

Mini-review

Molecules that function in the steps of fertilization

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1. Introduction

Fertilization, the union of mature male and female gametes, requires a precise series of cell–extracellular matrix and cell–cell interactions. It is therefore not surprising that many of the molecules with key roles in fertilization have signaling or adhesive functions. In many cases, the molecular mechanisms of germ cell interactions have important parallels in somatic tissue development and function.

Fig. 1 shows a generalized scheme for sperm–egg interactions divided into four sequential steps. After sperm have located the egg, they bind to the egg coat. This initial interaction is similar to the interactions that somatic cells have with their surrounding extracellular matrix. Sperm binding to the egg coat induces the exocytosis of the acrosomal vesicle (acrosome reaction). Sperm then penetrate the egg coat with the aid of enzymes formerly contained in the acrosomal vesicle as well as mechanical force generated by sperm motion. Once under the egg coat, sperm bind and fuse with the egg plasma membrane. This union activates the quiescent egg to commit its stored resources to the developmental program of a new individual. It should be noted that the events depicted in Fig. 1 will vary considerably in detail or may be completely absent in the fertilization of different species.

In this review, we examine each of the steps of fertilization with an emphasis on the underlying molecular mechanisms. This is not meant to be an exhaustive review of fertilization model systems and implicated

proteins. Rather, we survey the most well studied molecules of fertilization. For more extensive reviews or details on specific species see Yanagimachi [1], Vacquier [2] and Wassarman [3,4].

Most of what is currently known about fertilization comes from studies with marine invertebrates and mammals because of the ease of collecting gametes or the relevance to human fertility. However, important advances in our understanding of fertilization have also come from diverse systems such as green alga, flies, worms and frogs [5–8]. In fact, genetic approaches to the study of sperm–egg interactions in systems such as *Drosophila* and *C. elegans* promise to rapidly add to the list of molecules that mediate fertilization [6,7].

2. Getting sperm and egg together

Sperm must locate and move towards an egg often from a great distance. Thus, chemical signaling via sperm attractants plays a critical role in directing sperm toward an egg [2,9]. The best characterized sperm attractant is resact. This 14 amino-acid peptide is concentrated in the egg jelly of the sea urchin *Arbacia punctulata* and binds to receptors on sperm [10,11]. Sperm exposed to resact also show increased respiration, motility and metabolism of phospholipids [12]. In mammals, sperm must make their way through the complex twists and turns of the female reproductive tract. After passing through the uterus, the sperm of most mammalian species are stored for extended periods in the lower isthmus of the fallopian tubes under conditions that conserve sperm energy [13,14]. Exposure to the female reproductive tract induces sperm to undergo a process known as capacitation. Capacitation

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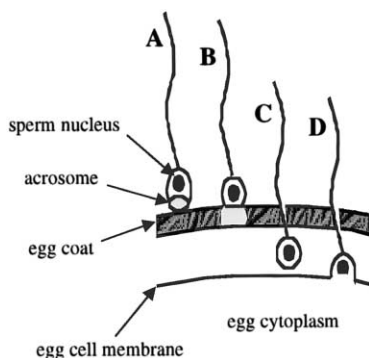


Fig. 1. The steps of gamete interactions combining features from various systems. (A) The sperm binds to the egg coat. (B) The acrosome reaction occurs. (C) The sperm penetrates the egg coat and binds to the egg cell membrane. (D) The membrane of the two gametes fuse.

refers to the final molecular and physiological changes that make the sperm 'competent' to fertilize an egg [15]. The regulation of sperm capacitation may synchronize sperm activity with ovulation [15,16]. Eventually, a few sperm make their way up the oviduct and contact the egg. Follicular fluids are thought to contain substances that attract sperm on this final leg of their journey to the egg [17–19].

3. Sperm interactions with the egg coat

Before sperm can interact with the egg plasma membrane, they must typically interact with an egg coat. The molecular composition of egg coats varies considerably between species [20]. Despite this diversity, most egg coats protect the early embryo from the environment, prevent the disaggregation of weakly adhering blastomeres, function in the block to polyspermy, and trigger sperm responses such as the acrosome reaction [21]. There are a large number of sperm molecules that are thought to mediate interactions with the egg coat (Table 1). Despite this disconcertingly large array of sperm molecules, a general theme of sperm–egg coat recognition is that carbohydrate-binding proteins on the sperm surface bind to glycosylated ligands on the egg coat [22].

Table 1
Sperm proteins that interact with the egg coat

Organism	Egg coat binding protein	References
Sea urchin	Polycystin-1 homologue	[26]
Mouse	β 1,4-Galactosyltransferase	[36]
Mouse	sp56	[37]
Human	Zona receptor kinase	[38]
Pig	Spermadhesins	[39]
Pig	Zonadhesin	[40,41]
Guinea pig	PH20	[55,56]
Rabbit	Sp17	[57]

In starfish and sea urchins, a loose and species non-specific binding of sperm at the egg jelly activates the acrosome reaction. The acrosome reaction includes the exocytosis of acrosomal vesicle contents and the extension of the acrosomal process which penetrates to the egg cell membrane [2,23]. Additionally, molecules released by the acrosome reaction, such as abalone lysin, aid in penetrating the egg coat [24,25]. Starfish egg jelly contains several molecules (ARIS, a sulfated polysaccharide, Co-ARIS, a steroid saponin, and asterosap, a heterogeneous mixture of small peptides) required for the acrosome reaction [2]. Sea urchin egg jelly contains a fucose sulfate polymer that binds a sperm molecule related to human polycystin-1 and induces the acrosome reaction [26,27].

In mammals, after traversing a layer of somatic cells and their associated extracellular matrix that surround the oocyte (the cumulus oophorus), sperm contact a thick egg coat known as the zona pellucida [3,28–30]. The zona pellucida is primarily composed of three glycoproteins; ZP1, ZP2 and ZP3. Purified ZP3 can inhibit sperm–egg binding in vitro while ZP1 and ZP2 have no effect [30]. Therefore, ZP3 is thought to be the primary zona pellucida receptor for sperm. If the *Zp3* gene is knocked out, no zona pellucida is formed and female mice are sterile [31–33]. Sperm binding to ZP3 requires the presence of O-linked oligosaccharides that exhibit a molecular heterogeneity and probably account for most species-specific sperm binding [34,35]. The identity of the ZP3 binding protein on sperm remains controversial. The best candidate molecules to mediate this interaction include β 1,4-galactosyltransferase (Gal-Tase) [36], sp56 [37], zona receptor kinase (ZRK) [38], spermadhesins [39] and zonadhesin [40,41]. GalTase is thought to function as a lectin and bind GlcNAc residues on the ZP3 O-linked oligosaccharides [42,43]. However, a problem with this hypothesis is that Gal-Tase knockout mice are fertile [44]. sp56 is most closely related to the α chain of complement 4B-binding protein and was thought to be a sperm peripheral membrane protein. The presence or absence of sp56 appeared to correlate with species-specific sperm–egg interactions [37]. The demonstration that sp56 is identical to AM67 (an acrosomal matrix protein) cast some doubt about this molecule's hypothesized role in fertilization since an intracellular localization is not consistent with an egg coat binding protein function [45]. ZRK protein apparently binds to ZP3 and inhibitors of tyrosine phosphorylation prevent the acrosome reaction [46]. However, the exact amino acid sequence of ZRK and its role in sperm–egg binding remain to be determined [47,48]. Spermadhesins have been primarily characterized in boar sperm and are composed of a mix of small sperm associated proteins that can bind zona pellucida glycoproteins [39,49]. Zonadhesin is a novel pig sperm transmembrane protein that was purified

Table 2
Sperm and egg proteins functioning in plasma membrane interactions

Organism	Molecule	Gamete	References
Abalone	18-kDa protein	Sperm	[58]
Sea urchin	Bindin	Sperm	[59,60]
Sea urchin	Bindin receptor	Egg	[64]
Nematode	SPE-9	Sperm	[65]
Guinea pig	Fertilin α and β	Sperm	[66,69]
Mouse	Cyritestin	Sperm	[67]
Mouse	Integrins $\alpha 6\beta 1$	Egg	[66]
Mouse	Tetraspanin – CD9	Egg	[72–75]
Rat	DE/CRISP-1	Sperm	[81]

based on its ability to bind zona pellucida proteins [41]. Sequence analysis of the zonadhesin cDNA revealed that it contains multiple cell adhesion molecule-like domains [40].

In order to reach the egg plasma membrane, sperm binding to the zona pellucida must be transient. ZP3 induced clustering of sperm receptors is thought to activate the acrosome reaction [50,51]. Hydrolytic enzymes released by the acrosome reaction and flagellar motility allow sperm to penetrate the zona pellucida [50,52]. Several acrosomal proteases have been identified [53,54]. Surprisingly, knockout mice for one of these proteases (acrosin) have no fertility defect in vivo. Acrosin may therefore encode a redundant function. Alternatively, acrosin could aid in the activation or dispersal of other molecules required for digestion or modification of the zona pellucida [54]. After the acrosome reaction, several other sperm proteins are thought to mediate secondary interactions with the zona pellucida. These proteins include PH20, a glycosyl phosphatidylinositol-anchored membrane protein with hyaluronidase activity that may also aid in sperm movement through the cumulus oophorus [55,56] and Sp17, a rabbit sperm autoantigen that binds to the zona pellucida and may contain a lectin binding domain [57].

4. Sperm–egg interactions at the plasma membrane and gamete fusion

After sperm have penetrated the egg coat, direct cell–cell interactions can take place that ultimately lead to the fusion of gametes. A list of sperm and egg molecules thought to be important in this step of fertilization can be found in Table 2. In abalone sperm, an 18-kDa protein is thought to mediate plasma membrane binding and fusion [58]. A component of the acrosomal vesicle from the sea urchin *Strongylocentrotus purpuratus* known as bindin becomes localized to the acrosomal process and mediates a species-specific binding to the egg vitelline membrane [59,60]. An 18-amino acid region of bindin may help mediate sperm–

egg membrane fusion [61]. The bindin receptor was thought to be a transmembrane glycoprotein that interacts with bindin through a domain that projects into the extracellular space of the egg and contains sequence similarity to the heat shock family of proteins [59]. Recent experiments have suggested that the originally predicted amino acid sequence and subcellular localization of this molecule were incorrect [62–64]. Despite new localization data, the true bindin receptor is still elusive.

In *C. elegans*, a sperm protein encoded by the *spe-9* gene is required for fertility [65]. The *spe-9* gene encodes a transmembrane protein that includes an extracellular region containing an array of 10 epidermal growth factor (EGF)-like motifs. A common feature of proteins that include EGF-like motifs is their involvement in adhesive and ligand–receptor interactions. These results are consistent with the hypothesis that the *spe-9* protein functions in gamete plasma membrane interactions during *C. elegans* fertilization.

In mammals, when sperm come in contact with the egg plasma membrane, several events are hypothesized to occur. First, sperm surface molecules such as fertilin (α subunit ADAM-1, β subunit ADAM-2) or cyritestin (ADAM-3) of the ADAM (*a disintegrin and metalloprotease*) family of proteins are thought to bind integrins $\alpha 6\beta 1$ in a complex with the integrin associated protein tetraspanin-CD-9 on the egg surface [66–70]. This binding allows the α subunit of fertilin to catalyze sperm–egg membrane fusion with a region of the protein that resembles the fusogenic portion of viral fusion proteins [71,72]. Gene knockout experiments for all of these molecules suggest that this model may require a fair amount of modification. Sperm from fertilin knockout mice are deficient in sperm–egg membrane adhesion and fusion [73,74]. However, these sperm also do not bind to the zona pellucida and have defects in sperm migration from the uterus into the oviduct. Sperm from cyritestin knockout mice are deficient in zona pellucida binding but not plasma membrane binding and fusion [75]. The model is further complicated by the fact that normal fertilization occurs with eggs lacking $\alpha 6\beta 1$ integrins [76]. ADAM proteins have been shown to participate in the maturation of other proteins by proteolytic cleavage [77]. It is possible that the primary function of fertilin and cyritestin is simply to modify other proteins that function directly in sperm–egg interactions. In contrast to the other knockout experiments, CD-9 knockout mice are deficient in gamete membrane fusion [78,79]. Another molecule implicated in sperm–egg fusion is DE/CRISP-1. DE/CRISP-1 is a member of a family of cysteine-rich secretory proteins. Purified DE/CRISP-1 [80] or anti-DE/CRISP-1 antibodies can inhibit sperm–egg fusion in vitro.

5. Egg activation

At about the time that sperm fuse with the previously dormant egg, a series of events termed egg activation take place. In addition to triggering cell divisions and embryonic development, egg activation leads to the cellular events required for the block to polyspermy [1]. In all species examined thus far, changes in intracellular Ca^{2+} are required for full egg activation [81,82]. At present there are primarily two competing models for Ca^{2+} signaling at fertilization [82,83]. The oldest model is that sperm activate a plasma membrane receptor to stimulate Ca^{2+} release through the IP_3 cascade. The more recent model, brought about by the success of intracytoplasmic sperm injection techniques for in vitro fertilization, suggests that Ca^{2+} release is activated by a soluble sperm factor released after gamete membrane fusion. Resolving the differences between these two controversial models will require either the identification of the egg surface receptor or the sperm factor required for Ca^{2+} release from a variety of species.

6. Perspectives

Although the basic steps of fertilization are well described (Fig. 1), there is still some confusion or lack of information concerning the molecules and molecular events that mediate these processes. These problems may be due to the diverse approaches and species used by researchers [34,84,85]. Also, the large number of candidate molecules involved in the different steps of sperm–egg interactions could indicate as yet unknown layers of complexity. Definitive genetic evidence demonstrating a direct requirement for sperm–egg interactions in vivo exists for only a few of the molecules of fertilization. Despite these issues, there will continue to be intense interest in the field of fertilization because it has important medical, social, and economic implications. Advances in the understanding of fertilization have led to novel immunological contraceptive strategies and treatments for human infertility [86–89]. Finally, concepts derived from studies investigating fertilization have a broad relevance to many cellular and developmental events. For instance, aspects of gamete interactions before and after fertilization have similarities to intercellular and intracellular signaling systems utilized by somatic cells [2,3]. Defects in any of these systems often play a role in the development and progression of human disease. Cell adhesion and membrane fusion events are important for tissue morphogenesis, immune and nervous system function and the transmission of infectious disease [90–92].

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