We recently identified a single family member homologue of syntaxin in the sea urchin. Syntaxin is present throughout development, and in rapidly dividing cleavage stage embryos it is present on numerous vesicles at the cell cortex. We hypothesized that syntaxin mediates essential membrane fusion events during early embryogenesis, reasoning that the vesicles and/or their contents are important for development. Here we show that functional inactivation of syntaxin with either *Botulinum* neurotoxin C1, which specifically proteolyzes syntaxin, or antibodies against syntaxin results in an inhibition of cell division. These observations suggest that syntaxin is essential for membrane fusion events critical for cell division.

**INTRODUCTION**

Cell division is a highly coordinated event requiring a variety of membrane fusion and fragmentation events. During mitosis in higher eukaryotes, for example, the nuclear envelope breaks down into nuclear membrane vesicles after chromosome condensation, and large cytoplasmic organelles such as the Golgi and endoplasmic reticulum (ER) are also believed to fragment (Lucocq and Warren, 1987; Warren, 1989). These fragmented organelle membranes then distribute equally into daughter cells and must reassemble with each other to reconstitute their respective organelles. In addition to the breakdown and reformation of the nuclear envelope, Golgi, and ER during the cell cycle, the cell also increases its membrane surface area during cell division (for review, see Rappaport, 1996).

What proteins mediate these essential membrane fusion events during cell division? A highly conserved set of membrane proteins have been identified that are involved in many types of intracellular fusion (Rothman, 1994; Sudhof, 1995). These proteins localize to both vesicle and target membranes, known as v- and t-soluble NSF attachment protein (SNAP) receptors (SNAREs), respectively, and appear to function throughout the secretory pathway as the minimal machinery driving membrane fusion (Fasshauer et al., 1998; Weber et al., 1998). Recently, single family member homologues of syntaxin (t-SNARE), vesicle-associated membrane protein (VAMP; v-SNARE), and the monomeric GTP-binding protein rab3 were identified in the sea urchin egg in association with cortical granules, secretory vesicles whose contents give rise to the fertilization envelope (Conner et al., 1997). Syntaxin, VAMP, and rab3 are also present throughout embryogenesis enriched in cells with elevated levels of regulated secretion (Conner and Wessel, manuscript in preparation). During the cleavage stage of this embryo, a period of cell division every 45–60 min, we find enrichment of these molecules on vesicles accumulating at the cortex of cells, suggesting that these vesicles may play an important role in cell division. Thus, we hypothesized that these proteins not only mediate the complex array of membrane fusion events of secretion, as previously documented (for review, see Ferro-Novick and Jahn, 1994; Bock and Scheller, 1997; Rothman and Sollner, 1997), but also function in the contribution of new membrane to the cell surface during division. Using the sea urchin embryo, which has a single detectable syntaxin homologue in early embryos, we test this hypothesis by inactivating syntaxin with the microinjection of *Botulinum* neurotoxin C1, which specifically proteolyzes syntaxin family members (Blasi et al., 1994; Schiavo et al., 1995; Walch-Solimena et al., 1995), and affinity-purified antibodies against syntaxin. We find that disruption of syntaxin inhibits cell division, whereas cells injected with toxin or antibodies that have been heat inactivated develop as normal. Thus, we conclude that functional syntaxin is required to mediate membrane fusion events during cell division. This further suggests that the molecular models that describe protein-mediated membrane fusion events for regulated exocytosis are applicable to membrane fusion events required for basic processes of cell division.

**MATERIALS AND METHODS**

**Animals**

Adult *Lytechinus variegatus* were obtained from Scott Services (Miami, FL) and Mele Enterprises (Duke University Marine Lab, Beaufort, NC). Gametes were obtained as described (McClay, 1986).

**Antibody Purification**

To affinity purify Fab fragment antibodies against syntaxin, a syntaxin-GST fusion protein was made using a nucleotide sequence
representing amino acids 1–265 (MRDL...KKFY) of the syntaxin cDNA clone (Conner et al., 1997) ligated into a pGEX-3A vector for fusion with GST and transformed into BL21(DE3) cells for overexpression. Syntaxin-GST fusion protein-expressing BL21(DE3) cells were induced at 23°C with 0.1 mM isopropyl-β-D-thiogalactopyranoside for 3 h. Cells were then pelleted by centrifugation at 4000 rpm for 10 min, resuspended in PBS, lysed with high pressure using a French press, and solubilized with 1% Triton X-100 for 30 min. Cellular debris was then pelleted at 10,000 g at 4°C for 20 min. The resulting supernatant was passed over a glutathione-agarose column (Sigma, St. Louis, MO), and the column was then washed with 10 column volumes of PBS. Syntaxin-GST fusion protein was specifically eluted with PBS containing 10 mM reduced glutathione (Sigma), and the purity of column eluant syntaxin-GST protein was verified by SDS-PAGE and immunoblot analysis. Affinity-purified syntaxin-GST protein was blotted to nitrocellulose in PBS and then blocked with preimmune sera for 10 min. The blot was then washed with PBS and incubated for 30 mins with syntaxin Fab fragment antiserum obtained using the Immunopure Fab preparation kit (Pierce, Rockford, IL), which previously had been conjugated to Oregon Green 488 using the FluoReporter labeling kit (Molecular Probes, Eugene, OR). The blots were then washed again with PBS, and the Oregon Green-labeled Fab fragment antibodies were eluted from the nitrocellulose with 100 mM glycine, pH 2.5, dialyzed extensively against PBS, and concentrated to 2 mg/ml using Ultrafree-4 centrifugal filters with a 10-kDa cutoff (Millipore, Bedford, MA). Protein concentration was determined using the Bradford method using BSA as a standard. Affinity-purified Fab fragment antibodies labeled with Oregon Green were tested by immunolocalization in thick sections of eggs (see below).

**Injections**

Eggs were fertilized and placed into a Kiehart chamber (Kiehart, 1982) in artificial seawater (ASW) (McCay, 1986). Fertilized eggs or a single blastomere of a two-cell-stage embryo was microinjected with various reagents. Botulimum neurotoxins A, C1, and E (BoNT-A, -C1, and -E; Wako Bioproducts, Richmond, VA) stock injection solutions were 1 mg/ml toxin in 200 mM NaCl and 50 mM sodium acetate, pH 6.0. BoNT-C1 was heat inactivated by incubation of the stock injection solution at 100°C for 10 min. BoNT-E was activated with 200 μg/ml trypsin at 37°C for 30 min. Trypsin was removed, selectively, by incubation with soybean trypsin inhibitor conjugated to agarose beads (Sigma) for 30 min at room temperature, trypsin-bound beads were then removed by centrifugation, and the supernatant was used subsequent to microinjection into cells for membrane labeling. The volume of oil containing DiOC₆(3) did not exceed 5% of the cell volume. FM1-43 (Molecular Probes) was resuspended in methanol at 1 μg/ml. It was then diluted in ASW to give a working concentration of 1 μM. To evaluate endocytosis, experimentally manipulated embryos were transferred to the FM1-43 in ASW and visualized after 15–45 min incubation at room temperature using confocal microscopy with a Zeiss LSM 410 microscope.

**Membrane Topology and Endocytosis**

3,3'-dihexyloxacarbocyanine iodide [DiOC₆(3)] (Molecular Probes) was resuspended in methanol at 1 mg/ml and then transferred to Hollywood safflower oil (Big Daddy Wesley’s, Beaufort, NC) by mixing 500 μl of the methanol/DiOC₆(3) solution with the 500 μl of safflower oil. DiOC₆(3) resuspended in safflower oil was then used for microinjection into cells for membrane labeling. The volume of oil containing DiOC₆(3) did not exceed 5% of the cell volume. FM1-43 (Molecular Probes) was resuspended in methanol at 1 mg/ml. It was then diluted in ASW to give a working concentration of 1 μM. To evaluate endocytosis, experimentally manipulated embryos were transferred to the FM3-43 in ASW and visualized after 15–45 min incubation at room temperature using confocal microscopy using a Zeiss LSM 410 microscope.

**Brefeldin A Treatment**

Eggs were fertilized in ASW and after 10 min were transferred to ASW containing brefeldin A (BFA; Calbiochem, La Jolla, CA) at the indicated concentrations (stock solution was 4 mg/ml in methanol) or ASW containing identical concentrations of methanol as that of the BFA-treated embryos as a control. The methanol in the ASW of the experimental and control samples was given 30 min at room temperature to evaporate before embryo transfer.

**RESULTS**

**Syntaxin Is Present in the Dividing Sea Urchin Embryo**

Because only a single sea urchin syntaxin family member is detectable throughout sea urchin embryogenesis, we asked whether syntaxin localizes in vivo to a distinct intracellular compartment of the secretory pathway like other syntaxin family members in a variety of other systems (Bennett et al., 1993; Dascher et al., 1994; Bock et al., 1997). By microinjection of detection levels (~200 nM, noninhibitory) of fluoro-
chrome-labeled affinity-purified antibodies against sea urchin syntaxin, we find syntaxin on intracellular vesicles enriched at the cell cortex of the fertilized egg (A and B) and cells of the dividing embryo (C–F). By increasing antibody injection 10-fold (−2 μM), syntaxin is found in association with ER, as seen in the four-cell-stage embryo (G and H). Immunolocalization in fixed sections confirms the pattern observed in vivo with syntaxin on vesicles at the cortex and in association with ER (I–K); however the syntaxin epitope at the cell cortex appears more accessible in fixed tissue, suggesting in vivo masking of the syntaxin epitope. Images visualized by indirect immunofluorescence using confocal microscopy. Oil droplets mark embryos injected with fluorochrome-labeled antibody. Bar, 50 μm.

Botulinum Neurotoxin C1 Blocks Cell Division in a Concentration-dependent Manner

To test the function of syntaxin during cell division, we microinjected BoNT-C1 into single cells to specifically inactive syntaxin by releasing the functional protein binding domains. cDNA sequence analysis and in vitro cleavage results indicate that the single sea urchin syntaxin family member contains the neurotoxin protease cleavage site (Schiavo et al., 1995; Conner et al., 1997; Coorssen et al., 1997). We find that both cytokinesis and karyokinesis are blocked in 22% of cells injected with 1.6 nM BoNT-C1 within one cell cycle after injection, whereas cell division is inhibited in 100% of cells injected with ≥5 nM BoNT-C1 (Figure 2, A–C). Maximal BoNT-C1 activity is supported at 37°C; however, these embryos were incubated at 23°C to retain maximal viability. Cells injected with either BoNT-A (45 nM; J–L) or BoNT-E (58 nM; M–O), which specifically proteolyze SNAP-25, also divide as normal. Injected cells are marked with an oil droplet. Bar, 50 μm.

Figure 1. In vivo immunolocalization of syntaxin by injecting detection levels of fluorochrome-labeled polyclonal antibodies (~200 nM) reveals syntaxin association with vesicles enriched at the cell cortex of the fertilized egg (A and B) and cells of the dividing embryo (C–F). By increasing antibody injection 10-fold (~2 μM), syntaxin is found in association with ER, as seen in the four-cell-stage embryo (G and H). Immunolocalization in fixed sections confirms the pattern observed in vivo with syntaxin on vesicles at the cortex and in association with ER (I–K); however the syntaxin epitope at the cell cortex appears more accessible in fixed tissue, suggesting in vivo masking of the syntaxin epitope. Images visualized by indirect immunofluorescence using confocal microscopy. Oil droplets mark embryos injected with fluorochrome-labeled antibody. Bar, 50 μm.

Figure 2. BoNT-C1, which specifically cleaves syntaxin, inhibits cell division in the sea urchin embryo. Injection of active BoNT-C1 blocks cells from dividing (5.3 nM; A–C), whereas uninjected cells or cells injected with either heat-inactivated BoNT-C1 (53 nM; G–I) or proteinase K (493 nM; G–I) develop normally. Cells injected with either BoNT-A (45 nM; J–L) or BoNT-E (58 nM; M–O), which specifically proteolyze SNAP-25, also divide as normal. Injected cells are marked with an oil droplet. Bar, 50 μm.
Table 1. BoNT-C1 inhibits cell division in a concentration dependent fashion

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Concentration (nM)</th>
<th>% cleavage</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>BoNT-A</td>
<td>5–45</td>
<td>7</td>
</tr>
<tr>
<td>BoNT-E</td>
<td>27–58</td>
<td>8</td>
</tr>
<tr>
<td>Inactivated BoNT-C1</td>
<td>5.3</td>
<td>5</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>493</td>
<td>5</td>
</tr>
<tr>
<td>BoNT-C1</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3.9</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>5.3</td>
<td>21</td>
</tr>
</tbody>
</table>

Blastomeres injected with a general proteinase, proteinase K, or heat-inactivated BoNT-C1 have no affects on cell division. However, as concentrations of BoNT-C1 are increased to ≥1.6 nM, we begin to see an inhibition in cell division, whereas uninjected cells divide as normal. Single blastomeres were injected at the two-cell stage, and cell division was assayed before the 16-cell stage.

BoNT-C1 has also been shown to proteolyze SNAP-25 (Foran et al., 1996; Williamson et al., 1996); thus to test the possibility that the BoNT-C1-induced phenotypes seen here result from the proteolysis of both syntaxin and SNAP-25, we injected either BoNT-A or -E, proteases that specifically target SNAP-25, into single cells of two-cell-stage embryos. We find that injection of either BoNT-A or -E has no affect on cell division at concentrations greater than that required by BoNT-C1 to block cell division (Table 1 and Figure 2). These observations suggest that the BoNT-C1-induced block in cell division is the result of the syntaxin-targeted toxin activity.

BoNT-C1 blocks synaptic vesicle fusion in the neuron by cleaving syntaxin, resulting in an accumulation of synaptic vesicles at the active zone of the synapse (Marsal et al., 1997; O’Connor et al., 1997). However, we suspected that in the rapidly dividing sea urchin embryo with a single syntaxin homologue that injection of BoNT-C1 could result in major changes in membrane topology of the cell that would lead to the block in cell division. To test this possibility, we injected the lipophilic dye DiOC₆(3), which labels any contacting membrane (Terasaki, 1998), into fertilized eggs, allowed them to divide, and then injected BoNT-C1 into single cells to ask whether gross morphological changes in cytoplasmic membrane could be detected. We find that although cells injected with BoNT-C1 are inhibited in cell division, there is no detectable difference in DiOC₆(3) membrane labeling patterns compared with toxin-free cells (Figure 3, F–H).

We also suspected that the observed effects of BoNT-C1 on cell division might be the result of impeding general membrane flow through the Golgi apparatus leading to a depletion of membrane-targeted vesicles. To test this possibility, we treated fertilized eggs with 10 μM BFA, a concentration well known for its ability to disassemble the Golgi apparatus by preventing anterograde vesicle transport from the ER but not the retrograde pathway (Lippincott-Schwartz et al., 1989; Klausner et al., 1992; Sciaky et al., 1997) in a variety of tissue culture cells (Sciaky et al., 1997; Kok et al., 1998; Zhang et al., 1998) and cultured sea urchin embryonic cells (Hwang and Lennarz, 1993). Surprisingly, treatment of newly fertilized eggs with 10–100 μM BFA has no observable effect on the timing or ability of the embryo to undergo cell division (Figure 3, D and E) compared with control embryos (Figure 3, I and J). The efficacy of BFA on blocking vesicle transport through the Golgi apparatus was tested by incubating unhatched sea urchin embryos in BFA to ask what concentration prevents secretion of the hatching enzyme. Sea urchin embryos are surrounded by a fertilization envelope during early development until the blastula stage, at which time they begin translating and secreting the hatching enzyme, which digests the envelope and allows the ciliated embryo to freely swim (Lepage and Gache, 1989; Lepage et al., 1992). We find that as low as 10 μM BFA prevents embryos from hatching out of the fertilization envelope (our unpublished results). The above results suggest that BoNT-C1 inhibits cell division by a specific syntaxin-mediated vesicle fusion effect and not simply the result of obstructing membrane flow through the Golgi apparatus.

Figure 3. Cell phenotypes resulting from BoNT-C1 treatment are not the result of gross membrane topological alterations or membrane flow blockage through the Golgi apparatus. Fertilized eggs, injected with the membrane marker DiOC₆(3) to reveal membrane topology, were allowed to develop and a single blastomere was then injected with BoNT-C1 (A and F). Cells injected with BoNT-C1 (5 nM) become inhibited in cell division (C and H); however, there is little difference in the topology of membranes between toxin-injected and uninjected cells (F–H). The contribution of Golgi-derived material for cell division was tested by treating fertilized eggs with BFA. Embryos treated with 10 μM BFA 10 min after insemination are unaffected in cell division (D and E) compared with control embryos (I and J). Bar, 50 μm.
Syntaxin and Cell Division

Additionally, some syntaxin family members have been shown to be involved in retrograde membrane traffic from the Golgi to the ER in yeast (Lewis and Pelham, 1996) and are also thought to participate in synaptic vesicle recycling (Walch-Solimena et al., 1995). Thus, we asked whether BoNT-C1 was in some way inhibiting the cell endocytic pathway, which might indirectly block cell division. To test this hypothesis, we asked whether neurotoxin-injected cells were still capable of endocytosing FM1-43, a membrane-impermeant lipophilic dye that fluoresces only when associated with membranes and has been shown useful in studying membrane dynamics in this embryo (Whalley et al., 1995). Single blastomeres of a two-cell-stage embryo were injected with BoNT-C1 and allowed to develop until a phenotypic difference in cell division was observed between injected and uninjected blastomeres, within 45 min to 1 h. We then transferred the embryos to ASW water containing FM1-43 to assay for endocytosis by looking for FM1-43-labeled endocytic vesicles. We find that toxin-injected cells are active in endocytosis, as evidenced by the accumulation of fluorescent vesicles in the cell cytoplasm (Figure 4, D–F), and no significant differences in endocytosis were apparent when compared with uninjected cells (Figure 4G). Moreover, we subsequently find FM1-43 fluorescent labeling in ER surrounding the cell nucleus (Figure 4, D and E), presumably by retrograde membrane traffic through the endosome and Golgi. These observations strongly suggest that the endocytic pathway is generally unaffected by treatment with BoNT-C1 and that cells are still capable of other membrane fusion events. Thus, we conclude that the toxin treatment is not simply affecting general metabolic processes or global membrane trafficking in the cell.

Finally, we also asked whether BoNT-C1 was simply blocking cell division indirectly by somehow preventing actin polymerization, thus preventing the formation of the contractile actin ring, which is required for cell division. However, phalloidin staining of toxin-injected embryos indicates this is not the case (our unpublished results).

**Botulinum Neurotoxin C1 Specifically Removes Syntaxin from Intracellular Vesicles**

BoNT-C1 cleaves syntaxin family members at an amino acid sequence specific site near the transmembrane domain at the C-terminus (Blasi et al., 1993; Schiaovo et al., 1995). Because the sea urchin syntaxin contains the conserved BoNT-C1 cleavage site, and BoNT-C1 cleaves sea urchin syntaxin in vitro (Coorssen et al., 1997), we hypothesized that antibodies to the N-terminal region of syntaxin might localize to vesicles in toxin-injected cells in vivo (Conner et al., 1997), because BoNT-C1 cleavage would release the syntaxin N-terminal region from the vesicle membrane. To test this hypothesis we injected a single blastomere of a two-cell embryo with BoNT-C1 and waited (45 min to 1 h) for a phenotypic difference in cell division between toxin-injected and uninjected cells. We then asked whether syntaxin localized to vesicles at the cell cortex by injecting fluorochrome-labeled antibodies against syntaxin (~200 nM). We find that in toxin-free cells, syntaxin localizes to vesicles enriched at the cell cortex, whereas in toxin-treated cells, vesicle-associated syntaxin signals are dramatically decreased (Figure 5). To quantify the effects of BoNT-C1 on syntaxin vesicle immunolocalization, we injection with fluorochrome-labeled antibodies against syntaxin (5–200 nM). We find that 6), arguing strongly that vesicle-associated syntaxin removal by BoNT-C1 is specific and that syntaxin removal does not stimulate rab3 loss from these same vesicles.

**Syntaxin Antibodies Inhibit Cell Division**

As an alternative approach to test the function of syntaxin in cell division, we injected affinity-purified antibodies against
recombinant sea urchin syntaxin into single blastomeres of a two-cell-stage embryo. Injection of monovalent Fab antibody fragments against syntaxin at 480 nM blocks cell division in a similar manner as that of BoNT-C1 (Figure 8, D–F). Antibody injection, like BoNT-C1 treatment, inhibits both karyokinesis and cytokinesis, and once injected cells have been inhibited, their development is halted, whereas un.injected blastomeres develop as normal. Injection of heat-inactivated affinity-purified Fab fragments has no affect on cell division (Figure 8, G–I), nor are any affects observed when single blastomeres are injected with nonrelevant Fab fragment antibodies (anti-rabbit IgG molecules at 580 nM; Figure 8, A–C). These observations argue that it is the specific inactivation of the syntaxin by antibodies that results in inhibited cell division, adding further evidence that functional syntaxin is required for cell division.

DISCUSSION

Does the syntaxin family of proteins have a general function during cell division? An essential role for syntaxin during embryogenesis has been recently implicated in *Drosophila*; female germ line mosaic mutants for syntaxin 1 do not

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**Figure 5.** BoNT-C1 removes syntaxin from intracellular vesicles. A single blastomere of a two-cell-stage embryo was injected with BoNT-C1 (5.3 nM). Once cell division was inhibited in the toxin-injected blastomere, daughter blastomeres originating from the uninjected cell and the toxin-injected blastomere were then injected with fluorescently labeled antibodies against syntaxin (E and F; injected blastomeres are marked with an oil droplet) to test syntaxin immunolocalization. Confocal sectioning of the embryo reveals a dramatic decrease in vesicle-associated syntaxin in the toxin-injected cell compared with control blastomeres (B–D). However, some vesicle-associated syntaxin can be seen in toxin-injected cells. Images were visualized by indirect immunofluorescence using confocal microscopy with a Zeiss LSM 410 microscope. Bar, 60 μm.

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**Figure 6.** BoNT-C1 dramatically reduces syntaxin vesicle localization. Cells treated with 3.9 and 5.3 nM BoNT-C1 have a ~30 and 70% decrease in syntaxin immunolocalization, respectively, when compared with untreated cells of the same embryo, whereas rab3 immunolocalization is unaffected by treatment with the toxin. Each bar represents the average relative immunolocalization of 15 confocal sections of three different embryos.

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**Figure 7.** Syntaxin and rab3 colocalize on intracellular vesicles in the sea urchin embryo. Fluorochrome-labeled Fab fragments affinity purified to syntaxin and rab3 were injected into fertilized eggs. A cell surface confocal section shows syntaxin (A) and rab3 (B) associated with vesicles at the cortex of the embryo. Image overlay reveals syntaxin (red) and rab3 (green) on the same vesicles (C, colocalization in yellow; boxed inset shows higher magnification), with corresponding bright-field image (D). Bar, 60 μm.
unaffected by BFA treatment, arguing that surface mem-

brane addition and the targeting of plasma membrane
proteins early in embryogenesis come from a Golgi-inde-
pendent membrane source or post-Golgi vesicles from
maternally derived vesicle stores.

Syntaxin, in cooperation with VAMP and SNAP-25, has
been shown to be involved in the formation of the mini-
mal core membrane fusion machinery (Weber et al., 1998).
Its role in neurotransmitter vesicle fusion has been exten-
sively studied by taking advantage of BoNT-C1 (Foran
et al., 1996; Marsal et al., 1997; O’Connor et al., 1997; Wil-
liamson and Neale, 1998), which specifically proteolyzes
syntaxin family members possessing the appropriate
cleavage site (Schiavo et al., 1995). Sea urchin syntaxin
cDNA analysis indicates that it possesses the BoNT-C1
cleavage site (Conner et al., 1997), the protein can be
cleaved in vitro by BoNT-C1 (Coorssen et al., 1997), and
here we have shown that the toxin specifically removes
syntaxin from vesicles enriched at the cortex and that
syntaxin-specific antibodies block cell division. However,
we are currently unable to test whether these vesicles are
blocked in their fusion ability by either treatment, because
we have no markers for the contents of these vesicles. It is
feasible that it is the vesicle contents in addition to the
inherent vesicle membrane proteins that are vital to cell
division, and thus we are interested in their identification.

Although BoNT-C1 specificity for some syntaxin family
members has been demonstrated (Schiavo et al., 1995),
reports exist that BoNT-C1 can proteolyze both SNAP-25
and syntaxin in permeabilized chromaffin cells (Foran
et al., 1996) and intact cultured neurons (Williamson
et al., 1996) with equal efficiency. SNAP-25 cleavage by
BoNT-C1 appears to occur at the C terminus, and al-
though the exact site of protease cleavage is unknown, it
is suspected that the protease recognizes a conserved
conformation. A highly conserved SNAP-25 family mem-
er has recently been cloned in the sea urchin sperm
(Schulz et al., 1998). Although we have been unable to
detect SNAP-25 in eggs with antibodies against sperm
SNAP-25, it is possible that the observed inhibition in cell
division may be the cumulative affects of BoNT-C1 pro-
teolysis of both syntaxin and SNAP-25. However, because
cells injected with either BoNT-A or -E develop normally,
we conclude that the BoNT-C1-induced phenotypes are
specific for syntaxin proteolysis.

In this study we find that syntaxin inhibition blocks
both cytokinesis and karyokinesis. However, it has been
appreciated for some time that cytokinesis is separable
from karyokinesis. For example, in the starfish, microin-
jection of antibodies against myosin results in blocking
cytokinesis by preventing cleavage furrow formation,
even though karyokinesis continues, as evidenced by the
appearance of multiple daughter nuclei (Mabuchi and
Okuno, 1977). More recently, selective inhibition of cyto-
kinesis is observed when embryos are exposed to the
natural marine toxins stypoldione from alga (O’Brien
et al., 1989) and pseudopterolide from soft coral (Grace
et al., 1992). These toxins are thought to target sulfhydryl-con-
taining proteins involved in the formation of the contrac-
tile ring, yet karyokinesis continues in the cells. These
studies focused on disruption of the cytoskeleton in cell
division in contrast to the present study, which examines
membrane dynamics. It is possible that if BoNT-C1 has

Figure 8. Syntaxin antibodies inhibit cell division. Cell division is
unaffected in blastomeres injected with non-relevant Fab fragment
antibodies at 580 nM (A–C, marked by an oil droplet; arrowhead).
Blastomeres are inhibited in cell division when injected with 480 nM
Fab fragment antibodies against syntaxin (D–F); however, cells in-
jected with up to 1.9 μM heat-inactivated syntaxin antibodies show
no affect on cell division (G–I). Bar, 50 μm.
targets on the ER, Golgi, or nuclear envelope, the introduction of the toxin could be disrupting homotypic membrane fusion events necessary for the reformation, fragmentation, or stability of these organelles during or after cell division. Thus, we hypothesize that treatment with syntaxin antibodies or BoNT-C1 could halt cell progression through the cell cycle at a checkpoint that monitors membrane status within the cell.

ACKNOWLEDGEMENTS

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