Molecules involved in acrosomal exocytosis and cortical granule exocytosis

Mengsi Lin¹, Qingwen Zhu¹, Jing Wang¹, Wenjie Yang², Haihua Fan², Jianfeng Yi², Maorong Jiang³

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

Received: 08 August 2017; Accepted: 30 August 2017; Published: 12 September 2017.
doi: 10.21037/biotarget.2017.08.05
View this article at: http://dx.doi.org/10.21037/biotarget.2017.08.05

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 Ca²⁺ 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 because 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 exocytic 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, intracellular 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 

© Biotarget. All rights reserved.  

Biotarget.amegroups.com  

Biotarget 2017;1:11
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 led 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 exocytic 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).

Syntaxis 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 secretary cargoes in multiple cell types. Desnos reported that MyoVa mediated secretory 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 pre-ovulatory 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.

**Acknowledgements**

**Funding:** This study was supported by grants from the National Natural Science Foundation of China (81101159),...
the Natural Science Foundation of Jiangsu Province, China (BK20151268), and Natural Science Research Project of young scientists of Nantong municipal commission of health and family planning (WQ2016048).

Footnote

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

References

2. Wassarman PM, Litscher ES. Towards the molecular basis of sperm and egg interaction during mammalian fertilization. Cells Tissues Organs 2001;168:36-45.
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.
44. 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.
56. Mandic SA, Skelin M, Johansson JU, et al. Munc18-1 and


C-terminal end of the SNARE complex. Elife 2016;5.
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.

doi: 10.21037/biotarget.2017.08.05