Cytoplasmic Loop Three of Cystic Fibrosis Transmembrane Conductance Regulator Contributes to Regulation of Chloride Channel Activity*

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To examine the contribution of the large cytoplasmic loops of the cystic fibrosis transmembrane conductance regulator (CFTR) to channel activity, the three point mutations (S945L, H949Y, G970R) were characterized that have been detected in the third cytoplasmic loop (CL3, residues 933–990) in patients with cystic fibrosis. Chinese hamster ovary cell lines stably expressing wild-type CFTR or mutant G970R-CFTR yielded poly peptides with apparent masses of 170 kDa as the major products, whereas the major products of mutants S945L-CFTR and H949Y-CFTR had apparent masses of 150 kDa. The 150-kDa forms of CFTR were sensitive to endoglycosidase H digestion, indicating that these mutations interfered with maturation of the protein. Increased levels of mature CFTR (170 kDa) could be obtained for mutant H949Y when cells were grown at a lower temperature (26 °C) or incubated in the presence of 10% glycerol. For all mutants, the open probability (P_o) of the CFTR channels was significantly altered. S945L-CFTR and G970R-CFTR showed a severe reduction in the P_o whereas the H949Y mutation doubled the P_o relative to wild-type. The changes in P_o predominantly resulted from an alteration of the mean burst durations which suggests that CL3 is involved in obtaining and/or maintaining stability of the open state. In addition, mutants S945L and G970R had current-voltage relationships that were not completely linear over the range ±80 mV, but showed slight outward rectification. The fact that CL3 mutations can have subtle effects on channel conductance indicates that this region may be physically close to the inner mouth of the pore.

Mutations in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR)1 chloride channel result in cystic fibrosis (CF) (Kerem et al., 1989), either by disrupting the biosynthetic processing of the molecule or through the production of a functionally impaired protein (Welsh and Smith, 1993). Current models predict that CFTR contains 12 hydrophobic transmembrane helices (TM), which assemble within the lipid bilayer to form the pore of the channel (Riordan et al., 1989). Linked to the TMs and protruding into the cell cytoplasm are three large domains, the two nucleotide binding folds (NBFs) which have Walker A and Walker B motifs (Walker et al., 1982) for interactions with ATP and the highly charged R-domain in consensus sequences for phosphorylation by the cAMP-dependent protein kinase (PKA) and protein kinase C. Functional studies showed that these cytoplasmic domains regulate the opening and closing of the channel through ATP binding and ATP hydrolysis as well as through kinase-mediated phosphorylation and phosphatase-mediated dephosphorylation (Anderson et al., 1991; Cheng et al., 1991; Tabcharani et al., 1991; Picciotto et al., 1992; Becq et al., 1994; Hwang et al., 1994; Carson and Welsh, 1995; Gunderson and Kopito, 1995; Wilkinson et al., 1996).

An additional set of cytoplasmic domains is constituted by the cytoplasmic loops (CLs), which connect the TMs on the intracellular side of the protein (Fig. 1). The CLs are predicted to be between 55 and 65 amino acids in length (Riordan et al., 1989) and are highly conserved among CFTRs expressed in different species (Diamond et al., 1991). Little is known regarding their contribution to CFTR function; however, since the CLs are very hydrophilic, it has been suggested that they might undergo intramolecular interactions with other charged portions of the CFTR molecule, or intermolecular interactions with adjacent CFTRs or additional molecules (Tao et al., 1996). The first line of evidence for the importance of CLs was provided by Xie et al. (1995) who found, using a 19-amino acid deletion mutant, that CL2 (predicted residues: 242–307) is involved in stabilizing the full conductance state of the CFTR channel. Subsequent studies showed that CL4 (predicted residues: 1035–1102), in contrast to the conductance effect of CL2, appears not to affect the pore properties of CFTR, but rather its responsiveness to regulatory stimuli (Seibert et al., 1996); sin-

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1 The abbreviations used are: CFTR, cystic fibrosis transmembrane conductance regulator; CF, cystic fibrosis; CHO, Chinese hamster ovary; CL, cytoplasmic loop; ER, endoplasmic reticulum; I-V, current-voltage; NBF, nucleotide binding fold; P_o, open state probability; PAG, polyacrylamide gel electrophoresis; PKA, cyclic AMP-dependent protein kinase; TM, transmembrane helix; TES, Tris(hydroxymethyl)trimethylene-2-aminoethanesulfonic acid; AMP-PNP, 5′-adenylyl-β,γ-imidodiphosphate.
Cytoplasmic Loop Three of CFTR

ingle-channel patch-clamp analysis demonstrated that channels with disease-associated point-mutations in CL4 have the same linear current-voltage relationship as wild-type CFTR, but reduced open probability. Thus, both CL2 and CL4 influence the overall chloride channel activity of CFTR, yet in different ways. The aim of the present study was to examine the functional significance of a previously uninvestigated domain, CL3 (predicted residues: 933–990, connecting TMs 8 and 9 of CFTR, Fig. 1), by characterizing the three different point-mutations that have been identified in CL3 from patients with CF (S945L (Claustres et al., 1993), H949Y (Ghanem et al., 1994), and G970R (Cuppens et al., 1993)).

MATERIALS AND METHODS

Construction, Expression, and Detection of CL3 Mutants—Mutagenesis was performed in the expression vectors pNUT-CFTR and pcDNA3-CFTR as described (Tabcharani et al., 1991; Seibert et al., 1996). The sequence of each polymerase chain reaction fragment was verified after insertion into the vector using the T7 Sequencing Kit (Pharmacia Biotech Inc.). Subconfluent COS-1, HEK-293, and Chinese hamster ovary (CHO) cells were transfected with the various cDNA constructs (2 μg/ml final concentration) using the calcium phosphate precipitation method of Chen and Okayama (1987). pcDNA3-CFTR transfected COS-1 and HEK-293 cells were maintained under standard growth conditions for 48 h before analysis. CHO cells transfected with pNUT-CFTR were split to various confluencies 48 h post-transfection and subsequently exposed to media containing 50 μg methotrexate.

Twelve days post-transfection, surviving colonies were picked and propagated in the selective media. CFTR expression was confirmed by Western blotting (Seibert et al., 1993). Cell surface labeling was carried out according to Loo and Clarke (1994), with the modification that sodium periodate and biotin-LC-hydrazide (Pierce) incubations were made at room temperature. Measurements of channel open probability were obtained at a membrane potential of −30 mV from recordings that lasted 5–11 min. To estimate the number of channels in the patch, CFTR channels were “locked open” with 1 mM AMP-PNP (Calbiochem) at the end of the experiment (Hwang et al., 1994). The mean durations of bursts of openings and the mean durations of closings between bursts were calculated for patches containing more than one CFTR channel as described (Seibert et al., 1996).

RESULTS

Processing Mutants in CL3—Three different point-mutations have been identified within CL3 (Fig. 1) of CFTRs from patients suffering from CF. To investigate their effects on protein processing, the mutations were reconstructed in a CFTR-containing pNUT vector (Chang et al., 1993) and stably expressed in CHO cells. Western blotting with the CFTR-specific monoclonal antibody M3A7 (Kartner et al., 1995), demonstrated that wild-type and G970R-mutant CFTRs yielded fully mature protein (170 kDa, band C) as the major product, whereas mutants S945L and H949Y yielded little of the mature form (Fig. 2). In these two cases, the predominant product corresponded to a molecular mass of 150 kDa (band B). Band B was sensitive to digestion with endoglycosidase H (data not shown), indicating that it was a core-glycosylated biosynthetic intermediate. The same results and relative protein maturation levels were observed when the mutations were reconstructed in pcDNA3-CFTR, transiently expressed in either HEK-293 or COS-1 cells, and screened by Western blotting (data not shown). These findings indicate that the S945L and H949Y mutations cause a defect in the biosynthetic processing pathways of CFTR maturation.

The most common mutation of CFTR (ΔPhe-508) associated with CF has been found to disrupt processing of the protein in a similar manner (Cheng et al., 1990; Sheppard et al., 1995). However, maturation of ΔPhe-508 is sensitive to the cell culture conditions; when cells were grown at reduced temperatures (Denning et al., 1992) or exposed to elevated levels of glycerol (Sato et al., 1996; Brown et al., 1996), an increase of mature ΔPhe-508 CFTR at the cell surface has been observed. To explore the temperature/glycerol sensitivity of the processing mutants in CL3, cells expressing these mutants were shifted to 26 °C for 48 h (Fig. 3A) or left at 37 °C for 48 h with the addition of 10% glycerol to the growth media (Fig. 3B). In comparison to a control sample which remained at 37 °C without glycerol, it was observed that for the severely affected mutant S945L these procedures did not improve maturation of CFTR. In the case of H949Y, however, the amount of protein in band C was further increased under both conditions, with a more striking effect observed with 10% glycerol. It has to be noted that many cells died upon exposure to glycerol. The extreme culture conditions did not induce the expression of endogenous CFTR in mock-transfected CHO cells.

Evaluation of CL3 Mutations by Iodide Efflux—Iodide efflux (Chang et al., 1993) was used to estimate whether the macroscopic anion permeability of cells expressing CL3 mutant CFTRs deviated from wild-type levels (Fig. 4). Upon exposure of iodide-loaded CHO cells to the agonist forskolin, there was cAMP-mediated efflux observed for cells transfected with wild-type CFTR, but no activation for mock-transfected cells. Very little stimulation occurred in cells expressing S945L-CFTR in agreement with the severe inhibition of protein maturation described above. H949Y-CFTR-containing cells exhibited iodide effluxes which were similar to wild-type, although there was much less mature protein than in wild-type expressing cells as judged by Western blotting. This suggested that the H949Y mutation may actually produce a hyperactive form of CFTR. For G970R-CFTR, a forskolin-induced efflux was almost absent, despite the large amounts of complex glycosylated protein observed in Western blotting. The same results of relative total cell activities were obtained when iodide effluxes were measured from COS-1 cells transiently expressing these mu-
It was apparent that G970R allowed the production of fully glycosylated CFTR, but that there was little anion channel activity in the cells expressing this mutant. To ensure that the glycosylated form of the protein did in fact reach the plasma membrane, surface labeling was carried out with the membrane-impermeant reagent biotin-LC-hydrazide (Bayer et al., 1988; Lisanti et al., 1989; Prince et al., 1994). Using this approach, it was observed that similar amounts of mature forms of G970R-CFTR and wild-type protein were biotinylated (Fig. 5). The lack of band B biotinylation served as an internal control that biotin-LC-hydrazide did not permeate the bilayer. This showed that G970R-CFTR did reach the cell surface and therefore must be severely impaired in function to explain the very low level of iodide efflux observed. The absence of function is likely to be the cause of the CF symptoms in patients affected by the G970R mutation.

**Mutational Analysis of Residue Gly-970**—The mutation of a Gly to an Arg at position 970 is a significant alteration since a small, uncharged amino acid is replaced by a bulky, positively charged amino acid. To investigate whether the functional effect observed was due to the introduction of a charge or due to the introduction of size, several other mutations were generated: Gly-970 was changed to either Ala (small residue), Met (bulky residue), Glu (negatively charged residue), or Lys (positively charged residue). The pcDNA3-CFTR constructs, containing the desired mutations, were transiently expressed in COS-1 cells, and the cells were subjected to iodide efflux experiments (Fig. 6A). The G970A and G970R alterations resulted in efflux levels similar to wild-type, suggesting that amino acid size was not the major factor determining disturbance of CFTR function. Introducing a negative charge (G970E) decreased iodide efflux while the introduction of a positive charge (G970K) again abolished CFTR function. Therefore, the loss of CFTR activity observed with the mutation G970R appears to be due to the presence of a positive charge at this site. To ensure that none of the observed functional effects of the different mutants were caused by misprocessing, Western blotting was carried out with monoclonal antibody M3A7. As shown in Fig. 6B, similar levels of mature CFTR were observed for all mutants.

**Patch-clamp Analysis of CL3 Mutants**—Iodide efflux measures...
urements suggested that mutations within CL3 have significant influences on overall CFTR function. To obtain a more detailed understanding of these effects, single-channel patch-clamp analysis was applied. All three CL3 mutants formed PKA- and ATP-dependent channels when stably expressed in CHO cells (Fig. 7). For each mutant, however, the level of channel activity was clearly different from wild-type CFTR (Fig. 7); both S945L and G970R channels showed a lower level of activity than wild-type, while for H949Y-CFTR activity appeared to be higher than wild-type. This was confirmed by channel mean open probability (P0) measurements; S945L-CFTR and G970R-CFTR had significantly lower mean P0 values than wild-type channels, and the mean P0 of H949Y channels was significantly greater than observed for wild-type (Fig. 8A). Analysis of channel burst kinetics indicated that for each mutant the alteration in P0 was mainly the result of a change in open burst duration (Fig. 8B), with a smaller change in interburst duration also contributing to the reduced P0 seen in S945L (Fig. 8C). The fact that CFTR channels with CL3 mutations can still be opened through PKA/ATP-mediated stimulation, but that the duration of their open states is significantly altered suggests that CL3 may be involved in obtaining and/or maintaining stability of the open state.

Two of the CL3 mutations studied also had subtle effects on chloride permeation through the CFTR channel. Under the applied recording conditions, wild-type channels had a linear single-channel current-voltage (I-V) relationship over the voltage range, but instead showed slight outward rectification (Fig. 9A and C). In S945L-CFTR, this outward rectification was due to a significant reduction in current at negative membrane potentials compared to the wild-type channel (Fig. 9, A and D), while in G970R outward rectification resulted from increased current at positive potentials (Fig. 9, C and D). In contrast, H949Y channels had a linear I-V relationship (Fig. 9B) with a conductance similar to wild-type (7.9 ± 0.1 pS; n = 7). The reasons for the outward rectification seen in both S945L and G970R are unclear; however, the fact that CL3 mutations can have subtle effects on channel conductance suggests that this region may be physically located close to the inner mouth of the CFTR chloride channel pore.

**DISCUSSION**

In this study we attempted to gain insights into the importance of the previously unexplored cytoplasmic loop CL3. The three identified CF-associated point-mutations of CL3 were reconstructed with the expectation that they might disrupt CFTR function since they were identified in patients suffering from CF. The most striking effect due to the amino acid substitutions was a drastically altered P0 of the mutant CFTRs relative to wild-type CFTR, with S945L and G970R decreasing the P0 of the channel and H949Y doubling its P0. These effects were largely the result of a change in the mean open time of the altered channels. Evidence has been put forward that ATP hydrolysis at NBF1 is associated with activation of CFTR, and ATP hydrolysis at NBF2 provides a mechanism for timing the duration of the open state (Gunderson and Kopito, 1994; Hwang et al., 1995; Carson and Welsh, 1995; Wilkinson et al., 1996). Within the framework of this model, the altered duration of the open state observed in the present study indicates that mutations in CL3 can affect events at NBF2 or affect communication from NBF2 to the pore. Mutations within CL3 can have opposite effects of either prolonging (H949Y) or dramatically decreasing (S945L, G970R) the duration of the open state. A similar finding was obtained for mutations within the NBF2 domain, with removal of Lys in the Walker A motif and removal of Asp in the Walker B motif prolonging activity after forskolin removal, whereas replacement of the conserved Gly-1349 markedly destabilizes the active state (Wilkinson et al., 1996).

CL3 mutations affect channel P0 mainly by altering the mean burst duration, whereas previous studies showed that other factors which influence CFTR channel activity, such as altered ATP concentrations (Winter et al., 1994) or removal of phosphorylation sites by site-directed mutagenesis (Chang et al., 1993; Rich et al., 1993) tend to affect the interburst duration much more than the burst duration. CL3 mutations also influence the mean closed time of CFTR; however, these changes are not very significant. The slightly altered conduc-
tances due to mutations S945L and G970R show that mutagenesis of residues in CL3 has a subtle influence on pore properties, suggesting that CL3 may contribute to a region around the inner mouth of the pore.

Notably, one of the CL3 mutations, H949Y, results in a channel which is more active than the one produced by nature. A similar effect was noted previously for the CF-associated NBF1 mutation P574H. Both Sheppard et al. (1995) and Champigny et al. (1995) found that this mutation decreases processing of CFTR, but increases the channel’s activity. As seen for H949Y, this hyperactivity is mainly due to an increase in the mean open time of the channel, whereas conductance of the channel is not affected by the mutation. The occurrence of substantial activity for these mutants correlates well with the observation that patients affected by the P574H and H949Y mutations suffer from a less severe form of CF and are pancreatic sufficient (Kerem et al., 1990; Ghanem et al., 1994). Residue substitutions which increase channel activity may eventually be helpful in gene or protein therapy by extracting maximal activity from channels which can be introduced into cells.

The magnitude of the differences in activity levels of each mutant relative to wild-type CFTR was more pronounced using iodide efflux assays compared to the $P_0$ measurements of single-channel patch-clamping. This may in part result from differences in CFTR activity in whole cells that have an intact cellular machinery versus excised patches, but more likely is due to limitations of the techniques themselves. Specifically, the wild-type-like iodide efflux level together with the low expression level of H949Y-CFTR suggest a very high activity for that mutant, which cannot be fully accounted for by the doubling of the $P_0$ observed in single-channel patch-clamping. However, iodide efflux may overestimate H949Y-CFTR activity if both wild-type CFTR expressing and H949Y-CFTR expressing cells reach an efflux plateau which masks a difference in the activity of their respective CFTR channels. This was observed by Rosenfeld et al. (1994) who showed that increasing expression of normal human CFTR cDNA in CF epithelial cells results in a progressive increase in the level of CFTR protein expression, but a limit in the level of cAMP-stimulated chloride secretion. Therefore, the overall iodide efflux can only be viewed as a qualitative estimate. To obtain true quantitative data, the initial slopes of the flux curves would have to be determined, which has thus far not been possible due to technical difficulties. Even if these difficulties are overcome, iodide efflux will always be a somewhat artificial system that uses iodide as a substitute for chloride; the two anions are handled somewhat differently by CFTR (Tabcharani et al., 1992). Thus,
iodide efflux provides an efficient tool to identify gross alterations in CFTR channel activity, but the quantitative details are best evaluated by more subtle techniques such as single-channel patch-clamping. However, for low activity channels such as G970R-CFTR, which give little iodide efflux, the $P_0$ may somewhat overestimate residual channel activity since only open channels can be observed electrophysiologically whereas channels which never open during the course of the experiment will be missed.

These results, together with those of Xie et al. (1995) and Seibert et al. (1996), attribute a significant role to the CLs in CFTR function. The observed effects are different for the various loops; CL2 is found to influence open channel properties by participating in the regulation of different subconductance states (Xie et al., 1995). In contrast, CL3 and CL4 appear to participate in the regulation of the channel opening and closing rather than changing the pore properties. More detailed studies of each loop will be necessary to obtain a deeper understanding of the overlap in function between the different CLs and their physical interaction with the regulatory domains and with each other. Tao et al. (1996) suggested that such interactions are likely to occur in view of the hydrophilic nature of the CLs. The significant decrease in CFTR function due to the G970R mutation is consistent with this point of view, since the loss of activity was due to the charge rather than size of the introduced Arg. This, and the observation that mature G970R-CFTR is properly trafficked to the plasma membrane, suggests that G970R affects activity through an altered electrostatic interaction within the protein or with other molecules rather than a gross change in CFTR structure.

An emerging theme from the investigation of CLs is the finding that many mutations in these regions cause misprocessing of the whole molecule. Two of the three amino acid substitutions in CL3 affect maturation of CFTR, which highlights the importance of the correct secondary structure of CL3 to allow the achievement of proper overall folding. The influence on folding may depend more on the location of the altered residue within the molecule rather than the specific residue change, since five different amino acids could be inserted at position 970 without inhibiting processing. If maturation is restrained by a mutation, then the consequence is severe since attempts to rescue the protein by lowering temperature or glycerol exposure do not improve the situation substantially. The only exception thus far is H949Y which already has some expression at the cell surface under normal growth conditions.

REFERENCES
Anderson, M. P., Berger, H. A., Rich, D. P., Gregory, R. J., Smith, A. E., and Welsh, M. J. (1991) Cell 67, 775–784
Bayer, E. A., Ben-Hur, H., and Wilchek, M. (1988) Anal. Biochem. 170, 271–281
Becq, P., Jensen, T. J., Chang, X.-B., Savuia, A., Rommens, J. M., Tsui, L.-C.,

Fig. 9. Chloride conductance properties of single CL3 mutant channels. A–C show mean single channel I-V relationships for each mutant (●) compared to wild-type (○) CFTR. Each point represents the mean ± S.E. (where this is larger than the size of the symbol) of data from 3–12 patches. $D$ shows the mean single-channel conductance for each mutant measured at both negative potentials (inward current; open bars) and positive potentials (outward current; hatched bars). * represents a significant difference from wild-type ($p < 0.01$, two-tailed t-test), indicating that S945L shows decreased inward (but not outward) currents and G970R has increased outward (but not inward) currents. All data are the mean ± S.E. from 6–15 patches.
Buchwald, M., Riordan, J. R., and Hanrahan, J. W. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9160–9164
Brown, C. R., Hong-Brown, L. Q., Biversi, J., Verkman, A. S., and Welch, W. J. (1996) Cell Stress Chap. 1, 117–125
Carson, M. R., and Welsh, M. J. (1995) Biophys. J. 69, 2443–2448
Carson, M. R., Travis, S. M., and Welsh, M. J. (1995) J. Biol. Chem. 270, 1711–1717
Champigny, G., Imler, J. L., Puchelle, E., Dalemans, W., Gribkoff, V., Hinzsny, J., Dott, K., Barbry, P., Pavirani, A., and Lazdunski, M. (1995) EMBO J. 14, 2417–2423
Chang, X.-B., Tabcharani, J. A., Hou, Y.-X., Jensen, T. J., Korttner, N., Alon, N., Hanrahan, J. W., and Riordan, J. R. (1995) J. Biol. Chem. 270, 11304–11311
Cheng, S. H., Gregory, R. J., Marshall, J., Paul, S., Souza, D. W., White, G. A., O’Riordan, C. R., and Smith, A. E. (1990) Cell 63, 827–834
Cheng, S. H., Rich, D. P., Marshall, J., Gregory, R. J., Welsh, M. J., and Smith, A. E. (1991) Cell 66, 1027–1036
Claustres, M., Laussel, M., Desgeorges, M., Giansily, M., Culard, J.-F., Razakatsara, G., and Demaille, J. (1993) Hum. Mol. Genet. 2, 1209–1213
Cuppens, H., Marynen, P., De Boeck, C., and Cassiman, J.-J. (1993) Genomics 18, 693–697
Denning, G. M., Anderson, M. P., Amara, J. F., Marshall, J., Smith, A. E., and Welsh, M. J. (1992) Nature 358, 761–764
Diamond, G., Scanlin, T. F., Zasloff, M. A., and Bevins, C. L. (1991) J. Biol. Chem. 266, 22761–22769
Ghanem, N., Costes, B., Girodon, E., Martin, J., Fanen, P., and Goossens, M. (1994) Genomics 21, 434–436
Gunderson, K. L., and Kopito, R. R. (1994) J. Biol. Chem. 269, 19349–19353
Gunderson, K. L., and Kopito, R. R. (1995) Cell 82, 231–239
Hwang, T.-C., Nagel, G., Nairn, A. C., and Gadsby, D. C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4698–4702
Kartner, N., Augustinas, O., Jensen, T. J., Naismith, A. L., and Riordan, J. R. (1992) Nat. Genet. 1, 321–327
Kerem, B.-S., Rommens, J. M., Buchanan, J. A., Markiewicz, D., Cox, T. K., Chakravarti, A., Buchwald, M., and Tsui, L.-C. (1989) Science 245, 1073–1080
Kerem, B., Zielenks, J., Markiewicz, D., Bozen, D., Gazit, E., Yahaf, J., Kennedy, D., Riordan, J. R., Collins, F. S., Rommens, J. R., and Tsui, L.-C. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 8447–8451
Lisanti, M. P., Le Bivc, A., Sargiacomo, M., and Rodriguez-Boulan, E. (1989) J. Cell Biol. 109, 2117–2127
Loo, T. W., and Clarke, D. M. (1994) J. Biol. Chem. 269, 28683–28689
Picciotto, M. R., Cohn, J. A., Bertuzzi, G., Greengard, P., and Nairn, A. C. (1992) J. Biol. Chem. 267, 12742–12752
Prince, L. S., Werkman, Jr., R. B., and Marchase, R. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5192–5196
Rich, D. P., Berger, H. A., Cheng, S. H., Travis, S. M., Saxena, M., Smith, A. E., and Welsh, M. J. (1995) J. Biol. Chem. 269, 20229–20237
Riordan, J. R., Rommens, J. M., Kerem, B.-S. Alon, N., Rozmahel, R., Graeza, Z., Zieleszk, J., Lok, S., Plavacic, N., Chou, J.-I., Drumm, M. L., Iannuzzi, M. C., Collins, F. S., and Tsui, L.-C. (1989) Science 245, 1066–1073
Rosenfeld, M. A., Rosenfeld, S. J., Dand, C., Banks, T. C., and Staf, R. G. (1994) Hum. Gene Ther. 5, 1121–1129
Sato, S., Ward, C. L., Krouse, M. E., Wine, J. J., and Kopito, R. R. (1996) J. Biol. Chem. 271, 635–638
Seibert, F. S., Linselled, P., Loo, T. W., Hanrahan, J. W., Clarke, D. M., and Riordan, J. R. (1996) J. Biol. Chem. 271, 15139–15145
Sheppard, D. N., Ostergaard, L. S., Winter, M. C., and Welsh, M. J. (1995) EMBO J. 14, 876–883
Tabcharani, J. A., Chang, X.-B., Riordan, J. R., and Hanrahan, J. W. (1991) Nature 352, 628–631
Tabcharani, J. A., Chang, X.-B., Riordan, J. R., and Hanrahan, J. W. (1992) Biophys. J. 62, 1–4
Tabcharani, J. A., Rommens, J. M., Hou, X.-Y., Chang, X.-B., Tsui, L.-C., Riordan, J. R., and Hanrahan, J. W. (1993) Nature 366, 79–82
Tao, T., Xie, J., Drumm, M. L., Zhao, J., Davis, P. B., and Ma, J. (1996) Biophys. J. 70, 743–753
Walker, J. E., Saraste, M., Runswick, M. J., and Gay, N. J. (1982) EMBO J. 1, 945–951
Welsh, M. J., and Smith, A. E. (1993) Cell 73, 1251–1254
Wilkinson, D. J., Mansoura, M. K., Watson, P. Y., Smit, L. S., Collins, F. S., and Dawson, D. C. (1996) J. Gen. Physiol. 107, 103–119
Winter, M. C., Sheppard, D. N., Carson, M. R., and Welsh, M. J. (1994) Biophys. J. 66, 1398–1403
Xie, J., Drumm, M. L., Ma, J., and Davis, P. B. (1995) J. Biol. Chem. 270, 28684–28691