Involvement of Rabphilin-3A in Cortical Granule Exocytosis in Mouse Eggs

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Abstract. Rabphilin-3A is a putative target protein for Rab3A, a member of the small GTP-binding protein superfamily that has been suggested to play a role in regulated exocytosis in presynapses. In this study we determined the expression and the function of Rabphilin-3A in mouse eggs at fertilization. Rabphilin-3A mRNA and protein were detected by reverse transcriptase-PCR and immunoblot analysis, respectively, in metaphase II mouse eggs. Immunofluorescence analysis showed that Rabphilin-3A protein was distributed in the cortical region in eggs. Sperm induces cortical granule (CG) exocytosis via an increase in cytosolic Ca2+ at fertilization. We microinjected the NH2- or COOH-terminal fragment of recombinant Rabphilin-3A into metaphase II eggs. Neither treatments altered the sperm-induced cytosolic Ca2+ increase, but both inhibited CG exocytosis in a dose-dependent manner. The NH2-terminal fragment was more effective than the COOH-terminal fragment. Full-length Rabphilin-3A did not affect CG exocytosis, but it attenuated the inhibition of CG exocytosis by the NH2-terminal fragment. These results show that Rabphilin-3A is involved in Ca2+-dependent CG exocytosis at fertilization in mouse eggs.

Cortical granule (CG) exocytosis in mammalian eggs is the first important event in fertilization, which functions to block polyspermy (9, 12, 38). Sperm induces an increase in cytosolic Ca2+ concentration ([Ca2+]i) (22) that triggers the fusion of CG vesicles to the plasma membrane. It has been established that the [Ca2+]i increase induced by sperm is essential for CG exocytosis (15, 33), but little is known about the intracellular signaling mechanism underlying this effect.

The Rab family of small GTP-binding proteins has been implicated in intracellular vesicle traffic such as exocytosis, endocytosis, and transcytosis (25, 27, 32, 34, 39). Particularly, Rab3A has been suggested to be involved in regulated exocytosis such as neurotransmitter release from presynapses (25). Recently, Rabphilin-3A, a putative target protein for Rab3A, was purified and its cDNA was sequenced (30, 31). Rabphilin-3A is a protein of 704 amino acids with a M, of 77,976 that binds Rab3A, and its cDNA was sequenced (30, 31). Rabphilin-3A is a protein of 704 amino acids with a M, of 77,976 that binds Rab3A at its NH2-terminal region. Rabphilin-3A has two repeated C2 domains at its COOH-terminal region. Rabphilin-3A has two repeated C2 domains at its COOH-terminal region. This C2 region was found in protein kinase C and synaptotagmin, which are known to bind Ca2+ and phospholipid through this region (36). These lines of evidence indicate that Rabphilin-3A may function with Rab3A as an intracellular signal in Ca2+-regulated exocytosis.

If the Rab–Rabphilin system is involved in Ca2+-regulated exocytosis, the system may function in CG exocytosis in eggs at fertilization. However, to date, Rabphilin-3A expression has been reported only in neurons and neuroendocrine cells such as adrenal chromaffin and PC12 cells (11, 17, 23). There have been no reports regarding Rab or Rabphilin expression in eggs. Thus, in the present study we investigated whether Rabphilin-3A is involved in the intracellular signaling pathway responsible for CG exocytosis at fertilization in mouse eggs.

Materials and Methods

Media

M2 medium (94.7 mM NaCl, 4.78 mM KCl, 1.71 mM CaCl2·H2O, 1.19 mM MgCl2·7H2O, 1.19 mM KH2PO4, 4 mM NaHCO3, 21.0 mM Hepes, 5.56 mM glucose, 0.33 mM pyruvic acid, 23.3 mM sodium-lactate, 0.05 mg/ml streptomycin, 100 IU/ml penicillin G, 4 mg/ml BSA), TYH medium (119.37 mM NaCl, 4.78 mM KCl, 12.6 mM CaCl2·H2O, 1.19 mM MgCl2·7H2O, 1.19 mM KH2PO4, 25.07 mM NaHCO3, 5.56 mM glucose, 1 mM pyruvic acid, 0.05 mg/ml streptomycin, 75 IU/ml penicillin G, 4 mg/ml BSA), and an intracellular-like medium (ICM; 120 mM KCI, 20 mM Hepes, 100 μM EGTA, 10 mM sodium-glycerophosphate, 200 μM PMSF, 1 mM DTT) were used in these experiments. Media were made with cell culture reagents and tis-
Preparation of Gametes

Unfertilized metaphase II eggs were collected from CD1 female mice (Charles River, Kanagawa, Japan) that were superovulated by intraperitoneal injection of 5 IU pregnant mare serum gonadotropin, followed 48 h later by 5 IU human chorionic gonadotropin. Eggs were collected from the ampulla of the oviduct 14–16 h later and placed in M2 medium covered by light mineral oil. The cumulus cells were removed by digestion (~5 min) with 0.3 mg/ml hyaluronidase (type IV-S). Only normal, mature eggs with a fully formed first polar body were used in these experiments. In some experiments, the zona pellucidae were removed by a brief exposure (~1 min) to acid Tyrode’s solution (pH 2.5). The eggs were then drawn into and expelled from a small bore pipette to ensure removal of the zona pellucidae. After each procedure, the eggs were thoroughly washed in M16 medium. Eggs were cultured in petri dishes in 100-μl drops of M16 under mineral oil and incubated in an atmosphere of 5% CO2 and 95% air at 37°C.

Spermatozoa were obtained from CD1 male mice (Charles River) of proven fertility. Spermatozoa were released from the cauda epididymis into TYH medium containing 0.3% BSA (fraction V; Sigma Chemical Co.). After incubation for 10 min in an atmosphere of 5% CO2 and 95% air at 37°C, the sperm concentration was adjusted to 1–2 × 10^6 spermatozoa per ml. The sperm suspension was overlain with paraffin oil and further incubated for 3 h.

Purification of GST–Rabphilin-3A

Recombinant Rabphilin-3A with glutathione-S-transferase (GST) was purified as previously described (29, 36). To produce the NH2-terminal (1–280 amino acids) and the COOH-terminal fragments (281–704 amino acids) of Rabphilin-3A, expression plasmids pGEX-2T-Rabphilin-3A-N and pGEX-2T-Rabphilin-3A-C were constructed as follows. The 0.84-kb cDNA fragment encoding the NH2-terminal fragment and the 1.3-kilobase cDNA fragment encoding the COOH-terminal fragment with the BamHI and KpnI sites upstream of the initiator methionine codon and downstream of the termination codon were synthesized by PCR. These fragments were digested by BamHI and inserted into the BamHI site of pGEX-2T. The NH2- and COOH-terminal fragments of Rabphilin-3A with GST were expressed in Escherichia coli and purified by glutathione-Sepharose 4B column chromatography. Characterization of these GST fusion proteins was described in detail previously (29). GTP-S–Rab3A bound to the GST NH2-terminal fragment of Rabphilin-3A, but did not bind to the GST C-terminal fragment; Ca2+ bound to the GST COOH-terminal fragment of Rabphilin-3A, but did not bind to the GST NH2-terminal fragment of Rabphilin-3A; and liposomes bound to the GST COOH-terminal fragment of Rabphilin-3A in a Ca2+-dependent manner, but did not bind to the GST NH2-terminal fragment. These samples were added to ICM solution after concentration using centricron membranes (final dose: 100 μM).

Purification of Hemagglutinin-tagged Rabphilin-3A

A DNA fragment encoding the hemagglutinin (HA) (YPYDVPDYA) epitope with the methionine codon was inserted into the BamHI site of pACYM1 Autographa california baculovirus transfer vector to express the HA-tagged fusion proteins (pACYM1-HA). To generate full-length (~704 amino acids), pACYM1–Rabphilin-3A), and deletion mutant constructs (1–280 aa, HA–Rabphilin-3A-N; 396–704 aa, Rabphilin-3A-C) of bovine brain Rabphilin-3A, cDNAs were made by PCR using specific oligonucleotide primers inserted into pACYM1–HA and expressed as fusion proteins with the NH2-terminal HA epitope using the insect/baculovirus system as described (21). Recombinant HA-tagged Rabphilin-3A was purified from the membrane fraction of overexpressing Sf9 cells by the same methods as those used for Rabphilin-3A without HA (29). HA-tagged Rabphilin-3A expressed endogenously in cells is known to function in Ca2+-regulated exocytosis (3, 16).

Generation of Rabphilin-3A Polyclonal Antibodies

Rabbit antisera were raised against the GST-binding NH2-terminal fragment and the COOH-terminal fragment of Rabphilin-3A (36). The anti-Rabphilin-3A monospecific antibodies were purified from the antisera by affinity column chromatography using the same protein fragments as ligands. Immunofluorescence and immunoblot analyses were carried out using these antibodies.

mRNA Extraction and Reverse Transcriptase–PCR

Metaphase II-arrested eggs were collected, immediately frozen in a liquid nitrogen, and stored at −80°C until mRNA extraction by oligo(dT)-cellulose (Micro mRNA Purification Kit; Pharmacia Biotech, Piscataway, NJ). The mRNA isolated from the eggs was then reverse transcribed into cDNA. The Rabphilin-3A oligonucleotides primers were synthesized on a DNA/RNA synthesizer (model 394; Applied Biosystems, Foster City, CA). The primer sequences and their corresponding locations in the nucleotide sequence of mouse Rabphilin-3A (11) were as follows: Rabphilin-3A-primer1, forward: 5'-GCAGAGAAGAGTGAAGCCATG-3' (181–201); reverse: 5'-CCACACGTGTGCAAGATCCT-3' (348–368); Rabphilin-3A-primer2, forward: 5'-GGAGAGGAGAAAGACCAATAG-3' (1092–1112); reverse: 5'-AGCCGACAGTGAAGCTTCAC-3' (1255–1275). These sequence data are available under EMBL/GenBank/DDJB accession number D29965.

Rabphilin-3A cDNAs were amplified singly. The PCR mixtures of Rabphilin-3A cDNA and respective primers were amplified using a program temperature control system (PC-700; Astec Co., Fukuoka, Japan). One cycle of PCR consisted of 30 s at 94°C, 90 s at 58°C, and 30 s at 72°C; a total of 35 cycles was performed. Mouse brain RNA samples were used as positive controls for Rabphilin-3A. These RNA samples were also amplified without reverse transcription as negative controls. Each PCR reaction mixture was subjected to 2% agarose gel electrophoresis, and the amplified products were visualized by staining with ethidium bromide for 30 min at room temperature.

To confirm the specificity of PCR, amplified cDNAs were digested by each restriction enzyme, and the digests were analyzed by 4.8% PAGE and stained with ethidium bromide. The bands were also identified by reverse transcriptase (RT)–PCR Southern blot analysis. Analysis consisted of the following steps. Fractionated DNA was transferred onto a nitrocellulose filter, and the filter was hybridized with 32P-maltoprim (Amersham Corp., Arlington Heights, IL)-labeled Rabphilin-3A cDNA probes. The filter was washed under high stringency conditions and exposed overnight to XAR-5 film (Eastman Kodak Co., Rochester, NY).

Immunoblot Analysis

About 500 eggs were collected in PBS (pH 7.4) containing 3 mg/ml of polyvinyl pyrrolidone and were solubilized by lysis buffer composed of 15% SDS. As positive controls for Rabphilin-3A, mouse brain and purified bovine Rabphilin-3A were prepared. Lysed samples were concentrated with microcon, and SDS-PAGE was performed using a microgel gel (30 × 30 × 1 mm). Samples were run through the stacking gel and separating gel at 10 mA per gel. The proteins were electrophoretically transferred onto a nitrocellulose membrane sheet, and the transferred proteins were immunoblotted using a polyclonal antibody against the NH2-terminal fragment of Rabphilin-3A. Biotinylated anti-rabbit IgG (goat, 1:4,000) was used as a secondary antibody. Finally, avidin-conjugated alkaline phosphatase was applied, and the filter was stained with 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium (BCIP/NBT; Bio Rad Laboratories, Richmond, CA).

Immunofluorescence

The metaphase II-arrested eggs were rinsed and fixed with Zamboni’s fixative (0.2% picric acid and 2% paraformaldehyde). Rabbit polyclonal antibodies against the NH2- and COOH-terminal fragments of Rabphilin-3A were applied as primary antibodies for 24 h at 4°C. Negative control experiments for each antibody included replacement of the primary antibody with a nonimmune rabbit IgG at the same concentration. After repeated washing in PBS, biotinylated anti-rabbit IgG (goat, 1:1,000) was applied for 24 h at 4°C. After repeated washing in PBS, avidin-FITC was applied for 30 min at 37°C. Unbound antibody was rinsed off, and the fluorescence of FITC was visualized by excitation at 488 nm with the argon laser in the confocal laser microscope. The confocal parameters of scan rate, aperture, gain, black level, and frames accumulated were the same for all samples.
Microinjection

Samples (10 μl, 50-100 μM, GST-bound or HA-tagged Rabphilin-3A) were microinjected into the cytoplasm of eggs using micromanipulators and a microinjector (Eppendorf, Tokyo, Japan). A fire-polished holding pipette and a beveled injection pipette with an outer 3-μm diameter was used. Injected eggs were used in [Ca2+]i and CG exocytosis experiments after a 30-min incubation in M16 medium.

Measurement of [Ca2+]i

[Ca2+]i was monitored with a digital imaging fluorescence microscope using the Ca2+-sensitive fluorescent dye, fura-2-AM (18, 33). Eggs were incubated at 37°C for 60 min in M16 medium containing 5 μM fura-2-AM (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). The eggs were then rinsed with TYH medium and placed on the microscope stage. The [Ca2+]i of eggs was measured at 7.5-s intervals with a digital imaging microscopic system (Mu-1000; Schlar Tec Corp., Osaka, Japan). [Ca2+]i was calculated by the ratio of the intensities of fluorescent emission at 510 nm with excitation at 340 and 380 nm. The fluorescent excitation beam was targeted on the cells, and then the emission fluorescence was recorded with a digital imaging analysis and a computer.

Measurement of Exocytosis in Living Eggs

As previously described (33), exocytosis in living eggs was measured using the membrane-impermeable membrane probe TMA-DPH (Molecular Probes, Eugene, OR). Zona-free eggs were plated in a 90-μl drop of TYH medium on glass coverslips that were sealed under a 1.0-cm hole in the coverslip. The drops were overlaid with oil, and 10 μl of 50 μM TMA-DPH was added into the drop at 37°C, to a final concentration of 5 μM. The 430-nm emission fluorescence of single eggs at 350-nm excitation was measured, and changes in the fluorescence intensity in individual eggs were analyzed with a digital imaging microscopy system (Mu-1000; Schlar Tec Corp.). Eggs were inseminated with spermatozoa, the final concentration of which was 1-2 × 105 spermatozoa per ml. After a spermatozoon attached to the plasma membrane of a zona-free egg, the 430-nm fluorescence of the egg at 350-nm excitation was measured. The percentage increase of fluorescence was represented as the total of exocytosis. Eggs were intermittently irradiated for 50 ms every 15 s to prevent UV damage.

Labeling of CGs with Lens Culinaris Agglutinin

Exocytosis of CGs was evaluated by Lens culinaris agglutinin (LCA) staining (2, 4). 30-40 min after fertilization, unfixed eggs were washed in M16 medium and incubated for 15 min in 10 μg/ml FITC-conjugated LCA (E-Y Laboratories, San Mateo, CA). These eggs were washed (five times, 5 min each) before examination by laser scanning confocal microscopy 2 h after fertilization. The CG exudate remained on the surface of the activated eggs after fertilization. The eggs were excited at 488 nm by the argon laser in the confocal laser microscope, and confocal optical images of FITC-LCA were analyzed. The confocal parameters of scan rate, aperture, gain, black level, and frames accumulated were the same for all samples. In control experiments, eggs were exposed to FITC-LCA in 100 mM α-methylmannoside for 10 min before incubation.

Data Analysis and Statistics

All experiments were repeated more than four times, and representative results are shown. The data shown in Fig. 6, b and c, are expressed as means ± SD of multiple determinations from the data of 30 eggs. The homoscedasticity of the data was analyzed by Bartlett’s test. The significance of differences was assessed by analysis of variance, followed by Scheffé’s multiple comparison, and a P value of <0.01 was taken to be significant.

Results

Expression of Rabphilin-3A mRNA in Metaphase II Eggs

RT-PCR was performed to detect Rabphilin-3A gene transcripts in unfertilized eggs. cDNA samples reverse transcribed from mRNA isolated from eggs were amplified using the two groups of synthesized Rabphilin-3A primers. Two amplified products of 188 and 181 bp were observed in Fig. 1, showing that Rabphilin-3A mRNA was expressed in metaphase II eggs. No amplified DNA fragments were observed when PCR was performed without prior reverse transcription (Fig. 1). This shows that the amplified fragments were generated from Rabphilin-3A mRNA and did not originate from genomic DNA. The specificity of the respective bands was determined by digesting the PCR products with restriction enzymes. The first fragment (188 bp) was separated to 121- and 67-bp fragments by ALV2 (Fig. 1). The second fragment (181 bp) was separated to 124- and 57-bp fragments by EcoRI (Fig. 1). Moreover, the sequences of 188- and 181-bp fragments were confirmed by Southern blot analysis and direct sequencing (not shown).

Presence of Rabphilin-3A Protein in Metaphase II Eggs

We determined the presence of Rabphilin-3A protein in metaphase II eggs by immunoblot analysis. A 78-kD band was specifically labeled with antibody against NH2-terminal of Rabphilin-3A (Fig. 2). As positive controls, similar bands were identified in mouse brain and bovine Rabphilin-3A protein (Fig. 2).

Distribution of Rabphilin-3A Protein in Metaphase II Eggs

Intracellular distribution of Rabphilin protein in metaphase II eggs was determined by immunofluorescence methods using a confocal microscope. Specific staining labeling with antibodies against the NH2- and COOH-terminal fragments of Rabphilin-3A was observed in eggs (Fig. 3). When primary antibodies were substituted with...
nonimmune rabbit IgG, no specific staining was observed (Fig. 3). Staining for both fragments was distributed in the cortical region in eggs. The staining intensity was not homogeneous. In some eggs, a staining-free region near the inside of the plasma membrane was observed.

**Effects of the GST NH₂ or COOH-terminal Rabphilin-3A Fragments on [Ca²⁺] and the Surface Area Increase due to Exocytosis**

To determine the function of Rabphilin-3A in the egg at fertilization, the GST-bound NH₂ or COOH-terminal fragments of Rabphilin-3A, or GST as a control, were microinjected into the egg cytoplasm. Then, the zona pellucidae of the treated eggs were removed, and the zona-free eggs were inseminated by spermatozoa. Exocytosis was evaluated by membrane surface area using the membrane dye TMA-DPH. In control eggs, repetitive increases in [Ca²⁺], and exocytosis were observed (Fig. 4). In eggs treated with the intracellular Ca²⁺ chelator BAPTA-AM as a negative control, both the increases in [Ca²⁺], and exocytosis were blocked (Fig. 4). Exocytosis at fertilization was blocked in respective eggs injected with the NH₂- or COOH-terminal fragments of Rabphilin-3A, but not in those injected with only GST (Fig. 4). The [Ca²⁺] changes in these three groups of eggs were not inhibited (Fig. 4). These results indicate that the NH₂- or COOH-terminal fragment of Rabphilin-3A specifically blocked a pathway downstream of Ca²⁺ mobilization.

**Effects of the GST NH₂ or COOH-terminal Rabphilin-3A Fragments on the Release of CG Contents**

Release of CG content was evaluated by LCA staining as another assay for CG exocytosis. FITC-LCA fluorescence was observed around the plasma membrane in inseminated eggs. This fluorescence represented CG exudate, and CG secretion was induced by sperm in almost all of the eggs at fertilization under the conditions used here. A GST-bound NH₂- or COOH-terminal fragment of Rabphilin-3A, or GST as a control, was injected into the egg cytoplasm, the treated eggs were inseminated by sperm, and optical images of LCA fluorescence were observed using a confocal laser microscope (Fig. 5 a). Intensities of LCA fluorescence in eggs were analyzed in respective groups. The increase of LCA fluorescence induced by fertilization was dose dependently inhibited by microinjection of the GST-bound NH₂- or COOH-terminal fragment of Rabphilin-3A (Fig. 5 b). The GST-bound NH₂-terminal fragment of Rabphilin-3A showed almost complete inhibition at concentrations in excess of 10 μM. The GST-bound COOH-terminal fragment of Rabphilin-3A showed almost complete inhibition at >40 μM. Injection of GST (100 μM) alone did not affect the release of CG contents (not shown).

**Effects of the HA-tagged Full-Length, NH₂ or COOH-terminal Rabphilin-3A on the Release of CG Contents**

Next, HA-tagged NH₂- or COOH-terminal fragments or full-length Rabphilin-3A were injected at the respective effective concentration. The increase of LCA fluorescence induced by fertilization was significantly inhibited by microinjection of the HA-tagged NH₂-terminal (10 μM) or COOH-terminal fragment (40 μM), but not by the full-length Rabphilin-3A (20 μM) (Fig. 5 c). The inhibition by the HA-tagged NH₂-terminal fragment of Rabphilin-3A...
whether Rabphilin-3A is specifically localized in CG vesicles or whether the Rabphilin-3A–free region corresponds to the CG-free region.

Next, we measured the sperm-induced [Ca$^{2+}$], mobilization and exocytosis in eggs after microinjection of deletion mutant proteins to determine the function of Rabphilin-3A. We used two assays for CG exocytosis in mouse eggs at fertilization, the change of plasma membrane areas and CG exudate using the membrane-labeling dye TMA-DPH and CG-labeling dye LCA, respectively. We have established the former method to measure dynamics of exocytosis in living mouse eggs (33) or in pituitary gonadotropes (19, 20). Two different types of Rabphilin-3A mutant proteins (GST fusion protein and HA-tagged protein) were used. We confirmed that these proteins have functions endogenously and in vitro as previously described (16, 29).

Rab3A is known to be involved specifically in Ca$^{2+}$-regulated exocytosis in presynapses (25). Rabphilin-3A has at least two functionally different domains, the NH$_2$-terminal Rab3A-binding domain and the COOH-terminal two C2-like domains interacting with Ca$^{2+}$ and phospholipid (36). In this study, microinjection of the COOH- or the NH$_2$-terminal fragment of recombinant Rabphilin-3A into cytoplasm of eggs dose dependently blocked sperm-induced CG exocytosis. The NH$_2$-terminal fragment was effective at a lower concentration than the COOH-terminal fragment. To competitively inhibit binding to the mobilized Ca$^{2+}$, a higher concentration of COOH-terminal fragment may be required. Moreover, the inhibition by the NH$_2$-terminal fragment of Rabphilin-3A was partially attenuated by the full-length molecule. These results indicate that NH$_2$- or COOH-terminal fragments of Rabphilin-3A blocked CG exocytosis by competing with intracellular native Rabphilin-3A. The full-length Rabphilin-3A did not affect CG release, because CG release at fertilization may be the maximal response. Our results suggest that Rabphilin-3A functions as a Ca$^{2+}$ sensor in Ca$^{2+}$-dependent CG exocytosis and communicates the sperm-induced signal to Rab3A.

In mouse eggs, maternal mRNAs, which are degraded at the two-cell stage, support meiosis, fertilization, and the first cleavage (1, 6, 28). We demonstrated the expression of Rabphilin-3A mRNA in metaphase II eggs by RT-PCR, and our results suggest that Rabphilin-3A may play an important role in fertilization. Moreover, we determined the presence and the localization of Rabphilin-3A protein in metaphase II eggs by immunoblot analysis and immunofluorescence analysis. Rabphilin-3A is known to be localized in synaptic vesicles in neurons (23). In eggs, Rabphilin-3A was distributed in the cortical region, corresponding to the distribution pattern of CG vesicles. Moreover, the distribution of Rabphilin-3A was heterogeneous in some eggs with the presence of a Rabphilin-3A–free area in the cortical region. It has been reported that the metaphase II mouse eggs have CG-free domains that are formed during egg maturation (5, 26). However, it is not yet clear
Rab3A but also Rab3B (35) and Rab3C (17). However, it is not clear whether Rabphilin-3A binds to Rab3D. In mouse eggs, Rabphilin-3A may function by binding to Rab3A or to Rab3D. Rab3D may also function through other intracellular signaling pathways in CG exocytosis. A mouse strain with a knockout mutation of Rab3A shows normal fertility (8). In the Rab3A-knockout mouse, Rab3D may function in CG exocytosis instead of Rab3A. On the other hand, while CG exocytosis causes the zona block to polyspermy, the plasma membrane block to polyspermy, which is not dependent on CG exocytosis, is present in mouse eggs (10). Thus, the membrane block may supplement the lack of the zona block in Rab3A-knockout mouse.

In conclusion, our results indicate that the Rab-Rabphilin system is involved in the signal transduction pathway downstream of the sperm-induced intracellular Ca\(^{2+}\) mobilization.

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