Syntaxin 1A inhibits regulated CFTR trafficking in Xenopus oocytes

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Syntaxin 1A inhibits regulated CFTR trafficking in Xenopus oocytes. Am. J. Physiol. 277 (Cell Physiol. 46): C174–C180, 1999.—The cystic fibrosis transmembrane conductance regulator (CFTR) is an epithelial cell Cl channel, whose gating activity and membrane trafficking are controlled by cAMP/protein kinase A (PKA)-mediated phosphorylation. CFTR Cl currents are regulated also by syntaxin 1A (A. P. Naren, D. J. Nelson, W. W. Xie, B. Jovov, J. Pevsner, M. K. Bennett, D. J. Benos, M. W. Quick, and K. L. Kirk. Nature 390: 302–305, 1997), a protein best known for its role in membrane trafficking and neurosecretion. To examine the mechanism of syntaxin 1A inhibition, we expressed these proteins in Xenopus oocytes and monitored agonist-induced changes in plasma membrane capacitance and cell surface fluorescence of CFTR that contains an external epitope tag. cAMP stimulation elicited large increases in membrane capacitance and in cell surface labeling of flag-tagged CFTR. Coexpression of CFTR with syntaxin 1A, but not syntaxin 3, inhibited cAMP-induced increases in membrane capacitance and plasma membrane CFTR content. Injection of botulinum toxin/C1 rapidly reversed syntaxin 1A’s effects on current and capacitance, indicating that they cannot be explained by an effect on CFTR synthesis. Functional expression of other integral membrane proteins, including Na-coupled glucose transporter hSGLT1, inwardly rectified K channel hIK1, P2Y2 nucleotide receptor, and viral hemagglutinin protein, was not affected by syntaxin 1A coexpression. These findings indicate that acute regulation of the number of CFTR Cl channels in plasma membranes is one mechanism by which cAMP/PKA regulates Cl currents. Inhibition of plasma membrane CFTR content by syntaxin 1A is consistent with the concept that syntaxin and other components of the SNARE machinery are involved in regulated trafficking of CFTR.

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Cystic fibrosis is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), which accounts for the cAMP-stimulated Cl conductance at the apical membranes of many secretory and absorptive epithelial cells (22). Biochemical and electrophysiological studies have demonstrated that CFTR Cl channel activity requires protein kinase A (PKA)-mediated phosphorylation of its R-domain (18, 31) and binding and hydrolysis of ATP at one or both nucleotide binding domains (1, 23, 26). In addition to facilitating CFTR Cl channel gating, cAMP/PKA stimulation of CFTR-expressing cells evokes changes in membrane trafficking. cAMP stimulates exocytosis and inhibits endocytosis, and these regulated trafficking events require the expression of wild-type CFTR (5). The close correlation observed between CFTR-dependent increases in Cl current and membrane capacitance suggests that CFTR resides in the membrane vesicles whose trafficking is influenced by cAMP-dependent agonists (25, 30). Accordingly, CI current stimulation by cAMP/PKA may arise from increases in channel open probability (P_o) and/or from increases in the number (N) of active CFTR Cl channels residing in the plasma membrane.

Results of recent studies have implicated a role for membrane traffic regulatory proteins in the control of CFTR Cl currents. Naren et al. (20) showed that syntaxin 1A is expressed in colonic secretory epithelial cells, and they detected an interaction between CFTR and syntaxin 1A using in vitro protein binding assays (21). Coexpression of syntaxin 1A with CFTR in Xenopus oocytes inhibited cAMP-stimulated Cl currents, and this inhibition could be reversed by the syntaxin binding protein, munc-18. They proposed that syntaxin 1A and munc-18 regulate CFTR, and thus CI secretion, at the apical membranes of secretory epithelial cells.

Using measurements of plasma membrane capacitance and cell surface expression of CFTR, we demonstrate here that syntaxin 1A expression interferes with cAMP/PKA-regulated CFTR trafficking. We used epitope-tagged CFTR to show that cAMP stimulation elicits an increase in plasma membrane CFTR content, which is paralleled by a cAMP-induced increase in plasma membrane capacitance. Syntaxin 1A coexpression selectively inhibited the cAMP-induced increase in membrane capacitance and reduced the amount of CFTR detected in the plasma membrane. This syntaxin-induced reduction in N is consistent with the role of this protein as a component of the membrane fusion machinery.
MATERIALS AND METHODS

Oocyte preparation. Oocyte isolations and RNA injections were performed as described previously (8, 11). Xenopus laevis was obtained from Xenopus 1 (Ann Arbor, MI). Oocytes were isolated surgically and follicular cells removed during a 60- to 90-min collagenase incubation (2 mg/ml, type 2; Worthington). Stage 5–6 oocytes were maintained in a modified Barth’s solution overnight before injection (50 nl vol) with cRNA for CFTR, syntaxin 1A, or syntaxin 3. Noninjected oocytes served as controls. Expression was allowed to proceed at 18°C for 1–3 days before current recordings or immunofluorescence measurements were performed.

Electrophysiology. The ND-96 solution utilized for the current measurements contained (in mM) 96 NaCl, 1 KCl, 1.8 CaCl2, 1.0 MgCl2, and 5 HEPES. Recordings of CFTR-dependent Cl current (Icl) and membrane capacitance (Cm) changes were performed as described in Ref. 30. Briefly, cells were impaled with two 3 M KCl-filled electrodes connected to a GeneClamp 500 amplifier (Axon Instruments, Foster City, CA) via Ag-AgCl pellet electrodes and referenced to the bath by a Ag-AgCl pellet. Membrane potentials were allowed to stabilize before current and capacitance recordings. The voltage clamp was controlled by an AD/DA interface board (AXOLAB 1100) using a PC-running software generated in the laboratory. Membrane capacitance was calculated from the time course of current decay during a 10-mV hyperpolarizing voltage pulse. The decay time constant was derived from a single exponential fit of the pulse currents, and Cm was calculated on-line as described (30). Steady-state membrane currents were recorded at ~60 mV; these inward currents reflect Cl flow through CFTR during stimulation by cAMP, as shown in prior studies (8, 11). Values of Icl and Cm were obtained at 2-s intervals; steady-state values are given in the figures.

Cell surface CFTR expression. We used immunofluorescence techniques to monitor the expression of CFTR in the oocyte plasma membrane. Cells were injected with cRNA encoding an epitope-tagged CFTR, which contains the flag epitope (DYKDDDK) inserted into the predicted fourth extracellular loop of CFTR, after amino acid position 901, and designated M2–901/CFTR. Prior studies have shown that this flag-tagged CFTR behaves identically to its wild-type counterpart during macroscopic current stimulation (13), and single channel measurements detect no difference in channel gating or PKA/ATP regulation relative to wild-type CFTR (24).

M2–901/CFTR-expressing oocytes were subjected to a staining protocol to quantitate CFTR protein expression at the cell surface using the monoclonal M2 antibody (Kodak). Oocytes were cooled rapidly to 0–4°C and incubated with M2 antibody overnight at this temperature (1.1,250 dilution). After they were washed with 5% FCS-ND-96, oocytes were incubated at 4°C in fluorescein-conjugated goat anti-mouse IgG (1:1,000 dilution) and then washed five times as above. This protocol avoids permeabilization or fixation conditions that might expose intracellular flag-CFTR to the M2 antibody. Thus the staining conditions and epitope position were selected to permit detection of M2–901/CFTR only when it is localized in the plasma membrane and the epitope is exposed at the cell surface. For some experiments, CFTR Cl currents were recorded from M2–901/CFTR-expressing oocytes before implementing the staining protocol. This permitted CFTR Cl currents and cell surface expression to be determined in the same cells. In other experiments, oocytes were fixed and permeabilized to assess total flag-CFTR labeling using ice-cold Caltag reagents (proprietary). Cells were immersed in 200 µl medium A for 15 min, washed once in 5% BSA-C (Aurion)/ND-96, and placed in 200 µl medium B for 30 min. After three washes in BSA-C/ND-96, they were subjected to the staining protocol described above.

Individual oocytes were scanned on a Molecular Dynamics multiprobe 2001 laser confocal microscope at >10 magnification using 10-µm optical sections. Sections were scanned at 512 × 512 pixels (488 nm laser, 510 nm primary beam splitter, 510 nm secondary beam splitter). Control (uninjected) oocytes were scanned to set the fluorescence background; the laser intensity and voltage were set accordingly. Image planes throughout the depth of the specimen were collected. A quantitative measure of protein expression at the surface of the oocyte was derived from a maximal intensity rendered image. The periphery of the image was delineated and the mean pixel intensity calculated.

Reagents. cRNAs were transcribed in vitro using the Message Machine kit (Ambion) from linearized plasmids. Rat syntaxin cDNAs were kindly provided by the laboratory of Dr. Richard Scheller (Stanford University, Stanford, CA). Their expression was confirmed by immunoprecipitation of 35-kDa proteins from oocytes expressing syntaxin 1A or syntaxin 3 (data not shown). Plasmids encoding the control proteins (see Fig. 5) were gifts of the laboratories of Drs. Ernst Wright (University of California, Los Angeles, CA), John Edelman (Vollum Institute), and David Julius (University of California, San Francisco, CA). Botulinum toxin/C1 (BoT/C1) was obtained from Wako Chemical Industries (Japan) and injected in 25-nl PBS. All results are expressed as means ± SE.

RESULTS

cAMP increases plasma membrane CFTR. Figure 1 shows composite images of intact, nonpermeabilized oocytes expressing epitope-tagged M2–901/CFTR (Fig. 1, B and C). A representative image from a control, noninjected oocyte is displayed in Fig. 1A. CFTR currents and cell surface fluorescence were determined 2–3 days after cRNA injection. In noninjected oocytes, background fluorescence was low (Fig. 1A). Oocytes expressing M2–901/CFTR under nonstimulated conditions showed low levels of surface staining that were slightly above background, suggesting a low level of CFTR expression in the plasma membrane in the absence of agonist stimulation (Fig. 1B). M2–901/ CFTR oocytes stimulated with forskolin/IBMX for 15 min showed a large and significant increase in surface fluorescence (Fig. 1C). The low fluorescence intensity detected in the absence of agonist suggests that the staining protocol does not permit access of the M2 antibody to intracellular epitope. In assays performed on permeabilized oocytes, the addition of forskolin/IBMX had no influence on fluorescence intensity using the same labeling conditions (CFTR basal, 25.1 ± 3.6; CFTR stimulated, 25.6 ± 3.8; n = 6). This result indicates that recognition of the flag epitope by the M2 antibody does not require CFTR stimulation.

Average data from three experiments in nonpermeabilized oocytes are provided in Fig. 1D. Also shown are the results from parallel measurements of steady-state current obtained before and after addition of forskolin/IBMX; these values are consistent with the magnitude of CFTR current stimulation observed in prior studies (8, 11). The immunofluorescence data indicate that the
increase in Cl current is paralleled by an increase in plasma membrane CFTR. For oocytes in the stimulation group, the staining protocol was initiated 15 min after addition of forskolin/IBMX, the time when Cl currents rise to their plateau level. Thus detection of the M2 epitope at the cell surface occurs within the time frame required for the cAMP-induced increases in Cl current and membrane capacitance (30). These findings are consistent with the idea that cAMP/PKA stimulation induces the insertion of CFTR-containing vesicles into the plasma membrane.

Syntaxin 1A inhibits cAMP/PKA-induced increases in plasma membrane capacitance and CFTR. The effects on $I_{\text{Cl}}$ and $C_m$ of syntaxin coexpression with CFTR are illustrated in Fig. 2. When syntaxin 1A was coexpressed with CFTR, Cl current stimulation by cAMP was inhibited, as reported by Naren et al. (20). In parallel with the syntaxin-induced reduction in Cl current, cAMP stimulation of membrane capacitance is also markedly inhibited. The inhibition of changes in both $I_{\text{Cl}}$ ($\Delta I_{\text{Cl}}$) and $C_m$ ($\Delta C_m$) is selective for syntaxin 1A. Coexpression of syntaxin 3 with CFTR did not affect these parameters significantly, suggesting that these effects are syntaxin isoform specific. This finding also eliminates translational competition between the CFTR and syntaxin cRNAs as an explanation of the inhibitory
effect of syntaxin 1A on the agonist-induced $\Delta I_{Cl}$ and $\Delta C_m$.

The effect of syntaxin 1A coexpression on CFTR detected at the oocyte surface during cAMP/PKA stimulation is illustrated in Fig. 3. As in Fig. 1C, these images were collected from oocytes stimulated with maximally effective concentrations of forskolin and IBMX (see legend to Fig. 3). The noninjected control oocyte illustrates the background level of cell surface staining in the presence of agonists (Fig. 3A). This low fluorescence intensity indicates that cAMP stimulation does not increase background fluorescence in the absence of CFTR expression (compare with Fig. 1A).

During cAMP stimulation, M2–901/CFTR was readily detected in the surface membrane (Fig. 3B), but coexpression with syntaxin 1A markedly reduced plasma membrane CFTR during cAMP stimulation (Fig. 3C). This result is consistent with the syntaxin 1A-induced decrease in cAMP-dependent $\Delta C_m$, shown in Fig. 2. As with the membrane capacitance and current responses, syntaxin 3 coexpression had no effect on cell surface CFTR staining (data not shown). Quantitation of the results from four experiments (Fig. 3D) shows that CFTR surface expression correlates with the magnitude of currents recorded from the same oocytes before antibody labeling. With syntaxin 1A coexpression, cell surface CFTR fluorescence intensity fell nearly to the level of the water-injected controls. Thus insertion of CFTR into the plasma membrane is required for activation of the CFTR $I_{Cl}$ currents recorded from these cells during stimulation, and syntaxin 1A reduces CFTR $I_{Cl}$ currents by decreasing the amount of CFTR in the plasma membrane during stimulation.

The effects of BoT/C1 on CFTR $\Delta I_{Cl}$ and $\Delta C_m$ with and without syntaxin 1A coexpression are shown in Fig. 4. We injected BoT/C1 60 min before recording the current and capacitance responses to forskolin/IBMX. BoT/C1 acutely reversed the syntaxin 1A inhibition of $I_{Cl}$ and $C_m$, presumably due to cleavage of syntaxin 1A. The toxin did not interfere with the $\Delta I_{Cl}$ or $\Delta C_m$ responses in oocytes injected with CFTR alone. This relatively rapid reversal of CFTR function argues against an effect of syntaxin on the level of CFTR protein expression as an explanation for the inhibitory effect on CFTR current or capacitance. For comparison, the half time for inhibition of CFTR currents in oocytes by brefeldin A is ~6 h (data not shown). This finding indicates that the steady-state rates of CFTR production and degradation in these cells are not sufficiently rapid for protein synthesis to account for the BoT/C1 reversal of syntaxin’s effects on CFTR.

Finally, we examined the influence of syntaxin coexpression on the activities of several other plasma membrane transport or receptor proteins. These con-
Control experiments were performed to examine the possibility that syntaxin 1A may block the functional expression of CFTR by generally interfering with the migration of proteins through the biosynthetic pathway leading to the plasma membrane. cRNA amounts were selected based on prior reports using these constructs (see references below), and the currents obtained are similar in magnitude. Coexpression of syntaxin 1A with the Na-dependent glucose transporter SGLT1 (12), the ATP-sensitive Ca-activated K channel IK1 (14), or the P2Y2 purinergic receptor (17) had no effect on the currents associated with activation of these proteins (Fig. 5, see legend for activation conditions). In addition, syntaxin 1A did not inhibit plasma membrane expression of the viral hemagglutinin (HA) protein at the oocyte surface, which was detected by immunofluorescence using techniques similar to those used for detection of cell surface M2–901/CFTR [background-subtracted HA fluorescence intensities: HA alone, 24.1 ± 4.1 (n = 6); HA + syntaxin 1A, 29.6 ± 5.0 (n = 5); cRNAs: 5 ng HA, 10 ng syntaxin 1A]. These data show that syntaxin 1A does not impair HA protein expression, even when the cRNA dose ratio is in favor of syntaxin 1A. In addition, relative protein expression level for hIK1 can be judged from the current, which is comparable with that obtained from CFTR (compare Figs. 2 and 5). Because the single-channel current amplitude and P_o of hIK1 are greater than those of CFTR (12), a similar macroscopic current requires the expression of less protein. Despite this, hIK1 currents were not inhibited by syntaxin 1A, even at five times the dose required for maximal inhibition of CFTR (see legends to Figs. 2 and 5). Accordingly, syntaxin 1A is not simply disrupting the expression and targeting of integral plasma membrane proteins, rather its inhibitory effect was specific for CFTR.

**DISCUSSION**

In a previous study (30), we showed that cAMP stimulation increased both Cl current and membrane capacitance in CFTR-expressing Xenopus oocytes. These effects were reversible with agonist washout and required CFTR expression. Changes in I\textsubscript{Cl} and C_m were closely correlated with one another as the agonist dose or CFTR expression level were varied, or when CFTR mutants with different ΔI\textsubscript{Cl} responses were expressed. The increase in cell surface area detected by ΔC_m was confirmed by morphometry of the oocyte surface in the same cells used for electrical recordings (30). This close correlation between ΔI\textsubscript{Cl} and ΔC_m suggests that CFTR activation by cAMP/PKA elicits reversible stimulation of membrane vesicle insertion into the plasma membrane, a process that is implicated in a variety of CFTR-expressing cells (4).

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**Fig. 4.** Effect of botulinum toxin C1 (BoT/C1) on current (I\textsubscript{Cl}) and capacitance (C_m) responses to cAMP stimulation. Oocytes were injected with 1 ng BoT/C1 60 min before recording. Experiment as in Fig. 2; results are from 3 experiments with at least 8 oocytes/group.

**Fig. 5.** Effect of syntaxin coexpression on activity of other plasma membrane proteins. Numbers in parentheses indicate cRNA amounts injected/oocyte. A: Na-coupled glucose transporter hSGLT1 (50 ng); syntaxin 1A (5 ng). Inward Na current at −100 mV evoked by addition of 5 mM α-methyl-d-glycoside to the bath. B: Inward rectifier K channel (hIK1; 50 ng); syntaxins 1A or 3 (10 ng). Inward K current at −100 mV evoked by bath addition of 1 µM ionomycin. C: current-voltage relationship for endogenous Cl current elicited by P2Y2 nucleotide receptor (5 ng) activation by bath addition of 100 µM UTP; syntaxin 1A (5 ng). See text for citations and other details.
The results obtained from oocytes expressing an epitope-tagged CFTR verify this concept. Incorporation of an extracellular flag epitope permits detection of CFTR protein at the surface of nonpermeabilized cells (13). This approach obviates problems of resolving protein localization in the plasma membrane, since standard immunofluorescence techniques do not have sufficient resolution to distinguish protein in 100-nm submembrane vesicles from that in the plasma membrane. CFTR bearing a flag tag in its fourth extracellular loop was not detected appreciably at the surface of oocytes under basal, nonstimulated conditions, when CFTR Cl currents are near zero (Fig. 1). cAMP stimulation increased plasma membrane CFTR sevenfold within the timeframe associated with stimulation of $I_{\text{Cl}}$ and $C_{m}$ ($\sim$15 min, Figs. 1 and 4). Control experiments indicated that M2 antibody staining of noninjected oocytes was not affected by cAMP stimulation, i.e., the surface labeling signal is CFTR and stimulation dependent. The correlations between cAMP-stimulated CFTR surface labeling signal is CFTR and stimulation dependent. The correlations between cAMP-stimulated CFTR insertion increased plasma membrane CFTR sevenfold within the timeframe associated with stimulation of $I_{\text{Cl}}$ and $C_{m}$ ($\sim$15 min, Figs. 1 and 4). Control experiments indicated that M2 antibody staining of noninjected oocytes was not affected by cAMP stimulation, i.e., the surface labeling signal is CFTR and stimulation dependent. The correlations between cAMP-stimulated CFTR current, capacitance, and cell surface labeling show that CFTR insertion by exocytosis is an important event in the stimulation of plasma membrane CFTR conductance of these cells by cAMP.

The oocyte has proven to be a useful model for studies of other transporter-related membrane-trafficking events. Previous studies include relocation of $\gamma$-aminobutyric acid transporters to the plasma membrane in response to protein kinase C activation (7) and regulation of the cell surface expression of several Na-dependent cotransport processes by protein kinases A and C (12). In addition, the influence of mutations in the amiloride-sensitive Na channel (ENaC), which produces hypertension by prolonging the plasma membrane residency time of ENaC, was characterized mechanistically using the oocyte expression system (28).

The biochemical events that control membrane insertion and retrieval processes at the plasma membrane are thought to conform to principles first identified for vesicle transit among compartments of the protein biosynthetic pathway (2, 29). Interactions among proteins that comprise the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein complex control the targeting, docking, fusion, and retrieval of membrane vesicles that contain secretory products or integral membrane proteins, like CFTR, that will become constituents of the plasma membrane. The physiological importance of SNARE proteins is exemplified in the highly regulated process of neurosecretion; our understanding of the actions of these proteins in membrane-trafficking events in epithelial cells is relatively meager.

Our findings implicate a role for syntaxin in regulated CFTR trafficking. Coexpression of CFTR with syntaxin 1A reduced the cAMP-dependent membrane insertion event that is detected as an increase in membrane capacitance (Fig. 2). Syntaxin also blocked the increase in plasma membrane CFTR that occurs during stimulation of its activity by cAMP/PKA (Fig. 3). CFTR was not detected at significant levels in the plasma membrane under basal conditions (Fig. 1), and it was not present appreciably at the cell surface during cAMP stimulation in cells coexpressing syntaxin 1A. These effects were specific for CFTR, as opposed to other integral membrane proteins examined, they were selective for this syntaxin isoform, and they could not be explained by an effect of syntaxin on CFTR synthesis. Taken together, these findings indicate that syntaxin 1A interferes with cAMP/PKA-stimulated trafficking of CFTR to the cell surface.

What is the mechanism of this effect? The results of Naren et al. (20, 21) suggest that CFTR Cl currents are inhibited due to a physical interaction between these proteins. A protein-protein interaction has been observed also between N-type Ca channels and syntaxin 1A (27), which may contribute to syntaxin-associated downregulation of Ca currents (3, 32). Assuming that physical interaction occurs between syntaxin 1A and CFTR in vivo, expressed syntaxin 1A may prevent CFTR insertion into the plasma membrane by binding with CFTR contained in vesicles that would normally fuse with the plasma membrane. Second, a CFTR-syntaxin 1A complex may be retrieved rapidly from the plasma membrane by endocytosis. Our measurements detect net membrane area ($C_{m}$) or steady-state plasma membrane CFTR and do not distinguish whether the effect of syntaxin is an insertion or retrieval of CFTR.

It is possible also that overexpressed syntaxin associates primarily not with CFTR but with other SNARE components to block the membrane fusion process. According to this view, syntaxin 1A overexpression would disrupt the stoichiometric interactions among SNARE proteins that are required for the plasma membrane fusion of CFTR-containing vesicles. This effect of expressed syntaxin has been observed previously. For example, exogenous expression of the Golgi t-SNARE, syntaxin 5, inhibited protein traffic from endoplasmic reticulum to Golgi, the step in which this syntaxin isoform functions (9). Likewise, expression of syntaxin 1A, but not 1B, blocked glucose-stimulated insulin secretion in pancreatic β cells (19). In MDCK cells, syntaxin 3 is apically localized, and its exogenous expression selectively inhibited apical targeting and apical recycling of the immunoglobulin receptor (16). These findings suggest that overexpression of a specific syntaxin isoform disrupts the pathway in which this isoform normally plays a role in membrane-trafficking events. If this is true, then our results suggest that endogenous SNARE proteins, including a Xenopus syntaxin 1A ortholog, are responsible for the plasma membrane CFTR insertion in oocytes. Expression of the core components of the SNARE machinery has been observed in the cortical granules of sea urchin oocytes (6). Accordingly, the SNARE fusion machinery would be employed to mediate the membrane insertion of CFTR, and an interaction of CFTR with SNARE proteins could explain how the expression of CFTR alone can confer cAMP-dependent membrane-trafficking events on cells where none was present before CFTR expression (5, 30).
The requirement for specific SNARE components in this process could also account for the apparent failure of certain cell types to support regulated CFTR trafficking events (10, 15). The demonstration that syntaxin 1A is expressed in colonic secretory cells (1) is consistent with the concept that this protein, together with other components of the SNARE machinery, mediates the cAMP/PKA-regulated insertion of CFTR into the apical membranes of these cells. Although our findings suggest that the SNARE machinery plays an important role in the regulated trafficking of CFTR at the plasma membrane, they do not rule out a direct regulation of CFTR’s gating (P<sub>n</sub>) that could result from a physical interaction with syntaxin. Accordingly, syntaxin could directly regulate CFTR Cl currents, perhaps in relation to SNARE protein-mediated membrane-trafficking reactions that control the amount of CFTR present in the plasma membrane.

K. W. Peters and J. Qi contributed equally to this work.

We thank Megan Weiss for technical assistance, Patricia Connelly for help with the manuscript, and the laboratory of Richard Scheller for syntaxin cDNAs. This research was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-50829 and by the Cystic Fibrosis Foundation Grants 97RO and 96PO.

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Received 19 February 1999; accepted in final form 5 April 1999.

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