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Mechanisms of sperm-egg interactions: between sugars and broken bonds

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Abstract

A model of the early events of mammalian fertilization emerged during the last 30 years. However, studies during the last decade have used newly available mouse models to readdress these processes. Here, we will consider these new data in light of the existing model and point to areas of reconciliation and of controversy.

“Make everything as simple as possible, but not simpler”

Attributed to Albert Einstein

Fertilization is one of the cellular mechanisms at the heart of sexual reproduction (the other being meiosis). The events by which sperm and egg unite are fundamental for our understanding of development and have translational value by forming the basis for many aspects of human infertility treatment and by guiding strategies for a new generation of contraceptive agents (2).

The mammalian egg that awaits fertilization within the oviduct is encased within an extracellular coat, or zona pellucida (ZP). The mouse ZP is composed of 3 proteins; ZP1, ZP2 and ZP3. These assemble into a filamentous structure that serves as a barrier to sperm. In order to contact and fuse with the egg plasma membrane, sperm must first penetrate the ZP. Sperm approaching the egg have completed capacitation, a behavioral reprogramming within the female reproductive tract that is required for sperm fertilization competence (reviewed in ref. 3, 4), and retain the acrosome, an exocytotic vesicle in the anterior head.

The current model of sperm-ZP interactions has penetrated to the level of standard texts in cell and developmental biology (5, 6). This model consists of the following steps (for review and citations not otherwise noted, see ref. 7). Once the sperm reach the site of fertilization, they bind to the ZP by specific interaction with ZP3. This conclusion was based on the determination that ZP3 exhibited two of the anticipated criteria of a sperm adhesion molecule: it bound directly to sperm (8, 9) and it inhibited sperm-ZP binding competitively (10). Because partially purified O-glycan derived from ZP3 recapitulates the activities of

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full-length ZP3 in binding to sperm directly and in inhibiting sperm adhesion to the ZP (11), it was concluded that the sperm adhesion domain of ZP3 is an O-linked oligosaccharide. Moreover, mutagenesis studies of heterologously expressed ZP3 suggested that the adhesion glycan is attached to ZP3 at serine(s) at positions 332 and/or 334 (12, 13). Once the sperm are attached, the ZP3 triggers exocytosis of the acrosomal vesicle (the acrosome reaction). These acrosome reacted sperm are then able to penetrate the ZP; fuse with the egg; and initiate cortical granule exocytosis with the consequent ZP-block to polyspermic fertilization. This block includes inactivation of the sperm adhesion and acrosome reaction-inducing functions of the ZP (10, 14, 15). Finally, as a consequence of cortical granule exocytosis, ZP2 undergoes proteolytic cleavage to ZP2f. This is proposed to be the mechanism underlying the decreased penetrability of the ZP by sperm following fertilization. A related proteolytic activity has been characterized in a number of animal species (16). It has also been postulated that cortical granule glycosidases converts ZP3 to ZP3f and inactivates sperm binding due to modification of adhesion ligand O-glycan (Fig. 1A). However, there is no direct evidence for the biochemical modification of ZP3 following fertilization.

This model is based largely on a reductionist biochemical approach, in which isolated ZP glycoproteins were examined in *in vitro* assays for their ability to mediate steps of sperm-egg interaction.

Recently, a different approach has been used to re-examine the mechanisms of fertilization. Transgenic and gene knock-out manipulations provide a means to study these events within the context of a modified ZP matrix, as opposed to the analysis of isolated, soluble proteins. An alternative model (the “supermolecular structure model”, Fig. 1B) has been put forward in which sperm interaction with the ZP is regulated by the structure of this matrix, rather than by specific proteins (1, 17, 18). As an alternative possibility, combining both models, access of the O-glycan to a glycosidase released during cortical granule exocytosis might be limited by the proteolytic cleavage state of ZP2 (Fig. 1C).

How do sperm bind to ZP?

The current model assigns to ZP3 all roles in the initial binding of sperm to the ZP of unfertilized eggs and in the failure of sperm to bind to the ZP after fertilization. In contrast, the proteolytic modification of ZP2 after fertilization is proposed to account for the transformation of the ZP into a structure that cannot be readily penetrated by sperm. It was known from earlier studies that ZP can bind sperm when full-length ZP2 is present and that conversion to ZP2f by proteolysis is associated with a state in which sperm cannot bind, irrespective of whether ZP2f conversion is triggered by fertilization or by artificial activation of eggs (19). However, this was interpreted as being due the temporally coincident but mechanistically distinct modifications of ZP2 and ZP3.

Can these functions be separated so neatly? In fact, previous studies with *Zp2^{-/-}* mice that express human ZP2 suggested a more complicated situation (17, 18). Gahlay et al. addressed this by generating mice that express a mutated ZP2 that lacks the proteolytic processing site and so cannot be converted to ZP2f. The ZP of these genetically modified mice do not

exhibit an inactivation of sperm binding activity following fertilization and, in contrast to the case in wild type mice, continue to bind sperm even at the 2-cell embryo stage (1). This is the case despite the fact that the ZP of these mutants contains wild type ZP3.

This shows that the ability of sperm to bind to ZP is regulated by the proteolytic processing state of ZP2. However, as discussed, ZP3 exhibits all the characteristics of an adhesion molecule that have been tested in vitro (reviewed in ref. 7). These results can be reconciled, as suggested, if sperm access to its cognate adhesion site on the ZP is regulated by the state of ZP2 proteolysis (18).

What do sperm recognize when they bind to the ZP?

The results of Gahlay et al. demonstrate that sperm binding to the ZP depends on the proteolytic cleavage state of ZP2 (1); however, these observations are silent regarding the molecular nature of that ZP binding site. The current model asserts that sperm adhesion is due to interaction with an O-linked oligosaccharide conjugated to ZP3 at Ser-332/334 and that this oligosaccharide chain is partially or completely cleaved by a cortical granule glycosidase that is released following sperm-egg fusion. However, evidence for the role of Ser-332/334 was based on heterologous expression studies and O-glycosylation of these residues in oocyte ZP3 were not detected by mass spectrometry (20, 21).

This question has now been re-addressed in an elegant series of experiments (1). A *Zp3* transgene with Ser332/334Ala mutations was introduced into *Zp3*^{-/-} mice (Fig. 2A), resulting in mice that were fertile in vivo and in vitro. Sperm were able to bind to the ZP of these eggs in vitro. These observations unequivocally indicate that Ser332/334 do not play an essential role in sperm-egg interaction. Where does this leave us with regard to the molecular nature of the sperm adhesion domain on the ZP?

There remains strong evidence that O-glycans from ZP3 have the anticipated characteristics of sperm adhesion domains (11). Ser-332/334 are not glycosylated, but O-glycans are detected elsewhere on mouse ZP3 by mass spectrometry, particularly at Thr155 and Thr162 (20, 21). The amino acid sequences flanking Thr155 residues are highly conserved in ZP3 from mammals, fish, amphibians and birds (Fig. 2B), including prolines at the -1 and +3 positions that are a signature of O-glycosylation sites (22, 23), and this Thr is predicted to be O-glycosylated in vertebrates (www.cbs.dtu.dk/services/NetOGlyc).

A model consistent with available data is that sperm bind to the ZP by interacting with a glycan, but this glycan is not conjugated to Ser332/334; and the ability of sperm to access this glycan is dependent on proteolytic cleavage state of ZP2. The current model asserts that the adhesion glycan is either modified or destroyed following fertilization, and posits the existence of an unidentified cortical granule glycosidase in this process. New results (1) suggest either that such a glycosidase does not exist and that the adhesion domain is occluded by conformational changes following fertilization; or that access of such a glycosidase to its O-glycan substrate is limited by the proteolytic cleavage state of ZP2 (Fig. 1C). This would place ZP2 as a functional switch in the ZP, reciprocally regulating the access of sperm receptors and of egg glycosidases to a glycan adhesion domain, depending on the proteolytic cleavage form that is present. Gahlay et al. have now provided an

experimental strategy that can be applied to other O-glycosylation sites on ZP3 and test the glycan model of sperm-ZP adhesion rigorously.

Does the ZP trigger acrosome reactions?

A central feature of the current model is that the ZP triggers acrosome reactions in bound sperm (reviewed by (7)). Confidence in the functional significance of this stimulated exocytosis is provided by observations that an acrosome reaction is also triggered in sea urchin sperm by the egg and that similar signaling pathways are evoked in mammalian and echinoderm sperm (24, 25). However, recent studies using genetically-modified ZP, including those of Gahlay et al., find that almost all of the sperm bound to the ZP after a 1 hr incubation have not undergone the acrosome reaction (1, 17).

Different experimental paradigms may account for these results. Sperm penetrate the ZP within minutes of contact. By 1 hr most eggs have been penetrated and the block to polyspermy has been initiated (26). Since the ability of the ZP to trigger acrosome reactions is inactivated after fertilization, it is expected that any sperm that associate with the ZP after this will remain acrosome intact. In order to resolve this, it will be necessary to examine the early interactions of sperm ($t < 5-10$ min) with these genetically modified ZP. Other differences, such as the source of ZP material (solubilized vs intact; ovarian oocytes vs ovulated eggs), also remain to be evaluated.

Final statements

Development of new models with mutated ZP has raised novel questions regarding the early events of fertilization. Some features of the present model, such as the role of ZP2 proteolysis in the control of sperm adhesion and the location along ZP3 of sperm adhesion glycans, require modification. At other points, results using mutant models are not inconsistent with the current model, such as the role of ZP3 O-glycans in sperm adhesion to the ZP. Key questions using these new, genetically manipulated models must be resolved. Does the mutation of ZP proteins affect structure? How is the zona block to polyspermy imposed, given that eggs with mutant ZP2 that cannot be proteolytically modified nevertheless effectively block polyspermy and lack perivitelline sperm (1, 18)? High resolution X-ray structures are available for domains of ZP3, but not yet for the region involved in sperm interaction (27). It is hoped that additional structural information will resolve these issues.

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Fig. 1.

Models of sperm binding to the zona pellucida. (A) The Glycan Model-Sperm bind by interacting selectively with O-glycans conjugated to ZP3 at Ser332/334. Sperm fail to bind after fertilization due to the enzymatic modification of the adhesion glycan by an egg glycosidase. (B) The Supermolecular Structure Model-The sperm binding site is not specified (shown as including elements of ZP2 and ZP3). Access to this binding site is regulated by proteolytic cleavage of ZP2, such that after fertilization sperm are restricted from this site and cannot bind to the ZP. (C) A Hybrid Model-Sperm bind to an O-glycan that is conjugated to ZP3 at a site other than Ser332/334. Sperm access to this glycan is regulated by the proteolytic cleavage state of ZP2. See text for details.

A

Mouse ZP3 (325) HGNC**SNSSSS**QFQIHGPRQWS
 * *
 332 334

B

Mouse (146) NVSSHPIQ**PTWVP**FRAT
 Human (147) NVSSQAIL**PTWL**PFRTT
 Chicken (174) NVSSHAIQ**PTWAP**FHST
 Frog (168) NVSSKAIK**PTWVP**FSST
 Zebrafish NVTSNALL**PTWL**PYGAT
 * * * *
 170 173

Fig. 2.

O-glycosylation sites on ZP3. (A) Mouse ZP3 residues 325–345 are shown, with proposed site of adhesion O-glycan conjugation to Ser-332 and Ser-334 indicated (green asterisk). Serines tested for a role in sperm binding by Gahlay et al. are shown (red lettering). (B) Mouse ZP3 residues 146–162 are shown, with predicted sites of O-glycosylation indicated (blue asterisk). This region is conserved between mouse, human and other vertebrates. Note the conservation of prolines at positions –1 and +3 relative to Thr-155, a pattern often seen at sites of O-glycosylation.