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Review

New insights into the molecular mechanisms of sperm-egg interaction

B. Nixon[a], R. J. Aitken[a,b,*] and E. A. McLaughlin[a,b]

[a] Reproductive Science Group, ARC Centre of Excellence in Biotechnology & Development, School of Environmental & Life Sciences, University of Newcastle, Callaghan, NSW 2308 (Australia), Fax: +61 2 4921 6308, e-mail: jaitken@mail.newcastle.edu.au
[b] ARC Centre of Excellence in Biotechnology & Development, University of Newcastle, Callaghan, NSW2308 (Australia)

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Abstract. At the moment of insemination millions of mammalian sperm cells are released into the female reproductive tract in order to find a single cell – the oocyte. The spermatozoa subsequently ignore the thousands of cells they make contact with during their journey to the site of fertilisation, until they reach the surface of the oocyte. At this point, they bind tenaciously to the acellular coat, known as the zona pellucida, that surrounds the oocyte and initiate the chain of cellular interactions that will culminate in fertilization. These exquisitely cell- and species-specific recognition events are among the most strategically important cellular interactions in biology. Understanding the cellular and molecular mechanisms that underpin them has implications for diagnosis of the aetiology of human infertility and the development of novel targets for fertility regulation. Herein, we describe two models indicating the plethora of highly orchestrated molecular interactions underlying successful sperm zona binding and sperm oocyte fusion.

Keywords. Capacitation, fertilisation, oocyte, spermatozoa, gamete.

Introduction

Despite the high level of specialisation achieved during spermatogenesis, mammalian spermatozoa leave the testis as functionally incompetent cells, possessing neither the capacity for forward progressive motility nor the ability to engage in the complex cascade of sperm-egg interactions that precede fertilisation. The sperm cell progressively acquires these physiological attributes during two subsequent phases of post-testicular maturation. The first of these occurs during sperm passage through the epididymis, a process characterised by dynamic remodelling of the protein and lipid composition of the sperm membrane and internal structures [1–3]. Although this endows spermatozoa with the potential to fertilise, this acquired ability is not realised until a further phase of functional maturation has occurred during the cells' ascent of the female genital tract to the site of fertilization in the oviduct [4]. This latter phase, known as capacitation, is accompanied by further surface modifications in addition to the activation of pivotal signalling pathways including a novel cAMP-dependent, protein kinase (PKA)-mediated upregulation of protein tyrosine phosphorylation [5, 6]. Collectively, these events culminate in the generation of fertilisation-competent spermatozoa.

* Corresponding author.

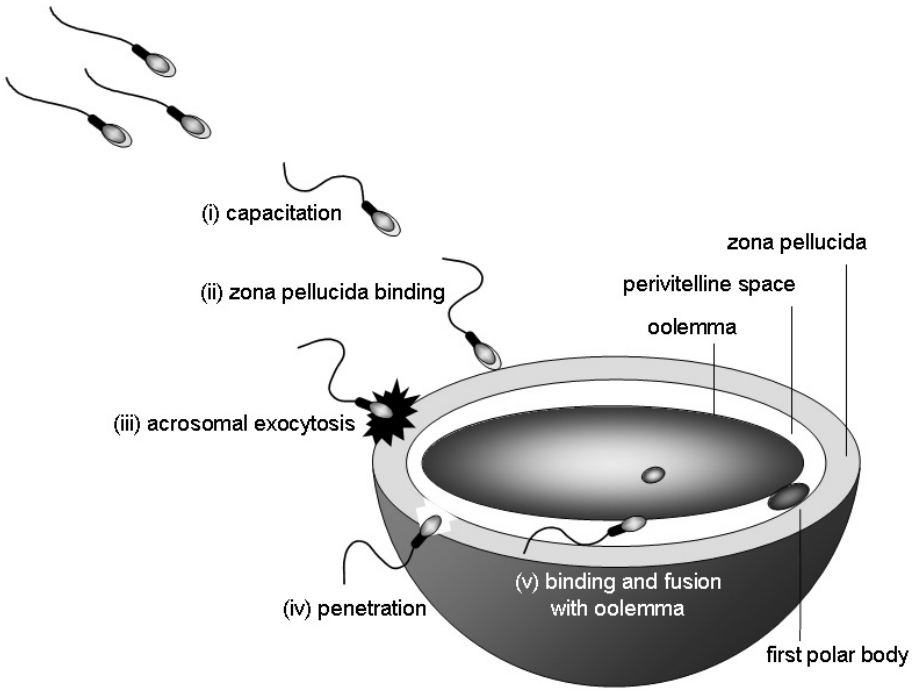

Figure 1. Mammalian fertilisation. Within the female reproductive tract (i) sperm undergo a series of surface and intracellular transformations, collectively termed capacitation, which enables them (ii) to bind to the zona pellucida (ZP) and (iii) undergo the acrosome reaction. (iv) The release of hydrolytic enzymes from the acrosome facilitates sperm passage through the ZP and (v) fusion with the oolemma.

Fertilisation, in turn, is initiated by a relatively species-specific recognition and binding event between complementary molecules that reside within the zona pellucida (ZP) and the apical aspect of the head of capacitated, acrosome intact spermatozoa. Subsequent cell-cell fusion is facilitated by the interaction of the oocyte plasma membrane with a highly localized area of the sperm plasma membrane overlying the equatorial segment (Fig. 1). Our current understanding of sperm-oocyte interaction has progressed substantially over the past 2 decades. These advances are due largely to the insights provided by the inhibition of this event *in vitro* through the judicious use of antibodies or antagonists against molecules of interest. Although such studies have identified a plethora of proteins that are putatively responsible for orchestrating these interactions, the validity of many of these candidates has been undermined by the generation of knockout mice in which candidate genes have been deleted. Thus the precise nature of this interaction and the molecules that mediate it remain controversial.

As summarised below, the advent of powerful systems biology approaches are currently gaining widespread use to gain further insight into the function of the respective gametes and the physical environment to which they are exposed on their passage to fertilisation. The information from these studies will be of great value in understanding the fundamental molecular events of fertilisation.

Sperm-zona interaction

The zona pellucida

The first barrier mammalian sperm encounter during fertilisation is the ZP, a porous extracellular matrix that is secreted by either the growing oocyte or a combination of the oocyte and surrounding granulosa cells, depending on the species (reviewed by [4,7–10]). In mice, the most widely studied mammalian model, the ZP comprises three major sulfated glycoproteins designated ZP1 (185–200 kDa), ZP2 (120–140 kDa) and ZP3 (83 kDa) [11]. In the human, there is also good evidence for a fourth zona glycoprotein, ZP4/B, which is thought to be dysfunctional in the mouse [12]. Current evidence suggests that the mouse zona is a non-covalently assembled structure composed of ZP2-ZP3 dimers that polymerize into filaments cross-linked by ZP1 [13, 14]. Determination of the primary amino acid sequence of ZP proteins has facilitated comprehensive examination of zona function [15–19]. Interestingly, targeted degradation of mouse ZP2 and ZP3 messenger RNA (mRNA) [20] or targeted disruption of the ZP3 gene by homologous recombination [21] has revealed that the absence of either of the two ZP filament proteins prevents assembly of the zona matrix.

As well as being essential structural components of the zona pellucida, ZP2 and ZP3 possess specific functions during the cascade of interactions between sperm and egg that culminate fertilisation. Mouse ZP3 functions as both a primary sperm receptor,

preferentially binding the plasma membrane region overlying the acrosome of acrosome-intact sperm and as an acrosome reaction-inducer during fertilization [4,11,22,23]. ZP2, in turn, has been postulated to serve as a secondary ligand during sperm-zona interaction by virtue of its ability to bind to the inner acrosomal membrane of acrosome-reacted sperm and thus ensures close contact between the penetrating spermatozoon and the zona matrix [24]. Other functions for the ZP include mediation of species specificity in gamete interaction [25], prevention of polyspermy by interaction with cortical granule enzymes released following fertilisation and protection of the developing embryo prior to implantation (reviewed by [8]). Prevailing evidence indicates that primary sperm-egg interaction is mediated by an intricate functional interplay between ZP carbohydrates and lectin-like proteins located on the surface of the sperm head [26,27]. The most widely accepted model of primary sperm-zona interaction emphasises the importance of O-linked carbohydrate moieties located near the carboxy terminus of the ZP3 glycoprotein [28–30]. This is consistent with the fact that female ZP3-null mice are infertile [21,31]. Furthermore, when transgenic mice expressing human ZP3 are bred with ZP3 null mice to produce ZP composed of mouse ZP1 and ZP2 and human ZP3, mouse but not human spermatozoa are able to bind to the chimeric zona [26,32]. Analysis of the glycosylation pattern of ZP3 derived from these transgenic mice indicates that the human ZP3 acquires the same complement of O-glycans as those observed in native mouse ZP3, emphasising the functional significance of O-linked carbohydrate residues [33]. Interestingly, comparative analyses have shown that ZP from different species possess distinct patterns of expression, distribution and structure of O-linked oligosaccharides, perhaps mediating the species specificity of gamete interaction (reviewed in [10]). However, in this context it is also noteworthy that studies of other mammals have shown that the initial interaction between sperm and egg does not just involve ZP3. In the pig for instance, sperm-binding activity resides in a heterodimer between the ZP1 and the ZP3 homologues [34]. Similarly, the ZP1 homologue, ZPB, has been shown to play a major role in sperm binding to the bovine zona [35] and that of the bonnet monkey and human [36,37].

While it is widely accepted that the principal bioactive component of mouse ZP3 is associated with its carbohydrate moieties, deciphering the functional significance of the diverse oligosaccharide ligand(s) remains an important focus [38,39]. Early studies, suggesting that galactose residues served as a critical determinant of sperm binding to ZP3 [28,29,40], have since been refuted [41,42] in favour of terminal N-acetylglucosamine [43], mannose, N-acetylgalactosamine and fucose, each of which has been demonstrated to inhibit sperm-zona binding *in vitro* (reviewed in [44]). Recently, beads coated with functional neoglycoproteins composed of mannose, N-acetylglucosamine (GlcNAc) or N-acetylgalactosamine were shown to mimic ZP3 and thus represent a valuable tool for determining the specificity of sperm-zona binding [45]. In addition, studies involving the use of targeted gene mutations to manipulate or eliminate the glycosyltransferase enzymes involved in the biosynthesis of ZP glycoconjugates also hold considerable promise for ascribing the physiological significance of specific glycan structures in sperm-ZP interaction. In this context, female transgenic mice bearing a null mutation for α1–3 galactosyltransferase (and therefore terminal Galα1–3Gal residues) produce oocytes that display normal sperm-binding characteristics [42], thus further discounting the involvement of galactose residues as originally proposed. However, similar approaches have also raised some doubt regarding the overall necessity of carbohydrates on zona pellucida glycoproteins for binding sperm. For instance, female mice that lack Core 2 GlcNAc transferase and therefore many of the O-glycans found in the zona are fertile [46]. Furthermore, mice that lack Mgat-I, the gene encoding the enzyme that initiates complex and hybrid-type glycan synthesis, produce oocytes that can be fertilised [47]. Notwithstanding these data, a small number of studies have also implicated the ZP protein backbone in sperm-zona interaction, particularly in the context of acrosomal exocytosis [9,48–50]. Notably, recent genetic data in mice have also drawn attention to the fact that the three-dimensional structure of the zona matrix, rather than a single protein (or carbohydrate), is important in mediating sperm binding [51,52]. Furthermore, it has recently been proposed that sperm are able to resolve gamete recognition into at least two distinct binding events. In this novel model, the binding of sperm to the ZP3-dependent ligand present in the zona matrix is actually preceded by adherence to a ZP3-independent ligand that is peripherally associated with the egg coat. Preliminary characterization of this putative ligand has indicated that is a 250-kDa, WGA-reactive glycoprotein that possesses a basic isoelectric point; however, its molecular identity remains to be resolved [53].

Sperm receptor molecules involved in ZP interaction

As a reflection of the complexity of the various models for sperm-zona interaction, it has also proven difficult to characterise the corresponding sperm surface molecules that mediate primary recognition and adhesion to this vestment. Several putative ZP

receptors have been identified through the use of a range of techniques, including analysis of mutations influencing fertility, development of inhibitory monoclonal antibodies, analysis of sperm autoantigens, ZP affinity columns, photoaffinity crosslinking and binding of radiolabelled ZP to sperm lysates (reviewed by [8]). Consistent with the notion that primary sperm-egg interaction is initiated by defined carbohydrate structures on ZP3, a number of the putative sperm receptors possess lectin-like affinity for specific sugar residues (reviewed by [8,54,55]). In the best-studied model, the mouse, these include, but are not limited to, β-1,4-galactosyltransferase (GalT I) [56–59], sp56 [54,60–62], an α-mannosidase [63] and zonadhesin [64,65]. However, despite a wealth of *in vitro* data implicating these molecules in zona interaction, the validity of each candidate has been subsequently compromised by further analyses. For instance, targeted disruption of the GalT I gene, perhaps the most widely studied of all ZP receptors (reviewed by [59]), does not result in infertility [66]. Although sperm from GalT I null mice bind poorly to ZP3 and fail to undergo a zona-induced acrosome reaction, they still possess the ability to bind to the ovulated egg coat *in vitro*. In fact, the prevailing evidence now indicates that neither this molecule nor any of the other putative candidates is uniquely responsible for directing the interaction between sperm and the ZP.

Such findings underscore the biological complexity of sperm-egg interaction and highlight the fact that this functional endpoint may require the coordinated action of several sperm proteins, each with a specific role at different stages of the recognition process. This, in turn, raises the possibility that the zona receptor is in fact a multimeric complex incorporating several discrete molecular entities. Such a notion has been proposed by Shur and colleagues who suggest that GalT I recognises ZP3 oligosaccharides in association with additional sperm surface receptors such as SED1, a protein that reportedly mediates the initial docking of sperm to the murine ZP [53]. Identification of additional key components of such a complex and the mechanisms by which it is assembled is the subject of ongoing investigations within a number of independent laboratories. In recognition of the fact that gamete interaction is predicated on spermatozoa acquiring a state of functional maturation during their post-testicular development, considerable attention has focused on identifying the changes in sperm surface architecture that accompany epididymal maturation and capacitation.

Epididymal Maturation

In the epididymis, a series of complex, sequential events transform immature spermatozoa into mature, motile cells with the potential to fertilize an oocyte. Notwithstanding recent reports to the contrary [67], the balance of evidence indicates that spermatozoa are transcriptionally inactive cells that are incapable of additional protein translation. Thus, their molecular transformation into fertilisation-competent cells must be underpinned by post-translational modifications of existing proteins and remodelling of the sperm surface. These changes are not intrinsic to the cells; rather, they appear to be a direct result of exposure to, and interaction with, the dynamic microenvironment encountered along the length of the epididymal tubule. This microenvironment is, in turn, generated by the secretory and resorptive functions of the epithelial cells that line this tubule. Although the characterisation of this microenvironment has been the subject of considerable study, the molecular basis of its contribution to sperm maturation remains to be fully resolved [68–72].

In mice, the ability of epididymal spermatozoa to bind to the ZP is first observed in the proximal corpus epididymis and appears to coincide with the acquisition of the potential for movement. However, unlike motility, sperm-zona interaction is dependent on the ability of the spermatozoa to undergo capacitation; non-capacitated cells cannot recognise the surface of the zona pellucida [73]. Intriguingly, acquisition of the competence to bind to the ZP is also temporally associated with the exposure of spermatozoa to large chaperone-laden 'dense bodies' in the epididymal lumen [74]. We hypothesise that the migration of molecular chaperones (and possibly other molecules) from these epididymal granules to the sperm surface completes the molecular machinery necessary to effect the subsequent orchestrated presentation of zona receptor molecules on the sperm surface during capacitation. However, the causative nature of this relationship remains the subject of ongoing investigations.

Capacitation

Capacitation represents the final phase of sperm maturation during which these cells realise their full potential for fertilisation. Biochemical analyses of this process have illustrated that one of the key attributes of a capacitated spermatozoon is that it has undergone a dramatic increase in tyrosine phosphorylation via an unusual cAMP-mediated pathway [5,6,75–81]. Immunocytochemical analysis of these tyrosine phosphorylated proteins has traditionally been achieved using fixed material and revealed a striking localisation on the sperm tail of most species that have been studied to date. Commensurate with these findings, phospho-proteomic analysis of capacitated human sperm has identified a number of phosphotyrosine

protein targets, including valosin-containing protein (VCP), a homologue of the SNARE-interacting protein NSF, and two members of the A kinase-anchoring protein (AKAP) family. Perhaps not surprisingly, most of the intracellular proteins modified by tyrosine phosphorylation during capacitation are components of the sperm exocytotic and motility machinery that are likely to play a role preparing the sperm to undergo acrosomal exocytosis and hyperactivation. However, the analysis of live, nonpermeabilised mouse spermatozoa has revealed an additional population of tyrosine-phosphorylated proteins on the surface of capacitated, but not uncapacitated spermatozoa [73]. Furthermore, this surface expression pattern appears confined to the plasma membrane overlying the acrosomal domain of the sperm head – an ideal position for these tyrosine-phosphorylated proteins to orchestrate the membrane remodelling events associated with sperm-ZP recognition (Fig. 2). Significantly, almost all mouse spermatozoa bound to the ZP demonstrate this pattern of phosphoprotein localisation, compared to fewer than 15% of the free-swimming population [73].

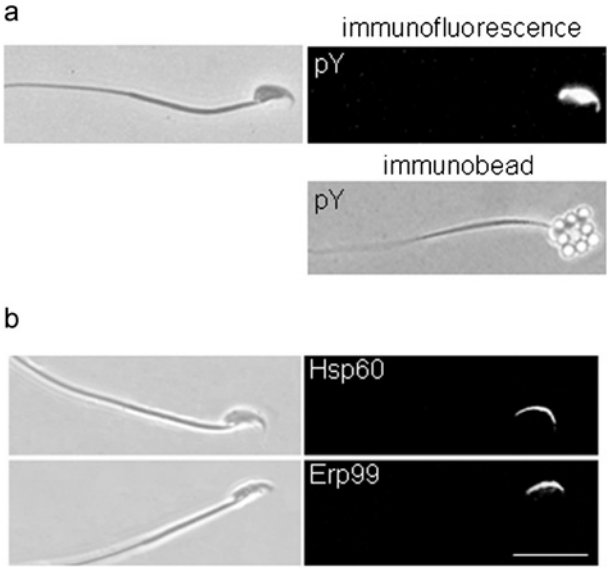

Figure 2. Surface localization of phosphotyrosine residues and molecular chaperones in capacitated mouse spermatozoa. Cauda epididymal sperm were incubated in physiological media optimised to promote capacitation [73]. Tyrosine phosphorylation and chaperone expression were then assessed in live spermatozoa by immunofluorescence or by incubation with anti-phosphotyrosine-coated beads (immunobead). Uncapacitated spermatozoa failed to demonstrate similar patterns of labelling. Scale bar represents 10 μm.

Although this intriguing expression pattern does not appear to be a universal correlate of capacitation in all species [82], it is not unique to mouse spermatozoa. For instance, recent quantitative studies of surface phosphotyrosine expression in boar spermatozoa has revealed a significant (threefold) increase in phosphotyrosine-associated fluorescence following capacitation [83]. This increase coincides with the exposure of several tyrosine-phosphorylated proteins on the outer leaflet of the sperm plasma membrane, at least two of which possess high affinity for the ZP [84,85]. In contrast, plasma membrane proteins isolated from freshly ejaculated sperm cells did not exhibit any ZP binding proteins, likely because these proteins were not tyrosine phosphorylated. However, the identity of these proteins remains to be elucidated. Interestingly, our own analysis of the repertoire of phosphoproteins that are uniquely expressed on the surface of capacitated mouse spermatozoa identified a set of molecular chaperone proteins consisting of heat shock protein (Hsp) 60, an 86-kDa isoform of Hsp90 and endoplasmin (Erp 99) [73,86]. Each of these proteins has in turn been localised to dense bodies within the proximal corpus epididymis and to the sperm surface overlying the anterior acrosome, the exact location where sperm-zona interaction is initiated [73,74]. Although such findings invite speculation that these chaperones may directly mediate sperm-egg interaction, our cumulative evidence argues against such a conclusion. Rather, we have put forward an alternative hypothesis whereby these proteins are responsible for chaperoning key recognition molecules to the site of sperm-oocyte interaction and/or orchestrating their assembly into a multimeric zona-receptor complex on the sperm surface.

The identification of chaperone-associated proteins remains an area of active investigation within our laboratory. Whilst we are using a number of conventional techniques to achieve this goal, a complementary approach has recently been published by Myles and colleagues [87] in which they have exploited the increasing power of mass-spectrometry-based proteomics to characterise the repertoire of membrane-associated proteins that populate the region of the sperm head that interacts with the oocyte. Whilst the complexity of such large-scale studies once presented significant problems, obtaining mass-spectrometry data no longer represents a limiting step. Instead, the main challenge is the effective purification of the desired subcellular fraction and the removal of background proteins. Acknowledging this problem, Myles and colleagues identified membrane proteins by vectorial labelling of the sperm surface and comparing this profile with that of proteins recovered in hybrid membrane vesicles released from the anterior sperm head following the acrosome reaction [87]. This approach has helped define the basic proteomic inventory of the anterior sperm head, the significance

of which is highlighted by the fact that among the 85 proteins identified, at least 9 have been implicated in fertilisation *in vivo* on the basis of gene knockout studies [87]. In principle, this inventory of proteins should facilitate rapid advances in our understanding of gamete interaction. However, before this information can be used to generate insights of physiological relevance, there is a need to address the important question of the temporal and spatial organisation of membrane-associated proteins in relation to the dynamic cellular changes that accompany capacitation. Furthermore, it should be appreciated that sperm-zona recognition and binding events may require a hierarchy of interactions involving molecules other than proteins.

In this context, recent work by Tanphaichitr and colleagues suggest that the sulfoglycolipid sulfogalactosylglycerolipid (SGG) may also play a key role in gamete interaction [88–95]. SGG (also known as seminolipid) is abundantly expressed in spermatozoa (constituting approximately 5–10 mol% of total sperm lipids depending on the species [94]). The saturated nature of its hydrocarbon chains (16:0/16:0), as well as strong intermolecular hydrogen-bonding networks, promotes the formation of ordered crystal-like SGG bilayers [96]. These properties also facilitate its interaction with cholesterol and saturated phospholipids [97] and thus inclusion in sperm lipid rafts (discussed below).

Lipid raft-associated sperm receptor molecules

Evidence for the involvement of SGG in sperm-ZP interaction has been advanced by the fact that pretreatment of human sperm with affinity-purified anti-SGG Fab markedly inhibits their interaction with the ZP in a concentration-dependent manner [90]. Furthermore, fluorescently labelled liposomes constructed of SGG apparently bind uniformly to isolated human ZP [90]. This interaction is believed to be mediated by virtue of an interaction between ZP glycans and the galactosyl sulfate head group of SGG. Commensurate with this hypothesis, liposomes constructed from either galactosylglycerolipid (the parent lipid of SGG) or phosphatidylserine (an alternative negatively charged lipid) failed to adhere to the ZP [90]. However, an apparent problem with this model for primary gamete interaction is the fact that the relatively small size of the galactosyl sulfate monosaccharide would restrict this moiety from projecting far beyond the sperm membrane layer. Nevertheless, to account for such steric constraints, Tanphaichitr and colleagues contend that SGG attachment is preceded by the anchoring of ZP glycans by arylsulfatase A (AS-A, also known as SLIP1) [88]. AS-A is a peripheral plasma membrane protein [95] that is adsorbed onto the surface of spermatozoa during their passage through the epididymis [93]. Significantly, AS-A has affinity for both SGG (which mediates its tight binding to the sperm surface) and also for the ZP [91–93,98–100]. It is proposed that AS-A binding, via interaction between its positively charged amino acids and sulfated sugar residues of the ZP glycans, facilitates the close alignment of the ZP glycans and the sperm plasma membrane for subsequent interaction with the galactosyl sulfate 'lawn' of SGG molecules. Although the carbohydrate–carbohydrate interactions between the galactosyl sulfate groups of SGG and ZP glycans are not strong, they may be stabilized by the concerted action of multiple SGG molecules in the lipid raft microdomains [88]. In this context, empirical studies have determined that the molar ratio of AS-A to SGG is 1:100 or less, and thus the majority of SGG molecules are not occupied by AS-A and therefore capable of ZP binding.

While such a model is attractive, it fails to account for the fact that AS-A-deficient male mice display normal fertility [101]. Nonetheless, two important findings emerge from this work. The first is the recognition of the growing importance of lipid rafts in sperm-ZP interactions (discussed below), and the second is a renewed appreciation of the role of sulfated residues in this interaction. In this context it has been known for some time that surface glycoproteins responsible for cellular adhesion in a variety of different systems are often sulfated [102]. Of particular interest is the fact that the components of the ZP [9], as well as the sperm surface [103], are also highly sulfated in nature. Furthermore, early studies have confirmed the relevance of sulfated moieties in sperm-zona adhesion. For instance, a range of synthetic sulfated substrates (such as arylsulfates, sulfated monosaccharides and ascorbate 2-sulfate), are capable of competitively inhibiting the fertilisation of hamster oocytes *in vitro* at the level of sperm-zona binding [104]. In addition, the exposure of spermatozoa to exogenous sulfatases, enzymes capable of desulfating biological macromolecules (such as cerebrosides, glycosaminoglycans and glycoproteins), eliminated their ZP binding potential. Although the nature of sulfated zona binding sites on spermatozoa remain to be fully elucidated, these early studies take on added significance in light of the recent report that male mice bearing a targeted deletion of Tpst-2 are infertile [105]. Tpst-2 is one of two closely related isoenzymes that mediate the tyrosine O-sulfation of a complex repertoire of substrates (including adhesion molecules, G-protein-coupled receptors, coagulation factors, serpins, extracellular matrix proteins and hormones) in both mice and humans [106]. Tpst-2 null mice have normal spermatogenesis and produce normal numbers of epididymal

sperm that appear indistinct from their wild-type counterparts in terms of their morphology, motility, ability to capacitate *in vitro* and undergo acrosome exocytosis in response to an agonist [105]. However, they are severely defective in terms of their ability to fertilise ZP-intact eggs. Significantly, *in vitro* fertilisation experiments designed to assess the nature of this defect revealed that the spermatozoa of Tpst-2 null males have reduced ability to adhere to the egg plasma membrane but are able to undergo membrane fusion with the egg [105]. While the targets for tyrosine O-sulfation in spermatozoa have yet to be investigated, it is noteworthy that sulfotyrosine residues share a high degree of structural similarity to that of the more widely studied phosphotyrosine residues. The extent of this molecular mimicry is evidenced by the fact that many of the commercially available antibodies routinely used to screen for phosphotyrosine proteins cross-react with sulfotyrosine [107]. Recognition of this potential for cross-reactivity encourages caution in the interpretation of the role of capacitation-associated tyrosine phosphorylation in sperm-zona interaction. Clearly, further investigation of the importance of tyrosine O-sulfation in relation to sperm function is warranted.

As referred to earlier, another potential avenue for the identification of sperm-zona receptor molecules is the study of the constituents of lipid rafts. These specialised liquid ordered microdomains are widely acknowledged as platforms for cell adhesion/signalling and molecule trafficking and sorting. A growing body of evidence supports the presence of lipid rafts within mammalian sperm membranes. Furthermore, a number of independent laboratories have drawn attention to the intriguing behaviour of these structures during sperm capacitation. In this regard, it has been shown that the capacitation of mouse and boar spermatozoa promotes a polarized redistribution of lipid raft markers and their coalescence within the apical portion of the sperm head. Such results invite speculation that lipid rafts may serve as a reservoir for the sperm protein machinery responsible for mediating sperm-zona interaction. This notion is consistent with the fact that mouse sperm lipid rafts contain a number of molecular chaperones [B. Nixon et al., unpublished observation], including those we have previously implicated in the assembly of a zona receptor complex [73]. The identification of similar chaperones as integral components of lipid rafts in other cell types [108–110] suggests that these proteins may fulfil an important general mechanism operating at the level of the plasma membrane through which cellular signalling/adhesion complexes are sorted and assembled. Indeed previous studies have implicated chaperones such as Hsp70 as playing important roles in maintaining the stability of lipid raft-associated signal transduction complexes in the rat forebrain and cerebellum following neural stress [108]. Conversely, it has also been suggested that lipid rafts regulate the functions of chaperones by providing a mechanism for the spatial constraint of their substrates [111], many of which form only low-affinity, transient interactions. Taken together, these results provide circumstantial evidence that the sperm lipid rafts provide a favourable environment for chaperones to mediate their conformational conversion and assembly of a functional receptor complex. This conclusion is consistent with the demonstration that that lipid rafts possess the ability to bind to homologous ZP in both the mouse [B. Nixon et al., unpublished observation] and pig [88]. In contrast, non-raft fractions do not appear to possess a similar affinity. Clearly, a comparative analysis of the composition of lipid rafts from capacitated and non-capacitated spermatozoa should yield important insights into the mechanisms underlying sperm-ZP interaction.

Proposed Model for sperm-zona pellucida interaction

Despite significant advances in our understanding of the initial interaction between sperm and the oocyte, it is clear that this fundamental recognition event remains largely enigmatic. The relevant literature features few reports that have adequately reconciled the ever-increasing number of putative candidate receptors and the conflicting models of sperm-zona interaction. On the basis of recent evidence, we propose that the study of sperm lipid rafts may hold the key to gaining further insight into the molecular events that underpin sperm-ZP interaction. It is our hypothesis that these specialised structures provide a mechanism for the recruitment, spatial constraint and assembly of a functional receptor complex. This notion is consistent with recent evidence that the proteomic composition of lipid rafts undergoes substantial changes in response to capacitation [112] and the fact that these microdomains are laden with a large number of putative zona-receptor molecules [113] in addition to the molecular machinery (chaperones) necessary for their productive assembly into a multimeric complex [B. Nixon et al. unpublished] (Fig. 3).

Sperm-oolemmal interaction

Unlike the well-characterised sperm receptor on the ZP, ZP3, the mechanisms by which acrosome-reacted spermatozoa bind to receptor molecules on the oocyte plasma membrane and initiate fusion with the oolem-

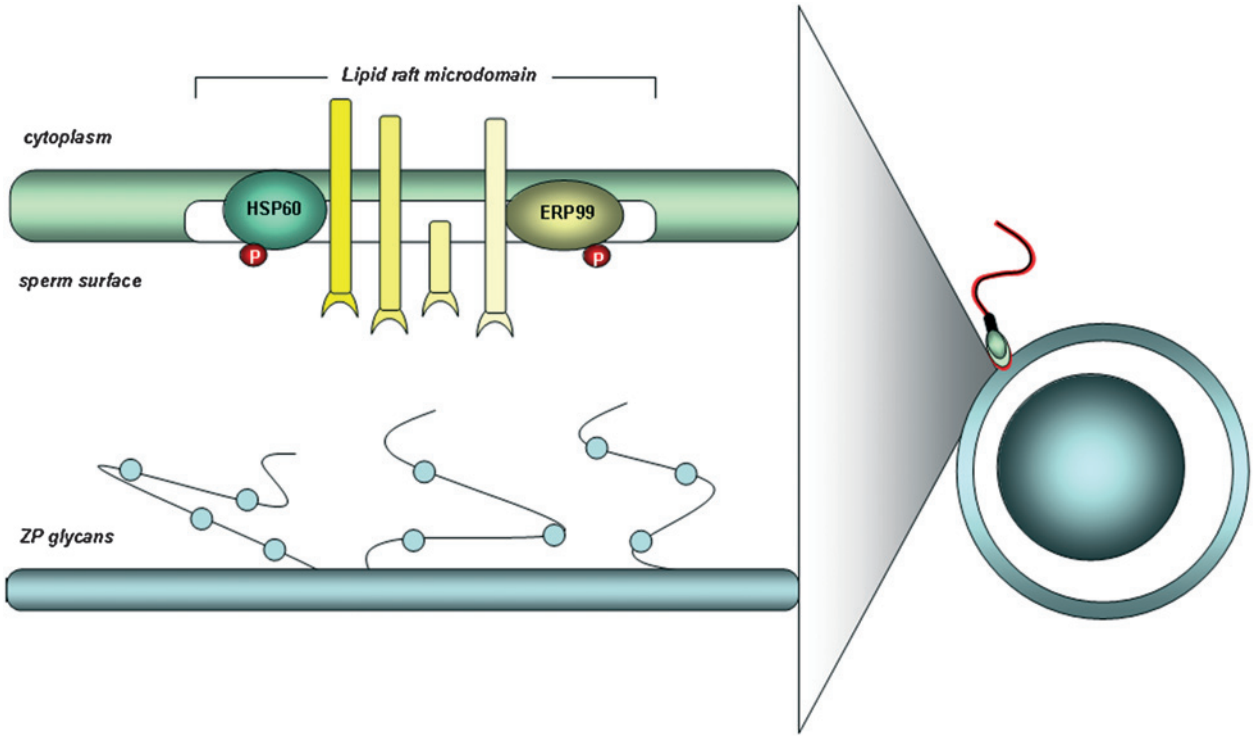

Figure 3. Model for mouse sperm-ZP interaction. It is proposed that lipid rafts serve as a reservoir for the recruitment of key zona recognition molecules and their delivery to the apical region of the sperm head during capacitation. This process coincides with the phosphorylation of molecular chaperone proteins (Hsp60 and Erp99) and their exposure on the sperm surface. The chaperones subsequently provide the molecular machinery to assemble a functional receptor complex, rendering the sperm competent to bind to the ZP. Initial docking is likely to be mediated by receptors with lectin-like affinity for ZP glycans followed by more specific interactions possibly involving interaction between the lipid, SGG seminolipid and sulfated residues within the zona matrix.

ma are still unresolved (Fig. 4). While the selective use of genomic and proteomic techniques has recently uncovered a plethora of molecules thought to play a role in sperm-oocyte interaction, the picture is still far from complete.

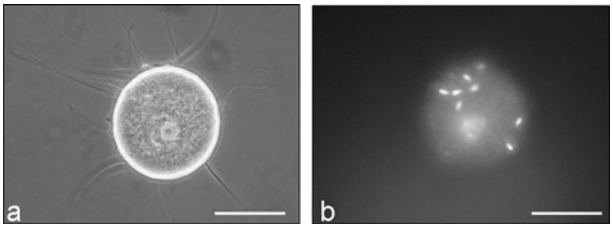

Figure 4. Murine sperm oocyte interaction. (*a*) Phase contrast photomicrograph of murine sperm bound to zona free oocyte and (*b*) fluorescent micrograph of DAPI-stained sperm nuclei fused with oocyte plasma membrane. Scale bar represents 50 μm.

Sperm proteins interacting with the oolemma

ADAM sperm proteins

ADAM, an acronym for proteins containing a disintegrin and metalloprotease domain, were initially thought to play an important role in fertilisation through molecular analysis of their structural domains [114,115]. Testicular ADAM proteins undergo some proteolytic processing, and only disintegrin, cysteine-rich domain and EGF (epidermal growth factor)-like repeat domains are expressed on the surface of spermatozoa. ADAM 2 (Fertilin β) was the first ADAM implicated in sperm-egg recognition following its identification as the antigen recognised by the antibody PH-30, which impedes fertilisation in guinea pigs [116]. Identification of ADAM 1 (fertilin α) as a member of an ADAM1/2 heterodimer [116–118] and ADAM 3 (cyritestin) in both mouse and monkey testes [119–121] resulted in large-scale molecular screening for other pertinent family members. To date, at least 39 ADAM-family members have been identified in vertebrates as well as invertebrate species such as *Drosophila melanogaster* and *Caenorhabditis elegans* [114]. The fact that approximately half of these are testis-specific or testes-enriched provides further support for a possible role in sperm-egg interaction.

Initial studies in the mouse demonstrated that synthetic peptides derived from key sequences contained within the disintegrin loops of ADAM 2 and ADAM 3 potently inhibited sperm-egg fusion but not sperm-

oolemmal binding *in vitro* [122]. However, the disintegrin loop peptides from ADAM-1 (fertilin α), ADAM-4 and ADAM-5 had no effect [123]. To test further the importance of these ADAMs, knockout mice with targeted deletions of either ADAM 2 or ADAM 3 were generated [124–126]. As expected, males were infertile; however, but surprisingly, sperm from both ADAM 2 and ADAM 3 knockout mice displayed a reduced capacity to bind to the plasma membrane of egg but retained the ability to fuse with the plasma membrane [124,125]. Furthermore, sperm from these males exhibited a reduced capacity to bind to the ZP [124–126], and ADAM 2 knockout sperm revealed an additional defect in female reproductive tract transit [124]. By contrast, migration of ADAM 3 knockout sperm from the uterus to the oviduct was normal [125,126]. The fact that both mating and sperm motility was comparable to the wild-type males in ADAM 2 null males suggests an important role for ADAM 2 in sperm interaction with the oviductal epithelia and ZP.

Attempts to attribute the observed fertility defects to the deletion of either ADAM 2 or ADAM 3 have been confounded by findings that ADAM 2 deletion also results in a lack of expression of ADAM 1 as well as severely reduced expression of ADAM 3. Similarly, ADAM 3 deletion results in a lack of ADAM 1 expression as well as approximately 50% reduction in ADAM 2 levels [125]. Since spermatozoa deficient in either ADAM 2 or ADAM 3 are capable of fertilising zona-free oocytes, albeit at a reduced rate, it is likely that these two ADAMs are not essential for egg membrane binding and fusion. It is possible that the observed reductions in egg membrane interactions as well as ZP interactions are attributable to alterations in the co-expression of various other cell surface proteins. Additionally, the role of ADAM 1, 3 and 5 in human fertilisation is debatable, as genomic analysis indicates that these transcripts are encoded by pseudogenes in primates [127–130]. An essential role for other ADAM proteins does however remain possible, given that several other ADAMS have now been detected on the surface of sperm in mouse and human [120,131–134].

Secretory protein CRISP-1

As sperm transit the epididymis, they are bathed in a carefully orchestrated sequence of epithelial cell-secreted proteins. Incorporation of some of these proteins into the spermatozoon membrane results in extensive remodelling of the sperm surface architecture. A series of epididymal-derived cysteine-rich secretory (CRISP) proteins identified across several mammalian species fulfil exactly this role. CRISP-1, also known as DE, is expressed in the epididymis in an androgen-dependent manner [135]. CRISP-2, also known as Tpx-1, is primarily expressed in the testis [136], while CRISP-3 exhibits variable tissue distribution [137–140]. The overall sequence homologies between CRISP family members ranges from approximately 30 to >80%, and all contain a cysteine-rich sequence at the carboxyl terminal.

CRISP-1 was identified as a possible mediator of sperm-egg interaction as a result of a series of observations primarily made in rats. Firstly, pre-incubation of sperm with antibodies against DE inhibits sperm-egg fusion during artificial insemination [141]. Similarly, anti-DE inhibits sperm-egg interaction when present in media during *in vitro* fertilisation (IVF) [142].

When purified from rat epididymal extracts, CRISP-1 binds to the membrane of both rat and mouse oocytes. Furthermore, this binding results in reduced levels of fertilisation during IVF [143,144]. Reduced fertility is also observed in male rats immunised with CRISP-1. Immunisation results in the presence of anti-CRISP-1 antibodies in epididymal and vas deferens fluids. Sperm from these males are able to bind to eggs but lack the capacity for fusion [145]. These studies found that whilst purified CRISP-1 inhibited rat sperm-egg fusion, it had no apparent effect on sperm-egg binding. Subsequent assays using synthetic peptides and other CRISPs support that the egg-interaction site of DE is within a 12-amino acid region corresponding to an evolutionary conserved domain (named Signature 2) [146].

Mouse SLLP1

More recently, a new candidate enzyme has emerged with a proposed role in sperm-oolemmal binding. Mouse sperm lysozyme-like protein (mSLLP1), a novel c-type lysozyme-like protein, has been located in the equatorial segment of human and mouse spermatozoa following the acrosome reaction and appears to play a role in sperm-egg binding and fertilization [147]. Initial experiments involving exposure of oocytes to both recombinant mSLLP1 and anti-mSLLP antibodies revealed a significant inhibition of sperm-oocyte binding. Interestingly, mSLLP was demonstrated to bind in a punctate fashion to the entire perivitelline membrane of zona-free oocytes except the amicrovillar region overlying the meiotic spindle – an area known not to fuse with murine spermatozoa [147].

Since mSLLP1 possesses putative N-acetylglucosamine binding residues, the authors speculate that mSLLP1 could bind to hyaluronan and/or related molecules in the perivitelline space and signal to the oolemma that sperm have entered the perivitelline space, possibly triggering the signalling events for

sperm phagocytosis. As mSLLP1 binding sites disappear after fertilisation, the authors also postulate a role in the development of the block to polyspermy [147]. Production of null mice will further assess the importance of this protein in sperm oocyte recognition.

Oxidoreductase ERp57 (Pdi3a)

Originally thought to be located exclusively in the endoplasmic reticulum with roles in protein folding, members of the protein disulfide isomerase (PDI) family with multiple cellular activities have recently been confirmed as cell surface proteins [148]. Several PDI family members were identified as sperm membrane components during a global proteomic screen of murine spermatozoa [87], and PDI, ERp57, ERp72 and P5 were localised on the plasma membrane overlying the equatorial segment, integral to sperm-oocyte fusion [149]. Preincubation of capacitated spermatozoa with membrane-impermeable inhibitors of PDI activity substantially reduced rates of gamete fusion [149], while the use of specific antibodies to inhibit sperm-egg fusion revealed a particular role for ERp57 (Pdi3a) in mammalian gamete interaction [149].

Since in some viral membrane fusion systems activation of the fusion protein requires a thiol-disulfide exchange, it is possible that a similar mechanism is functioning in sperm-egg fusion. Thus, gamete fusion may require a sperm surface-associated disulfide isomerase, which could trigger a protein-refolding step, in the path to cell-cell fusion. Key proteins with uncharacterised roles in sperm-egg fusion, the sperm surface protein Izumo [150] and the oocyte tetraspanin proteins CD9, CD81 and CD151 [151,152] (discussed below) all possess disulfides in their extracellular domains and may be the target substrates of ERp57.

Izumo

In 2005, Inoue and colleagues used the polyclonal antibody (OBF13) [150,153] to identify successfully a sperm protein with a key role in mouse and human sperm-oocyte fusion. Located on the inner acrosomal membrane and equatorial segment, Izumo is a novel member of the immunoglobulin superfamily [150] with unknown function. Initial *in vitro* antibody inhibition studies [153] indicated a vital role for this protein in sperm-oocyte interaction, and this has been confirmed by *in vivo* knockout studies. *Izumo*$^{-/-}$ females and *Izumo*$^{-/+}$ males exhibited normal fertility. *Izumo*$^{-/-}$ males, however, were sterile despite normal mating behaviour, ejaculation and sperm migration to the site of fertilisation. In addition, sperm from *Izumo*$^{-/-}$ males were capable of binding to and penetrating the ZP and subsequently binding to but not fusing with the oolemma. Hence, sterility in these null mice was specifically due to a failure to fuse with the oocyte plasma membrane.

The extracellular immunoglobulin domain of Izumo possesses a putative glycosylation site and two cysteine residues thought to form a disulfide bridge [150]. In light of the previously outlined importance of the PDI ERp57, the presence of this putative disulfide bond raises the possibility that it is cleaved during sperm-egg fusion, initiating a series of conformational changes that, in a mechanism similar to the action of viral fusogens, promotes sperm-egg fusion.

Egg proteins interacting with spermatozoa

Throughout investigations into sperm-egg interaction, multiple classes of proteins found on the egg surface have been proposed as potential candidates for either sperm binding and/or fusion partners. Proteins formerly and currently of interest include the integrins, in particular $\alpha_6\beta_1$, glycophosphatidylinositol-anchored proteins and latterly tetraspanins.

Integrins

Initially, integrins were identified as possible mediators of sperm-egg binding and fusion following the detection of ADAM proteins containing disintegrin domains on the sperm surface. The role of integrins on the egg surface thus received much attention as studies aimed to dissect two main issues: (i) whether integrins participate in gamete interactions, and (ii) if so, whether sperm ADAMs were indeed the ligands for these integrins.

Integrins are a family of cell adhesion molecules that, in various systems, mediate cell-cell as well as cell-extracellular matrix interactions [154]. They exist as heterodimers for which at least 18 α and 8 β subunits have been identified. Different subunit combinations give rise to a total of 24 different integrins. Based on sequence homologies between α subunits as well as general ligand-binding characteristics, these 24 different integrins can be divided into 6 subfamilies. Of these 24 family members, at least 6 different integrins have been reported to interact with at least 8 different ADAMs [155–164].

Initial results obtained from a series of *in vitro* experiments pointed to a possible role for egg integrins in gamete membrane fusion. The integrin of primary interest, $\alpha_6\beta_1$, was implicated as a receptor for both ADAM 2 and ADAM 3 [156–158,165]. However, studies utilising knockout animals to validate these findings have so far been unable to support a role for egg integrins in gamete membrane fusion.

Eggs from multiple strains of null mice lacking a variety of subunit knockout combinations exhibit normal fertility [166]. Nonetheless, it remains possible that integrins contribute in one way or another to gamete interaction but may not be essential in the mouse or human [166,167].

Glycophosphatidylinositol (GPI)-anchored proteins

GPI-anchored proteins are attached to membranes via a GPI lipid anchor and were first implicated in sperm-egg interaction when Coonrod and colleagues [168] utilised phosphatidylinositol-specific phospholipase C (PI-PLC) to specifically cleave GPI-anchored proteins (GPI-APs) from the surface of sperm or eggs. This enzymatic treatment of zona-free oocytes caused a reduction in sperm-egg binding and a complete block of sperm-egg fusion [168], suggesting a role for GPI protein(s) in this process. In keeping with this concept, PI-PLC-mediated cleavage of GPI-APs significantly reduced sperm-egg binding and fusion in both the mouse and the human sperm/hamster oocyte model [156].

To confirm the importance of GPI-APs on the egg surface, Alfieri et al. [169] tested to see whether GPI-APs were required for fertilisation *in vivo*. Through use of the *Cre/loxP* system, an oocyte-specific knockout of the Pig-A gene, an enzyme involved in GPI anchor biosynthesis, was developed. In these *in vivo* studies, null females were found to be infertile, with a minority of eggs (>1%) fertilised *in vivo* despite the presence of multiple spermatozoa in the perivitelline space. Following up this observation, zona-free eggs from GPI knockout females were also inseminated *in vitro*. With no significant reduction observed in sperm-oolemmal binding, infertility in this instance appeared to be entirely due to defects in sperm-oocyte fusion, in contrast to those observed by Coonrod [168,169].

Coonrod et al. [156,168] further demonstrated that PI-PLC-induced infertility was associated with the release of a 70-kDa (pI 5) and 35–45-kDa (pI 5.5) protein cluster from the egg surface. The 70-kDa protein was believed to be CD55 [169]; however CD55 is unlikely to be the egg surface GPI-AP of interest, as CD55-null mice exhibit normal fertility [170]. Advances in this area are now dependent on identification of the 35–45-kDa protein cluster, or the discovery of previously undetected GPI-APs on the egg surface.

Tetraspanins

Tetraspanins were first discovered in 1990, although the broad functional importance of this family has only recently been appreciated. Widely expressed in eukaryotic organisms [171–173], tetraspanins are a family of related proteins that, as their name suggests, span the plasma membrane of eukaryotic cells four times. With both the N and C termini residing in the intracellular environment, the structure results in two extracellular loops, EC1 and EC2 [174]. While extracellular loop EC1 is small, EC2 is large and contains both a constant and a variable domain [175]. Members of this family typically contain 4–6 conserved extracellular cysteine residues linked into 2–3 disulfide bonds. In addition to these conserved cysteines, tetraspanins also typically contain conserved polar amino acids within transmembrane domains 1, 3 and 4.

A crucial aspect of tetraspanin biology is their proposed role in membrane organisation [176] and as mediators of protein interactions at the primary, secondary and tertiary level [177]. Primary interactions are direct and detergent stable. Integrins, immunoglobulin superfamily members and membrane-anchored growth factors are all classes of protein with which tetraspanins form primary interactions [177]. Tetraspanins also form secondary interactions with other tetraspanins. CD81 is known to interact with CD9 and CD151. Many of the proteins that interact with tetraspanins such as CD9P-1 and EWI-2 [178,179], also interact with each other. The result is a large network of proteins linked by either direct or indirect associations with tetraspanins [177]. The sum of these interactions is referred to as the tetraspanin web [177,180]. Tetraspanin webs give rise to tetraspanin-enriched microdomains. The lipid composition within these microdomains is unique and is distinguishable from classic membrane rafts by several features [176]. These specific microdomains provide a means by which tetraspanins regulate critical cellular functions that require cooperation of multiple proteins [181]. Multiple reports indicate that the tetraspanins CD9 and CD81, as well as these microdomains are implicated in sperm-egg interaction [177].

CD9 was first implicated in fertilisation when Chen et al. [182] observed that antibodies raised against this tetraspanin inhibited sperm-egg binding and fusion in a dose-dependent manner *in vitro* [182]. An unequivocal role was later established when three different laboratories generated mice deficient in CD9 [183–185]. Otherwise, normal CD9 null females exhibited severely reduced fertility attributable to a lack of fusibility of their CD9-deficient oocytes. Whilst no significant differences were observed for sperm-binding ability between control and CD9-deficient eggs, CD9-deficient eggs rarely fused with spermatozoa during either *in vivo* or *in vitro* fertilisation studies. Despite their lack of fusibility, the use of intracytoplasmic sperm injection (ICSI) to bypass this defect in fusion resulted in viable embryos that developed to term [186].

These observations raised obvious and fundamental questions regarding the role of CD9 in gamete fusion. Does CD9 function directly as binding partner for proteins on the sperm surface? Does this molecule mediate the formation and stabilization of a receptor complex within the egg membrane? Recent studies on tetraspanins have identified the large extracellular loop, EC2, as a region of functional significance [176]. Preincubation of sperm with recombinant EC2 had no effect on sperm binding or fusion, whereas preincubation of eggs with EC2 resulted in an inhibition of fusion [187]. This indicated that the EC2 domain of CD9 plays a significant role in the interaction of CD9 with other egg membrane proteins, and that the presence of the EC2 construct impedes sperm-egg fusion by disrupting these interactions.

However, it remains possible that the EC2 domain of egg CD9 interacts directly, in trans, with a ligand on the sperm surface. Studies by Waterhouse et al. (2002) revealed that the pregnancy-specific glycoprotein 17 (PSG17) binds directly to macrophages in a CD9-dependent manner. It was established that residues within the EC2 loop of CD9 play a key role in this interaction [188]. Interestingly, PSG17 results in substantial competitive inhibition of sperm-egg fusion when eggs are preincubated in its presence [189]. Although PSG17 is not expressed on the surface of mammalian sperm, it is an example of a system whereby a sperm antigen could possibly bind directly to CD9. Despite the precedence of direct interaction of PSG17 with macrophage CD9, it does remain possible that, like the bacterially expressed mouse EC2 construct, PSG17 binding to EC2 simply impedes CD9 interactions with other egg membrane proteins, thereby disrupting receptor complex formation.

Through mRNA microinjection, it has been possible to identify regions of the EC2 loop essential to CD9 function during sperm-oocyte fusion. Infertility in CD9-deficient eggs can be rescued via injection of polyadenylated mouse CD9 mRNA at the egg germinal vesicle phase [190]. Expression of the exogenous transcript restores fusibility in the otherwise CD9-deficient eggs. Fertility can also be restored, up to approximately 90% of that for wild-type eggs, via injection of human CD9 [190]. Similarly, injection with mouse CD81 restores fertility up to approximately 50% [190]. More recently it has been demonstrated that fertility in CD81 null mice is also compromised due to a deficiency in sperm-oocyte fusion [191]. Analysis of hepatitis virus binding to CD81 had previously demonstrated that a single amino acid (F186) substitution in the EC2 domain of CD81 dramatically affects virus binding [192].

To determine whether the equivalent region of CD9 was also important, two mutant CD9 mRNA constructs were generated and tested for their capacity to restore fusion. The mutant CD9 proteins contained a substitution of either amino acid residue 174 from phenylalanine to alanine (F→A) or substitution of amino acid residues 173–175 from serine, phenylalanine and glutamine to alanine, alanine, alanine (SFQ→AAA) [187]. The ability of the CD9 constructs to restore fertility in CD9-deficient eggs is either greatly reduced in the case of the F→A (174) mutant or completely abolished in the SFQ→AAA (173–75) mutant [187]. Substantial data exists supporting the correct folding of the SFQ mutant [187], suggesting that these results were not attributable to protein misfolding. This same SFQ mutation in a GST construct of the CD9 EC2 also effectively abolishes the binding of the EC2 loop to PSG17-coated beads [189]. These findings clearly identify the SFQ sequence of EC2 as a critical CD9 domain during sperm-egg interaction.

With an unequivocal role for CD9 in murine sperm-egg interaction established, studies to gain an understanding of the protein interactions mediated by this tetraspanin are ongoing. Generation of double knockout mice indicated that $CD9^{-/-}$/$CD81^{-/-}$ KO mice are completely infertile and that CD9 and CD81 play complementary roles in sperm-oocyte fusion [191]. These authors also demonstrated that one of the two common partners of the CD9 and CD81 tetraspanins, EWI-F, a member of a novel subfamily of proteins with immunoglobulin Ig domains [178], is present on freshly ovulated oocytes and may play a crucial role in sperm oocyte fusion [191]. It has been long known that human oocytes express several integrins, including the α_6 and $ß_1$ subunit [193,194], and that the $ß_1$ integrin may have a role in fertilisation [194]. However, a complementary role for tetraspanins in conjunction with integrins in fertilisation has only recently been shown. Ziyyat and colleagues [152] demonstrated that CD9 controls the formation of integrin $\alpha_6ß_1$-containing, clusters on the oocyte plasma membrane, both in human and mouse, and using function blocking antibodies showed a role for the $\alpha_6ß_1$ integrin and the tetraspanins CD9 and CD151 in human sperm-oocyte fusion. Finally, a recent paper demonstrated that CD9 is enriched on the microvillar membrane of mouse oocytes and co-immunoprecipitates with two Ig superfamily cis partners, EWI-2 and EWI-F [195]. The authors speculate that, as $CD9^{-/-}$ oocytes have an abnormal microvillar shape and distribution, the microvilli could act as a platform to concentrate adhesion/fusion proteins. In addition, CD9 may dictate a membrane protrusion with a low radius of curvature of the microvilli, allowing capture of the sperm cell, and physically locating the sperm in close contact with the oocyte plasma membrane [195].

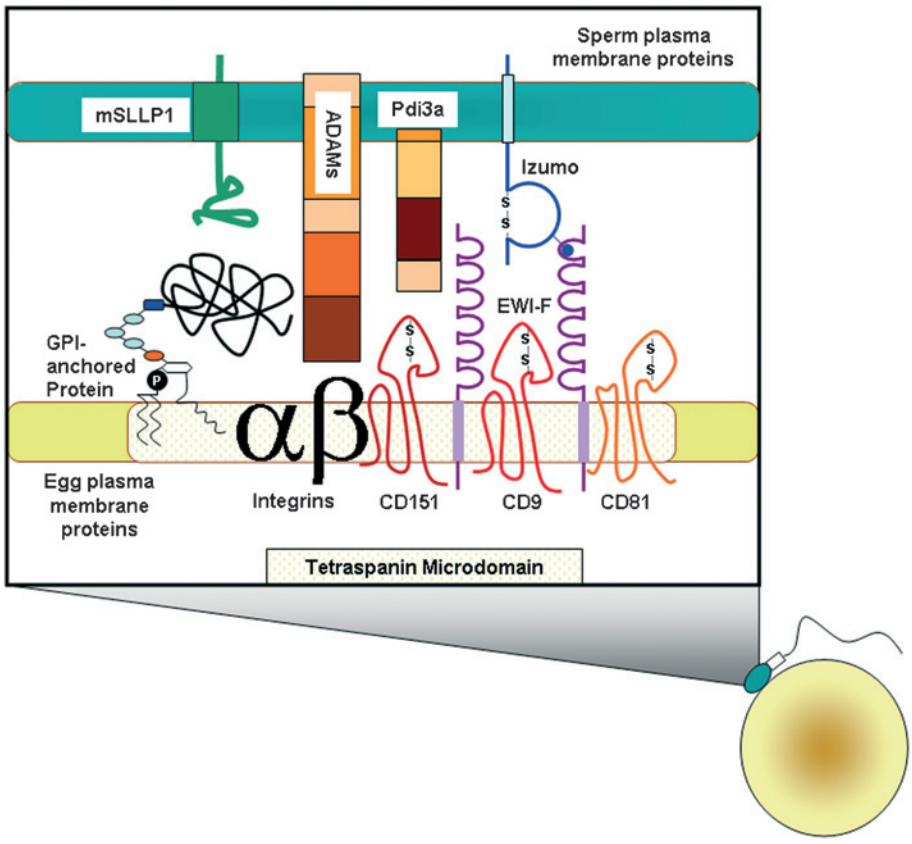

Figure 5. Model for sperm oolemmal interaction. It is proposed that microdomains within the oolemma forming a CD9, CD81 and CD151 receptor web of key molecular players including glycophosphatidylinositol (GPI)-linked proteins, integrins and ADAM proteins are responsible for sperm-oocyte binding and facilitating the chaperone Pdi3a to refold Izumo, leading to cell-cell fusion.

Proposed Model for sperm oolemmal binding and fusion

Recent research in conjunction with genomic and proteomic techniques has uncovered a number of cell surface molecules with putative roles in the sperm-oolemmal interaction; however, the picture remains incomplete. We propose a model (see Fig. 5) in which, similar to viral cell fusion processes, sperm surface receptors bind to oocyte surface receptors and initiate protein refolding mediated through thiol exchange. Thus, close proximity of the sperm and oolemma, modulated by CD9 and co-partner proteins, leads to the interaction of sperm membrane components (like Izumo) with molecular complexes mediated through tetraspanin webs within lipid raft microdomains, resulting in cell-cell fusion (Fig. 5).

Conclusions

Resolving the molecular basis of sperm-egg interaction is of immense strategic importance to the development of novel methods for fertility regulation and the diagnosis of human infertility. Fertilisation is, by its nature, an exquisitely cell- and species-specific event involving an intricate cascade of cell-cell interactions and culminating in the fusion of the fertilising spermatozoon with the vitelline membrane of the egg. New technologies in such key areas as functional genomics and proteomics should significantly advance our understanding of this process and, in the wake of this knowledge, open up new opportunities for pharmacological regulation.

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1 Nikolopoulou, M., Soucek, D. A. and Vary, J. C. (1985) Changes in the lipid content of boar sperm plasma membranes during epididymal maturation. Biochim. Biophys. Acta 815, 486 – 498.
2 Haidl, G. and Opper, C. (1997) Changes in lipids and membrane anisotropy in human spermatozoa during epididymal maturation. Hum. Reprod. 12, 2720 – 2723.
3 Toshimori, K. (1998) Maturation of mammalian spermatozoa: modifications of the acrosome and plasma membrane leading to fertilization. Cell Tissue Res. 293, 177 – 187.
4 Yanagimachi, R. (1994) Fertility of mammalian spermatozoa: its development and relativity. Zygote 2, 371 – 372.
5 Visconti, P. E., Bailey, J. L., Moore, G. D., Pan, D., Olds-Clarke, P. and Kopf, G. S. (1995) Capacitation of mouse spermatozoa. I. Correlation between the capacitation state and protein tyrosine phosphorylation. Development 121, 1129 – 1137.
6 Visconti, P. E., Moore, G. D., Bailey, J. L., Leclerc, P., Connors, S. A., Pan, D., Olds-Clarke, P. and Kopf, G. S.

(1995) Capacitation of mouse spermatozoa. II. Protein tyrosine phosphorylation and capacitation are regulated by a cAMP-dependent pathway. Development 121, 1139 – 1150.
7 Wassarman, P. M. (1990) Profile of a mammalian sperm receptor. Development 108, 1 – 17.
8 McLeskey, S. B., Dowds, C., Carballada, R., White, R. R. and Saling, P. M. (1998) Molecules involved in mammalian sperm-egg interaction. Int. Rev. Cytol. 177, 57 – 113.
9 Prasad, S. V., Skinner, S. M., Carino, C., Wang, N., Cartwright, J. and Dunbar, B. S. (2000) Structure and function of the proteins of the mammalian Zona pellucida. Cells Tissues Organs 166, 148 – 164.
10 Sinowatz, F., Kolle, S. and Topfer-Petersen, E. (2001) Biosynthesis and expression of zona pellucida glycoproteins in mammals. Cells Tissues Organs 168, 24 – 35.
11 Bleil, J. D. and Wassarman, P. M. (1980) Structure and function of the zona pellucida: identification and characterization of the proteins of the mouse oocyte's zona pellucida. Dev. Biol. 76, 185 – 202.
12 Lefievre, L., Conner, S. J., Salpekar, A., Olufowobi, O., Ashton, P., Pavlovic, B., Lenton, W., Afnan, M., Brewis, I. A., Monk, M. et al. (2004) Four zona pellucida glycoproteins are expressed in the human. Hum. Reprod. 19, 1580 – 1586.
13 Greve, J. M. and Wassarman, P. M. (1985) Mouse egg extracellular coat is a matrix of interconnected filaments possessing a structural repeat. J. Mol. Biol. 181, 253 – 264.
14 Wassarman, P. M. and Mortillo, S. (1991) Structure of the mouse egg extracellular coat, the zona pellucida. Int. Rev. Cytol. 130, 85 – 110.
15 Ringuette, M. J., Sobieski, D. A., Chamow, S. M. and Dean, J. (1986) Oocyte-specific gene expression: molecular characterization of a cDNA coding for ZP-3, the sperm receptor of the mouse zona pellucida. Proc. Natl. Acad. Sci. USA 83, 4341 – 4345.
16 Ringuette, M. J., Chamberlin, M. E., Baur, A. W., Sobieski, D. A. and Dean, J. (1988) Molecular analysis of cDNA coding for ZP3, a sperm binding protein of the mouse zona pellucida. Dev. Biol. 127, 287 – 295.
17 Kinloch, R. A., Roller, R. J., Fimiani, C. M., Wassarman, D. A. and Wassarman, P. M. (1988) Primary structure of the mouse sperm receptor polypeptide determined by genomic cloning. Proc. Natl. Acad. Sci. USA 85, 6409 – 6413.
18 Liang, L. F., Chamow, S. M. and Dean, J. (1990) Oocyte-specific expression of mouse Zp-2: developmental regulation of the zona pellucida genes. Mol. Cell. Biol. 10, 1507 – 1515.
19 Epifano, O., Liang, L. F. and Dean, J. (1995) Mouse Zp1 encodes a zona pellucida protein homologous to egg envelope proteins in mammals and fish. J. Biol. Chem. 270, 27254 – 27258.
20 Tong, Z. B., Nelson, L. M. and Dean, J. (1995) Inhibition of zona pellucida gene expression by antisense oligonucleotides injected into mouse oocytes. J. Biol. Chem. 270, 849 – 853.
21 Liu, C., Litscher, E. S., Mortillo, S., Sakai, Y., Kinloch, R. A., Stewart, C. L. and Wassarman, P. M. (1996) Targeted disruption of the mZP3 gene results in production of eggs lacking a zona pellucida and infertility in female mice. Proc. Natl. Acad. Sci. USA 93, 5431 – 5436.
22 Bleil, J. D. and Wassarman, P. M. (1986) Autoradiographic visualization of the mouse egg's sperm receptor bound to sperm. J. Cell Biol. 102, 1363 – 1371.
23 Vazquez, M. H., Phillips, D. M. and Wassarman, P. M. (1989) Interaction of mouse sperm with purified sperm receptors covalently linked to silica beads. J. Cell Sci. 92 (Pt 4), 713 – 722.
24 Bleil, J. D., Greve, J. M. and Wassarman, P. M. (1988) Identification of a secondary sperm receptor in the mouse egg zona pellucida: role in maintenance of binding of acrosome-reacted sperm to eggs. Dev. Biol. 128, 376 – 385.
25 Vieira, A. and Miller, D. J. (2006) Gamete interaction: is it species-specific? Mol. Reprod. & Dev. 73, 1422 – 1429.
26 Hoodbhoy, T. and Dean, J. (2004) Insights into the molecular basis of sperm-egg recognition in mammals. Reproduction 127, 417 – 422.
27 Gwatkin, R. B. and Williams, D. T. (1977) Receptor activity of the hamster and mouse solubilized zona pellucida before and after the zona reaction. J. Reprod. Fertil. 49, 55 – 59.
28 Florman, H. M. and Wassarman, P. M. (1985) O-linked oligosaccharides of mouse egg ZP3 account for its sperm receptor activity. Cell 41, 313 – 324.
29 Litscher, E. S., Juntunen, K., Seppo, A., Penttila, L., Niemela, R., Renkonen, O. and Wassarman, P. M. (1995) Oligosaccharide constructs with defined structures that inhibit binding of mouse sperm to unfertilized eggs in vitro. Biochemistry 34, 4662 – 4669.
30 Wassarman, P. M. (1999) Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis, and fusion. Cell 96, 175 – 183.
31 Rankin, T., Familari, M., Lee, E., Ginsberg, A., Dwyer, N., Blanchette-Mackie, J., Drago, J., Westphal, H. and Dean, J. (1996) Mice homozygous for an insertional mutation in the Zp3 gene lack a zona pellucida and are infertile. Development 122, 2903 – 2910.
32 Rankin, T. L., Tong, Z. B., Castle, P. E., Lee, E., Gore-Langton, R., Nelson, L. M. and Dean, J. (1998) Human ZP3 restores fertility in Zp3 null mice without affecting order-specific sperm binding. Development 125, 2415 – 2424.
33 Dell, A., Chalabi, S., Easton, R. L., Haslam, S. M., Sutton-Smith, M., Patankar, M. S., Lattanzio, F., Panico, M., Morris, H. R. and Clark, G. F. (2003) Murine and human zona pellucida 3 derived from mouse eggs express identical O-glycans. Proc. Natl. Acad. Sci. USA 100, 15631 – 15636.
34 Yurewicz, E. C., Sacco, A. G., Gupta, S. K., Xu, N. and Gage, D. A. (1998) Hetero-oligomerization-dependent binding of pig oocyte zona pellucida glycoproteins ZPB and ZPC to boar sperm membrane vesicles. J. Biol. Chem. 273, 7488 – 7494.
35 Yonezawa, N., Fukui, N., Kuno, M., Shinoda, M., Goko, S., Mitsui, S. and Nakano, M. (2001) Molecular cloning of bovine zona pellucida glycoproteins ZPA and ZPB and analysis for sperm-binding component of the zona. Eur. J. of Biochem. 268, 3587 – 3594.
36 Govind, C. K., Gahlay, G. K., Choudhury, S. and Gupta, S. K. (2001) Purified and refolded recombinant bonnet monkey (Macaca radiata) zona pellucida glycoprotein-B expressed in Escherichia coli binds to spermatozoa. Biol. Reprod. 64, 1147 – 1152.
37 Govind, C. K., Hasegawa, A., Koyama, K. and Gupta, S. K. (2000) Delineation of a conserved B cell epitope on bonnet monkey (Macaca radiata) and human zona pellucida glycoprotein-B by monoclonal antibodies demonstrating inhibition of sperm-egg binding. Biol. Reprod. 62, 67 – 75.
38 Easton, R. L., Patankar, M. S., Lattanzio, F. A., Leaven, T. H., Morris, H. R., Clark, G. F. and Dell, A. (2000) Structural analysis of murine zona pellucida glycans. Evidence for the expression of core 2-type O-glycans and the Sd(a) antigen. J. Biol. Chem. 275, 7731 – 7742.
39 Diekman, A. B. (2003) Glycoconjugates in sperm function and gamete interactions: how much sugar does it take to sweet-talk the egg? Cell. Mol. Life Sci. 60, 298 – 308.
40 Bleil, J. D. and Wassarman, P. M. (1988) Galactose at the nonreducing terminus of O-linked oligosaccharides of mouse egg zona pellucida glycoprotein ZP3 is essential for the glycoprotein's sperm receptor activity. Proc. Natl. Acad. Sci. USA 85, 6778 – 6782.
41 Nagdas, S. K., Araki, Y., Chayko, C. A., Orgebin-Crist, M. C. and Tulsiani, D. R. (1994) O-linked trisaccharide and N-linked poly-N-acetyllactosaminyl glycans are present on mouse ZP2 and ZP3. Biol. Reprod. 51, 262 – 272.
42 Thall, A. D., Maly, P. and Lowe, J. B. (1995) Oocyte Gal alpha 1,3Gal epitopes implicated in sperm adhesion to the zona pellucida glycoprotein ZP3 are not required for fertilization in the mouse. J. Biol. Chem. 270, 21437 – 21440.
43 Miller, D. J., Macek, M. B. and Shur, B. D. (1992) Complementarity between sperm surface beta-1,4-galactosyltransferase and egg-coat ZP3 mediates sperm-egg binding. Nature 357, 589 – 593.

44 Benoff, S. (1997) Carbohydrates and fertilization: an overview. Mol. Hum. Reprod. 3, 599 – 637.

45 Bendahmane, M. and Tulsiani, D. R. (2003) Capacitated acrosome-intact mouse spermatozoa bind to Sepharose beads coated with functional neoglycoproteins. Arch. Biochem. Biophys. 415, 203 – 212.

46 Ellies, L. G., Tsuboi, S., Petryniak, B., Lowe, J. B., Fukuda, M. and Marth, J. D. (1998) Core 2 oligosaccharide biosynthesis distinguishes between selectin ligands essential for leukocyte homing and inflammation. Immunity 9, 881 – 890.

47 Shi, S., Williams, S. A., Seppo, A., Kurniawan, H., Chen, W., Ye, Z., Marth, J. D. and Stanley, P. (2004) Inactivation of the Mgat1 gene in oocytes impairs oogenesis, but embryos lacking complex and hybrid N-glycans develop and implant. [Erratum appears in Mol. Cell. Biol. 2005 Feb; 25(3): 1214. Mol. Cell. Biol. 24, 9920 – 9929.

48 Florman, H. M., Bechtol, K. B. and Wassarman, P. M. (1984) Enzymatic dissection of the functions of the mouse egg's receptor for sperm. Dev. Biol. 106, 243 – 255.

49 Chapman, N., Kessopoulou, E., Andrews, P., Hornby, D. and Barratt, C. R. (1998) The polypeptide backbone of recombinant human zona pellucida glycoprotein-3 initiates acrosomal exocytosis in human spermatozoa in vitro. Biochem. J. 330 (Pt 2), 839 – 845.

50 Hinsch, E., Aires, V. A., Hedrich, F., Oehninger, S. and Hinsch, K. D. (2005) A synthetic decapeptide from a conserved ZP3 protein domain induces the G protein-regulated acrosome reaction in bovine spermatozoa. Theriogenology 63, 1682 – 1694.

51 Rankin, T. L., Coleman, J. S., Epifano, O., Hoodbhoy, T., Turner, S. G., Castle, P. E., Lee, E., Gore-Langton, R. and Dean, J. (2003) Fertility and taxon-specific sperm binding persist after replacement of mouse sperm receptors with human homologs. Dev. Cell 5, 33 – 43.

52 Dean, J. (2004) Reassessing the molecular biology of sperm-egg recognition with mouse genetics. Bioessays 26, 29 – 38.

53 Shur, B. D., Rodeheffer, C., Ensslin, M. A., Lyng, R. and Raymond, A. (2006) Identification of novel gamete receptors that mediate sperm adhesion to the egg coat. Mol. Cell. Endocrinol. 250, 137 – 148.

54 Wassarman, P. M. (1992) Mouse gamete adhesion molecules. Biol. Reprod. 46, 186 – 91.

55 Topfer-Petersen, E. (1999) Carbohydrate-based interactions on the route of a spermatozoon to fertilization. Hum. Reprod. Update 5, 314 – 329.

56 Shur, B. D. and Bennett, D. (1979) A specific defect in galactosyltransferase regulation on sperm bearing mutant alleles of the T/t locus. Dev. Biol. 71, 243 – 259.

57 Shur, B. D. and Hall, N. G. (1982) A role for mouse sperm surface galactosyltransferase in sperm binding to the egg zona pellucida. J. Cell Biol. 95, 574 – 579.

58 Lopez, L. C., Bayna, E. M., Litoff, D., Shaper, N. L., Shaper, J. H. and Shur, B. D. (1985) Receptor function of mouse sperm surface galactosyltransferase during fertilization. J. Cell Biol. 101, 1501 – 1510.

59 Nixon, B., Lu, Q., Wassler, M. J., Foote, C. I., Ensslin, M. A. and Shur, B. D. (2001) Galactosyltransferase function during mammalian fertilization. Cells Tissues Organs 168, 46 – 57.

60 Cheng, A., Le, T., Palacios, M., Bookbinder, L. H., Wassarman, P. M., Suzuki, F. and Bleil, J. D. (1994) Sperm-egg recognition in the mouse: characterization of sp56, a sperm protein having specific affinity for ZP3. J. Cell Biol. 125, 867 – 878.

61 Bookbinder, L. H., Cheng, A. and Bleil, J. D. (1995) Tissue- and species-specific expression of sp56, a mouse sperm fertilization protein. Science 269, 86 – 89.

62 Cohen, N. and Wassarman, P. M. (2001) Association of egg zona pellucida glycoprotein mZP3 with sperm protein sp56 during fertilization in mice. Int. J. Dev. Biol. 45, 569 – 576.

63 Cornwall, G. A., Tulsiani, D. R. and Orgebin-Crist, M. C. (1991) Inhibition of the mouse sperm surface alpha-D-mannosidase inhibits sperm-egg binding in vitro. Biol. Reprod. 44, 913 – 921.

64 Gao, Z. and Garbers, D. L. (1998) Species diversity in the structure of zonadhesin, a sperm-specific membrane protein containing multiple cell adhesion molecule-like domains. J. Biol. Chem. 273, 3415 – 3421.

65 Topfer-Petersen, E., Romero, A., Varela, P. F., Ekhlasi-Hundrieser, M., Dostalova, Z., Sanz, L. and Calvete, J. J. (1998) Spermadhesins: a new protein family. Facts, hypotheses and perspectives. Andrologia 30, 217 – 224.

66 Lu, Q., Hasty, P. and Shur, B. D. (1997) Targeted mutation in beta1,4-galactosyltransferase leads to pituitary insufficiency and neonatal lethality. Dev. Biol. 181, 257 – 267.

67 Gur, Y. and Breitbart, H. (2006) Mammalian sperm translate nuclear-encoded proteins by mitochondrial-type ribosomes. Genes Dev. 20, 411 – 416.

68 Johnston, D. S., Jelinsky, S. A., Bang, H. J., DiCandeloro, P., Wilson, E., Kopf, G. S. and Turner, T. T. (2005) The mouse epididymal transcriptome: transcriptional profiling of segmental gene expression in the epididymis. Biol. of Reprod. 73, 404 – 413.

69 Turner, T. T., Johnston, D. S. and Jelinsky, S. A. (2006) Epididymal genomics and the search for a male contraceptive. Mol. Cell. Endocrinol. 250, 178 – 183.

70 Jervis, K. M. and Robaire, B. (2001) Dynamic changes in gene expression along the rat epididymis. Biol. Reprod. 65, 696 – 703.

71 Chaurand, P., Fouchecourt, S., DaGue, B. B., Xu, B. J., Reyzer, M. L., Orgebin-Crist, M. C. and Caprioli, R. M. (2003) Profiling and imaging proteins in the mouse epididymis by imaging mass spectrometry. Proteomics 3, 2221 – 2239.

72 Gatti, J. L., Castella, S., Dacheux, F., Ecroyd, H., Metayer, S., Thimon, V. and Dacheux, J. L. (2004) Post-testicular sperm environment and fertility. Animal Reprod. Sci. 82 – 83, 321 – 339.

73 Asquith, K. L., Baleato, R. M., McLaughlin, E. A., Nixon, B. and Aitken, R. J. (2004) Tyrosine phosphorylation activates surface chaperones facilitating sperm-zona recognition. J. Cell Sci. 117, 3645 – 3657.

74 Asquith, K. L., Harman, A. J., McLaughlin, E. A., Nixon, B. and Aitken, R. J. (2005) Localization and significance of molecular chaperones, heat shock protein 1, and tumor rejection antigen gp96 in the male reproductive tract and during capacitation and acrosome reaction. Biol. Reprod. 72, 328 – 337.

75 Aitken, R. J., Paterson, M., Fisher, H., Buckingham, D. W. and van Duin, M. (1995) Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. J. Cell Sci. 108 (Pt 5), 2017 – 2025.

76 Galantino-Homer, H. L., Visconti, P. E. and Kopf, G. S. (1997) Regulation of protein tyrosine phosphorylation during bovine sperm capacitation by a cyclic adenosine 3'5'-monophosphate-dependent pathway. Biol. Reprod. 56, 707 – 719.

77 Pukazhenthi, B. S., Long, J. A., Wildt, D. E., Ottinger, M. A., Armstrong, D. L. and Howard, J. (1998) Regulation of sperm function by protein tyrosine phosphorylation in diverse wild felid species. J. Androl. 19, 675 – 685.

78 Kalab, P., Peknicova, J., Geussova, G. and Moos, J. (1998) Regulation of protein tyrosine phosphorylation in boar sperm through a cAMP-dependent pathway. Mol. Reprod. Dev. 51, 304 – 314.

79 Mahony, M. C. and Gwathmey, T. (1999) Protein tyrosine phosphorylation during hyperactivated motility of cynomolgus monkey (Macaca fascicularis) spermatozoa. Biol. Reprod. 60, 1239 – 1243.

80 Lewis, B. and Aitken, R. J. (2001) A redox-regulated tyrosine phosphorylation cascade in rat spermatozoa. J. Androl. 22, 611 – 622.

81 Pommer, A. C., Rutllant, J. and Meyers, S. A. (2003) Phosphorylation of protein tyrosine residues in fresh and cryopre-

served stallion spermatozoa under capacitating conditions. Biol. Reprod. 68, 1208 – 1214.
82 Liu, D. Y., Clarke, G. N. and Baker, H. W. (2006) Tyrosine phosphorylation on capacitated human sperm tail detected by immunofluorescence correlates strongly with sperm-zona pellucida (ZP) binding but not with the ZP-induced acrosome reaction. Hum. Reprod. 21, 1002 – 1008.
83 Piehler, E., Petrunkina, A. M., Ekhlasi-Hundrieser, M. and Topfer-Petersen, E. (2006) Dynamic quantification of the tyrosine phosphorylation of the sperm surface proteins during capacitation. Cytometry 69A, 1062 – 1070.
84 Flesch, F. M., Colenbrander, B., van Golde, L. M. and Gadella, B. M. (1999) Capacitation induces tyrosine phosphorylation of proteins in the boar sperm plasma membrane. Biochem. Biophys. Res. Commun. 262, 787 – 792.
85 Flesch, F. M., Wijnand, E., van de Lest, C. H., Colenbrander, B., van Golde, L. M. and Gadella, B. M. (2001) Capacitation dependent activation of tyrosine phosphorylation generates two sperm head plasma membrane proteins with high primary binding affinity for the zona pellucida. Mol. Reprod. Dev. 60, 107 – 115.
86 Ecroyd, H., Jones, R. C. and Aitken, R. J. (2003) Tyrosine phosphorylation of HSP-90 during mammalian sperm capacitation. Biol. Reprod. 69, 1801 – 1807.
87 Stein, K. K., Go, J. C., Lane, W. S., Primakoff, P. and Myles, D. G. (2006) Proteomic analysis of sperm regions that mediate sperm-egg interactions. Proteomics 6, 3533 – 3543.
88 Bou Khalil, M., Chakrabandhu, K., Xu, H., Weerachatyanukul, W., Buhr, M., Berger, T., Carmona, E., Vuong, N., Kumarathasan, P., Wong, P. T. et al. (2006) Sperm capacitation induces an increase in lipid rafts having zona pellucida binding ability and containing sulfogalactosylglycerolipid. Dev. Biol. 290, 220 – 235.
89 White, D., Weerachatyanukul, W., Gadella, B., Kamolvarin, N., Attar, M. and Tanphaichitr, N. (2000) Role of sperm sulfogalactosylglycerolipid in mouse sperm-zona pellucida binding. Biol. Reprod. 63, 147 – 155.
90 Weerachatyanukul, W., Rattanachaiyanont, M., Carmona, E., Furimsky, A., Mai, A., Shoushtarian, A., Sirichotiyakul, S., Ballakier, H., Leader, A. and Tanphaichitr, N. (2001) Sulfogalactosylglycerolipid is involved in human gamete interaction. Mol. Reprod. Dev. 60, 569 – 578.
91 Tantibhedhyangkul, J., Weerachatyanukul, W., Carmona, E., Xu, H., Anupriwan, A., Michaud, D. and Tanphaichitr, N. (2002) Role of sperm surface arylsulfatase A in mouse sperm-zona pellucida binding. Biol. Reprod. 67, 212 – 219.
92 Carmona, E., Weerachatyanukul, W., Soboloff, T., Fluharty, A. L., White, D., Promdee, L., Ekker, M., Berger, T., Buhr, M. and Tanphaichitr, N. (2002) Arylsulfatase a is present on the pig sperm surface and is involved in sperm-zona pellucida binding. Dev. Biol. 247, 182 – 196.
93 Carmona, E., Weerachatyanukul, W., Xu, H., Fluharty, A., Anupriwan, A., Shoushtarian, A., Chakrabandhu, K. and Tanphaichitr, N. (2002) Binding of arylsulfatase A to mouse sperm inhibits gamete interaction and induces the acrosome reaction. Biol. Reprod. 66, 1820 – 1827.
94 Tanphaichitr, N., Haebe, J., Leader, A., Carmona, E., Harris, J. D., da Silva, S. M., Antunes, T. T., Chakrabandhu, K. and Leveille, M. C. (2003) Towards a more precise assay of sperm function in egg binding. J. Obstet. Gynaecol. 25, 461 – 470.
95 Weerachatyanukul, W., Xu, H., Anupriwan, A., Carmona, E., Wade, M., Hermo, L., da Silva, S. M., Rippstein, P., Sobhon, P., Sretarugsa, P. et al. (2003) Acquisition of arylsulfatase A onto the mouse sperm surface during epididymal transit. Biol. Reprod. 69, 1183 – 1192.
96 Attar, M., Kates, M., Bou Khalil, M., Carrier, D., Wong, P. T. and Tanphaichitr, N. (2000) A Fourier-transform infrared study of the interaction between germ-cell specific sulfogalactosylglycerolipid and dimyristoylglycerophosphocholine. Chem. Phys. Lipids 106, 101 – 114.
97 Weerachatyanukul, W., Probodh, I., Kongmanas, K., Tanphaichitr, N. and Johnston, L. J. (2007) Visualizing the localization of sulfoglycolipids in lipid raft domains in model membranes and sperm membrane extracts. Biochim. Biophys. Acta. 1768, 299 – 310.
98 Tanphaichitr, N., Tayabali, A., Gradil, C., Juneja, S., Leveille, M. C. and Lingwood, C. A. (1992) Role of a germ cell-specific sulfolipid-immobilizing protein (SLIP1) in mouse in vivo fertilization. Mol. Reprod. Dev. 32, 17 – 22.
99 Tanphaichitr, N., Smith, J., Mongkolsirikieart, S., Gradil, C. and Lingwood, C. A. (1993) Role of a gamete-specific sulfoglycolipid immobilizing protein on mouse sperm-egg binding. Dev. Biol. 156, 164 – 175.
100 Rattanachaiyanont, M., Weerachatyanukul, W., Leveille, M. C., Taylor, T., D'Amours, D., Rivers, D., Leader, A. and Tanphaichitr, N. (2001) Anti-SLIP1-reactive proteins exist on human spermatozoa and are involved in zona pellucida binding. Mol. Hum. Reprod. 7, 633 – 640.
101 Hess, B., Saftig, P., Hartmann, D., Coenen, R., Lullmann-Rauch, R., Goebel, H. H., Evers, M., von Figura, K., D'Hooge, R., Nagels, G., et al. (1996) Phenotype of arylsulfatase A-deficient mice: relationship to human metachromatic leukodystrophy. Proc. Nat. Acad. Sci. USA 93, 14821 – 14826.
102 Rapraeger, A. C. and Epel, D. (1981) The appearance of an extracellular arylsulfatase during morphogenesis of the sea urchin Strongylocentrotus purpuratus. Dev. Biol. 88, 269 – 278.
103 Murray, R. K., Narsimhan, R., Levine, M. and Pinteric, L. (1980) Galactoglycerolipids of mammalian testis, spermatozoa, and nervous tissue. In: Cell Surface Glycolipids, pp. 105 – 125, Sweeley, C. C. (Ed.), American Chemical Society, Washington, D. C.
104 Ahuja, K. K. and Gilburt, D. J. (1985) Involvement of sperm sulphatases in early sperm-zona interactions in the hamster. J. Cell Sci. 78, 247 – 261.
105 Borghei, A., Ouyang, Y. B., Westmuckett, A. D., Marcello, M. R., Landel, C. P., Evans, J. P. and Moore, K. L. (2006) Targeted disruption of tyrosylprotein sulfotransferase-2, an enzyme that catalyzes post-translational protein tyrosine O-sulfation, causes male infertility. J. Biol. Chem. 281, 9423 – 9431.
106 Beisswanger, R., Corbeil, D., Vannier, C., Thiele, C., Dohrmann, U., Kellner, R., Ashman, K., Niehrs, C. and Huttner, W. B. (1998) Existence of distinct tyrosylprotein sulfotransferase genes: molecular characterization of tyrosylprotein sulfotransferase-2. Proc. Natl. Acad. Sci. USA 95, 11134 – 11139.
107 Lingwood, C., Mylvaganam, M., Minhas, F., Binnington, B., Branch, D. R. and Pomes, R. (2005) The sulfogalactose moiety of sulfoglycosphingolipids serves as a mimic of tyrosine phosphate in many recognition processes. Prediction and demonstration of Src homology 2 domain/sulfogalactose binding. J. Biol. Chem. 280, 12542 – 12547.
108 Chen, E. H. and Olson, E. N. (2005) Unveiling the mechanisms of cell-cell fusion. Science 308, 369 – 373.
109 Broquet, A. H., Thomas, G., Masliah, J., Trugnan, G. and Bachelet, M. (2003) Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release. J. Biol. Chem. 278, 21601 – 21606.
110 Shah, M., Patel, K., Fried, V. A. and Sehgal, P. B. (2002) Interactions of STAT3 with caveolin-1 and heat shock protein 90 in plasma membrane raft and cytosolic complexes. Preservation of cytokine signaling during fever. J. Biol. Chem. 277, 45662 – 45669.
111 Elhyany, S., Assa-Kunik, E., Tsory, S., Muller, T., Fedida, S., Segal, S. and Fishman, D. (2004) The integrity of cholesterol-enriched microdomains is essential for the constitutive high activity of protein kinase B in tumour cells. Biochem. Soc. Trans. 32, 837 – 839.
112 Thaler, C. D., Thomas, M. and Ramalie, J. R. (2006) Reorganization of mouse sperm lipid rafts by capacitation. Mol. Reprod. Dev. 73, 1541 – 1549.

113 Sleight, S. B., Miranda, P. V., Plaskett, N. W., Maier, B., Lysiak, J., Scrable, H., Herr, J. C. and Visconti, P. E. (2005) Isolation and proteomic analysis of mouse sperm detergent-resistant membrane fractions: evidence for dissociation of lipid rafts during capacitation. Biol. Reprod. 73, 721 – 729.
114 Primakoff, P. and Myles, D. G. (2000) The ADAM gene family: surface proteins with adhesion and protease activity. Trends Genet. 16, 83 – 87.
115 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.
116 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.
117 Waters, S. I. and White, J. M. (1997) Biochemical and molecular characterization of bovine fertilin alpha and beta (ADAM 1 and ADAM 2): a candidate sperm-egg binding/fusion complex. Biol. Reprod. 56, 1245 – 1254.
118 Cho, C., Ge, H., Branciforte, D., Primakoff, P. and Myles, D. G. (2000) Analysis of mouse fertilin in wild-type and fertilin beta(−/−) sperm: evidence for C-terminal modification, alpha/beta dimerization, and lack of essential role of fertilin alpha in sperm-egg fusion. Dev. Biol. 222, 289 – 295.
119 Barker, H. L., Perry, A. C., Jones, R. and Hall, L. (1994) Sequence and expression of a monkey testicular transcript encoding tMDC I, a novel member of the metalloproteinase-like, disintegrin-like, cysteine-rich (MDC) protein family. Biochim. Biophys. Acta. 1218, 429 – 431.
120 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.
121 Heinlein, U. A.O., Wallat, S., Senftleben, A. and Lemaire, L (1996) Male germ cell-expressed mouse gene TAZ83 encodes a putative, cysteine rich transmembrane protein (cyritestin) sharing homologies with snake toxins and sperm-egg fusion proteins. Dev. Growth Differ. 36, 49 – 58.
122 McLaughlin, E. A., Frayne, J., Bloomerg, G. and Hall, L. (2001) Do fertilin beta and cyritestin play a major role in mammalian sperm–oolemma interactions? A critical re-evaluation of the use of peptide mimics in identifying specific oocyte recognition protiens. Mol. Hum. Reprod. 7, 313 – 317.
123 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.
124 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.
125 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.
126 Shamsadin, R., Adham, I. M., Nayernia, K., Heinlein, U. A., Oberwinkler, H. and Engel, W. (1999) Male mice deficient for germ-cell cyritestin are infertile. Biol. Reprod. 61, 1445 – 1451.
127 Jury, J. A., Frayne, J. and Hall, L. (1998) Sequence analysis of a variety of primate fertilin alpha genes: evidence for non-functional genes in the gorilla and man. Mol. Reprod. Dev. 51, 92 – 97.
128 Frayne, J. and Hall, L. (1998) The gene for the human tMDC I sperm surface protein is non-functional: implications for its proposed role in mammalian sperm-egg recognition. Biochem. J. 334 (Pt 1), 171 – 176.
129 Hall, L. and Frayne, J. (1999) Non-functional fertility genes in humans: contributory factors in reduced male fertility? Hum. Fertil. (Camb) 2, 36 – 41.
130 Frayne, J., Dimsey, E. A., Jury, J. A. and Hall, L. (1999) Transcripts encoding the sperm surface protein tMDC II are non-functional in the human. Biochem. J. 341 (Pt 3), 771 – 775.
131 Choi, I., Woo, J. M., Hong, S., Jung, Y. K., Kim, D., H. and Cho, C. (2003) Identification and characterization of ADAM32 with testis-predominant gene expression. Gene 304, 151 – 162.
132 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.
133 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.
134 Frayne, J., Hurd, E. A. and Hall, L. (2002) Human tMDC III: a sperm protein with a potential role in oocyte recognition. Mol. Hum. Reprod. 8, 817 – 822.
135 Cameo, M. S. and Blaquier, J. A. (1976) Androgen-controlled specific proteins in rat epididymis. J. Endocrinol. 69, 47 – 55.
136 Kasahara, M., Gutknecht, J., Brew, K., Spurr, N. and Goodfellow, P. N. (1989) Cloning and mapping of a testis-specific gene with sequence similarity to a sperm-coating glycoprotein gene. Genomics 5, 527 – 534.
137 Mizuki, N. and Kasahara, M. (1992) Mouse submandibular glands express an androgen-regulated transcript encoding an acidic epididymal glycoprotein-like molecule. Mol. Cell. Endocrinol. 89, 25 – 32.
138 Haendler, B., Kratzschmar, J., Theuring, F. and Schleuning, W. D. (1993) Transcripts for cysteine-rich secretory protein-1 (CRISP-1; DE/AEG) and the novel related CRISP-3 are expressed under androgen control in the mouse salivary gland. Endocrinology 133, 192 – 198.
139 Kratzschmar, J., Haendler, B., Eberspaecher, U., Roosterman, D., Donner, P. and Schleuning, W. D. (1996) The human cysteine-rich secretory protein (CRISP) family. Primary structure and tissue distribution of CRISP-1, CRISP-2 and CRISP-3. Eur. J. Biochem. 236, 827 – 836.
140 Schambony, A., Gentzel, M., Wolfes, H., Raida, M., Neumann, U. and Topfer-Petersen, E. (1998) Equine CRISP-3: primary structure and expression in the male genital tract. Biochim. Biophys. Acta. 1387, 206 – 216.
141 Cuasnicu, P. S., Gonzalez Echeverria, F., Piazza, A. D., Cameo, M. S. and Blaquier, J. A. (1984) Antibodies against epididymal glycoproteins block fertilizing ability in rat. J. Reprod. Fertil. 72, 467 – 471.
142 Cuasnicu, P. S., Conesa, D. and Rockwerger, L (1990) Potential contraceptive use of an epididymal protein that particpates in fertilsation. In: A. N. J., Griffin, D., Speiler, J. M., and Waites, G. M. H. (eds.), Gamete Interactions: Prospects for Immunocontraception, Wiley-Liss, New York. pp. 143 – 153.
143 Rochwerger, L., Cohen, D. J. and Cuasnicu, P. S. (1992) Mammalian sperm-egg fusion: the rat egg has complementary sites for a sperm protein that mediates gamete fusion. Dev. Biol. 153, 83 – 90.
144 Cohen, D. J., Ellerman, D. A. and Cuasnicu, P. S. (2000) Mammalian sperm-egg fusion: evidence that epididymal protein DE plays a role in mouse gamete fusion. Biol. Reprod. 63, 462 – 468.
145 Ellerman, D. A., Brantua, V. S., Martinez, S. P., Cohen, D. J., Conesa, D. and Cuasnicu, P. S. (1998) Potential contraceptive use of epididymal proteins: immunization of male rats with epididymal protein DE inhibits sperm fusion ability. Biol. Reprod. 59, 1029 – 1036.
146 Ellerman, D. A., Cohen, D. J., Da Ros, V. G., Morgenfeld, M. M., Busso, D. and Cuasnicu, P. S. (2006) Sperm protein

'DE' mediates gamete fusion through an evolutionarily conserved site of the CRISP family. Dev. Biol. 297, 228 – 237.
147 Herrero, M. B., Mandal, A., Digilio, L. C., Coonrod, S. A., Maier, B. and Herr, J. C. (2005) Mouse SLLP1, a sperm lysozyme-like protein involved in sperm-egg binding and fertilization. Dev. Biol. 284, 126 – 142.
148 Turano, C., Coppari, S., Altieri, F. and Ferraro, A. (2002) Proteins of the PDI family: unpredicted non-ER locations and functions. J. Cell Physiol. 193, 154 – 163.
149 Ellerman, D. A., Myles, D. G. and Primakoff, P. (2006) A role for sperm surface protein disulfide isomerase activity in gamete fusion: evidence for the participation of ERp57. Dev. Cell 10, 831 – 837.
150 Inoue, N., Ikawa, M., Isotani, A. and Okabe, M. (2005) The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. Nature 434, 234 – 238.
151 Rubinstein, E., Ziyyat, A., Wolf, J. P., Le Naour, F. and Boucheix, C. (2006) The molecular players of sperm-egg fusion in mammals. Semin. Cell Dev. Biol. 17, 254 – 263.
152 Ziyyat, A., Rubinstein, E., Monier-Gavelle, F., Barraud, V., Kulski, O., Prenant, M., Boucheix, C., Bomsel, M. and Wolf, J. P. (2006) CD9 controls the formation of clusters that contain tetraspanins and the integrin alpha 6 beta 1, which are involved in human and mouse gamete fusion. J. Cell Sci. 119, 416 – 424.
153 Okabe, M., Yagasaki, M., Oda, H., Matzno, S., Kohama, Y. and Mimura, T. (1988) Effect of a monoclonal anti-mouse sperm antibody (OBF13) on the interaction of mouse sperm with zona-free mouse and hamster eggs. J. Reprod. Immunol. 13, 211 – 219.
154 Evans, J. P. (2002) The molecular basis of sperm-oocyte membrane interactions during mammalian fertilization. Hum. Reprod. Update 8, 297 – 311.
155 Eto, K., Huet, C., Tarui, T., Kupriyanov, S., Liu, H. Z., Puzon-McLaughlin, W., Zhang, X. P., Sheppard, D., Engvall, E. and Takada, Y. (2002) Functional classification of ADAMs based on a conserved motif for binding to integrin alpha 9beta 1: implications for sperm-egg binding and other cell interactions. J. Biol. Chem. 277, 17804 – 17810.
156 Coonrod, S. A., Naaby-Hansen, S., Shetty, J., Shibahara, H., Chen, M., White, J. M. and Herr, J. C. (1999b) 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.
157 Bigler, D., Takahashi, Y., Chen, M. S., Almeida, E. A., Osbourne, L. and White, J. M. (2000) Sequence-specific interaction between the disintegrin domain of mouse ADAM 2 (fertilin beta) and murine eggs. Role of the alpha(6) integrin subunit. J. Biol. Chem. 275, 11576 – 11584.
158 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.
159 Nath, D., Slocombe, P. M., Webster, A., Stephens, P. E., Docherty, A. J. and Murphy, G. (2000) Meltrin gamma(A-DAM-9) mediates cellular adhesion through alpha(6)beta(1)integrin, leading to a marked induction of fibroblast cell motility. J. Cell Sci. 113 (Pt 12), 2319 – 2328.
160 Nath, D., Slocombe, P. M., Stephens, P. E., Warn, A., Hutchinson, G. R., Yamada, K. M., Docherty, A. J. and Murphy, G. (1999) Interaction of metargidin (ADAM-15) with alphavbeta3 and alpha5beta1 integrins on different haemopoietic cells. J. Cell Sci. 112 (Pt 4), 579 – 587.
161 Zhang, X. P., Kamata, T., Yokoyama, K., Puzon-McLaughlin, W. and Takada, Y. (1998) Specific interaction of the recombinant disintegrin-like domain of MDC-15 (metargidin, ADAM-15) with integrin alphavbeta3. J. Biol. Chem. 273, 7345 – 7350.
162 Cal, S., Freije, J. M., Lopez, J. M., Takada, Y. and Lopez-Otin, C. (2000) ADAM 23/MDC3, a human disintegrin that promotes cell adhesion via interaction with the alphavbeta3 integrin through an RGD-independent mechanism. Mol. Biol. Cell 11, 1457 – 1469.
163 Zhou, M., Graham, R., Russell, G. and Croucher, P. I. (2001) MDC-9 (ADAM-9/Meltrin gamma) functions as an adhesion molecule by binding the alpha(v)beta(5) integrin. Biochem Biophys. Res. Commun. 280, 574 – 580.
164 Bridges, L. C., Tani, P. H., Hanson, K. R., Roberts, C. M., Judkins, M. B. and Bowditch, R. D. (2002) The lymphocyte metalloprotease MDC-L (ADAM 28) is a ligand for the integrin alpha4beta1. J. Biol. Chem. 277, 3784 – 3792.
165 Almeida, E. A., Huovila, A. P., Sutherland, A. E., Stephens, L. E., Calarco, P. G., Shaw, L. M., Mercurio, A. M., Sonnenberg, A., Primakoff, P. and Myles, D. G. (1995) Mouse egg integrin alpha 6 beta 1 functions as a sperm receptor. Cell 81, 1095 – 1104.
166 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.
167 Sengoku, K., Takuma, N., Miyamoto, T., Horikawa, M. and Ishikawa, M. (2004) Integrins are not involved in the process of human sperm-oolemmal fusion. Hum. Reprod. 19, 639 – 644.
168 Coonrod, S., Naaby-Hansen, S., Shetty, J. and Herr, J. (1999a) PI-PLC releases a 25 – 40 kDa protein cluster from the hamster oolemma and affects the sperm penetration assay. Mol. Hum. Reprod. 5, 1027 – 1033.
169 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.
170 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.
171 Fradkin, L. G., Kamphorst, J. T., DiAntonio, A., Goodman, C. S. and Noordermeer, J. N. (2002) Genomewide analysis of the Drosophila tetraspanins reveals a subset with similar function in the formation of the embryonic synapse. Proc. Natl. Acad. Sci. USA 99, 13663 – 13668.
172 Todres, E., Nardi, J. B. and Robertson, H. M. (2000) The tetraspanin superfamily in insects. Insect. Mol. Biol. 9, 581 – 590.
173 Gourgues, M., Clergeot, P. H., Veneault, C., Cots, J., Sibuet, S., Brunet-Simon, A., Levis, C., Langin, T. and Lebrun, M. H. (2002) A new class of tetraspanins in fungi. Biochem. Biophys. Res. Commun. 297, 1197 – 1204.
174 Boucheix, C., Benoit, P., Frachet, P., Billard, M., Worthington, R. E., Gagnon, J. and Uzan, G. (1991) Molecular cloning of the CD9 antigen. A new family of cell surface proteins. J. Biol. Chem. 266, 117 – 122.
175 Seigneuret, M., Delaguillaumie, A., Lagaudriere-Gesbert, C. and Conjeaud, H. (2001) Structure of the tetraspanin main extracellular domain. A partially conserved fold with a structurally variable domain insertion. J. Biol. Chem. 276, 40055 – 40064.
176 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.
177 Hemler, M. E. (2005) Tetraspanin functions and associated microdomains. Nat. Rev. Mol. Cell Biol. 6, 801 – 811.
178 Charrin, S., Le Naour, F., Oualid, M., Billard, M., Faure, G., Hanash, S. M., Boucheix, C. and Rubinstein, E. (2001) The major CD9 and CD81 molecular partner. Identification and characterization of the complexes. J. Biol. Chem. 276, 14329 – 14337.
179 Charrin, S., Le Naour, F., Labas, V., Billard, M., Le Caer, J. P., Emile, J. F., Petit, M. A., Boucheix, C. and Rubinstein, E.

(2003) EWI-2 is a new component of the tetraspanin web in hepatocytes and lymphoid cells. Biochem. J. 373, 409 – 421.
180 Boucheix, C. and Rubinstein, E. (2001) Tetraspanins. Cell. Mol. Life Sci. 58, 1189 – 1205.
181 Stein, K. K., Primakoff, P. and Myles, D. (2004) Sperm-egg fusion: events at the plasma membrane. J. Cell Sci. 117, 6269 – 6274.
182 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.
183 Kaji, K. and Kudo, A. (2004) The mechanism of sperm-oocyte fusion in mammals. Reproduction 127, 423 – 429.
184 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.
185 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.
186 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.
187 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.
188 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.
189 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.
190 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.
191 Rubinstein, E., Ziyyat, A., Prenant, M., Wrobel, E., Wolf, J. P., Levy, S., Le Naour, F. and Boucheix, C. (2006) Reduced fertility of female mice lacking CD81. Dev. Biol. 290, 351 – 358.
192 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.
193 Fusi, F. M., Vignali, M., Busacca, M. and Bronson, R. A. (1992) Evidence for the presence of an integrin cell adhesion receptor on the oolemma of unfertilized human oocytes. Mol. Reprod. Dev. 31, 215 – 222.
194 Ji, Y. Z., Wolf, J. P., Jouannet, P. and Bomsel, M. (1998) Human gamete fusion can bypass beta1 integrin requirement. Hum. Reprod. 13, 682 – 689.
195 Runge, K. E., Evans, J. E., He, Z. Y., Gupta, S., McDonald, K. L., Stahlberg, H., Primakoff, P. and Myles, D. G. (2007) Oocyte CD9 is enriched on the microvillar membrane and required for normal microvillar shape and distribution. Dev. Biol. 304, 317 – 325.