Sperm-egg fusion: events at the plasma membrane

Kathryn K. Stein[1,*], Paul Primakoff[2] and Diana Myles[1,‡]

[1]Section of Molecular and Cellular Biology and [2]Department of Cell Biology and Human Anatomy, School of Medicine, University of California Davis, Davis, CA 95616, USA

*Present address: Laboratory of Biochemistry and Genetics, NIDDK, National Institutes of Health, Bethesda, MD 20892, USA
‡Author for correspondence (e-mail: dgmyles@ucdavis.edu)

Journal of Cell Science 117, 6269-6274 Published by The Company of Biologists 2004
doi:10.1242/jcs.01598

Summary

Sperm-egg fusion is a cell-cell membrane fusion event essential for the propagation of sexually reproducing organisms. In gamete fusion, as in other fusion events, such as virus-cell and intracellular vesicle fusion, membrane fusion is a two-step process. Attachment of two membranes through cell-surface molecules is followed by the physical merger of the plasma membrane lipids. Recent progress has demonstrated an essential role for an oocyte tetraspanin, CD9, in mouse sperm-egg fusion, and a specific molecular site crucial for CD9 function has been identified. Absence of glycosylphosphatidylinositol-anchored proteins on the oocyte surface also results in loss of oocyte fusion competence in this gamete. These discoveries provide a strong starting point for the identification of additional proteins that have roles in sperm-egg fusion.

Key words: Membrane fusion, Fertilization, Cell-cell fusion, Sperm, Egg

Introduction

In contrast to cell division, which occurs ubiquitously and frequently during the development of an organism, the fusion of two cells is a rare event, restricted to specific cell types. Here, we discuss the basic strategies for uniting the membranes and cytoplasms of two cells and focus on sperm-egg fusion in mammals as a representative example.

Membrane fusion in animal systems occurs in three contexts: (1) virus-cell fusion, necessary for infectivity of certain viruses; (2) intracellular vesicle fusion, crucial for protein trafficking and exocytosis; and (3) cell-cell fusion. Although relatively little is known about cell-cell fusion, virus-cell fusion and intracellular fusion are better studied (reviewed by Jahn et al., 2003). Although virus-cell fusion and intracellular fusion occur in different cellular environments and require different proteins, some important mechanistic concepts are shared and might also apply to cell-cell fusion.

The membrane fusion process can be divided into three key events. First, membrane recognition (attachment): initial membrane contact is achieved through protein-protein-mediated or protein-carbohydrate-mediated binding of the two membranes. This step ensures target specificity and is referred to as attachment. Second, membrane apposition: the activity of fusion proteins brings the two membranes even closer together. In many systems, this is accomplished by a fusion protein spanning the intermembrane space and physically linking the two membranes (through protein-lipid or protein-protein interactions). One of a variety of factors then induces an irreversible conformational change in which the fusion protein folds back on itself. This shape change results in a hinge-like motion that draws the two membrane-inserted ends of the protein very close together, pulling the membranes with it (Smith and Helenius, 2004). Third, lipid mixing: once membranes are very close, lipid mixing will occur between the proximal membrane leaflets and then the distal leaflets, leading to cytoplasmic continuity between the two cells (Jahn and Grubmuller, 2002). It is not clear whether proteins are necessary for this step, because liposomes can be induced to fuse without proteins. In some viral fusion systems, the fusion peptide is thought to actively disrupt the organization of the lipid in the membrane, which facilitates the fusion event (Tamm et al., 2003). Throughout the following discussion, we refer to proteins thought to mediate step one as ‘attachment’ proteins and steps two and three as ‘fusion’ proteins.

The fusion of two cells is a critical biological event that is required for fertilization in sexually reproducing organisms and for tissue organization during development. It is likely to incorporate the three fundamental steps mentioned above and be mediated by proteins that share features with those involved in other types of membrane fusion. A significant effort has been made to identify putative attachment and fusion proteins in cell-cell fusion systems, particularly in *Drosophila* myoblast fusion and mammalian (primarily mouse) sperm-oocyte fusion. Below, we concentrate on mouse sperm-egg fusion and discuss recent advances in this field, while drawing on our understanding of other fusion systems in order to inform the development of new models.

Attachment and fusion proteins in virus-cell and cell-cell fusion

Attachment proteins are responsible for the initial interaction between two membranes. In virus-cell fusion, virus-receptor interactions are mediated by carbohydrate moieties or cell adhesion domains on proteins or other molecules in the plasma membrane (Bomsel and Alfsen, 2003; Dimitrov, 2004). In many cases, multiple proteins participate in a single virus-cell attachment event, producing a complex interaction that occurs in a limited time frame. Several putative cell-cell attachment proteins share these characteristics. The best-characterized

candidates are the four immunoglobulin (Ig)-superfamily members involved in *Drosophila* myoblast fusion: Sns, Hbs, Duf and Rst (Taylor, 2002). Each of these proteins contains several Ig-like domains, which are well-defined cell-cell adhesion domains. Null mutations in the *sns* or *rst* and *duf* gene, or overexpression of *hbs*, leads to defects in myoblast fusion at the attachment stage. Cells expressing Duf aggregate in vitro with cells expressing Sns and Hbs, and it is likely that these proteins function cooperatively in attachment in vivo. In *Chlamydomonas*, *fus1* mutants are unable to attach to the mating process of the opposite mating type and fusion is never observed (Misamore et al., 2003). Fus1 is a single-pass transmembrane protein sequence that has similarity within five Ig-like repeats to bacterial invasins and intimins, which mediate the adhesion step that precedes bacterial invasion of host cells. The *Caenorhabditis elegans* sperm protein SPE-9 contains ten epidermal growth factor (EGF) repeats in its extracellular domain and is likely to act as an attachment factor in gamete fusion (Singson et al., 1998). All of these proteins contain cell adhesion domains, and optimal function of the myoblast fusion candidates requires the participation of several proteins. The similarities between cell-cell and viral-cell attachment proteins probably stem from their common function and might provide predictive criteria with which to evaluate candidates as for sperm-egg attachment proteins.

Fusion proteins directly mediate the mixing of two membrane bilayers. In virus-cell fusion, a fusion protein typically contains a single transmembrane domain and a fusion peptide – a sequence of 10-30 residues that form an amphiphilic domain at the N-terminus (class I) or within the protein (class II) that is crucial for fusion (Chernomordik and Kozlov, 2003; Jahn et al., 2003). Putative cell-cell fusion proteins are diverse in structure and few match the portrait of a canonical viral fusion protein. Whether this is because an alternative fusion mechanism is used for each case of cell-cell fusion or because these particular proteins do not represent the fusion protein, per se, is not yet clear. A strong candidate for a 'true' fusion protein is the *C. elegans* protein EFF-1, which is necessary (Mohler et al., 2002) and sufficient (Shemer et al., 2004) for most epithelial cell fusion events in the developing worm. EFF-1 is unique in cell-cell fusion systems because it contains a putative fusion peptide within the protein and is thus similar to a class II viral fusagen (Shemer and Podbilewicz, 2003). In *Saccharomyces cerevisiae*, an absence of the pentaspan protein Prm1p on both mating types yields closely apposed (separated by 8 nm), but unfused, membranes in more than half of the mating pairs (Heiman and Walter, 2000). In *Drosophila* myoblast fusion, the only proteins currently shown to have a direct role in fusion are cytoplasmic (Loner, Myoblast city and Rols/Ants) and are probably important for cytoskeletal rearrangement that occurs following attachment and prior to membrane fusion (Taylor, 2003).

The process of fertilization

Mammalian fertilization is a multistep process that culminates in the fusion of the sperm and oocyte plasma membranes (reviewed by Primakoff and Myles, 2002). Mature gametes meet in the oviduct and the sperm penetrates two oocyte barriers – the cumulus cell layer and the zona pellucida (ZP), which is the oocyte extracellular matrix. Binding to the ZP signals the sperm to exocytose its single giant secretory vesicle, the acrosome (Bleil and Wassarman, 1983), and this exocytosis must occur for sperm to become fusion competent. The exocytosis of the acrosome (called the acrosome reaction) results in significant remodeling of the sperm surface. The outer acrosomal and anterior head plasma membranes vesiculate and are lost from the sperm, exposing a new surface membrane (the inner acrosomal membrane) and changing the morphology of the cell. Additionally, the contents of the acrosome contain hydrolytic enzymes, which might modify proteins on the entire sperm surface and prime the sperm for cell-cell attachment and fusion.

Fusion is topologically confined to a specific region of each gamete, which might reflect a unique protein population, lipid organization or composition, or membrane morphology of that region. The sperm plasma membrane overlying the acrosome that does not participate in the acrosome reaction is termed the equatorial region, and the fusion process is apparently initiated in this region of the sperm (Yanagimachi, 1988). The oocyte plasma membrane can be divided into two surface domains: the microvillar-free region, which overlies the meiotic spindle; and the microvillar-rich region, which covers the rest of the oocyte surface. Sperm-oocyte fusion occurs predominantly (Johnson et al., 1975) or exclusively (Ebensperger and Barros, 1984) in the microvillar-rich region. Shortly after fusion, a global exocytic event occurs in the oocyte and the contents of cortical secretory granules are released into the extracellular milieu. Included in these granules are enzymes that modify the ZP in a way that prevents further sperm penetration through this matrix. The oocyte plasma membrane rapidly becomes refractory to a second fusion event, which indicates that the fusion machinery is tightly regulated. How the membrane block to polyspermy is established is not known, but this depends upon incorporation of the sperm membrane into the oocyte plasma membrane, a post-fusion increase in intracellular calcium concentration, and actin polymerization (Maleszewski et al., 1996; McAvey et al., 2002). Fusion competence might be disabled by removal or inactivation of key proteins, or by a chemical or physical block that renders another fusion event less probable.

Sperm-oocyte attachment and fusion

The identification of putative sperm-egg attachment and fusion proteins is a principal goal of ongoing research programs. To date, the primary approach of gamete fusion studies has been to use cell biological strategies to identify candidates for attachment and fusion proteins. Following identification of these candidates, experiments typically involve insemination of superovulated oocytes in the presence of reagents predicted to inhibit the fertilization process. Typically, the ZP is removed from the oocyte by chemical or enzymatic methods in order to focus studies on events that occur at the plasma membrane. Promising candidates can then be pursued by targeted gene deletion in the mouse. Here, we limit our discussion to proteins that have been tested by a gene-knockout approach, which provides crucial in vivo data for evaluation of protein function. Discussion of other candidates can be found elsewhere (Kaji and Kudo, 2004; Cuasnicu et al., 2001; Talbot et al., 2003).

A barrier to the identification of attachment candidates is the lack of a physiologically meaningful in vitro sperm-oocyte

binding assay (Talbot et al., 2003). Most of the sperm bound in the typical adhesion assay cannot progress to fusion. Also, physiologically relevant attachment at the level of the plasma membrane might be difficult to measure owing to the use of various methods of ZP removal (Evans et al., 1997a; Evans et al., 1997b; Yamagata et al., 2002). Specifically, the ZP may not be completely removed by some methods and one cannot distinguish whether sperm are binding to residual ZP or to the plasma membrane, which limits data interpretation (Yamagata et al., 2002). Additionally, sperm in ZP-free conditions must undergo a 'spontaneous' acrosome reaction and these sperm may not be physiologically equivalent to those experiencing a ZP-induced acrosome reaction. Variation in washing protocols to remove 'loosely bound' sperm also prevents reliable quantification of sperm binding and comparison between results obtained in different laboratories. Thus, there is no suitable method currently in use to measure binding, and the only definitive in vitro indicator of a defect in attachment is a defect in fusion. For this reason, we consider the attachment and fusion candidates as one group.

The egg

CD9

CD9 is a member of the tetraspanin protein family and is abundantly expressed on the mouse egg surface. Tetraspanins are found in many species (from *C. elegans* to humans), in virtually all cell and tissue types, and have an important role in membrane organization (Hemler, 2003). Tetraspanins are proposed to mediate several 'levels' of protein interactions, including primary (detergent-stable) interactions with proteins such as integrins, Ig-superfamily members, and membrane-anchored growth factors and secondary interactions with other tetraspanins. Many of these proteins interact with other proteins, and the sum of these interactions is termed the 'tetraspanin web' (Boucheix and Rubinstein, 2001), a very large network of proteins linked by associations (direct and indirect) with tetraspanins. The tetraspanin web is associated with a tetraspanin-enriched microdomain of unique lipid composition that can be differentiated from a classical membrane raft by several criteria (Hemler, 2003). In these microdomains, tetraspanins are poised to regulate critical cellular events that require the cooperative function of several proteins in a specialized cellular domain.

The two closely related tetraspanins CD9 and CD81 are known to be important in membrane fusion events: human CD81 is a co-receptor for the hepatitis C virus (Cormier et al., 2004) and both CD9 and CD81 have been implicated in mammalian myoblast fusion (Schwander et al., 2003; Tachibana and Hemler, 1999) and monocyte/macrophage fusion in mice (Takeda et al., 2003). An unequivocal role for CD9 in sperm-oocyte fusion was established in 2000, when mice with deletions in this gene were generated in three laboratories (Kaji et al., 2000; Le Naour et al., 2000; Miyado et al., 2000). The mice are healthy and viable, but fertility in the females is severely reduced. The *Cd9*$^{-/-}$ females exhibit normal oogenesis and produce mature oocytes in numbers comparable with those of the wild type (Miyado et al., 2000). After mating, many sperm are found in the perivitelline space, indicating that early sperm-oocyte interactions proceed normally and infertility results from a failure of attachment or fusion at the plasma membrane (Kaji et al., 2000; Miyado et al., 2000). The role of CD81 in sperm-egg interactions has not been clarified. CD81 is expressed at low levels on the oocyte (Takahashi et al., 2001). *Cd81*$^{-/-}$ mice have defects in reproduction after several generations of backcrossing (see Deng et al., 2000) but the details are not known because no formal study has been published.

A fundamental question regarding CD9 in sperm-egg fusion is whether it functions in cis with proteins in the oocyte membrane or in trans as an adhesion partner for a sperm protein. CD9 exhibits an orientation in the membrane characteristic of a tetraspanin in which the N- and C-termini are cytoplasmic and two extracellular loops, one small (EC1) and one large (EC2), project into the extracellular space. Emerging evidence for a functional role for EC2 in many tetraspanins (Hemler, 2003) prompted investigators to concentrate on this domain of CD9. The cis or trans function of CD9 was tested in sperm-egg fusion assays by preincubation of a bacterially expressed mouse EC2 construct with either sperm or oocytes prior to insemination (Zhu et al., 2002). Fusion inhibition was observed only when oocytes were pre-incubated with the EC2 construct. Thus, a cis interaction of CD9 with one or more additional proteins on the egg plasma membrane is important for fusion and is consistent with an anticipated role for CD9 as an organizer of the tetraspanin web on the oocyte surface.

Although an interaction in cis is suggested by the experiments with the EC2 domain, CD9 might also work in trans. There is precedence for a trans interaction between CD81 in hepatocytes and hepatitis C virus (Bartosch et al., 2003; Cormier et al., 2004). Recent experiments have demonstrated that the pregnancy-specific glycoprotein 17 (PSG17), a soluble member of the carcinoembryonic antigen (CEA) subfamily of the Ig superfamily, binds to macrophages in trans in a CD9-dependent manner (Waterhouse et al., 2002). PSG17 and CD9 interact directly in vitro and this interaction is dependent on key residues in the EC2 loop (see below) (Ellerman et al., 2003). When eggs are pre-incubated with PSG17, sperm-egg fusion is substantially inhibited (Ellerman et al., 2003). PSG17 itself (or a membrane-anchored PSG17 isoform) is not expressed on sperm and thus is not likely to mediate sperm-oocyte interaction in vivo. However, at least one related protein in the CEA subfamily, sperad, has been reported on guinea pig sperm (Quill and Garbers, 1996). A mouse homolog of this protein will be a good candidate to test for a role in fusion. Although these findings are also compatible with an exclusively cis function for CD9, they raise the possibility that CD9 has an additional, trans role as a receptor for sperm.

Studies of hepatitis virus binding to CD81 showed that a single amino acid (F186) in the EC2 loop is important for cell-virus interactions (Higginbottom et al., 2000). In tests to examine whether the equivalent region of CD9 is critical in gamete fusion, *Cd9*$^{-/-}$ oocytes were injected with mRNA encoding wild-type CD9 or either of two CD9 mutants, F174→A or SFQ173-175→AAA, matured in vitro and inseminated with wild-type sperm (Zhu et al., 2002). Wild-type CD9 rescues fusion in 55% of the oocytes, which otherwise cannot fuse with sperm. The mutants have little (F→A) or no (SFQ→AAA) effect. In similar experiments, mouse CD81 is only moderately effective at rescuing the *Cd9*$^{-/-}$ oocytes (Kaji

et al., 2002). Thus, an initial molecular dissection of the EC2 loop identified a specific site essential for sperm-egg fusion.

Studies of CD9 in other systems indicate a role for this protein in the regulation of cell fusion rather than in directly mediating membrane mixing. In contrast to the requirement for CD9 in sperm-oocyte fusion, in other cell types CD9 and CD81 are important for maintaining a fusion-inactive state. *Cd9*$^{-/-}$*Cd81*$^{-/-}$ double-knockout mice have a high incidence of multinucleated giant cells, which result from cell-cell fusion of monocytes/macrophages (Takeda et al., 2003). Consistent with this finding is the observation that anti-CD9 and anti-CD81 antibodies promote monocyte fusion, although they did not affect adhesion. Therefore, in different cell types (egg versus monocyte/macrophage), the absence of CD9 has opposite effects on the fusion competence. These results might mean that CD9 has cell-type-specific partners that are the key modulators of fusion or that different cell types have different core membrane-fusion machineries that have distinct interactions with CD9.

Glycosylphosphatidylinositol-anchored proteins

Glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-APs) are a class of extracellular glycoproteins that are attached to membranes by a GPI lipid anchor rather than through a transmembrane domain. In vitro studies using phosphatidylinositol-specific phospholipase C, an enzyme that cleaves the GPI anchor, have implicated this class of proteins as having a role in gamete fusion (Coonrod et al., 1999). Oocyte-specific knockout of Pig-A, an enzyme acting in GPI anchor biosynthesis, causes female mice to be infertile owing to a defect in fertilization (Alfieri et al., 2003). Examination of cells in the oviduct 24 hours after mating show that, in wild-type animals, 84% of healthy cells are two-cell embryos, whereas only 1% of cells in the tissue-specific knockouts progress to the two-cell stage. Many of the knockout eggs have sperm in the perivitelline space after mating, indicating that penetration of the cumulus layer and the ZP occurs normally. In vitro, sperm are unable to fuse with ZP-free oocytes from knockout females.

The defect in these oocytes might be due to one of several factors. The oocytes could lack one or more GPI-APs critical for fusion. CD55 has recently been identified as an egg GPI-anchored protein, but mice that have targeted deletions in this gene are healthy and fertile, eliminating a role for this protein in sperm-oocyte fusion (Alfieri et al., 2003; Sun et al., 1999). Identification of prospective candidates in the literature or a proteomic cataloging of oocyte GPI-APs will be useful to address their possible function. The absence of GPI-APs might also result in perturbations in the oocyte plasma membrane. GPI-anchored proteins are enriched in classic lipid rafts, and the loss of GPI-APs could lead to a disruption of these specialized domains.

Integrins

The presence of a disintegrin domain in sperm proteins of the ADAM (for ‘a disintegrin and metalloprotease’) family, together with a series of in vitro experiments, initially pointed to a role for egg integrins in plasma membrane fusion events. However, in vivo tests of a role for integrins have not supported the initial in vitro findings. Knockout experiments using members of the integrin family have so far shown no role for any integrin known to be expressed on the oocyte or known to act as an ADAM receptor – all $\beta1$ integrins, including α subunits 2, 3, 5, 6, 9 and v, $\alpha v\beta3$ integrin and $\alpha v\beta5$ integrin (He et al., 2003; Miller et al., 2000). All female knockout mice lacking these integrins on their eggs have normal fertility in vivo and/or (where applicable) in vitro. Blocking the function of additional integrins in varying combination also does not affect fusion. Although it is still possible that a role for some oocyte integrin in sperm-egg fusion will be found, further consideration of this hypothesis must await new evidence.

The sperm

ADAMs

ADAM-family proteins are ideal candidates for attachment proteins on sperm owing to the presence of a transmembrane domain and two cell-cell adhesion domains – the disintegrin and cysteine-rich domains (Iba et al., 2000; Primakoff and Myles, 2000). The founding ADAM-family members, subunits of the heterodimer fertilin (ADAM1b-ADAM2), were originally identified in a monoclonal antibody screen for guinea pig sperm proteins with a role in gamete fusion (Primakoff et al., 1987). To date, 39 ADAM-family members have been identified. About half of these are testis-specific or testis-enriched proteins, which indicates that this family of proteins might have important roles in sperm function.

The best-studied sperm ADAMs are fertilin β (ADAM2) and cyritestin (ADAM3). Initial experiments in the mouse system showed that peptides from the disintegrin loop of these two ADAMs potently inhibit sperm-oocyte fusion in vitro, whereas the same regions of ADAM1, ADAM4 and ADAM5 are ineffective (Yuan et al., 1997). Targeted deletion of *Adam2* or *Adam3* yields infertile male mice (Cho et al., 1998; Nishimura et al., 2001; Shamsadin et al., 1999). However, both mouse lines show defects in the fertilization process upstream of gamete membrane interactions. *Adam2*$^{-/-}$ sperm exhibit defective transit through the female reproductive tract (Cho et al., 1998). *Adam2*$^{-/-}$ and *Adam3*$^{-/-}$ mice exhibit defective ZP binding (Cho et al., 1998; Nishimura et al., 2001; Shamsadin et al., 1999). In vitro tests of ZP-free oocytes found that *Adam3*$^{-/-}$ sperm bind to the oocyte plasma membrane at very low levels (9% compared with wild type), but fuse at a normal rate (Nishimura et al., 2001). This finding eliminates a required role for cyritestin in physiologically relevant plasma membrane binding or fusion. Such a conclusion is supported by recent data showing that ADAM3 is released from the cell during the acrosome reaction and thus may not be present on the sperm surface as an integral protein at the time of plasma membrane binding (Kim et al., 2004). *Adam2*$^{-/-}$ sperm show a similar reduction in plasma membrane binding, and a 50% decrease in fusion under these in vitro conditions (Cho et al., 1998). The *Adam2*$^{-/-}$*Adam3*$^{-/-}$ double-knockout mice mimic *Adam2*$^{-/-}$ sperm (Nishimura et al., 2001). ADAM2 is not essential for plasma membrane binding or fusion, but could have a contributing role in this process. A molecular dissection of this protein might provide a better understanding of its direct role, if any, in fusion.

Finally, one other factor complicates the interpretation of these data. *Adam2*$^{-/-}$ and *Adam3*$^{-/-}$ sperm do not lack only the

genetically targeted protein. Through an as-yet-undetermined mechanism, the levels of at least two other ADAMs (ADAM3, ADAM1b) are reduced on *Adam2*$^{-/-}$ sperm, and it is possible that the loss extends to other classes of proteins (Nishimura et al., 2001). *Adam3*$^{-/-}$ sperm show a similar, although less-severe, phenotype. An essential role for an ADAM protein in fusion is still possible, because several other ADAMs are expressed on sperm (Brachvogel et al., 2002; Choi et al., 2003; Wolfsberg et al., 1995; Zhu et al., 2001). Recombinant protein and disintegrin peptide loop inhibition studies show a somewhat specific and potent inhibition of fusion (Chen and Sampson, 1999; Yuan et al., 1997). These peptides may inhibit the interaction of some other ADAM with the oocyte.

Perspectives

Progress in the past few years has seen the identification of several proteins important for sperm-egg fusion, although many unresolved questions persist. In order to make advances in the identification of attachment proteins, establishment of a reliable binding assay would be a significant advantage. Improvement upon the present assay might be achieved by the use of green fluorescent protein (GFP)-acrosin sperm (Nakanishi et al., 1999), making it possible to distinguish between nonspecific (acrosome-intact) sperm binding to the oocyte plasma membrane and the binding of acrosome-reacted sperm. A more thorough understanding of the role of CD9 should be sought, including the identification of associated proteins on the oocyte and a possible sperm ligand. In light of recent studies (Cherukuri et al., 2004), domains outside of EC2, especially palmitoylation sites, might also be examined. The presence of Ig-like domains on attachment proteins in other fusion systems and a putative role for a PSG-17-like protein in sperm-egg interactions encourage a close examination of gamete proteins containing Ig domains. The mouse sperm transcriptome has recently been published (Schultz et al., 2003) and is likely to be useful in this capacity. Last, recent technological advances have made genetic mutant screens in the mouse a plausible method by which to identify mutants defective in a biological process (Branda and Dymecki, 2004; Lessard et al., 2004). It is likely that, in the future, 'forward genetics' will provide a powerful approach to identifying candidates for gamete attachment and fusion proteins on sperm and oocytes.

References

Alfieri, J. A., Martin, A. D., Takeda, J., Kondoh, G., Myles, D. G. and Primakoff, P. (2003). Infertility in female mice with an oocyte-specific knockout of GPI-anchored proteins. *J. Cell Sci.* **116**, 2149-2155.

Bartosch, B., Vitelli, A., Granier, C., Goujon, C., Dubuisson, J., Pascale, S., Scarselli, E., Cortese, R., Nicosia, A. and Cosset, F. L. (2003). Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. *J. Biol. Chem.* **278**, 41624-41630.

Bleil, J. D. and Wassarman, P. M. (1983). Sperm-egg interactions in the mouse: sequence of events and induction of the acrosome reaction by a zona pellucida glycoprotein. *Dev. Biol.* **95**, 317-324.

Bomsel, M. and Alfsen, A. (2003). Entry of viruses through the epithelial barrier: pathogenic trickery. *Nat. Rev. Mol. Cell Biol.* **4**, 57-68.

Boucheix, C. and Rubinstein, E. (2001). Tetraspanins. *Cell Mol. Life Sci.* **58**, 1189-1205.

Brachvogel, B., Reichenberg, D., Beyer, S., Jehn, B., von der Mark, K. and Bielke, W. (2002). Molecular cloning and expression analysis of a novel member of the Disintegrin and Metalloprotease-Domain (ADAM) family. *Gene* **288**, 203-210.

Branda, C. S. and Dymecki, S. M. (2004). Talking about a revolution: the impact of site-specific recombinases on genetic analyses in mice. *Dev. Cell* **6**, 7-28.

Chen, H. and Sampson, N. S. (1999). Mediation of sperm-egg fusion: evidence that mouse egg alpha6beta1 integrin is the receptor for sperm fertilinbeta. *Chem. Biol.* **6**, 1-10.

Chernomordik, L. V. and Kozlov, M. M. (2003). Protein-lipid interplay in fusion and fission of biological membranes. *Annu. Rev. Biochem.* **72**, 175-207.

Cherukuri, A., Carter, R. H., Brooks, S., Bornmann, W., Finn, R., Dowd, C. S. and Pierce, S. K. (2004). B cell signaling is regulated by induced palmitoylation of CD81. *J. Biol. Chem.* **279**, 31973-31982.

Cho, C., Bunch, D. O., Faure, J. E., Goulding, E. H., Eddy, E. M., Primakoff, P. and Myles, D. G. (1998). Fertilization defects in sperm from mice lacking fertilin beta. *Science* **281**, 1857-1859.

Choi, I., Woo, J. M., Hong, S., Jung, Y. K., Kim do, H. and Cho, C. (2003). Identification and characterization of ADAM32 with testis-predominant gene expression. *Gene* **304**, 151-162.

Coonrod, S. A., Naaby-Hansen, S., Shetty, J., Shibahara, H., Chen, M., White, J. M. and Herr, J. C. (1999). Treatment of mouse oocytes with PI-PLC releases 70-kDa (pI 5) and 35- to 45-kDa (pI 5.5) protein clusters from the egg surface and inhibits sperm-oolemma binding and fusion. *Dev. Biol.* **207**, 334-349.

Cormier, E. G., Tsamis, F., Kajumo, F., Durso, R. J., Gardner, J. P. and Dragic, T. (2004). CD81 is an entry coreceptor for hepatitis C virus. *Proc. Natl. Acad. Sci. USA* **101**, 7270-7274.

Cuasnicu, P. S., Ellerman, D. A., Cohen, D. J., Busso, D., Morgenfeld, M. M. and da Ros, V. G. (2001). Molecular mechanisms involved in mammalian gamete fusion. *Arch. Med. Res.* **32**, 614-618.

Deng, J., Yeung, V. P., Tsitoura, D., DeKruyff, R. H., Umetsu, D. T. and Levy, S. (2000). Allergen-induced airway hyperreactivity is diminished in CD81-deficient mice. *J. Immunol.* **165**, 5054-5061.

Dimitrov, D. S. (2004). Virus entry: molecular mechanisms and biomedical applications. *Nat. Rev. Microbiol.* **2**, 109-122.

Ebensperger, C. and Barros, C. (1984). Changes at the hamster oocyte surface from the germinal vesicle stage to ovulation. *Gamete Res.* **9**, 387-397.

Ellerman, D. A., Ha, C., Primakoff, P., Myles, D. G. and Dveksler, G. S. (2003). Direct binding of the ligand PSG17 to CD9 requires a CD9 site essential for sperm-egg fusion. *Mol. Biol. Cell* **14**, 5098-5103.

Evans, J. P., Kopf, G. S. and Schultz, R. M. (1997a). Characterization of the binding of recombinant mouse sperm fertilin beta subunit to mouse eggs: evidence for adhesive activity via an egg beta1 integrin-mediated interaction. *Dev. Biol.* **187**, 79-93.

Evans, J. P., Schultz, R. M. and Kopf, G. S. (1997b). Characterization of the binding of recombinant mouse sperm fertilin alpha subunit to mouse eggs: evidence for function as a cell adhesion molecule in sperm-egg binding. *Dev. Biol.* **187**, 94-106.

He, Z. Y., Brakebusch, C., Fassler, R., Kreidberg, J. A., Primakoff, P. and Myles, D. G. (2003). None of the integrins known to be present on the mouse egg or to be ADAM receptors are essential for sperm-egg binding and fusion. *Dev. Biol.* **254**, 226-237.

Heiman, M. G. and Walter, P. (2000). Prm1p, a pheromone-regulated multispanning membrane protein, facilitates plasma membrane fusion during yeast mating. *J. Cell Biol.* **151**, 719-730.

Hemler, M. E. (2003). Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu. Rev. Cell Dev. Biol.* **19**, 397-422.

Higginbottom, A., Quinn, E. R., Kuo, C. C., Flint, M., Wilson, L. H., Bianchi, E., Nicosia, A., Monk, P. N., McKeating, J. A. and Levy, S. (2000). Identification of amino acid residues in CD81 critical for interaction with hepatitis C virus envelope glycoprotein E2. *J. Virol.* **74**, 3642-3649.

Iba, K., Albrechtsen, R., Gilpin, B., Frohlich, C., Loechel, F., Zolkiewska, A., Ishiguro, K., Kojima, T., Liu, W., Langford, J. K. et al. (2000). The cysteine-rich domain of human ADAM 12 supports cell adhesion through syndecans and triggers signaling events that lead to beta1 integrin-dependent cell spreading. *J. Cell Biol.* **149**, 1143-1156.

Jahn, R. and Grubmuller, H. (2002). Membrane fusion. *Curr. Opin. Cell Biol.* **14**, 488-495.

Jahn, R., Lang, T. and Sudhof, T. C. (2003). Membrane fusion. *Cell* **112**, 519-533.

Johnson, M. H., Eager, D., Muggleton-Harris, A. and Grave, H. M. (1975).

Mosaicism in organisation concanavalin A receptors on surface membrane of mouse egg. *Nature* **257**, 321-322.

Kaji, K. and Kudo, A. (2004). The mechanism of sperm-oocyte fusion in mammals. *Reproduction* **127**, 423-429.

Kaji, K., Oda, S., Shikano, T., Ohnuki, T., Uematsu, Y., Sakagami, J., Tada, N., Miyazaki, S. and Kudo, A. (2000). The gamete fusion process is defective in eggs of Cd9-deficient mice. *Nat. Genet.* **24**, 279-282.

Kaji, K., Oda, S., Miyazaki, S. and Kudo, A. (2002). Infertility of CD9-deficient mouse eggs is reversed by mouse CD9, human CD9, or mouse CD81; polyadenylated mRNA injection developed for molecular analysis of sperm-egg fusion. *Dev. Biol.* **247**, 327-334.

Kim, E., Nishimura, H., Iwase, S., Yamagata, K., Kashiwabara, S. and Baba, T. (2004). Synthesis, processing, and subcellular localization of mouse ADAM3 during spermatogenesis and epididymal sperm transport. *J. Reprod. Dev.* **50**, 571-578.

Le Naour, F., Rubinstein, E., Jasmin, C., Prenant, M. and Boucheix, C. (2000). Severely reduced female fertility in CD9-deficient mice. *Science* **287**, 319-321.

Lessard, C., Pendola, J. K., Hartford, S. A., Schimenti, J. C., Handel, M. A. and Eppig, J. J. (2004). New mouse genetic models for human contraceptive development. *Cytogenet. Genome Res.* **105**, 222-227.

Maleszewski, M., Kimura, Y. and Yanagimachi, R. (1996). Sperm membrane incorporation into oolemma contributes to the oolemma block to sperm penetration: evidence based on intracytoplasmic sperm injection experiments in the mouse. *Mol. Reprod. Dev.* **44**, 256-259.

McAvey, B. A., Wortzman, G. B., Williams, C. J. and Evans, J. P. (2002). Involvement of calcium signaling and the actin cytoskeleton in the membrane block to polyspermy in mouse eggs. *Biol. Reprod.* **67**, 1342-1352.

Miller, B. J., Georges-Labouesse, E., Primakoff, P. and Myles, D. G. (2000). Normal fertilization occurs with eggs lacking the integrin alpha6beta1 and is CD9-dependent. *J. Cell Biol.* **149**, 1289-1296.

Misamore, M. J., Gupta, S. and Snell, W. J. (2003). The *Chlamydomonas* Fus1 protein is present on the mating type plus fusion organelle and required for a critical membrane adhesion event during fusion with minus gametes. *Mol. Biol. Cell* **14**, 2530-2542.

Miyado, K., Yamada, G., Yamada, S., Hasuwa, H., Nakamura, Y., Ryu, F., Suzuki, K., Kosai, K., Inoue, K., Ogura, A. et al. (2000). Requirement of CD9 on the egg plasma membrane for fertilization. *Science* **287**, 321-324.

Mohler, W. A., Shemer, G., del Campo, J. J., Valansi, C., Opoku-Serebuoh, E., Scranton, V., Assaf, N., White, J. G. and Podbilewicz, B. (2002). The type I membrane protein EFF-1 is essential for developmental cell fusion. *Dev. Cell* **2**, 355-362.

Nakanishi, T., Ikawa, M., Yamada, S., Parvinen, M., Baba, T., Nishimune, Y. and Okabe, M. (1999). Real-time observation of acrosomal dispersal from mouse sperm using GFP as a marker protein. *FEBS Lett.* **449**, 277-283.

Nishimura, H., Cho, C., Branciforte, D. R., Myles, D. G. and Primakoff, P. (2001). Analysis of loss of adhesive function in sperm lacking cyritestin or fertilin beta. *Dev. Biol.* **233**, 204-213.

Primakoff, P. and Myles, D. G. (2000). The ADAM gene family: surface proteins with adhesion and protease activity. *Trends Genet.* **16**, 83-87.

Primakoff, P. and Myles, D. G. (2002). Penetration, adhesion and fusion in mammalian sperm-egg interaction. *Science* **296**, 2183-2185.

Primakoff, P., Hyatt, H. and Tredick-Kline, J. (1987). Identification and purification of a sperm surface protein with a potential role in sperm-egg membrane fusion. *J. Cell Biol.* **104**, 141-149.

Quill, T. A. and Garbers, D. L. (1996). Sperad is a novel sperm-specific plasma membrane protein homologous to a family of cell adhesion proteins. *J. Biol. Chem.* **271**, 33509-33514.

Schultz, N., Hamra, F. K. and Garbers, D. L. (2003). A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. *Proc. Natl. Acad. Sci. USA* **100**, 12201-12206.

Schwander, M., Leu, M., Stumm, M., Dorchies, O. M., Ruegg, U. T., Schittny, J. and Muller, U. (2003). Beta1 integrins regulate myoblast fusion and sarcomere assembly. *Dev. Cell* **4**, 673-685.

Shamsadin, R., Adham, I. M., Nayernia, K., Heinlein, U. A. O., Oberwinkler, H. and Engel, W. (1999). Male mice deficient for germ-cell cyritestin are infertile. *Biol. Reprod.* **61**, 1445-1451.

Shemer, G. and Podbilewicz, B. (2003). The story of cell fusion: big lessons from little worms. *Bioessays* **25**, 672-682.

Shemer, G., Suissa, M., Kolotuev, I., Nguyen, K. C., Hall, D. H. and Podbilewicz, B. (2004). EFF-1 is sufficient to initiate and execute tissue-specific cell fusion in *C. elegans*. *Curr. Biol.* **14**, 1587-1591.

Singson, A., Mercer, K. B. and L'Hernault, S. W. (1998). The *C. elegans* spe-9 gene encodes a sperm transmembrane protein that contains EGF-like repeats and is required for fertilization. *Cell* **93**, 71-79.

Smith, A. E. and Helenius, A. (2004). How viruses enter animal cells. *Science* **304**, 237-242.

Sun, X., Funk, C. D., Deng, C., Sahu, A., Lambris, J. D. and Song, W. C. (1999). Role of decay-accelerating factor in regulating complement activation on the erythrocyte surface as revealed by gene targeting. *Proc. Natl. Acad. Sci. USA* **96**, 628-633.

Tachibana, I. and Hemler, M. E. (1999). Role of transmembrane 4 superfamily (TM4SF) proteins CD9 and CD81 in muscle cell fusion and myotube maintenance. *J. Cell Biol.* **146**, 893-904.

Takahashi, Y., Bigler, D., Ito, Y. and White, J. M. (2001). Sequence-specific interaction between the disintegrin domain of mouse ADAM 3 and murine eggs: role of beta1 integrin-associated proteins CD9, CD81, and CD98. *Mol. Biol. Cell* **12**, 809-820.

Takeda, Y., Tachibana, I., Miyado, K., Kobayashi, M., Miyazaki, T., Funakoshi, T., Kimura, H., Yamane, H., Saito, Y., Goto, H. et al. (2003). Tetraspanins CD9 and CD81 function to prevent the fusion of mononuclear phagocytes. *J. Cell Biol.* **161**, 945-956.

Talbot, P., Shur, B. D. and Myles, D. G. (2003). Cell adhesion and fertilization: steps in oocyte transport, sperm-zona pellucida interactions, and sperm-egg fusion. *Biol. Reprod.* **68**, 1-9.

Tamm, L. K., Crane, J. and Kiessling, V. (2003). Membrane fusion: a structural perspective on the interplay of lipids and proteins. *Curr. Opin. Struct. Biol.* **13**, 453-466.

Taylor, M. V. (2002). Muscle differentiation: how two cells become one. *Curr. Biol.* **12**, R224-R228.

Taylor, M. V. (2003). Muscle differentiation: signalling cell fusion. *Curr. Biol.* **13**, R964-R966.

Waterhouse, R., Ha, C. and Dveksler, G. S. (2002). Murine CD9 is the receptor for pregnancy-specific glycoprotein 17. *J. Exp. Med.* **195**, 277-282.

Wolfsberg, T. G., Straight, P. D., Gerena, R. L., Huovila, A. P., Primakoff, P., Myles, D. G. and White, J. M. (1995). ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. *Dev. Biol.* **169**, 378-383.

Yamagata, K., Nakanishi, T., Ikawa, M., Yamaguchi, R., Moss, S. B. and Okabe, M. (2002). Sperm from the calmegin-deficient mouse have normal abilities for binding and fusion to the egg plasma membrane. *Dev. Biol.* **250**, 348-357.

Yanagimachi, R. (1988). Sperm-egg fusion. In *Current Topics in Membranes and Transport*, Vol. 32 (ed. F. Bronner), pp. 3-43. San Diego, CA: Academic Press.

Yuan, R., Primakoff, P. and Myles, D. G. (1997). A role for the disintegrin domain of cyritestin, a sperm surface protein belonging to the ADAM family, in mouse sperm-egg plasma membrane adhesion and fusion. *J. Cell Biol.* **137**, 105-112.

Zhu, G. Z., Myles, D. G. and Primakoff, P. (2001). Testase 1 (ADAM 24) a plasma membrane-anchored sperm protease implicated in sperm function during epididymal maturation or fertilization. *J. Cell Sci.* **114**, 1787-1794.

Zhu, G. Z., Miller, B. J., Boucheix, C., Rubinstein, E., Liu, C. C., Hynes, R. O., Myles, D. G. and Primakoff, P. (2002). Residues SFQ (173-175) in the large extracellular loop of CD9 are required for gamete fusion. *Development* **129**, 1995-2002.