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Proteome of the porosome complex in human airway epithelia: Interaction with the cystic fibrosis transmembrane conductance regulator (CFTR)

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Abstract

The surface of the airways is coated with a thin film of mucus composed primarily of mucin, which is under continuous motion via ciliary action. Mucin not only serves to lubricate the airways epithelia, but also functions as a trap for foreign particles and pathogens, thereby assisting in keeping the airways clean and free of particulate matter and infections. Altered mucin secretion especially increased mucin viscosity, results in mucin stagnation due to the inability of the cilia to propel them, leading to infections and diseases such as cystic fibrosis (CF). Since porosomes have been demonstrated to be the secretory portals at the cell plasma membrane in cells, their presence, structure, and composition in the mucin-secreting human airway epithelial cell line Calu-3 expressing CF transmembrane receptor (CFTR), were investigated. Atomic force microscopy (AFM) of Calu-3 cells demonstrates the presence of approximately 100 nm in diameter porosome openings at the plasma membrane surface. Electron microscopy confirms the AFM results, and tandem mass spectrometry and immunoanalysis performed on isolated Calu-3 porosomes, reveal the association of CFTR with the porosome complex. These new findings will facilitate understanding of CFTR–porosome interactions influencing mucous secretion, and provide critical insights into the etiology of CF disease.

Biological significance—In the present study, the porosome proteome in human airway epithelia has been determined. The interaction between the cystic fibrosis transmembrane conductance regulator (CFTR) and the porosome complex in the human airway epithelia is further demonstrated. The possible regulation by CFTR on the quality of mucus secretion via the porosome complex at the cell plasma membrane is hypothesized. These new findings will facilitate understanding of CFTR–porosome interactions influencing mucous secretion, and provide critical insights into the etiology of CF disease.

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Conflict of interest

The authors declare no competing financial interests or conflicts.

Keywords

Human airway epithelia porosome; proteome; Porosome–CFTR interaction; Mucus secretion; Tandem mass spectrometry

1. Introduction

It is well established that cellular organelles called *porosomes*, are secretory portals present at the cell plasma membrane in neurons, exocrine, endocrine, and neuroendocrine cells, where membrane-bound secretory vesicles transiently dock and fuse to expel intravesicular contents from cells during secretion [1–10]. Porosomes have been immunoisolated from a number of cells, including the exocrine pancreas [5,6] and neurons [3]. The morphology, composition, and reconstitution of porosomes in the exocrine pancreas and in neurons are well documented [2–11], and the 3D contour map of the assembly of proteins within the structure has also been determined in great detail [10]. This new understanding of the secretory machinery in cells now provides a platform to address diseases resulting from secretory defects. In the current study, the structure and composition of the porosome in the mucin-secreting human airway epithelial cell line Calu-3 expressing cystic fibrosis (CF) transmembrane regulator (CFTR) were determined to better understand cystic fibrosis disease.

The cystic fibrosis transmembrane conductance regulator (CFTR) is a plasma membrane chloride selective cyclic AMP-activated ion channel, localized at the apical membrane of secretory epithelial cells, including the conducting airways [12]. Besides mediating the secretion of Cl^- , CFTR also regulates several other transport proteins, including K^+ channels, aquaporin water channels, anion exchangers, the membrane fusion protein syntaxin-1A, and sodium bicarbonate transporters [13–25]. Accordingly, studies show that CFTR and its associated proteins are present in large macromolecular signaling complexes via scaffolding proteins containing PDZ domains [12,24,26]. In cells, a number of proteins contain a conserved 80–100 amino acid sequence, called the PDZ domain, that mediates protein–protein interactions by binding to short peptide sequences of target proteins [12]. PDZ is an acronym for the first letters of three proteins originally discovered to share this domain: postsynaptic density protein (PSD95), drosophila disk large tumor suppressor (Dlg1), and the zonula occludens-1 protein (zo-1) [26]. The C-terminus of CFTR in humans contains the sequence Asp–Thr–Arg–Leu, that mediates binding to several PDZ domain proteins [12]. In addition, CFTR has several other regions that mediate protein–protein interactions, such as a domain at its N-terminus that binds to syntaxin-1A and SNAP-23 [14,22]. CFTR also contains a protein phosphatase-2A (PP2A)-binding, and an AMP kinase (AMPK)-binding domain [12]. Similarly, CFTR has a regulatory domain that is a substrate to both protein kinases A (PKA) and C (PKC) [27]. These interactions facilitate CFTR to form large CFTR-associated macromolecular signaling complexes at the plasma membrane.

CF as a disease was first identified as cysts observed in the pancreas and the highly viscous mucus found in the lung of patients. However, since discovery that these observed defects are a result of a dysfunction of the CFTR chloride channel [28,29], there has been little progress in our understanding of the link between CFTR dysfunction and the secretion of such highly viscous mucin in the lung of CF patients [30]. The surface of the airways is coated with a thin film of mucous composed of essentially mucin, salt, proteases, antioxidants, and antibodies [30,31]. Mucin lubricates, traps foreign particles and pathogens, and assists in the clearance of foreign particles from the airways via ciliary transport [30,31]. The major composition of the mucus layer are mucins, which are large glycoprotein biopolymers, synthesized and secreted by specialized cells of the airway epithelium called

goblet cells and submucosal mucus secreting cells [32,33]. Mucins are O-linked glycoproteins containing long stretches of polypeptides rich in serine and threonine residues, which serve as sites for the covalent attachment of a variety of O-glycans, many of which are sialylated or sulfated. Consequently, mucins are polyionic and typically 70% of their mass is represented by carbohydrate [31]. The glycan chains, which are usually clustered in the central domains of mucin, confer important structural and functional properties to the molecule such as hydration via ion and water binding, as well as confer resistance to proteases [31]. In addition to glycosylated domains, a subset of mucins possess cysteine-rich domains for disulfide-mediated polymerization; critical for gel formation. Hence, both hydration and disulfide-mediated polymerization are critical for regulating viscosity of the mucus layer. Therefore, a key property of mucus is its appropriate viscosity that enables its movement by the underlying cilia. Secretion of more viscous mucus disallows its proper transport, resulting in chronic and fatal airway disease such as CF [31].

Similar to other secretory cells that undergo secretory vesicle volume increase during secretion [34–44], goblet cells of the airway epithelia that store mucin in a dehydrated state within membrane-bound secretory granules are no exception. Since vesicle swelling is a requirement for cell secretion [40], and both ion channels and water channels or aquaporins regulate this process [41,43], altered chloride transport would impair secretory vesicle hydration and optimal release. Furthermore, recent studies in mice lacking functional CFTR [30] showed that these animals secrete highly viscous mucous that adhered to the epithelium. Since CFTR is known to interact with syntaxin-1A, chloride channel CLC-3, and aquaporins [13–25], which are components of the porosome complex [1,2,5–7,45], the possible interactions between CFTR and the porosome in goblet cells were hypothesized and tested in the present study. Results from the study demonstrate the presence of approximately 100 nm in size porosomes and microvilli at the surface of the plasma membrane in Calu-3 cells. Proteomic analysis of isolated porosomes using mass spectrometry demonstrates the presence of CFTR as well as several proteins found in the neuronal porosome complex, including Syntaxin-1A, actin, rabs, heterotrimeric G-protein, and the GTPase activating protein GAP. Immunoblot analysis of the isolated porosome complex, further confirms its association with CFTR, reflecting important implication in both normal mucus secretion in the airway epithelium in health, and in the impaired state in CF disease.

2. Materials and methods

2.1. Calu-3 cell culture

Airway epithelial Calu-3 cells are derived from a human lung adenocarcinoma [46]. The cells were grown in Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F-12) (Invitrogen) containing 15% fetal bovine serum. The cells were incubated in a humidified atmosphere at 37 °C and 5% CO₂.

2.2. HEK cell culture and transfection for CFTR expression

HEK 293 cells were cultured in Dulbecco's Modified Eagle's (DMEM) (Invitrogen) supplemented with 10% fetal bovine serum. All cells were maintained in a humidified atmosphere containing 5% CO₂ at 37 °C. Cells were transiently transfected using Lipofectamine 2000 (Invitrogen). Transfections involving co-expression were carried out at 2.5:1 plasmid molar ratio. Two days after transfection, the cells were harvested for use in the study.

2.3. Atomic force microscopy

Intact aldehyde-fixed Calu-3 cells grown on glass coverslips were used for imaging using the AFM. Cells were partially dehydrated by 10 min of air-drying at RT, and imaged using a modification of our previously published procedure [47]. A Nanoscope IIIa AFM from Digital Instruments (Santa Barbara, CA) was used for imaging. Images were obtained using the “tapping” mode in air, and aluminum coated silicon tips with a spring constant of 40 N m⁻¹, and an imaging force of <200 pN, line frequencies of 1–2 Hz, with 256 lines per image, and constant image gains. The topographical dimensions of cellular structures were analyzed using the Nanoscope IIIa4.43r8 software supplied by Digital Instruments.

2.4. Electron microscopy

Transmission electron microscopy of Calu-3 cells was performed as described in a previously published procedure [47], on both resting and stimulated (10 μM forskolin for 10 min) Calu-3 cells. Briefly, resting and stimulated cells were fixed in 2% glutaraldehyde/2% paraformaldehyde in ice-cold PBS for 24 h, washed, embedded in 2% SeaPrep agarose, followed by post-fixation for 1 h at 4 °C using 1% OsO₄ in 0.1 M cacodylate buffer. Finally, the samples were dehydrated in a graded series of ethanol, through propylene oxide, and infiltrated and embedded in Spurr's resin. Ultrathin sections were cut with a diamond knife, retrieved onto 200 mesh nickel thin-bar grids, and contrasted with alcoholic uranyl acetate and lead citrate. Grids were viewed with a JEOL 1400 transmission electron microscope (JEOL USA, Inc., Peabody, MA) operating at 60 or 80 kV, and digital images were acquired with an AMT-XR611 11 megapixel ccd camera (Advanced Microscopy Techniques, Danvers, MA).

2.5. Immunoisolation of the porosome complex from Calu-3 cells

The establishment of continuity between the secretory vesicle membrane and the membrane at the porosome base requires the participation of specific membrane proteins called SNAREs. At the nerve terminal for example, target membrane proteins SNAP-25 and syntaxin, collectively called t-SNAREs present at the base of the neuronal porosome complex, and synaptic vesicle-associated protein v-SNARE, are involved in fusion of synaptic vesicles at the porosome base [9]. Both SNAP-25 and SNAP-23 are expressed in Calu-3 cells, and syntaxin-1A which interacts with SNAP-25 has also been reported with CFTR [14]. SNAP-25 antibody was employed to immunoisolate porosomes from Calu-3 cells. To isolate the Calu-3 porosome complex, SNAP-25 specific antibody conjugated to protein A-sepharose® was utilized. For each immunoisolation, 1 mg of Triton–Lubrol-solubilized Calu-3 cells was used. The Triton/Lubrol solubilization buffer contained 0.5% Lubrol, 0.5% Triton X-100, 1 mM benzamidine, 5 mM Mg-ATP, and 5 mM EDTA in PBS at pH 7.5, supplemented with protease inhibitor mix (Sigma, St. Louis, MO). Ten micrograms of SNAP-25 antibody conjugated to the protein A-sepharose® was incubated with 1 mg of the solubilized cells for 1 h at room temperature followed by three washes of 10 volumes of wash buffer (500 mM NaCl, 10 mM Tris, 2 mM EDTA, pH 7.5). The immunoprecipitated sample attached to the immunosepharose beads was eluted using low pH buffer (pH 3.5) to dissociate the porosome complex from the antibody bound to the bead, and the eluted sample was immediately returned to neutral pH in a total volume of 300 μl. Eighty microliters of the sample was aliquoted and resuspended in Laemmli [48] reducing sample preparation buffer, boiled for 2 min, and used for SDS-PAGE and Western blot analysis. Two hundred microliters of the sample was aliquoted and stored at –80 °C for proteomics using mass spectrometry [11], and the remaining 20 μl was used for determining porosome size and charge using Zetasizer and zeta potential measurements [43].

2.6. Western blot analysis

Isolated Calu-3 porosomes in Laemmli buffer and 5 µg of total lysate of Calu-3 and HEK cells expressing CFTR also in Laemmli buffer were resolved in a 12.5% SDS-PAGE, followed by electrotransfer to either PVDF membrane or 0.2 mm nitrocellulose sheets. The membrane or nitrocellulose was incubated for 1 h at room temperature in blocking buffer (5% nonfat milk in phosphate buffered saline or PBS containing 0.1% Triton X-100 and 0.02% NaN₃) and immunoblotted for 2 h at room temperature with antibodies against CFTR (monoclonal antibody against CFTR #217 was from Cystic Fibrosis Foundation Therapeutics, Bethesda, MD), and porosome associated proteins syntaxin-1A, actin, SNAP-25, SNAP-23, Gai3, and vimentin (Santa Cruz Biotechnology Inc., Santa Cruz, CA), at a dilution of 1:1000 in blocking buffer. The immunoblotted nitrocellulose sheets were washed in PBS containing 0.1% Triton X-100, prior to incubation for 1 h at room temperature in horseradish peroxidase-conjugated secondary antibodies at a dilution of 1:5000 in blocking buffer. The immunoblots were washed in PBS containing 0.1% Triton X-100 and processed for enhanced chemiluminescence and exposure to X-Omat-AR film. The exposed films were then developed and photographed.

2.7. Immunofluorescence microscopy

To determine the distribution of CFTR and the porosome-associated protein Gai3 in Calu-3 cells, immunofluorescent studies were carried out according to published procedures [49]. To determine the position of the cell nucleus, cells were exposed to 50 µg/ml of the nuclear stain NucBlue™ Live Cell Stain (Molecular Probes, Life Technologies, Carlsbad, CA). Images were taken using an immunofluorescence FSX100 Olympus microscope.

2.8. Photon correlation spectroscopy (PCS) on isolated porosomes from Calu-3 cells

Calu-3 porosome size was determined using photon correlation spectroscopy (PCS). PCS is a well-known technique for the measurement of nm to µm size particles and macromolecules. PCS measurements were performed in a Zetasizer Nano ZS (Malvern Instruments, UK). In a typical experiment, the size distribution of isolated porosomes was determined using built-in software provided by Malvern Instruments. Prior to determination of the porosome hydrodynamic radius, calibration of the instrument was performed using latex spheres of known size. In PCS, subtle fluctuations in the sample scattering intensity are correlated across microsecond time scales. The correlation function was calculated, from which the diffusion coefficient was determined. Using the Stokes–Einstein equation, hydrodynamic radius can be acquired from the diffusion coefficient. The intensity size distribution, which is obtained as a plot of the relative intensity of light scattered by particles in various size classes, is then calculated from a correlation function using built-in software.

2.9. Tryptic digestion of isolated Calu-3 porosomes; LC–MS/MS analysis and database search

For proteomics analysis, aliquots of purified Calu-3 porosomes were reduced, cysteine-blocked with 25 mM methyl methanethiosulfonate (MMTS) and then digested with trypsin (1:10 w/w) overnight at 37 °C [50–54]. Digested porosome sample was analyzed by nanoLC–MS/MS. The tryptic peptides were separated on a reversed-phase C18 column with a 90 min gradient using the Dionex Ultimate™ HPLC system (Thermo Scientific, Barrington, IL). Then the MS and MS/MS spectra were acquired on an Applied Biosystems QSTAR XL mass analyzer using information dependent acquisition mode. A MS scan was performed from m/z 400–1500 for 1 s followed by product ion scans on two most intense multiply charged ions. Peaklists were submitted to Mascot server to search against the NCBI nr database for human sequences with methylthio (C) used as a fixed modification and oxidation (M), N-acetylation (protein N terminus) as variable modifications. Secondary

analysis was next performed using Scaffold (Proteome Software, Portland, OR). Minimum protein identification probability was set at 95% with 2 unique peptides at 95% minimum peptide identification probability.

3. Results and discussion

3.1. AFM demonstrates that Calu-3 cells possess porosomes or secretory portals for mucus secretion at the cell plasma membrane

The AFM was instrumental in the discovery of the porosome nearly two decades ago, first in the acinar cells of the exocrine pancreas [2,5–7], followed by chromaffin cells in the adrenal medulla [45], GH secreting cells of the pituitary [1], at the nerve terminal in brain tissue [3,4,10,11,55], in astrocytes [56,57], β -cells of the endocrine pancreas [58], hair cells of the inner ear [59], and in RBL-2H3 and BMMC cells [60], among others. Similarly in the current study, AFM was utilized to image at high resolution the surface topology of aldehyde-fixed and semi-dry Calu-3 cells. It is nearly impossible to image live Calu-3 cells since the secreted mucous adheres to the cantilever tip. Hence aldehyde-fixed cells that confirm extra rigidity to the cell surface were used for imaging using the AFM. High resolution AFM micrographs (Fig. 1) of the surface topology of aldehyde-fixed and semi-dry Calu-3 cells demonstrate the presence of approximately 100 nm in diameter porosome openings (102.4 ± 3.0), and similar in diameter (96 ± 3.3) microvilli, at the cell plasma membrane. Nearly the entire cell surface exposed to the medium was found covered with the microvilli, making it extremely difficult to image at high resolution the interspersed porosome openings despite the fact that the AFM images the surface topology of the cell at nanometer resolution and in three dimensions. The presence of dense microvilli at the plasma membrane also precluded imaging any intracellular structures such as the nucleus or the cytoskeleton underlying the cell plasma membrane. However, in certain areas of the cell surface devoid of microvilli or porosome openings, cytoskeletal structures (Fig. 1B) underlying the cell plasma membrane were observed.

3.2. Electron microscopy confirms the presence of microvilli and porosomes at the plasma membrane of Calu-3 cells

To further determine the porosome structure in Calu-3 cells, TEM was performed. In confirmation with AFM studies, results from the TEM study demonstrate the presence of dense microvilli at the cell plasma membrane (Fig. 2A), with the occasional presence of porosome structures (Fig. 2B–D). The two main reasons for observing so few porosome structures at the cell plasma membrane in the TEM micrographs of Calu-3 cells are that TEM images are in two dimensions as opposed to 3D in AFM micrographs; and second, the likelihood of obtaining a sagittal section through a 100 nm porosome complex scattered at the cell plasma membrane would be extremely rare. However, careful examination of the electron micrographs demonstrates the presence of cup-shaped porosomes at the cell plasma membrane. In Fig. 2D, one porosome is seen which has undergone a sagittal section, and a neighboring porosome to the left that has undergone a sagittal section at its base. Additionally, Calu-3 cells demonstrate the presence of electron-dense secretory products at the mouth of the porosome complex opening to the outside.

3.3. Immunoanalysis demonstrates the association of CFTR with the porosome complex in Calu-3 cells

Immunisolated Calu-3 porosome complexes demonstrate a particle size of approximately 300 nm (Fig. 3A) using PCS. Furthermore as hypothesized, immunoblot analysis of the isolated Calu-3 porosome complex (Fig. 3B) demonstrates the co-association of CFTR with the porosome that contains among other proteins Gai3, actin, and vimentin (Fig. 3C). Similarly, when CFTR was immunisolated from solubilized Calu-3 cells, the porosome

complex was also immunisolated with CFTR, as demonstrated by the presence of porosome-associated proteins syntaxin-1A, SNAP-25, SNAP-23, actin, and vimentin (Fig. 3D).

3.4. Mass spectrometry on the isolated Calu-3 porosome complex confirms the association of CFTR

Mass spectrometry on the immunisolated porosome complex from Calu-3 cells (Table 1), demonstrates the presence of various porosome-associated proteins (Table 2) as well as CFTR. In addition to assisting in identifying the porosome proteome in Calu-3 cells, results from this mass spectrometry study further confirm the interaction between CFTR and the porosome complex in the cell determined using immunoprecipitation and immunoblot analysis (Fig. 3).

3.5. Immunofluorescence microscopy demonstrates the interaction between CFTR and the porosome complex in Calu-3 cells

To further test the interaction between CFTR and the porosome in Calu-3 cells, double-labeling immunofluorescence microscopy using antibodies against CFTR and the porosome-associated GTP-binding protein Gai3 protein was performed (Fig. 4). CFTR and Gai3 immunoreactivity were found both at the cell plasma membrane and in the cytosol, especially the perinuclear region of the cell. On merger of the CFTR and the Gai3 immunostained micrographs, it was evident that besides the presence of independent CFTR and Gai3 immuno punctate regions, extensive co-distribution of both proteins is observed, including at the cell plasma membrane (Fig. 4). In the cytosol, the co-distribution of CFTR and Gai3 protein may represent CFTR-carrying vesicles, and at the cell plasma membrane, the co-presence of CFTR with the porosome complex. At the level of resolution afforded by conventional wide-field fluorescence, the results support CFTR and Gai3 proteins to co-distribute, further confirming results of the immunocolocalization of CFTR with the porosome-associated GTP-binding Gai3 protein in Calu-3 cells (Fig. 4).

In conclusion, we report for the first time that the human airway epithelial cell line Calu-3 known to express CFTR, possesses secretory portals at the cell plasma membrane called porosomes (Fig. 5). Determination of the Calu-3 cell porosome proteome demonstrates the presence of CFTR among other known porosome proteins. This new finding that porosomes in the human airway epithelia interact with CFTR will facilitate understanding of CFTR–porosome interactions influencing cellular secretion and provide critical insights into the etiology and treatment of CF disease. Further studies using CFTR knockout and siRNA [61] on mucin secretion will help establish the molecular mechanism of porosome–CFTR interaction in regulation of mucin secretion.

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Fig. 1.

AFM micrographs demonstrating the presence of microvilli and interspersed porosomes at plasma membrane in Calu-3 cells. Microvilli measuring on average 96 nm in thickness (mean ± SEM; 96 ± 3.3 , $n = 50$) are densely packed at the cell plasma membrane exposed to the medium, and demonstrated both in low (A–C) and high (D–H) resolution AFM images. Interspersed among the microvilli are the 102 nm in diameter openings (mean ± SEM; 102 ± 3 , $n = 50$) of possibly the secretory portals or porosomes shown in figure G (red and green arrowheads). Similarly, the microvilli shown in figure H (red and green arrowheads) demonstrate some that appear coiled around each other, possibly as a consequence of secreted mucus.

Fig. 2.

Representative electron micrographs of Calu-3 cells in culture demonstrating the presence of microvilli (MV) and porosomes (P) at the cell plasma membrane. (A) Calu-3 cells demonstrating the presence of dense microvilli and porosomes at the cell plasma membrane. (B–D) Note the flask-shaped porosomes measuring nearly 100 nm in diameter (E) and from 200 to 300 nm in depth, with openings to the cell surface (red arrowhead). In (C), what appears to be mucus is found at the opening of the porosome to the cell exterior. Of the two porosomes depicted in (D), the one to the center appears to be sectioned right through the center of the organelle, whereas the porosome to the left appears to have been sectioned at its base. (E) Similar to the AFM images in Fig. 1, the microvilli measure on average 92 nm in diameter.

Fig. 3.

Photon correlation spectroscopy (PCS) demonstrates the immunoprecipitated porosome complex from Calu-3 cells measuring on average 271.2 nm, and both immunoprecipitation and immunoblot analysis demonstrate the interaction of CFTR with the porosome complex in this cell. (A) PCS on isolated porosomes from Calu-3 cells demonstrates average size of 271.2 nm, and net charge of -14.4 mV. (B) Immunoblot analysis using CFTR-specific antibody of CFTR-expressing HEK cell proteins resolved using SDS-PAGE, followed by electrotransfer to nitrocellulose membrane, demonstrates the presence of a 180 kDa band representing CFTR (lane 1, positive control). SDS-PAGE resolved immunoprecipitated porosome complexes also demonstrate immunopositive for CFTR (lane 2). (C) Immunoblot analysis of the total Calu-3 cell homogenate (CH) and isolated porosome complex (P), demonstrates the presence of porosome proteins actin, Gai3, and vimentin. Note the enriched presence of the proteins in the porosome complex. (D) Similarly, immunoprecipitated CFTR complex using the CFTR-specific antibody, results in pull-down of porosome associated proteins such as syntaxin-1A (present as 70 kDa t-/v-SNARE complex), SNAP-25 (present as 70 kDa t-/v-SNARE complex), SNAP-23 (present as 68 kDa t-/v-SNARE complex), and actin.

Fig. 4.

Immunofluorescence microscopy demonstrating the co-distribution of CFTR (green) with porosome-associated GTP-binding protein Gai3 (red) in Calu-3 cells. Note the punctate immunostaining for CFTR and Gai3-specific antibody in both the cytosol and the plasma membrane. To determine co-distribution of CFTR (green) with porosome-associated Gai3 protein (red), the images are merged (Merge), expressing yellow at co-localization sites. The nucleus is labeled blue in the merged micrograph. Cells imaged using brightfield microscopy (BF) are demonstrated in the lower right micrograph. In the cytosol, the co-distribution of CFTR and Gai3 protein may represent CFTR-carrying vesicles, and at the cell plasma membrane, the co-presence of CFTR with the porosome complex, at the level of resolution afforded by conventional wide-field fluorescence.

Fig. 5. Schematic drawing showing interactions between the cystic fibrosis transmembrane conductance regulator (CFTR) and the porosome complex at the cell plasma membrane in human airway epithelia. The possible regulation by CFTR on the quality of mucus secreted via the porosome complex, such as highly viscous mucus resulting from CFTR defects is hypothesized.

Table 1

Major proteins identified in Calu-3 cell porosome proteome by LC-MS/MS and Western Blot [*] analysis. SNAP-25 immunisolated porosome complexes were obtained using 1% Triton-Lubrol-solubilized Calu-3 cells.

Gene symbol	MW	Calu-3 cell porosome proteins identified using LC-MS/MS	Calu-3 porosome proteins identified using western blots
ACTB	42 kDa	Actin, cytoplasmic 1	*
VIME	54 kDa	Vimentin	
ANXA2	39 kDa	Annexin 2A	
FLNA	283 kDa	Filamin-A	
GNAI	40 kDa	Guanine nucleotide binding protein G(i) alpha inhibiting activity	*
TBB5	50 kDa	Tubulin beta chain	
TBA1A	50 kDa	Tubulin alpha-1A chain	
STX1A	33 kDa	Syntaxin-1A	*
PROF1	15 kDa	Profilin-1	
VDAC1	31 kDa	Voltage-dependent anion-selective channel protein 1	
EZRI	69 kDa	Ezrin	
SPTN1	285 kDa	Spectrin alpha chain	
SPTB2	275 kDa	Spectrin beta chain	
ANXA3	36 kDa	Annexin A3	
TAGL2	23 kDa	Transgelin-2	
VDAC3	31 kDa	Voltage-dependent anion-selective channel protein 3	
CLIC1	27 kDa	Chloride intracellular channel protein 1	
RAB1A	23 kDa	Ras-related protein Rab-1A	
RAB3C	26 kDa	Ras-related protein Rab-3C	
MYL6	17 kDa	Myosin light polypeptide 6	
MYH9	227 kDa	Myosin-9	
CALM	17 kDa	Calmodulin	
RTN4	130 kDa	Reticulon-4	
G3BP1	52 kDa	Ras GTPase-activating protein-binding protein 1	
ARF3	21 kDa	ADP-ribosylation factor 3	
MOES	68 kDa	Moesin	
COF1	19 kDa	Cofilin-1	
ASAP2		Arf-GAP with SH domain, ANK repeat and PH domain-containing protein 2	
P4R3A		Serine/threonine-protein phosphatase 4	
RASA1	117 kDa	Ras GTPase-activating protein 1	
PTN14	136 kDa	Tyrosine-protein phosphatase non-receptor type 14	
CFTR	168 kDa	Cystic fibrosis transmembrane conductance regulator	
CLC3	85 kDa	Chloride channel protein 3	*
SNP25	25 kDa	Synaptosomal-associated protein 25	*

Table 2

Porosome proteins common to both Calu-3 cells and neurons.

Gene symbol	MW	Some proteins common to Calu-3 porosome and the neuronal porosome complex
ACTB	42 kDa	Actin, cytoplasmic 1
GNA	40 kDa	Guanine nucleotide-binding G-protein subunit alpha
RAB3	25 kDa	Ras-related protein Rab-3
RTN4	126 kDa	Reticulon-4
SNP25	25 kDa	Synaptosomal-associated protein 25
STX1A	33 kDa	Syntaxin-1A
TBA1A	50 kDa	Tubulin alpha-1A chain Tubulin beta chain Spectrin beta GTPase activating protein Myosin heavy polypeptide 1