Review Article



# Molecules involved in acrosomal exocytosis and cortical granule exocytosis

**Mengsi Lin[1], Qingwen Zhu[1], Jing Wang[1], Wenjie Yang[2], Haihua Fan[2], Jianfeng Yi[2], Maorong Jiang[3]**

[1]Department of Prenatal Diagnosis, Maternal and Child Health Care Hospital of Nantong, Nantong, 226018, China; [2]Medical College of Nantong University, Nantong, 226001, China; [3]Laboratory Animals Center, Nantong University, Nantong 226001, China

*Contributions:* (I) Conception and design: M Jiang; (II) Administrative support: M Jiang; (III) Provision of study materials or patients: M Lin, Q Zhu; (IV) Collection and assembly of data: M Lin, Q Zhu, J Wang; (V) Data analysis and interpretation: M Lin, Q Zhu, J Wang, W Yang, H Fan, J Yi; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*Correspondence to:* Maorong Jiang. Laboratory Animals Center, Nantong University, 19 Qixiu Road, Nantong 226001, China. Email: jiangmr@ntu.edu.cn.

**Abstract:** At fertilization, the acrosome reaction and cortical reaction are crucial process to block polyspermy and the prevention of triploidy. Although molecules involved in trigger secretion are various in different exocytosis events, the two processes may share the similar exocytosis mechanism. Exocytosis is an accurate regulated process that consists of multiple stages such as recruitment, targeting, tethering and docking of secretory vesicles with the plasma membrane, priming of the fusion machinery and calcium-triggered membrane fusion. After fusion, the membrane around the secretory vesicle is incorporated into the plasma membrane and the granule releases its contents. The proteins involved in these processes belong to several highly conserved families: Rab GTPases, SNAREs (soluble NSF-attachment protein receptors), α-SNAP (α-NSF attachment protein), NSF (N-ethylmaleimide-sensitive factor), Munc13, Munc18, complexins and synaptotagmins. This review discussed that molecules believed to participate in the secretory function and their involvement in acrosome and cortical granule exocytosis.

**Keywords:** Acrosome reaction; cortical reaction; exocytosis; polyspermy; SNARE

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## Background

Mammalian fertilization is the fusion of a human oocyte and sperm, which includes a series of coordinated events. Firstly, fertilizable oocytes are ovulated along with the first polar body, zona pellucida (ZP), and the cumulus oophorus (1,2). In the next several hours, the sperm sustain capacitation in fallopian tube. This process enhances its viability and makes its membrane in unstable condition in preparation for the acrosomal reaction. Once the sperm penetrates the secondary oocyte and contact with the oocyte's ZP, the acrosome reaction occurs in the ampulla of the tuba uterina. In several mammalian species (mouse, pig and human), acrosomal exocytosis is triggered during gamete contact by ZP3, or ZP (3,4). ZP3 induces a continuous enrichment of the internal $Ca^{2+}$ concentration, afterwards causing the acrosome reaction (5). The acrosomal reaction takes place in the acrosome of the sperm when being close to the egg, including the fusion of the acrosome membrane with the sperm's plasma membrane and the exocytosis of the acrosome.

When the spermatozoon swim through the ZP, the cortical reaction takes place (6). The cortical granules are released after cortical reaction initiation during fertilization to prevent polyspermy, the fusion of multiple sperm with one egg. The cortical granules contain a series of enzymes that modify and harden the ZP, which is a proteinaceous matrix surrounding the oocyte (7). As a result, the exocytosis of cortical granules reduces the binding affinity of sperm through cleavage of the ZP protein ZP2 and makes the ZP impermeable to additional sperm (8). In order to make one oocyte fertilized by merely one sperm, the acrosome reaction and cortical reaction are fundamental. They

functions on the foundation of same molecules involved in endocellular membrane fusion and exocytosis in multiple types of cells (9).

SNARE (Soluble N-ethylmaleimide-sensitive factor attachment protein receptor) is the vital regulative proteins in every membrane fusion case (10-12). SNARE proteins are a large protein superfamily consisting of at least 38 members based on the latest data. To date, large numbers of scientists have offered insights into the mechanism by which SNAREs drive specific membrane fusion. SNAREs can be classified into v (vesicle)-SNARE and t (target)-SNARE. V-SNARE and t-SNARE are related to the transport vesicles and acceptor or target compartments, respectively. In the end, both SNARE molecules form a SNARE complex. V-SNARE consists of VAMP (vesicle- associated membrane protein) and synaptotagmin, which were existed on the membranes of vesicle. T-SNARE is composed of syntaxin and SNAP-25 (synaptosome-associated protein of 25 kDa), which were expressed on the membranes of target (13-15).

Membrane fusion is also associated with other protein molecules (16). It was reported that Rab GTPases (Rabs) and other small GTP-binding protein were involved in the process (17). Rabs bind to proper t-SNAREs on target membranes and lead to the docking of vesicles to target membranes. Moreover, Sec1/Munc18-1 (SM), synaptotagmin, complexin, and $Ca^{2+}$ proved to be important for exocytosis in eukaryotic cells (18-22).

In this review, we attempted to discuss recent progress in order to understand the role of exocytosis related proteins involved in the acrosome reaction and cortical reaction, and to analyze their function in human fertilization.

## Molecules involved in acrosomal reaction

### *SNARE proteins*

Various SNARE proteins are required to dock and fuse the interacting membranes in sperm during acrosome reaction (23). As a consequence, SNAREs localized to specific organelle membranes and mediate membrane fusion (24). Meanwhile, SNAREs decide the specificity of cargo transfer to the target membrane. Only when T-SNAREs and v-SNAREs assemble into a stable SNARE complex, they can provide the force that is necessary for membrane fusion (23,25). However, the regulation of acrosome reaction relies on the accurate cooperation between SNAREs and their interactional proteins (23,26,27).

SNAREs in a *cis* configuration in sperm are actors as ternary complexes, different to the SNAREs in other cells. Due to the fact that sperm can merely fertilize the oocyte once, the successful procedure partly depends on the accurate spatial and temporal regulation of the acrosome reaction. At first, SNAREs are inactive actors in *cis* complexes on plasma and acrosomal membranes. The activation of Rab3A took place after $Ca^{2+}$ enters into the cytoplasm, afterwards triggering the locking of the acrosome to the plasma membrane (28,29). Subsequently, N-ethylmaleimide-sensitive factor (NSF)/α-SNAP can disassemble *cis* SNARE complexes on both membranes. NSF is presented in the acrosome of several mammalian spermatozoa. However, the active NSF is required for $Ca^{2+}$-dependent exocytosis of permeabilized sperm (30). The disassembly of *cis* SNARE complexes is mediated by NSF. NSF is a chaperone-like ATPase, and its ATPase activity is regulated by α-SNAP (31). At this time, under the help of SNAREs and synaptotagmin, through inositol 1, 4, 5-trisphosphate (IP3)-sensitive $Ca^{2+}$ channels, $Ca^{2+}$ can be released from inside the acrosome to trigger the eventual part of membrane fusion (32). Based on recent research, SNAP-23/Syntaxin3/VAMP2 could form a trimeric SNARE complex by the induction of acrosome exocytosis (33). $Ca^{2+}$ induced acrosome reaction was completely inhibited by the addition of a specific antibody against SNAP-23, suggesting that SNAP-23 was involved in the exocytotic process. In addition, studies had manifested that treatment with Botulinum neurotoxin type A (BoNT/A), -E, -F, and -C inhibited the expression of SNAP-25, VAMP2, syntaxin 1A, and syntaxin 1B, resulted in a $Zn^{2+}$ dependent inhibition of $Ca^{2+}$ induced acrosomal release (9,34). In summary, the above listed proteins in SNAREs family are involved in the acrosome reaction.

### *Rab proteins*

The Ras superfamily of monomeric G proteins includes Rab family, in which nearly 70 types of Rabs have been identified in humans so far. It was observed that the Rab GTPases regulation was involved in many processes of membrane transit, including vesicle formation and movement in cytoskeletal networks (35,36). During membrane fusion, Rab proteins, which are peripheral membrane proteins, firstly recognize and attach to the compartments which are going to fuse (37,38). Consequently, Rab proteins via a lipid group covalently bound to GDP/GTP docked to a membrane. Similar to other GTPases, Rab proteins have two conformations, including an inactive form bound to GDP, and an active form bound to GTP (39,40). Meanwhile,

Rabs can switch between inactive form and active form. The conversion from GDP-bound to GTP-bound form is catalyzed by a GDP/GTP exchange factor (GEF). Rab was activated after the conversion was finished. However, Rabs inactivation was also regulated by a GTPase-activating protein (GAP), which increased the inherent GTP hydrolysis of Rabs (41,42).

Rabs have various isoforms, and each Rab isoform has numerous effectors which functions variously (43). The Rab protein is to be effective because of the specific binding of the effector to the Rab protein. Conversely, the Rab protein switches to the inactive state cause of effector dissociation from the Rab protein. In the end, the Rabs was recycled back to its original membrane after membrane fusion and effector dissociation. During dissociation, the GDP dissociation inhibitor (GDI) can inhibit the exchange of GDP to GTP and deliver Rab to its original membrane by binding the prenyl groups of the inactive.

Rab3 is one of proteins that were the most studied in exocytotic cells in Rab family. During the acrosome reaction, it was shown that the Rab3A permeated through membrane triggered acrosomal exocytosis in the virtual absence of $Ca^{2+}$ (28,44). Once Rab3A is activated, NSF and α-SNAP disassemble the *cis* complexes, and subsequently the free SNARE reassembles in loose *trans* complexes (45). Nevertheless, when the Rab3A translocated into the cytoplasm, it can trigger secretion by activating downstream of the Rab3A effectors. The synthetic peptides of the Rab3A effector domain have played a similar function in acrosome exocytosis (45). These peptides can trigger or block acrosome exocytosis based on the given condition (28,29,46).

In addition, Rab27 is also the Rab subfamilies implicated in regulated exocytosis (47-49). The recruitment and attachment of secretory vesicles to the plasma membrane could be controlled by Rab27 interacting with its effectors (50). Nevertheless, unlike other Rab families in driving endocellular membrane transit, the persistent cycle of Rab27 and Rab3 in sperm between their motionless GDP-bound and dynamic GTP-bound forms do not exist. On the contrary, the activation state and localization of Rab27 and Rab3 in sperm are coupled to exocytotic stimulus (51). As a result, a large proportion of resting sperm contains these Rab proteins in their inactive GDP-bound form. During the exocytotic cascade, coupled to acrosomal swelling, Rab27 and Rab3A are activated (conversion from GDP-bound to GTP-bound form) in react to exocytosis (52,53). Beyond that, several scientists observed that a chimeric protein consisting of the amino-terminal portion of Rab3A fused to the carboxyl-terminal portion of Rab22A bears the repressing capacity of Rab3A (54). Recombinant, full-length Rab22A has no measurable influence on the calcium-triggered acrosome reaction, which suggests that the effects elicited by the chimera are attributable to the Rab3A portion of the molecule. Rab3A-22A halts exocytosis after swelling, intra-acrosomal calcium mobilization and the opening of fusion pores but prior to the vesiculation of membranes and release of acrosomal contents. In other words, Rab3A-22A stabilizes fusion pores and prevents their expansion. In general, Rab proteins have a vital effect on acrosome exocytosis.

### *Munc18-1*

Munc18-1 (mammalian uncoordinated18-1), which was first discovered in the nematode worm C. elegans, has multiple roles in exocytosis as a member of the SM family. So far, it proves to be important in the dynamic process of trans-SNARE complex formation and stability during human sperm acrosomal exocytosis (55). Munc18-1 binds syntaxin directly to form a syntaxin/munc18 complex, and regulate the availability of syntaxin for SNARE complex assembly (56-59). This interaction with SNARE core was proved to be stabilized in the conserved $H_{abc}$ domain of syntaxin (60,61). Sequestration of free syntaxin with recombinant Munc18-1 (55) or an excess of a-SNAP (62) halts the acrosome reaction exocytotic cascade before it reaches this stage. At the stage before $Ca^{2+}$ efflux from inside of acrosomal, endogenous Munc18-1 played a trigger effect in acrosomal exocytosis to activate membrane fusion (55). But the integration of gene recombinant Munc18-1 with the inactive endogenous syntaxin 1 is associated with the depression of acrosomal exocytosis. It turned out to be valid based on the discovery that syntaxin held toxin-sensitive properties when recombinant Munc18-1 existed (63). Moreover, at syntaxin-1 two different conformational states, Munc18-1 could regulate the N-terminal domain of syntaxin-1 (64). Munc18-1 is also closely responsible for the transfer of syntaxin to target compartments (65). It was reported that Munc18-1 not only bound a closed form of syntaxin 1 to block SNARE complex formation, but also could release syntaxin assembled from SNARE complexes of different compositions (66). In sperm, α-SNAP/NSF could disassemble *cis*-SNARE complexes (31,33,62), while α-SNAP/NSF action could be resisted by tetanus toxin (TeTx)-resistant *trans* complexes (67). Intact SNAREs are essential for the anchoring of the acrosome to the membrane of plasma (33). When the active Munc18-1 is absent,

 

*trans* complexes could not be formed, otherwise at toxin-sensitive states, they can be still sensitive to α-SNAP/NSF to allow the SNAREs to cycle. Furthermore, previous research showed that Munc18-1 increased the fusion rate of relative SNARE proteins (55).

### *Calcium*

Although activated secretion are various in diverse exocytosis issues, the level of intracellular $Ca^{2+}$ for the activation of secretion will rise in most cases (68,69). Hence, the scientific community has reached the consensus that $Ca^{2+}$ is necessary for the acrosome reaction, which is needed to penetrate the egg's plasma membrane for capacitation (70,71). Multiple different $Ca^{2+}$ channels have been verified to control sperm function. When acrosomal exocytosis was induced by $Ca^{2+}$ (72), $Ca^{2+}$ influx into the sperm cell leaded to the dissociation of complexin 2 (73) and allowed the participation of complementary R-SNARE to the completion of acrosomal exocytosis. Increased intracellular $Ca^{2+}$ levels indirectly cause a conformational conversion of the *trans*-SNARE complex into the *cis*-SNARE complex during acrosomal exocytosis, thus inducing the fusion of the two docked membranes (71). The complex exocytotic process released the acrosomal contents including $Ca^{2+}$, enzymes, and modifies membrane constituents, and turned the inner membrane of acrosomal to the extracellular medium (74). $Ca^{2+}$ levels are balanced between influx from the extracellular matrix and efflux from intracellular reserves (75). $Ca^{2+}$ influx through this channel in turn could activate a $Ca^{2+}$-sensitive phospholipase C (PLC) to generate inositol trisphosphate (IP3) and diacylglycerol, and could also activate IP3 receptors which empty $Ca^{2+}$ stores and evoke the sustained $Ca^{2+}$ elevation (76,77).

## Molecules involved in cortical reaction

Cortical reaction is a $Ca^{2+}$-dependent exocytotic process, which is initiated during fertilization. The content of secretory granules is released into the perivitelline space after fertilization immediately, to establish a permanent barrier to sperm entry for preventing polyspermy. There are a lot of molecules involved in cortical exocytosis, just like acrosomal exocytosis.

### *SNARE proteins*

There are many convincing evidence in several mammalian species that the interactions between SNARE proteins participated in the exocytosis of cortical granules, which specialized secretory vesicles within egg's cortex to fuse with the egg's plasma membrane. Subsequently, the exocytosis of the contents of cortical granules can modify the matrix in the cell exterior to prevent additional spermatozoa from going through the newly fertilized egg (78,79). However, such as in mouse, unfertilized oocytes contained the SNAP-25, a t-SNARE protein, which was fundamental for the cortical reaction. Meanwhile, in case of the SNAP-25 selectively cleaved by botulinum neurotoxin A, it could cause the depressing of cortical granule exocytosis inducing by sperm (80,81).

Syntaxins also exists in oocyte in mouse (82). Syntaxins were members of integrated membrane Q-SNARE proteins that were involved in membrane fusion (83,84). It was reported that syntaxin 4 was specifically expressed in oocytes in metaphase II and co-localized with cortical granules. Likewise, syntaxin 4 also took part in membrane fusion between the vesicle membrane, plasma membrane, and exocytosis during the cortical reaction (82,85). In addition, an up-to-date study in the field of porcine oocytes revealed that SNAP-23 and VAMP1 were associated with anchoring cortical granules to the oolemma (8). The interactions between t-SNARE proteins and v-SNARE proteins, coupled with the molecule complexin, are significant for retaining cortical granules in the cortex of the oocytes before exocytosis (8). The existence of SNARE complex-composing proteins syntaxin 2/VAMP1/SNAP-23 in the restricted cortical reaction during meiotic maturation, and the increasing of the co-localization of SNARE proteins with cortical granules at the oocyte's surface were both to further support for the idea that SNARE proteins were involved in the cortical reaction. Further evidence was the consistent presence of complexin at the oolemma and the SNARE core protein complex were stabilized by complexin (86,87).

All of the above, the interaction between SNARE and complexin may play an important role in the temporary arresting of the docked CGs at the oolemma, in order to further prevent the spontaneous fusion of granule membrane with the oolemma (88-91). As well, syntaxins bind synaptotagmin in a $Ca^{2+}$-dependent fashion via carboxy-terminal H3 domain. Nevertheless, the cytosolic $Ca^{2+}$ levels as the fertilization-dependent increasing probably release complexin from the SNARE complex and thereby allow the initiation of the SNARE-mediated cortical reaction.

The principle of action named "SNARE hypothesis" was

 

firstly put forward by Jahn *et al.* (11). Two t-SNAREs proteins and one v-SNARE proteins are observed to form the ternary *trans*-SNARE complex. The formation of ternary complex was considered to push the fusing membranes tightly together and trigger lipid bilayer fusion. So far, α-SNAP and NSF is necessary for the disassembling of SNARE complex. It was reported that cortical granule exocytosis can be compressed by blocking α-SNAP and NSF function. These findings unveiled the mechanism that α-SNAP and NSF take part in the early stage of a round cycle of SNARE complex. Thus, α-SNAP and NSF could disassemble the *cis*-SNARE complex and make their constituents obtainable to assemble the *trans*-SNARE complex for bilayer fusion in the process of cortical reaction.

### *Rab proteins*

Membrane trafficking, cell growth, and differentiation are inseparable from Rab family members. The Rab proteins are involved in the most phases of membrane system including the nucleus membrane and the plasma membrane in intracellular. It is well understood that membrane fusion is the essential proceed for cortical granule exocytosis. Rab3A, a member of small GTP-binding Rab family (92), was broadly expressed in the cytoplasm in maturational mouse oocytes at all stages (93). Not like Rab3A, Rab27A was specifically distributed in the cortex of oocytes from germinal vesicle (GV) to MII stages. Following cortical granule exocytosis, Rab3A associated selectively with cortical granules in eggs and oocytes (93,94). Rab3A have multiple function in regulating CG migration and peripheral spindle, polarity establishment, and asymmetric division. So, it can be said Rab3A is a marker of cortical granules and participates in cortical reaction. However, Rab27A had an important effect on regulating CG exocytosis following MII-stage oocyte activation. Above results indicate Rab proteins involved in oocyte CG exocytosis.

### *Myosin-Va (MyoVa)*

MyoVa, encoded by the *MYO5A* gene, is an actin-based motor molecular. It is involved in secretion on the heels of prior observations (95-98). Previous research had demonstrated that the translocation of CG was independent of microtubules. However, Rab27a has been proved to interact with MyoVa to form a complex together, and to regulate the transport of secretory cargoes in multiple cell types. Desnos reported that MyoVa mediated secretary granule docking and partial loss of MyoVa function by using of a truncated construct could reduce granule exocytosis (99). Cheeseman *et al.* further justified the participation of MyoVa in the translocation of CGs (100). In summary, MyoVa plays an important role in CGs trafficking and involves certainly in cortical reaction.

### *Calcium*

The trigger exocytosis of CGs is necessary to prevent polyspermy at fertilization. Besides, CGs release in mature eggs is an event of $Ca^{2+}$-triggered exocytosis, and similar to those in other somatic cells (101,102). Therefore, $Ca^{2+}$-dependent proteins are very important for cortical reaction. In mammalian eggs, $Ca^{2+}$ triggers egg activation through $Ca^{2+}$-dependent protein effectors, such as protein kinases (PK), calmodulin (CaM), and other specific proteins involved in exocytosis (103,104). Elevation of cytoplasmic $Ca^{2+}$ is considered to be needed for the exocytotic case to take place. In contrast, due to absent or unavailable ability to release and respond to increases in intracellular $Ca^{2+}$, the CGs release or CGs exocytosis could not be undergo in preovulatory oocytes (105-108). The calcium chelator-BAPTA (1,2-bis (o-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid) could compress the CGs exocytosis (109-111). The using of BAPTA was the good evidence for the role of $Ca^{2+}$ involved in cortical reaction. But, there were also special case in sea urchin eggs. It was reported that mastoparan induced CG exocytosis in a $Ca^{2+}$-independent (112).

## Summary

Acrosome reaction and cortical granule exocytosis at fertilization is necessary processes for blocking polyspermy. In this review, we discussed retrospectively the function of molecules in the secretory exocytosis and their involvement in acrosome or cortical granule exocytosis, regardless of other involved molecules which is absent in this review. Despite the difference of the biology of acrosome and cortical granules, the two types of vesicle also shared similarities. In summary, the similarities included a dependence on a transient calcium exposure for exocytosis, and numbers of molecules implicated in regulating exocytosis.

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## Footnote

*Conflicts of Interest*: The authors have no conflicts of interest to declare.

## References

1. Arnoult C, Zeng Y, Florman HM. ZP3-dependent activation of sperm cation channels regulates acrosomal secretion during mammalian fertilization. J Cell Biol 1996;134:637-45.
2. Wassarman PM, Litscher ES. Towards the molecular basis of sperm and egg interaction during mammalian fertilization. Cells Tissues Organs 2001;168:36-45.
3. McLeskey SB, Dowds C, Carballada R, et al. Molecules involved in mammalian sperm-egg interaction. Int Rev Cytol 1998;177:57-113.
4. Wassarman PM. Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis, and fusion. Cell 1999;96:175-83.
5. O'Toole CM, Arnoult C, Darszon A, et al. Ca(2+) entry through store-operated channels in mouse sperm is initiated by egg ZP3 and drives the acrosome reaction. Mol Biol Cell 2000;11:1571-84.
6. Ducibella T, LeFevre L. Study of protein kinase C antagonists on cortical granule exocytosis and cell-cycle resumption in fertilized mouse eggs. Mol Reprod Dev 1997;46:216-26.
7. Apparicio M, Mostachio GQ, Motheo TF, et al. Distribution of cortical granules and meiotic maturation of canine oocytes in bi-phasic systems. Reprod Fertil Dev 2015;27:1082-7.
8. Liu M. The biology and dynamics of mammalian cortical granules. Reprod Biol Endocrinol 2011;9:149.
9. Tomes CN. The proteins of exocytosis: lessons from the sperm model. Biochem J 2015;465:359-70.
10. Wickner W, Schekman R. Membrane fusion. Nat Struct Mol Biol 2008;15:658-64.
11. Jahn R, Scheller RH. SNAREs--engines for membrane fusion. Nat Rev Mol Cell Biol 2006;7:631-43.
12. Rizo J, Sudhof TC. The membrane fusion enigma: SNAREs, Sec1/Munc18 proteins, and their accomplices--guilty as charged? Annu Rev Cell Dev Biol 2012;28:279-308.
13. Hess DT, Slater TM, Wilson MC, et al. The 25 kDa synaptosomal-associated protein SNAP-25 is the major methionine-rich polypeptide in rapid axonal transport and a major substrate for palmitoylation in adult CNS. J Neurosci 1992;12:4634-41.
14. Oyler GA, Higgins GA, Hart RA, et al. The identification of a novel synaptosomal-associated protein, SNAP-25, differentially expressed by neuronal subpopulations. J Cell Biol 1989;109:3039-52.
15. A snare for the weak. Nat Biotechnol 2000;18:1025.
16. Whiteside TL. Stimulatory role of exosomes in the context of therapeutic anti-cancer vaccines. Biotarget 2017;1:5.
17. Novick P, Zerial M. The diversity of Rab proteins in vesicle transport. Curr Opin Cell Biol 1997;9:496-504.
18. Schaub JR, Lu X, Doneske B, et al. Hemifusion arrest by complexin is relieved by Ca2+-synaptotagmin I. Nat Struct Mol Biol 2006;13:748-50.
19. Steinhardt RA, Epel D, Carroll EJ, Jr., et al. Is calcium ionophore a universal activator for unfertilised eggs? Nature 1974;252:41-3.
20. Sudhof TC, Rothman JE. Membrane fusion: grappling with SNARE and SM proteins. Science 2009;323:474-7.
21. Tang J, Maximov A, Shin OH, et al. A complexin/synaptotagmin 1 switch controls fast synaptic vesicle exocytosis. Cell 2006;126:1175-87.
22. Wood TC, Wildt DE. Effect of the quality of the cumulus-oocyte complex in the domestic cat on the ability of oocytes to mature, fertilize and develop into blastocysts in vitro. J Reprod Fertil 1997;110:355-60.
23. Tomes CN, Michaut M, De Blas G, et al. SNARE complex assembly is required for human sperm acrosome reaction. Dev Biol 2002;243:326-38.
24. Sollner TH. Regulated exocytosis and SNARE function (Review). Mol Membr Biol 2003;20:209-20.
25. Fasshauer D, Sutton RB, Brunger AT, et al. Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. Proc Natl Acad Sci U S A 1998;95:15781-6.
26. Ramalho-Santos J, Moreno RD, Sutovsky P, et al. SNAREs in mammalian sperm: possible implications for fertilization. Dev Biol 2000;223:54-69.
27. De Blas GA, Roggero CM, Tomes CN, et al. Dynamics of SNARE assembly and disassembly during sperm acrosomal exocytosis. PLoS Biol 2005;3:e323.
28. Yunes R, Michaut M, Tomes C, et al. Rab3A triggers the acrosome reaction in permeabilized human spermatozoa. Biol Reprod 2000;62:1084-9.
29. Iida H, Yoshinaga Y, Tanaka S, et al. Identification of

 

Rab3A GTPase as an acrosome-associated small GTP-binding protein in rat sperm. Dev Biol 1999;211:144-55.
30. Ramalho-Santos J, Schatten G. Presence of N-ethyl maleimide sensitive factor (NSF) on the acrosome of mammalian sperm. Arch Androl 2004;50:163-8.
31. Tomes CN, De Blas GA, Michaut MA, et al. alpha-SNAP and NSF are required in a priming step during the human sperm acrosome reaction. Mol Hum Reprod 2005;11:43-51.
32. Chen YA, Scales SJ, Patel SM, et al. SNARE complex formation is triggered by Ca2+ and drives membrane fusion. Cell 1999;97:165-74.
33. Zanetti N, Mayorga LS. Acrosomal swelling and membrane docking are required for hybrid vesicle formation during the human sperm acrosome reaction. Biol Reprod 2009;81:396-405.
34. Pellizzari R, Rossetto O, Schiavo G, et al. Tetanus and botulinum neurotoxins: mechanism of action and therapeutic uses. Philos Trans R Soc Lond B Biol Sci 1999;354:259-68.
35. Fukuda M. Regulation of secretory vesicle traffic by Rab small GTPases. Cell Mol Life Sci 2008;65:2801-13.
36. Ungermann C, Price A, Wickner W. A new role for a SNARE protein as a regulator of the Ypt7/Rab-dependent stage of docking. Proc Natl Acad Sci U S A 2000;97:8889-91.
37. Barr FA. Review series: Rab GTPases and membrane identity: causal or inconsequential? J Cell Biol 2013;202:191-9.
38. Ohya T, Miaczynska M, Coskun U, et al. Reconstitution of Rab- and SNARE-dependent membrane fusion by synthetic endosomes. Nature 2009;459:1091-7.
39. Zick M, Wickner W. Improved reconstitution of yeast vacuole fusion with physiological SNARE concentrations reveals an asymmetric Rab(GTP) requirement. Mol Biol Cell 2016;27:2590-7.
40. Gibieza P, Prekeris R. Rab GTPases and cell division. Small GTPases 2017:1-9.
41. Fukuda M. How can mammalian Rab small GTPases be comprehensively analyzed?: Development of new tools to comprehensively analyze mammalian Rabs in membrane traffic. Histol Histopathol 2010;25:1473-80.
42. Erdman RA, Maltese WA. Different Rab GTPases associate preferentially with alpha or beta GDP-dissociation inhibitors. Biochem Biophys Res Commun 2001;282:4-9.
43. Itoh T, Fukuda M. Rab small GTPases and their effectors. Tanpakushitsu Kakusan Koso 2008;53:2065-70.
44. Belmonte SA, Lopez CI, Roggero CM, et al. Cholesterol content regulates acrosomal exocytosis by enhancing Rab3A plasma membrane association. Dev Biol 2005;285:393-408.
45. Bustos MA, Roggero CM, De la Iglesia PX, et al. GTP-bound Rab3A exhibits consecutive positive and negative roles during human sperm dense-core granule exocytosis. J Mol Cell Biol 2014;6:286-98.
46. Garde J, Roldan ER. rab 3-peptide stimulates exocytosis of the ram sperm acrosome via interaction with cyclic AMP and phospholipase A2 metabolites. FEBS Lett 1996;391:263-8.
47. Burgoyne RD, Clague MJ. Calcium and calmodulin in membrane fusion. Biochim Biophys Acta 2003;1641:137-43.
48. Tsuboi T, Fukuda M. Rab3A and Rab27A cooperatively regulate the docking step of dense-core vesicle exocytosis in PC12 cells. J Cell Sci 2006;119:2196-203.
49. Pavlos NJ, Gronborg M, Riedel D, et al. Quantitative analysis of synaptic vesicle Rabs uncovers distinct yet overlapping roles for Rab3a and Rab27b in Ca2+-triggered exocytosis. J Neurosci 2010;30:13441-53.
50. Tsuboi T, Fukuda M. The C2B domain of rabphilin directly interacts with SNAP-25 and regulates the docking step of dense core vesicle exocytosis in PC12 cells. J Biol Chem 2005;280:39253-9.
51. Bustos MA, Lucchesi O, Ruete MC, et al. Rab27 and Rab3 sequentially regulate human sperm dense-core granule exocytosis. Proc Natl Acad Sci U S A 2012;109:E2057-66.
52. Branham MT, Bustos MA, De Blas GA, et al. Epac activates the small G proteins Rap1 and Rab3A to achieve exocytosis. J Biol Chem 2009;284:24825-39.
53. Ruete MC, Lucchesi O, Bustos MA, et al. Epac, Rap and Rab3 act in concert to mobilize calcium from sperm's acrosome during exocytosis. Cell Commun Signal 2014;12:43.
54. Quevedo MF, Lucchesi O, Bustos MA, et al. The Rab3A-22A Chimera Prevents Sperm Exocytosis by Stabilizing Open Fusion Pores. J Biol Chem 2016;291:23101-11.
55. Rodriguez F, Zanetti MN, Mayorga LS, et al. Munc18-1 controls SNARE protein complex assembly during human sperm acrosomal exocytosis. J Biol Chem 2012;287:43825-39.
56. Dulubova I, Sugita S, Hill S, et al. A conformational switch in syntaxin during exocytosis: role of munc18. EMBO J 1999;18:4372-82.
57. Dawidowski D, Cafiso DS. Munc18-1 and the Syntaxin-1 N Terminus Regulate Open-Closed States in a t-SNARE Complex. Structure 2016;24:392-400.
58. Rathore SS, Bend EG, Yu H, et al. Syntaxin N-terminal peptide motif is an initiation factor for the assembly of the SNARE-Sec1/Munc18 membrane fusion complex. Proc Natl Acad Sci U S A 2010;107:22399-406.
59. Mandic SA, Skelin M, Johansson JU, et al. Munc18-1 and

 

Munc18-2 proteins modulate beta-cell Ca2+ sensitivity and kinetics of insulin exocytosis differently. J Biol Chem 2011;286:28026-40.
60. Zhou P, Pang ZP, Yang X, et al. Syntaxin-1 N-peptide and Habc-domain perform distinct essential functions in synaptic vesicle fusion. EMBO J 2013;32:159-71.
61. Shen J, Tareste DC, Paumet F, et al. Selective activation of cognate SNAREpins by Sec1/Munc18 proteins. Cell 2007;128:183-95.
62. Rodriguez F, Bustos MA, Zanetti MN, et al. alpha-SNAP prevents docking of the acrosome during sperm exocytosis because it sequesters monomeric syntaxin. PLoS One 2011;6:e21925.
63. Graham ME, Barclay JW, Burgoyne RD. Syntaxin/Munc18 interactions in the late events during vesicle fusion and release in exocytosis. J Biol Chem 2004;279:32751-60.
64. Burkhardt P, Hattendorf DA, Weis WI, et al. Munc18a controls SNARE assembly through its interaction with the syntaxin N-peptide. EMBO J 2008;27:923-33.
65. Xia T, Tong J, Rathore SS, et al. Network motif comparison rationalizes Sec1/Munc18-SNARE regulation mechanism in exocytosis. BMC Syst Biol 2012;6:19.
66. Jorgacevski J, Potokar M, Grilc S, et al. Munc18-1 tuning of vesicle merger and fusion pore properties. J Neurosci 2011;31:9055-66.
67. Berruti G, Paiardi C. Acrosome biogenesis: Revisiting old questions to yield new insights. Spermatogenesis 2011;1:95-8.
68. Scheenen WJ, Wollheim CB, Pozzan T, et al. Ca2+ depletion from granules inhibits exocytosis. A study with insulin-secreting cells. J Biol Chem 1998;273:19002-8.
69. Tucker WC, Weber T, Chapman ER. Reconstitution of Ca2+-regulated membrane fusion by synaptotagmin and SNAREs. Science 2004;304:435-8.
70. Alvarez L, Dai L, Friedrich BM, et al. The rate of change in Ca(2+) concentration controls sperm chemotaxis. J Cell Biol 2012;196:653-63.
71. De Blas G, Michaut M, Trevino CL, et al. The intraacrosomal calcium pool plays a direct role in acrosomal exocytosis. J Biol Chem 2002;277:49326-31.
72. Tahara M, Coorssen JR, Timmers K, et al. Calcium can disrupt the SNARE protein complex on sea urchin egg secretory vesicles without irreversibly blocking fusion. J Biol Chem 1998;273:33667-73.
73. Tsai PS, Brewis IA, van Maaren J, et al. Involvement of complexin 2 in docking, locking and unlocking of different SNARE complexes during sperm capacitation and induced acrosomal exocytosis. PLoS One 2012;7:e32603.
74. Yanagimachi R. Fertility of mammalian spermatozoa: its development and relativity. Zygote 1994;2:371-2.
75. Darszon A, Nishigaki T, Beltran C, et al. Calcium channels in the development, maturation, and function of spermatozoa. Physiol Rev 2011;91:1305-55.
76. Kirichok Y, Navarro B, Clapham DE. Whole-cell patch-clamp measurements of spermatozoa reveal an alkaline-activated Ca2+ channel. Nature 2006;439:737-40.
77. Cuesta M, Pelaz L, Perez C, et al. Fat-soluble vitamin deficiencies after bariatric surgery could be misleading if they are not appropriately adjusted. Nutr Hosp 2014;30:118-23.
78. Ducibella T. The cortical reaction and development of activation competence in mammalian oocytes. Hum Reprod Update 1996;2:29-42.
79. Hart NH, Yu SF. Cortical granule exocytosis and cell surface reorganization in eggs of Brachydanio. J Exp Zool 1980;213:137-59.
80. Ikebuchi Y, Masumoto N, Matsuoka T, et al. SNAP-25 is essential for cortical granule exocytosis in mouse eggs. Am J Physiol 1998;274:C1496-500.
81. Yao Y, Ferrer-Montiel AV, Montal M, et al. Activation of store-operated Ca2+ current in Xenopus oocytes requires SNAP-25 but not a diffusible messenger. Cell 1999;98:475-85.
82. Tsai PS, van Haeften T, Gadella BM. Preparation of the cortical reaction: maturation-dependent migration of SNARE proteins, clathrin, and complexin to the porcine oocyte's surface blocks membrane traffic until fertilization. Biol Reprod 2011;84:327-35.
83. Fasshauer D, Eliason WK, Brunger AT, et al. Identification of a minimal core of the synaptic SNARE complex sufficient for reversible assembly and disassembly. Biochemistry 1998;37:10354-62.
84. Weber T, Zemelman BV, McNew JA, et al. SNAREpins: minimal machinery for membrane fusion. Cell 1998;92:759-72.
85. Ducibella T, Kurasawa S, Duffy P, et al. Regulation of the polyspermy block in the mouse egg: maturation-dependent differences in cortical granule exocytosis and zona pellucida modifications induced by inositol 1,4,5-trisphosphate and an activator of protein kinase C. Biol Reprod 1993;48:1251-7.
86. Chen X, Tomchick DR, Kovrigin E, et al. Three-dimensional structure of the complexin/SNARE complex. Neuron 2002;33:397-409.
87. Choi UB, Zhao M, Zhang Y, et al. Complexin induces a conformational change at the membrane-proximal

 

C-terminal end of the SNARE complex. Elife 2016;5.
88. Liu J, Guo T, Wei Y, et al. Complexin is able to bind to SNARE core complexes in different assembled states with distinct affinity. Biochem Biophys Res Commun 2006;347:413-9.
89. Liu J, Guo T, Wu J, et al. Overexpression of complexin in PC12 cells inhibits exocytosis by preventing SNARE complex recycling. Biochemistry (Mosc) 2007;72:439-44.
90. Maximov A, Tang J, Yang X, et al. Complexin controls the force transfer from SNARE complexes to membranes in fusion. Science 2009;323:516-21.
91. Tadokoro S, Nakanishi M, Hirashima N. Complexin II regulates degranulation in RBL-2H3 cells by interacting with SNARE complex containing syntaxin-3. Cell Immunol 2010;261:51-6.
92. Lledo PM, Johannes L, Vernier P, et al. Rab3 proteins: key players in the control of exocytosis. Trends Neurosci 1994;17:426-32.
93. Conner S, Wessel GM. rab3 mediates cortical granule exocytosis in the sea urchin egg. Dev Biol 1998;203:334-44.
94. Masumoto N, Sasaki T, Tahara M, et al. Involvement of Rabphilin-3A in cortical granule exocytosis in mouse eggs. J Cell Biol 1996;135:1741-7.
95. McGinnis LA, Lee HJ, Robinson DN, et al. MAPK3/1 (ERK1/2) and Myosin Light Chain Kinase in Mammalian Eggs Affect Myosin-II Function and Regulate the Metaphase II State in a Calcium- and Zinc-Dependent Manner. Biol Reprod 2015;92:146.
96. Woolner S, O'Brien LL, Wiese C, et al. Myosin-10 and actin filaments are essential for mitotic spindle function. J Cell Biol 2008;182:77-88.
97. Kogel T, Gerdes HH. Roles of myosin Va and Rab3D in membrane remodeling of immature secretory granules. Cell Mol Neurobiol 2010;30:1303-8.
98. Rodriguez OC, Cheney RE. Human myosin-Vc is a novel class V myosin expressed in epithelial cells. J Cell Sci 2002;115:991-1004.
99. Desnos C, Huet S, Fanget I, et al. Myosin va mediates docking of secretory granules at the plasma membrane. J Neurosci 2007;27:10636-45.
100. Cheeseman LP, Boulanger J, Bond LM, et al. Two pathways regulate cortical granule translocation to prevent polyspermy in mouse oocytes. Nat Commun 2016;7:13726.
101. Abbott AL, Fissore RA, Ducibella T. Incompetence of preovulatory mouse oocytes to undergo cortical granule exocytosis following induced calcium oscillations. Dev Biol 1999;207:38-48.
102. Satouh Y, Nozawa K, Yamagata K, et al. Viable offspring after imaging of Ca2+ oscillations and visualization of the cortical reaction in mouse eggs. Biol Reprod 2017;96:563-75.
103. Wang WH, Sun QY, Hosoe M, et al. Quantified analysis of cortical granule distribution and exocytosis of porcine oocytes during meiotic maturation and activation. Biol Reprod 1997;56:1376-82.
104. Ducibella T, Duffy P, Buetow J. Quantification and localization of cortical granules during oogenesis in the mouse. Biol Reprod 1994;50:467-73.
105. Jones KT, Carroll J, Whittingham DG. Ionomycin, thapsigargin, ryanodine, and sperm induced Ca2+ release increase during meiotic maturation of mouse oocytes. J Biol Chem 1995;270:6671-7.
106. Stricker SA. Comparative biology of calcium signaling during fertilization and egg activation in animals. Dev Biol 1999;211:157-76.
107. Jones KT. Ca2+ oscillations in the activation of the egg and development of the embryo in mammals. Int J Dev Biol 1998;42:1-10.
108. Jaffe LA, Kado RT, Muncy L. Propagating potassium and chloride conductances during activation and fertilization of the egg of the frog, Rana pipiens. J Physiol 1985;368:227-42.
109. Chiba K, Kado RT, Jaffe LA. Development of calcium release mechanisms during starfish oocyte maturation. Dev Biol 1990;140:300-6.
110. Berg LK, Wessel GM. Cortical granules of the sea urchin translocate early in oocyte maturation. Development 1997;124:1845-50.
111. Whittingham DG, Siracusa G. The involvement of calcium in the activation of mammalian oocytes. Exp Cell Res 1978;113:311-7.
112. Lopez-Godinez J, Garambullo TI, Martinez-Cadena G, et al. Mastoparan induces Ca2+-independent cortical granule exocytosis in sea urchin eggs. Biochem Biophys Res Commun 2003;301:13-6.

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