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Membrane rafts regulate phospholipase B activation in murine sperm

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It is intuitive that fertilization—the start of life—involves communication between a sperm cell and an egg. It has been known that to become able to fertilize an egg, a sperm must first communicate with stimuli in the female tract. For example, sterol removal from the plasma membrane is required for sperm to undergo membrane fusion during acrosome exocytosis (AE). However, how membrane lipid changes were transduced into initiation of AE remained unclear. Recently, we found that sperm phospholipase B (PLB) is activated in response to sterol removal and released into the extracellular fluid by proteolytic cleavage. The resultant active PLB fragment can stimulate initiation of AE without other physiological stimulation. These results provide a possible mechanism for how AE is triggered, a critical question given recent data from others that show that AE is induced prior to contact with the egg's extracellular covering, the zona pellucida.

Keywords: fertilization, sperm, acrosome exocytosis, membrane rafts, phospholipase B

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Mammalian sperm are unable to fertilize an egg immediately after ejaculation, but acquire the ability during migration through the female reproductive tract. This process, collectively referred to as "capacitation"[1], is a prerequisite to initiate acrosome exocytosis (AE), which is itself necessary for a sperm to pass through the covering of the egg, known as the zona pellucida (ZP). Removal of sterols from the sperm plasma membrane is one of the initial triggers for the onset of capacitation.[2,3] This was demonstrated by evidence that exposure of sperm to cholesterol acceptors, such as BSA or 2-hydroxypropyl-β-cyclodextrin, triggers activation of the signaling cascades inherent to capacitation and potentiates responsiveness to initiate AE.[4] Despite the strict requirement for sterol removal prior to undergoing fusion of the plasma membrane overlying the acrosome (APM) with the outer acrosomal membrane, the molecular mechanism(s) by which sterol removal is transduced into the initiation of AE, have remained unknown.

Membrane rafts are membrane domains highly enriched in specific lipids and proteins, and play important roles in the regulation of diverse cellular processes including communication between cells and the extracellular environment.[5] Using live murine sperm, we previously demonstrated that the APM is a macrodomain highly enriched in sterols and the ganglioside G_{M1}.[6] This membrane region is itself composed of multiple, dynamic domains,[7] consistent with our biochemical results demonstrating that murine sperm possess at least 3 raft sub-types differing in lipid and protein composition.[8] Our recent proteomic analysis of sperm rafts identified phospholipase B (PLB) in all of the raft sub-types.[9] PLB is a membrane-anchored phospholipase that possesses calcium-independent PLA_1, PLA_2, and lysophospholipase activities.[10] Although PLB expression has been reported in sperm,[11] the physiological functions of PLB remained unclear. We recently reported that sperm PLB is localized in both the APM and acrosomal membranes where membrane fusion occurs.[12] Furthermore, sperm PLB is activated by sterol removal from the APM, providing an important link in

the communication of the sperm with its external environment and playing an important role in fertilization.[12]

Of note, we found that activated PLB is released into the extracellular fluid,[12] suggesting involvement of proteolytic cleavage. A study with human epidermis reported that PLB underwent limited proteolysis in response to exogenous trypsin or autolysis, producing a soluble 97 kDa fragment and a 140 kDa membrane-bound fragment,[13] consistent with tryptic treatment of recombinant PLB.[13] Unlike these studies, our results showed that sperm PLB can be proteolytically cleaved into 27 kDa and 50 kDa soluble fragments. This disparity might result from the difference in experimental conditions (artificial proteolysis vs. physiological response), or that PLB might differ in susceptibility to proteolysis between tissues or species. Autolysis of rat intestinal tissues produced 90 and 130 kDa PLB fragments,[11] whereas only a 140 kDa fragment was produced in guinea pig.[14] Interestingly, a recent study with a pathogenic fungus showed that N-linked glycosylation of PLB is important for its stability and protection from proteolysis.[15] Using software-based prediction, comparison of N-linked glycosylation sites among guinea pig, rat, and mouse PLB revealed that the number and location of potential glycosylation sites differed among these species (data not shown). Therefore, it is possible that the status of N-linked glycosylation might contribute to the cleavage pattern of PLB by limiting the accessibility of proteases to a given proteolytic site.

It was a long-standing view that capacitated sperm undergo AE when they bind to the ZP, in a process historically called the "acrosome reaction." However, this dogma has been strongly challenged in recent years. Studies using sperm expressing EGFP in the acrosome first suggested that membrane fusion resulted in a highly regulated form of exocytosis as opposed to a quick, binary transition from "intact" to "reacted"[16]. This same group recently reported evidence for the transient exposure of acrosomal matrix proteins in guinea pig sperm during capacitation,[17] suggesting the occurrence of multiple membrane fusion events during the course of capacitation. At the same time, another group demonstrated that sperm began AE before they encountered the ZP during the process of in vitro fertilization.[18] Together, these findings suggest that the induction of AE begins much earlier, as the sperm communicate with one or more stimuli within the female reproductive tract.

Our data suggest a possible mechanism for how this communication is transduced into a change in sperm function, through stimulation of AE. For example, incubation of sperm with either medium from capacitated sperm or with active fragments of recombinant PLB resulted in AE,[12] suggesting a role for PLB in stimulating membrane fusions. There is still much more to learn about the mechanisms leading to exocytosis, including whether it represents a "kiss and run" mechanism in which fusion pores are formed and rapidly closed without further dilatation,[19,20] or whether individual fusion events simply sum and merge into each other. In any case, the accumulated results of recent studies lead us to realize that acrosome exocytosis and fertilization involve not just communication between egg and sperm, but between sperm and other factors in the female reproductive tract and PLB plays an important role in this molecular conversation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Chang MC. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. Nature 1951; 168:697-8; PMID:14882325; http://dx.doi.org/10.1038/168697b0
2. Davis BK. Influence of serum albumin on the fertilizing ability in vitro of rat spermatozoa. Proc Soc Exp Biol Med 1976; 151:240-3; PMID:1250865; http://dx.doi.org/10.3181/00379727-151-39182
3. Davis BK, Byrne R, Hungund B. Studies on the mechanism of capacitation. II. Evidence for lipid transfer between plasma membrane of rat sperm and serum albumin during capacitation in vitro. Biochim Biophys Acta 1979; 558:257-66; PMID:508748; http://dx.doi.org/10.1016/0005-2736(79)90260-8
4. Visconti PE, Galantino-Homer H, Ning X, Moore GD, Valenzuela JP, Jorgez CJ, Alvarez JG, Kopf GS. Cholesterol efflux-mediated signal transduction in mammalian sperm. beta-cyclodextrins initiate transmembrane signaling leading to an increase in protein tyrosine phosphorylation and capacitation. J Biol Chem 1999; 274:3235-42; PMID:9915865; http://dx.doi.org/10.1074/jbc.274.5.3235
5. Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 2000; 1:31-9; PMID:11413487; http://dx.doi.org/10.1038/35036052
6. Selvaraj V, Asano A, Buttke DE, McElwee JL, Nelson JL, Wolff CA, Merdiushev T, Fornés MW, Cohen AW, Lisanti MP, et al. Segregation of micron-scale membrane sub-domains in live murine sperm. J Cell Physiol 2006; 206:636-46; PMID:16222699; http://dx.doi.org/10.1002/jcp.20504
7. Selvaraj V, Asano A, Buttke DE, Sengupta P, Weiss RS, Travis AJ. Mechanisms underlying the micron-scale segregation of sterols and GM1 in live mammalian sperm. J Cell Physiol 2009; 218:522-36; PMID:19012288; http://dx.doi.org/10.1002/jcp.21624
8. Asano A, Selvaraj V, Buttke DE, Nelson JL, Green KM, Evans JE, Travis AJ. Biochemical characterization of membrane fractions in murine sperm: identification of three distinct sub-types of membrane rafts. J Cell Physiol 2009; 218:537-48; PMID:19006178; http://dx.doi.org/10.1002/jcp.21623
9. Asano A, Nelson JL, Zhang S, Travis AJ. Characterization of the proteomes associating with three distinct membrane raft sub-types in murine sperm. Proteomics 2010; 10:3494-505; PMID:20815087; http://dx.doi.org/10.1002/pmic.201000002
10. Ghannoum MA. Potential role of phospholipases in virulence and fungal pathogenesis. Clin Microbiol Rev 2000; 13:122-43; PMID:10627494; http://dx.doi.org/10.1128/CMR.13.1.122-143.2000
11. Takemori H, Zolotaryov FN, Ting L, Urbain T, Komatsubara T, Hatano O, Okamoto M, Tojo H. Identification of functional domains of rat intestinal phospholipase B/lipase. Its cDNA cloning, expression, and tissue distribution. J Biol Chem 1998; 273:2222-31; PMID:9442065; http://dx.doi.org/10.1074/jbc.273.4.2222
12. Asano A, Nelson-Harrington JL, Travis AJ, Phospholipase B. Phospholipase B is activated in response to sterol removal and stimulates acrosome exocytosis in murine sperm. J Biol Chem 2013; 288:28104-15; PMID:23943622; http://dx.doi.org/10.1074/jbc.M113.450981
13. Maury E, Prévost MC, Nauze M, Redoulès D, Tarroux R, Charvéron M, Salles JP, Perret B, Chap H, Gassama-Diagne A. Human epidermis is a novel site of phospholipase B expression. Biochem Biophys Res Commun 2002; 295:362-9; PMID:12150957; http://dx.doi.org/10.1016/S0006-291X(02)00657-5
14. Delagebeaudeuf C, Gassama-Diagne A, Nauze M, Ragab A, Li RY, Capdevielle J, Ferrara P, Fauvel J, Chap H. Ectopic epididymal expression of guinea pig intestinal phospholipase B. Possible role in sperm maturation and activation by limited proteolytic digestion. J Biol Chem 1998; 273:13407-14; PMID:9593672; http://dx.doi.org/10.1074/jbc.273.22.13407
15. Turner KM, Wright LC, Sorrell TC, Djordjevic JT. N-linked glycosylation sites affect secretion of cryptococcal phospholipase B1, irrespective of glycosylphosphatidylinositol anchoring. Biochim Biophys Acta 2006; 1760:1569-79; PMID:16919392; http://dx.doi.org/10.1016/j.bbagen.2006.07.002
16. Kim KS, Gerton GL. Differential release of soluble and matrix components: evidence for intermediate states of secretion during spontaneous acrosomal exocytosis in mouse sperm. Dev Biol 2003; 264:141-52; PMID:14623237; http://dx.doi.org/10.1016/j.ydbio.2003.08.006
17. Kim KS, Foster JA, Kvasnicka KW, Gerton GL. Transitional states of acrosomal exocytosis and proteolytic processing of the acrosomal matrix in guinea pig sperm. Mol Reprod Dev 2011; 78:930-41; PMID:21919109; http://dx.doi.org/10.1002/mrd.21387

18. Jin M, Fujiwara E, Kakiuchi Y, Okabe M, Satouh Y, Baba SA, Chiba K, Hirohashi N. Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. Proc Natl Acad Sci U S A 2011; 108:4892-6; PMID:21383182; http://dx.doi.org/10.1073/pnas.1018202108

19. Tardif S, Cormier N. Role of zonadhesin during sperm-egg interaction: a species-specific acrosomal molecule with multiple functions. Mol Hum Reprod 2011; 17:661-8; PMID:21602212; http://dx.doi.org/10.1093/molehr/gar039

20. Harper CV, Cummerson JA, White MR, Publicover SJ, Johnson PM. Dynamic resolution of acrosomal exocytosis in human sperm. J Cell Sci 2008; 121:2130-5; PMID:18522990; http://dx.doi.org/10.1242/jcs.030379