Effect of Cystic Fibrosis-associated Mutations in the Fourth Intracellular Loop of Cystic Fibrosis Transmembrane Conductance Regulator*

(Received for publication, March 25, 1996, and in revised form, June 11, 1996)

Joseph F. Cotten, Lynda S. Ostedgaard, Mark R. Carson, and Michael J. Welsh‡

From the Howard Hughes Medical Institute and Departments of Internal Medicine and Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242

The cystic fibrosis transmembrane conductance regulator (CFTR) contains multiple membrane spanning sequences that form a Cl− channel pore and cytosolic domains that control the opening and closing of the channel. The fourth intracellular loop (ICL4), which connects the tenth and eleventh transmembrane spans, has a primary sequence that is highly conserved across species, is the site of a preserved sequence motif in the ABC transporter family, and contains a relatively large number of missense mutations associated with cystic fibrosis (CF). To investigate the role of ICL4 in CFTR function and to learn how CF mutations in this region disrupt function, we studied several CF-associated ICL4 mutants. We found that most ICL4 mutants disrupted the biosynthetic processing of CFTR, although not as severely as the most common ΔF508 mutation. The mutations had no discernible effect on the channel’s pore properties; but some altered gating behavior, the response to increasing concentrations of ATP, and stimulation in response to pyrophosphate. These effects on activity were similar to those observed with mutations in the nucleotide-binding domains, suggesting that ICL4 might help couple activity of the nucleotide-binding domains to gating of the Cl− channel pore. The data also explain how these mutations cause a loss of CFTR function and suggest that some patients with mutations in ICL4 may have a milder clinical phenotype because they retain partial activity of CFTR at the cell membrane.

The cystic fibrosis transmembrane conductance regulator (CFTR)2 is a phosphorylation-regulated, ATP-dependent Cl− channel (for review, see Refs. 1 and 2) that belongs to the ATP Binding Cassette (ABC) transporter family (3). Like other members of this family, CFTR has two nucleotide-binding domains (NBD1 and NBD2) and two membrane-spanning domains (MSD1 and MSD2). In CFTR, each MSD is composed of six putative transmembrane segments (M1–6 and M7–12, respectively) and their connecting intracellular and extracellular loops. CFTR also contains a unique R domain which is involved in phosphorylation-dependent regulation of Cl− channel activity.

Mutations in the gene encoding CFTR cause the common genetic disease cystic fibrosis (CF) (4). Although CF-associated missense mutations have been discovered throughout the coding region of the CFTR gene (5), certain regions appear to have a relatively high frequency of missense mutations; for example, M1, M6, and specific regions of NBD1 are the sites of many missense mutations. The study of CF-associated missense mutations in these regions has helped elucidate the function of CFTR and has shown how the mutations disrupt function in CF. Studies of mutations in M1 and M6 have shown that these regions contribute to the Cl−-conducting pore (6, 7), and studies of mutations in NBD1 have helped investigators understand ATP-dependent gating and biosynthesis of CFTR (8–14). Examination of the distribution of CF-associated missense mutations shows that the fourth intracellular loop (ICL4) which lies between M10 and M11 is another region that contains many missense mutations: at least 19 CF-associated missense mutations have been discovered in this loop (Fig. 1) (15–20). Interestingly, one residue within ICL4, R1066, has been reported to have four separate CF-associated mutations: R1066C, R1066H, R1066L, and R1066S.

Inspection of CFTR sequences from various species including human, rat, mouse, bovine, ovine, frog, and shark shows substantial sequence conservation throughout the MSDs, particularly within the intracellular loops. Evolutionary maintenance of these residues suggests that they have an important function. Additional support for an important function came from a recent study by Manavalan et al. (21) who compared the length, sequence, and predicted secondary structure of the intra- and extracellular loops of the MSDs of various ABC transporters. Their study showed that the length of the intracellular loops was conserved, and they uncovered a consensus sequence in ICL4 (Fig. 1). This consensus sequence encompasses the cluster of CF-causing mutations in ICL4.

Based on these observations, we hypothesized that ICL4 may play an important role in the structure and function of CFTR.

2 R1066S (C. Férec, I. Quere, C. Verlingue, O. Raguenes, M.-P. Audrezet, and B. Mercier, personal communication), F1074L (T. Casals, M. D. Ramos, J. Giménez, V. Nunes, and X. Estivill, personal communication), K1060T (T. Casals, M. D. Ramos, J. Giménez, V. Nunes, and X. Estivill, personal communication), T1086I (T. Bienvenu, S. Bousquet, C. Herbulet, C. Beldjord, and J.-C. Kaplan, personal communication), and R1070W (M. Macek, S. Sedriks, S. Kiesewetter, and G. R. Cutting, personal communication).
To test this hypothesis and to learn how CF-associated mutations in ICL4 disrupt function, we constructed several of the CF-associated mutants in this region by site-directed mutagenesis, expressed them in heterologous cells, and studied their processing and function.

EXPERIMENTAL PROCEDURES

Site-directed Mutagenesis and Transfection—CFTR mutants were constructed in the vaccinia virus expression plasmid pTM-CFTR (22) by the method of Kunkel (23). Mutations were verified by restriction enzyme analysis, DNA sequencing around the site of mutation, and enzyme analysis, DNA sequencing around the site of mutation, and by the method of Kunkel (23). Mutations were verified by restriction enzyme analysis, DNA sequencing around the site of mutation, and enzyme analysis, DNA sequencing around the site of mutation, and by the method of Kunkel (23).

Results are presented as means ± S.E. for n observations. Statistical significance was determined using a log likelihood ratio test or an unpaired Student's t test where appropriate. p values < 0.05 were considered statistically significant.

RESULTS

Processing of ICL4 Mutants—Many CF-associated mutations cause a loss of CFTR Cl⁻ channel function by disrupting biosynthetic processing such that the mutant protein is not delivered to the cell membrane (see Ref. 29, for a review). Processing of CFTR can be assessed by examining its glycosylation status. Electrophoresis of wild-type CFTR resolves two bands: a broad, slowly migrating band (band C) that represents immature protein, and a more rapidly migrating band (band B) that represents immature protein. For whole cell and excised macropatch data, replayed records were filtered at 1 kHz using a variable 8-pole Bessel filter (Frequency Devices Inc., Haverhill, MA) and digitized at 2 kHz. For single channel analysis, replayed data were filtered at 1 kHz using a variable 8-pole Bessel filter, digitized at 5 kHz, and digitally filtered at 500 Hz. Event lists for single channel analysis were created using a half-height transition protocol; transitions less than 1 ms in duration were excluded from analysis.

To derive open and closed time constants, single channel open and closed time histograms were plotted with a logarithmic abscissa with 10 bins/decade and were fit with both one and two component exponential functions using the maximum likelihood method, with a lower fitting limit of 2.5 ms. To determine if the two component function fit statistically better than a one component fit, the log likelihood ratio test was used and considered significant at a value of 2.0 or greater. Burst analysis was performed as described previously using the pClamp 6.0 software package (28). We used a t₀ (the time which delineates interburst from intraburst closures) of 20 ms as derived from single channel closed time histograms. A t₀ of 20 ms was found suitable for all ICL4 mutants and CFTR. Regions of data from patches containing greater than one active channel with no superimposed openings were used for burst duration analysis as described previously (28).

Processing of ICL4 Mutants—Many CF-associated mutations cause a loss of CFTR Cl⁻ channel function by disrupting biosynthetic processing such that the mutant protein is not delivered to the cell membrane (see Ref. 29, for a review). Processing of CFTR can be assessed by examining its glycosylation status. Electrophoresis of wild-type CFTR resolves two bands: a broad, slowly migrating band (band C) that represents immature protein, and a more rapidly migrating band (band B) that represents immature protein. For whole cell and excised macropatch data, replayed records were filtered at 1 kHz using a variable 8-pole Bessel filter (Frequency Devices Inc., Haverhill, MA) and digitized at 2 kHz. For single channel analysis, replayed data were filtered at 1 kHz using a variable 8-pole Bessel filter, digitized at 5 kHz, and digitally filtered at 500 Hz. Event lists for single channel analysis were created using a half-height transition protocol; transitions less than 1 ms in duration were excluded from analysis.

To derive open and closed time constants, single channel open and closed time histograms were plotted with a logarithmic abscissa with 10 bins/decade and were fit with both one and two component exponential functions using the maximum likelihood method, with a lower fitting limit of 2.5 ms. To determine if the two component function fit statistically better than a one component fit, the log likelihood ratio test was used and considered significant at a value of 2.0 or greater. Burst analysis was performed as described previously using the pClamp 6.0 software package (28). We used a t₀ (the time which delineates interburst from intraburst closures) of 20 ms as derived from single channel closed time histograms. A t₀ of 20 ms was found suitable for all ICL4 mutants and CFTR. Regions of data from patches containing greater than one active channel with no superimposed openings were used for burst duration analysis as described previously (28).

Results are presented as means ± S.E. for n observations. Statistical significance was determined using a log likelihood ratio test or an unpaired Student's t test where appropriate. p values < 0.05 were considered statistically significant.

RESULTS

Processing of ICL4 Mutants—Many CF-associated mutations cause a loss of CFTR Cl⁻ channel function by disrupting biosynthetic processing such that the mutant protein is not delivered to the cell membrane (see Ref. 29, for a review). Processing of CFTR can be assessed by examining its glycosylation status. Electrophoresis of wild-type CFTR resolves two bands: a broad, slowly migrating band (band C) that represents immature protein, and a more rapidly migrating band (band B) that represents immature protein. For whole cell and excised macropatch data, replayed records were filtered at 1 kHz using a variable 8-pole Bessel filter (Frequency Devices Inc., Haverhill, MA) and digitized at 2 kHz. For single channel analysis, replayed data were filtered at 1 kHz using a variable 8-pole Bessel filter, digitized at 5 kHz, and digitally filtered at 500 Hz. Event lists for single channel analysis were created using a half-height transition protocol; transitions less than 1 ms in duration were excluded from analysis.

To derive open and closed time constants, single channel open and closed time histograms were plotted with a logarithmic abscissa with 10 bins/decade and were fit with both one and two component exponential functions using the maximum likelihood method, with a lower fitting limit of 2.5 ms. To determine if the two component function fit statistically better than a one component fit, the log likelihood ratio test was used and considered significant at a value of 2.0 or greater. Burst analysis was performed as described previously using the pClamp 6.0 software package (28). We used a t₀ (the time which delineates interburst from intraburst closures) of 20 ms as derived from single channel closed time histograms. A t₀ of 20 ms was found suitable for all ICL4 mutants and CFTR. Regions of data from patches containing greater than one active channel with no superimposed openings were used for burst duration analysis as described previously (28).

Results are presented as means ± S.E. for n observations. Statistical significance was determined using a log likelihood ratio test or an unpaired Student's t test where appropriate. p values < 0.05 were considered statistically significant.

RESULTS

Processing of ICL4 Mutants—Many CF-associated mutations cause a loss of CFTR Cl⁻ channel function by disrupting biosynthetic processing such that the mutant protein is not delivered to the cell membrane (see Ref. 29, for a review). Processing of CFTR can be assessed by examining its glycosylation status. Electrophoresis of wild-type CFTR resolves two bands: a broad, slowly migrating band (band C) that represents immature protein, and a more rapidly migrating band (band B) that represents immature protein. For whole cell and excised macropatch data, replayed records were filtered at 1 kHz using a variable 8-pole Bessel filter (Frequency Devices Inc., Haverhill, MA) and digitized at 2 kHz. For single channel analysis, replayed data were filtered at 1 kHz using a variable 8-pole Bessel filter, digitized at 5 kHz, and digitally filtered at 500 Hz. Event lists for single channel analysis were created using a half-height transition protocol; transitions less than 1 ms in duration were excluded from analysis.

To derive open and closed time constants, single channel open and closed time histograms were plotted with a logarithmic abscissa with 10 bins/decade and were fit with both one and two component exponential functions using the maximum likelihood method, with a lower fitting limit of 2.5 ms. To determine if the two component function fit statistically better than a one component fit, the log likelihood ratio test was used and considered significant at a value of 2.0 or greater. Burst analysis was performed as described previously using the pClamp 6.0 software package (28). We used a t₀ (the time which delineates interburst from intraburst closures) of 20 ms as derived from single channel closed time histograms. A t₀ of 20 ms was found suitable for all ICL4 mutants and CFTR. Regions of data from patches containing greater than one active channel with no superimposed openings were used for burst duration analysis as described previously (28).

Results are presented as means ± S.E. for n observations. Statistical significance was determined using a log likelihood ratio test or an unpaired Student's t test where appropriate. p values < 0.05 were considered statistically significant.
selected F1052V, which lies outside the cluster of mutations and the ABC transporter consensus sequence, because we thought it would likely have a functional defect since it is associated with CF yet is processed like wild-type protein.

We initially used the whole cell patch-clamp technique to test if mutants form regulated Cl\(^{-}\) channels. We found that cells expressing all of the ICL4 mutants (F1052V, R1066C, R1066H, R1066L, and A1067T) generated cAMP-stimulated Cl\(^{-}\) selective currents that showed time- and voltage-independent behavior identical to that of wild-type CFTR (data not shown). These data indicate that CFTR variants bearing mutations in ICL4 are able to form regulated Cl\(^{-}\) channels with several properties similar to those of wild-type protein. However, the fact that each of the ICL4 mutants is associated with CF suggested that in addition to defective processing they possess functional characteristics different from that of the wild-type protein.

Conductive Properties of ICL4 Mutants—A region of increased hydrophobicity lies in the middle of ICL4 (Fig. 1). It is possible that such a region might be associated with the plasma membrane where it could contribute to the formation of the channel pore. An emerging theme in the construction of ion channels is that intra- and extracellular loops fold back into the plasma membrane and line the permeation pathway (30, 31). Therefore, we asked whether the ICL4 mutations altered the conductive properties of the channel using excised, inside-out patches of membrane.

Fig. 3 shows representative single-channel traces from the mutants. Mutations did not alter single-channel conductance: wild-type, 8.9 ± 0.3 pS; F1052V, 9.6 ± 0.2 pS; R1066C, 8.9 ± 0.4 pS; R1066H, 8.5 ± 0.7; R1066C, 8.6 ± 0.3; and A1067T, 9.2 ± 0.2 pS. Using excised patches of membrane, we also examined the relative anion permeability and conductivity sequence for 3 of the mutants. Table I shows that F1052V, R1066L, and A1067T did not alter the relative permeability or conductivity sequence for Cl\(^{-}\), Br\(^{-}\), or I\(^{-}\). These data suggest that the mutations in ICL4 did not alter the conductive properties of CFTR Cl\(^{-}\) channels.

Regulation and Gating of ICL4 Mutants—In agreement with our whole cell data, excised patch studies demonstrated that all of the mutants were both PKA- and ATP-dependent (data not shown). However, some of the ICL4 mutants had readily apparent alterations in gating. Fig. 3 shows single channel tracings selected to illustrate these differences. The most noticeable differences compared to wild-type were that the R1066C channels had longer closed times between bursts of activity. In addition, the Arg-1066 mutants and F1052V appeared to have bursts of activity with altered durations. The differences in gating were quantified in Figs. 4 and 5. Interestingly, mutation of Arg-1066 to cysteine reduced open state probability (P\(_{o}\)) (Figs. 3 and 4A), yet when this same residue was mutated to histidine or leucine there was no effect on P\(_{o}\). The decrease in P\(_{o}\) of R1066C was not due to a decrease in burst duration, but was instead due to an increased long closed time between bursts of activity (Figs. 3 and 5C). In contrast to the cysteine mutation, mutation of Arg-1066 to histidine did not significantly alter any of the kinetics and mutation to leucine produced a small decrease in burst duration and increase in fast closed time. Mutation of the adjacent residue Ala-1067 to threonine produced a different pattern; P\(_{o}\) was decreased primarily because of a decrease in burst duration.

These findings show that ICL4 mutations altered channel gating without affecting Cl\(^{-}\) permeation. These effects are similar to the effect of mutations in the NBDs of CFTR (10, 28, 32), suggesting the possibility of a functional interaction between these CFTR domains. Therefore we speculated that ICL4 mutations might disrupt or modify some aspect of NBD-mediated gating; i.e. they might alter the interaction with ATP or they might affect the way the channel responds to agents whose effect is mediated through the NBDs.

To test this hypothesis we first examined the effect of increasing concentrations of ATP on channel activity. We studied...
R1066L and A1067T because they showed altered gating (Figs. 4 and 5); we did not study R1066C because it was difficult to study in excised, inside-out membrane patches, possibly because of its very low Po and poor processing. Fig. 6 shows that as the concentration of ATP increased, the Po of wild-type and mutant CFTR increased. R1066L had a response identical to that of wild-type CFTR. The maximum Po of A1067T was decreased, but the shape of the concentration Po curve mirrored that of wild-type. As we have previously reported (8), the shape of the curves did not fit simple Michaelis kinetics. This pattern of response for A1067T is similar to what we have found with the NBD mutants G551D, G1244E, and G1349D (8). The decrease in maximum Po without a change in the shape of the dose-response curve suggests that in A1067T ATP binding may be unaltered but that a step distal to binding may be affected.

To investigate further a potential interaction between ICL4 and the NBDs, we examined the effect of PPi on several ICL4 mutants. Previous studies have shown that pyrophosphate (PPi) stimulates CFTR Cl\textsuperscript{−} channels through an interaction with the NBDs (33, 34). PPi increases the Po of wild-type CFTR by prolonging the burst duration and by decreasing the inter-burst interval, effects which suggest that it interacts primarily with NBD2 (34). Fig. 7A shows that application of PPi to the cytosolic surface of an excised macropatch reversibly increased the activity of R1066L channels. However, Fig. 7B shows that the response of R1066L and F1052V to PPi was less than that of wild-type CFTR. This result suggested that ICL4 mutations may have altered an effect of PPi which is mediated through the NBDs, and perhaps specifically NBD2. To begin to isolate the effect of PPi to a specific NBD and to test further our hypothesis, we examined the response of several NBD mutants to PPi. We found that two NBD1 mutants, K464A and G551S, had a normal or increased response to PPi (Fig. 7C). In contrast, mutation of two analogous residues in NBD2, K1250M

TABLE I

| n  | P\textsubscript{Br}/P\textsubscript{CL} | G\textsubscript{Br}/G\textsubscript{CL} | G\textsubscript{Cl}/G\textsubscript{CL} | G\textsubscript{I}/G\textsubscript{CL} |
|----|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| 3  | 1.29 ± 0.07                      | 1.00                             | 0.56 ± 0.13                       | 0.35 ± 0.06                       |
| 2  | 1.41                             | 1.00                             | 0.50                             | 1.00                             |
| 4  | 1.36 ± 0.07                      | 1.00                             | 0.88 ± 0.11                       | 0.40 ± 0.11                       |
| 4  | 1.29 ± 0.15                      | 1.00                             | 0.66 ± 0.04                       | 0.43 ± 0.06                       |

Fig. 4. Open state probability (Po) (A) and burst duration (B) for wild-type CFTR and several ICL4 mutants. Measurements were made in the presence of 1 mM ATP and 75 nM PKA with membrane potential clamped at −80 mV. Burst durations were determined as described under “Experimental Procedures.” Data are mean ± S.E. of (6/5) measurements for Po and burst duration, respectively: wild-type (19/18), F1052V (6/5) R1066C (3/3), R1066H (6/7), R1066L (12/5), and A1067T (9/3). Asterisks indicate p < 0.05 relative to wild-type.

Fig. 5. Open and closed time constants for wild-type CFTR and ICL4 mutants. Time constants were plotted and fit as described under “Experimental Procedures.” t\textsubscript{o} refers to open time constant, t\textsubscript{cf} refers to fast closed time constant, and t\textsubscript{cs} refers to slow closed time constant. n = at least three for each, except n = 2 for t\textsubscript{cs} for R1066C. Asterisks indicate p < 0.05 relative to wild-type.
Measurements were made with membrane potential clamped at -80 mV. Each of these NBD1 and NBD2 mutations inhibit CFTR current to a similar extent.

**DISCUSSION**

ICL4 of CFTR has a primary sequence that is highly preserved across species from elasmobranch to amphibian to mammals; it is the site of a conserved motif in the ABC transporter family, and it contains a relatively large number of residues that are sites of CF-associated missense mutations. Our data show that CF-associated mutations in ICL4 can disrupt the biosynthesis of CFTR and that some mutations altered gating of the channel. These considerations indicate that ICL4 plays an important role in determining the structure and function of CFTR.

Previous studies have shown that CF-associated mutations can disrupt the processing of mutant CFTR so that it fails to escape from the endoplasmic reticulum and traffic to the plasma membrane. This is the primary defect in the most common CF mutation, ΔF508 (22). Previous reports of defective processing have focused on CF-associated missense mutations in the NBDs (10, 13, 22, 35). The molecular basis for misprocessing of mutant CFTR is not well understood; presumably it results from altered folding and/or structure of the mutant protein with consequent recognition as abnormal and degradation by the cellular quality control system. Our results showing that ICL4 mutations disrupt processing suggest that ICL4 plays a critical structural role in the protein. ICL4 mutations could cause misprocessing because they disrupt the structure of ICL4 itself, or they could have an indirect effect by altering the structure of an associated part of the protein. More profound changes in ICL2 can also disrupt processing. Delaney et al. (36) found that deletion of exon 5 (residues 163–193) disrupted processing and hence Cl\(^{-}\) channel activity. Xie et al. (37) reported that deletion of 19 residues from ICL2 (residues E267 to M285) produced a similar effect. However, when that channel was incorporated into lipid bilayers it produced regulated Cl\(^{-}\) channels. In addition, when Chang et al. (38) introduced a glycosylation site into ICL4 at Val-1056 they found that the amount of protein decreased.

In addition to their effect on processing, ICL4 mutations altered channel function. However, we could discern no relationship between the effect on processing and the effect on function. For example, the mutant F1052V was processed normally but had dramatically altered function, whereas the R1066H mutation had a dramatic effect on processing but little discernible effect on function. Moreover, mutation of Arg-1066 to three different residues, histidine, leucine, and cysteine, each decreased processing to roughly similar extents, yet had different effects on function. Clearly the requirements for channel processing and function are different. This conclusion is consistent with observations on NBD mutations; for example, the ΔF508 mutation is severely misprocessed yet retains approximately one-third of its function; whereas the G551D mutation is processed correctly but has very little function (9, 12, 14, 35, 39).

How might ICL4 participate in the Cl\(^{-}\) channel function of CFTR? Our whole cell and excised patch data revealed no alteration of conductive properties, suggesting that ICL4 does not contribute directly to the conduction pore. Instead, ICL4 mutations altered the gating behavior. Because NBD mutations also alter gating, the data suggested the possibility of an interaction between ICL4 and the NBDs. The fact that ICL4 and NBD2 mutants reduced the response to PPi, and the finding that G1349D and A1067T altered the effect of increasing concentrations of ATP in a similar way further suggest some interaction between ICL4 and the NBDs, particularly NBD2.

In CFTR, some mechanism must exist for interaction between the NBDs, which control channel gating, and the MSDs which form the channel pore. There is evidence for such an interaction in other ABC transporters. In P-glycoprotein, transported substrate interacts with the MSDs and stimulates ATPase activity by the NBDs. Conversely, ATP hydrolysis by
the NBDs stimulates drug efflux (40). Recent work by Loo and Clarke (41) provided biochemical evidence for an interaction between each NBD and its respective MSD in P-glycoprotein.

We speculate that in CFTR, ICL4 and probably other intracellular loops may link the NBDs to the MSDs. In this way the intracellular loops might couple the activity of the NBDs to the gating of the channel. This function for the ICLs could explain their evolutionary conservation in CFTR from different species and in other ABC transporters. There is precedent for such speculation in P-glycoprotein, where mutagenesis of the intracellular loops can alter substrate specificity (42, 43). In addition, in several prokaryotic ABC transporters, where individual domains are encoded by separate genes, it has been suggested that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).

These data also have implications for CF. First, an understanding of how particular CF-causing mutations disrupt CFTR function may prove helpful in the design and development of suitable therapies. For example, processing of several of the ICL4 mutants was not completely disrupted suggesting that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).

Data also have implications for CF. First, an understanding of how particular CF-causing mutations disrupt CFTR function may prove helpful in the design and development of suitable therapies. For example, processing of several of the ICL4 mutants was not completely disrupted suggesting that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).

Richardson et al. (46) provided biochemical evidence for an interaction between each NBD and its respective MSD in P-glycoprotein.

We speculate that in CFTR, ICL4 and probably other intracellular loops may link the NBDs to the MSDs. In this way the intracellular loops might couple the activity of the NBDs to the gating of the channel. This function for the ICLs could explain their evolutionary conservation in CFTR from different species and in other ABC transporters. There is precedent for such speculation in P-glycoprotein, where mutagenesis of the intracellular loops can alter substrate specificity (42, 43). In addition, in several prokaryotic ABC transporters, where individual domains are encoded by separate genes, it has been suggested that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).

These data also have implications for CF. First, an understanding of how particular CF-causing mutations disrupt CFTR function may prove helpful in the design and development of suitable therapies. For example, processing of several of the ICL4 mutants was not completely disrupted suggesting that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).

Richardson et al. (46) provided biochemical evidence for an interaction between each NBD and its respective MSD in P-glycoprotein.

We speculate that in CFTR, ICL4 and probably other intracellular loops may link the NBDs to the MSDs. In this way the intracellular loops might couple the activity of the NBDs to the gating of the channel. This function for the ICLs could explain their evolutionary conservation in CFTR from different species and in other ABC transporters. There is precedent for such speculation in P-glycoprotein, where mutagenesis of the intracellular loops can alter substrate specificity (42, 43). In addition, in several prokaryotic ABC transporters, where individual domains are encoded by separate genes, it has been suggested that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).

These data also have implications for CF. First, an understanding of how particular CF-causing mutations disrupt CFTR function may prove helpful in the design and development of suitable therapies. For example, processing of several of the ICL4 mutants was not completely disrupted suggesting that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).

Richardson et al. (46) provided biochemical evidence for an interaction between each NBD and its respective MSD in P-glycoprotein.

We speculate that in CFTR, ICL4 and probably other intracellular loops may link the NBDs to the MSDs. In this way the intracellular loops might couple the activity of the NBDs to the gating of the channel. This function for the ICLs could explain their evolutionary conservation in CFTR from different species and in other ABC transporters. There is precedent for such speculation in P-glycoprotein, where mutagenesis of the intracellular loops can alter substrate specificity (42, 43). In addition, in several prokaryotic ABC transporters, where individual domains are encoded by separate genes, it has been suggested that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).

These data also have implications for CF. First, an understanding of how particular CF-causing mutations disrupt CFTR function may prove helpful in the design and development of suitable therapies. For example, processing of several of the ICL4 mutants was not completely disrupted suggesting that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).

Richardson et al. (46) provided biochemical evidence for an interaction between each NBD and its respective MSD in P-glycoprotein.

We speculate that in CFTR, ICL4 and probably other intracellular loops may link the NBDs to the MSDs. In this way the intracellular loops might couple the activity of the NBDs to the gating of the channel. This function for the ICLs could explain their evolutionary conservation in CFTR from different species and in other ABC transporters. There is precedent for such speculation in P-glycoprotein, where mutagenesis of the intracellular loops can alter substrate specificity (42, 43). In addition, in several prokaryotic ABC transporters, where individual domains are encoded by separate genes, it has been suggested that a conserved sequence motif in a hydrophilic cytosolic loop of the membrane-spanning subunit is the site of interaction with the subunit containing the NBD (44, 45).
Effect of Cystic Fibrosis-associated Mutations in the Fourth Intracellular Loop of Cystic Fibrosis Transmembrane Conductance Regulator
Joseph F. Cotten, Lynda S. Ostedgaard, Mark R. Carson and Michael J. Welsh

J. Biol. Chem. 1996, 271:21279-21284.
doi: 10.1074/jbc.271.35.21279

Access the most updated version of this article at http://www.jbc.org/content/271/35/21279

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 17 of which can be accessed free at http://www.jbc.org/content/271/35/21279.full.html#ref-list-1