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SPERM DISINTEGRINS, EGG INTEGRINS, AND OTHER CELL ADHESION MOLECULES OF MAMMALIAN GAMETE PLASMA MEMBRANE INTERACTIONS

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TABLE OF CONTENTS

1. ABSTRACT

The cell-cell interactions that occur between sperm and egg involve not only the binding but also the fusion of the gamete plasma membranes. Numerous studies, carried out decades ago and more recently, have implicated several different molecules on both the sperm and egg as being involved in gamete membrane interactions. The sperm proteins that have received the most attention recently have homology to disintegrins, which are proteins in snake venoms that can interact with integrins. These sperm disintegrin-like proteins are members of a molecular family, known as ADAM's (for A Disintegrin and A Metalloprotease) or MDC's (for Metalloprotease, Disintegrin, Cysteine-rich). This review will focus on the molecules that have been implicated in mediating mammalian sperm-egg binding or the fusion of the gamete membranes. The molecules that will be discussed include three members of the ADAM/MDC family on sperm (fertilin alpha, fertilin beta, and cyritestin), integrins on eggs (alpha-6/beta-1 and others), and a number of other egg and sperm molecules, novel and characterized in other systems, that have been implicated in these processes.

2. THE STEPS OF FERTILIZATION

Fertilization takes place in a series of discrete steps (figure 1). The sperm actually interacts with the egg on three separate levels: first with the cumulus cells and the hyaluronic acid extracellular matrix (ECM) in which they are embedded (not pictured), secondly with the egg's own ECM, called the *zona pellucida* (ZP), and finally with egg plasma membrane (figure 1). Sperm-ZP interactions are important, because the binding of the sperm to specific ZP glycoproteins (ZP3 in the mouse) induces the sperm to undergo the "acrosome reaction," the exocytosis of the acrosome vesicle on the head of the sperm (1). The acrosome reaction has two important results. First, enzymes released from the acrosome allow the sperm to penetrate the ZP to gain access to the perivitelline space. Secondly, new portions of the sperm membrane are exposed or modified upon the acrosome reaction, including the inner acrosomal membrane and the equatorial segment (figure 2A), regions of the sperm head that can participate in initial gamete membrane binding or subsequent sperm-egg membrane fusion (1-3). The acrosome reaction is absolutely required for sperm-egg plasma membrane interactions to occur, as only acrosome-reacted sperm can bind and fuse with the egg plasma membrane (1). (It should be noted that the acrosome reaction can occur spontaneously in a portion of a population of sperm during capacitation (4), making *in vitro* fertilization of ZP-free eggs possible in experimental systems, such as the mouse; see Section 3.3.)

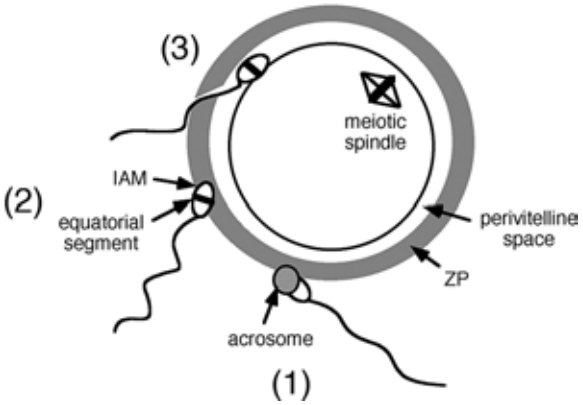

Figure 1. The steps of fertilization. This diagram shows a generic mammalian egg, with the meiotic spindle off to one side of the egg cytoplasm, and surrounded by the egg's ECM, known as the zona pellucida (ZP). The perivitelline space lies between the ZP and the egg plasma membrane. Step (1): (at the bottom of the diagram) The sperm first interacts with the egg's ZP. This interaction induces the sperm to undergo the acrosome reaction. Step (2): The release of enzymes from the acrosome allow the sperm the penetrate the ZP. The acrosome reaction has also revealed the inner acrosomal membrane (IAM) at the sperm head's anterior, and the equatorial segment. Step (3): Sperm-egg membrane interactions, binding to and fusion with the egg plasma membrane, occur in the perivitelline space.

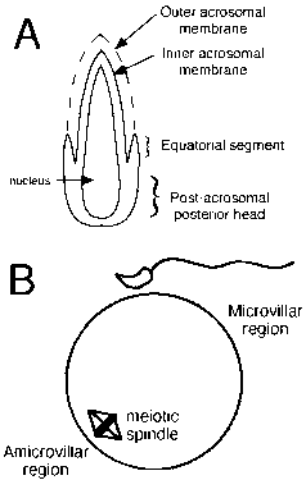

Figure 2. Spatial restrictions on where sperm-egg membrane interactions occur. Panel A show a diagram of section through the head of an acrosome-reacted sperm (this section runs perpendicular to the tail for rodent sperm, and tangentially for other species). The light gray dotted lines shows where the outer acrosomal was prior to the acrosome reaction. The locations of the inner acrosomal membrane (exposed following the dispersal of the outer acrosomal membrane), the equatorial segment, and the posterior head are shown. Panel B shows a rodent egg, with the amicrovillar region overlying the meiotic spindle, and the microvillar region (shaded).

Gamete plasma membrane interactions occur in the perivitelline space, between the membrane and the ZP (figure 1). Several cell biological aspects of these interactions should be noted. The interactions of the gamete plasma membranes appear to involve multiple ligands and receptors (detailed below), frequently noted as analogous to leukocyte-endothelial interactions (5,6). In addition, the sperm and egg not only adhere to each other but also go on to undergo membrane fusion, ultimately making one cell (the zygote) from two. Finally, the sperm-egg membrane interactions lead to a series of signal transduction events in the egg, known as collectively as egg activation. The events associated with egg activation include the initiation of oscillations in intracellular calcium concentration, the exit from meiosis, the entry into the first embryonic mitosis, and the formation of a block to polyspermy via the release of ZP-modifying enzymes from the egg's cortical granules. At present, it is unclear if it is sperm-egg binding (via a receptor-effector mechanism), sperm-egg fusion (by the diffusion of an egg-activating factor from the sperm cytoplasm to the egg cytoplasm), or a combination of both processes that induce egg activation. This review will not address the mechanism by which egg activation occurs, but the reader is referred to several excellent reviews for supplemental information (1,7-11). (NOTE: Throughout this review, I will refer to "receptors" on eggs for ligands on sperm. Use of this term is not meant to imply that these receptors are actually involved in signal transduction from the sperm to the egg, as this has not been definitively demonstrated for any of the molecules discussed here.)

It is important to note that mammalian sperm-egg membrane interactions occur in a spatially restricted manner. As described above, the acrosome-reacted sperm interacts with the egg plasma membrane via its inner acrosomal membrane and the equatorial segment (figure 2A). Following binding via these domains, membrane fusion occurs via the equatorial segment and the posterior head of the sperm (1). (The reader is referred to figures 15 and 19 in (1) for illustrations of sperm head morphologies in different species.) Rodent eggs are also polarized with an asymmetrically localized region for sperm interactions. Sperm bind to and fuse with a limited portion of the rodent egg plasma membrane, known as the microvillar domain, located away from the meiotic spindle (figure 2B). The eggs from non-rodent species do not have this asymmetric localization of microvilli on the plasma membrane (12), although they may have more subtle asymmetries and it is unknown if such asymmetries could affect sperm binding and fusion sites.

3. METHODS USED TO STUDY SPERM-EGG INTERACTIONS

Experimental methods that have been used to study cell adhesion have also been used to study sperm-egg interactions. The "cell adhesion assay" used is *in vitro* fertilization (IVF), with sperm-egg binding and fusion assayed as endpoints (see Sections 3.1 and 3.2). Reagents, such as antibodies, synthetic peptides, and reduced. (In Panel B, the majority of the amicrovillar region is out of the plane of

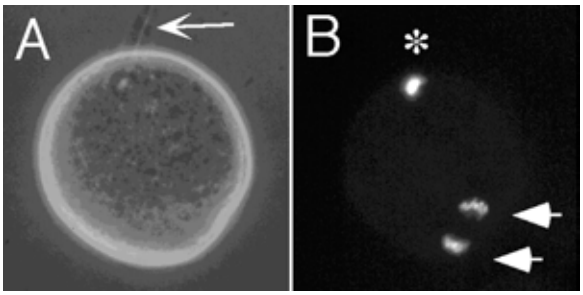

Figure 3. DAPI staining to reveal sperm decondensation in the egg cytoplasm. A ZP-free egg was inseminated for 45 minutes, then fixed and stained with 2 ug/ml DAPI. Panel A shows the phase contrast image, with the sperm tail visible outside the plane of focus (arrow). Panel B shows the swollen DAPI-stained sperm nucleus (asterisk) within the egg cytoplasm. The egg has exited from metaphase; the segregating chromosomes in anaphase are evident (arrowheads).

Figure 4. Domains of proteins in the ADAM/MDC family. This diagram shows the typical domain structure identified from the amino acid sequences deduced from cDNA clones of ADAM/MDC family members (amino-terminus to the left). These domains include a signal sequence (not labeled), a prodomain, a metalloprotease domain (MP), a disintegrin domain, a cysteine-rich domain (Cys-rich), an EGF-like repeat (EGF), a transmembrane domain (TM), and a cytoplasmic tail.

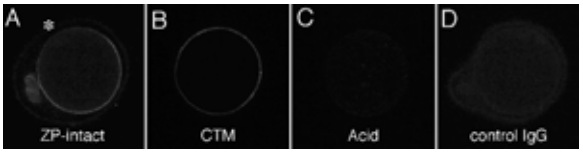

Figure 5. Staining of eggs with the anti-alpha-6 integrin subunit mAb GoH3. Live mouse eggs (ZP-intact [Panels A and D] or with the ZP removed by either treatment with chymotrypsin combined with mechanical shearing [CTM, Panel B] or by incubation in acidic medium to solubilize the ZP proteins [acid; Panel C]) were incubated in medium containing 0.5 mg/ml of the mAb GoH3 IgG (or, in Panel D, a nonimmune rat IgG) for 45 mintues. The eggs were then washed and fixed (3.7% paraformaldehyde in PBS), stained with an anti-rat fluorescent secondary antibody, and then viewed by confocal microscopy. The GoH3 antibody binds to the plasma membrane of ZP-intact eggs (Panel A) and of CTM-treated ZP-free eggs (Panel B). In Panel A, the intensity of labelling over the amicrovillar region is the GoH3 antibody does not bind to the plasma membrane of the acid-treated ZP-free eggs, suggesting that the 10-15 second exposure to acidic medium (pH 1.5) destroys the epitopes for this antibody. However, it should be noted that although acid-treated eggs lack the GoH3 epitope, they are still capable of binding sperm, recombinant fertilin beta, and recombinant fertilin alpha (16,68).

focus.) However, purified proteins, are tested in IVF assays to see if they can perturb the interactions between gametes. Several sperm molecules that participate in fertilization have been identified by a method similar to the method that identified integrins: making a battery of monoclonal antibodies to intact sperm (e.g., (13)) and then testing the abilities of these antibodies to perturb sperm-cumulus cell complex interactions, sperm-ZP interactions, or sperm-egg membrane interactions.

3.1. Measuring and detecting sperm-egg fusion

There are two measures of sperm-egg fusion: (a) the percentage of eggs in a sample that have fused with at least one sperm (also called the "fertilization rate"); and (b) average number of sperm fused per egg (this measurement, also called the "fertilization index," is an indicator of the extent of polyspermy). Sperm-egg fusion is easily detected because the sperm head decondenses inside the egg cytoplasm. In some species, the swollen sperm head in the egg cytoplasm is visible by phase contrast microscopy. The inseminated eggs can be also stained with a DNA-staining dye, making the decondensing sperm DNA, with the associated sperm tail outside the egg, readily apparent (figure 3). Eggs can also be loaded with a cell-permeable DNA-staining dye prior to fertilization such that, upon penetration of the egg by a sperm, the dye transfers from the egg cytoplasm to stain the sperm DNA (14).

3.2. Measuring and detecting sperm-egg binding

Sperm-egg binding (measured as the average number of sperm bound per egg) is an operational definition: the number of sperm that stay attached to the egg plasma membrane after a specific, controlled series of washes. Consequently, the actual sperm-egg binding observed in an experiment is highly dependent on numerous variables in the washing protocol, including the diameter of the pipet used to transfer the eggs between wash drops, the medium the eggs are washed in, and the pressure used to blow the eggs out of the pipet (which is done by mouth pipetting). Unfortunately, these variables make it virtually impossible to compare results on sperm-egg binding from one study to another. It should also be noted that the sperm that are defined as bound to the egg must be in the proper orientation, bound by the appropriate regions of the sperm head to the appropriate region of the egg plasma membrane (See figure 2 and Section 2).

It must be emphasized that binding is a prerequisite for fusion. This fact affects the interpretation of results from IVF experiments and thus must be kept in mind when considering the data in the literature. Some experimental treatments (e.g., antibody, peptide) reduce the average number of sperm fused per egg without affecting sperm-egg binding; such a result implicates the molecule of interest specifically in the fusion process (e.g., (15)). Other experimental treatments reduce the average number of sperm bound per egg without affecting sperm-egg fusion (i.e., the few sperm that were able to bind are still capable of fusing with the egg membrane); this result suggests that the molecule of interest participates in the binding of sperm to egg. A third possibility is that decreases in both sperm-egg binding and sperm-egg fusion are observed. In this

instance, it is possible (and likely) that sperm-egg fusion is decreased as a downstream effect of a substantial decrease in the prerequisite step, sperm-egg binding, although a specific effect on fusion is also a formal possibility. Thus, such a result makes it somewhat more difficult to dissect out the roles for particular molecules. One can conclude that sperm-egg binding was affected, but one cannot state conclusively that sperm-egg fusion was specifically affected, because the observed decrease in sperm-egg fusion may be a consequence of reduced sperm-egg binding.

3.3. IVF assays: ZP-intact and ZP-free eggs, and homologous versus heterologous systems

IVF experiments can be performed with either ZP-intact eggs or eggs from which the ZP have been removed (ZP-free eggs). Both methods have advantages and disadvantages. Inseminating ZP-intact eggs is less artificial, because ZP-removal treatments can modify the egg plasma membrane (see Section 5.2 and reference (16)). However, the disadvantage of using ZP-intact eggs is that it is very difficult to assess sperm-egg membrane binding. Sperm inseminating a ZP-intact egg are trapped within and under the ZP and thus cannot be washed off the egg plasma membrane. Therefore, it is virtually impossible to define a sperm as "bound" (i.e., staying bound after a series of defined washes; see Section 3.2) to the membrane of a ZP-intact egg. For assessment of sperm-egg binding, ZP-free eggs are used. However, the concentration of sperm that is used to inseminate ZP-free eggs must be carefully controlled, because the insemination of ZP-free eggs can result in a high incidence of polyspermy as ZP-free eggs lack the ECM that serves as the block to polyspermy after penetration of the egg by the first sperm (1). The observation of inhibition of sperm-egg binding in the presence of a relatively high sperm concentration could be indicative of a potent inhibitory effect of a reagent. On the other hand, inseminating ZP-free eggs with a low concentration of sperm could conceivably reveal more subtle inhibitory effects.

It should also be noted that heterologous systems (i.e., sperm from one species and eggs from another species) are occasionally used. ZP-free hamster eggs are commonly used, because they are capable of fusing with sperm from nearly every mammalian species tested (3). This is frequently referred to as the "zona-free hamster egg penetration test." It is not clear how biologically relevant the zona-free hamster egg penetration test is. The promiscuity of hamster egg fusion suggests that this process may occur via a mechanism that is unique to the hamster egg, and some studies provide evidence of this. For example, an anti-sperm monoclonal antibody was found to inhibit the fusion of hamster eggs with guinea pig sperm, but have no effect on the fusion of guinea pig eggs with guinea pig sperm (17). Furthermore, since human eggs inherit a centrosome from the fertilizing sperm but hamster eggs do not, the hamster eggs may be inappropriate for studying some causes of human male-factor fertility (18). Nevertheless, the zona-free hamster penetration test has proved to be extremely useful, particularly in studies of human sperm and sperm from other species for which homologous eggs are not readily obtainable.

4. SPERM DISINTEGRINS

4.1. General information on ADAM/MDC family members

The sperm protein fertilin, originally known as PH-30, is perhaps the best-characterized molecule that mediates sperm-egg membrane interactions. The characterization of this sperm protein led to the identification of a new molecular family of proteins that has been termed ADAM's (for A Disintegrin and A Metalloprotease domain) (19) or MDC's (for Metalloprotease/Disintegrin/Cysteine-rich) (20-22). The three sperm disintegrins that I will discuss, fertilin beta, fertilin alpha, and cyritestin, are members of this molecular family.

The first members of the ADAM/MDC family were reported six years ago, including EAP-1, a protein expressed in the apical surface of epididymal epithelium in an androgen-dependent manner (20), MDC, a candidate breast cancer tumor suppressor (23), and guinea pig fertilin alpha and beta (24,25). Since then, several experimental approaches, including PCR with degenerate primers (26-33), cDNA library screens (19), and genetic studies (34) have identified many more members of this family in several mammalian species, *Xenopus laevis*, *Drosophila*, and *C. elegans*. Several members of the ADAM/MDC family are expressed by spermatogenic cells, and some of these appear to be testis-specific. Other ADAM/MDC members show much wider tissue distribution. Since a number of good reviews have been published discussing the traits of these proteins, the various family members, and their possible functions (35-38), I will not go into the ADAM/MDC family at length here. However, several features of these proteins should be highlighted with respect to their potential roles in mediating sperm-egg interactions.

The names "ADAM" and "MDC" reflect the conserved domain structure observed in the family members that have been cloned and characterized. These domains include a signal sequence, a prodomain, a metalloprotease domain, a disintegrin domain, a cysteine-rich domain, an EGF-like repeat, a transmembrane domain, and a short cytoplasmic tail (figure 4). This domain structure is partially shared by the snake venom metalloproteases (SVMP's), which include a metalloprotease domain, a disintegrin domain, and/or a cysteine-rich domain, depending on their size (39). Class II SVMP's have a metalloprotease domain and a disintegrin domain. Class III SVMP's have a metalloprotease domain, a disintegrin domain and a cysteine-rich domain.

All ADAM/MDC proteins have disintegrin domains of approximately 80-90 amino acids in length. These domains have homology to the disintegrin domains found in SVMP's (39,40). It should be noted that the disintegrin domains in class III SVMP's and ADAM/MDC proteins have been called "disintegrin-like domains" (41),

because they differ from the "true" disintegrin domains in the smaller class II SVMP's in two ways. First, the disintegrin-like domains of ADAM/MDC proteins and class III SVMP's have two extra cysteine residues that are lacking in class II SVMP's, suggesting that the disulfide bonding pattern and the three-dimensional structures of the disintegrin-like domains might differ from those of true disintegrin domains (42-44). Second, class II SVMP's have RGD sequences within their disintegrin domains which is believed to be presented at the end of an extended loop, based on NMR structures of smaller snake venom disintegrins (that lack a metalloprotease domain) (42,43). In contrast, ADAM/MDC proteins (with one exception (45)) and class III SVMP's lack the RGD consensus sequence that is present in class II SVMP's and integrin ligands such as fibronectin, vitronectin, and fibrinogen. The RGD-containing disintegrins bind to platelet integrins (such as alpha-IIb/beta-3 and alpha-v/beta-3) and disrupt the binding of fibrinogen, thus preventing platelet aggregation and clot formation. The identification of similar disintegrin domains in ADAM/MDC proteins has led to the hypothesis that a disintegrin domain-containing protein on sperm (a) could function as a cell adhesion molecule to mediate sperm-egg binding, and (b) could bind to an egg integrin.

4.2. Fertilin beta (ADAM2)

As noted above, fertilin is the best-characterized candidate molecule that mediates sperm-egg membrane interactions. Fertilin was originally identified in a screen of a battery of monoclonal antibodies made against the surfaces of guinea pig sperm. Several of these anti-sperm surface antibodies were found to bind the posterior head (13), a region of the sperm head involved in sperm-egg fusion (1). One of these antibodies that bound to the posterior head, known as PH-30, inhibited sperm-egg fusion of ZP-free guinea pig eggs (46). The antigen of the monoclonal antibody (mAb) PH-30 was subsequently named fertilin to reflect its role in fertilization (47). Fertilin is a heterodimeric protein (46,48,49), and may form higher order oligomers (49). The two subunits that comprise fertilin, known as fertilin alpha and fertilin beta, are both members of the ADAM/MDC family of proteins (see Section 4.1). Fertilin beta homologues from seven species (guinea pig, mouse, macaque, human, bovine, rat, rabbit) have been cloned.

Both fertilin alpha and fertilin beta are proteolytically processed during sperm development, fertilin alpha in the testis and fertilin beta during epididymal transit (48); the sizes of the proteins vary slightly by species (48-53). Based on studies in the guinea pig, it appears that a trypsin-like serine protease(s) is responsible for the proteolytic processing of fertilin beta, whereas fertilin alpha is cleaved intracellularly, possibly by subtilysin-type pro-protein convertase (54). In the guinea pig, proteolysis of fertilin coincides with its appearance in the posterior head of the sperm during epididymal transit (55). Slightly different localizations for fertilin beta have been reported in different species, including the equatorial region and the inner acrosomal membrane (49,51,52,56). These differences could be due to several factors, including species-to-species variations, antibodies, and experimental techniques.

Both fertilin alpha and beta are cleaved between the metalloprotease domain and the disintegrin domain based on N-terminal amino acid sequence data (49,54) or on the size of the protein observed on mature sperm (51,52). Thus, the forms of these proteins on mature sperm have only the disintegrin domain, the cysteine-rich domain, and the EGF-like repeat on their extracellular surface. (NOTE: It was originally believed that fertilin alpha was cleaved such that it only had the last 20 amino acids of the disintegrin domain (24). However, more recent work has shown that guinea pig and bull sperm actually have the complete disintegrin domain of fertilin alpha on their surfaces (49,54).

In the position of the R-G-D tripeptide in snake venom disintegrins, the consensus sequence in fertilin beta (based on cDNA clones from seven species) is X-D/E-E, where X is any amino acid (table 1). Thus, this was proposed to be the tripeptide sequence active in sperm-egg interactions. It should also be noted that the sequence E-C-D that follows the X-D/E-E is completely conserved in all fertilin beta homologues and in some other ADAM/MDC's, prompting the suggestion that this sequence is involved in sperm-egg interactions (27). Beads coated with synthetic peptides corresponding to the fertilin beta disintegrin loop bind to guinea pig eggs (47). More importantly, these and similar peptides inhibit gamete interactions in homologous systems (guinea pig (47) and mouse (52,57,58)) and a heterologous one (hamster eggs and human sperm (59)). However, it is not known precisely which amino acids (X-D/E-E and/or ECD) comprise the active site of the fertilin beta disintegrin loop (see table 1) because several variations of these peptides inhibited sperm-egg binding in these assays: (a) peptides that include the full X-D/E-E-C-D sequence (47,52,59), (b) peptides that include the X-D/E-E but truncate (47,58) or point-mutate (57) the E-C-D sequence, and (c) peptides that include only the E-C-D sequence (60). The identification of the functional determinants (e.g., the X-D/E-E sequence and/or the E-C-D sequence) within the fertilin beta disintegrin domain will be a focus of future studies.

Other data, in addition to these synthetic peptide studies, indicate that fertilin beta functions as a cell adhesion molecule. A bacterially-expressed recombinant form of extracellular portion of mouse fertilin beta (hereafter referred to as recombinant fertilin beta) binds to the microvillar region of mouse eggs (figure 2B) in a divalent cation-dependent manner (16), and this divalent cation dependence is similar to the divalent cation dependence of mouse sperm binding (58). The binding of recombinant fertilin beta can be inhibited with synthetic peptides corresponding to the disintegrin domain (16). Incubation of mouse eggs in recombinant fertilin beta prior to insemination inhibits sperm-egg binding during IVF (16). Likewise, incubation of sperm in antibodies against the disintegrin domain of mouse fertilin beta prior to insemination also inhibits sperm-egg binding and fusion (52). Anti-fertilin beta antibodies also reduced the

Table 1. Comparison of the disintegrin loops of different ADAM/MDC family members **AU: Changes OK?**

MOLECULE	CONSENSUS SEQUENCE	REFERENCE
Snake disintegrin consensus	**C-F---G--CR--RGD------C-G-S—C**	
Mouse fertilin beta (ADAM2)	CKLKRKGEVCRLAQDECDVTEYCNGTSEVC	(19,58)
Guinea pig fertilin beta	CEFKTKGEVCRESTDECDLPEYCNGSSGAC	(25)
Rabbit fertilin beta	CTFKEKGQSCRPPVGECDLFEYCNGTSALC	(50)
Bovine fertilin beta	CAFIPKGHICRGSTDECDLHEYCNGSSAAC	(49)
Rat fertilin beta	CNLKAKGELCRPANQECDVTEYCNGTSEVC	(51)
Macaque fertilin beta	CLFMSQERVCRPSFDECDLPEYCNGTSASC	(27)
Human fertilin beta	CLFMSKERMCRPSFEECDLPEYCNGSSASC	(143,144)
FERTILIN BETA CONSENSUS	**C----*--*CR---*ECDL-EYCNG**--C**	
Mouse fertilin alpha(ADAM1)	CTFKKKGSLCRPAEDVCDLPEYCDGSTQEC	(19)
Guinea pig fertilin alpha	CQYKNSGYLCRPSVGPCDLPEYCTGQSGKC	(25)
Rabbit fertilin alpha	CKYRRKGFLCRSIGRNCDLPEYCSGKSASC	(50)
Bovine fertilin alpha	CQYERTGRSCRPASGECDLPEFCLGTSGEC	(49)
Rat fertilin alpha	CTFKKKGTLCRPAEDVCDLPEYCNGITGEC	(51)
Macaque fertilin alpha	CTFRRKGFLCRPTQDECDLPEYCDGSSAEC	(27)
Tamarin fertilin alpha	CTFRRKGFLCRPTQDECDLPEYCDGSSAEC	(145)
Baboon fertilin alpha	CTFRRKGFLCRPTQDECDLPEYCDGSSAEC	(145)
Orangutan fertilin alpha	CKFQRKGYPCRPSSRSCDLPEFCNGTSALC	(145)
FERTILIN ALPHA CONSENSUS	**C---*-G--CR-----CDLPE*C-G-*--C**	
Mouse cyritestin (ADAM3)	CTIAERGRLCRKSKDQCDFPEFCNGETEGC	(19,76)
Macaque cyritestin	CTIYARGHVCRKSIDMCDFPEYCNGTSEFC	(75)
Human cyritestin	CTIHERGHVCRKSVDMCDFPEYCNGTSEFC	(146)
CYRITESTIN CONSENSUS	**CTI--RGH*CRKS-D-CDFPE*CNG-SEFC**	
Mouse ADAM4	CKFAPTGTICRDKNGICDLPEYCSGASEHC	(19)
Mouse ADAM5	CTVKMNDVVCRKSVDECDLLEYCNGLDPYC	(19)
Human ADAM20	CKFLPSGTLCRQQVGECDLPEWCNGTSHQC	(72)

incidence of fertilization of rabbit eggs *in vitro* (56). A recombinant form of mouse fertilin beta that lacks the disintegrin domain is unable to inhibit sperm-egg binding during IVF (61). Finally, the fertilin beta gene has been disrupted by homologous recombination with a targeting gene construct corresponding to the exon encoding the fertilin beta disintegrin domain (62). Sperm from males homozygous for this disrupted fertilin beta gene show greatly reduced ability to bind to egg plasma membranes, although the few sperm that are able to bind are capable of sperm-egg fusion, and egg activation occurs normally (62).

There are several interesting questions that remain to be resolved regarding fertilin beta's role in fertilization. First, the phenotype of the fertilin beta knock-out is complex, with multiple facets of sperm function affected (reduced levels of sperm transit from the uterus to the oviduct, reduced sperm-ZP binding, reduced sperm-egg binding (62)), all of which can contribute to male infertility. It is unclear why disruption of the fertilin beta gene affects sperm function on so many levels, but additional insights are certain to come in the future. A second issue to resolve is whether fertilin beta is involved in sperm-egg binding only or possibly in fusion as well. One anti-fertilin beta antibody, the mAb PH-30, inhibits guinea pig sperm-egg fusion (46), and a subsequent review article notes that the mAb PH-30 had no effect on sperm-egg binding (63). However, other reagents, including synthetic peptides (52,58), recombinant fertilin beta (16), and anti-fertilin beta antibodies (52), perturb mouse sperm-egg binding (with varying downstream effects on sperm-egg fusion; see Section 3.2). This could be a species difference (guinea pig versus mouse), or possibly the mAb PH-30 binds to a specific epitope of fertilin beta that participates in gamete fusion. Finally, a few pieces of recent data suggest that fertilin beta and fertilin alpha could be involved in sperm-ZP binding as well as sperm-egg membrane binding. As noted above, sperm from fertilin beta knock-out mice show reduced ZP binding (62). Furthermore, a report by Hardy et al. (56) showed that sperm showed reduced binding to the ZP of ZP-intact eggs in the presence of anti-fertilin beta and anti-fertilin alpha antibodies. In agreement with this, recombinant fertilin beta and recombinant fertilin alpha reduce the fertilization rates of ZP-intact eggs (61), although sperm-ZP binding was not directly examined in this study. These observations, however, fall far short of confirming a role for fertilin alpha and beta in sperm-ZP binding; the possible role of these two sperm proteins in ZP binding needs to be examined more closely.

4.3. Fertilin alpha (ADAM1)

Somewhat less is known about fertilin alpha as compared to fertilin beta. Initial data suggested that fertilin alpha on mature sperm lacked the disintegrin domain (24), and thus fertilin beta garnered much of the early attention. However, fertilin alpha has since been found to have an intact disintegrin domain on sperm from two different species (49,54). Fertilin alpha also has two noteworthy features not shared by fertilin beta and cyritestin. First, fertilin alpha and a subset of other ADAM/MDC proteins have the consensus active site (HEXXHXXGXXHE) required for enzymatic activity (64) within their metalloprotease domain. However, it is not clear if fertilin alpha possesses or exerts metalloprotease activity (54). In contrast, fertilin beta and cyritestin lack this consensus sequence. Second, in some species, fertilin alpha has a short sequence within its cysteine-rich domain that has similarity to viral fusion peptides. Fertilin beta and

cyritestin lack putative fusion peptides. Viral fusion peptides are hydrophobic stretches of about 20 amino acids that are believed to insert into lipid bilayers to facilitate bilayer mixing leading to membrane fusion (65-67). Thus, these two domains implicate that fertilin alpha could be involved in both sperm-egg binding and in sperm-egg fusion.

Although the story is less complete than that for fertilin beta, there are experimental data suggest that fertilin alpha functions as a cell adhesion molecule during fertilization. A bacterially-expressed form of the extracellular portion of mouse fertilin alpha binds to the microvillar region of mouse eggs and inhibits sperm-egg binding (61). Surprisingly, a truncated form of recombinant fertilin alpha that lacks the most of the disintegrin domain, corresponding to the initial N-terminal sequence data of guinea pig fertilin alpha (24), is also able to bind to eggs and inhibit sperm-egg binding (61,68). However, the disintegrin-lacking form of recombinant fertilin alpha is less effective at inhibiting sperm-egg binding than is recombinant fertilin beta (68) and recombinant fertilin alpha that includes the disintegrin domain (61). It should be emphasized that truncation of the disintegrin domain could affect the folding of the fertilin alpha protein, and thus these studies indicate that the fertilin alpha disintegrin domain is involved in either interacting with egg binding sites or in maintaining proper folding (61). Synthetic peptides corresponding to the disintegrin loop of fertilin alpha (AEDVCDLP; see table 1) have a slight inhibitory effect on sperm-egg binding and fusion (52). The effect is modest compared to fertilin beta and cyritestin peptides, and, moreover, background levels of inhibition of binding with a scrambled peptide control were similar to those for the AEDVCDLP peptide (52).

Taken together, these data suggest that multiple domains of fertilin alpha could participate in sperm-egg interactions. The disintegrin loop of fertilin alpha (AEDVCDLP) could be involved (52), but reagents that include additional portions of molecule appear to be more effective at inhibiting sperm-egg binding than is this synthetic peptide (61). This raises interesting questions about what protein(s) on the egg surface could serve as binding sites for fertilin alpha. For example, the disintegrin domain could interact with an egg integrin(s) and the other domains (cysteine-rich domain and/or EGF-like repeat) could interact with a completely different receptor(s). It is also possible that the putative fusion peptide interacts with lipids in the membrane (69-71) (see below). These studies of different fertilin alpha domains also highlight an important difference between fertilin alpha and fertilin beta. Multiple portions of fertilin alpha (i.e,. the cysteine-rich domain or the EGF-like repeat as well as the disintegrin domain) appear to be involved in sperm-egg interactions; in contrast, the disintegrin domain of fertilin beta appears to be critical for its role in sperm-egg binding (61).

The putative fusion peptide within the cysteine-rich domain implicates fertilin alpha as a candidate to mediate the process of sperm-egg fusion. Another member of the ADAM/MDC family, meltrin alpha, has been implicated in myoblast fusion (28). However, two different fertilin alpha reagents reduce sperm-egg binding (52,61) and thus it is unclear whether the resulting decrease in sperm-egg fusion is due to a direct effect on fusion or if it is simply a downstream effect of reduced sperm-egg binding (see Section 3.2). Synthetic peptides corresponding to the putative fusion peptide of guinea pig fertilin alpha bind to lipid vesicles and/or induce lipid vesicle fusion (69-71), although it is questionable how specific this interaction is. Two studies used the amino acid sequence of the putative fusion peptide of guinea pig fertilin alpha in the correct order (KLICTGISSIPPIRALFAAIQPH (69,71)), while another appears to have used the amino acid sequence in the reverse order (HPIQIAAPLARIPPISSIGYCILK) and compared this peptide to the fusion peptide from HIV2 gp41 in the correct order (70). Additional work is needed to clarify the specificity of the interactions of the putative fusion peptide from fertilin alpha with lipid vesicles.

It should also be noted that the putative fusion peptide is not particularly well-conserved in different fertilin alpha homologues. Similarly aligned amino acid sequences of mouse, rabbit, macaque, and bovine fertilin alpha are not well conserved nor are they as hydrophobic as the putative fusion peptide in guinea pig fertilin alpha (50). However, it is possible that the three-dimensional structure rather than the primary sequence is critical. Studies of synthetic peptides corresponding to the putative fusion peptide of guinea pig fertilin alpha and a mutated form (replacing two prolines with two alanines) revealed that the two prolines in the middle of this region are important for the peptides to have the abilities to assume a beta-structure in the presence of lipid vesicles and to induce lipid vesicle fusion (71). These prolines could be significant since this residue is known to introduce a kink in a polypeptide chain. Interestingly, the first of these two prolines is completely conserved in all species' homologues of fertilin alpha, in mouse meltrin alpha (28), and in human ADAM20 (72) (see below), but not in other ADAM/MDC family members. It is also possible that fusion peptides are in different regions of the molecule, as analysis of bovine fertilin alpha reveals that there is a beta sheet with a hydrophobic side in a different region of the cysteine-rich domain (49). Nonetheless, whether fertilin alpha from any species has a functional fusion peptide remains to be experimentally demonstrated.

Finally, it is worth noting that a functional fertilin alpha gene has yet to be identified in humans. Instead, the fertilin alpha-like "pseudogene" in both humans and gorillas contains numerous insertions, deletions, and termination codons (73,74). The recently identified human ADAM20 shares several traits with fertilin alpha, including the consensus active site for metalloprotease activity and a putative fusion peptide, and it has been speculated that ADAM20 may function in place of fertilin alpha (72). However, fertilin alpha participates in sperm-egg binding in the mouse, as do several other proteins, including fertilin beta and cyritestin. If such redundancy also exists in

sperm-egg binding in the human, then a functional fertilin alpha protein on the human sperm surface would not be required for successful sperm-egg binding since other sperm proteins could also conceivably mediate in this process. It is not known what role fertilin alpha has in other tissues where it is expressed (35); ADAM20 is apparently expressed in at least two other cell types/tissues (B-cells and placenta), albeit at lower levels than in testis (72).

4.4. Cyritestin (ADAM3, tMDCI)

Cyritestin was originally identified in monkey by a PCR cloning strategy to identify ADAM/MDC family members (75), and in mouse in a screen of a testis cDNA library as a cDNA that hybridized to testis polyA+ RNA, but not liver or kidney polyA+ RNA (76). The name of the protein refers to its molecular composition and its expression pattern (Cysteine-rich, testicular). Cyritestin is expressed as a larger precursor (110 kD) which appears to be proteolytically processed to a smaller, mature form (55 kD) as sperm in the testis are released into the lumen of the seminiferous tubules (known as testicular sperm) and are then transported to the epididymis (known as epididymal sperm). Results from immunoblotting of epididymal sperm extracts with different antibodies suggest that processing could be either complete (52,77) or partial (52) in the mouse; processing appears to be complete in the monkey (53). It is not known if cyritestin is complexed with another ADAM/MDC family member, as fertilin alpha and beta are dimerized in guinea pig and bull sperm (46,48,49). Cyritestin has been observed localized to the inner acrosomal membrane of acrosome-reacted or permeabilized mouse sperm (77) and to the equatorial region of live acrosome-intact and acrosome-reacted mouse sperm (52).

Cyritestin has the characteristic domain structure of ADAM/MDC family members. There is no active site consensus sequence in its metalloprotease domain, suggesting that this domain is enzymatically inactive, and a hydropathy plot suggests that cyritestin lacks a putative fusion peptide in the cysteine-rich domain (76). There is evidence, however, that the disintegrin domain of cyritestin is involved in sperm-egg interactions. Synthetic peptides corresponding to the disintegrin loop of cyritestin, both as a 12-mer RKSKDQCDFPEF (78) and an 8-mer SKDQCDFP (52), are effective at inhibiting sperm-egg interactions and reducing the incidence of fertilization. Fluorescently-conjugated RKSKDQCDFPEF peptides were also shown to bind to ZP-free mouse eggs (78), although it is difficult to discern if binding is limited to the microvillar region of the plasma membrane. It is worth noting that cyritestin peptides were by far the most effective at inhibiting sperm-egg binding in a comparative study of synthetic peptides from five different ADAM/MDC family members, including fertilin beta (52). Preincubation of sperm with antibodies to the cyritestin disintegrin domain (a 23-mer peptide, spanning amino acids 453-475) also reduced sperm-egg binding and fusion during IVF; in these experiments, anti-cyritestin and anti-fertilin beta disintegrin domain antibodies were comparably effective (52).

5. EGG INTEGRINS AS POSSIBLE RECEPTORS FOR SPERM AND SPERM DISINTEGRINS

5.1. Integrins on mammalian eggs

The identification of a disintegrin domain in guinea pig fertilin beta (24) led to the hypothesis that this sperm protein bound to an integrin on the egg surface. There are now at least three sperm disintegrins to consider: fertilin beta, fertilin alpha, and cyritestin (16,47,52,57,58,61,78). Since the disintegrin domains of fertilin alpha and cyritestin have only recently been implicated in sperm-egg interactions, most studies to date have focused only on fertilin beta as a potential sperm ligand for an egg integrin, although the other sperm disintegrins are also candidates for interacting with egg integrins.

The first data implicating integrins in sperm-egg membrane interactions came from a report that RGD peptides inhibited the interactions of human and hamster sperm and ZP-free hamster eggs (79), which since has also been observed with human sperm and human eggs (80). RGD peptides, however, do not have a significant inhibitory effect on mouse gamete interactions (57,58). In contrast, peptides corresponding to the disintegrin domains of fertilin beta (52,58,81) and cyritestin (52,78) inhibit the interactions of mouse gametes much more effectively than do RGD peptides. It is possible that there is a unique molecule on the hamster egg (and possibly human egg) that is very sensitive to inhibition by RGD peptides (e.g., a molecule that binds RGD peptides very effectively). It should be noted that the disintegrin loops of human fertilin beta and cyritestin do not contain an RGD sequence (table 1).

Several integrin alpha and beta integrins are expressed by mammalian eggs including alpha-2, alpha-3, alpha-4, alpha-5, alpha-6, alpha-v, alpha-M, beta-1, beta-2, and beta-3 (57,82-87). Moreover, anti-integrin antibodies have been reported to perturb sperm-egg interactions. A limited study with human sperm and hamster eggs reported inhibitory effects with anti-alpha-2, anti-alpha-4, and anti-alpha-5 antibodies (87). The alpha-M/beta-2 integrin has also been implicated in studies that used an alpha-M/beta-2 ligand, C3b, and antibodies to this ligand to inhibit fertilization (85) (see Section 6.3).

The egg integrin subunits that have received the most attention are alpha-6 and beta-1, which have been studied almost exclusively in the mouse. Both subunits co-immunoprecipitate from surface-labeled mouse eggs, suggesting that they are paired with each other on the egg plasma membrane (82). It is possible that beta-1 is paired with other alpha subunits as well (82,86). Alpha-6 appears to only be paired with beta-1 (82). (Beta-4, the other common partner of alpha-6, does not appear to be expressed by eggs (88) or co-immunoprecipitate with alpha-6 (82).) Both alpha-6 and beta-1 are localized on the microvillar surface of mouse eggs by immunocytochemistry (57,82,86). Immuno-electron microscopy reveals that alpha-6 and beta-1 are both present on the amicrovillar region, although the density of alpha-6

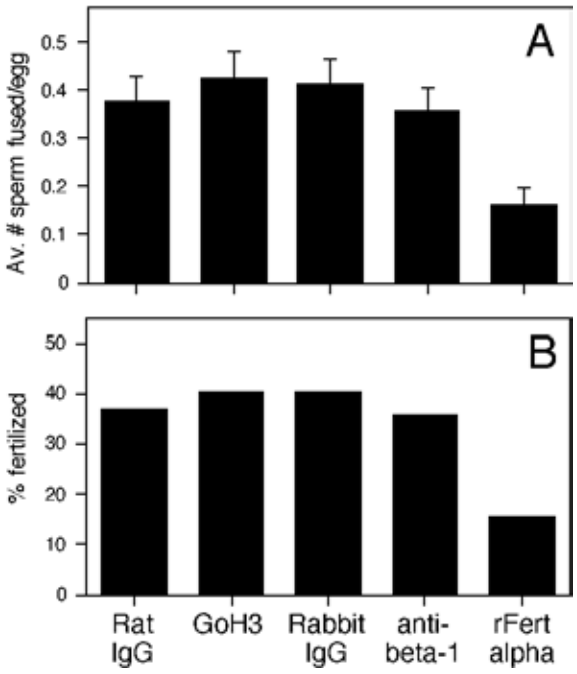

Figure 6. Effects of two anti-integrin antibodies on the incidence of fertilization of ZP-intact eggs during IVF. ZP-intact eggs were collected and incubated in a 5 ul drop of medium containing 0.5 mg/ml of control rat IgG, the anti-alpha-6 mAb GoH3 (as used in (16,57)), control rabbit IgG, an anti-beta-1 polyclonal antibody (as used in (16,57)), or recombinant fertilin alpha (alpha-DCE, which has been shown previously to inhibit IVF of ZP-intact eggs (61)). After 60 minutes, a sperm suspension was added such that the final volume of the medium drop was 10 ul and the final sperm concentration was 1,500,000-2,000,000/ml, as described in (61), resulting in a final concentration of 0.25 mg/ml of protein present during IVF. (NOTE: Small medium volumes are used to conserve antibody. Since IVF of ZP-intact eggs in somewhat inefficient in these small volumes, the control fertilization rates can be 50% or less.) The eggs were then inseminated for three hours, then fixed and stained with DAPI to visualize the sperm in the egg cytoplasm (see figure 3). Panel A shows the average number of sperm fused per egg; Panel B shows the percentage of eggs fertilized. The data in the graphs represent the results of four separate experiments, with 20-25 eggs examined per group per experiment for a total of 92-95 total eggs per group. These experiments show that neither the mAb GoH3 nor the anti-beta-1 polyclonal antibody was effective at inhibiting the fertilization of ZP-intact eggs. In contrast, eggs treated with recombinant fertilin alpha show a statistically significant (ANOVA) difference in both the average number of sperm fused per egg and in the percentage of eggs fertilized, as previously shown (61).

is slightly lower on the amicrovillar region than on the microvillar region (82).

5.2. Alpha-6/beta-1 integrins as receptors for sperm and for sperm disintegrins

There are several pieces of evidence that suggest that the alpha-6/beta-1 integrin can support sperm binding. First, as noted above, both integrin subunits are localized to the microvillar region of mouse eggs, the region to which sperm bind. Second, the anti-alpha-6 mAb GoH3 and an anti-beta-1 polyclonal antibody inhibit mouse sperm binding to ZP-free eggs during IVF (16,57). (In the mouse, sperm-egg fusion does not appear to be inhibited, as the sperm that are able to bind go on to undergo fusion (57). In the human, sperm-egg fusion was partially inhibited by an anti-beta-1 antibody and by GRGDTP peptides; sperm-egg binding was not examined in this study of human gamete interactions (80).) Finally, cells that have been transfected with alpha-6 integrin cDNA also support sperm binding, and this binding can be inhibited by the mAb GoH3 (57). Since these data implicate alpha-6 and beta-1 on eggs as being involved in sperm-egg interactions, the next issue to address is what ligand(s) on sperm could they be binding. Fertilin beta has been examined the most extensively as a possible ligand for alpha-6/beta-1, although, as noted above, there are other sperm disintegrins (fertilin alpha, cyritestin, and potentially others) that merit consideration as candidate integrin ligands on sperm.

The hypothesis that a beta-1 integrin binds fertilin beta is supported by the observation that the binding of recombinant fertilin beta to mouse eggs is reduced in the presence of an anti-beta-1 integrin antibody (16), the same antibody that inhibits sperm binding (16,57). Moreover, the binding of recombinant fertilin beta to mouse eggs is reduced in the presence of synthetic peptides corresponding to the disintegrin domain of mouse fertilin beta (16), suggesting that recombinant fertilin beta interacts with egg plasma membrane receptor(s) via its disintegrin domain. While these data implicate a beta-1 integrin as a receptor for the disintegrin domain of fertilin beta, it should be emphasized that this could be an oversimplified picture of receptor-ligand interactions during fertilization. There is evidence that other integrin ligands such as vitronectin (see Section 6.2) and other sperm disintegrins (see Sections 4.3 and 4.4) could also be involved in sperm-egg interactions and thus could also be a ligand for a beta-1 integrin.

The data that support the hypothesis that an alpha-6 integrin binds fertilin beta are the following. Peptides corresponding to the disintegrin domain of fertilin beta have been reported to reduce the binding of the anti-alpha-6 mAb GoH3 (57), suggesting that the peptides and the antibody bind to the same sites on eggs. Furthermore, results presented at the 1997 ASCB meeting demonstrate that fluorescent beads coated with protein(s) immunoprecipitated from sperm extracts with anti-fertilin beta antibodies bind to eggs and this binding can be inhibited with the GoH3 monoclonal antibody (89). There are, however, caveats to this finding that should be noted. First, the molecular composition of the anti-fertilin beta immunoprecipitates has not been fully characterized. This complex could include fertilin alpha (46,49) and possibly other proteins (90). Second, the mAb GoH3 had no effect on the binding of recombinant fertilin beta to eggs (16),

Table 2. Extracellular Matrix Protein Reagents Examined in IVF Assays

REAGENT	CONCENTRATION	EFFECT ON SPERM-EGG BINDING	EFFECT ON SPERM-EGG FUSION	REFERENCE
Anti-FN antibodies				
• monoclonal	0.2 mg/ml	reduced	reduced	(103)
• polyclonal	0.5 mg/ml	reduced	reduced	(103)
Anti-FN polyclonal antibodies	20% dilution	reduced	none	(104)
RGD peptides[1]	30-250 μM	reduced	reduced	(79)
Echistatin[2]	0.5-10 μg/ml	reduced	none	(147)
GRGDdSP[3]	1-100 μM	none	reduced	(107)
GdRGDSP[4]	1-100 μM	reduced	reduced	(107)
Vitronectin	0.1 μM	increased	none	(107)
Vitronectin	10 μM	reduced	reduced	(107)

[1] RGDV, GRGDS, GRGDTP, [2] Echistatin is an RGD-containing snake venom disintegrin, [3] GRGDdSP blocks fibrnonectin-specific receptors, [4] GdRGDSP blocks fibrnonectin and vitronectin receptors

suggesting that recombinant fertilin beta could be binding to eggs via another site (e.g., a different beta-1 integrin or a completely different molecule). This result does not necessarily rule out alpha-6 as a binding site for fertilin beta. Recombinant fertilin beta was expressed in bacteria and therefore its conformation is likely to be different from native fertilin beta on sperm. However, it is worth noting that certain data indicate that recombinant fertilin beta has at least some conformational similarities with fertilin beta on sperm. Recombinant fertilin beta inhibits sperm-egg binding, presumably via competing with fertilin beta on sperm for egg binding sites, and QDE-containing peptides inhibit the binding of recombinant fertilin beta, suggesting that the disintegrin domain of recombinant fertilin beta is functional in mediating interactions of the protein with egg binding site(s).

Mice that are heterozygous for beta-1 and alpha-6 gene disruptions are fertile, although the eggs from these animals are likely to have both integrin subunits due to maternally contributed mRNA (91). (The fertility of homozygous animals cannot be assessed, as beta-1 -/- embryos die around the time of implantation (92,93), and alpha-6 -/- pups die shortly after birth with severe epithelial defects (94).) However, a different study demonstrated that mouse eggs that have virtually no binding sites for the anti-alpha-6 mAb GoH3 on their surface are still capable of binding sperm and recombinant fertilin beta (16) and recombinant fertilin alpha (68). A particular ZP removal treatment (brief exposure to low pH) appears to adversely affect the GoH3 alpha-6 epitope, as GoH3 immunoreactivity is not detected on these eggs (figure 5C), even with a sensitive luminometric immunoassay. In contrast, GoH3 immunoreactivity is easily detected on ZP-free eggs generated by brief exposure to chymotrypsin (figure 5B) (16). What is the fate of the "low GoH3 eggs" in IVF? They can be fertilized perfectly well, and they support the binding sperm, recombinant fertilin beta and recombinant fertilin alpha (16,68). In addition, recent experiments in my lab using ZP-intact eggs (thus avoiding side effects of ZP removal treatments) show that the mAb GoH3 binds to the egg plasma membrane but does not reduce the incidence of fertilization (figures 5A and 6), in agreement with the finding that GoH3 only perturbs sperm-egg binding and not sperm-egg fusion (57). Taken together, these observations suggest that an alpha-6 integrin can bind to fertilin beta, but also that another molecule(s) on the egg surface (i.e., other than the alpha-6 integrin epitope identified by the mAb GoH3) can mediate the binding of sperm and recombinant fertilin beta. These egg molecules remain to be identified, and could include other alpha-6 epitopes, other integrins, or non-integrin molecules. Furthermore, while the antibody studies are compelling, it should be emphasized that a direct interaction between fertilin beta (or any sperm protein for that matter) with alpha-6/beta-1 has yet to be shown.

6. OTHER MOLECULES INVOLVED IN GAMETE INTERACTIONS

It must be stressed that sperm-egg membrane interactions are likely to involve more that sperm disintegrins and egg integrins. In order to ensure that the reader does not come away with an oversimplified view of how fertilization occurs, some other important molecules that have been implicated in this process must be introduced.

6.1. DE (CRISP-1, ARP)

DE, a 37 kD glycoprotein, is a sperm-associated protein that is synthesized and secreted from the epididymal epithelium in an androgen-dependent manner and is added the surface of sperm head during epididymal transit (95). This protein is also known as CRISP-1, a member of a family of cysteine-rich secretory proteins (96), and as AEG (acidic epididymal glycoprotein) (97). DE has been identified in the rat, mouse, and human by molecular cloning. DE is localized to the dorsal region of the rat sperm head, and translocates to equatorial segment during capacitation, a sperm maturational process that occurs in the female reproductive tract (or can be mimicked *in vitro*) that renders the sperm capable of fertilizing an egg (98). Anti-DE antibodies inhibit fertilization in vivo (99) and sperm penetration of ZP-free rat eggs (100). DE protein purified from epididymal extracts binds to the microvillar region of the rat egg, and inhibits sperm-egg fusion in IVF of ZP-free eggs, but has no effect on sperm-egg binding (15). This is particularly interesting because several other proteins or antibodies also affect sperm-egg binding,

making it difficult to say whether there is any specific effect on sperm-egg fusion (see Section 3.2). DE is one of the few reagents that affects sperm-egg fusion specifically. The human DE-like cDNA is known as AEG-related protein (ARP) (101) and shares 40% homology with DE. While there are conflicting reports on whether DE/ARP is present on human sperm (101,102), recent results show that penetration of ZP-free hamster eggs by human sperm is reduced in the presence of anti-ARP antibodies, but sperm-egg binding is unaffected (D. Cohen and P. Cuasnicu, personal communication). It is not known what the egg receptor for DE is.

6.2. Extracellular matrix proteins

Several extracellular matrix proteins have been observed in human sperm, including fibronectin (103,104), laminin (105), and vitronectin (105-107). The sources of these sperm-associated extracellular matrix proteins have not been definitively determined. Fibronectin could associate with the surface of sperm during spermatogenesis in the testis (108), during epididymal transit (109), or during mixture with seminal plasma (110). Vitronectin mRNA has been detected in Northern blots of human testis (106), although the seminal plasma is another potential source (111). Fibronectin and vitronectin are of particular interest because of their localization. Fibronectin has been reported to be associated with the equatorial segment (104,109,112,113), although other studies observed a uniform distribution over the entire sperm (103,105). Vitronectin appears to be localized in the acrosome of acrosome-intact sperm and released during the acrosome reaction (105,106). Laminin has been reported to be localized on the sperm tail (105) or on the acrosomal membranes (114).

Various reagents, including anti-ECM protein antibodies, soluble ECM proteins, and peptides, have been used in experiments to examine if fibronectin or vitronectin participate in sperm-egg interactions; a summary of these is presented in table 2. Results from these studies suggest a possible role for fibronectin or vitronectin in sperm-egg binding, although it is not known if the reduction in sperm-egg binding observed with these reagents is sufficient to lead to a reduction in sperm-egg fusion. Interestingly, an increase in sperm-egg binding is observed with lower concentrations of vitronectin, whereas a decrease is observed in higher concentrations (107). The reduced sperm-egg binding may be due to agglutination of sperm in the higher concentrations, or perhaps is due to vitronectin serving as a "bridge" between a sperm vitronectin receptor and an egg vitronectin receptor. A similar effect has been observed with complement components C3b and C1q (see Section 6.3). Moreover, several studies have reported a correlation between the presence of extracellular matrix proteins and integrins on the sperm surface and the fertility of the sperm donors (105,115,116), the capacitation or acrosome status of the sperm (105,117,118), or ability of the sperm to fertilize zona-free hamster eggs (119), although other studies dispute the conclusion that these proteins are associated with sperm quality (105,120). Finally, it is unclear if laminin present on sperm is capable of mediating any part of sperm-egg membrane interactions. While it is theoretically possible that laminin on the sperm could serve as a ligand on alpha-6/beta-1 on the egg, recent results presented at the 1995 and 1997 ASCB meetings suggest that laminin binds to eggs under different conditions than do sperm (81,89).

6.3. Complement components and their receptors

Multiple components of the complement pathway and their receptors have been suggested to be involved in sperm-egg interactions, as well as in protection of the sperm from complement attack in the female reproductive tract. Two different soluble complement components have been examined. C3b, which is proteolytic product of the precursor protein C3, binds to MCP (Membrane Cofactor Protein; also called CD46) and to the alpha-M/beta-2 integrin. C1q, a component of the C1 complex, binds to a specific receptor, known as C1qR. C1qR (121) and MCP (122) have been reported to be present on human sperm. The other protein to which C3b binds, the alpha-M/beta-2 integrin, is present on human eggs (85), and C1qR is present on hamster eggs (121). The effects of dimeric C3b on IVF led to the hypothesis that C3b functions as a "bridge," bringing sperm and egg together by binding to MCP on the sperm and to alpha-M/beta-2 on the egg. Low concentrations of dimeric C3b (10 nM-1 uM) actually increased the incidence of fertilization, whereas high concentrations (10-20 uM) of C3b and anti-C3 antibodies inhibited IVF of hamster egg by human sperm. Similar concentration-dependent effects were observed with treating sperm with C1q prior to IVF (121). Anti-C1qR antibodies (121), anti-C3 antibodies (85), and anti-MCP antibodies (85,122) have a moderate inhibitory effect on human sperm-hamster egg binding and fusion during IVF. CD59 (also known as protectin) is complement regulatory protein that has been proposed to be involved in sperm-egg interactions as well, based on the observation of inhibition of human sperm binding and fusing with hamster eggs in the presence of anti-CD59 antibodies (121).

It is also worth noting that an extract from the sperm acrosome cleaves C3 to C3b in vitro, and that C3b binds to the equatorial segment of human sperm (85). By immuno-electron microscopy, MCP immunoreactivity is present on acrosome-reacted (but not acrosome-intact) human sperm, and appears to be localized on the inner acrosomal membrane, the equatorial segment, or perhaps in the acrosomal matrix (122). Thus, the acrosome reaction could be doing two things: (a) cleaving C3 to produce C3b, and (b) exposing MCP on the surface of the acrosome-reacted sperm. The mouse homologue of MCP was recently cloned and determined to be expressed in the testis (123), which could lead to new experimental studies of this protein and its role in fertilization.

6.4. Other molecules

There are a number of molecules that are characterized to varying degrees but that hold considerable promise. First, there is a 94 kD egg protein that is lost from the surfaces of hamster egg upon treatment with trypsin or chymotrypsin (124). Such protease treatment reduces the ability of eggs to bind and fuse with sperm (125) and to

bind recombinant fertilin beta (16) and recombinant fertilin alpha (68). The loss of this trypsin/chymotrypsin-sensitive 94 kD protein correlates with a decrease in the egg's ability to bind and fuse with sperm, and the reappearance of this protein correlates with the recovery of the egg's ability to be fertilized (126). Based on these data, this 94 kD egg protein is a candidate for mediating gamete interactions. Unfortunately, egg molecules can be extremely difficult to characterize due to a paucity of material (25 ng of protein per mouse egg, and 20-50 eggs can be retrieved per superovulated mouse). Other candidate molecules include the antigens of antibodies that inhibit sperm-egg binding or fusion (127-131). Many of these antigens are uncharacterized, but potentially interesting ones are egg surface antigens (131) and sperm antigens that are exposed upon the occurrence of the acrosome reaction (130) or capacitation (132). Genetic analysis of *t* haplotype mice is also likely to produce important findings. The *t* haplotype is an inverted variant of the *t* complex, the proximal portion of mouse chromosome 17 (133). Male mice with two copies of the *t* haplotype are sterile, producing sperm that have motility defects and are unable to fuse with the egg plasma membrane (134). New studies of the *t* haplotype utilizing hybrid sterility analysis has mapped the region responsible for the fusion defect to a distance of 1 cM (135) and in the future could identify molecule(s) involved in gamete membrane interactions. Finally, a membrane glycoprotein that binds sulfated glycolipids known as SLIP1 has recently been reported to be present on the egg plasma membrane and to be involved in sperm-egg membrane interactions (D. White and N. Tanphaichitr, personal communication), possibly by binding to sulfogalactosylglycerolipid present on the sperm surface (136).

7. SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

Studies of fertilization are likely to impact several areas of cell biology and vice versa. The mechanism that initiates the signal transduction events of egg activation will continue to be an exciting area of research, with a focus on whether it involves a traditional receptor-ligand interaction, the introduction of an egg-activiating molecule(s) from the sperm into the egg cytoplasm, or both (7,8,137). Insights into membrane fusion events have been provided by extensive studies of influenza virus, by recent breakthroughs in the understanding of HIV's use of chemokine receptors as coreceptors for fusion, and by research into other cell-cell fusion events, such as the fusion of myoblasts in cell culture or in *Drosophila* embryos (65,67,138). These fields are likely to contribute much to what we know about sperm-egg fusion, and, likewise, what is learned about molecules such as fertilin alpha, DE and other molecules is likely to extend what is known about membrane fusion in general. Moreover, the field of cell adhesion is likely to learn much from the adhesion events between gametes, including new insights into the ADAM/MDC proteins, the identification of new roles for integrins, and possibly completely novel cell adhesion molecules.

It should also be noted that I have only discussed the molecules that are implicated in mammalian fertilization. Fertilization is studied in a wide variety of species, and significant advances have been made in a number of experimental systems, particularly sea urchin and frog; the reader is referred to articles that highlight some of these other species (139-141). Interestingly, an ADAM/MDC family member on *Xenopus* sperm has been implicated in sperm-egg binding (33) and in egg activation (142).

We are left with a long list of molecules that could mediate sperm-egg interactions. There are at least three sperm disintegrins (fertilin alpha, fertilin beta, and cyritestin) that can mediate sperm-egg binding, and there are multiple integrins on eggs. An alpha-6/beta-1 integrin appears to be able to function as a receptor for fertilin beta (57), but it should be noted that other sperm disintegrins and perhaps other integrin ligands on sperm could bind to this receptor. Moreover, it is possible that fertilin beta could bind to other receptors as well (16). Fertilin alpha is also likely to bind multiple receptors, as both its disintegrin domain and other portions of its extracellular domain appear to participate in interactions with the egg plasma membrane (61). Finally, while numerous molecules mediating sperm-egg binding have been identified, there are significantly fewer candidates to mediate sperm-egg fusion, although some molecules (fertilin alpha, DE (15), and products of genes within the *t* haplotype (135), among others) are definite possibilities for mediating this process.

Why are sperm-egg interactions so complicated? It appears, from the large number of molecules addressed above, that gamete binding and fusion are going to involve many different molecular interactions. This might well be anticipated for an event as critical for the survival of a species as fertilization is. Furthermore, the adhesion events between sperm and egg are occurring essentially under flow conditions with sperm's own tail providing the motive force, and thus are somewhat analogous to the flow conditions experienced by leukocytes interacting with the endothelium (5,6). This raises interesting questions about the biochemistry and the biophysics of the adhesion events that will be a focus of future research. The question of whether these molecules provide redundant, back-up mechanisms or whether the molecular interactions need to occur in a specific, orderly fashion will need to be addressed. Such findings could impact our understanding of how normal, healthy reproduction takes place, as well as help develop optimal artificial reproductive technologies to promote the occurrence of fertilization and new ways to perturb the process for contraceptive purposes.

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