

# Syntaxin, VAMP, and Rab3 are Selectively Expressed During Sea Urchin Embryogenesis

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**ABSTRACT** SNARE and rab protein family members were originally identified in terminally differentiated cell types. These proteins are phylogenetically conserved and while compelling evidence demonstrates their involvement in the secretory pathway, their exact function is debated. We recently identified SNARE protein family members in the sea urchin egg and provided evidence that rab3 functions in the exocytosis of cortical granules. Here we tested the hypothesis that these same proteins might also be present throughout embryogenesis to mediate membrane fusion events. We provide evidence that the sea urchin possesses a low complexity of gene family members of syntaxin, VAMP, and rab3 and that these proteins are not only present during development, but are enriched in regions of the embryo with active secretory roles. We found accumulation of each family member in the apical and basal aspects of cleaving blastomeres, indicative of bidirectional secretion into the extraembryonic environment and blastocoel. Elevated levels of syntaxin, VAMP, and rab3 were also found in the mesodermally derived pigment cells that invade and move within the ectoderm. These cells likely rely on SNARE and rab proteins to enable mobility by mediating the secretion of enzymes that break adhesion to neighboring cells and the extracellular matrix. In addition, these secretory proteins are enriched in the gut following gastrulation. Thus, we conclude that VAMP, syntaxin, and rab3 mediate a variety of secretory events that is important for development. *Mol. Reprod. Dev.* 58:22–29, 2001.

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lization envelope. This extracellular matrix forms the permanent block to polyspermy (Just, 1919) and is quickly followed by a continuous flow of secretory events that create a multilayered extraembryonic environment (Matese et al., 1997). Rapid cell division ensues in this embryo, necessitating the timely addition of membrane surface area (reviewed by Rappaport, 1996), the targeting of molecules to the plasma membrane to regulate cell adhesion to both the extracellular matrix (ECM) and neighboring cells, as well as the secretion of signaling molecules required for proper cell differentiation within the embryo (Wessel and Wikramanayake, 1999). As the embryo forms a blastula, vectorial transport of different cell surface and ECM molecules establishes distinct basal and apical cell polarity. For example, secretion basally leads to the deposition of ECM into the blastocoel, essential for cell migration and invagination (Wessel and Wikramanayake, 1999), while apical secretion of the hatching enzyme digests the fertilization envelope and allows the newly ciliated embryo to swim freely throughout its environment (Lepage and Gache, 1990). The embryo then begins reorganizing itself at gastrulation with the ingression of primary mesenchyme cells into the blastocoel and the invagination of the vegetal plate to establish the three primary germ layers (ecto-, meso-, endoderm). This rapid reorganization requires that links with neighboring cells and ECM be broken while adhesion to new substrates of the blastocoel be established (McClay et al., 1992), further adding to the spatio-temporal complexity of the secretory events and changes in cell surfaces required for proper embryogenesis. Several major morphological changes ensue that include formation of the larval skeleton, the result of extensive mesenchymal cell ECM secretions, and maturation of the gut with its associated secretion of digestive enzymes.

These fundamental processes of protein secretion, targeting of cell surface protein, and membrane addition are tightly regulated through the secretory path-

## INTRODUCTION

The rapidly changing embryo continually remodels its extracellular environment as well as its cell surfaces to initiate mechanisms of cell signaling, to establish cell polarity, and enable cell migration, and, ultimately, to differentiate. All of these processes must be integrally linked to the intracellular secretory pathway and be under tight spatio-temporal regulation for proper embryogenesis. Fertilization results in the first major secretory event of development with the exocytosis of cortical granule contents. In the sea urchin, this process modifies the vitelline layer of the egg and creates the ferti-

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way. Which proteins regulate the vesicular trafficking in the final steps of the cell secretory pathway? A convergence of studies that include the genetics of secretion in yeast (Ferro-Novick and Jahn, 1994), in vitro intra-Golgi trafficking in mammalian cells (Rothman and Sollner, 1997), and synaptic vesicle secretion in the vertebrate nervous system (Bock and Scheller, 1997) has led to the identification of two classes of molecules thought to be essential in regulating vesicle transport and the dynamics of membrane fusion (Hay and Scheller, 1997; Novick and Zerial, 1997). One class consists of integral membrane proteins localized to both the vesicle and target membranes, known as v- and t-SNAREs (soluble NSF-attachment protein [SNAP] receptors) respectively, that act to form the core vesicle docking and fusion machinery (Rothman and Sollner, 1997; Weber et al., 1998; Chen et al., 1999). Monomeric GTP-binding proteins known as rabs, belonging to the *ras* superfamily, make up the second class of proteins and are thought to regulate v-/t-SNARE interactions as well as the fusion event (Aridor and Balch, 1996; Johannes et al., 1996; Rybin et al., 1996; Geppert et al., 1997; Lupashin and Waters, 1997).

Recently, homologs belonging to both classes of molecules were identified in the sea urchin egg (Conner et al., 1997). The v-SNARE, VAMP/synaptobrevin, and the t-SNARE, syntaxin, were found enriched at the cortex of the unfertilized egg, present at the appropriate time and place to mediate cortical granule exocytosis (Conner et al., 1997). In addition, studies using a VAMP-specific protease (*Tetanus* toxin) have implicated a functional role for VAMP in this process (Bi et al., 1995; Avery et al., 1997). Likewise, a rab3 homolog was found associated with cortical granules (Conner et al., 1997) and inhibition of its function blocked cortical granule exocytosis suggesting that rab3 functions at a step following vesicle docking (Conner and Wessel, 1998). Although cortical granules are unique to eggs and oocytes, we postulated that these same proteins which regulate cortical granule exocytosis would also function in secretory processes essential for embryonic development. Here we report that VAMP, syntaxin, and rab3 are present throughout the development of the sea urchin embryo. They are enriched in cells known to be active in secretion like cells that produce the skeleton, and the cells of the developing gut. In addition, these molecules reveal significant secretory activity in pigment cells, consistent with their highly invasive behavior. Thus, the localization of these proteins indicates important developmental events that require secretion or changes in cell surface molecules.

## MATERIALS AND METHODS

### Animals and Reagents

Adult *Strongylocentrotus purpuratus* were obtained from Marinus (Long Beach, CA) and *Lytechinus variegatus* were obtained from Scott Services (Miami, FL). Gametes were obtained and fertilized as described

(McClay, 1986). Antibodies used in this study were generated as described (Conner et al., 1997).

### Electrophoresis and Immunoblot Analysis

Eggs, embryos, and cell fractions were subjected to SDS-PAGE and immunoblot analysis essentially as described (Towbin et al., 1979). Samples for analysis were pelleted, resuspended in SDS-PAGE sample buffer containing 10 mM DTT and a protease inhibitor cocktail (consisting of a final concentration per ml of aprotinin, 1 TIU; benzamidine, 10  $\mu$ g; soybean trypsin inhibitor, 10  $\mu$ g; antipain, 1  $\mu$ g; leupeptin, 1  $\mu$ g; bestatin, 0.5  $\mu$ g; E-64, 1  $\mu$ g; phosphoramidon, 1  $\mu$ g; phenylmethylsulfonyl fluoride, 10  $\mu$ g; chymostatin, 1  $\mu$ g; pepstatin, 1  $\mu$ g), and denatured for 3 min at 100°C. The proteins were resolved on an acrylamide gel and either stained with Coomassie Blue or transferred to nitrocellulose for immunolabeling as described (Towbin et al., 1979). For immunolabeling, blots were washed twice for a total of 1 hr in blotto buffer (50 mM Tris, pH 7.5, 0.9% NaCl, 0.05% Tween-20, and 3% nonfat dry milk) and then incubated for 1 hr in blotto containing diluted antibody (see Immunolocalization Assays In Situ, below). The blots were then washed three times in blotto over 30 min and incubated in blotto with goat anti-rabbit antibodies conjugated to alkaline phosphatase (Sigma, St. Louis, MO) diluted 32,000 $\times$ . The blots were washed in blotto three more times over 30 min, then washed in blotto without milk. Immunolabel signals were detected by BCIP/NBT colorimetric development as described (Harlow and Lane, 1988; Promega Corporation, Madison, WI). Control immunoblots using preimmune antisera and secondary antibody alone show no signal or cross-reactivity (data not shown).

### Immunolocalization Assays In Situ

Immunofluorescence localization was performed on sections of embryos (fertilized eggs to the gastrula stage) and in whole mounts (pluteus stage embryos) that were fixed and processed as previously described (Laidlaw and Wessel, 1994). Primary antibodies were diluted between 1/50 (~20  $\mu$ g/ml) and 1/10,000 (~0.1  $\mu$ g/ml), while the secondary antibody (Cy3-conjugated affinity-purified goat anti-rabbit IgG; Kirkegaard & Perry Labs, Gaithersburg, MD) was diluted 1/100. In some cases, embryos were treated to remove hyalin, which sometimes caused non-selective binding of antibodies, prior to fixation. In these cases, embryos were treated with hyalin-extraction media as described (McClay, 1986). Control experiments for these immunolabeling protocols include use of normal rabbit serum, irrelevant immune serum, and competition of the immune serum with the original fusion protein used to generate the antibody. Each of these experiments failed to show any significant immunolabel. For double immunolocalization experiments using the pigment cell-specific monoclonal antibody Sp1/20.3.1 (Gibson and Burke, 1985), secondary antibodies included FITC-conjugated affinity-purified goat anti-mouse IgG and rhodamine-conjugated affinity-purified goat anti-

rabbit IgG that were both diluted 1/100 (Kirkegaard & Perry Labs, Gaithersburg, MD). Controls for double immunolocalization experiments with two different secondary antibodies showed no cross-reactivity with sea urchin proteins and species specificity of the secondary antibody. All immunofluorescent signals were recorded by confocal microscopy with a Zeiss LSM 410.

### In Situ RNA Hybridization

Ovaries, eggs, and embryos from *L. variegatus* were fixed in 2% glutaraldehyde and were prepared for the whole mount in situ RNA hybridization as described (Ransick et al., 1993). Template cDNAs used in these experiments were as described (Conner et al., 1997). Digoxigenin-labeled antisense transcripts were synthesized by first linearizing each plasmid with *Bam*HI and then transcribing the template using T7 RNA polymerase. A sense probe was synthesized by linearizing each plasmid with *Hind*III and then transcribing the template using T3 RNA polymerase (enzymes from Life Technologies, Gaithersburg, MD). Antisense or sense strand probe was then hybridized to target embryos overnight at  $T_m - 5^\circ\text{C}$ . The unbound probe was washed as described (Ransick et al., 1993) and the bound probe was detected with an anti-digoxigenin antibody conjugated to alkaline phosphatase (Boehringer Mannheim, Indianapolis, IN). Images were recorded with a Spot Camera (Diagnostic Instruments, Sterling Heights, MI) attached to a Zeiss Axioplan.

### Genomic DNA Gel Blot Hybridizations

Genomic DNA was isolated from the sperm as described (Hendrickson et al., 1988) and was digested with either *Bam*HI, *Eco*RI, *Hind*III, *Xba*I, *Xho*I. Ten micrograms of each digest was then loaded onto a 0.8% agarose gel and the DNA fragments were resolved, blotted, and hybridized as described (Xiang et al., 1991). Hybridization conditions of low stringency (allowing 30% mismatch) were used to detect family members with divergent sequences. The probes used here for hybridization were identical to the previously isolated cDNAs for sea urchin rab3, syntaxin, and VAMP (Conner et al., 1997).

## RESULTS

### The Sea Urchin Possesses a Low Complexity of Gene Family Members for VAMP, Syntaxin, and Rab3

Previously we identified cDNAs that encoded members of the SNARE complex (Conner et al., 1997). In extensive PCR and low stringency hybridization screens for cDNAs encoding VAMP and syntaxin, as well as for the GTPase, rab3, we were able to identify clones encoding only a single member of each family. To determine the complexity of these gene families in this animal, we probed genomic DNA gel blots using low stringency conditions to detect potentially divergent family members. Examination of genomic DNA isolated from four males, analyzed with five different restriction

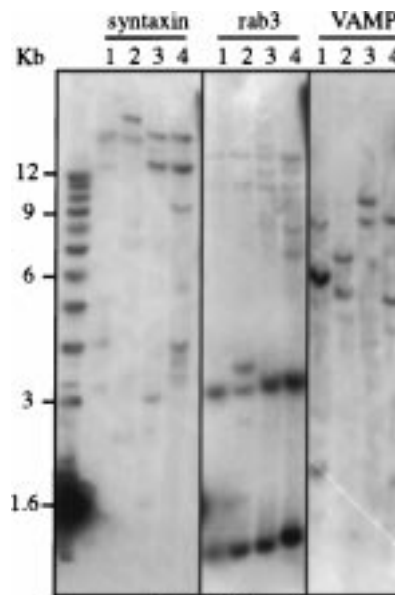
enzymes (one representative shown), suggests that the sea urchin *Strongylocentrotus purpuratus* possesses single VAMP and rab3 genes and at most two syntaxin genes (Fig. 1). Were other representatives present in the genome, their sequences would be expected to be greater than 30% divergent, and likely not detectable by our immunological and RNA analysis approaches.

### SNARE Proteins are Present in the Developing Embryo

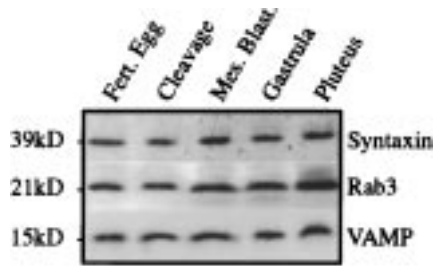
To test if syntaxin, VAMP, and rab3 are present in the developing sea urchin, we used antibodies generated against bacterially expressed recombinant sea urchin proteins (Conner et al., 1997). Immunoblot analysis indicates that all three proteins are present at all stages in the developing embryo and are of an appropriate size for each family member: syntaxin, 39 kDa, rab3, 21 kDa, and VAMP, 15 kDa (Fig. 2). Furthermore, each protein accumulates to roughly equal levels throughout development.

### Cells With Active Secretory Roles Have Enriched VAMP, Syntaxin, and Rab3 Expression

Since immunoblot analysis indicated that syntaxin, VAMP, and rab3 are present throughout development, immunolocalizations were performed in whole-mount and thick-sectioned embryos to test the hypothesis that enriched expression of these molecules would coincide with regions of the embryo with active secretory roles. Immunolocalization in the newly fertilized egg indi-



**Fig. 1.** Genomic DNA gel blot of 10  $\mu\text{g}$  of DNA from four different males (1–4), digested with *Hind*III (rab3 and syntaxin) and *Bam*HI (VAMP). Comparable results are obtained for syntaxin, rab3, or VAMP when DNA is digested with other restriction enzymes including *Bam*HI, *Eco*RI, and *Hind*III (data not shown). The low complexity of bands seen, even under low stringency hybridization, is indicative of 1 or 2 genes present in the genome.

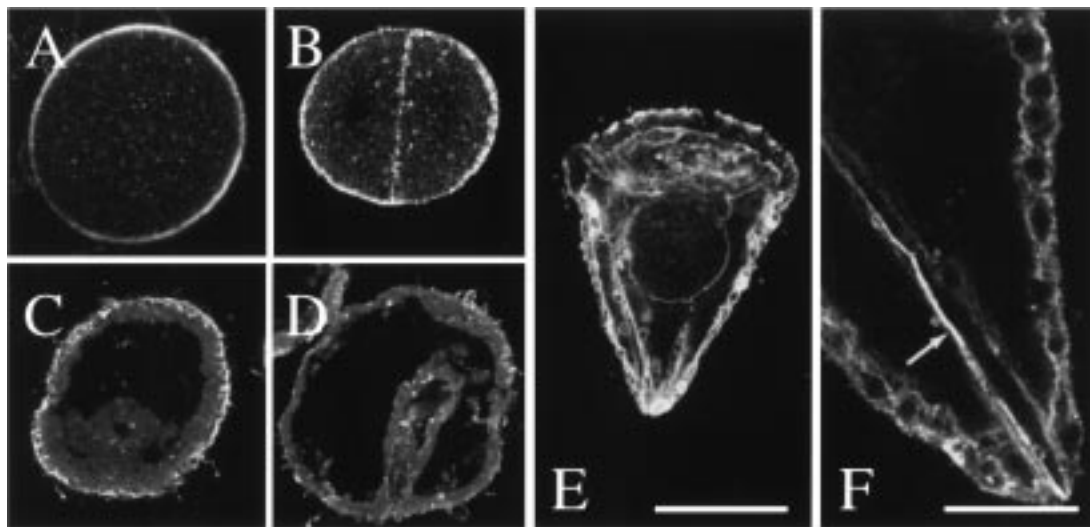


**Fig. 2.** Immunoblot analysis using antibodies generated against sea urchin syntaxin, rab3, and VAMP indicates that they are present at similar levels throughout the development of the embryo.

indicates that syntaxin and VAMP localize to the cortex (Figs. 3A and 5A), while rab3 is found associated with vesicles at the zygote cortex and in the cytoplasm (Fig. 4A), some of which are known to be endocytic in origin (Conner and Wessel, 1998). As the embryo begins to divide, we find syntaxin, VAMP, and rab3 associated with vesicles surrounding the cortex of dividing cells (Figs. 3B/4B/5B). In fertilized eggs and early embryos, label is also seen in the fertilization envelope which we believe to be true SNARE labeling. Although the fertilization envelope is not composed of a lipid bilayer, we find FM1-43 (a membrane impermeant lipophilic fluorescent dye) labeling in the forming fertilization envelope that results from tiny plasma membrane attachments (with associated SNAREs) that break off and remain associated with the vitelline layer as it lifts from the plasma membrane (S. Conner, M. Terasaki, and G. Wessel, in preparation). These membrane frag-

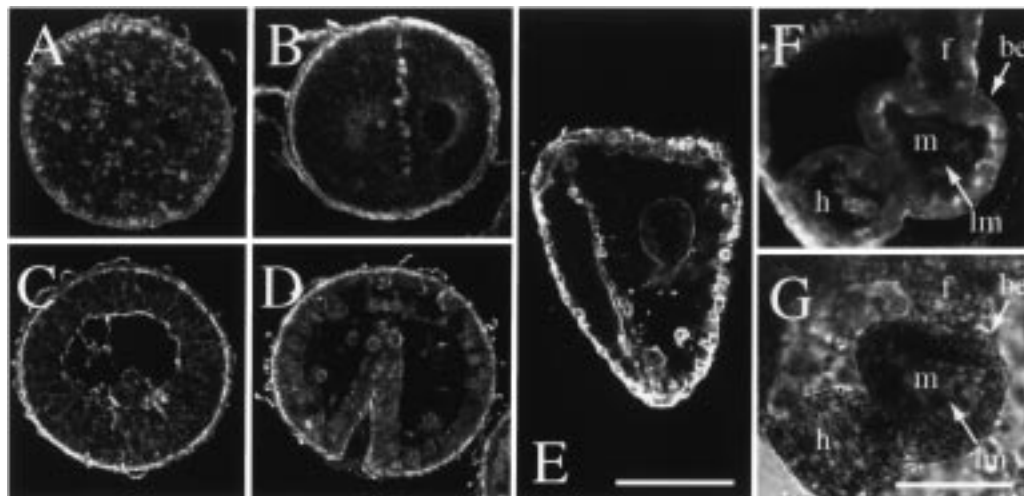
ments contain SNAREs and retain the immunolabel, though now in the envelope. At the blastula stage when primary mesenchyme cells begin ingressing into the blastocoel, all three molecules are enriched at the apical surface of epithelial cells and in cells at the vegetal plate, including the ingressing primary mesenchyme cells (Figs. 3C/4C/5C). With the establishment of the three primary germ layers during gastrulation, all three proteins are enriched in the apical aspect of both the ectoderm and the endoderm of the invaginating gut; however, the immunolabel in the basal region of these cells is lost (Figs. 3D/4D/5D) perhaps reflecting a decreased level of ECM secretion in the blastocoel. In the feeding larvae, these proteins are enriched in cells of the ectoderm, cells along the growing spicule, and in cells of the gut (Figs. 3E/4E,F/5E,F). Thus, VAMP, syntaxin, and rab3 co-localize with cell tissues known to be active in secretion.

Unexpectedly, we also found an enrichment of VAMP, syntaxin, and rab3 in developing pigment cells. Pigment cells are derived from secondary mesenchyme, invade the ectoderm, and are apparent in the larval stage by the accumulation of echinochrome (see Giudice, 1986). To identify pigment cells prior to their pigment accumulation, we used a monoclonal antibody specific for pigment cells (Gibson and Burke, 1985) and performed a co-immunolocalization experiment with either VAMP, syntaxin, or rab3. The result is that all three proteins co-localize with pigment cells (Fig. 6, shown with VAMP). We also note that each of these proteins (VAMP, rab3, syntaxin, and the pigment cell epitope) accumulate in pigment cells well prior to the pigment (compare arrows in Fig. 6); however, the immunolabel



**Fig. 3.** Syntaxin is enriched in regions of the embryo with active secretory roles. Syntaxin is enriched at the plasma membrane of the fertilized egg (A) and immunolocalizes to vesicles at the apical plasma membrane and the cleavage plane in the dividing embryo (B). Syntaxin is enriched at the apical surface of ectoderm cells and in vegetal plate cells of the mesenchyme blastula (C), while the gastrula shows enrichment at the apical surface of cells in the developing gut (D). In

the pluteus, syntaxin is enriched in the ectoderm, the gut, and the cells lining the developing skeleton (E). Greater magnification shows syntaxin enrichment along the skeleton (F). All immunofluorescent images (Figs. 3–5) were recorded by confocal microscopy with a Zeiss LSM 410. Embryos shown in C and D were treated to remove hyalin prior to fixation. bar, 50  $\mu$ m (A–E), 25  $\mu$ m (F).



**Fig. 4.** Rab3 is present throughout the development of the sea urchin embryo. In the fertilized egg, rab3 is found on vesicles at the cortex and in the cytoplasm (A) and associates with vesicles at the cleavage plane in the dividing embryo (B). In the mesenchyme blastula, rab3 is enriched in vegetal plate cells and at the apical surface of ectodermal cells (C), while in the gastrula rab3 also localizes to the apical surface of the endoderm of the forming gut (D). The ectoderm,

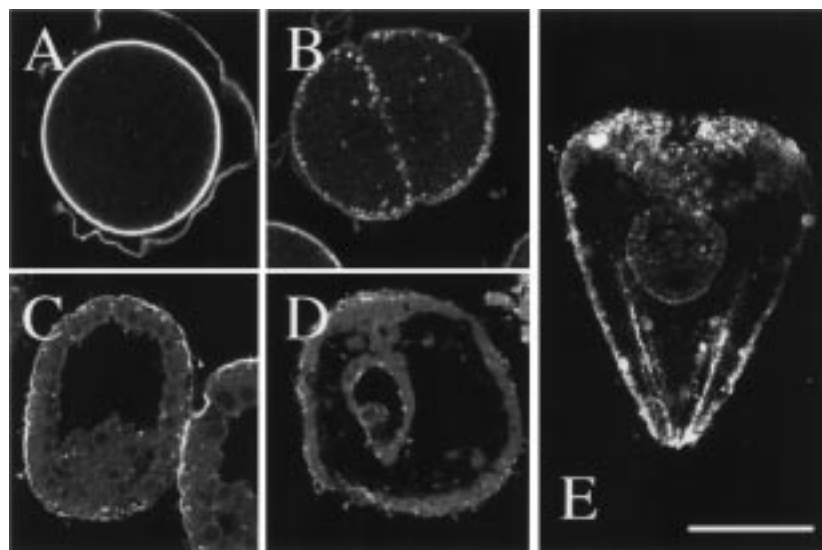
is dramatically decreased in cells that have accumulated pigment (Fig. 6 arrowhead).

#### Differential mRNA Accumulation in the Embryo

RNA hybridizations in situ to identify syntaxin, VAMP, and rab3 mRNA suggest that the transcriptional activity of these genes changes during embryogenesis. mRNA encoding VAMP (Fig. 7A), syntaxin, and rab3 (data not shown) is highly enriched in eggs

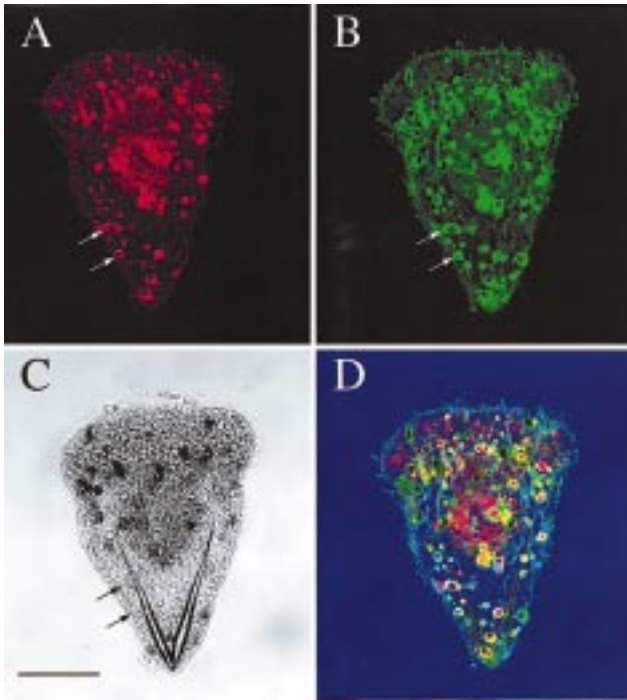
and oocytes, though no signal is detectable in the somatic tissues of the ovary (Fig. 7A). In cleavage stage embryos, mRNA levels for all three proteins are low with a uniform distribution. In contrast, the elevated hybridization signal is seen later in development for VAMP and syntaxin, especially in mesodermal cells and the developing and mature gut (Fig. 7B–D). Rab3 mRNA expression is similar to that of both VAMP and syntaxin; however, mesodermal cells show more significant levels of rab3 mRNA. Rab3 mRNA is found in

gut, and cells lining the skeleton of the pluteus are enriched with rab3 (E). Higher magnification of the gut shows rab3 at both the basal and apical regions of the endoderm (F) and for comparison, the brightfield image is shown (G). Fluorescent images visualized by confocal microscopy. h, hindgut; m, midgut; f, foregut; lm, lumen; be, basal endoderm; bar, 50  $\mu$ m (A–E), 25  $\mu$ m (F,G).



**Fig. 5.** Immunolocalization of VAMP indicates that it is present in the sea urchin embryo and enriched in regions of the embryo where higher levels of secretion are anticipated. Following fertilization, VAMP is enriched at the cortex of the zygote (A), and as the embryo begins to divide, VAMP localizes to vesicles at both the cortex and cleavage plane (B). VAMP immunolabel is observed at the apical cortex of cells at the mesenchyme blastula stage and, during gas-

trulation, the apical regions of both ectoderm and endoderm of the forming gut show enriched VAMP immunolabel (D). The feeding larva shows VAMP enrichment in the gut, the ectoderm, and the cells along the developing skeleton (E). Fluorescent images visualized by confocal microscopy and embryos shown in C and D were treated to remove hyalin prior to fixation. bar, 50  $\mu$ m.

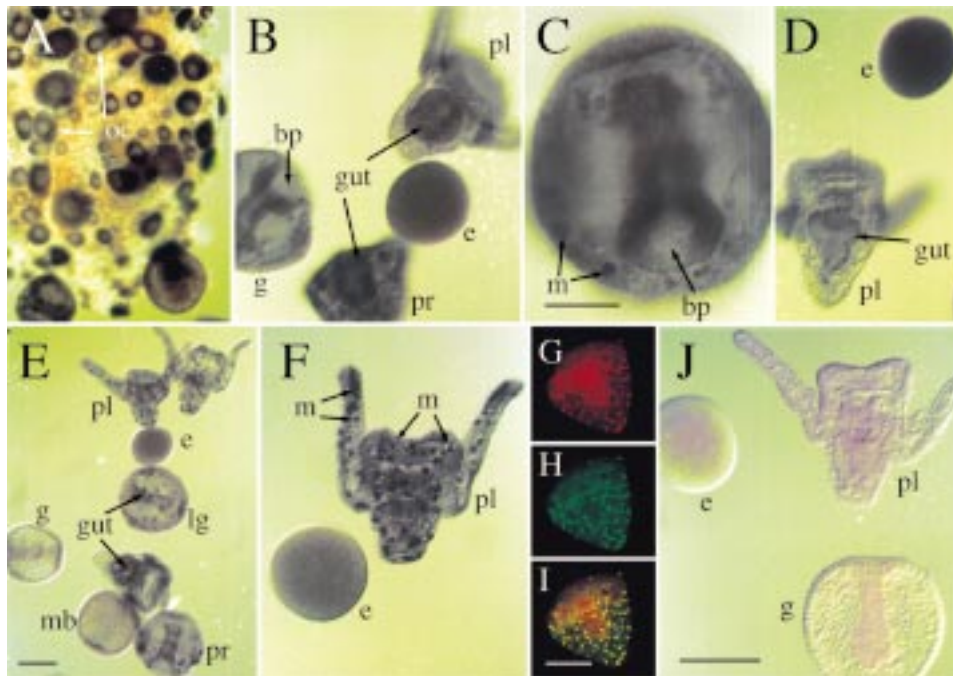


**Fig. 6.** VAMP co-localization with pigment cells in sea urchin larva. Representative of syntaxin and rab3, VAMP immunolocalization (A), immunolocalization of mAb that is specific for pigment cells (B), with the corresponding brightfield image with dark spots indicative of pigment cells (C). Overlay of the three images indicates that VAMP co-localizes with pigment cells (D). Arrows indicate cells which have not accumulated pigment, but are positive for the pigment cell-specific marker and VAMP, while arrowheads indicate cells that have accumulated pigment but have decreased VAMP immunolabel. These images are representative of syntaxin and rab3. bar, 50  $\mu$ m.

cells of the gut of the early gastrula (Fig. 7E), and mesodermal cells do not display significant RNA hybridization until late in gastrulation (Fig. 7E), coincident with the appearance of pigment cells, and it remains at high levels in the larval stage (Fig. 7F). It is likely that at least some of the mesodermal cells that show enriched rab3 mRNA give rise to pigment cells, and rab3 coimmunolocalization with pigment cells is shown (Fig. 7G–I). A representative sense strand hybridization control is shown indicating background nonspecific RNA hybridization levels (Fig. 7J).

### DISCUSSION

Are SNARE and rab3 protein family members required for development? Syntaxin mRNA is expressed



**Fig. 7.** In situ RNA hybridizations show differential gene expression for VAMP, syntaxin, and rab3. Representative of syntaxin and rab3, VAMP mRNA is highly enriched in developing oocytes (oc) and eggs (e) as compared to the somatic tissue of the ovary (A). VAMP and syntaxin share nearly identical mRNA expression patterns, both displaying label around the blastopore (bp), in the developing gut of the gastrula (g) and prism stage (pr) embryos, the mature gut of the pluteus (pl), and in mesodermal (m) cells (B/C, VAMP; D, syntaxin). Note that there is an asymmetric accumulation of the label around the

blastopore, apparent in B and C. Rab3 mRNA expression mimics that of both syntaxin and VAMP; however, enriched mesodermal cell expression is observed beginning with late gastrula (lg) to pluteus, coincident with the differentiation of pigment cells, but not seen in mesenchyme blastula (mb) or earlier gastrula (E,F). Immunolocalization of a mAb that identifies pigment cells (G), rab3 (H), and their colocalization (I). Representative image of a sense control hybridization (J). Images were recorded with a Spot Camera attached to a Zeiss Axioplan. bar, 100  $\mu$ m (A,E), 75  $\mu$ m (B,D,F,J), 25  $\mu$ m (C), and 50  $\mu$ m (G–I).

during all stages of *Drosophila* development (Schulze and Bellen, 1996) and mutagenesis studies demonstrate that syntaxin null mutants are lethal. Germ line mosaic mutants also show defects in cellularization following the syncytial blastoderm stage (Burgess et al., 1997). In *Arabidopsis*, a cytokinesis-specific syntaxin, KNOLLE, localizes to the plane of cell division and when mutated, cell division is impaired (Lauber et al., 1997). Moreover, we recently found that specific inactivation of syntaxin with antibodies or neurotoxin in early sea urchin embryos causes cessation of cell division (Conner and Wessel, 1999). Together, these results provide evidence that syntaxin-mediated membrane trafficking is required for the rapid cell divisions of early development. Similarly, synaptobrevin/VAMP mRNA is found very early in *Drosophila* development at times well before neurogenesis and is highly enriched in cells of the gut and malpighian tubules (Chin et al., 1993), cell types predicted to have high secretory levels. Here we find that sea urchin syntaxin and VAMP share similar expression patterns throughout embryogenesis, with elevated levels in the apical region of most cells with enrichment in the nascent and feeding gut, as well as in cells aligning the skeleton. Although a direct functional role for VAMP has not been tested during sea urchin embryogenesis, cumulatively, these results argue that SNARE protein family members play an important role in membrane trafficking of developing embryos across phylogeny, and especially across those regions and times with specialized secretory roles and rapid cell division.

Rabs are the largest group of monomeric GTP-binding proteins of the *ras* superfamily (see Zerial and Huber, 1995) and while rab family members have been shown to play an important role in vesicle trafficking throughout the secretory pathway in terminally differentiated cell types, it is unclear if they function in regulating fusion events in development. Rab3 family members are believed to function in the final steps of vesicle transport and fusion (Bean and Scheller, 1997), though their expression is not ubiquitous in vertebrates. Instead rab3 is enriched in cells with highly specialized secretory roles like the neuron (Fischer von Mollard et al., 1990). Recently, we have found a developmental requirement for rab3 in the sea urchin embryo: injection of antibodies against rab3 or rab3 effector domain peptides into single cells resulted in cell cycle inhibition (Conner and Wessel, 2000), arguing that rab3 plays an essential role in regulating membrane fusion events during cell division. Here we find that sea urchin rab3, like that of syntaxin and VAMP, is present in many cell types of the developing embryo and is highly enriched in cells aligning the growing skeleton and maturing gut. Thus we believe that rab3 plays a functional role in secretion in these cells. However, recently rab5 which is known to function in vesicle docking and fusion during endocytosis in mammalian fibroblasts, was also shown to modulate rac-independent lamellipodia formation and cell migration (Spaargaren and Bos, 1999). This raises the possibility

that other rab family members, including rab3, may have functions in addition to membrane trafficking. Surprisingly, inactivation of the rab3A gene in mice by targeted gene recombination results in viable offspring that only show defects in the ability of their neurons to respond to repetitive stimulation (Castillo et al., 1997; Geppert et al., 1997). Since vertebrates possess multiple rab3 isoforms (rab3 A–D), it is possible that another rab3 isoform is functionally redundant to retain viability. In contrast, the sea urchin appears to have a single rab3 family member homolog which likely functions to regulate secretory vesicle fusion events in both terminally differentiated cells as well as many cell types during development.

Both from our original cDNA screens (Conner et al., 1997) and from the genomic DNA gel blots presented here, we believe that the sea urchin has a low complexity of genes for rab3, VAMP, and syntaxin with one or at most two different isoforms. Since humans and rodents possess multiple isoforms of each protein, these single members in the sea urchin must have either broader functional capabilities or an increased number of regulatory players to direct specific membrane fusion events. This is compatible with what is seen in *Drosophila* which also possesses a single syntaxin gene. However, since syntaxin isoforms can be up to 70% divergent in sequence, it is possible that the sea urchin has other genes that encode molecules that function as a syntaxin, but whose sequence is too divergent for us to detect. With the apparent singular dependence of SNARE and rab3 members shown here, we have an ability to target inactivation without redundancy to examine their functional contribution to development.

Although we do not know the function of the SNARE/rab positive vesicles shown here in the embryos, several roles are possible. The intense labeling in the apical regions of cells in early embryos could, for example, indicate vesicles that secrete components of the extra-embryonic matrix—the fibropellins (Burke et al., 1991) and hyalin (Wessel et al., 1998). The labeling in the basal aspect would likely indicate secretion of the extracellular matrix in forming and remodeling the basal lamina and blastocoel. However, another consideration of these SNARE/rab3-positive vesicles is the membrane proteins that they are likely to deliver to the cell surface. Already these vesicles are postulated to bear membrane molecules needed in cell division (Conner and Wessel, 1999) but with the rapidly changing cell surface of this embryo (Wessel and Wikramanayake, 1999), one could also envision a plethora of receptors, adhesion molecules, and ligands carried with the membrane of these vesicles that would function in development.

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