
Structure of the Cystic Fibrosis Transmembrane Conductance Regulator

Fiona L.L. Stratford Christine E. Bear

Programme in Structural Biology and Biochemistry, Research Institute, Hospital for Sick Children, and Departments of Physiology and Biochemistry, University of Toronto, Toronto, Canada

Abstract

CFTR is a distinctive member of the ATP-binding cassette (ABC) superfamily of membrane transport proteins. As expected, on the basis of this designation, CFTR is a multidomain membrane protein which utilizes cellular ATP to regulate the flux of its substrate through the membrane. Furthermore, as for related family members, the conformation of the membrane-spanning domains (MSDs) is regulated by nucleotide interactions with both cytosolic nucleotide-binding domains (NBD1 and NBD2). CFTR is distinct in that it functions as a channel and its MSDs form a pore through which chloride ions can be conducted. A further distinction relates to the presence of a unique regulatory domain, the 'R' domain, which contributes to the regulation of channel opening and closing when phosphorylated. To date, mutagenesis studies have guided much of our understanding of the molecular basis of the chloride permeation pathway and its regulation by the NBDs and the 'R' domain. However, recent X-ray crystallization studies of prokaryotic ABC proteins and the crystal structure of CFTR-NBD1 provide the first high resolution models of the molecular basis for ATP binding and permit further modelling of the domain-domain interactions essential for activity of CFTR.

CFTR is a multidomain membrane protein possessing an internal duplication like other related members of the ATP-binding cassette (ABC) superfamily of transport proteins [1, 2]. The CFTR protein is composed of two halves,

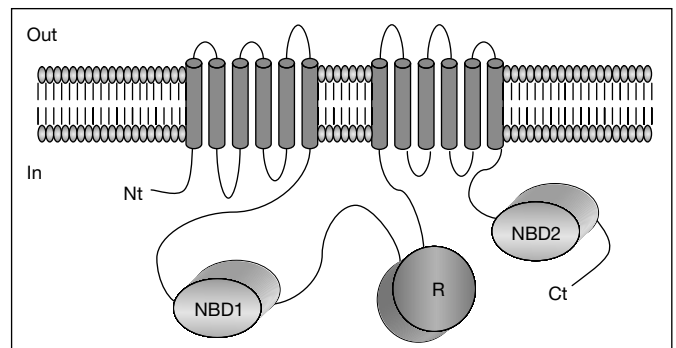


Fig. 1. The putative secondary structure of CFTR, highlighting the two MSDs, two NBDs (NBD1 + 2) and the regulatory (R) domain.

each possessing a membrane-spanning domain (MSD), comprised of several helical membrane segments (probably six) with intervening extracellular and intracellular loops followed by a cytosolic nucleotide-binding domain (NBD) (fig. 1). In the first half of the molecule, the putative NBD (NBD1) is followed by a large cytoplasmic domain called the 'R' domain, which contains multiple serine residues that are phosphorylated by protein kinase A and protein kinase C to regulate CFTR channel function [3, 4]. The 'R' domain connects the first and the second half of the molecule, with the second half of the molecule comprised of the second MSD (MSD2) and the second NBD (NBD2).

CFTR is the only member of the ABC superfamily that functions as a chloride channel. The regulation of CFTR channel function exhibits several intriguing properties. For example, channel activity by CFTR exhibits obligatory regulation by phosphorylation of its large cytosolic regulatory 'R' domain, a structure that is unique to CFTR in the ABC family. CFTR distinguished itself as a chloride channel in that it also possesses intrinsic ATP-binding and ATPase activities which are involved in opening and closing of the gate at the mouth of the channel. However, the molecular mechanism underlying ATP-dependent channel gating in CFTR remains an area of active investigation. In the following review, we will discuss the current biochemical and structural findings which guide our understanding of the mechanism of action of CFTR.

Quaternary Structure of CFTR: Monomer or Dimer?

There has been considerable interest regarding the quaternary structure of CFTR which mediates its chloride channel activity. Biochemical studies of cellular and purified CFTR suggest that CFTR is monomeric. Differentially tagged versions of CFTR fail to show co-immunoprecipitation from whole cell homogenates in a variety of detergents, arguing that in studies representative of the total cellular pool of CFTR, there is little detectable association between CFTR molecules [5, 6]. Gel filtration studies of detergent solubilized CFTR derived from whole cell homogenates showed that CFTR migrated primarily as a monomer [7]. In biochemical studies, we found that monomers of CFTR can be purified and functionally reconstituted, and were capable of mediating chloride electrodiffusion. Furthermore, detailed single channel electrophysiological studies revealed that purified monomeric CFTR can mediate regulated chloride channel and ATPase activity (a function discussed in depth in subsequent paragraphs) [8].

Recent structural determinations of prokaryotic and eukaryotic ABC proteins support the idea that the minimal functional unit of CFTR is monomeric. X-ray crystal structures of prokaryotic ABC proteins; including the lipid A transporter, MsbA [9] and the vitamin B₁₂ transporter, BtuCD [10] reveal multi-subunit proteins comprised of two MSDs and two NBDs. These essential components of the functional proteins are contained within a single CFTR polypeptide. Finally, low resolution structures of CFTR and P-glycoprotein generated by electron crystallography

provide further evidence that these purified proteins are monomeric [11, 12].

However, biochemical studies of CFTR localized exclusively at the cell surface suggest that it may exist as a dimer in the plasma membrane. CFTR-containing complexes, the size of dimers, can be cross-linked at the surface of mammalian cells using cross-linkers with short spacer arms of approximately 11 Å, consistent with the close contact of these proteins [7]. Li et al. [13] recently reported that CFTR dimers could be cross-linked along with signalling molecules in the apical membrane of Calu-3 epithelial cells. Therefore, these findings suggest that while dimeric association of CFTR molecules is not necessary for channel function, it may have an impact on trafficking to and/or interaction with other proteins at the cell surface.

Molecular Basis for Anion Permeation Through CFTR

Chloride Ion Permeation

The molecular basis for chloride permeation through CFTR remains poorly understood. On the basis of the structures of prokaryotic ABC proteins [9, 10], one would predict that the translocation path for chloride ions would be formed at the interface between the two MSDs (MSD1 and MSD2) of CFTR (each comprised of six putative helical segments). The sixth putative transmembrane (TM6) helix within MSD1 has been studied most extensively through mutagenesis and/or by monitoring the accessibility of substituted cysteine residues along its length using sulphhydryl reagents [14, 15]. Multiple residues appear to contribute to anion binding in TM6, including amino acids 334–344. Studies by Gong et al. [15] and Smith et al. [16] highlight the potential importance of the positively charged residues: arginine at position 334 and lysine, at position 335, in forming an external vestibule which attracts anions toward the selectivity filter. Gong and Linsdell [15, 17] have performed elegant studies that suggest that the anion selectivity filter may be confined to a relatively narrow segment within the pore (including residues: F337–S341 of TM6) K95, a positively charged residue, lines the relatively wide inner vestibule of the CFTR pore and likely attracts chloride ions (and possibly other organic anions into the pore) [18]. Mutations in comparable regions of TM5 and TM2–4 led to minor or negligible effects on the above biophysical properties arguing that TM1, TM6 and to a minor degree, TM5 contribute to the chloride permeation path, whereas TM2–4 likely exert a supportive role [19].

The contribution of MSD2 to anion conduction through CFTR is somewhat less clear. Recently, we found that purified and reconstituted MSD2 can dimerize to reconstitute a chloride-selective pore [20], arguing that MSD2, as well as MSD1, possesses structural features important for chloride conduction. In fact in site-directed mutagenesis studies, Gupta et al. [21] reported that mutations in TM12 (T1134, M1137, S1141) caused minor effects on the anion selectivity and unitary conductance of the channel. Similarly, mutations at S1118 in TM11 and T1134 in TM12 altered the affinity and/or voltage dependence of channel blockade by: DPC or NPPB [22]. In summary, studies to date suggest that the anion conductance path is comprised of multiple segments interspersed throughout the putative membrane domain.

Permeation of Other Physiologically Significant Anions

Patch clamp studies have revealed that CFTR can directly mediate the conduction of bicarbonate ion although the relative permeability of bicarbonate ion is significantly less than that of chloride ion ($P_{\text{HCO}_3^-}/P_{\text{Cl}^-} \sim 0.1$) [23, 24]. Studies by Reddy and Quinton [25] and subsequently by Shcheynikov et al. [26] support the concept that the pore of CFTR may exist in two forms, one form which may be bicarbonate permeant and the other, relatively chloride permeant. However, the two groups favour different regulatory mechanisms whereby selectivity may be altered. The evidence reported by Reddy and Quinton from studies using perfused human sweat ducts, support a model wherein the relative chloride and the bicarbonate-conducting functions of CFTR can be differentially regulated by the intracellular amino acid, glutamate and nucleotides. On the other hand, in *Xenopus* oocyte expression studies, Shcheynikov et al. determined that external chloride ion concentrations played a pivotal role in modulating the relative Cl^- : HCO_3^- permeability, wherein a reduction of extracellular chloride ion concentration to 20–30 mM led to an increase in the relative HCO_3^- permeability. To summarize, although the mechanism is unclear at present, the anion selectivity of the CFTR pore appears to be regulated, possibly reflecting long range conformational changes induced by anion binding to internal and/or external binding sites.

Linsdell and Hanrahan [27] were the first to show that glutathione (and other large organic anions) could be conducted through membranes expressing CFTR although their permeability relative to that of chloride ions is very low, i.e. $P_{\text{GSH}}/P_{\text{Cl}^-} \sim 0.08$. Furthermore, these authors reported that glutathione conductance exhibited a distinctive dependence on nucleotide interaction. In our recent work, we also determined that the nucleotide requirements

for glutathione conductance through purified CFTR were different from those required for chloride conductance [28]. These findings complement those discussed for bicarbonate ion above and suggest that the structure of the conductance pore through CFTR may be regulated by distinct ligand interactions.

Molecular Basis for Regulation by Phosphorylation

It is well known that phosphorylation of the ‘R’ domain of CFTR by PKA is absolutely required for nucleotide-dependent channel activity of CFTR [29]. There are ten dibasic consensus sites for PKA phosphorylation on the ‘R’ domain and it has been proposed that upon phosphorylation, they act in concert to mediate changes in the conformation and/or activity of other domains, as no single phosphoserine residue appears to be critical for function. However, the mechanisms underlying phosphorylation-dependent regulation remains unclear.

Electrophysiological studies suggest that the functional ‘R’ domain likely extends from a residue located somewhere between position 634–673 to residue 835 of CFTR [30, 31]. Deletion mutants of the ‘R’ domain and the functional analysis of isolated peptides derived from the ‘R’ domain reveal that there may be structures responsible for inhibition or stimulation within the ‘R’ domain. Deletion of residues 760–783 led to constitutive, phosphorylation-independent activity of CFTR, suggesting that this region may have an inhibitory effect on CFTR channel gating [32]. On the other hand, studies by Winter and Welsh [31] showed that addition of a PKA-phosphorylated ‘R’ domain peptide, corresponding to residues 708–831 caused activation of the CFTR channel (missing this region), suggesting that this large peptide includes regions which can engage in stimulatory interactions. As this large ‘R’ domain peptide enhanced nucleotide-dependent gating to the channel open state, primarily by enhancing the nucleotide affinity of the activity, it was suggested that it interacts with the NBDs and modifies them. Currently, however, it remains unclear whether the ‘R’ domain may also interact with other regions of the protein, i.e. the MSDs to mediate or modify channel gating transitions. Analyses by circular dichroism and NMR showed that this same peptide is largely unstructured, prompting speculation that the ‘R’ domain likely interacts with multiple regions of the protein simultaneously.

Molecular Basis for Regulation by Nucleotides

Functional Regulation of CFTR by Nucleotides:

Biochemical Studies of the ATPase Activity of CFTR

Purified, reconstituted full length CFTR exhibits a low level of intrinsic ATPase activity [33]. To date, experimental data suggest that the ATPase activity of CFTR (as for other ABC proteins) is mediated by an intramolecular complex between NBD1 and NBD2 [34]. For example, recombinant NBD1 and NBD2 proteins of CFTR functionally interact to generate significantly greater ATPase activity than either individual domain [34]. In the context of the full length CFTR protein, a mutation in either the ABC signature motif of NBD1 (G551D) or in the Walker A:ATP binding consensus motif of NBD2 (K1250A) completely abrogates ATPase activity [33, 35], pointing to the functional significance of NBD heterodimerization and the importance of these particular motifs.

Functional Regulation of CFTR by Nucleotides:

Electrophysiological Studies of the Role of Nucleotides in Channel Gating

CFTR channel opening and closing, i.e. gating, has been linked to nucleotide binding and ATPase activity by one or both of its NBDs [36]. There have been many studies of nucleotide-dependent gating of CFTR [35, 37–40] and certain general concepts emerge from such studies. First, opening of the CFTR channel is likely regulated by nucleotide binding and/or events secondary to ATP binding. It has been suggested that channel closure may be linked (directly or indirectly) to ATPase activity at one or more sites in CFTR. Mutations that decrease ATPase activity, such as mutation of the Walker A lysine residue in NBD2 (K1250A) and mutations which disrupt the Walker B motif of NBD2; D1370N and E1371S, lead to prolonged channel open times with a significant decrease in the rate of channel closure [35, 36]. These studies argue that there is a relationship between the ATPase activity and the rate of channel closing.

Vergani et al. [36] have incorporated the biochemical and electrophysiological studies described above to generate a model for the molecular basis underlying channel gating. The model proposes that nucleotide interaction with two sites, one containing the Walker A of NBD1 and the other, the Walker A of NBD2, leads to effective interaction of the two NBD domains and conformational changes associated with chloride channel gating to the open state. Subsequent hydrolysis of nucleotide at one or both sites returns the protein to its ground level and the channel gate resumes the closed conductance state. This model resem-

bles the ‘ATP Switch’ model recently proposed by Higgins and Linton [42] for ABC proteins in general, wherein the power stroke associated with transporter function and substrate translocation is conferred by nucleotide binding and induction of a ‘closed’ nucleotide dimer. Subsequent nucleotide hydrolysis ‘opens’ the nucleotide dimer and results in restoration of the basal conformation of the membrane domains. The above model has yet to be rigorously tested for CFTR.

Structural Studies: NBDs of Prokaryotic ABC Proteins

Crystal structures of prokaryotic proteins have provided useful tools to model the structural basis for CFTR NBD interactions [43–45]. These NBDs share a common three-dimensional fold and nucleotide-binding site architecture. The NBD is divided roughly into two sub-domains, termed lobes I and II [46]. The NBDs contain several conserved motifs which are involved in ATP binding and hydrolysis. These are the Walker A, Walker B, ABC signature motif and the Q loop and H (or Switch) loop (named, respectively, for glutamine and histidine residues) [10]. The N terminus of the α -helix in lobe I contains the Walker A motif. Lobe II is mostly α -helical and contains the ABC signature motif. Both lobes are connected by a shared β -sheet that forms an interlobe interface and contains the Walker B motif [43].

Several studies have shown that ABC NBDs work in homodimeric or heterodimeric pairs and that dimeric assembly is essential for ABC ATPase function [47, 48]. The crystal structure of the ATPase domain of Rad50 in complex with ATP [43] was the first to reveal a head to tail arrangement of the two NBD domains. In this structure, two functional active sites were formed at the dimer interface by juxtaposition of the ABC signature motif of one monomer with the Walker A and B motifs of the other monomer. Biochemical and crystal structure models [10, 44, 47] suggest that this relative orientation of the dimer interface is conserved amongst several ABC proteins (fig. 2a).

It is predicted that the two NBDs of CFTR also interact in a head-tail orientation (fig. 2b). However, unlike the prokaryotic NBDs, the NBDs of CFTR are structurally asymmetrical (fig. 2c) and hence interaction of CFTR NBDs may confer one rather than two catalytic sites. NBD1 possesses the canonical ABC signature motif but the Walker A motif is modified, i.e. S/T substituted for T/S [49]. It also lacks conservation of two residues implicated in interaction with the hydrolytic water, a Walker B glutamate [48] and a histidine residue [44]. NBD2 possesses sequence conservation in canonical Walker A, B and H motifs, but lacks conservation within the ABC signature

motif. Molecular modelling of the NBDs of CFTR, using prokaryotic NBD dimers as a template, predicts that site 1 comprises a ‘conventional’ catalytic site (comprising the Walker A, B motifs of NBD2 and the ABC signature motif of NBD1). Site 2, comprising the Walker A, B of NBD1 with the Walker C of NBD2 forms a ‘non-conventional’ site [49].

Structural Studies of CFTR-NBD1

Lewis et al. [50, 51] recently determined high-resolution crystal structures for monomeric mouse (m) and human (h) CFTR NBD1 (fig. 3, 4). Overall, the core tertiary structures for both CFTR NBD1s were similar to other ABC transporters NBDs, however they differed from typical ABC domains in having two major insertional regions. mNBD1 contains an insertion of two short α -helices (H1b and H1c) separated by a flexible linker region between β -strands S1 and S2 (fig. 3). This insertion in mNBD1 results in an altered binding geometry for the base and ribose. Interestingly, in the hNBD1 structure [51], this insertion, is reorientated such that the canonical base stacking interaction is restored, suggested that this region may be somewhat flexible. Furthermore, the C-termini of mNBD1 and hNBD1 include a long α -helix (H9B) that is not present in other ABC-NBD domain structures. The authors suggest that this region may correspond to a neighbouring, but distinct domain, the ‘R’ domain, as it contains one (Ser 660) of multiple phosphorylation sites [50]. This extension is also flexible as its’ orientation in the mNBD and hNBD structures is displaced by 180°.

The crystal structures of mNBD1 and hNBD1 revealed the nucleotide-binding site of this domain. As in other ABC NBDs, the following residues formed hydrogen bonds with the phosphates and/or coordinated the Mg^{2+} ion: Lys464 and Thr465 (Walker A), Asp572 (Walker B) and Gln493 (Q loop). As displayed in figure 4a, b, the regions involved in nucleotide interaction line up at the surface of the domain. However, there were no structural changes induced by nucleotide binding in NBD1, possibly reflecting the absence of NBD2, its functional partner in ATP hydrolysis.

Lewis et al. [50] then tested the prediction that the catalytic site(s) formed by the CFTR-NBDs requires interaction between NBD1 and NBD2 as a labile nucleotide ‘sandwich’ by creating a model of an NBD1-NBD2 heterodimer, docking the mNBD1 structure and a homology model of NBD2 onto the only available dimeric structure of the E171Q mutant MJ0796 homodimer [44]. However, in this heterodimeric model, there are severe main chain steric clashes between NBD1 and NBD2 [50]. Specifically, the C-terminal α -helix H9b of mNBD1 is thrust directly into

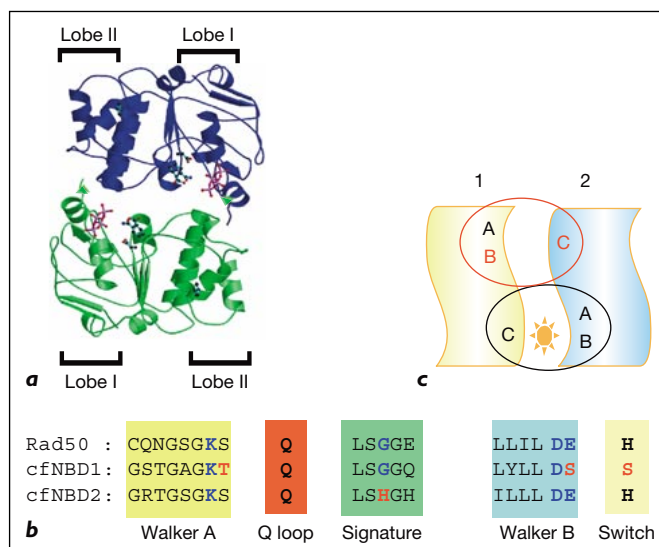


Fig. 2. NBDs – primary and tertiary structure. **a** The BtuD dimer solved by Locher et al. [10] and rendered by G.D. Smith using Molscript. Each monomer possesses two lobes which interact in a head to tail orientation within the dimeric complex. Pyrophosphate is ‘sandwiched’ within the two NBDs at two distinct sites. **b** Alignment of the key nucleotide binding motifs in Rad50 with CFTR-NBD1 and CFTR-NBD2. Modified from Locher et al. [10]. **c** Cartoon of the putative interaction between the two NBDs of CFTR in a head-to-tail orientation. The circled star indicates the site (site 1) at which the canonical nucleotide binding motifs A, B and signature are conserved with the rest of the family. The empty circle indicates the site (site 2) at which the motifs are degenerate.

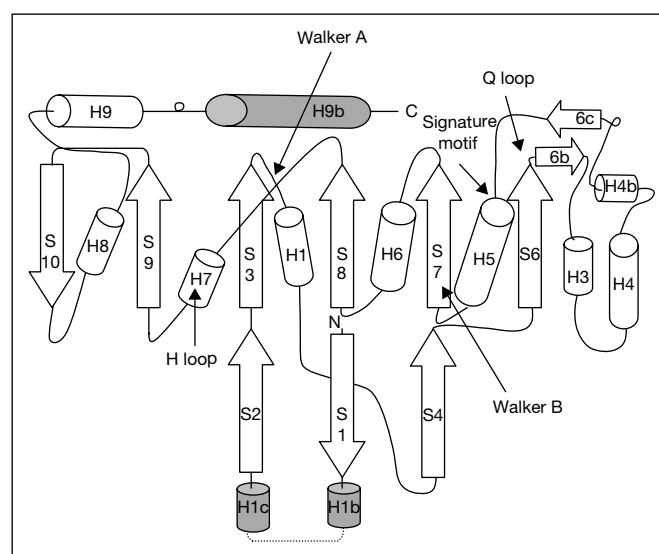
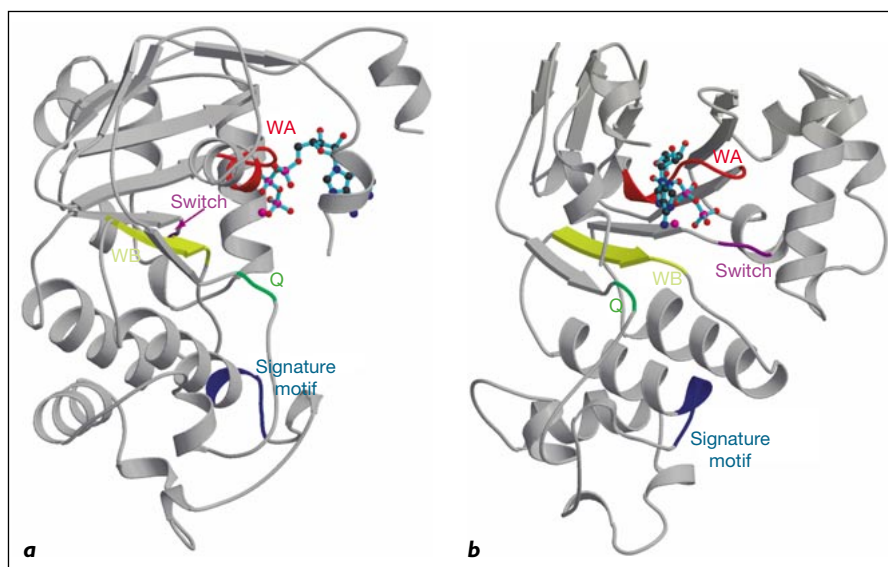


Fig. 3. Topology diagram of mNBD1. Regions of mNBD1 that are different from previous ABC structures are shown in grey. Modified from Lewis et al. [50].

Fig. 4. Two aspects of a ribbon diagram of mNBD1 adapted from Lewis et al. [50]. **a** ATP shown in ball and stick representation. Key region interacting with ATP: the Walker A motif (red) has been shown. The location of degenerate Walker B (yellow) motif of NBD1 is also shown. The signature motif (blue) is spatially separated from the one ATP bound to this protein. **b** The model has been rotated by 90° in order to show the linear arrangement of all of these motifs. Models were generated by Lewis et al. [50] and rendered by G.D. Smith using Molscript.



the opposing NBD2. The steric hindrance conferred by this region is apparent in the model rendered in figure 4a. These authors suggest there are several ways this conflict may be resolved, possibly through a conformational change in NBD1 upon interdomain interaction or through displacement of clashing segments upon phosphorylation [50].

Structural Studies of Intact Prokaryotic ABC Proteins by Three-Dimensional X-Ray Crystallography

Recently, high-resolution crystal structures have been reported for three intact prokaryotic ABC proteins, the lipid flippase MsbA proteins [from *Vibrio cholera* (VC-MsbA) and *Escherichia coli* (EC-MsbA)] and the vitamin B₁₂ transporter (BtuCD), [9, 10, 52]. These structures reveal three structural features critical for ABC transporter function: the translocation pathway in the membrane, the association of the cytosolic NBDs and the transmission interface, or connection between the membrane incorporated and cytosolic domains.

In all three protein structures, the translocation pathway is located at the interface of the two MSDs. However, the MSDs of BtuCD appear to interact in a tighter complex than those of VC-MsbA. For both BtuCD and VC-MsbA, the MSDs and the NBDs are connected via a helical loop or a ‘U’-like structure extending from the membrane or intracytosolic domains, respectively. The NBD structures of these proteins share the conserved architecture of two subdomains, lobe I containing the Walker A and lobe II con-

taining the conserved signature motif sequence. In BtuCD, two ATP binding/hydrolysis sites are formed in the presence of cyclotetranadate molecules (mimicking the α - and β -phosphates of ATP). The cyclotetranadate molecules are sandwiched at the interface of two NBD subunits arranged in a head to tail orientation as found in other ABC transporters [10]. The NBDs of VC-MsbA are generally oriented relative to one another in manner similar to the BtuCD structure [9]. However, in the absence of nucleotide or analogues/mimetics, their interaction is relatively open, postulated to represent the catalytically inactive conformation.

Locher et al. [10] showed that helical segments (L1-L2) extend from the membrane domains of BtuCD to provide a docking site for the NBDs (fig. 5). The L1-L2 helical segments are bisected by a glycine residue, which confers a bend critical in creating the interface. Locher et al. [10] aligned this loop region in BtuD with regions in MSD1 (residues 248–261) and MSD2 (1056–1069) of CFTR, which are the sites of many disease-causing mutations [53, 54]. Interestingly, the phenylalanine residue (F508) which is deleted in most cases of cystic fibrosis, aligns with a residue in the NBD:BtuD located at the L1, L2-NBD interface [10]. The crystal structure of human NBD1 lacking F508 was recently published by Lewis et al. [51]. Although deletion of F508 caused only minimal perturbations in the structure or folding of the domain, it did lead to a change in the local topography on the surface of the NBD in the same region modelled to interact with the MSD. These findings

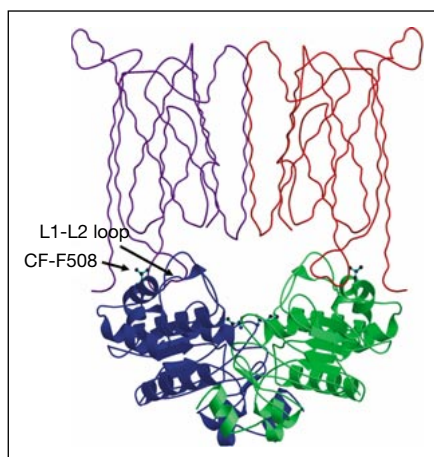


Fig. 5. Model of BtuCD structure generated by Locher et al. [10] and rendered by G.D. Smith using Molscript. Backbone of membrane-spanning helices (BtuC) drawn in purple and red. Ribbon diagrams of NBDs (BtuD) drawn in blue and green. The location of the L1-L2 loop in the MSD, forming the interaction between the MSDs and the NBDs has been shown. The putative position of F508 (deleted in most cases of cystic fibrosis) in the NBD is rendered as a ball and stick model (arrow). Figure rendered by D.G. Smith.

corroborate biochemical studies of the intact mutant protein that suggest the molecular basis for defective folding of $\Delta F508$ -CFTR lies in the perturbation of domain-domain interactions throughout the full length molecule [55–57].

Structural Studies of Intact Eukaryotic ABC Proteins by Electron Crystallography

Recently, several low resolution structures of full length eukaryotic ABC transporters have been determined [11, 12, 58, 59]. The first three-dimensional structure of an ABC protein (at 8 Å resolution) was determined for P-glycoprotein by Rosenberg et al. [11] allowing detection of the orientation of helices within the membrane domain of the intact transporter as well as the relative orientation of the NBDs. As predicted, 12 cylindrical densities, oriented in two symmetrical bundles (corresponding to MSD1 and MSD2) were observed and these densities could be modeled as α -helices within and extending beyond the membrane. The helical extensions connect with the NBDs and likely relate to the intracytoplasmic domains described for VC-MsbA by Chang [9]. The resolution was not sufficient to permit identification of the particular helices. Similarly,

the regions corresponding to the NBDs lack sufficient resolution to permit definition of β -strands or helices. However, the MJ0796 NBD structure with ATP bound [44] and the BtuCD NBD structure [10] could be superimposed on the NBDs of P-glycoprotein, suggesting that the NBDs of P-glycoprotein may associate in an identical manner as these prokaryotic NBDs. Most recently, Rosenberg et al. [12] determined a low resolution structure of CFTR by electron crystallography of negatively stained two-dimensional crystals. The overall features of the CFTR structure resembled that of P-glycoprotein [11]. The two-dimensional crystal studies of CFTR in the presence of the non-hydrolyzable ATP analogue: MgAMP-PNP, reported two quite distinct conformations which the authors ascribe to the nucleotide-bound and unbound states. These inspiring advances in structural studies of ABC proteins and CFTR in particular promise a future in which we will eventually have high resolution models with which to determine the molecular mechanisms of activity.

Conclusions

The tremendous recent progress in structural determination of ABC protein domains and intact ABC proteins has stimulated excitement and optimism that we may be able to generate meaningful models of the molecular mechanisms underlying CFTR function soon. Within the next few years we hope to understand the molecular basis for ATPase activity by the NBDs of CFTR, the nature of the interaction of ‘R’ domain with the NBDs and in the longer term, the structural basis for the regulation of the membrane-spanning, pore-forming region of CFTR by these cytoplasmic domains. Once these models are in place, understanding the consequences of cystic fibrosis disease-causing mutations on CFTR structure and function and development of structure-based therapeutics will become realistic goals.

Acknowledgements

The structural models rendered in this paper were prepared by G. David Smith (Emeritus Scientist, Programme in Structural Biology and Biochemistry, Research Institute, Hospital for Sick Children). The work performed in the author’s laboratory was possible through the generous support by the Canadian Cystic Fibrosis Foundation.

References

- Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsky N, Chou JL, et al: Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. *Science* 1989; 245:1066–1073.
- Higgins CF, Linton KJ: Structural biology: The xyz of ABC transporters. *Science* 2001; 293:1782–1784.
- Seibert FS, Loo TW, Clarke DM, Riordan JR: Cystic fibrosis: Channel catalytic and folding properties of the CFTR protein. *J Bioenerg Biomembr* 1997;29:429–442.
- Chappe V, Hinkson DA, Howell LD, Evagelidis A, Liao J, Chang XB, Riordan JR, Hanrahan JW: Stimulatory and inhibitory protein kinase C consensus sequences regulate the cystic fibrosis transmembrane conductance regulator. *Proc Natl Acad Sci USA* 2004; 101:390–395.
- Chen JH, Chang XB, Aleksandrov AA, Riordan JR: CFTR is a monomer: Biochemical and functional evidence. *J Membr Biol* 2002;188:55–71.
- Marshall J, Fang S, Ostedgaard LS, O'Riordan CR, Ferrara D, Amara JF, Hoppe HT, Scheule RK, Welsh MJ, Smith AE, et al: Stoichiometry of recombinant cystic fibrosis transmembrane conductance regulator in epithelial cells and its functional reconstitution into cells in vitro. *J Biol Chem* 1994;269:2987–2995.
- Ramjeesingh M, Kidd JF, Huan LJ, Wang Y, Bear CE: Dimeric cystic fibrosis transmembrane conductance regulator exists in the plasma membrane. *Biochem J* 2003;374(pt 3): 793–797.
- Ramjeesingh M, Li C, Kogan I, Wang Y, Huan LJ, Bear CE: A monomer is the minimum functional unit required for channel and ATPase activity of the cystic fibrosis transmembrane conductance regulator. *Biochemistry* 2001;40:10700–10706.
- Chang G: Structure of MsbA from *Vibrio cholerae*: A multidrug resistance ABC transporter homolog in a closed conformation. *J Mol Biol* 2003;330:419–430.
- Locher KP, Lee AT, Rees DC: The *E. coli* BtuCD structure: A framework for ABC transporter architecture and mechanism. *Science* 2002;296:1091–1098.
- Rosenberg MF, Callaghan R, Modok S, Higgins CF, Ford RC: Three-dimensional structure of P-glycoprotein: The transmembrane regions adopt an asymmetric configuration in the nucleotide-bound state. *J Biol Chem* 2005;280:2857–2862.
- Rosenberg MF, Kamis AB, Aleksandrov LA, Ford RC, Riordan JR: Purification and crystallization of the cystic fibrosis transmembrane conductance regulator (CFTR). *J Biol Chem* 2004;279:39051–39057.
- Li C, Roy K, Dandridge K, Naren AP: Molecular assembly of cystic fibrosis transmembrane conductance regulator in plasma membrane. *J Biol Chem* 2004;279: 24673–24684.
- Akabas MH: Probing CFTR channel structure and function using the substituted-cysteine-accessibility method. *Methods Mol Med* 2002;70:159–174.
- Gong X, Linsdell P: Molecular determinants and role of an anion binding site in the external mouth of the CFTR chloride channel pore. *J Physiol* 2003;549(pt 2):387–397.
- Smith SS, Liu X, Zhang ZR, Sun F, Kriewall TE, McCarty NA, Dawson DC: CFTR: Covalent and noncovalent modification suggests a role for fixed charges in anion conduction. *J Gen Physiol* 2001;118:407–431.
- Gong X, Linsdell P: Maximization of the rate of chloride conduction in the CFTR channel pore by ion-ion interactions. *Arch Biochem Biophys* 2004;426:78–82.
- Linsdell P: Location of a common inhibitor binding site in the cytoplasmic vestibule of the CFTR chloride channel pore. *J Biol Chem* epub January 2005.
- Ge N, Muise CN, Gong X, Linsdell P: Direct comparison of the functional roles played by different membrane spanning regions in the cystic fibrosis transmembrane conductance regulator chloride channel pore. *J Biol Chem* 2004;279:55283–55289.
- Ramjeesingh M, Ugwu F, Li C, Dhani S, Huan LJ, Wang Y, Bear CE: Stable dimeric assembly of the second membrane-spanning domain of CFTR reconstitutes a chloride-selective domain. *Biochem J* 2003;375(pt 3):633–641.
- Gupta J, Evagelidis A, Hanrahan JW, Linsdell P: Asymmetric structure of the cystic fibrosis transmembrane conductance regulator chloride channel pore suggested by mutagenesis of the twelfth transmembrane region. *Biochemistry* 2001;40:6620–6627.
- Zhang ZR, Zeltwanger S, McCarty NA: Direct comparison of NPPB and DPC as probes of CFTR expressed in *Xenopus* oocytes. *J Membr Biol* 2000;175:35–52.
- Linsdell P, Tabcharani JA, Rommens JM, Hou YX, Chang XB, Tsui LC, Riordan JR, Hanrahan JW: Permeability of wild-type and mutant cystic fibrosis transmembrane conductance regulator chloride channels to polyatomic anions. *J Gen Physiol* 1997;110: 355–364.
- Illek B, Yankaskas JR, Machen TE: cAMP and genistein stimulate HCO_3^- conductance through CFTR in human airway epithelia. *Am J Physiol* 1997;272:L752–L761.
- Reddy MM, Quinton PM: Control of dynamic CFTR selectivity by glutamate and ATP in epithelial cells. *Nature* 2003;423:756–760.
- Shcheynikov N, Kim KH, Kim KM, Dorwart MR, Ko SB, Goto H, Naruse S, Thomas PJ, Muallem S: Dynamic control of cystic fibrosis transmembrane conductance regulator $\text{Cl}^-/\text{HCO}_3^-$ selectivity by external Cl^- . *J Biol Chem* 2004;279:21857–21865.
- Linsdell P, Hanrahan JW: Glutathione permeability of CFTR. *Am J Physiol* 1998;275(1 pt 1):C323–C326.
- Kogan I, Ramjeesingh M, Li C, Kidd JF, Wang Y, Leslie EM, Cole SP, Bear CE: CFTR directly mediates nucleotide-regulated glutathione flux. *EMBO J* 2003;22:1981–1989.
- Hanrahan JW, Tabcharani JA, Becq F, Mathews CJ, Augustinas O, Jensen TJ, Chang XB, Riordan JR: Function and dysfunction of the CFTR chloride channel. *Soc Gen Physiol* 1995;Ser 50:125–137.
- Chan KW, Csanady L, Seto-Young D, Nairn AC, Gadsby DC: Severed molecules functionally define the boundaries of the cystic fibrosis transmembrane conductance regulator's NH_2 -terminal nucleotide binding domain. *J Gen Physiol* 2000;116:163–180.
- Winter MC, Welsh MJ: Stimulation of CFTR activity by its phosphorylated R domain. *Nature* 1997;389:294–296.
- Xie J, Adams LM, Zhao J, Gerken TA, Davis PB, Ma J: A short segment of the R domain of cystic fibrosis transmembrane conductance regulator contains channel stimulatory and inhibitory activities that are separable by sequence modification. *J Biol Chem* 2002; 277:23019–23027.
- Li C, Ramjeesingh M, Wang W, Garami E, Hewryk M, Lee D, Rommens JM, Galley K, Bear CE: ATPase activity of the cystic fibrosis transmembrane conductance regulator. *J Biol Chem* 1996; 271:28463–28468.
- Kidd JF, Ramjeesingh M, Stratford F, Huan LJ, Bear CE: A heteromeric complex of the two nucleotide binding domains of cystic fibrosis transmembrane conductance regulator (CFTR) mediates ATPase activity. *J Biol Chem* 2004;279:41664–41669.
- Ramjeesingh M, Li C, Garami E, Huan LJ, Galley K, Wang Y, Bear CE: Walker mutations reveal loose relationship between catalytic and channel-gating activities of purified CFTR (cystic fibrosis transmembrane conductance regulator). *Biochemistry* 1999;38: 1463–1468.
- Vergani P, Nairn AC, Gadsby DC: On the mechanism of MgATP -dependent gating of CFTR Cl^- channels. *J Gen Physiol* 2003;121: 17–36.
- Gunderson KL, Kopito RR: Conformational states of CFTR associated with channel gating: The role ATP binding and hydrolysis. *Cell* 1995;82:231–239.
- Aleksandrov AA, Chang X, Aleksandrov L, Riordan JR: The non-hydrolytic pathway of cystic fibrosis transmembrane conductance regulator ion channel gating. *J Physiol* 2000; 528(pt 2):259–265.
- Schultz BD, Venglarik CJ, Bridges RJ, Frizzell RA: Regulation of CFTR Cl^- channel gating by ADP and ATP analogues. *J Gen Physiol* 1995;105:329–361.
- Berger AL, Ikuma M, Welsh MJ: Normal gating of CFTR requires ATP binding to both nucleotide-binding domains and hydrolysis at the second nucleotide-binding domain. *Proc Natl Acad Sci USA* 2005;102:455–460.
- Ikuma M, Welsh MJ: Regulation of CFTR Cl^- channel gating by ATP binding and hydrolysis. *Proc Natl Acad Sci USA* 2000;97:8675–8680.
- Higgins CF, Linton KJ: The ATP switch model for ABC transporters. *Nat Struct Mol Biol* 2004;11:918–926.
- Hopfner KP, Karcher A, Shin DS, Craig L, Arthur LM, Carney JP, Tainer JA: Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. *Cell* 2000;101:789–800.

- 44 Smith PC, Karpowich N, Millen L, Moody JE, Rosen J, Thomas PJ, Hunt JF: ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer. *Mol Cell* 2002;10:139–149.
- 45 Chen J, Lu G, Lin J, Davidson AL, Quijcho FA: A tweezers-like motion of the ATP-binding cassette dimer in an ABC transport cycle. *Mol Cell* 2003;12:651–661.
- 46 Hung LW, Wang IX, Nikaido K, Liu PQ, Ames GF, Kim SH: Crystal structure of the ATP-binding subunit of an ABC transporter. *Nature* 1998;396:703–707.
- 47 Fetsch EE, Davidson AL: Vanadate-catalyzed photocleavage of the signature motif of an ATP-binding cassette (ABC) transporter. *Proc Natl Acad Sci USA* 2002;99:9685–9690.
- 48 Moody JE, Millen L, Binns D, Hunt JF, Thomas PJ: Cooperative ATP-dependent association of the nucleotide binding cassettes during the catalytic cycle of ATP-binding cassette transporters. *J Biol Chem* 2002;277:21111–21114.
- 49 Callebaut I, Eudes R, Mornon JP, Lehn P: Nucleotide-binding domains of human cystic fibrosis transmembrane conductance regulator: Detailed sequence analysis and three-dimensional modeling of the heterodimer. *Cell Mol Life Sci* 2004;61:230–242.
- 50 Lewis HA, Buchanan SG, Burley SK, Connors K, Dickey M, Dorwart M, Fowler R, Gao X, Guggino WB, Hendrickson WA, Hunt JF, Kearins MC, Lorimer D, Maloney PC, Post KW, Rajashankar KR, Rutter ME, Sauder JM, Shriver S, Thibodeau PH, Thomas PJ, Zhang M, Zhao X, Emtage S: Structure of nucleotide-binding domain 1 of the cystic fibrosis transmembrane conductance regulator. *EMBO J* 2004;23:282–293.
- 51 Lewis HA, Zhao X, Wang C, Sauder JM, Rooney I, Noland BW, Lorimer D, Kearins MC, Connors K, Condon B, Maloney PC, Guggino WB, Hunt JF, Emtage S: Impact of the delta F508 mutation in NBD1 of human CFTR on domain folding and structure. *J Biol Chem* 2005;280:1346–1353.
- 52 Chang G, Roth CB: Structure of MsbA from *E. coli*: A homolog of the multidrug resistance ATP binding cassette (ABC) transporters. *Science* 2001;293:1793–1800.
- 53 Cotten JF, Ostedgaard LS, Carson MR, Welsh MJ: Effect of cystic fibrosis-associated mutations in the fourth intracellular loop of cystic fibrosis transmembrane conductance regulator. *J Biol Chem* 1996;271:21279–21284.
- 54 Seibert FS, Linsdell P, Loo TW, Hanrahan JW, Clarke DM, Riordan JR: Disease-associated mutations in the fourth cytoplasmic loop of cystic fibrosis transmembrane conductance regulator compromise biosynthetic processing and chloride channel activity. *J Biol Chem* 1996;271:15139–15145.
- 55 Cheng SH, Gregory RJ, Marshall J, Paul S, Souza DW, White GA, O’Riordan CR, Smith AE: Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* 1990;63:827–834.
- 56 Thibodeau PH, Brautigam CA, Machius M, Thomas PJ: Side chain and backbone contributions of Phe508 to CFTR folding. *Nat Struct Mol Biol* 2005;12:10–16.
- 57 Du K, Sharma M, Lukacs GL: The deltaF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. *Nat Struct Mol Biol* 2005;12:17–25.
- 58 Lee JY, Urbatsch IL, Senior AE, Wilkens S: Projection structure of P-glycoprotein by electron microscopy evidence for a closed conformation of the nucleotide binding domains. *J Biol Chem* 2002;277:40125–40131.
- 59 Velarde G, Ford RC, Rosenberg MF, Powis SJ: Three-dimensional structure of transporter associated with antigen processing (TAP) obtained by single particle image analysis. *J Biol Chem* 2001;276:46054–46063.

Christine E. Bear
 Programme in Structural Biology and
 Biochemistry
 Research Institute
 Hospital for Sick Children
 555 University Avenue
 Toronto (Canada)
 E-Mail bear@sickkids.on.ca