

# Fusion of Membranes During the Acrosome Reaction: A Tale of Two SNAREs

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**ABSTRACT** During spermiogenesis, hydrolytic enzymes are sorted from the Golgi apparatus to the acrosome, a supranuclear megavesicle. At fertilization, the enzymatic content of the acrosome is released by exocytosis when a portion of the plasma membrane enveloping the sperm head fuses with the outer membrane of the acrosome. Membrane fusion involves the interaction of a specific pair of proteins, called SNAREs (for soluble N-ethylmaleimide sensitive factor attachment protein receptor). v-SNARE is presumably associated with the membrane of the acrosomal vesicle. Target t-SNARE is associated with the plasma membrane. The interaction of v-SNARE and t-SNARE requires two additional proteins: Rab proteins, members of a family of small GTPases related to the Ras proteins, and a complex of two proteins, NSF-SNAP, recruited by the interacting v-SNARE-tSNARE pair. Syntaxin 2, a v-SNARE member, and Rab3A, a member of the Rab GTPases, have been localized in the acrosome of rodent sperm. *Mol. Reprod. Dev.* 57:309–310, 2000. © 2000 Wiley-Liss, Inc.

A paper from Katafuchi et al. in this issue of *Molecular Reproduction and Development* (p 375–383) reports the localization of syntaxin 2, a member of the SNAREs (for soluble N-ethylmaleimide sensitive factor attachment protein receptor) in the acrosomal region of rat, mouse, and hamster sperm. Members of the SNARE family mediate the fusion of vesicle and target membranes as defined by a general model known as the SNARE hypothesis (Rothman, 1994), that includes the participation of members of the family of small GTPases as well as effector and regulatory proteins. In fact, Rab3A, a small GTPase, was reported to be associated with the acrosome of rat and mouse sperm (Iida et al., 1999; Ward et al., 1999).

How do syntaxin 2 and Rab3A fit into the SNARE model of vesicle fusion with respect to the acrosome reaction? Let's go back to the Golgi apparatus, the site of glycosylation and sorting of acrosomal enzymes during spermiogenesis, to appreciate the potential significance of SNAREs in the acrosomal reaction. Budding transporting vesicles derived from the endoplasmic reticulum-called COPII vesicles because they are coated with polymerized coatamer protein subunits with the help of the small (about 20 kDa) GTPase ARF1 (for ADP ribosylation factor)—carry the future acrosomal enzymes to the Golgi apparatus. ARFs bound to

ADP is soluble and inactive. The active GTP-bound form of ARF binds strongly to membranes in the presence of regulators and effectors. A 200 amino acid domain on ARF, called Sec7, catalyzes the exchange of GDP for GTP. The activation of ARF-GTPases causes changes in the distribution of lipids and proteins in the associated membrane, leading to altered structure and function of the membrane, including the implementation of signaling pathways (see a recent review, Donaldson and Jackson, 2000).

COPII transporting vesicles shed their coat and fuse with the *cis* face of the Golgi stack and shuttle across the stack. COPI vesicles, with a different coat assembled and disassembled by the same GTP-dependent process, exit from the opposite *trans* face of the stack towards a post-Golgi site, presumably to the acrosomal vesicle during spermiogenesis. A significant event during vesicle uncoating is the unmasking of v-SNARE membrane proteins to enable fusion with t-SNARE partners on the target membranes. Vesicles can move in a retrograde direction from the Golgi to the endoplasmic reticulum (Barlowe, 2000).

The SNARE hypothesis poses important questions with respect to spermiogenesis: Does the same SNARE molecular mechanism operate during the gradual build up of the acrosome in spermatids? Do all proteins entering the Golgi stack have the same common acrosomal fate or can some of them return to the endoplasmic reticulum by a retrograde flow mechanism (Kluperman, 2000; Pelham and Rothman, 2000)? It is rather encouraging to see that the attractive process of molecular “acrosomogenesis” is now being revisited in light of the contemporary view of the Golgi apparatus. The use of *in vitro* systems, where early spermatids can be cocultured with Sertoli cells, should provide an experimental system for dissecting both the vectorial transport and molecular components of COPI and COPII vesicles during acrosomogenesis. In fact, a useful experimental approach could represent the visual tracking of artificial protein aggregates migrating from the Golgi- to-acrosome in developing spermatids *in vitro*. A precedent of this technical approach has

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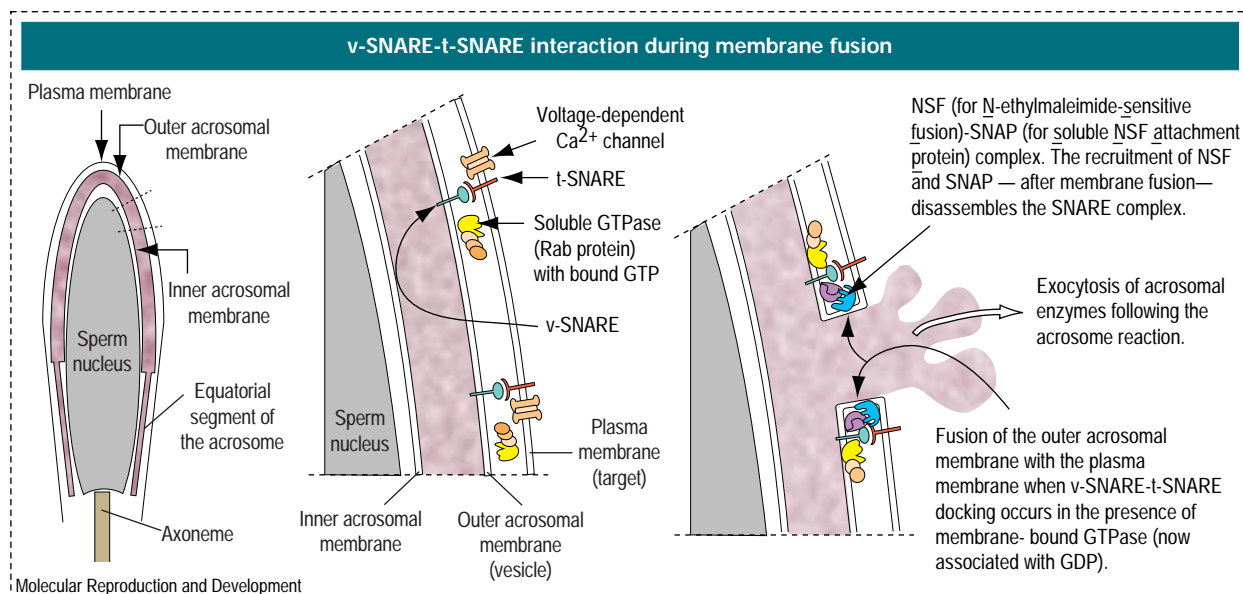


Fig. 1.

been reported in another system (Volchuk et al., 2000). The major key issues to be addressed are: What directs the sorting of hydrolytic enzymes to accumulate in the acrosome? When do the tethers and docking proteins appear during spermiogenesis? What prevents a premature fusion of outer acrosomal and plasma membranes prior to the acrosome reaction?

Acrosome-plasma membrane fusion is central to fertilization and seems to adhere to the basic principles of the SNARE hypothesis. Two acrosomal players have so far been identified: the SNARE member syntaxin 2 and the GTPase member Rab3A. The basic steps of acrosome membrane-plasma membrane fusion are depicted in Fig. 1, but the validation and extension of the illustrated model awaits further identification of additional players in the membrane fusion process. What is potentially significant is the finding of direct binding of syntaxin to the voltage dependent  $\text{Ca}^{2+}$  channel observed in neuronal presynaptic membranes. This observation can be related to a G-protein-promoted increase in intracellular calcium observed at fertilization during acrosomal exocytosis (Florman et al., 1989). Although a general model supporting an operational SNARE hypothesis is beginning to emerge during the acrosomal reaction, several issues remain unresolved. Perhaps a high priority is the identification

of effector and regulatory molecules that mediate the activity of the members of the family of ARF-GTPases (Schimoller et al., 1998) following post -vSNARE-tSNARE docking, probably leading to the exocytosis of acrosomal enzymes.

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