The PDZ-binding Chloride Channel ClC-3B Localizes to the Golgi and Associates with Cystic Fibrosis Transmembrane Conductance Regulator-interacting PDZ Proteins

Martina Gentzsch, Liying Cui, April Mengos, Xiu-bao Chang, Jey-Hsin Chen, and John R. Riordan

From the Mayo Clinic Scottsdale, S. C. Johnson Medical Research Center, Scottsdale, Arizona 85259

Chloride channels mediate a broad range of cellular functions. Among the several different families of proteins that constitute these channels, the neurotransmitter ligand-gated \( \gamma \)-aminobutyric acid (GABA) and glycine and voltage-gated ClC classes are the largest and best characterized (1). The ClC family is especially diverse with members in all organisms including at least nine in mammals. Mutations in several of these are disease-causing (2). ClC-1, ClC-2, and ClC-K channels within one branch of the mammalian family reside in the plasma membrane of cells in different tissue types whereas ClC-3 through ClC-7 form another large branch and reside primarily in membranes of intracellular organelles with the highly homologous ClC-3, -4, -5 channels occupying the endosomal/lysosomal compartments. These three may all provide negative charge shunts that increase the rate of acidification of these compartments by proton ATPases (1, 3, 4). A similar function has been suggested for bacterial ClC channels in extreme acid resistance response (5).

Recently, a splice variant of human ClC-3 was discovered that has a PDZ domain-binding C terminus (6). When heterologously expressed, this form, termed ClC-3B, was detectable mainly in a general intracellular localization. However, when co-expressed with the PDZ domain, EB50, a small amount of the channel protein was detected on the surface at the leading edge of spreading cells. Furthermore, EB50-mediated association with the other known PDZ-binding chloride channel, CFTR, was reported as was an ability of CFTR to activate ClC-3B. The CFTR chloride channel is distinct from the other families of chloride channels and belongs to the large family of ABC transport proteins (7). CFTR resides in the apical membrane of epithelial cells where it is crucial to ion and fluid secretion and reabsorption (8). Hence the possibility of ClC-3B trafficking to the plasma membrane and either being influenced by CFTR when it is present or potentially substituting for it when it is absent or dysfunctional in cells of CF patients is of great importance to understanding or influencing the disease. For this reason we have carefully examined the subcellular localization of heterologously and endogenously expressed ClC-3B as well as its possible PDZ protein-mediated interaction with CFTR. We found that ClC-3B is localized to the Golgi and differs in its location from the late endosomal isoform ClC-3A and from most CFTR, present at the plasma membrane. Both ClC-3B and CFTR interact with PDZ domains of GOPC, EB50, and PDZK1 and promotes the association with the other known PDZ-binding chloride channel, CFTR, specifically with TC10; by Charest et al. (20) and termed PIST for PDZ domain-interacting specifically with TC10; by Charest et al. (20) and termed FIG for Fused In Glioblastoma; and by Cheng et al. (19) and

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The abbreviations used are: PDZ, PSD-95/discs large/ZO-1; EB50; ERM-binding phosphoprotein 50; CFTR, cystic fibrosis transmembrane conductance regulator; GIPC, GIPC-interacting protein, C terminus; GOPC, Golgi-associated PDZ and coiled-coil motif-containing protein; GRASP, Golgi reassembly stacking protein; GST, glutathione S-transferase; Mint, Munc18-interacting protein; NKCC, sodium potassium chloride cotransporter 1; HA, hemagglutinin, mAb, monoclonal antibody; SNARE, soluble NSF attachment protein receptors.
ciation of the two PDZ-binding chloride channels. Despite this the only co-localization of CFTR and CIC-3B that we detected is at the Golgi where a small amount of CFTR resides. CIC-3A and CIC-3B were found to interact with each other, most likely as heterodimers as described for other CIC channels.

EXPERIMENTAL PROCEDURES

Plasmid Construction—Full-length cDNAs of CIC-3A and CIC-3B were amplified by PCR using a human pancreas cDNA library (Clontech) as template. PCR fragments were first introduced into the EcoRI site of pBluescript and then subcloned into pcDNA3. A c-Myc tag was added to the CIC-3B open reading frame at the N terminus; CIC-3A was tagged at its N terminus with amino acid residues 723–729 of the MRPI protein as an epitope recognized by mouse monoclonal antibody 42.4 (9). Partial cDNA fragments encoding different PDZ domains of EBPD50, PDZK1, and GOPC were subcloned into pGEX-5X-3 (Amersham Biosciences) to generate glutathione S-transferase (GST) fusion proteins. The boundaries of the expressed polypeptides containing different PDZ domains were amino acid residues 1–129 for PDZ domain 1 of EBPD50, amino acid residues 133 to the C-terminal end for PDZ domain 2 of EBPD50, amino acid residues 1–140 for PDZ domain 1 of PDZK1, amino acid residues 127–237 for PDZ domain 2 of PDZK1, amino acid residues 231–371 for PDZ domain 3 of PDZK1, amino acid residues 368 to the C-terminal end for PDZ domain 4 of PDZK1, and amino acid residues 281–383 for the PDZ domain of GOPC. A cDNA fragment encoding the C-terminal amino acid residues 1440–1480 of CIC-3B was subcloned into pGEX-5X-3 vector to produce a fusion of GST and the C terminus of CIC-3B.

Cell Culture and Transfection—BHK-21, HEK293T, Calu-3, Capan-1, CFPAC-1, TS4, Caco-2, MDCK, and FANC-1 cells were obtained from American Type Culture Collection (ATCC) and grown at 37 °C in 5% CO2. BHK-21 cells were transiently transfected with LipofectAMINE Plus Reagent (Invitrogen) or stably transfected using calcium phosphate (10). For transient expression cells were transfected with cDNAs of CFTR, ClC-3A, ClC-3B, EBP50, or PDZK1 subcloned into pEGFP-Endo (Clontech). Cells were examined on a Leica TCS 4D confocal microscope (Leica Microsystems) according to the manufacturer’s instructions.

Cell Lysis and Membrane Preparation—Cells were washed in ice-cold phosphate-buffered saline and lysed with Nonidet P-40 lysis buffer (1% Nonidet P-40, 150 mM NaCl, 50 mM Tris, pH 7.4, 10 mM Na2MoO4) at 4 °C for 30 min. Protease inhibitors were added to Nonidet P-40 lysis buffer as described earlier (12). Cell lysates were centrifuged at maximal speed in an Eppendorf at 4 °C, and supernatants were collected for further experiments. Scrapings from human bronchus were extracted with 2% Nonidet P-40 buffer and sonicated briefly before centrifugation. Scrapings from colon were first ground in a homogenizer in extraction buffer and then processed exactly as the bronchial scrapings. The preparation of membranes from cultured cells was performed exactly as described earlier (13).

Primary Antibodies—Mouse monoclonal antibodies recognizing CIC-3A and CIC-3B were raised against conjugated peptides derived from C-terminal sequences of CIC-3A (RHMAQTANQPASIMFN) and CIC-3B (KQHVEPLAPPWHYNKKR). Initial screens of hybridomas from C-terminal sequences of ClC-3A (RHMAQTANQPASIMFN) and ClC-3B (KQHVEPLAPPWHYNKKR) were performed by Western blotting of lysates or membranes from cells heterologously expressing ClC-3A or ClC-3B. The derived monoclonal antibodies 34.1 and 69.16 recognized CIC-3A and CIC-3B, respectively. Additionally, CIC-3A and CIC-3B were detected with antibodies 42.4 and 9E10 directed against the C-terminal tag. 34.1 was detected with antibody 596, 528, or 570, FLAG-tagged PDZK1 with antibody anti-FLAG M2 (Sigma), HA-tagged GOPC, and HA-tagged EBPD50 with the antibody 16B12 (Babco). The antibodies from the following manufacturers were used to detect proteins by immunofluorescence: Giantin, Babco; Golgin, Molecular Probes, GM130, BD Transduction Laboratories; γ-Adaptin, Santa Cruz Biotechnology; Calnexin, StressGen; EEA1, BD Transduc-

termed CAL for CFTR-associated ligand. We have used the term GOPC, which seems most descriptive and does not derive from one of the proteins with which it interacts.
of these electrophoretic bands (Fig. 1C). N-glycosidase F caused the 130-kDa and 90-kDa bands to collapse to a band slightly smaller than the latter in the case of both isoforms. Endoglycosidase H had a similar effect on the 90-kDa bands, indicating they are glycosylated species. Their decrease in size corresponds to ~5 kDa, consistent with the removal of two core oligosaccharide chains, probably from the two consensus N-glycosylation sites in the protein sequence (Fig. 1A). The 130-kDa bands were unaffected by endoglycosidase H as expected if they had acquired complex oligosaccharide chains. Thus both forms of ClC-3 are N-glycosylated membrane proteins that would be synthesized on membrane-bound ribosomes and transported to the Golgi where the glycosyl transferases that assemble complex oligosaccharide chains are located.

**Differential Localization of ClC-3A and ClC-3B**—In previous work, ClC-3A has been most definitely localized to late endosomes or lysosomes (4) and to synaptic vesicles in the brain (3). When expressed in BHK cells ClC-3A co-localizes extensively with the late endosomal marker RhoB, and to a lesser extent with the early endosomal marker EEA-1 (Fig. 2A). This localization is distinct from that of calnexin in the ER and several Golgi markers including giantin, GM130, and γ-adaptin.

**FIG. 1. Expression of ClC-3A and ClC-3B.** A, sequence alignment of ClC-3A and ClC-3B. ClC-3A and ClC-3B share identical sequences with the exception of the last 47 amino acids. ClC-3B has at its C-terminal end a PDZ-binding motif highlighted in green. α-Helices crossing the membrane partially or completely are underlined (B to R), and residues important for chloride selectivity are shown in red. These predictions were derived from an alignment with prokaryotic ClC channels, whose structures have been determined (16). Two potential N-glycosylation sites are shown in blue. A shorter form of ClC-3A has been described (37). The N-terminal end of the short form is shown with an arrow. B, heterologous expression of ClC-3A and ClC-3B in BHK-21 and HEK293T cells. Cells were transiently or stably transfected as described under “Experimental Procedures.” ClC-3A was detected with antibody 42.4, which recognizes the N-terminal MRP1 tag (BHK-21 transient) or with antibody 69.16, which recognizes the N-terminal c-Myc-tag or with antibody 9E10, which recognizes the C-terminal tail (BHK-21 stable and HEK293T stable). In the first lane of each panel, the same amount of protein was loaded from cells not expressing ClC-3A or ClC-3B (control).
distinct contrast ClC-3B did co-localize with these markers, especially giantin, GM130, and γ-adaptin (Fig. 2B). Of the endosomal markers there was some co-localization only with EEA-1, suggesting that some of both isoforms of the ClC-3 channel may occupy the early endosome compartment, possibly even as heterodimers (see below). The primarily distinct localization of the two channels; however, is emphasized when each is compared with the same marker. With giantin, for example, there is nearly complete correspondence with ClC-3B but very little with ClC-3A, even though some of it is situated contiguous with that compartment. This is not surprising of course since there is extensive traffic between the trans-Golgi network.
were detected by Western blotting using the same antibodies. Co-immunoprecipitated ClC-3A and ClC-3B were subjected to immunoprecipitation of ClC-3A or ClC-3B using antibodies from BHK-21 cells transiently expressing ClC-3A and ClC-3B. Membranes from BHK-21 cells transiently expressing ClC-3A and ClC-3B were subjected to immunoprecipitation of ClC-3A or ClC-3B using antibodies 42.4 or 9E10, respectively. Co-immunoprecipitated ClC-3A and ClC-3B were detected by Western blotting using the same antibodies. B, influence of ClC-3A and ClC-3B co-expression on the localization of each. ClC-3A and ClC-3B were transiently co-expressed in BHK-21 cells. ClC-3A was visualized with antibody 62.4 conjugated to Alexa 568, and ClC-3B was detected using antibody 61.16 conjugated to Alexa 488.

Endogenous ClC-3B Expression and Localization in Epithelia—Since we were primarily interested in its possible relationship to CFTR and relevance to cystic fibrosis, we examined the presence of ClC-3B in epithelial cells. First epithelial scrapings from human bronchus and colon were homogenized in detergent (Nonidet P-40) and immunoprecipitated with a mAb to ClC-3B, 69.16. After SDS-PAGE analysis under fully reducing conditions, a Western blot was probed with the same antibody (Fig. 4A). Distinct ClC-3B bands appeared in colonic samples from all three individuals tested and one bronchial sample; the other two bronchial samples gave weak signals but these observations clearly confirm ClC-3B expression in epithelial tissue affected in cystic fibrosis. We then examined expression in a number of epithelial cell lines and found positive signals of variable intensity in Western blots of lysates of all (Fig. 4B). ClC-3B appears most highly expressed in two of the lines, which also contain large amounts of CFTR viz. Calu-3 derived from submucosal glands and Capan-1, a pancreatic ductal cell line. Confocal immunofluorescence of Calu-3 cells grown on permeable supports at an air-liquid interface revealed ClC-3B in intracellular vesicular structures (Fig. 4C) as when it was heterologously expressed. This distinct population of vesicles is similar to those stained with the Golgi markers, giantin and golgin and entirely distinct from several apical and basolateral markers which clearly demarcate these portions of the cell surface. Thus, as when heterologously expressed, the principal residences of ClC-3B (Golgi) and CFTR (apical) are different.

ClC-3B Interactions with PDZ Proteins—The PDZ binding capacity of ClC-3B led to the idea that it might associate with and even be influenced by CFTR, possibly via EBPs (6). We tested the ability of ClC-3B to bind to the individual PDZ domains of three different PDZ proteins, EBPs, PDZK, and GOPC, known to interact with CFTR (17–19). GST fusions with each of the PDZ domains were incubated with detergent lysates of BHK cells expressing CFTR and ClC-3B. Both channel proteins bound preferentially to the first PDZ domain of EBP50 and to a much lesser extent to the second (Fig. 5A). This result is different from that of Ogura et al. (6) who reported that ClC-3B preferred PDZ2 of EBP50 and hence that ClC-3B and CFTR might be coupled by EBP50. The C-terminal sequences of both channels indicate they should bind class I PDZ domains (15). When similar experiments were performed with the four PDZ domains of PDZK1, ClC-3B appeared to bind only the first (Fig. 5B). CFTR, however, bound strongly to both domains 1 and 3. Hence at least in principle, a ternary complex could form with ClC-3B at PDZ1 and CFTR at PDZ3 of PDZK1. Since we found that ClC-3B resides primarily in the Golgi it seemed reasonable that it might bind to the Golgi-associated coiled-coil PDZ protein, GOPC, which binds CFTR (19). Fig. 5C confirms that the single PDZ domain of GOPC binds ClC-3B as well as CFTR.

Since the three PDZ proteins interact with both channel proteins, we examined the influence of each of the PDZ proteins on the localization of ClC-3B and CFTR (Fig. 6). Consistent with the results of Cheng et al. (19), EBP50 overexpression had little effect on the expression of CFTR, which is already at the cell surface. ClC-3B, however, was shifted from its presence just in the Golgi to somewhat more peripheral locations in the cell but was not detectible in the plasma membrane. PDZK1 overexpression similarly had little effect on CFTR, which is reasonable because PDZK1 and most CFTR are located in nearly the same place even when not co-expressed. On ClC-3B, however, PDZK1 had a stronger impact than EBP50, causing even more of that channel to move toward the cell periphery, although apparently not entirely to the plasma membrane. GOPC overexpression has a much more dramatic effect on both channels, causing them to become condensed with it in a focal...
Golgi location. The apparent large reduction in the amount of both channel proteins on co-expression with GOPC but not EBP50 or PDZK1 is confirmed by Western blots of whole cell lysates (Fig. 7). While these findings clearly show that the Golgi PDZ protein, GOPC, interacts strongly with the Golgi chloride channel, ClC-3B, this interaction cannot be entirely responsible for their co-localization since CFTR also is bound by GOPC but resides mostly at the cell surface.

Fig. 4. Endogenous expression of ClC-3B in epithelial cells. A, CIC-3B in human colonic and bronchial epithelia. CIC-3B was immunoprecipitated from lysates of human colonic and bronchial epithelia using antibody 69.16 and detected with the same antibody by Western blotting. B, CIC-3B and CFTR in epithelial cell lines. Membranes were prepared from epithelial cell lines and separated by 6% SDS-PAGE. CIC-3B was detected using antibody 69.16, and CFTR was detected using antibody 596. C, localization of CIC-3B in polarized Calu-3 cells. Calu-3 cells were grown at an air-liquid interface and stained for immunofluorescence microscopy as described under “Experimental Procedures.” MUC1, EBP50, and CFTR localize to the apical membrane. Immunofluorescence of sodium, potassium ATPase (NaK-ATPase), sodium potassium chloride cotransporter 1 (NKCC), and Zonula occludens protein 1 (ZO-1) demonstrate polarization of the cells. Nuclei were stained with propidium iodide and are shown in blue in the CFTR panel.

Fig. 5. Abilities of GST fusions of PDZ domains of EBP50 (A), PDZK1 (B), and GOPC (C) to interact with CIC-3B and CFTR. GST fusions with PDZ domains of EBP50, PDZK1, and GOPC were bound to glutathione-Sepharose beads and incubated with lysates from BHK-21 cells expressing CIC-3B or CFTR. The beads were washed, and bound proteins eluted. Lysates from cells expressing CIC-3B or CFTR were loaded as a positive control. Individual or combined PDZ domains employed are indicated above each lane. The high molecular weight CIC-3B bands near the top of the gels reflect strong aggregation in non-ionic detergent.

Golgi PDZ protein, GOPC, interacts strongly with the Golgi chloride channel, ClC-3B, this interaction cannot be entirely responsible for their co-localization since CFTR also is bound by GOPC but resides mostly at the cell surface.
ClC-3B Interaction with CFTR—Since PDZK1 bound the two channel proteins at different PDZ domains it seemed possible that it might couple them. To test this possibility two types of experiments were performed. In the first, both ClC-3B and CFTR could be co-immunoprecipitated with PDZK1 (Fig. 8A). Second, we compared the ability of a GST fusion with the C-terminal 40 amino acids of CFTR to pull-down ClC-3B from lysates of cells in which it was overexpressed alone or together with PDZK1 or one of the other PDZ proteins. Fig. 8B shows that interaction between the CFTR tail and ClC-3B is greatly enhanced by PDZK1. The low level of association without overexpressed PDZK1 may reflect the action of endogenous PDZ proteins or interaction between these two integral membrane proteins mediated by other means. In this assay, GOPC did not appear to cause increased association (Fig. 8B) despite its strong self-association via its C-terminal coiled-coil domain (11, 20). Similarly, EBP50, which also self-associates, did not appear to mediate interaction of ClC-3B with the tail of CFTR (Fig. 8B). This result is consistent with the fact that both channel proteins are bound primarily by the same PDZ domain of EBP50 (Fig. 5A). Overall, these results show that PDZK1 can mediate interactions between ClC-3B and CFTR. However, in our experiments the two channels are seen to co-localize only in the Golgi where a very small portion of cellular CFTR resides (Fig. 9). Although PDZK1 seems to cause some movement of ClC-3B from the Golgi, the co-localization with CFTR at the surface shown by Ogura et al. (6) was not detected in our experiments. This does not exclude the possibility that it may occur under different conditions. Nevertheless, the most consistent observation is of the co-localization of much of the ClC-3B pool with a small proportion of the total CFTR pool in a Golgi compartment.

DISCUSSION

Since CFTR and ClC-3B, members of every different protein families, are both chloride channels with C termini that bind class I PDZ domains, it is reasonable to ask what may be the significance, if any, of this common feature. This is of particular interest from the perspective of the proposal of Ogura et al. (6) that the two channel proteins may interact via the PDZ protein, EBP50, enabling CFTR to regulate the ClC-3B channel. The sites of localization of CFTR in cells are reasonably well established (21–23) as are certain aspects of its trafficking (22, 23). The bulk of the protein resides at the apical plasma membrane of epithelial cells in which it is endogenously expressed with small but detectable amounts associated with intracellular membranes in the secretory pathway (22). When expressed heterologously in non-polar mammalian cells, most of the mature protein is at the plasma membrane but significant amounts are intracellular, most in the ER (14). These major
features of CFTR localization have been observed by many investigators and are amply illustrated in Figs. 4C, 6, and 9.

The only novel feature of CFTR localization arose from our focus on the Golgi because of the finding that ClC-3B is present primarily at the Golgi. A small but readily detectable amount of CFTR, when heterologously expressed, co-localized with ClC-3B at the Golgi (Fig. 9). This is worthy of note only because most investigators have not reported on CFTR in the Golgi and those who have (22) emphasized a lesser amount there than in the ER. Indeed, in Calu-3 epithelial cells in which CFTR is endogenously expressed, only weak signals are detected in any intracellular compartment (Fig. 4).

In contrast to CFTR, localization of the newly discovered ClC-3B has not previously been extensively characterized. The original work of Ogura et al. (6) showed only that most of the protein was intracellular with a small amount appearing at the leading surface of spreading cells in which EBP50 was also overexpressed. We have now shown that virtually all of the ClC-3B either heterologously expressed in BHK or HEK 293 cells or endogenously expressed in Calu-3 epithelial cells resides in the Golgi, separate from but nearly contiguous with ClC-3A, which is in late endosomes (Fig. 2). Both ClC-3 isoforms must at least transit the Golgi since they acquire complex N-linked oligosaccharide chains (Fig. 1C). ClC-3A has been found to reside in endosomal compartments (3, 4). The fact that ClC-3B remains Golgi-associated whereas ClC-3A does not would seem likely due to the PDZ-binding capacity of the former. Several Golgi-associated PDZ proteins are known including the GRASPs (24), Mints (25), GIPC (26), and GOPC (27). We focused on GOPC since Cheng et al. (19) recently have shown that it binds and influences the localization and turnover of CFTR. We confirmed these findings with CFTR and found that overexpression of GOPC had a similar influence on ClC-3B (Figs. 6 and 7). Although the mechanisms involved are not yet clear, these responses of the two PDZ-binding channel proteins is consistent with a growing body of evidence that GOPC plays an important role in vesicular trafficking in the secretory and endocytic pathways. It was discovered by its binding to the C terminus of a member of the frizzled family of cell surface WNT receptors and co-localized with a portion of the population of these receptors at the Golgi (27). Independently, the second coiled-coil domain of GOPC was found to bind TC-10, a member of the Rho-GTPase family involved in the regulation of the endocytic pathway (11). The same domain also interacts with the Golgi membrane protein, syntaxin 6, suggesting a relationship of GOPC with SNARE-mediated membrane recognition and fusion (20). Very recently the PDZ domain of GOPC was found to interact with a neurodegenerative mouse mutant of the PDZ-binding GluR2 glutamate ion channel, and its C-terminal coiled-coil domain with Beclin1, a factor

![Fig. 8. Interaction of CIC-3B and CFTR. A, co-immunoprecipitation of PDZK1 and CIC-3B or CFTR. PDZK1 was immunoprecipitated from solubilized membranes prepared from BHK-21 cells expressing CIC-3B or CFTR alone or with PDZK1. CIC-3B or CFTR were detected by immunoblotting. B, influence of overexpression of PDZK1, GOPC, and EBP50 on the association of CIC-3B with the C-terminal tail of CFTR. A fusion of the last 40 amino acids of CFTR (1440–1480) and GST was bound to glutathione-Sepharose beads and incubated with lysates or solubilized membranes from BHK-21 cells expressing CIC-3B with or without PDZK1 or EBP50. To avoid the down-regulation of CIC-3B in the presence of co-expressed GOPC two different membrane preparations from cells overexpressing CIC-3B or GOPC were combined and solubilized to test the influence of GOPC on the association of CIC-3B with the C terminus of CFTR.

![Fig. 9. Co-localization of a small pool of CFTR with CIC-3B, CFTR, CIC-3B, and EBP50, PDZK1, or GOPC and were transiently expressed in BHK-21 cells. CFTR and CIC-3B were visualized by immunofluorescence as described under "Experimental Procedures."
promoting autophagy where cellular constituents are trafficked to lysosomes and degraded (28). Hence, GOPC appears capable of more generally shifting the balance of PDZ-binding membrane proteins in the degradative direction. The other important clue to GOPC function comes from the knock-out of its gene in mice which results in failure of acrosome formation, a Golgi-dependent function, during spermatogenesis without detectable effect on other tissues (29).

While GOPC, which is known to self-associate via its coiled-coil domains (11, 27) binds and co-localizes with some CTER and CIC-3B molecules, we did not find evidence that it promoted association between them (Fig. 8). Under special circumstances favoring dimerization instead of interactions with components of the trafficking machinery, GOPC could theoretically promote association of CIC-3B and CTER. However, its influence seems more likely to be on the fate of each individually under normal circumstances, in that both are severely knocked down by its overexpression. Other Golgi PDZ proteins, including those mentioned above, also have been proposed to play a role in the trafficking and biosynthetic sorting of other PDZ-binding proteins (24–26) and may also with these chloride channels. How such a multiplicity of potential interactions within a specific organelle are regulated is not yet understood.

Similarly, PDZ protein binding alone clearly does not entirely determine the major localizations of CTER and CIC-3B in cells since, in addition to GOPC, they both bind EBP50 and PDZK1, which reside primarily at the cell surface. Interactions of CTER with these promotes its endocytic recycling and, hence, residence in the apical membrane of epithelial cells (30). CTER and CIC-3B bind to different PDZ domains of PDZK1 and it is able to promote association of the two channels (Fig. 8). PDZK1, which normally has a very similar localization as CTER, some does redistribution of CIC-3B toward more peripheral regions of cells (Figs. 6 and 9), but we have been unable to detect any in the plasma membrane on co-expression with either PDZK1 or EBP50. Neither PDZK1 nor EBP50 knocked down the amount of CIC3B or CTER as GOPC did.

As mentioned above, while promotion of cell surface retention of CTER is attributed to interaction with PDZ proteins such as EBP50 at that location (30), this clearly does not occur with CIC-3B. Additional factors must be at play in determining the primary steady-state localization of the two channel proteins. Although CIC-3A was not the primary focus of our experiments it was necessary to precisely correlate its localization with that of a number of markers of different intracellular membrane compartments to clearly distinguish it from CIC-3B. This result (Fig. 2) showed a late endosomal localization in excellent agreement with the findings of Strobrawa et al. (3) and Li et al. (4). This is significant for at least two reasons. First, there have been several claims of heterologously expressed CIC-3A at the cell surface (31, 32). However, these findings when made with functional assays of channel activity may have reflected endogenous channels (33) and when made by immunofluorescence in some cases used a commercial antibody that recognized proteins other than CIC-3A (1). Li et al. (4) have reported that small amounts of an N-terminally truncated version of CIC-3A does reach the cell surface. Both swallowing (31) and calcium/calmodulin kinase (32) activated chloride channels attributed to CIC-3A in some of these studies are not altered in cells from mice in which the CIC-3 gene was knocked out (3).

Second, since there is new evidence that CIC-3A functions as an anion shunt to increase the rate of endosomal acidification (3, 4), CIC-3B, which is identical in sequence except at the C terminus, probably has a similar function in Golgi membranes.

Overall, our present study has clearly established that the CIC-3B isoform is a Golgi channel where it may function as an anion shunt during acidification as CIC-3A does in late endosomes (3, 4). While this possibility remains to be rigorously tested, if confirmed it would also fit well with this general function of the other members of the CIC-3, -4, -5 branch of the mammalian CIC family (1). Our findings do not preclude the possibility that a small amount of CIC-3B might traffic to the cell surface and perhaps even be influenced by CTER under some circumstances as suggested by Ogura et al. (6). Mohamed-Sian et al. (34) have reported immunolocalization of CIC-4 to the apical surface of epithelial cells in intestines. Appearance of CIC-3B at the cell surface could require the involvement of a β-subunit as is the case with CIC-Ka, where barttin plays this role (35) or interference with recognition of a PY internalization and degradation motif, as is the case with CIC-5 (36). However, our findings do not support the idea that interactions with subplasma membrane PDZ proteins such as EBP50 (or PDZK1) bring about either cell surface localization or association with CTER at the plasma membrane.

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REFERENCES

1. Jentsch, T. J., Stein, V., Weinreich, F., and Zdebik, A. A. (2002) Physiol. Rev. 82, 503–558.
2. Estevez, R., and Jentsch, T. (2002) Curr. Opin. Struct. Biol. 12, 531.
3. Strobrawa, S. M., Breiderthoff, T., Takamori, S., Engel, D., Schweizer, M., Zdebik, A. A., Boel, M. R., Ruether, K., Jahn, H., Draughn, A., Jahn, R., and Jentsch, T. J. (2001) Neuron 29, 185–196.
4. Li, X., Wang, T., Zhao, Z., and Weimann, S. A. (2002) An. J. Physiol. Cell Physiol. 282, C1483–C1491.
5. Iyer, R., Iverson, T. M., Accardi, A., and Miller, C. (2002) Nature 419, 715–718.
6. Ogura, T., Furukawa, T., Toyozaki, T., Yamada, K., Zheng, Y. J., Katayama, Y., Nakaya, H., and Inagaki, N. (2002) FASEB J. 16, 863–865.
7. Hanrahan, J. H., Gentzsch, M., and Riordan, J. R. (2002) in ABC Proteins from Bacteria to Man (Holland, B., Cole, P. S. P., Kuchar, K., and Higgins, C. F., eds) pp. 589–618, Academic Press Ltd., London.
8. Prizzell, R. A. (ed) (1999) Physiology of Cystic Fibrosis Vol. 79.
9. Hou, Y.-X., Cui, L., Riordan, J. R., and Chang, X.-B. (2000) J. Biol. Chem. 275, 26290–26297.
10. Chen, C., and Oyakawa, H. (1987) Mol. Cell. Biol. 7, 2745–2752.
11. Neudauer, C. L., Jolberty, G., and Macara, I. G. (2001) Biochem. Biophys. Res. Commun. 290, 541–547.
12. Jensen, T. C., Luo, M. A., Pind, S., Williams, D. B., Goldberg, A. L., and Riordan, J. R. (1995) Cell 83, 129–135.
13. Aleksandrov, L., Menges, A., Chang, X.-b., Aleksandrov, A., and Riordan, J. R. (2001) J. Biol. Chem. 276, 12913–12923.
14. Luo, M. A., Jensen, T. C., Cui, L., Hou, Y.-X., Chang, X.-B., and Riordan, J. R. (1998) EMBO J. 17, 6879–6887.
15. Hung, A. Y., and Sheng, M. (2002) J. Biol. Chem. 277, 5699–5702.
16. Dutzler, R., Campbell, E. B., Cadene, M., Chait, B. T., and MacKinnon, R. (2002) Nature 415, 287–294.
17. Short, D. R., Trotter, K. W., Recek, D., Kreda, S. M., Bretsch, A., Boucher, R. C., Stutts, M. J., and Milgram, S. L. (1998) J. Biol. Chem. 273, 19797–19801.
18. Wang, S., Yue, H., Derin, R. B., Guggino, W. B., and Li, M. (2000) Cell 103, 169–179.
19. Cheng, J., Moyer, B. D., Milewski, M., Loffing, J., Ikeda, M., Mickel, J. E., Costing, G. R., Li, M., Stanton, B. A., and Guggino, W. B. (2002) J. Biol. Chem. 277, 35260–35269.
20. Charest, A., Lane, K., McMahon, K., and Housman, D. E. (2001) J. Biol. Chem. 276, 29456–29456.
21. Kartesz, N., Augustainas, O., Jensen, T. J., Naismith, A. L., and Riordan, J. R. (1992) Nat. Genet. 1, 321–327.
22. Bannyahe, S. I., Bannyahe, G. L., Fish, K. N., Moyer, B. D., Riordan, J. R., and Balch, W. E. (2000) Traffic 2, 802–870.
23. Yoo, J. S., Moyer, B. D., Bannyahe, S., Yoo, H. M., Riordan, J. R., and Balch, W. E. (2002) J. Biol. Chem. 277, 11401–11409.
24. Ku, A., Zhong, C., Lane, W. S., and Derynck, R. (2000) EMBO J. 19, 6427–6439.
25. Biederer, T., Cao, X., Sudhof, T. C., and Liu, X. (2002) J. Neurosci. 22, 7340–7351.
26. Liu, T. F., Kandal, G., and Setahuri, V. (2001) J. Biol. Chem. 276, 35768–35777.
27. Yao, R., Maeda, T., Takada, S., and Noda, T. (2001) Biochem. Biophys. Res.
32. Huang, P., Liu, J., Di, A., Robinson, N. C., Musch, M. W., Kaetzel, M. A., and Nelson, D. J. (2001) J. Biol. Chem. 276, 20093–20100

33. Weylandt, R. H., Valverde, M. A., Nobles, M., Raguz, S., Amey, J. S., Diaz, M., Nastrucci, C., Higgins, C. F., and Sardini, A. (2001) J. Biol. Chem. 276, 17461–17467

34. Mohammad-Panah, R., Ackerley, C., Rommens, J., Choudhury, M., Wang, Y., and Bear, C. E. (2002) J. Biol. Chem. 277, 566–574

35. Estévez, R., Boettger, T., Stein, V., Birkenhager, R., Otto, E., Hildebrandt, F., and Jentsch, T. J. (2001) Nature 414, 558–561

36. Schwake, M., Friedrich, T., and Jentsch, T. J. (2001) J. Biol. Chem. 276, 12049–12054

37. Shimada, K., Li, X., Xu, G., Nowak, D. E., Shewalter, L. A., and Weinman, S. A. (2000) Am. J. Physiol. Gastrointest. Liver Physiol. 279, G268–G276
The PDZ-binding Chloride Channel ClC-3B Localizes to the Golgi and Associates with Cystic Fibrosis Transmembrane Conductance Regulator-interacting PDZ Proteins
Martina Gentzsch, Liying Cui, April Mengos, Xiu-bao Chang, Jey-Hsin Chen and John R. Riordan

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