

Evidence for Direct Membrane Retrieval Following Cortical Granule Exocytosis in *Xenopus* Oocytes and Eggs

WILLIAM M. BEMENT,^{1,2*} HELENE BENINK,¹ CRAIG A. MANDATO,¹
AND BRAD B. SWELSTAD¹

¹Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706

²Program in Cellular and Molecular Biology, University of Wisconsin,
Madison, Wisconsin 53706

ABSTRACT Rapid exocytosis is typically followed by rapid resorption of exocytosed membrane; however, whether membrane retrieval occurs via indirect endocytosis of numerous small vesicles or direct resealing of the original, larger exocytotic vesicles is controversial. Here we show that cortical granule (CG) exocytosis in *Xenopus* oocytes and eggs is followed by rapid formation of endosomes as large as the CGs. Large endosomes are translucent, and their formation has the same developmental and pharmacological profile as CG exocytosis. Time course analyses show that large endosomes are not derived from small endosomes. Large endosome formation is triggered by stimuli that do not trigger increases in intracellular-free calcium and is insensitive to perturbation of microtubules by treatment with nocodazole. Perturbation of the f-actin cytoskeleton with latrunculin, however, sharply reduces large endosome formation. We conclude that CG membrane is directly retrieved in *Xenopus* oocytes and eggs and suggest that this retrieval is not directly dependent on an increase in intracellular-free calcium, but is dependent on the actin cytoskeleton. *J. Exp. Zool.* 286:767-775, 2000. © 2000 Wiley-Liss, Inc.

Regulated exocytosis is a feature common to a variety of cell types, including professional secretory cells such as neurons and neuroendocrine cells, as well as vertebrate and invertebrate eggs that employ a slow block to polyspermy. The cellular consequences of regulated exocytosis are considerable. In the space of milliseconds to seconds, secretory granules fuse en masse with the plasma membrane and release their contents into the extracellular space. In the absence of rapid membrane resorption mechanisms, the cell is faced with a twofold or greater increase in surface area that occurs either locally, as in neurons, or globally, as in certain neuroendocrine cells and eggs.

It has long been thought that membrane derived from secretory granules is retrieved following regulated exocytosis by classic, relatively slow endocytotic mechanisms involving formation and internalization of small (50–100 nm) clathrin-coated vesicles. For example, electron microscopy analysis of membrane retrieval following synaptic vesicle exocytosis in amphibian neuromuscular junctions has been proposed to be accomplished by coated vesicles (Heuser et

al., '74). Similarly, membrane retrieval following cortical granule exocytosis in sea urchin eggs has also been attributed to clathrin-mediated endocytosis (Fisher and Rehman, '83). However, recent studies have provided evidence for membrane retrieval by a mechanism distinctly different than established endocytotic pathways. Thomas et al. ('94) studied exocytosis and endocytosis in isolated pituitary cells and found, by analyzing plasma membrane capacitance changes following the release of caged calcium, that membrane retrieval following exocytosis occurs via formation of endosomes considerably larger than classic endosomes. Whalley et al. ('95) studied exocytosis and endocytosis in sea urchin eggs and showed, using a variety of microscopy approaches, that exocytosis of cortical secretory granules (CGs) triggered forma-

Grant sponsor: Department of Zoology, University of Wisconsin, Madison; Grant sponsor: National Science Foundation; Grant number: MCB 9630860; Grant sponsor: National Institutes of Health; Grant number: GM 52932-0142.

*Correspondence to: William M. Bement, Department of Zoology, University of Wisconsin, 1117 West Johnson Street, Madison, WI 53706. E-mail: wmbement@vms2.macc.wisc.edu

Received 17 August 1999; Accepted 26 October 1999

tion of endosomes as large or larger than the CGs themselves. These large endosomes formed with spatial and temporal kinetics that paralleled CG exocytosis. Moreover, partial inhibition of CG content release following exocytosis resulted in the formation of large endosomes containing material that ultrastructurally resembled the original cortical granule contents.

Based on their results, Thomas et al. ('94) and Whalley et al. ('95) proposed a new endocytotic mechanism, namely, the immediate resealing of secretory vesicles following release of their contents. Such "direct membrane retrieval" is an attractive notion, as it provides a simple and rapid means to recover secretory granule membranes in their entirety. Here we have analyzed endocytosis following CG exocytosis in *Xenopus* oocytes and eggs. This system has three important features: First, the cortical granules themselves are extremely large (~0.5–3.0 μm in diameter; Grey et al., '74; Campanella and Andreuccetti, '77) relative to endosomes derived from either receptor-mediated endocytosis or pinocytosis (~50–100 nm in diameter). Assuming that direct membrane retrieval occurs, it should be possible to distinguish such vesicles from traditional endosomes by light microscopy. Second, the ease of oocyte and egg preparation permits simple labeling experiments that allow the direct observation of different endosomal markers. Third, studies on the development of "activation competence" in the *Xenopus* system have demonstrated that the ability of different pharmacological agents to trigger CG exocytosis varies as a function of the meiotic state (Bement, '92). Specifically, calcium ionophores such as A23187 trigger CG exocytosis in metaphase-II-arrested *Xenopus* eggs, but not in prophase-I arrested oocytes (Campanella et al., '84; Charbonneau and Grey, '84). In contrast, the protein kinase C agonist, phorbol 12-myristate 13-acetate (PMA), triggers CG exocytosis in both oocytes and eggs in a calcium-independent manner (Bement and Capco, '89, '90; Grandin and Charbonneau, '91).

Using this system, we provide evidence for resealing of CG membranes following exocytosis. Further, this direct membrane retrieval occurs in response to PMA treatment, indicating that the retrieval mechanism is not dependent on an increase in intracellular-free calcium. Finally, we demonstrate that direct membrane retrieval is apparently dependent on the f-actin cytoskeleton.

MATERIALS AND METHODS

Xenopus oocytes and eggs

Xenopus oocytes and eggs were obtained as previously described (Bement and Capco, '89). Briefly, oocytes were obtained surgically from adult *Xenopus* females, subjected to collagenase (Gibco, Grand Island, NY) treatment (0.8–1.0% for 1 hr), followed by extensive washing and manual removal of the follicle cells. Eggs were obtained by treating defolliculated oocytes for 8–14 hr at 18°C in 10 $\mu\text{g}/\text{ml}$ progesterone. For all experiments, comparisons were made using oocytes or eggs obtained from a single female. Each experiment represents oocytes or eggs from a different female.

Purification and Fluorescence Labeling of *Xenopus* Vitellogenin

Vitellogenin (Vtg) was obtained according to the protocol of Opresko ('91). Adult female *Xenopus* were injected with β -estradiol on day 1, and again on day 10. On day 15, frogs were anesthetized with benzocaine, and exsanguinated by cardiac incision. Blood was allowed to coagulate for 4 hr at room temperature, and plasma was collected following centrifugation at 2,500g for 15 min at 4°C. Vtg was selectively precipitated by the sequential addition of EDTA and MgCl_2 , as described (Opresko, '91), pelleted by centrifugation, resuspended in 1 M NaCl, 50 mM Tris (pH 7.5), reprecipitated, resuspended, and then analyzed by SDS-PAGE. Vtg purity confirmed to be 95% or greater by SDS-PAGE was snap frozen in liquid nitrogen and stored at -80° until use. For fluorescence labeling, Vtg was thawed and centrifuged at 100,000g for 1 hr to pellet aggregated material. Oregon green or Texas red labeling of Vtg was performed using kits (Molecular Probes, Eugene, OR) according to the instructions. The efficiency of labeling was checked by SDS-PAGE whereas the ability of the fluorescently modified vitellogenin (Vtg) to bind specifically to its receptor was checked by the incubation of oocytes in fluorescent Vtg with increasing concentrations of unlabeled Vtg.

Endocytosis assays

Oocytes or eggs were incubated in fluorescein dextran (F-dex; 3,000 molecular weight, Molecular Probes) at a final concentration of 2 mM in 1 \times OR2 (82.5 mM NaCl, 2.5 mM KCl, 1 mM CaCl_2 , 1 mM MgCl_2 , 1 mM Na_2HPO_4 , 5 mM HEPES, pH 7.4). Concentrations lower than 2 mM did not permit the visualization of small endosomes (i.e., pinocytotic vesicles), whereas higher concentrations

made the experiments prohibitively expensive. To visualize endosomes, oocytes and eggs were rapidly washed (to remove F-dex) after being subjected to the treatments indicated in Results, and then mounted in OR-2 and examined using a 100 \times objective on a Nikon Optiphot 2 fluorescence video microscope. In the experiments involving cytoskeleton perturbation, oocytes were subjected to the pretreatments indicated in Results, and then induced to undergo exocytosis by incubation in OR-2 containing 300 nM PMA and 2 mM fluorescent dextran (Molecular Probes), as well as the cytoskeletal drug in question.

For the quantification of endosomes, ten fields (each was 336 μm^2) from three different oocytes or eggs were videotaped, and the number of endosomes in each field was quantified and averaged. Quantitative analysis showed that endosomes sorted into two distinct classes based on size (unpublished results): for each oocyte or egg, the number of large endosomes per 336 μm^2 field were averaged, and these averages were used to calculate the mean \pm SEM for each experiment.

To visualize receptor-mediated endosomes, oocytes were incubated in fluorescent Vtg at a final concentration of 5 $\mu\text{g}/\text{ml}$ for 10 min. Longer incubations did not appreciably increase fluorescence signal from endosomes, but did result in the appearance of larger, irregular structures, presumably sorting endosomes. Although all possible combinations of fluorescent Vtg and dextran were tried to allow simultaneous visualization of receptor-mediated endosomes and large endosomes, we were unable to obtain images showing both structures in the same field of view. Consequently, the images comparing receptor-mediated endosomes to large endosomes are from different oocytes.

Cortical granule exocytosis analysis

We were unable to immunolocalize CGs, and the spectrophotometric assay previously employed to monitor CG exocytosis (Bement and Capco, '89) proved unsuitable for oocytes treated with latrunculin, as oocytes tended to lyse. Consequently, CG exocytosis was followed indirectly by quantifying the elevation of the fertilization envelope, which results from CG exocytosis. Fertilization envelope elevation was detected using a dissecting microscope; the envelope appeared as a translucent layer surrounding the oocytes.

Pharmacological treatments

PMA (Calbiochem, La Jolla, CA) was stored at -20°C in a stock solution of 1 mM in DMSO and

diluted to a final concentration of 300 nM immediately before use. This concentration has previously been shown to trigger cortical granule exocytosis in *Xenopus* oocytes and eggs (Bement and Capco, '89). A23187 (Calbiochem) was stored at -20°C in a stock solution of 10 mM in ethanol and diluted to a final concentration of 10 μM immediately before use. Latrunculin B and nocodazole (Calbiochem) were stored at -20°C in DMSO stock solutions of 10 mM and 20 mM, respectively. Oocytes were preincubated in 5 μM latrunculin or 20 μM nocodazole for 1 hr prior to exposure to PMA; these concentrations have previously been shown to disrupt f-actin-dependent processes and result in microtubule depolymerization in *Xenopus* oocytes, respectively (Canman and Bement, '97).

RESULTS

Induction of exocytosis in Xenopus eggs results in the formation of large, translucent endosomes

Endosome formation was assessed by incubating *Xenopus* oocytes or eggs in the presence of 2 mM fluorescein-dextran (F-dex; see Materials and Methods). Because this probe does not pass the plasma membrane by diffusion, it is a marker for bulk endocytotic mechanisms such as pinocytosis. Full grown (i.e., prophase-I arrested) *Xenopus* oocytes were incubated in fluorescein-dextran (F-dex) and, to induce CG exocytosis, the protein kinase C agonist, phorbol 12-myristate 13-acetate (PMA) at a concentration of 300 nM for 15 min (Bement and Capco, '89). Oocytes were then rapidly washed to remove F-dex and viewed by epifluorescence video microscopy. In oocytes treated with PMA, large endosomes were present, as judged by the presence of large (~ 2 μm diam), ovoid fluorescent structures just under the plasma membrane (Fig. 1). Analysis of the same field of view by differential interference contrast microscopy (DIC) showed that these large endosomes were translucent, as would be expected if they were derived from rapid resealing of CGs following exocytosis. In contrast, the surrounding yolk and pigment granules were relatively opaque.

To obtain a comparison of these large endosomes to smaller endosomes derived from receptor-mediated endocytosis, *Xenopus* oocytes were incubated with F-dex and PMA or with fluorescent Vtg. Vtg is a well-known substrate for receptor-mediated endocytosis in *Xenopus* oocytes (Opresko, '91). Fluorescently-labeled Vtg was incorporated into endosomes that were considerably

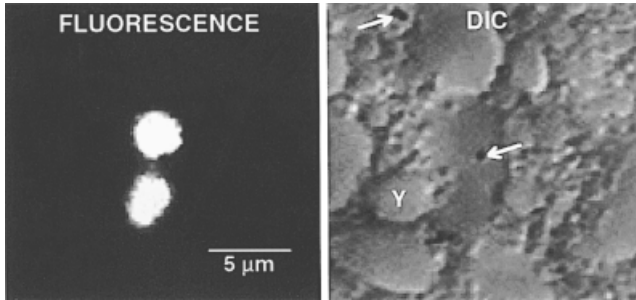


Fig. 1. Translucent endosomes form in *Xenopus* oocytes induced to undergo cortical granule exocytosis. Micrograph showing the same field of view using fluorescence and DIC microscopy of a *Xenopus* oocyte incubated in F-dex and 300 nM PMA to induce exocytosis. Two large endosomes are present, as determined by fluorescence microscopy, while the corresponding DIC image shows that these endosomes are translucent. Arrows, pigment granules; Y, yolk granule.

smaller ($<0.2 \mu\text{m}$) than those formed in response to PMA treatment ($>0.5 \mu\text{m}$; Fig. 2).

Large endosome formation has the same developmental and pharmacological profile as CG exocytosis

CG exocytosis in *Xenopus* eggs (that is, metaphase-II arrested eggs, the developmental stage that follows full-grown oocytes) can be triggered by several different “activating” stimuli, including those that result in increases in intracellular-free calcium, such as A23187 or pricking with

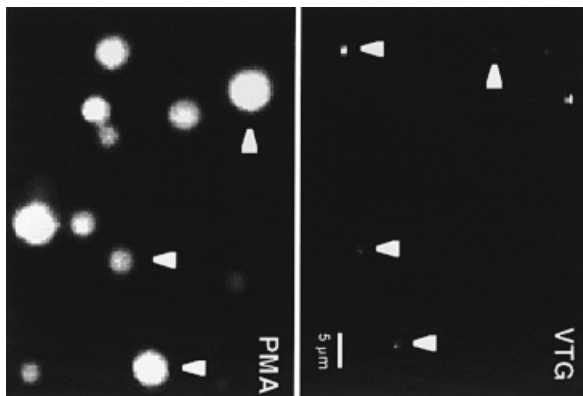


Fig. 2. PMA-induced endosomes are much larger than receptor-mediated endosomes. Fluorescence micrograph showing the size comparison of receptor mediated endosomes and large endosomes formed following cortical granule exocytosis. Receptor-mediated endosomes, detected by incubation of oocytes in fluorescent vitellogenin (Vtg) are very small (less than $0.2 \mu\text{m}$). In contrast, large endosomes, detected after incubation of oocytes in F-dex and 300 nM PMA (PMA), are typically greater than $0.5 \mu\text{m}$ in diameter. Both images are shown at the same magnification.

a needle (Charbonneau and Grey, '84), or by treatments which activate protein kinase C, such as PMA or diacylglycerol (Bement and Capco, '89). To quantify the effects of such known CG exocytotic stimuli, *Xenopus* eggs were incubated in F-dex + DMSO (the vehicle for PMA) as a control, F-dex + A23187, or F-dex + PMA. Both A23187 and PMA resulted in the formation of many large endosomes in eggs, while control eggs, which do not undergo CG exocytosis, showed no endosomes (Fig. 3A, B). The endosomes ($0.5\text{--}3 \mu\text{m}$) were as large or larger than CGs (Grey et al., '74; Campanella and Andreucetti, '77), again suggesting that the large endosomes are derived from direct resealing of CG membranes following exocytosis. Furthermore, the number of large visible endosomes is consistent with previous quantifications of CGs (see Discussion). Prick-activation of eggs also resulted in large endosome formation (not shown).

To further test whether the formation of large endosomes results from CG exocytosis, rather than as a result of some other process triggered by egg activation, full-grown oocytes were treated with F-dex and DMSO, F-dex and A23187, or F-dex and PMA. A23187 triggers CG exocytosis in eggs, but not oocytes (Campanella et al., '84; Charbonneau and Grey, '84), while PMA triggers CG exocytosis in both oocytes and eggs (Bement and Capco, '89). As with eggs, controls showed no large endosomes, while PMA-treated oocytes possessed numerous large endosomes (Fig. 4A, B). In contrast to eggs, however, A23187 treatment of oocytes did not trigger large endosome formation (Fig. 4A, B).

Time course analysis of large endosome formation

The translucence and large size of the endosomes that formed following CG exocytosis suggested that these structures formed by direct resealing of CG membranes following fusion with the plasma membrane. However, it remained possible that large endosomes might be derived from the rapid formation and subsequent fusion of numerous small endosomes. To test this possibility, oocytes were incubated in F-Dex and PMA, washed at increasing times intervals, and then analyzed by fluorescence microscopy. PMA and oocytes were used rather than A23187 and eggs because the exocytotic response in the former is slower than the latter (Bement and Capco, '89), permitting the greater resolution of processes that follow exocytosis. At the earliest time points

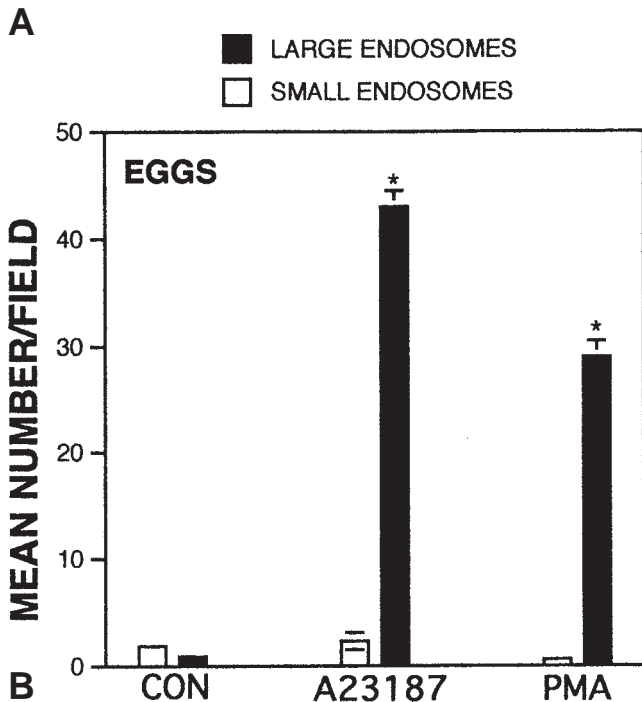
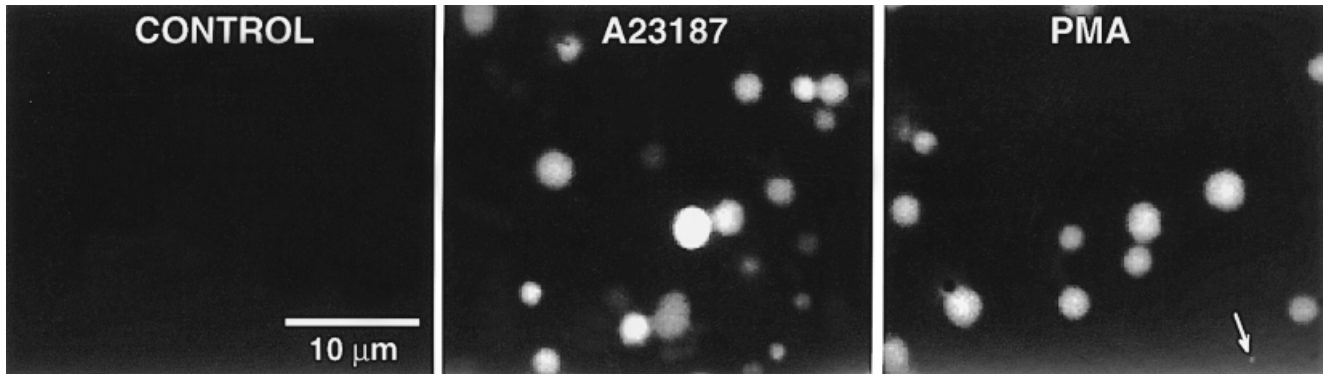


Fig. 3. Agents that trigger cortical granule exocytosis in *Xenopus* eggs trigger large endosome formation. **A.** Fluorescence micrographs of *Xenopus* eggs treated with DMSO (control), 10 μ M of the calcium ionophore A23187 (A23187), or 300 nM PMA (PMA) for 15 min in the presence of F-dex. A23187 and PMA, but not DMSO, trigger the formation of large endosomes. A small endosome (<0.5 μ m diam) is also present (arrow in PMA panel). **B.** Quantification of small (<0.5 μ m diam) and large (greater than 0.5 μ m diam) endosomes in eggs treated with DMSO, A23187, or PMA per 336 μ m² field. Results shown are the mean \pm S.E.M. from three independent experiments; * $P < 0.05$; one-tailed Student's *t*-test.

where endosomes were visible (90 sec), large endosomes were often observed in the complete absence of small endosomes (Fig. 5). The failure to observe small endosomes was not due to insufficient fluorescence signal from small endosomes since: (1) the 2 mM concentration of F-Dex was sufficient to detect receptor mediated endosomes (see Materials and Methods), and (2) small endosomes could be observed in the same field as large endosomes in later time points of the same experiment (e.g., arrow in Fig. 3A).

Large endosome formation is actin-dependent and microtubule-independent

Because the cytoskeleton has been implicated in endocytosis, we sought to assess the potential role of actin filaments and microtubules in large endosome formation. To test the role of actin fila-

ments or microtubules, oocytes were preincubated for 1 hr in 5 μ M latrunculin B or 20 μ M nocodazole, respectively. Control oocytes were preincubated in DMSO, the vehicle for both latrunculin and nocodazole, for 1 hr. Following preincubation, oocytes were transferred to a medium containing the appropriate drug, F-Dex, and PMA for 15 min, and then processed for fluorescence analysis of endosome formation. Nocodazole pretreatment had no significant effect on large endosome formation. In contrast, latrunculin treatment significantly reduced the number of large endosomes (Fig. 6).

Two distinct mechanisms could explain the observed inhibition of large endosome formation by latrunculin. This inhibition might result from either inhibition of the endocytotic process itself or the inhibition of CG exocytosis. To distinguish be-

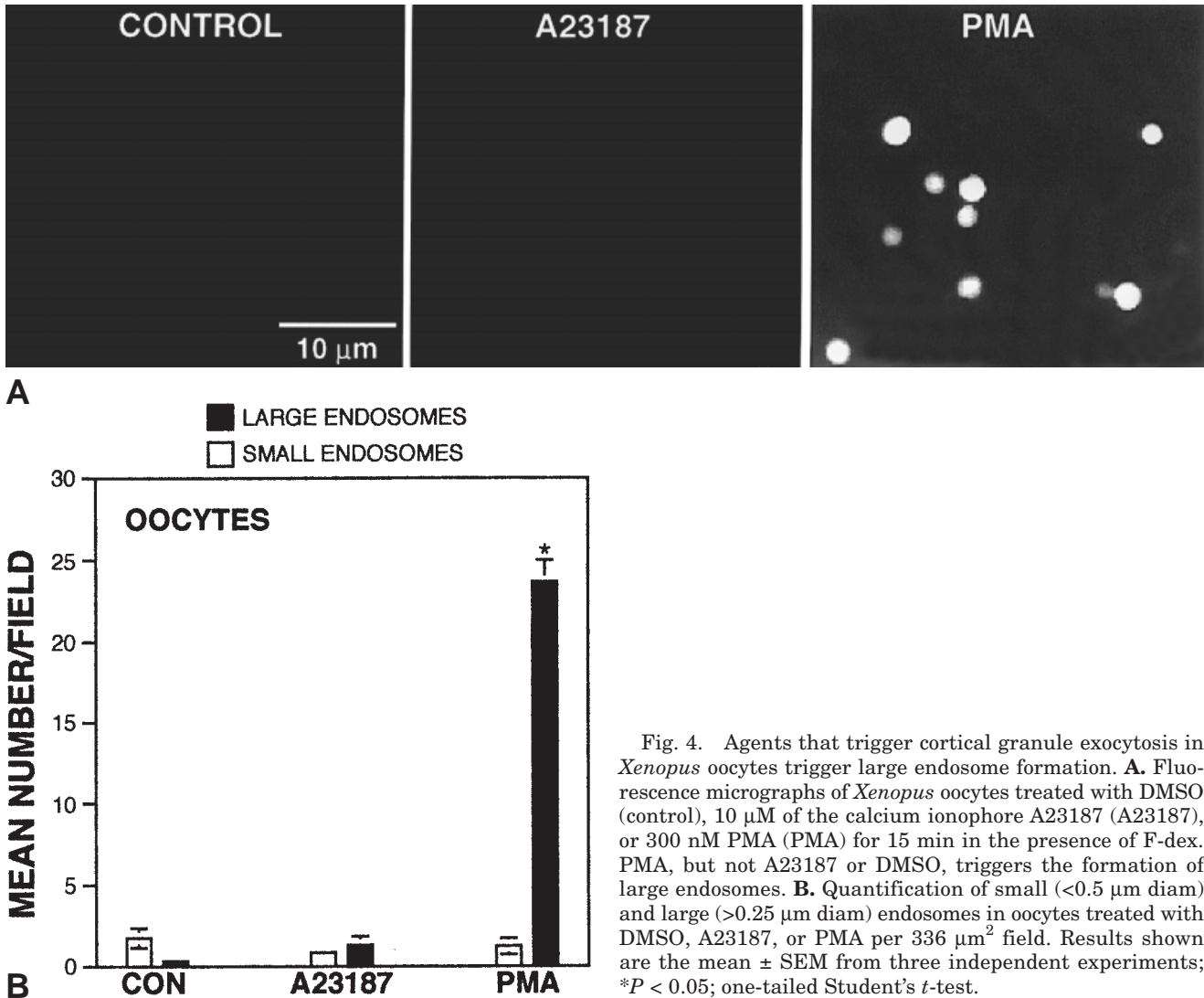


Fig. 4. Agents that trigger cortical granule exocytosis in *Xenopus* oocytes trigger large endosome formation. **A.** Fluorescence micrographs of *Xenopus* oocytes treated with DMSO (control), 10 μM of the calcium ionophore A23187 (A23187), or 300 nM PMA (PMA) for 15 min in the presence of F-dex. PMA, but not A23187 or DMSO, triggers the formation of large endosomes. **B.** Quantification of small (<0.5 μm diam) and large (>0.25 μm diam) endosomes in oocytes treated with DMSO, A23187, or PMA per 336 μm² field. Results shown are the mean ± SEM from three independent experiments; **P* < 0.05; one-tailed Student's *t*-test.

tween these possibilities, we monitored CG exocytosis in control oocytes and oocytes treated with either latrunculin or nocodazole by following fertilization envelope elevation in oocytes. The fertilization envelope forms as the result of the exocytosis of cortical granule materials, which modify the extracellular matrix such that it lifts off the surface of the cell, forming a translucent layer that can be observed with a dissecting microscope. Neither latrunculin nor nocodazole significantly inhibited fertilization envelope elevation relative to controls (Fig. 7), indicating that latrunculin inhibits large endosome formation by inhibiting the endocytotic process itself.

DISCUSSION

The goal of this study was to assess the mechanisms of membrane resorption following regulated

exocytosis in *Xenopus* oocytes and eggs. In particular, we sought to test the "direct membrane retrieval" hypothesis recently proposed to account for membrane resorption in pituitary cells (Thomas et al., '94) and sea urchin eggs (Whalley et al., '95). Because means to specifically follow individual secretory granule membranes before, during, and after exocytosis are not yet available, efforts to demonstrate that a given class of endosome is derived from a given class of secretory granule must necessarily be indirect.

Five lines of evidence indicate that the observed endosomes form as a result of CG exocytosis. The first line of evidence is based on the size of the endosomes formed following CG exocytosis. The endosomes are approximately as large as the reported sizes of the CGs themselves (~0.5–3.0 μm; e.g., Grey et al., '74; Campanella and Andreucetti,

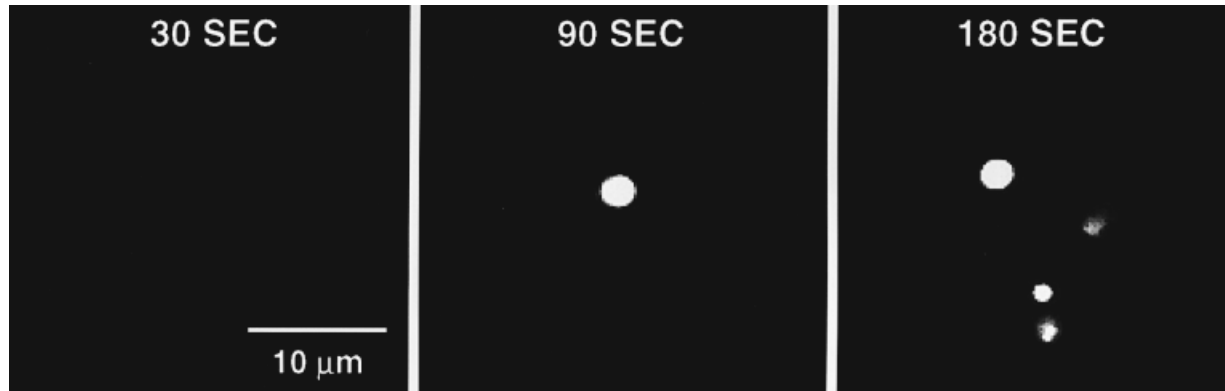


Fig. 5. Time course analysis of large endosome formation. Oocytes were incubated in F-dex and PMA for the indicated amount of time, washed, and then viewed by fluorescence

microscopy. By 90 seconds (90 sec), a large endosome can be seen, even though no small endosomes are present.

'77) and are clearly much larger (at least 4–10 times) than vitellogenin-based endosomes, which are ~50–100 nm in diameter (e.g., Fig. 1A in Wall and Patel, '89). The second line of evidence is based on the translucence of the large endosomes. Simultaneous imaging of the large endosomes by fluorescence and DIC microscopy shows that these

structures are virtually invisible by DIC, revealed only as areas where cytoplasm and other opaque organelles are excluded. This is exactly what one would predict if the endosomes were formed by rapid resealing, since they would contain only what is present in the extracellular medium. The third line of evidence comes from simple quantification of the number of large endosomes seen following activation of eggs: 43 per 336 μm^2 field, or

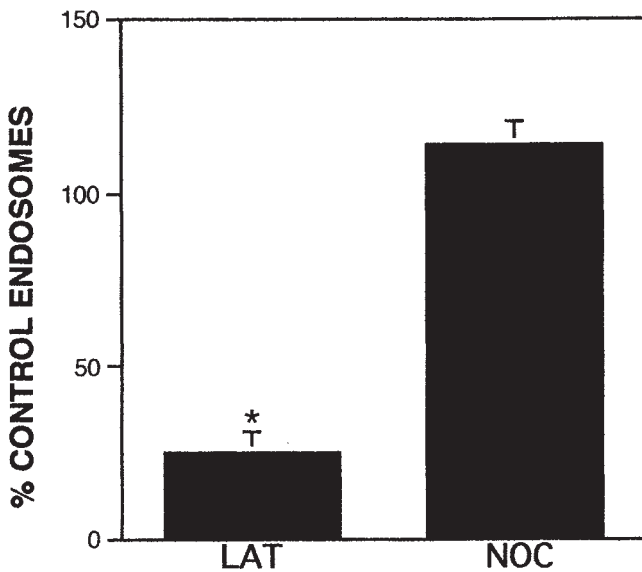


Fig. 6. Large endosome formation requires f-actin but not microtubules. Oocytes were treated with 20 μM nocodazole (NOC) or 5 μM latrunculin B (LAT) for 1 hr and then exposed to 300 nM PMA and the appropriate drug in the presence of F-dex. After 15 min oocytes were washed, and the average number of large endosomes per 336 μm^2 field was quantified, averaged, and compared to the average number of large endosomes in control oocytes treated with DMSO and then exposed to PMA. Results shown are mean \pm SEM from three independent experiments; * $P < 0.05$; one-tailed Student's *t*-test.

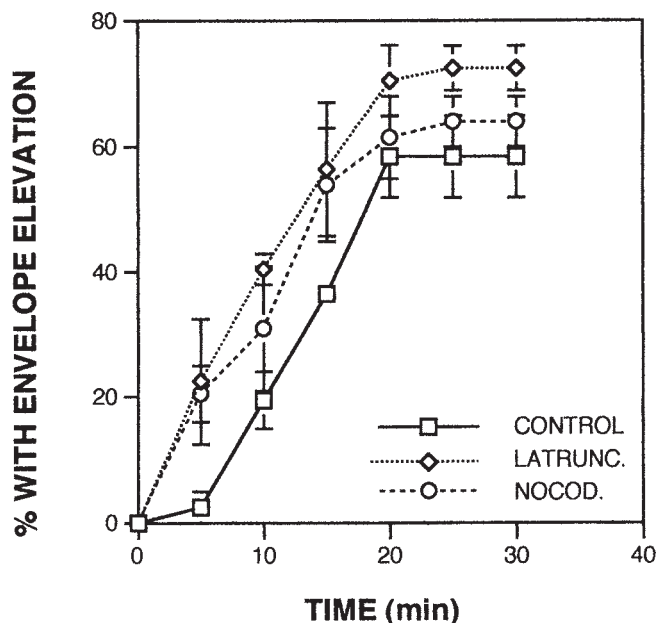


Fig. 7. Neither latrunculin nor nocodazole inhibit fertilization envelope elevation. Oocytes were treated with latrunculin, nocodazole, or DMSO as in Figure 6 and then exposed to 300 nM PMA and monitored for elevation of the fertilization envelope, as a marker for CG exocytosis (see text). The number of oocytes displaying an elevated fertilization envelope was scored every 5 min.

0.13 endosomes/ μm^2 , a number remarkably close to that measured by electron microscopy for CGs in intact eggs (0.1 CG/ μm^2 ; Peres and Bernardini, '95). The fourth line of evidence is based on a simple developmental and pharmacological analysis. Eggs undergo CG exocytosis in response to either A23187 or PMA, but oocytes are only responsive to PMA (Campanella et al., '84; Charbonneau and Grey, '84; Bement and Capco, '89).

Consistent with the supposition that the large endosomes are formed as a result of CG exocytosis followed by direct retrieval, PMA, but not A23187, results in large endosome formation in oocytes. Finally, the time course analysis showed that large endosomes could form in the absence of small endosomes, indicating that they are not generated by the fusion of smaller vesicles. Nor can it be argued that small vesicles label too poorly with F-dex to be seen in our assays, since vesicles the size of Vtg-induced endosomes were observed in many samples (e.g., Figs. 3A and 4B). Based on these lines of evidence, we conclude that large endosome formation following CG exocytosis is operative not only in pituitary cells (Thomas et al., '94) and invertebrate eggs (Whalley et al., '95), but also in vertebrate eggs.

The extension of the findings of Thomas et al. ('94) and Whalley et al. ('95), to vertebrate eggs is important, but this study also provides insight into potential mechanisms for direct membrane retrieval. In particular, the fact that PMA treatment triggers large endosome formation is quite surprising, since it has been shown that PMA treatment of *Xenopus* eggs does not trigger an increase in intracellular-free calcium, but nevertheless triggers CG exocytosis (Bement and Capco, '90; Grandin and Charbonneau, '91). Furthermore, intracellular calcium elevation in *Xenopus* oocytes does not trigger CG exocytosis (Campanella et al., '84; Charbonneau and Grey, '84), while PMA treatment does (Bement and Capco, '89). Thus, based on our demonstration that large endosomes form in PMA-treated oocytes and eggs, it follows that the mechanism that triggers direct membrane retrieval is not strictly dependent on an increase in intracellular-free calcium.

Since direct membrane retrieval occurs in response to exocytotic stimuli that both do (A23187) and don't (PMA) trigger an increase in intracellular-free calcium, we propose that the resealing of secretory granules is directly entrained to the fusion event itself, rather than requiring a specific intracellular signal. That is, we suggest that the CG membrane is somehow "hardwired" to rapidly

reseal after fusion with the plasma membrane. In principle, this hardwiring could be achieved by having the secretory granule membranes supported by some kind of membrane skeleton that lends it rigidity, such that after the fusion and release of CG contents, resealing is energetically favored.

It is also possible that resealing is driven by an ATP-dependent process. For example, the sensitivity of large endosome formation to latrunculin treatment suggests that the f-actin membrane cytoskeleton is involved. Consistent with this possibility, Becker and Hart ('99) have recently localized both f-actin and myosin-II to "cortical crypts," structures that form following the cortical granule release in zebrafish eggs. They proposed that such crypts are resealed by a purse string composed of f-actin and myosin-II. It is also possible that unconventional myosins participate in the closure, such as has been proposed for phagocytosis (Swanson et al., '99; Voigt et al., '99).

Finally, the observation that neither endocytosis nor exocytosis are significantly altered by microtubule depolymerization is interesting, in that microtubule depolymerization significantly accelerates PMA-induced cortical flow in oocytes (Canman and Bement, '97). Because microtubules are thought to be responsible for controlling membrane insertion during processes such as cytokinesis (e.g., Danilchik et al., '98), one simple explanation for the effects of microtubule depolymerization on cortical flow is a reduction in the amount of new membrane insertion into the plasma membrane. That is, global insertion of new membrane would be expected to reduce the overall cortical tension, and thereby slow cortical flow. The fact that we saw no apparent effect of microtubule depolymerization on either endo- or exocytosis implies that either our assays did not detect the relevant membrane pool or that microtubules are not regulating cortical flow via membrane dynamics.

ACKNOWLEDGMENTS

The original observation of large endosome formation in *Xenopus* oocytes was made by B.B.S., Jesse Johanson, and Damon Fairfield in an experimental laboratory class funded by the Department of Zoology, University of Wisconsin, Madison. Further work was supported by grants from the National Science Foundation (MCB 9630860) and the National Institutes of Health (GM 52932-01A2) to W.M.B. and by a Howard Hughes Scholarship to B.B.S. C.A.M. is funded by a National Science and Engineering Research Council (NSERC) postdoctoral fellowship.

LITERATURE CITED

- Becker KA, Hart NH. 1999. Reorganization of filamentous actin and myosin-II in zebrafish eggs correlates temporally and spatially with cortical granule exocytosis. *J Cell Sci* 112:97–110.
- Bement WM. 1992. Signal transduction by calcium and protein kinase C during egg activation. *J Exp Zool* 263:382–397.
- Bement WM, Capco DG. 1989. Activators of protein kinase C trigger cortical granule exocytosis, cortical contraction, and cleavage furrow formation in *Xenopus laevis* oocytes and eggs. *J Cell Biol* 108:885–892.
- Bement WM, Capco DG. 1990. Protein kinase C acts downstream of calcium at entry into the first mitotic interphase of *Xenopus laevis*. *Cell Regul* 1:315–326.
- Campanella C, Andreuccetti P. 1977. Ultrastructural observations on cortical endoplasmic reticulum and on residual cortical granules in the egg of *Xenopus laevis*. *Dev Biol* 56:1–10.
- Campanella C, Andreuccetti P, Taddei C, Talevi R. 1984. The modifications of cortical endoplasmic reticulum during in vitro maturation of *Xenopus laevis* oocytes and its involvement in cortical granule exocytosis. *J Exp Zool* 229:283–293.
- Canman JC, Bement WM. 1997. Microtubules suppress actomyosin-based cortical flow in *Xenopus* oocytes. *J Cell Sci* 110:1907–1917.
- Charbonneau M, Grey RD. 1984. The onset of activation responsiveness during maturation coincides with the formation of the cortical endoplasmic reticulum in oocytes of *Xenopus laevis*. *Dev Biol* 102:90–97.
- Danilchik MV, Funk WC, Brown EE, Larkin K. 1998. Requirement for microtubules in new membrane formation during cytokinesis of *Xenopus* embryos. *Dev Biol* 194:47–60.
- Fisher GW, Rebhun LI. 1983. Sea urchin egg cortical granule exocytosis is followed by a burst of membrane retrieval via uptake into coated vesicles. *Dev Biol* 99:456–472.
- Grandin N, Charbonneau M. 1991. Intracellular pH and intracellular-free calcium responses to protein kinase C activators and inhibitors in *Xenopus* eggs. *Development* 112:461–470.
- Grey RD, Wolf DP, Hedrick JL. 1974. Formation and structure of the fertilization envelope in *Xenopus laevis*. *Dev Biol* 36:44–61.
- Heuser JE, Reese TS, Landis DM. 1974. Functional changes in frog neuromuscular junctions studied with freeze-frac-ture. *Neurocytol* 3:109–131.
- Opresko LK. 1991. Vitellogenin uptake and in vitro culture of oocytes. *Methods Cell Biol* 36:117–132.
- Peres A, Bernardini G. 1995. The effective membrane capacity of *Xenopus* eggs: its relations with membrane conductance and cortical granule exocytosis. *Pflugers Arch* 404:266–272.
- Swanson JA, Johnson MT, Beningo K, Post P, Mooseker M, Araki N. 1999. A contractile activity that closes phagosomes in macrophages. *J Cell Sci* 112:307–316.
- Thomas P, Lee AK, Wong JG, Almers W. 1994. A triggered mechanism retrieves membrane in seconds after Ca(2+)-stimulated exocytosis in single pituitary cells. *J Cell Biol* 124:667–675.
- Wall DA, Patel S. 1989. Multivesicular bodies play a key role in vitellogenin endocytosis by *Xenopus* oocytes. *Dev Biol* 119:275–289.
- Whalley T, Terasaki M, Cho MS, Vogel SS. 1995. Direct membrane retrieval into large vesicles after exocytosis in sea urchin eggs. *J Cell Biol* 131:1183–1192.
- Voigt H, Olivo JC, Sansonetti P, Guillen N. 1999. Myosin IB from *Entamoeba histolytica* is involved in phagocytosis of human erythrocytes. *J Cell Sci* 112:1191–1201.