A Role for Myosin 1e in Cortical Granule Exocytosis in Xenopus Oocytes*

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Xenopus oocytes undergo dynamic structural changes during maturation and fertilization. Among these, cortical granule exocytosis and compensatory endocytosis provide effective models to study membrane trafficking. This study documents an important role for myosin1e in cortical granule exocytosis. Myosin1e is expressed at the earliest stage that cortical granule exocytosis can be detected in oocytes. Prior to exocytosis, myosin1e relocates to the surface of cortical granules. Overexpression of myosin1e augments the kinetics of cortical granule exocytosis, whereas tail-derived fragments of myosin1e inhibit this secretory event (but not constitutive exocytosis). Finally, intracellular injection of myosin1e antibody inhibits cortical granule exocytosis. Further experiments identified cysteine string proteins as interacting partners for myosin1e. As constituents of the membrane of cortical granules, cysteine string proteins are also essential for cortical granule exocytosis. Future investigation of the link between myosin1e and cysteine string proteins should help to clarify basic mechanisms of regulated exocytosis.

A recent analysis concluded that the eukaryotic myosin superfamily includes 37 discrete types of myosins that are distinguished by the auxiliary domains linked to the ATP/actin-binding core of these motor proteins (1). Although the role of vertebrate, skeletal muscle myosins is well established, the specific function of the majority of the non-muscle, unconventional myosins remains unclear. To investigate the role of individual myosins in dynamic cellular events, we initiated studies using immature oocytes and eggs of Xenopus laevis (2, 3). These cells have several useful advantages. (i) They harbor mRNA encoding a variety of unconventional myosins (3). (ii) They undergo distinctive structural and functional changes in response to specific signaling events, including hormone-induced maturation or the induction of cortical granule exocytosis (4–9). (iii) The large size and relative ease of manipulation of these cells facilitate biochemical and ultrastructural studies, as well as dynamic imaging experiments.

In a recent study, we observed that myosin 1c (Myo1c)² is involved in the compression of actin coats that surround the large endosomes that are produced in the aftermath of cortical granule exocytosis in Xenopus eggs (3). Because a second type 1 myosin, myosin 1e (Myo1e; see ref. 10 for nomenclature), is present in Xenopus oocytes, we were interested whether this motor protein also has a role during cortical granule exocytosis or the ensuing process of compensatory endocytosis. Myo1e, like other myosins-1, has an NH₂-terminal actin and ATP-binding motor domain, followed by a central calmodulin-binding IQ domain and a COOH-terminal tail. The tail is composed of a basic MyTH1 (Myosin Tail Homology 1) domain that binds anionic phospholipids, and a glycine-proline-rich MyTH2 region of unknown function (11). In addition, the tails of myosins 1e and 1f include a COOH-terminal Src homology 3 (SH3) domain that presumably is involved in protein-protein interactions (12, 13).

Here we show that Xenopus Myo1e rapidly relocates to the surface of cortical granules in response to stimuli that trigger exocytosis. We also demonstrate that disruption of Myo1e function leads to a suppression of cortical granule exocytosis. We also show that Myo1e binds to cysteine string protein (csp), an established component of cortical granules (14) that is associated with secretory organelles in many other systems, including neurons (15, 16). These results directly link this type 1 myosin to the control of regulated exocytosis and suggest that Myo1e functions at least in part via interaction with csp.

EXPERIMENTAL PROCEDURES

Isolation and Culturing of Oocytes—Stage II–VI oocytes (17) of X. laevis were isolated from fragments of ovary by treatment (2–4 h at ~22 °C) in a solution containing collagenase (3–5 mg ml⁻¹) and then in a potassium phosphate solution (which facilitates the removal of the follicle cell layer) followed by culturing in Barth’s solution (in mM: NaCl, 88; KCl, 1; NaHCO₃, 2.4; Hepes, 15; CaNO₃, 0.3; CaCl₂, 0.41; MgSO₄, 0.82 with enrofloxacin at 10 μg/ml) as described (18). Except for Fig. 1, all experiments used stage V–VI oocytes.

Affinity Purification of Myo1e Antibody—Rabbit antisera were produced against the deduced NH₂-terminal peptide

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² The abbreviations used are: Myo1c, myosin 1c; Myo1e, myosin 1e; Csp, cysteine string protein; MyTH, myosin tail homology; SH3, Src homology 3; PMA, phorbol 12-myristate 13-acetate; MBP, maltose-binding protein; eGFP, enhanced green fluorescent protein.
released cortical granule lectin (typically, 1–1.5 mg per oocyte; see Ref. 18) was detected principally as a broad band between 35 and 50 kDa. Where indicated, each oocyte was extracted for immunoblot analysis of Myo1e or Myo1e constructs as described for csp in Ref. 20. For Fig. 6, oocytes were injected with mRNA encoding eGFP-Myo1e (~50 ng), and the next day a single oocyte was mounted on the stage of a Zeiss Axiosvert 100 M microscope (with Bio-Rad 1024 × 1024 lasersharp confocal software using a numerical aperture 1.4, ×63 objective lens) in a solution containing 0.1 mg ml⁻¹ wheat germ agglutinin (to suppress cortical granule lectin actinera, a kind gift from Dr. J. Hedrick, University of California, Davis). The extract of Xenopus brain that served as a control in Fig. 1 was prepared by homogenizing whole frog brain in 0.3 M sucrose and centrifuging 10 min at 1,000 × g. The supernatant from this centrifugation was dispersed using concentrated electrophoresis sample buffer and used for immunoblot analysis.

Dynamic Confocal Fluorescence Microscopy and Image Analysis—Experiments were performed as described previously (3, 19). Briefly, oocytes were injected with mRNA encoding eGFP-Myo1e (~50 ng), and the next day a single oocyte was mounted on the stage of a Zeiss Axiovert 100 M microscope (with Bio-Rad 1024 × 1024 lasersharp confocal software using a numerical aperture 1.4, ×63 objective lens) in a solution containing 0.1 mg ml⁻¹ wheat germ agglutinin (to suppress cortical flow), the extracellular marker, neutral, Texas Red dextran (0.1 mM; 3,000 kDa; Molecular Probes), and 50 mM PMA. The time-lapse images were collected at 2-s intervals from a single optical
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FIGURE 1. Developmental expression of Myo1e, cortical granule lectin, and csp in frog oocytes and eggs. Detergent extracts were prepared from oocytes (and eggs) staged according to Ref. 17. Extract from a single oocyte (or egg) was resolved electrophoretically (along with the equivalent of 50 μg of frog brain protein) for immunoblot analysis as described under “Experimental Procedures.” Approximate masses of these proteins (cgl is cortical granule lectin) are given in kDa.

plane immediately beneath the plasma membrane. Movies were assembled and analyzed using Volocity (Improvision).

Immunoprecipitation Experiments—Extracts were prepared by homogenizing oocytes in 10 volumes of buffer A (with 190 Triton X-100) and centrifuging for 1 min at 15,000 × g. The lipid at the top of the extract was removed, and the centrifugation and lipid aspiration steps were repeated (2–3 times) to obtain a lipid-free solution. Extract equivalent to 20–25 oocytes was incubated at 4°C for 12–16 h with 5–7 μg of Myo1e antibody (and either no antibody or Myo 1d antibody as a control) or 5 μl of csp antibody (prepared as in Ref. 22) followed by 1 h with 15 μl of protein A/G-agarose (Santa Cruz Biotechnology). The agarose beads were washed three times with buffer A and suspended in sample buffer for SDS-PAGE and immunoblot analysis as described in Ref. 20. Attempts to determine whether calmodulin co-immunoprecipitates with Myo1e were hampered by the lack of a suitable calmodulin antibody.

Pulldown Experiments—Full-length Xenopus Csp was fused to maltose-binding protein (MBP) and the recombinant fusion protein was harvested from bacterial lysates by binding to amylose resin as described in the supplier’s instructions (New England Biolabs). The equivalent of ~0.1 mg of MBP-Csp or MBP alone (bound to the amylose resin) was incubated for 12–16 h at 4°C with an oocyte extract prepared as for the immunoprecipitations (above). Alternatively, full-length Myo1e (or Myo1c) was translated in a wheat germ extract (Promega), and 20 μl of the in vitro translation reaction was diluted into 180 μl of buffer A (without Triton X-100) and incubated 12–16 h at 4°C with ~0.1 mg of MBP-csp or MBP alone. The resins were washed three times with 0.5 ml of buffer A and suspended in sample buffer for immunoblot analysis. Myo1c was detected using the M2 monoclonal antibody (23).

RESULTS

Evidence that Myo1e is expressed in Xenopus oocytes was initially obtained by affinity-purifying antibody against the deduced NH₂- or COOH-terminal peptides of Xenopus Myo1e and using these antibodies for immunoblot analysis of oocyte extracts. The data reveal (Fig. 1) that Myo1e is detectable not only in fully grown stage VI oocytes (staged according to Ref. 17) but in every stage examined (stage II–VI and eggs). The increased expression of Myo1e in later stage oocytes largely parallels the expression of two other proteins: the Csp (a protein associated principally with the membrane of cortical granules in these cells; see Ref. 14), and the major cargo protein of cortical granules, cortical granule lectin (24, 25) (Fig. 1, cgl). Concurrently, both Myo1e and Csp are detectably expressed in Xenopus brain, whereas cortical granule lectin is not (Fig. 1). As a counterpoint to the Myo1e results, we observed that even though oocytes contain mRNA for myosins 1d and 1f (3), they do not detectably express either of these myosins, as judged by immunoblot analysis (data not shown).

Although type 1 myosins are predominantly globular proteins with no predicted transmembrane segments, their primary structure includes the MyTH1 domain, a region that binds to anionic phospholipids and is important for the association of these proteins with membranes (11). Thus, we were interested in determining the relative distribution of Myo1e between cytosolic and membrane compartments in the oocyte. As a first step, we used subcellular fractionation. Previous work (14) showed that when oocytes were gently disrupted in an isosmotic solution, both the plasma membrane and cortical granules (along with pigment granules and yolk platelets) sedimented during a low g centrifugation. By subjecting oocytes to this fractionation scheme, we found >90% of the endogenous Myo1e in the low speed supernatant (Fig. 2A). At the same time, all of the detectable β-tubulin was in the low speed supernatant, whereas ~90% of the csp was in the low speed pellet (Fig. 2). After ultracentrifugation of the low speed supernatant, the Myo1e remained in the high speed supernatant (as did the tubulin; Fig. 2A; we did not probe for csp after the ultracentrifugation). These results indicate that >90% of the Myo1e in oocytes resides in a soluble (cytosolic) compartment, whereas <10% associates with the structures that sediment at low g forces.

To extend the subcellular fractionation results, we used immunofluorescence microscopy to assess the distribution of Myo1e in stage VI oocytes. The image in Fig. 2B, panel H, reveals that at low magnification most of the immunoreactive Myo1e is detected in the cortical rim of the oocyte, where it yields a diffuse staining along with scattered brighter puncta. This cortical distribution of Myo1e immunoreactivity is similar to what is observed both for Csp (Fig. 2B, panel D) (14) and cortical granule lectin (which is detected using fluorescent D. biflorus lectin; Fig. 2B, panel A). At higher magnification (Fig. 2B, panel B), the labeling for cortical granule lectin reveals a prominent band of fluorescence that extends less than 10 μm into the interior of the oocyte and shows greatest intensity close to the plasma membrane. At the same magnification, Csp immunostaining also appears strongest in the region closest to the plasma membrane (Fig. 2B, panel E). However, in contrast to the lectin signal (Fig. 2B, panel B), the Csp immunostaining extends at least 20 μm beneath the plasma membrane (Fig. 2B, panel E).

This trend is further accented for the Myo1e immunostaining, which appears as a diffuse layer extending ~50 μm into the oocyte (Fig. 2B, panel I). When visualized by confocal fluorescence microscopy, the staining for cortical granule lectin in an en face view reveals a cluster of spherical structures (~0.5–2 μm diameter) that correspond to cortical granules (Fig. 2B, panel C). In a similar en face view, Csp immunostaining is evident as a ring around many of the cortical gran-
To investigate whether Myo1e exhibits dynamic behavior during cortical granule exocytosis, we monitored the distribution of eGFP-Myo1e using time-lapse confocal microscopy. In this approach, exocytosis is triggered (by the application of PMA) in the presence of extracellular Texas Red dextran. Following exocytosis, the extracellular dextran diffuses into the lumen of the fused granules, and by focusing in a single optical plane ~1 μm beneath the plasma membrane, the entry of the dextran produces red fluorescent disks (3, 19). In the current experiments, eGFP-Myo1e was detected at the plasma membrane of oocytes prior to the triggering of exocytosis (data not shown), but it was not associated with any other structures in the cortex of the cell (Fig. 3A, 00:00). However, following the induction of cortical granule exocytosis, eGFP-Myo1e prominently encircled the cortical granules that had undergone exocytosis, as judged by the disc of fluorescent red dextran that was present in the same focal plane (Fig. 3A, 01:10). Quantitative analysis revealed that >98% (175 of 178) of the cortical granules that underwent exocytosis had an associated ring of eGFP-Myo1e. This result strongly suggests that Myo1e participates in events that are linked to cortical granule exocytosis or the ensuing compensatory endocytosis.

Remarkably, the live imaging also revealed that in the presence of PMA, eGFP-Myo1e often localized to circular compartments that lacked dextran but were of the correct dimension and in the proper location to be cortical granules (Fig. 3A, 01:10). The simplest explanation of this observation is that the eGFP-Myo1e relocalizes to the surface of cortical granules prior to (or during) exocytosis. Further analysis of time-lapse images confirmed this interpretation. As shown (Fig. 3B and supplemental movie 1), eGFP-Myo1e forms annular structures well in advance of the appearance of the disks of red dextran. Quantification of the recruitment time of eGFP-Myo1e relative to dextran incorporation revealed that the formation of rings of eGFP-Myo1e preceded the appearance of the red dextran by 8.6 ± 0.65 s (mean ± S.E.; n = 178).

To ascertain whether Myo1e has a role in cortical granule exocytosis, we first evaluated the impact on this process of overexpression of the IQT region of Myo1e (see schematic in Fig. 4C). This approach relies on the fact that similar deletion constructs exert a dominant negative impact on events mediated by other myosins (3, 26, 27). Representative results (Fig. 4A) indi-
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To extend the results of Fig. 4, we evaluated whether further truncation of the Myo1e IQT yields constructs (see schematic in Fig. 4C) that still inhibit cortical granule exocytosis. By eliminating the calmodulin-binding IQ region, we tested the effect of the Myo1e tail. Representative results (Fig. 5A) indicate that high level expression of the Myo1e tail also inhibits cortical granule exocytosis. In n = 11 cells in which the Myo1e tail was expressed (to a mean level 9.9-fold above the immunoblot signal for endogenous Myo1e), cortical granule exocytosis was inhibited by 96 ± 2% relative to control. Next, we removed both the MyTH1 and MyTH2 domains, thereby leaving only the SH3 domain of Myo1e. This construct is highly expressed in oocytes (the signals for the SH3 domains in Fig. 5B exceed those of the full-length Myo1e by >27-fold), but the SH3 domain only partially inhibits (a mean reduction of 43 ± 7%) cortical granule exocytosis (Fig. 5B; this figure shows the greatest inhibition obtained with the SH3 domain). Thus, although the inhibitory efficacy of Myo1e-IQT is preserved in the tail construct, it is appreciably weaker in the isolated SH3 domain. Finally, note that none of the constructs of Fig. 4C affected the subcellular distribution of csp (judged using the fractionation procedure of Fig. 2A; data not shown).

As another means for perturbing cortical granule exocytosis, we investigated the effect of injecting oocytes with affinity-purified Myo1e antibody. Initially, oocytes were injected with either the affinity purified N-end or C-end antibody. Alone, these antibodies had no effect on cortical granule exocytosis, even in cells injected with 0.5 mg of purified antibody (data not shown). However, injection of oocytes with a mixture of the N-end and C-end antibody led to a nearly complete inhibition of cortical granule exocytosis (Fig. 6). These results suggest that antibodies targeting both ends of Myo1e are necessary either for the steric occlusion of Myo1e function or for the effective formation of immune complexes that prevent Myo1e from participating in exocytosis.

We also explored the possibility of inhibiting Myo1e expression in oocytes by using Myo1e-specific morpholino antisense oligonucleotides (this strategy is frequently effective for blunting protein expression in Xenopus embryos and other cells; see Refs. 28, 29). However, because of what appears to be a relatively slow turnover of Myo1e in oocytes (data not shown), the morpholino strategy did not result in a significant reduction of the endogenous Myo1e (data not shown). Instead, we asked whether overexpression of full-length Myo1e would enhance the kinetics of cortical granule exocytosis. For these experiments, we modified the standard cortical granule exocytosis assay (which uses 50 nM PMA and a 30-min collection period during which ~80% of the available cortical granule lectin in the cell is secreted into the medium surrounding the oocyte (14)) by using 20 nM PMA and a 20-min collection period. A representative experiment (Fig. 7) reveals that cells overexpressing full-length Myo1e exhibit a significant increase in the amount of cortical granule lectin that is discharged during this 20-min period. Overall, oocytes that overexpress Myo1e...
released 32 ± 6% (this difference is significant at \( p < 0.01 \) for \( n = 15 \) oocytes) more cortical granule lectin than control cells. Thus, in contrast to the significant inhibition of cortical granule exocytosis observed in oocytes expressing the tail or IQT fragments of Myo1e, overexpression of the full-length myosin enhances exocytosis.

Although the preceding data support a functional role for Myo1e in cortical granule exocytosis, we were also interested whether Myo1e participates in constitutive exocytosis. To answer this question, we overexpressed Myo1e-IQT in oocytes (under the same conditions that block cortical granule exocytosis, as shown in Fig. 4), and we monitored the constitutive secretion of alkaline phosphatase (as in Ref. 21). The results (Fig. 8) reveal that Myo1e-IQT does not inhibit the constitutive secretion of alkaline phosphatase. This finding supports the conclusion that Myo1e functions preferentially in the regulated secretory pathway.

The data of Fig. 3 indicate that PMA triggers eGFP-Myo1e to re-localize to cortical granules. Although type 1 myosins bind effectively to anionic phospholipids in bilayer membranes (11), it is assumed (30) that distinct docking/effector proteins confer specificity of type 1 myosins for binding to biological membranes. Thus, we sought to identify prospective binding partners for Myo1e on cortical granules. This search took into account...
account the following considerations. First, the evidence linking Myo1e to cortical granule exocytosis (Figs. 3–7) suggests that Myo1e might interact with one or more cortical granule proteins that participate in exocytosis. In this context, it is noteworthy that membranes of regulated secretory organelles (particularly, synaptic vesicles) include no more than ~10 distinct classes of proteins (31). Of these ~10 classes of proteins, oocytes contain no detectable synaptophysin, SV2A or -B, or synapsins (as judged by immunoblot analysis using antibodies that detect these proteins in Xenopus brain extracts; data not shown). Concurrently, there remains little evidence linking proteins like synaptogyrin and the SCAMPs (secretory carrier-associated membrane proteins) to steps in the exocytotic cascade (32). Although Csp and synaptotagmin I are present in Xenopus oocytes (14, 33), we have been unable to detect the most common vesicular-soluble, N-ethylmaleimide-sensitive factor attachment receptors synaptobrevins I–III in oocytes (data not shown). Thus, our search for prospective Myo1e docking proteins focused on Csp and synaptotagmin I. Using Myo1e antibody for immunoprecipitations, we tested for co-immunoprecipitation of Csp or synaptotagmin I. These experiments revealed that Csp co-immunoprecipitated with Myo1e (Fig. 9). However, there was no detectable co-immunoprecipitation of synaptotagmin I (data not shown; as additional controls, neither β-tubulin nor myristoylated, alanine-rich, protein kinase C substrate co-immunoprecipitated in these experiments). Concurrently, immunoprecipitation of Csp also culminated in co-immunoprecipitation of Myo1e (Fig. 9). These results are suggestive of a link between csp and Myo1e during cortical granule exocytosis.

To extend the observations of Fig. 9, we first investigated whether the Myo1e that is present in oocyte extracts binds to immobilized, recombinant Csp. The recombinant Csp was prepared as a fusion to MBP, and the fusion construct was bound to amylose resin. Results in Fig. 10A indicate that Myo1e in an oocyte extract binds to recombinant MBP-Csp but not to MBP alone. Because the results in Figs. 9 and 10A could arise either from a direct association of Csp and Myo1e, or via an indirect interaction (mediated by some other protein in the oocyte extract), we next tested whether Myo1e binds to Csp in the absence of oocyte extract. These experiments were hampered by the poor recovery of intact Myo1e from bacterial expression systems. To circumvent this problem, we used in vitro translation to prepare Myo1e (as well as Myo1c and the Myo1e-head). Data in Fig. 10B indicate that in vitro translated Myo1e binds to MBP-Csp but not to MBP alone. Concomitantly, neither Myo1c nor the Myo1e-head binds to MBP-Csp (or to MBP; Fig. 10B). These results are consistent with a direct (and selective) interaction between Csp and Myo1e and support the conclusion from earlier experiments that determinants within the tail of Myo1e are important for exocytosis in oocytes.

**DISCUSSION**

Several observations support the conclusion that Myo1e participates in cortical granule exocytosis in frog oocytes. First, this protein is expressed as early as stage II in these cells. By this stage, oocytes are...
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already capable of secreting cortical granule lectin in response to PMA (18) indicating that they possess the molecular machinery needed for regulated exocytosis. Second, cytosolic Myo1e relocates in a stimulus-dependent fashion to the surface of cortical granules. It arrives there prior to the onset of exocytosis, implying that it may contribute to a pivotal step in the exocytotic cascade. Third, overexpression of recombinant constructs of Myo1e (which delete the motor domain or the motor domain plus the IQ region) leads to a prominent inhibition of cortical granule exocytosis (but not constitutive exocytosis). Fourth, injection of oocytes with antibody targeting the NH₂ and COOH termini of Myo1e inhibits cortical granule exocytosis. Fifth, overexpression of full-length Myo1e enhances the kinetics of cortical granule exocytosis. Collectively, these observations raise interesting questions both about the specific role of Myo1e in regulated exocytosis, as well as the mechanism of its re-localization to cortical granules.

To date, abundant evidence points to the involvement of certain unconventional myosins (particularly, type V myosins) in the transport of secretory organelles within the actin-rich cortex of cells (34–38). Thus, one possibility is that Myo1e contributes to a late stage transport step that immediately precedes (on a time scale of seconds; see Fig. 3) cortical granule exocytosis. For this hypothesis to be tenable, we need to know whether cortical granules are situated at a sufficient distance from the plasma membrane to require a pre-exocytotic transport step. In this context, it is interesting that an electron microscopic study revealed that the sub-plasmalemmal distribution of Xenopus cortical granules changes during the transition of an immature oocyte into a meiotically mature egg (9). Thus, during the early stages of progesterone-induced maturation, few cortical granules were within 200 nm of the plasma membrane. However, after maturation, most granules were <200 nm from the plasma membrane (9). Although these results indicate that cortical granules undergo a re-distribution during maturation, the large size of these organelles (diameters up to ~3 μm) makes it difficult to determine with accuracy the minimum distance between the membrane of individual cortical granules and the plasma membrane. This uncertainty derives from the fact that when oblate structures of this diameter approach a relatively planar surface (i.e. the plasma membrane), only a small fraction of the cortical granules will be sampled where their membrane makes its closest approach to the plasma membrane (for instance, when a 100 nm thick transverse section of the cortex of an oocyte is visualized in the electron microscope, fewer than 10% of the cortical granules will be sampled at their closest approach to the plasma membrane). Despite this caveat, if we assume that cortical granules are typically situated ~200 nm from the plasma membrane, then a transport step clearly becomes an essential prerequisite for exocytosis. In fact, although information is not yet available for Myo1e, the step size of Myo1c (estimated at 5–6 nm; see Ref. 39) suggests that multiple duty cycles of Myo1e would be necessary to close a 200 nm gap between a cortical granule and the plasma membrane.

Although Myo1e may be involved in a “pre-exocytotic” stage of granule transport, we cannot exclude other possible functions for this protein. For instance, Myo1e may contribute to a pre-exocytotic “bending” of the plasma membrane. Early descriptions of an inward dimpling of the plasma membrane during exocytosis included evidence for cytoskeletal elements connecting the target membranes (discussed in Ref. 40 and see recent reviews of the possible contributions of membrane bending and hemi-fusion to exocytosis; see Refs. 41, 42). However, there has been little further characterization of these cytoskeletal elements or their functional significance. It may be of interest to pursue this issue, both in the context of our results and data from several other groups that suggest that certain myosins have roles in exocytosis that are unrelated to organelle transport (43–45). However, in addition to membrane bending, other prospective functions for these “exocytotic” myosins include roles in the re-distribution of proteins on the secretory granule surface or as contributors to the dilation of the fusion pore (something that may be important in cells with secretory organelles as large as cortical granules). Clearly, additional work will be needed to clarify the role(s) of myosins in exocytosis.

The dynamic imaging data indicate that Myo1e binds to the cortical granule surface on a time scale that is consistent with
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this motor protein having a role in regulated exocytosis. At the same time, the protein interaction data suggest that Csp, a protein associated with the cortical granule surface (14), may play a role as a docking or effector protein for Myo1e. In fact, the identification of docking proteins for type 1 myosins has been an elusive task (30) and has led to the possibility that the membrane association of these proteins relies principally, if not exclusively, on specific lipid targets in the membrane (46). However, it is plausible that the apparent interaction between Myo1e and Csp goes beyond a stabilization of the membrane (lipid) binding of Myo1e. Recent investigations (20) indicate that Csp, in conjunction with a 70-kDa cognate heat shock protein (Hsc70), plays an important role in cortical granule exocytosis in oocytes. Thus, although the precise contribution of Csp (and Hsc70) to the exocytotic sequence remains to be clarified, the current experiments suggest that Csp function is likely to be intertwined with that of Myo1e, and possibly serve as a model for understanding the role of myosins in exocytosis.

Prior studies of vertebrate Myo1e revealed that this myosin isoform was concentrated in the cytoplasm and in elongated structures at sites of intercellular contact that contained actin (and α-actinin but not vinculin (47). In addition, Myo1e was found at intercellular adherens-type junctions that were induced by constitutively active Cdc42 (48). This targeting did not rely on the SH3 domain, but it was disrupted upon deletion of the COOH-terminal ~180 residues of Myo1e implying that this portion of the Myo1e tail was essential for its subcellular distribution (48). These latter observations offer certain parallels to the current work in that overexpression of the SH3 domain of Myo1e only weakly interferes with cortical granule exocytosis, whereas the complete tail region is an effective inhibitor of exocytosis. The most likely explanation for these results is that the MyTH1 or MyTH2 regions in the tail of Myo1e include determinants that stabilize its interaction with specific targets in the oocyte that are important not only for exocytosis but also for the subcellular localization of Myo1e. In a separate study, Myo1e was found preferentially in the cuticular plate, an actin meshwork that anchors the stereocilia in auditory and vestibular epithelia (49), and a very recent investigation implicated Myo1e in constitutive endocytosis (50). Although our data argue for an exocytotic function of Myo1e in oocytes, we cannot exclude a role in endocytosis. Regardless, in view of the diverse cell types in which Myo1e is expressed, it will be important to determine whether common mechanisms underlie the subcellular distribution and function of this motor protein.

We recently reported that Myo1c participates in the compensatory endocytosis that follows cortical granule exocytosis (3). Specifically, Myo1c is required for tight coupling of dynamic actin to the membranes of exocytosing cortical granules. Together with the current results, these findings indicate that different myosins 1 can play distinctly different roles on the same membrane compartment within a very short time span. Presumably, at least some of the variation in the roles played by these two myosins reflects differences in the structure of their tail region and associated light chains; Myo1c has three IQ motifs and three associated calmodulin light chains, in contrast to Myo1e, which has a single IQ motif and associated calmodulin. In addition, Myo1c lacks the MYTH2 and SH3 domains found in the tail of Myo1e (11).

In summary, this study indicates that Myo1e plays a vital role during regulated exocytosis (but not constitutive exocytosis) in frog oocytes. It will be interesting to determine whether Myo1e contributes to regulated secretion in other cell types and to learn more about the molecular role of Myo1e in this process.

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