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.

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”, 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 GM1.6 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 PLA1, PLA2, 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.²

Of note, we found that activated PLB is released into the extracellular fluid,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, consistent with trypsin 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, 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”¹⁶¹⁷. 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, 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,¹⁹²⁰ 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.

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