Cystic Fibrosis Transmembrane Regulator Regulates Uptake of Sphingoid Base Phosphates and Lysophosphatidic Acid

MODULATION OF CELLULAR ACTIVITY OF SPHINGOSINE 1-PHOSPHATE

Sphingolipids have been implicated in the regulation of cell growth, differentiation, and programmed cell death. Sphingosine 1-phosphate (SPP) has recently emerged as an important lipid messenger and a ligand for the endothelial differentiation gene receptor family of proteins through which it mediates its biologic effects. Recent studies in Saccharomyces cerevisiae in our laboratory implicated the yeast oligomycin resistance gene (YOR1), a member of the ATP binding cassette family of proteins, in the transport of SPP. The cystic fibrosis transmembrane regulator is a unique member of the ATP binding cassette transporter family and has high homology with YOR1. We therefore set out to investigate if this member of the family can regulate SPP transport. We demonstrate that C127/cystic fibrosis transmembrane regulator (CFTR) cells, expressing wild type CFTR, exhibited significantly higher uptake of sphingosine 1-phosphate than either cells expressing a mutant CFTR C127/ΔF508 or C127/mock-transfected cells. This effect was specific, dose-dependent, and competed off by dihydrosphingosine 1-phosphate and lysophosphatidic acid. There was no difference in uptake of sphingosine, C16-ceramide, sphingomyelin, lysophosphoinositol, phosphatidylinositol, or phosphatic acid among the different cell lines. Pretreatment with forskolin or isobutylmethylxanthine to stimulate cAMP did not affect the uptake in any of the cell lines. Moreover, we found that mitogen-activated protein kinase activation by SPP was less responsive in C127/CFTR as compared with C127/mock-transfected cells, suggesting that uptake of SPP by CFTR may divert it from interacting with its cell surface receptors and attenuate signaling functions. Taken together, these data implicate CFTR in uptake of SPP and the related phosphorylated lipids dihydrosphingosine 1-phosphate and lysophosphatidic acid. This uptake influences the availability of SPP to modulate biologic activity via endothelial differentiation gene receptors. These studies may have important implications to cystic fibrosis.

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Sphingolipids have been implicated in the regulation of cell growth, differentiation, and programmed cell death. Sphingosine 1-phosphate (SPP) has recently emerged as an important lipid messenger and a ligand for the endothelial differentiation gene receptor family of proteins through which it mediates its biologic effects. Recent studies in Saccharomyces cerevisiae in our laboratory implicated the yeast oligomycin resistance gene (YOR1), a member of the ATP binding cassette family of proteins, in the transport of SPP. The cystic fibrosis transmembrane regulator is a unique member of the ATP binding cassette transporter family and has high homology with YOR1. We therefore set out to investigate if this member of the family can regulate SPP transport. We demonstrate that C127/cystic fibrosis transmembrane regulator (CFTR) cells, expressing wild type CFTR, exhibited significantly higher uptake of sphingosine 1-phosphate than either cells expressing a mutant CFTR C127/ΔF508 or C127/mock-transfected cells. This effect was specific, dose-dependent, and competed off by dihydrosphingosine 1-phosphate and lysophosphatidic acid. There was no difference in uptake of sphingosine, C16-ceramide, sphingomyelin, lysophosphoinositol, phosphatidylinositol, or phosphatic acid among the different cell lines. Pretreatment with forskolin or isobutylmethylxanthine to stimulate cAMP did not affect the uptake in any of the cell lines. Moreover, we found that mitogen-activated protein kinase activation by SPP was less responsive in C127/CFTR as compared with C127/mock-transfected cells, suggesting that uptake of SPP by CFTR may divert it from interacting with its cell surface receptors and attenuate signaling functions. Taken together, these data implicate CFTR in uptake of SPP and the related phosphorylated lipids dihydrosphingosine 1-phosphate and lysophosphatidic acid. This uptake influences the availability of SPP to modulate biologic activity via endothelial differentiation gene receptors. These studies may have important implications to cystic fibrosis.

** The abbreviations used are: SPP, sphingosine 1-phosphate; dhSPP, dihydrosphingosine 1-phosphate; LPA, lysophosphatidic acid; EDG, endothelial differentiation gene; MAP, mitogen-activated protein; MRP, mitogen-activated protein kinase; ABC, ATP binding cassette; CFTR, cystic fibrosis transmembrane regulator; ERK, extracellular signal-regulated kinase; PDGF, platelet-derived growth factor; IBMX, 3-isobutyl-1-methylxanthine; PCR, polymerase chain reaction; ET, reverse transcription; wt, wild type; PBS, phosphate-buffered saline; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum.

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There is a high percentage of homology between Yor1 and CFTR based on amino acid sequence and hydropathy profile: 28 and 38.7%, respectively. Because of these similarities, we wondered if CFTR is involved in sphingolipid transport.

In this report we demonstrate that C127 murine epithelial cells transfected with wild type CFTR (C127/CFTR) exhibit significantly greater uptake of sphingosine 1-phosphate (SPP), dihydrosphingosine 1-phosphate (dhSPP), and lysophosphatidic acid (LPA) than C127/mock cells transfected with the vector only (C127/mock) or cells transfected with the ΔF508 mutant CFTR (C127/ΔF508). This effect is specific and dose-dependent. Pretreatment with forskolin or 3-isobutyl-1-methylxanthine (IBMX) did not affect the uptake in any of the cell lines. Moreover, MAP kinase activation by SPP was less responsive in C127/CFTR cells as compared with C127/mock cells. Therefore, we propose that CFTR regulates the uptake and cellular activity of SPP, dhSPP, and LPA.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture**—Murine C127 epithelial cells stably transfected with the wild-type human CFTR cDNA, the ΔF508 mutant CFTR, and a mock-transfected control line were from the laboratory of Dr. Jon Cohn. It has been demonstrated, using both molecular and functional approaches, that C127/CFTR wt and C127/CFTR ΔF508 cells express normal and mutated CFTR, respectively (12).

The cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies, Inc.) supplemented with 10% fetal bovine serum (Life Technologies, Inc.), 1% penicillin/streptomycin (Life Technologies, Inc.), 400 μg/ml Geneticin G418 (Sigma) at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. Quiescence was achieved by placing the 60–70% confluent cell cultures in DMEM with 0.1% FBS for 24–48 h.

**Lipid Uptake Studies**—C127 CFTR wt, CFTR ΔF508, and mock cells were seeded in 24-well plates and cultured as described earlier at 37 °C to obtain a final density of 8 × 10⁴ to 1.0 × 10⁵ cells/well. The cells were then incubated for 0–60 min with or without one of the following radiolabeled lipids: [3H]rhodamine-labeled sphingosine 1-phosphate (5 μCi), [3H]dihydrosphingosine 1-phosphate (5 μCi), [3H]sphingosine 1-phosphate (5 μCi), [3H]palmitic acid-1-ceramide (1 μCi), [3H]lysophosphatidic acid (1 μCi), [3H]lysophosphatidylcholine (5 μCi), [3H]lysophosphatidylcholine (5 μCi), [3H]lysophosphatidylinositol (5 μCi), or palmitic acid (5 μCi) at 37 °C in growth medium containing G418. The incubation with SPP was done with a mixture of cold SPP (0.01 μM, 0.5 μM, 1 μM) and [3H]SPP (5 μCi). At the desired time points, the medium was removed and cells washed with PBS. The cells were resuspended in 0.5 ml of PBS and counted in a liquid scintillation counter. In each experiment, two wells were kept for protein determination. The counts were adjusted to the amount of protein.

In additional experiments, cells were pretreated with or without IBMX (500 μM) or forskolin (30 μM) for 30 min prior to incubation with [3H]dihydrosphingosine 1-phosphate (0.5, 1, and 50 μM) for 0–60 min.

**Phospho-MAPK Immunoblots**—C127 wt and mock-transfected cells were seeded at a density of 2–4 × 10⁴ cells/well in T6 well plates. Quiescent cells were stimulated with SPP (0.1, 1, 2.5, and 5 μM) or PDGF-AB (10 ng/ml) for 10 min. In some cases cells were pretreated with PD098059 (10 ng/ml) for 60 min prior to stimulation with SPP. Cells were suspended in 150 μl of Laemmli sample buffer (13) and homogenized by repeatedly (three times) passing through a 0.24-mm gauge syringe. Protein samples (20–25 μg) were loaded on a 12% Ready-gel (Bio-Rad) and subjected to SDS-polyacrylamide gel electrophoresis. The separated proteins in the gel were transferred to polyvinylidene difluoride membranes (Bio-Rad) and immunoblotted with rabbit polyclonal pThr202/pTyr204-specific MAPK antibodies that specifically recognize Tyr202-phosphorylated (but not phosphoethanolated) p44MAPK and p44mAPK (New England Biolabs). The phospho-MAPK antibody was used at a 1:1000 dilution, whereas the control antibody, which recognizes total MAPK, was used at 1:2000 dilution. The membranes were incubated overnight with the antibodies in antibody buffer (PBS, 0.1% Tween 20, and 5% nonfat milk), washed, and exposed to anti-rabbit horseradish peroxidase-conjugated IgG (1:2500) in antibody buffer for 1 h. Immunoreactive bands were revealed using the ECL detection kit (Amersham Pharmacia Biotech) and subjected to autoradiography using Kodak Biomax-MR film.

**Growth Studies**—C127/CFTR wt and C127/mock cells were seeded at a density of 7 × 10⁴ cells/well at 15% confluency in DMEM supplemented with 0.1, 2, 5, or 10% fetal bovine serum. Duplexes were done for each well. At 24, 48, 72, and 96 h, the cells were washed with PBS, released with trypsin/EDTA, incubated with trypan blue, and counted using a hemocytometer.

**Isolation of RNA and RT-PCR**—Total RNAs were isolated from C127/wt CFTR and C127/mock-transfected cells with the RNeasy Mini kit (Qiagen) as described by the manufacturer. The integrity of RNA was confirmed by running them on denaturing agarose gel stained with ethidium bromide as described previously (14). Total RNA (1 μg) was then used in reverse transcriptase reactions to generate complementary DNA (cDNA) using Promega’s avian myeloblastosis virus reverse transcriptase with random primers (Promega) as described by the manufacturer. The resulting total cDNA was used in PCR using Taq polymerase (Qiagen) to measure the mRNA levels of EDG-1, -3, and -5 receptor genes. The primers (Integrated DNA Technologies) used for PCR amplification were: 5’-AGCCCTCTGCGAC-TCTTACCA-3’ and 5’-TTGGACCCACATACATCAATG-3’ for EDG-1 (15); 5’-CAGGGAGCAGTATGTGCTG-3’ and 5’-GGGTTACGGCGA-TTGAG-3’ for EDG-3 (15); 5’-CATTGGCATAGGGCCACACC-3’ and 5’-TGACTAGACAGCGCCACACC-3’ for EDG-5 (15); 5’-TGTC-CCAATGTGGCCACTA and 5’-GAAGATGTCGAATGTCC for rRNA (16).

PCR reactions were performed for 30 cycles with denaturation at 95 °C for 45 s, annealing at 58 °C for 45 s, and elongation at 72 °C for 50 s. PCR products (10 μl) were analyzed by agarose gel electrophoresis after staining with ethidium bromide. For EDG-5 we increased the number of cycles to 35, decreased the annealing temperature to 55 °C, and loaded 22 μl of PCR product. The mRNA levels of EDG-1, -3, and -5 receptor genes were normalized to the rRNA levels used as internal controls. The linearity of amplification for EDG-1, -3, and -5 r-RNA genes was determined separately by using increasing concentrations of cDNAs on each PCR.

**Results**

**CFTR Regulates Uptake of SPP**—To determine if CFTR affects the uptake of SPP, we evaluated the uptake of SPP in two lines of C127 murine cells: C127/CFTR cells transfected with wild type CFTR, and C127/mock cells transfected with empty vector. C127/CFTR cells exhibited significantly greater time-dependent uptake of [3H]SPP (1 μM) as compared with mock cells (Fig. 1A) whereby the difference in the uptake of [3H]SPP occurred at 15 min and continued to increase until 60 min. Therefore, the overexpression of CFTR enhances the uptake of SPP by cells.

The most common mutation in cystic fibrosis involves deletion of phenylalanine at position 508 and is named ΔF508, where the CFTR protein does not reach the cell membrane. We examined the uptake of SPP in C127/CFTR and C127/ΔF508 cells. Interestingly, the C127/ΔF508 cells demonstrated significantly lower uptake of [3H]SPP as compared with the C127/CFTR cells and appeared to resemble the mock-transfected cells (Fig. 1B).

Next, a dose-response study of [3H]SPP uptake was undertaken. Uptake of SPP in C127/CFTR cells increased with increasing concentration of SPP and reached a plateau at around 80 μM, possibly saturating the uptake mechanism (Fig. 1C). Taken together, these results demonstrate a time- and concentration-dependent uptake of [3H]SPP induced by CFTR overexpression.

**CFTR Regulates Uptake of dhSPP and LPA**—Further inves...
CFTR Regulates Uptake of Sphingoid Base Phosphates and LPA

**Experimental Procedures**

Effects of CFTR expression on uptake of SPP. A, C127/CFTR cells and C127/mock cells were evaluated for their ability to take up [3H]SPP as described under “Experimental Procedures” over the indicated time range. B, C127/CFTR cells and C127/AF508 cells were evaluated for [3H]SPP (1 μM) over time. C, C127/CFTR cells were evaluated for uptake with increasing concentrations of [3H]SPP. These data are representative of three separate experiments performed in duplicate.

Fig. 1. Effects of CFTR expression on uptake of SPP. A, C127/CFTR cells and C127/mutant cells were evaluated for their ability to take up [3H]SPP as described under “Experimental Procedures” over the indicated time range. B, C127/CFTR cells and C127/mock cells were evaluated for [3H]SPP (1 μM) over time. C, C127/CFTR cells were evaluated for uptake with increasing concentrations of [3H]SPP. These data are representative of three separate experiments performed in duplicate.

Tigations were performed to characterize SPP uptake by the CFTR protein. dhSPP, which lacks the double bond at the C4–C5 position of SPP, is a structurally related sphingolipid and a SPP receptor agonist. dhSPP has been shown to be as potent as SPP in activation of DNA synthesis, extracellular signal-regulated kinase (ERK) activation, and cell migration (17). We wondered if it was also taken up by CFTR as well. Similar to SPP, the uptake of [3H]dhSPP (1 μM) by C127/CFTR cells was significantly greater as compared with mock and mutant cells (Fig. 2A).

LPA and SPP are also structurally related lipid mediators that share the same receptor family, namely the EDG family of proteins, albeit with different specificities. We therefore next tested whether LPA was taken up as was SPP in C127/CFTR cells. The uptake of [3H]LPA in C127/CFTR cells was significantly greater as compared with mock and mutant cells at 1 μM (Fig. 2B). Similar to SPP, the uptake of LPA between the three cell lines was significantly different at 15 min of incubation.

Further investigation was aimed at determining the relationship between the uptake of SPP and that of dhSPP and of LPA. For these studies, cells were incubated with 100 μM [3H]SPP and the uptake of SPP was evaluated in the presence of increasing concentrations of dhSPP or of LPA. The results showed that the uptake of SPP was competed off by dhSPP in a concentration-dependent manner (Fig. 2C), suggesting that dhSPP and SPP probably share the same mechanism of uptake. Similarly, LPA was able to compete off the uptake of SPP in a dose-dependent manner (Fig. 2D).

**CFTR Does Not Regulate the Uptake of Other Sphingolipid Metabolites**—To examine whether the difference in uptake between cells expressing CFTR and the other cell lines was specific to SPP, we investigated the uptake of other sphingolipid compounds in C127/CFTR, C127/mock cells, and C127/AF508. All three cell lines showed a rapid but similar uptake of [3H]sphingosine, [3H]C16-ceramide, [14C]sphingomyelin, [3H]palmitic acid, [3H]lyso-phosphatidylcholine, [3H]lysosphingomyelin, and [3H]phosphatidylcholine (Table I). It is likely that incorporation into the membrane lipid bilayer accounts for some of the uptake of these lipids, especially sphingomyelin and phosphatidylcholine, which form a significant component of the cellular membrane. CFTR did not affect the uptake of any of these lipids, indicating that the effect of CFTR on uptake of [3H]SPP, [3H]dhSPP, and [3H]LPA is specific.

**Effects of Forskolin and IBMX on Uptake of [3H]Sphingosine 1-Phosphate**—CFTR forms a chloride channel that is activated by protein kinase A and is therefore regulated by cAMP. Forskolin, an adenylate cyclase activator, and IBMX, an inhibitor of phosphodiesterase, stimulate chloride secretion through G-protein-coupled CFTR through cAMP pathway. To evaluate if the uptake of dhSPP by CFTR is cAMP-dependent, we incubated cells with forskolin (20 μM) and IBMX (2 mM) and tested whether the uptake of dhSPP was affected. Pretreatment of C127/CFTR cells with or without IBMX and forskolin did not affect the uptake of [3H]dhSPP (data not shown). These data indicate that uptake of dhSPP may be regulated by the CFTR protein, and that this regulation is not through the cAMP-dependent chloride channel.

**Role of CFTR in Activation of MAP Kinase by Sphingosine 1-Phosphate**—It has been recently demonstrated that SPP is a ligand for the EDG-1 receptor using a highly sensitive radioligand binding assay (2). dhSPP competitively inhibited [3H]SPP binding with potency almost as great as that of unlabeled SPP (2, 18), suggesting that dhSPP and SPP both act through the same members of the EDG-receptor family. There is also increasing evidence that many cellular effects elicited by SPP are mediated by the EDG receptor family (EDG-1, EDG-3, EDG-5, and EDG-8). Mitogen-activated protein kinases p42mapk and p44mapk are activated in cells stimulated with epidermal growth factor and other agents. Exogenous SPP rapidly stimulates the activation of the MAP kinase pathway by a pertussis toxin-sensitive mechanism (19), suggesting the involvement of G protein-coupled EDG receptors. In Swiss 3T3 cells (20), sphingosine and SPP are growth stimulatory at low
concentrations via mobilization of calcium stores and activation of the MAP kinase pathway. dhSPP was as potent as SPP in p38 MAP kinase and ERK activation (21).

To test the MAP kinase pathway in the C127-transfected cells, we examined the effects of overexpression of CFTR on the activation of MAPK. Since the uptake of SPP, dhSPP, and LPA by CFTR was similar, we elected to focus the rest of our studies on the biologic aspects of SPP response in the different cell lines. First, we demonstrated that platelet-derived growth factor PDGF-AB (10 ng/ml, 10 min) stimulated p42mapk and p44mapk equally in C127/CFTR and C127/mock cells (Fig. 3A). Next, phosphorylation of MAP kinase in response to SPP in these cells was examined. Fig. 3 (B and C) shows that SPP activated p42mapk and p44mapk in a dose-dependent manner in both C127/CFTR and C127/mock cells. However, SPP-dependent activation of p42/p44 MAPK was significantly higher in C127/mock as compared with C127/CFTR. The difference was observed at low concentrations starting at 1 μM and became more pronounced at higher concentrations (1.75 times). Indeed, at 1 and 2.5 μM, SPP induced MAPK phosphorylation by 1.6- and 1.7-fold, whereas it barely exerted an effect in the C127/CFTR cells (Fig. 3C). PD098059, a known inhibitor of the ERK-2 signaling pathway, diminished SPP-stimulated activation of p42mapk and p44mapk seen on immunoblots (Fig. 3D), indicating that this stimulation by SPP is specific to the MAP kinase pathway. Those results demonstrate that overexpression of CFTR interferes with the SPP/EDG/MAPK pathway, possibly through diverting SPP to intracellular pools.

Expression of EDG Receptors in C127 Epithelial Cells—It was of interest to determine whether the SPP receptors EDG-1,
EDG-3, and EDG-5 are expressed in C127 mouse mammary epithelial cells and whether the expression level is similar in C127/CFTR and C127/mock. Serial dilutions of cDNAs were used in RT-PCR to confirm the linearity of PCR amplifications. The mRNA levels of EDG-3 and EDG-5 receptor genes were normalized to rRNA levels used as internal controls in these cells. RT-PCR analysis clearly demonstrated that mRNA levels of EDG-3 and EDG-5 receptor genes were similar in C127/CFTR and C127/mock cells (Fig. 4). The EDG-1 mRNA levels, however, were undetectable in either cell line.

**DISCUSSION**

These results show that CFTR enhances the uptake of SPP. This uptake was specific and dose-dependent. dhSPP and LPA, structurally related lipids, were also selectively taken up by C127/CFTR and were able to compete with the uptake of SPP. Cells overexpressing the CFTR protein, despite expressing equal amounts of EDG receptors, demonstrated decreased SPP-mediated MAP kinase activation and grew slower than mock-transfected cells.

Two important implications of this study are that CFTR regulates uptake of SPP and the structurally related lipids dhSPP and LPA, and that this uptake modulates the response of cells to these lipids.
lular effects of SPP in cells that may or may not express CFTR.

These data may, however, have important implications on the pathogenesis of cystic fibrosis. Cystic fibrosis is characterized by recurrent and chronic bacterial infection and inflammation. Chronic infection and angiogenesis are related in that one leads to the other, and a cycle of inflammation and angiogenesis is perpetuated. Excessive angiogenesis has been noted in cystic fibrosis tissues (25). Several proangiogenic molecules have been described including vascular endothelial cell growth factor (26). Vascular endothelial growth factor elevation is related to airway infection; serum vascular endothelial cell growth factor decreases with treatment of pulmonary exacerbation (26). It is conceivable that, in cystic fibrosis, no functional CFTR exists and consequently there is diminished uptake of SPP, allowing SPP to be more available to stimulate excessive angiogenesis in response to inflammation.

How CFTR regulates uptake of SPP may be related to its chloride channel activity. The CFTR protein is an epithelial Cl−/H+ channel stimulated by ATP and protein kinase A. In addition CFTR also acts as a regulator of outwardly rectifying Cl−/H+ channel, calcium-activated Cl− channels, and sodium channels (27). It is not absolutely clear whether those latter functions are due to direct action of CFTR or to indirect regulation of separate channels. Therefore, it is feasible that CFTR may transport or may regulate the transport of other, as yet unrecognized, substrates in addition to Cl− ions, such as SPP.

Cl− secretion is stimulated either by agents that increase cAMP, or it can be stimulated by intracellular Ca2+. The former pathway is defective in cystic fibrosis; however, the latter pathway is intact in epithelia of cystic fibrosis patients. In the mouse model of cystic fibrosis, it appears that Ca2+-mediated Cl secretion allows the animal to compensate and avoid the respiratory disease. SPP is known to cause increases in cytosolic Ca2+, and this is believed to be mediated by EDG receptor action. In the absence of CFTR, and with presumably more endogenous SPP available for EDG receptor binding, it is surprising that this mechanism of Cl− secretion does not take over. It is possible to propose exogenous SPP as a potential agent to overcome the defect in Cl secretion in CFTR, albeit this would be in conflict with its potentially deleterious effects on angiogenesis and inflammation as hypothesized above. It is conceivable that the effects of SPP on angiogenesis and on calcium mobilization could be dissociated such that the former could be blocked and the latter enhanced.

Our yeast molecular genetic work led us to the discovery of YOR1 as a fumonisin resistant gene and to identify its function as a SPP transporter. YOR1 belongs to class 2 of the ABC transporter family of proteins, the same subclass of the ABC

FIG. 4. Expression of EDG-1, EDG-3, and EDG-5 mRNAs in C127/mock and C127/CFTFR cells. A, mRNA levels of EDG-1 and EDG-3 were detected by RT-PCR in C127/mock and C127/CFTFR cells (lanes 2 and 3 and lanes 4 and 5, respectively) as described under “Experimental Procedures.” B, mRNA levels of EDG-5 were detected in C127/mock and C127/CFTFR transfectants (lanes 2 and 3, respectively). C, the mRNA levels of EDG-3 and EDG-5 were normalized to that of rRNA in these cells (lanes 2 and 3). Lane 1 contains HaeIII-digested Y174 DNA used as molecular size marker.

FIG. 5. Growth assay of C127-transfected cells. C127/CFTFR and C127/mock cells were grown in different concentrations of FBS, and the growth rate was evaluated at several time points. A, growth rate at 48 h of C127/CFTFR and C127/mock in different concentrations of fetal bovine serum. B, time course of growth assay of C127/CFTFR and C127/mock cells in medium containing 5% FBS.
transporter family as CFTR. This is based on the spacing between two domains, Walker A and Walker B, in nucleotide binding domain 1 of these transporters (28). Similarly to CFTR, YOR1 is demonstrated to localize to the S. cerevisiae plasma membrane. It is interesting that a deletion of a phenylalanine residue in YOR1 at position 670 (ΔF670 YOR1) led to an unstable mutant protein (29) that appeared to be retained in the endoplasmic reticulum, comparable to the effects of the ΔF508 mutation in the CFTR leading to cystic fibrosis. This study underscores the potentially important role of yeast genetics in the elucidation of novel pathways that are relevant to human disease.

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