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Role of Acrosomal Matrix in Mammalian Fertilization

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포유류 수정과정에서 정자 첨체기질의 기능

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SUMMARY

Sperm competent for fertilization can become capacitated, bind to the zona pellucida (ZP) of an egg in a specific manner, and complete acrosomal exocytosis. Failure to carry out these functions results in infertility. Although the interactions between the ZP and the plasma membrane overlying the sperm acrosome have been considered important for sperm-egg recognition and signalling, recent results have prompted a reassessment of current paradigms concerning these interactions. In this review, we're going to discuss about the roles of the acrosomal matrix, the particulate component of the acrosomal contents, in fertilization. The general hypothesis is that acrosomal exocytosis leads to the exposure of acrosomal matrix proteins that become de-facto extracellular matrix (ECM) on the surface of the sperm head, and that the dynamic interactions of this newly-exposed sperm ECM with the egg ECM (the ZP) govern sperm-egg recognition and sperm penetration of the ZP. Informations from these experiments may provide new ways to address the poor ZP binding of sperm from some human infertility patients and may offer new avenues for contraception through the disruption of purposeful sperm-ZP binding.

(Key words: sperm, acrosome reaction, acrosomal exocytosis, fertilization)

The Prevailing View: The Acrosome Reaction Model

1. Acrosomal Dynamics as a Two-state or Binary Reaction

The Acrosome Reaction Model emphasizes the acrosome-intact and acrosome-reacted states of spermatozoa and minimizes the importance of intermediates between these two extremes. To use computer parlance, this is a binary or digital system; the acrosome is either 'on' or 'off' (i.e., intact or reacted). This model does not provide a role for acrosomal matrix proteins in sperm-ZP interactions. As explained by Yanagimachi (1994), this model proposes that the OAM and PM fuse simultaneously in multiple places, allowing for the rapid release of the acrosomal components thought, principally, to be enzymes. The vesiculated intermediate is considered to be short-lived; the acrosomal matrix components either dissipate or are shed with

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vesiculated membranes from the sperm surface, leaving the bare inner acrosomal membrane.

2. Spontaneous Secretion

Acrosomes can be lost from spermatozoa via normal physiological events, such as those occurring during fertilization(so-called true acrosome reactions), or they may become detached through mechanical shearing or other processes such as occurs when moribund or dead spermatozoa degenerate (so-called false acrosome reactions)(Bedford; 1970). Acrosomal secretion can also occur without a stimulant such as the ZP, but the Acrosome Reaction Model categorizes these spontaneous acrosome reactions as false, non-physiological, or spurious. However, the occurrence of spontaneous acrosome reactions is not accidental, but greatly increases as a function of capacitation, a poorly defined maturation process that normally takes place within the female reproductive tract but can be mimicked experimentally. Furthermore, the incidence of spontaneous acrosome reactions is dependent upon the species, the animal strain, medium composition, state of epididymal storage, pre- and post-ejaculation conditions, and immunological condition of the spermatozoa (Yanagimachi, 1994). Importantly, Yanagimachi points out that spermatozoa that have undergone spontaneous acrosome reactions are still capable of fertilizing ZP-free eggs.

3. Sperm-ZP Pellucida Interactions and Acrosomal Status

The Acrosome Reaction Model assumes that sperm-ZP interactions are governed by the acrosomal status of the sperm cell. Building on the pioneering mouse sperm studies of Saling et al. (1979) and others examining the cells from various species (see Yanagimachi (1994) for references), the concept developed that the acrosomes must be intact for sperm-ZP adhesion. One motive to revisit the Acrosome Reaction Model is the body of literature suggesting that the "acrosome-intact" requirement cannot be generalized to all species. For example, Myles et al., (1987) convincingly demonstrated that guinea pig spermatozoa are capable of adhesion to the ZP in both acrosome-intact and acrosome-reacted states. In an extension of this study Schroer et al., (2000) reported that guinea pig sperm penetrate the cumulus in an acrosome-intact state but that the acrosome reaction is completed on or near the surface of the ZP; acrosome -intact sperm were observed on the ZP but these were not tightly bound. Human sperm cells were also observed to adhere to eggs in the acrosome -intact and acrosome-reacted states (Morales et al., 1989). Recently, acrosome-reacted spermatozoa were recovered from the perivitelline space of fertilized rabbit eggs and used successfully to re-inseminate other eggs *in vitro*, indicating that rabbit spermatozoa do not need intact acrosomes to be competent for ZP adhesion, ZP penetration, and fertilization (Valdivia et al., 1999). In addition, the conclusion that adhesion to the extracellular matrix (ZP) surrounding an egg requires that sperm be acrosome-intact has been applied primarily to mammals. In species such as the sea urchin, spermatozoa must initiate acrosomal exocytosis in order to enable them to adhere to the vitelline layer, the sea urchin equivalent of the ZP, via the acrosomal matrix protein bindin (Vacquier and Moy, 1977).

Because it is extremely difficult to determine the definitive acrosomal status of sperm cells that adhere to extracellular coats surrounding eggs, the classification of the acrosome-intact and acrosome -reacted states have generally necessitated special assays. However, each assay measures a different parameter of the acrosome. Some protocols demonstrate the presence of specific components inside the acrosome, on the OAM, or on the PM (Cross and Meizel, 1989; Larson and Miller, 1999), but these approaches do not address membrane integrity. Other dye-binding assays measure various parameters, such as pH or ionic gradients (Lee and

Storey, 1985), but these assays may be compromised by non-specific binding to acrosomal components or by difficulties with dye loading. Even inspection by light microscopy of spermatozoa which have very large acrosomes, such as the guinea pig, has pitfalls. Because of the underlying and particulate matrix inside the acrosome, an apparently 'intact' acrosome may actually possess points of membrane fusion or rupture that cannot be readily detected by phase-contrast microscopy. Similarly, membranes are dynamic; once a sperm sample has been processed for an acrosomal status assay, the assay might not truly represent the state of the acrosome at the time of intervention. Finally, the data from different experiments that use alternative assays may not be directly comarable because one assay might report a spermatozoon as acrosome-intact while another method would categorize the same spermatozoon as acrosome-reacted.

4. Adhesion to the Zona Pellucida.

The verdict that the acrosome must be intact for a sperm to adhere to the ZP leads to the presumption that the PM overlying the acrosome contains a protein that recognizes and binds a component of the ZP. The ZP3 glycoprotein was identified as the probable ligand since soluble ZP3 blocks the adhesion of spermatozoa to unfertilized mouse eggs (Bleil and Wassarman, 1980). Another ZP glycoprotein, ZP2, does not affect the initial adhesion of spermatozoa to ZP, but does interfere with maintenance of adhesion when the acrosome reaction is detected to occur on the ZP (Bleil et al., 1988). In the Acrosome Reaction Model, the consequences of sperm-ZP adhesion include the stimulation of the acrosome reaction to enable the release of proteins (hydrolases) necessary for penetration of the ZP and the unmasking of some mechanism for the continued attachment to the ZP as the sperm cell penetrates this extracellular egg coat. Some investigators, neglecting the existence of the acrosomal matrix, have proposed that molecules on the inner acrosomal membrane may mediate the secondary adhesion. However, the Acrosome Reaction Model does not adequately address how a sperm cell can efficiently adhere to and yet simultaneously pass through the ZP to reach the oolemma.

5. Zona Pellucida Recognition Proteins

Over the years many candidates have been proposed to act as ZP-binding proteins or signalling receptors on the mammalian sperm PM. Without precluding a role for any of these candidates in sperm-ZP interactions, we will focus this study on mouse sperm protein sp56 (Bleil and Wassarman, 1990). The deduced amino acid sequence of sp56 demonstrated that this protein is a member of the complement regulatory protein superfamily and is most closely related to complement 4b-binding protein (C4BP), (Bookbinder et al., 1995). A non-conventional method to visualize immuno-colloidal gold particles on surface replicas was used to localize sp56 on whole mounts of capacitated spermatozoa, leading to the questionable conclusion that sp56 is an extracellular, peripheral PM protein (Suzuki-Toyota et al., 1995). However, when we identified AM67, the guinea pig orthologue of sp56, as a component of the intracellular AM, we revisited the issue of sp56 localization and demonstrated by conventional immunoelectron microscopic procedures that mouse sp56 is definitely an intra-acrosomal protein (Foster et al., 1997). As viewed from the Acrosome Reaction Model, the fact that sp56 is not a PM protein would discount it as a protein important in the initial adhesion of spermatozoa to the ZP. However, as described below, the Acrosomal Exocytosis Model envisions an important functional role for this acrosomal matrix protein as a ZP-binding protein.

6. Zona Pellucida-stimulated Secretion

Besides blocking sperm-ZP adhesion, ZP3 is re-

ported to induce acrosome reactions (Bleil and Wassarman, 1983). Thus, ZP3 can act not only as a ligand for a binding protein on the sperm surface, but this ZP subunit can also transmit a signal through a molecule on the sperm surface to stimulate acrosomal secretion.

7. Zona Pellucida Penetration

Following adhesion to the ZP, the sperm must pass through the ZP to reach the oolemma. Several penetration mechanisms have been proposed including the use of mechanical force (Bedford, 1988; Green, 1987). The most popularly held belief is that ZP penetration is mediated by a sperm protease. This assumption is based on the observations that mammalian fertilization can be blocked by trypsin inhibitors. However, some of the effects of protease inhibitors are clearly due an inhibition of sperm-ZP adhesion (Bleil et al., 1988; Fraser, 1982; Liu and Baker, 1993; Saling, 1981). Contrary to long-standing expectations, the acrosomal serine protease acrosin is not a requisite ZP hydrolase since male mice null for the acrosin gene are completely fertile (Baba et al., 1994). Alternatively, the penetration mechanism could involve the use of proteins that disassemble the ZP in a restricted area by a non-catalytic process as has been found for the abalone (Lewis et al., 1982). If any of these mechanisms are to function, the proteins must originate in the sperm head; a likely source is the AM.

An Alternative Paradigm: The Acrosomal Exocytosis Model

1. The Acrosomal Exocytosis Model Departs in Several Key Ways from the Acrosome Reaction Model

① The alternative paradigm does not require nor does it preclude that the acrosomal membranes must be completely intact (no membrane fusion) at the time of the initial interaction with the ZP.

② Transitional intermediates of acrosomal exocytosis are recognized to exist ephemerally and are functionally important in the fertilization process.

③ Sperm capacitation prepares sperm for exocytosis and leads to increased rates of initiating physiologically relevant, spontaneous acrosomal exocytosis.

④ Specific ligands (e.g., the ZP) or pharmacological agents (e.g., ionophores, progesterone) can greatly accelerate the incidence of acrosomal exocytosis compared to the spontaneous rate by stimulating the fusion of the PM and OAM through a signal transduction cascade. This is in contrast to the Acrosome Reaction Model which posits that the ZP serves to initiate sperm secretion.

⑤ Spontaneous acrosomal exocytosis is physiologically relevant and represents a slower, but mechanistically similar, version of the ligand-accelerated process.

⑥ Compartments of the acrosome, including the soluble acrosomal proteins and AM as well as distinguishable morphological domains may influence the interaction of a spermatozoon with the ZP.

The model proposes that transitional intermediates of exocytosis represent capacitated spermatozoa whose OAM and PM have partially fused in limited areas, exposing the acrosomal contents at the sperm surface. Some of the exposed components on the outer perimeter of the AM come in contact with the ZP and mediate gamete adhesion. Acrosomal components assist the penetration of the spermatozoa through the ZP by the restricted disassembly of this structure either enzymatically or stoichiometrically. As a consequence of exposure to the external milieu, acrosomal components are gradually dispersed as a result of their inherent solubility properties or are released following proteolytic processing of the acrosomal matrix. In the

microenvironment at the periphery of the exposed acrosomal matrix, the acidic intra-acrosomal pH rises to approach the neutral conditions of the surrounding milieu, leading to the localized activation of acrosomal proteases (e.g., acrosin) which act to process and disperse the AM. Meanwhile, the (proximal) perinuclear acrosomal matrix is processed more slowly, perhaps as a result of the localized concentration of protease inhibitors that have yet to diffuse away. Thus, the acrosomal matrix dissolves from the outer zone to the inner recesses in a manner analogous to the release of drugs from "time-release" capsules. Following the dissolution of the acrosomal matrix from the outer margins, the freshly exposed, underlying acrosomal matrix materials can then reinitiate the ZP adhesion and start the cycle over again. In this continuously variable (analog) manner, the sperm cell can then ratchet its way through the ZP, leaving a clean penetration slit in its wake.

2. Mouse Sperm Protein sp56 is an Acrosomal Matrix Protein

We previously demonstrated that mouse sperm ZP-binding protein sp56 is an intra-acrosomal protein (Foster et al., 1997). Based upon the homology between mouse sp56 and guinea pig acrosomal matrix protein AM67, we tested the hypothesis that sp56 is part of the acrosomal matrix (Kim et al., 2001a), a structure that had yet to be demonstrated to exist in mouse sperm. To test this hypothesis, we prepared affinity-purified peptide-specific polyclonal antisera against two mouse sp56 peptides, CPTPDMEKIKIVSERRDF and VYKLFLEIERLEHQKEK. The antibodies, designated as anti-CPT and anti-VYK, respectively, identified a major immunoreactive protein with a molecular weight of 67,000. Minor species of 43,000 and 31,000 M_r were also detected by both antibodies; a band representing an 18,000~20,000 M_r protein was also detected by the C-terminal peptide-specific anti-VYK (Kim et al., 2001a). The respective peptides completely blocked the reactivity of each antibody. Anti-guinea pig AM67 also detected a protein of the same size in mouse and guinea pig. Monoclonal antibody 7C5 against sp56 (Cheng et al., 1994) only detected unreduced sp56. Like guinea pig AM67 (Foster et al., 1997), sp56 was found as a large molecular weight, disulfide-linked multimer in its native state. We also found that sp56 first appeared in late meiotic cells and accumulated during spermiogenesis, the haploid stage of spermatogenic cell development. The forms of sp56 in pachytene spermatocytes and spermatids had molecular weights higher than the sperm form; the size differences were apparently due to alterations in carbohydrate side-chains. The sp56 complex could not be solubilized by non-ionic detergent Triton X-100 but remained associated with the dorsal surface of the mouse sperm head, demonstrating that mouse sperm possess an acrosomal matrix containing a particulate form of sp56 (Kim et al., 2001a).

3. The Differential Time-Release Hypothesis

The second hypothesis states that each specific acrosomal protein has a different rate of release from sperm that is dependent upon its intrinsic properties and interactions with other components. The first corollary of this hypothesis is that soluble components of the acrosomal contents are quickly released from sperm following induction of acrosomal exocytosis. We initially tested this hypothesis by examining materials released from guinea pig sperm undergoing acrosomal exocytosis in response to ionophore A23187 and comparing these with the proteins remaining associated with the sperm (Kim et al., 2001b).

The second corollary of the Differential Time-Release Hypothesis states that AM components remain associated with sperm for a prolonged period of time but may undergo post-translational

modifications coincident with their release from the sperm surface ECM. Guinea pig AM67 was apparently not processed as a consequence of acrosomal exocytosis. Proacrosin was detected in immunoblots of mature sperm, but was not detected in either the pellet or supernatant of A23187-treated sperm, suggesting that the protein had been modified during acrosomal exocytosis. Consistent with this conclusion, protease activity consistent with the activation of proacrosin to lower molecular weights forms of acrosin were detected in the soluble acrosomal components released from the sperm stimulated to undergo acrosomal exocytosis with A23187. Furthermore, AM50 was proteolytically processed to AM50AR as a consequence of its release from the sperm, consistent with the results of others (Westbrook-Case et al., 1994). We have determined that processing of AM50 to AM50AR occurred in the amino-terminal region of the protein after arginines (data not shown). This cleavage pattern is consistent with proteolysis by acrosin, suggesting that this protease may function in the dispersion of the acrosomal matrix during acrosomal exocytosis. These data demonstrate that AM components are sequentially released during acrosomal exocytosis.

Like guinea pig AM50, mouse sp56 is converted to a lower molecular weight form coincident with its release from the sperm into the supernatant during the course of spontaneous acrosomal exocytosis. Apparently, proteolysis cleaves the C-terminal end of the sp56 monomer because anti-VYK no longer recognizes the same band detected by anti-CPT. We suggest that this proteolytic event is important for maintaining the function of sp56 as a ZP-binding protein of the AM. Experiments proposed below will examine this issue further.

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