro can initiate binding to the zona when they have already acrosome-reacted in suspension. After the acrosome reaction, the PH-20 protein, originally on the posterior head plasma membrane of guinea pig sperm, has migrated to the inner acrosomal membrane (IAM) [11]. Acrosome-reacted sperm bind via the IAM to the zona. Experiments with function-blocking (and control nonfunction-blocking) monoclonal antibodies (mAbs) to guinea pig PH-20 show that anti-PH-20 function-blocking mAbs reduce the number of acrosome-reacted sperm bound to the zona by about 90% [1]. Secondary binding can also be blocked > 90% by polyclonal antibodies to PH-20 or their *Fab* fragments. Treating acrosome-reacted sperm with a phosphatidylinositol-specific phospholipase C (PI-PLC), which cleaves PH-20 from its GPI anchor, results in the specific removal of about half the surface PH-20. Such PI-PLC-treated, PH-20-depleted sperm are inhibited 55–60% in secondary binding, again suggesting a required role for PH-20 in this step. The function of PH-20 in secondary sperm-zona binding does not require its hyaluronidase activity and could be an activity of PH-20's C-terminal domain, which is proteolytically cleaved from the N-terminal domain during acrosome reaction (Fig. 2) [30]. The activity of PH-20 that makes it required in secondary binding is unknown [30], and secondary binding remains a poorly understood step in fertilization.

Secondary binding of sperm to the zona pellucida is followed by the penetration of these acrosome-reacted sperm through the zona and into the perivitelline space, the narrow space in mammalian eggs between the zona and the egg plasma membrane.

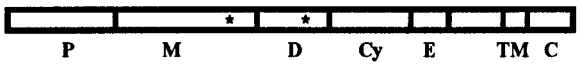
SPERM SURFACE FUNCTION DURING EGG PLASMA MEMBRANE BINDING AND FUSION

Observations of Sperm-Egg Membrane Binding and Fusion

The final function of the sperm cell is to fuse with the egg plasma membrane. When fusion occurs, the sperm ceases to be a sperm and becomes part of a newly formed cell, the zygote. Observations of both living and fixed gametes [31, 32] reveal a pattern in which a sperm can be seen to approach the egg plasma membrane and initiate interaction via the tip of its head (binding via the inner acrosomal membrane). The sperm then pivots on the site of initial adhesion and comes to lie flat against the egg membrane, in contact with the egg surface via first the side and back of its head, and subsequently along most of the length of the tail. Membrane fusion begins in the equatorial and/or posterior head regions and then continues to occur throughout the length of the tail, so that these regions of the sperm plasma membrane become incorporated into the plasma membrane of the new zygote. Only the anterior part of the sperm head is excluded, and because fusion is limited to acrosome-reacted sperm, this means that the inner acrosomal membrane is the region of the sperm membrane that does not fuse with the egg plasma membrane. Instead, the inner acrosomal membrane becomes incorporated into the egg cytoplasm in a membrane vesicle that is a hybrid of egg plasma membrane and sperm IAM. Because the sperm plasma membrane is divided into separate domains and membrane proteins are localized to these separate domains, it is probable that more than one sperm surface protein is involved in this series of events.

Sperm Surface Protein Fertilin

Our initial studies have focused on fertilin, a protein that is localized to the equatorial region (mouse) or posterior



P prodomain
M metalloprotease
D disintegrin
Cy cysteine rich
E EGF-like
TM transmembrane
C cytoplasmic

* active sites in metalloprotease and disintegrin domains

FIG. 3. Multidomain structural organization common to the precursor form of members of the ADAM family of proteins, including the α and β subunits of fertilin. Both fertilin α and β are processed to a mature form as are other family members. Related soluble snake proteins include a variable number of domains with the longest snake proteins including pro-, metalloprotease, disintegrin, and cysteine-rich domains.

head region (guinea pig) of the sperm plasma membrane and, therefore, is in an appropriate domain to be involved in the initial steps of sperm-egg fusion. Fertilin is a heterodimer composed of two transmembrane subunits: fertilin α and fertilin β [2, 33]. Both subunits are made as precursor proteins that are processed during sperm maturation [34] and are members of a newly identified family of transmembrane proteins that are multidomain proteins (Fig. 3). We have named this new family of transmembrane proteins ADAMs because they contain *A Disintegrin And Metalloprotease* domain [35]. They are closely related to a family of soluble snake venom peptides and proteins that have a similar domain structure but lack the transmembrane region [36].

Fertilin first appears on the cell surface of guinea pig spermatogenic cells relatively late in spermatogenesis. When it is expressed in the sperm plasma membrane it is already restricted in its distribution: it appears on the whole spermatid head and the cytoplasmic lobe, but is absent from the posterior tail membrane [6]. As sperm are released into the seminiferous tubules, the cytoplasmic lobe remains behind as a residual body, and when guinea pig testicular sperm enter the epididymis, fertilin is restricted to the whole head surface. During epididymal passage, the pattern of localization of guinea pig fertilin becomes further restricted, moving from the anterior head region so that it is localized exclusively in the posterior head domain [8].

The fertilin α and β subunits in guinea pig are processed at different times in sperm development. Processing of fertilin α occurs in the testis. The processing of fertilin β occurs later in the epididymis [8, 22] and accompanies the change in the pattern of localization (unpublished results).

Role of Fertilin β in Sperm Binding and Fusion

Several lines of evidence indicate a required role for fertilin β in sperm-egg fusion. Two mAbs (PH-30 and PH-1 mAbs) that recognize different epitopes on the β subunit of guinea pig fertilin were tested in an in vitro fusion assay [2]. One of the mAbs, PH-30, was function-blocking and showed a dose-dependent inhibition of sperm-egg fusion. The other mAb, PH-1, was a nonfunction-blocking mAb that served as a control antibody and had no effect on fusion.

During proteolytic processing, the fertilin β precursor is cleaved between the metalloprotease and disintegrin domain. Thus, mature fertilin β on cauda sperm has an N-terminal disintegrin domain. We tested to determine whether the fertilin β disintegrin domain functions in sperm-egg binding and fusion. As in other ADAMs, the disintegrin domain of fertilin β has high sequence homology with the disintegrin class of soluble snake venom peptides from pit vipers [36]. The soluble snake disintegrins bind to the platelet integrin αIIbβ3 (GPIIbIIIa), thereby preventing fibrinogen binding and platelet aggregation, and contributing to bleeding of the victim. Nuclear magnetic resonance (NMR) structural analysis of the short snake venom polypeptides had shown that their active site region, containing the signature RGD sequence, was located at the tip of a flexible loop with the base of the loop formed by disulfide bonds [36, 37]. Comparison of the snake disintegrin sequence with the fertilin β subunit sequence is shown in Figure 4. In guinea pig fertilin β, the sequence TDE is in the same position as RGD in the snake disintegrins. TDE-containing peptides with additional residues from this region of fertilin β (Fig. 4) have been tested for their effects in fusion assays with guinea pig gametes (Fig. 5). These TDE-containing peptides potently inhibit the fusion of sperm with the egg, presumably by preventing a step in sperm-egg membrane binding that leads to membrane fusion (Fig. 5) [38]. Similar results have been obtained with mouse peptides from the same active site loop of the mouse fertilin β sequence ([39–41], unpublished results).

Depending on the conditions used in these various experiments, the peptide mimetics of the fertilin β active binding site and also antibodies directed against fertilin β showed variability in their ability to inhibit sperm-egg plasma membrane *binding* even when they strongly inhibited sperm-egg fusion. Considering the somewhat artificial conditions of in vitro fusion assays (cumulus and zona-free eggs, presence of acrosome-intact sperm at the egg plasma membrane), we do not regard this as a surprising result. Sperm in vitro may bind to the egg by various mechanisms, some of which may not be functional in fusion and some of which may be intermediate (sequential) steps in the binding and fusion process. Our interpretation of these results is that binding of sperm to the egg plasma membrane via

Loop sequences from the disintegrin active site

C R I P ***R G D*** - M P D D R C - Kistrin
C R A S ***M S E*** C D P A E H C - Jararhagin
C R E S ***T D E*** C D L P E Y C - GP fertilin β
C R L A ***Q D E*** C D V E T Y C - mouse fertilin β

Inhibiting peptide sequences - guinea pig

cyc C S T D E C [1]
S T D E C D L P [1]

Inhibiting peptide sequences - mouse

C R L A Q D E C D V E T Y C [2]
cyc C A Q D E C [3]

FIG. 4. Sequences between the two cysteines that form a loop in the soluble snake disintegrin peptides that have been analyzed by NMR, including kistrin [37]. This region of most of the short snake disintegrins contains the signature *RGD* sequence found in many integrin ligands. The RGD sequence is substituted by other amino acids in the longer snake disintegrins like jararhagin [58] and mammalian disintegrin domains (found in ADAM family members). ADAMs also contain an extra cysteine following the position of the RGD substitutions. Mimetic peptides were made from this region of guinea pig and mouse fertilin β and tested by in vitro sperm binding and fusion assays. [1] [38], [2] [39], [3] [40].

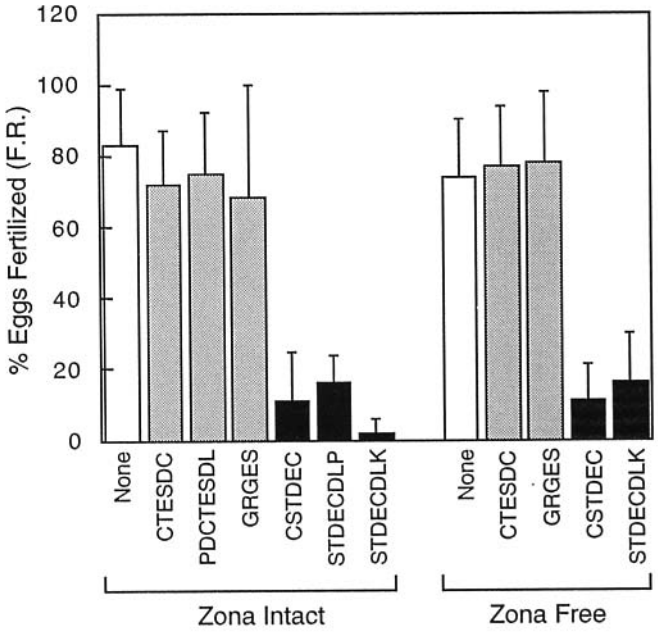


FIG. 5. Composite of experiments showing ability of mimetic peptides from guinea pig fertilin β disintegrin loop to inhibit the fertilization rate. Similar results were observed for inhibition of fertilization index (the average number of sperm fused per egg). Peptides in these experiments were tested at a concentration of either 250 μM or 500 μM.

fertilin β is a binding step leading to sperm-egg fusion, but sperm may bind to the egg plasma membrane via other interactions that do not necessarily lead to fusion.

Potential Functions of Fertilin α

Although all ADAM family members have a metalloprotease domain, only a minority have the correct residues in the metalloprotease active site that would allow them to have metalloprotease activity [42]. The precursor form of fertilin α contains a consensus sequence for an enzyme active site in the metalloprotease domain. (This active site is defined by the consensus sequence HEXXHXXGXXH.) In addition, in fertilin α, the final His in the sequence is followed by an Asp, defining the fertilin α metalloprotease as a reprolysin metalloprotease, distinct from other related proteases (astacins and matrixins) [43, 44]. The conserved sequence for a metalloprotease-active site is not present in fertilin β. Because processing of fertilin α occurs in the testis, this presumably active metalloprotease domain is no longer part of the fertilin molecule on mature sperm. Its presence in fertilin α in the testis does mean that it may function in the testis, either as a surface enzyme before processing or as a soluble enzyme.

Our original determination of the N-terminal sequence of processed guinea pig fertilin α indicated that the processing resulted in removal of most of the disintegrin domain, including the potential binding site region [33]. Recently reanalysis of the processed guinea pig fertilin α (C. Blobel, personal communication) and fertilin α from cow (S. Waters and J. White, personal communication) indicates that (as with fertilin β) processing occurs at the junction between the metalloprotease and the disintegrin domains. This leaves open the possibility that fertilin α also functions in binding sperm to the egg plasma membrane.

In addition, the fertilin α subunit contains an intriguing hydrophobic region (a putative "fusion peptide") that we have proposed could be directly involved in the fusion process. In guinea pig fertilin α, this region is residues 90–111, which can be modeled as a sided alpha helix with all the large hydrophobic residues on one face of the helix [33]. The hypothesis for participation of fertilin α in sperm-egg fusion is based on the model for fusion of enveloped viruses with host cell membranes. In many cases, viral fusion proteins contain a hydrophobic stretch of amino acids (a fusion peptide) that also can be modeled as an alpha helix with the hydrophobic amino acids organized along one face of the helix. This peptide remains cryptic until a trigger for fusion occurs: then there is a conformational change that allows the hydrophobic fusion peptide to become inserted into the host cell membrane, causing the formation of a fusion pore. The fusion pore can then expand to bring about membrane confluence.

We have suggested that in a similar way the fertilin β subunit could bind sperm to an integrin in the egg plasma membrane and bring about a conformational change in the fertilin α subunit, revealing the hydrophobic fusion peptide. This would then allow an association between the two lipid bilayers of sperm and egg plasma membranes, creating a fusion pore that would then expand to fuse the two membranes together. In support of this hypothesis, a synthetic peptide representing the proposed fusion peptide of guinea pig fertilin α can bind to lipid vesicles and induce fusion between large unilamellar vesicles [45]. However, in fertilin α from other species including the cow (S. Waters and J. White, personal communication), monkey [46], and rabbit (M. Holland, personal communication), the sequences corresponding to the putative fusion peptides of guinea pig and mouse fertilin α are not as hydrophobic and appear not to be amphipathic helixes. In these cases, it is possible that other regions of the fertilin α molecule act to promote fusion (S. Waters and J. White, personal communication), but such possibilities remain to be tested. Thus, there is considerable uncertainty about the hypothesis that fertilin α contains a fusion peptide, and further biochemical and genetic experiments probing fertilin α function in membrane fusion are needed.

Other Members of the ADAM Family and Their Potential Functions in Somatic Tissues

Fertilin α and β belong to the ADAM family, a growing family of transmembrane proteins with the same protein domain organization (Fig. 3). Additional members of this family have been found in testis [33, 35, 47–49] and in other male reproductive tissues, i.e., the epididymal epithelium [50]. Two of the ADAM proteins, fertilin β and cyritestin, show testis-specific expression by reverse transcriptase polymerase chain reaction [35]. Newly discovered family members show a wide distribution in somatic tissues or specific expression in one or a few somatic tissues [35, 42, 43, 51–54]. New ADAMs have been found in several mammalian species as well as nonmammalian species, e.g., *Xenopus laevis* (C. Blobel, personal communication) and *Drosophila melanogaster* [55].

Five members of this family of genes have been mapped to mouse chromosomes [47, 56] and one to a human chromosome [54]. The five mouse genes are found on four different chromosomes as follows: *Ftna* (fertilin α), chromosome 5; *Ftnb* (fertilin β), chromosome 14; *Adam-4* (ADAM 4 protein), chromosome 9; *Adam-5* (ADAM 5 protein), chromosome 8 [56]; and *Cyrn-1* (cyritestin), chromosome 8 [47]. By comparative mapping information, the human metalloprotease/disintegrin-like (MDC) gene that has been

mapped to the human chromosome 17q21 [54] is also localized to a chromosomal region distinct from the five mouse genes [56]. Both the human MDC gene [54] and fertilin β (unpublished results) have multiple small exons, indicating that the primordial gene may have arisen from exon shuffling and undergone subsequent duplication and widespread dispersion.

The number of ADAM family members is growing rapidly. In most cases thus far, only cDNA sequences have been reported, and the roles of the corresponding proteins remain to be studied. The multidomain nature of these proteins means that they may have more than one type of function. In those ADAMs that have an active metalloprotease site, the enzyme could function as a soluble protease (after cleavage from the membrane) or as a membrane-bound enzyme acting on substrates in the same membrane or the membranes of adjacent cells. Because the sequence motif in the disintegrin domain required for adhesive function has not been determined, some or all of these proteins could function in cell adhesion (presumably by binding to integrins on the surface of other cells). The cytoplasmic domain in many of these proteins has an unusual proline-rich sequence. Potential SH3 ligand domains have been identified in at least one protein, MDC9 [51], leading to a hypothesis that in some instances these proteins could serve as signal transduction molecules in the cells in which they are expressed. They could also be signaling ligands, binding to integrins on adjacent cells, leading to the stimulation of signaling pathways through occupancy of the integrin binding site and integrin clustering. It has been suggested that this latter type of signalling occurs in mammalian egg activation [31], in which integrins occur on the egg surface and can bind sperm, possibly via fertilin [39]. Recently it has been demonstrated that an RGD-containing peptide can activate *Xenopus* eggs [57], although its binding to a *Xenopus* egg integrin has not yet been documented.

One protein that may serve a function similar to that of fertilin is an ADAM family member, expressed in fusing myoblasts, and named meltrin α [53]. Meltrin α shows a limited tissue distribution: it is found in muscles and bones of neonates, but in adults it was detected only in bone, which has multinucleated osteoclasts. It was not detected in adult brain, liver, heart, or muscle by Northern blot analysis. The meltrin α sequence encodes an active metalloprotease site as well as a hydrophobic region similar to the putative fusion peptide of fertilin α (see above). Changes in molecular size of the protein during cell differentiation are consistent with the processing of the protein to remove the pro- and metalloprotease domains as seen for fertilin subunits. Using the C2 myoblast cell line, experiments were carried out to alter the amount of expressed meltrin α by transfecting C2 cells with either a vector containing the processed meltrin α sequence or an antisense construct. The formation of myotubes by cell fusion was directly affected by the level of expressed (processed) meltrin α: cells transfected with the vector containing the mature protein sequence resulted in a higher fusion index, while antisense transfected cells with lower meltrin α expression resulted in a lower fusion index (compared to appropriate controls) [53].

Another ADAM recently found in *Drosophila* indicates that members of this family have a signaling activity during development of the nervous system [55]. A new *Drosophila* gene (*kuz*) from the ADAM family has been identified by isolating loss of function mutants whose phenotype indicates a function in multiple processes in developing neural cells. The Kuz protein acts as a neural development-promoting factor as well as inhibiting the developmental potential of cells neighboring neural precursors (lateral inhibition). Because the protein has high homology (43% amino acid identity) with an ADAM family bovine metalloprotease purified from brain, the results with Kuz raise the possibility that this protein could also be involved in mammalian neurogenesis.

Involvement of ADAM Family Members Other Than Fertilin in Mammalian Fertilization

In addition to fertilin α and β, at least 10 additional ADAM family members ([35] and C. Blobel, personal communication) are expressed in mouse testis. We have begun to study these other ADAMs by asking if the additional family members are associated with sperm and if they function in sperm binding to the egg plasma membrane before fusion. Preliminary evidence indicates that, in addition to fertilin, at least one other family member, cyritestin, may participate in a binding step that precedes sperm fusion. Disintegrin domain active site peptides and antibodies to the disintegrin active site in cyritestin are able to inhibit sperm binding and fusion ([41] and unpublished results). These results indicate that sperm-egg fusion may be a multistep process and that the disintegrin binding sites of different ADAMs may participate in more than one of these binding steps. Uncovering this unexpected complexity in the process may prove to be the key to understanding it.

Summary and Perspective

Once sperm have reached the cumulus-enclosed oocyte, the process of fertilization involves a series of penetrations, punctuated by transitory bindings (at the zona surface), culminating in sperm binding to and fusing with the egg plasma membrane. The sperm cell has surface molecules deployed in specific domains that it rearranges as fertilization proceeds. The actual regulatory or functional significance of these membrane protein localizations and migrations is not well understood. The penetration of sperm through the cumulus cell layer to the zona seems to be a simple process requiring sperm motility and the plasma membrane protein PH-20, which has a hyaluronidase activity. This apparent simplicity may be real or may reflect our complete ignorance of other features of this process. Sperm, having penetrated the next barrier, the zona, bind to and fuse with the egg. The discovery of fertilin, the first sperm protein suggested to function in fusion, has led to the description of a large new gene family (ADAM family). Fertilin and at least one other ADAM family member, cyritestin, have required roles in binding sperm to the egg before fusion. These findings suggest that the binding and fusion process may work through other molecules, still to be identified, and proceed through a (potentially complex) sequence of steps. Fertilin α has been proposed to have a fusion peptide and promote membrane fusion, but so far evidence to support this proposal is scant. Thus, the actual protein(s) that acts to make the sperm and egg lipid bilayers fuse may be unknown.

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