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Minireview

Sequence homologies between nucleotide binding regions of CFTR and G-proteins suggest structural and functional similarities

Parthasarathy Manavalan[a,*], Dorr G. Dearborn[b], John M. McPherson[a], Alan E. Smith[a]

[a]*Department of Biotherapeutic Product Development, Genzyme Corporation, One Mountain Road, Framingham, MA 01701, USA*
[b]*Departments of Pediatrics and Biochemistry, Case Western Reserve University, Cleveland, OH 44106, USA*

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Abstract **Sequence homology between the α-subunits of G-proteins and other GTP-binding proteins and certain regions within the nucleotide binding domains (NBDs) of cystic fibrosis transmembrane conductance regulator (CFTR) indicates that these protein structures may be similar. A sequence alignment of the NBDs of CFTR and NBDs from other membrane transporters, forms the basis of a structural model. This model predicts that one of the conserved sequences GGQR, within which a number of CF mutations occur, forms part of the nucleotide binding pocket and serves as an ON/OFF conformational switch as observed in GTP binding proteins. Furthermore, based on subtle sequence differences between the first and second NBDs of CFTR and from structure–activity data, we suggest that the nucleotide binding site environments of the two NBDs are different.**

Key words: Cystic fibrosis transmembrane conductance regulator; Nucleotide binding; Conformational switch

1. Introduction

Cystic fibrosis transmembrane conductance regulator (CFTR) is a membrane protein and functions as a chloride channel regulated by both protein kinase A mediated phosporylation and binding of ATP [1]. Mutations in the gene coding CFTR causes cystic fibrosis (CF), a common lethal genetic disease characterized by the defective epithelial chloride transport [2]. CFTR belongs to a superfamily of transport related proteins [3] that include multiple drug resistance glycoprotein (MDR), α-factor pheromone export system (STE6), MHC-linked peptide transporter system (HAM1, RING4, etc.) hemolysin and a number of periplasmic permeases (HisP, MalB, etc.). These transporter proteins, in addition to having a membrane associated component as a common structural element, share one or more ATP/nucleotide binding domains (NBD). Hyde et al. [4] proposed a model of the NBD of CFTR based on a comparison with the crystal structure of another ATP binding protein, adenylate kinase (ADK) [5]. Mimura et al. [6] modeled the NBD of another transport related protein, His P. The latter model was based on the three dimensional structures of both ATP- and GTP- binding proteins.

Although there is evidence indicating that hydrolysis of ATP is required for the channel activity of CFTR [7], it is not clear how hydrolysis is involved in signal transduction. The presence of two NBDs in CFTR also raises the question whether the domains are functionally different. Regarding signal transmission, Ames and Lecar [8] recently proposed that the conserved region (GGQR) is located some distance from the nucleotide binding pocket and acts as a linker region in transmitting signals from the nucleotide binding pocket to other domains of the protein. However, we note that the sequence motif GGQR is observed in G-related proteins as an integral portion of the nucleotide binding pocket [9]. Our sequence comparison of the NBDs of transport related proteins with other ATP- and GTP-binding proteins has revealed a remarkable level of sequence conservation between NBDs and G-related proteins [10,11] in certain regions. In this minireview we summarize the sequence and structural similarities between the NBDs of CFTR and G-related proteins and present an alternative model for the NBD regions of CFTR. We also characterize the functional regions involved in conformational switching and discuss their roles in signaling mechanism.

2. Sequence comparisons between CFTR and other transport-related molecules and G-proteins

The sequence alignment shows four sequence motifs shared by the NBDs of transport related proteins and GTP binding proteins (Fig. 1). The first region contains the Walker A sequence (GXXXXGKS/T) which is characteristic of many ATP- and GTP-binding proteins and is also observed in CFTR and G-related proteins. This region is often called the P-loop or loop L1 (loop L1 in Fig. 1). The second conserved sequence has the motif G-G-Q-/R/K/Q and is labeled loop L4 in Fig. 1. This motif has been defined as switch II region for transducin-α [12]. The motif GGQR which is found in all G-proteins including the ADP-ribosylating factor family, is also conserved in CFTR and other transport related proteins, but surprisingly its conservation between G-proteins and ATP-dependent transporters has not been noted previously. However, this sequence motif is not obvious in the other ATP and GTP binding proteins (although the GQ of ras p21 is readily aligned; Fig. 1).

The third conserved sequence is the Walker B motif, containing four hydrophobic amino acids followed by D/E, characteristic of many ATP-binding proteins. Walker B is also observed in CFTR, ADK and other transporters included in this class. G-Proteins and GTP-binding proteins ras p21 and EF-Tu have the hydrophobic stretch of the Walker B motif but they do not have the negatively charged residue at the C-terminal end. Interestingly, GK which is highly homologous to ADK also does not have the charged residue at the C-terminal end. On the

*Corresponding author. Fax: (1) (508) 872-9080.

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other hand, the ADP-ribosylation factor family (ARL2) has the Walker B motif including the charged residue (Fig. 1). A fourth conserved motif AXSAXD immediately after the Walker B sequence was also noted shared between by the G proteins and certain membrane transporters such as MDR, STE6 but this motif is not apparent in CFTR.

Another important sequence pattern that is conserved in all G-proteins is the motif R-X-X-T. This motif which is located 23 residues up-stream from the GGQR motif has been called switch I region (Fig. 1) for transducin-α [12] and is involved in binding to GTP. In NBD1 of CFTR, switch I role may be played by Arg-487 (Fig. 1). Alternatively, the motif R-X-X-S (516–519, not shown in Fig. 1) found 33 residues up stream from GGQR motif may perform this role. However, this motif is not conserved in all of these transport proteins.

3. Topological orientations of functional regions in G-proteins and CFTR

The three-dimensional structure of NBDs are most likely composed of a core parallel β-sheet structure surrounded by α-helices. The prediction of this core structure involves consideration of the number of β-strands, their location along the sequence and their ordering and orientation in three-dimensional space.

G-proteins are homologous to GTP-binding ras p21 and EF-Tu in that they all share three functionally important sequence motifs (GXGXXGK, DXXG and NXXD) which have been shown to be involved in the regulation of GTP/GDP binding and hydrolysis [11]. This predicts that the core β-sheet structure of the major nucleotide binding regions of G-proteins are likely to have a structure similar to ras p21 or EF-Tu. This is confirmed by inspection of the tertiary structure of p21, EF-Tu and the G protein transducin-α as determined by X-ray crystallography [12]. The locations of these β-strands for G-proteins based on the crystal structure of transducin-α are shown in Fig. 1. Accordingly, loop L1, which has the conserved Walker A GXGXXGK motif would follow β-stand A and loop L4 which includes the DXXG and the overlapping GGQ/R/K/Q motifs defined here would connect β-stand C and β-strand

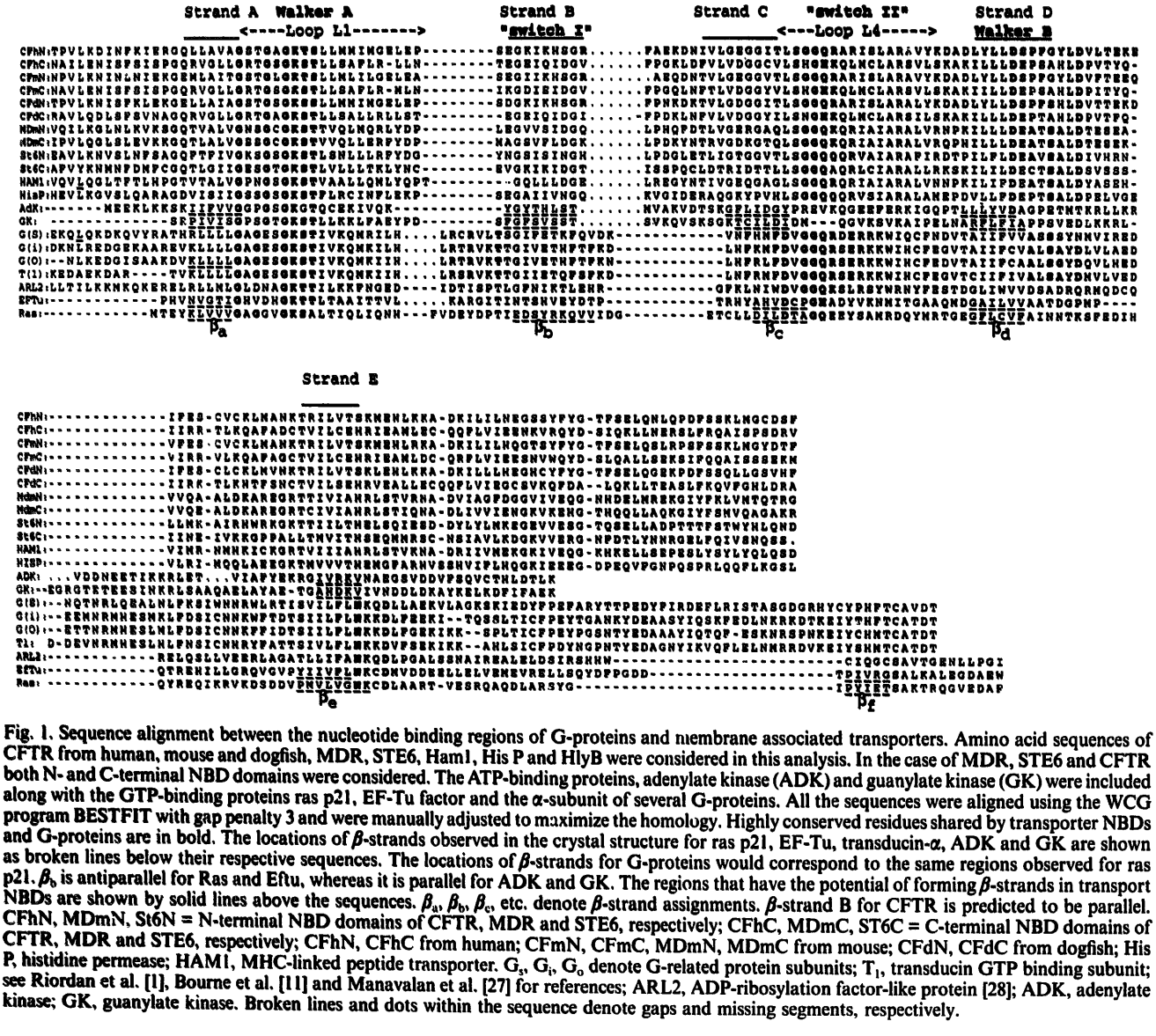

Fig. 1. Sequence alignment between the nucleotide binding regions of G-proteins and membrane associated transporters. Amino acid sequences of CFTR from human, mouse and dogfish, MDR, STE6, Ham1, His P and HlyB were considered in this analysis. In the case of MDR, STE6 and CFTR both N- and C-terminal NBD domains were considered. The ATP-binding proteins, adenylate kinase (ADK) and guanylate kinase (GK) were included along with the GTP-binding proteins ras p21, EF-Tu factor and the α-subunit of several G-proteins. All the sequences were aligned using the WCG program BESTFIT with gap penalty 3 and were manually adjusted to maximize the homology. Highly conserved residues shared by transporter NBDs and G-proteins are in bold. The locations of β-strands observed in the crystal structure for ras p21, EF-Tu, transducin-α, ADK and GK are shown as broken lines below their respective sequences. The locations of β-strands for G-proteins would correspond to the same regions observed for ras p21. β_b is antiparallel for Ras and Eftu, whereas it is parallel for ADK and GK. The regions that have the potential of forming β-strands in transport NBDs are shown by solid lines above the sequences. β_a, β_b, β_c, etc. denote β-strand assignments. β-strand B for CFTR is predicted to be parallel. CFhN, MDmN, St6N = N-terminal NBD domains of CFTR, MDR and STE6, respectively; CFhC, MDmC, ST6C = C-terminal NBD domains of CFTR, MDR and STE6, respectively; CFhN, CFhC from human; CFmN, CFmC, MDmN, MDmC from mouse; CFdN, CFdC from dogfish; His P, histidine permease; HAM1, MHC-linked peptide transporter. G_s, G_i, G_o denote G-related protein subunits; T_1, transducin GTP binding subunit; see Riordan et al. [1], Bourne et al. [11] and Manavalan et al. [27] for references; ARL2, ADP-ribosylation factor-like protein [28]; ADK, adenylate kinase; GK, guanylate kinase. Broken lines and dots within the sequence denote gaps and missing segments, respectively.

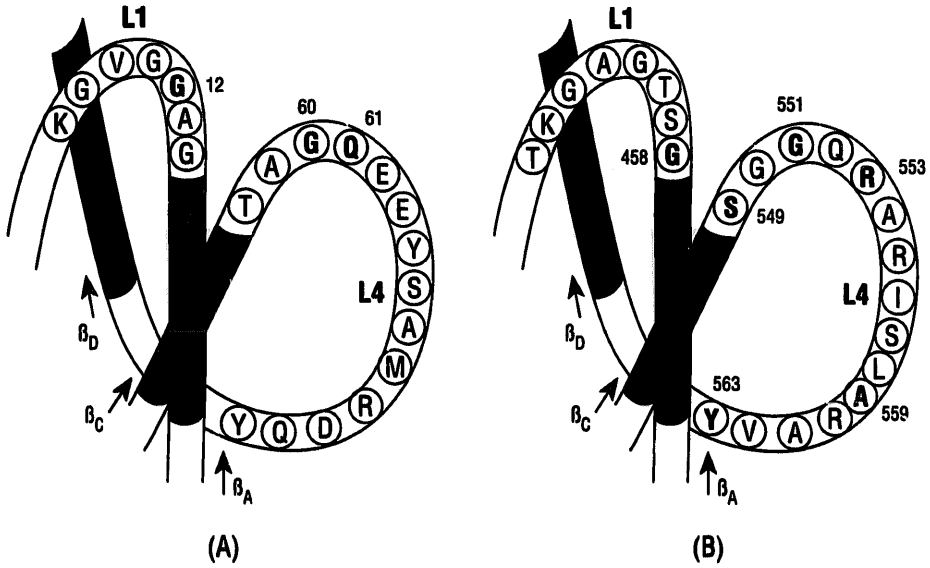

Fig. 2. A schematic representation showing β-strands A, C and D and the mutational sites in the connecting loops L1 and L4 which are involved in phosphate binding. Shaded bars represent β-strands. (A) Model for ras p21, EF-Tu and G-proteins (adapted from Pai et al. [20]. The residues shown in the loops correspond to ras. The mutations are: *L1:* G12 → V,D; *L4:* G60 → L; Q61 → L. (B) Model for transport NBDs. The residues and the mutations correspond to CFTR. The mutations are: *L1:* G458 → V; *L4:* S549 → I,R; G551 → D; A559 → T; Y563 → N.

D. Sequence homology between G-proteins and NBDs in both loop L1 and loop L4 regions would strongly suggest that NBDs would also have β-strand A β-strand C, β-strand D and β-strand E at similar locations. These four regions are labelled strand A, strand C, strand D and strand E, respectively in Fig. 1. The assignment of these four β-stands are in agreement with the prediction made by Hyde et al. [4].

The crystal structures of EF-Tu, ras p21 and transducin-α complexed with GTP and GDP indicate that critical residues involved in binding to GTP/GDP are located in loop L1 and loop L4 regions. Based on our prediction, we propose that the same loops also form the nucleotide binding sites for CFTR and other transporters in this class. This model would place the loop L4 in juxtaposition to loop L1 (Fig. 2) where it can directly interact with bound nucleotide. This is in contrast to the model proposed by Ames and Lecar [8]. According to their model, loop L4 region does not interact with loop L1 and is located distantly from the nucleotide binding site.

4. Structure–activity data

There are a number of mutations in the NBDs of CFTR that cause CF or impair the biological function of CFTR [13–14]. There is a high incidence of missense mutations within the N-terminal NBD and they are clustered essentially in three major locations. The first mutation region is in the vicinity of the Walker A motif (loop L1, Fig. 1). It is well documented that both for ATP and GTP binding proteins this region is critical and is involved in binding the phosphate moieties of a nucleotide. More specifically, the invariant Lys/Arg residue in loop L1 is involved in binding to α and β phosphates while the adjacent Ser/Thr residue interacts with Mg^{2+} ion. Table 1 depicts specific residues that are likely to interact with nucleotides for different ATP/GTP binding proteins. Table 1 also includes the putative nucleotide binding site residues from NBD1 and NBD2 of CFTR. Consistent with their playing a major role in protein function, mutation at most of these sites causes CF or affects the channel activity of CFTR.

The second region associated with CF mutations is in the vicinity of Phe-508 and the homology for this region with any other protein is not apparent. The third mutation region precedes the Walker B motif. According to our structural prediction, this latter region is in the loop L4 that connects β-strand C and β-strand D of CFTR as seen in G-proteins (Fig. 1). Mutations in this region also drastically affect the catalytic activity of GTP/ATP binding proteins. For example, the carboxylate group of aspartic acid or a hydroxyl group of Ser/Thr in loop L4 is involved in coordinating Mg^{2+} ion in GTP binding proteins. The corresponding residue for CFTR may be Thr-547 or Ser-549 (Glu-543 is also close to this region).

Interestingly, the residues in the loop L4 region (the so-called 'switch II' region) have also been shown to be critical for the proper functioning of GTP-binding proteins [9,11,12,15]. The loop L4 which interacts with the phosphoryl binding loop L1 has been shown to play a role in switching the protein from its GDP bound to its GTP bound conformation (Fig. 2A). The substitution of Leu for the most conserved residue Gln at this site significantly reduces the catalytic activity of $G_{s\alpha}$(Gln-227 → Leu) as well as ras p21 (Gln-61 → Leu). Similarly, the substitution of Ala for Gly in the adjacent residues (Gly-226 → Ala for $G_{s\alpha}$ and Gly-60 → Ala for Ras p21) substantially reduces the flexibility of L4, and thereby prevents the signal transmission initiated by binding GTP. CF related mutations have also been observed at the equivalent site where Gly has been replaced by Asp in both the NBDs (Gly-551 → Asp and Gly-1349 → Asp) of CFTR (Fig. 2B). The corresponding STE6 missense mutations also result in dramatically reduced STE6 function [16]. Other mutations in this region that are associated with CF are Ser-549 → Ile, or Arg or Asn and Ala-559 → Thr [13,14,17]. This consistency in the structure-activity data

between NBDs and ras p21 and G-proteins would strongly suggest that the orientation of this loop as well as the assignments of β-strand C and β-strand D are similar for both transport-NBDs and GTP binding proteins.

5. Catalytic roles of switch I and switch II regions

The involvement of switch I and switch II regions in binding to the γ phosphate of GTP and in catalytic activity is more clear from the crystal structures of $G_{t\alpha}$-protein complexed with GDP [12] and GTPγS [18]. Crystal structure of $G_{t\alpha}$–GTPγS shows that the structural changes in the switch I region are induced by hydrogen bonds between the γ-phosphate of GTP and Thr-177 and to a lesser extent with Arg-174 (switch I region). Changes in the switch II region are initiated by a hydrogen bond between Gly-199 and the γ-phosphate of GTP. More recently, Coleman et al. [19] determined the crystal structures of $G_{i\alpha 1}$ protein complexed with GTPγS and GDP-ALF4 and showed specific roles of Arg-178 and Gln-204 in transition state stabilization. Gln-204 of $G_{i\alpha 1}$ protein (Gln-61 in ras p21; [20]) stabilizes and orients the hydrolytic water molecules to the trigonal γ-phosphate in the transition state and Arg-178 promotes bond cleavage by stabilizing the developing negative charge in the oxygen atoms of the γ-phosphate.

Based on the structure–activity data and homologies in switch I and switch II regions, we suggest that these regions in CFTR play a similar role in ATP binding and hydrolysis. More specifically, Gln-552 in switch II region and Arg-516 in switch I region (or Arg-487) may be involved in transition state stabilization. Gly-551 may interact with the γ-phosphate of ATP. The presence of all these critical residues in NBD1 together with the recent report [21] that a Gln residue at a similar location in the ATP hydrolyzing RecA protein may be critical for the binding of ATP leads us to suggest that NBD1 may indeed hydrolyze ATP.

6. Functional roles of the NBDs in the activation of CFTR

While the two NBDs in CFTR have very similar sequences and equivalent Walker A and B motifs, other key residues involved in transition state stabilization and catalytic activity are different, suggesting different functional roles for the NBDs. The R-X-X-T sequence (switch I), which is important in both ribose and α- and γ-phosphate coordination in transducin-α, is present in NBD1 but not in NBD2. The GGQR sequence (switch II) of NBD1 is consistent with the consensus motif GGQ/R/K/Q, but in NBD2 the corresponding sequence is HGHK. The region around β-strand B, which is involved in binding to nucleotides in many ATP/GTP binding proteins [5,11] is also different for the two NBDs (Fig. 1). In NBD1, the sequence around β-strand B is KIKHSGR (481–487) and in NBD2 the corresponding sequence is EIQIDGV (1266–1272).

The absence of Gln and other key residue in NBD2 suggests that the binding affinity of ATP/ADP or the rate of ATP hydrolysis at NBD2 may be different from NBD1. Alternatively, ATP hydrolysis may only occur in NBD1 and NBD2 may have a role in nucleotide exchange related especially to ADP.

Structure–activity data from two laboratories [22,23] are consistent with such complementary roles for the NBDs of CFTR. These two studies reported mutated residues at similar locations in each NBD and measured the channel activity of CFTR. The structure–activity analysis indicates that mutation in the L1 region of the first NBD (K464 to M, Q, A, R) drastically reduced the chloride channel activity, whereas the equivalent mutation in NBD2 (K1250) enhanced chloride channel activity by abolishing the inhibitory effect of ADP on chloride conductance [22]. Similar effects on chloride current and ADP inhibition were observed when D572 and D1370 were mutated to Asn. Smit et al. [23] also found opposing effects in the activation of CFTR chloride channel by 3-isobutyl-1-methylxanthine (IBMX) when the similar homologous NBD1 and NBD2 mutations were introduced. The NBD1 mutations (K464Q, D572N) showed a decrease in sensitivity to IBMX activation while the equivalent NBD2 mutations (K1250Q, D1370N) produced an increase in IBMX sensitivity. These results together with our sequence analysis data suggest that the nature of nucleotide binding and the subsequent conformational changes may differ for the two domains.

7. Nucleotide regulated molecular switch for signal transduction

G- or GTP-binding proteins function as a molecular switch that regulates the opening and closing of signal transduction. All signal transducing GTP-binding proteins bind and hydrolyze GTP, properties which are crucial to their function as a molecular switch [11]. The GTP-bound form is the active conformation and turns on the signal and the hydrolysis of GTP to GDP bound form shifts the conformation to an inactive form, i.e. turns off signal transduction.

The signal transduction mechanism is more complex in CFTR because of the presence of two NBDs. Based on the chloride channel activity data, Gunderson and Kopito [24] proposed that binding of ATP at both NBD1 and NBD2 of CFTR is required to open the channel and ATP hydrolysis at at least one of these sites is necessary for transition to the closed state. Furthermore, they also demonstrated that orthovandate, a transition state analog, and poorly hydrolyzable ATP analogs do not open the channel but in the presence of ATP they all

Table 1
Residues interacting with phosphate moieties and Mg^{2+} ion in GTP/ATP binding proteins and the potential sites in CFTR

Protein	Phosphate moiety			Mg^{2+} ion
	PO4(α)	PO4(β)	PO4(γ)	
H-ras 21	G15, A18	V14, G15, K16, S17	G13, T35, G60	S17, T35, D33, D57 thru H_2O
EF-Tu	G23, T26, Y47	G23, K24, T25	K24, T62, G84, H85	T25, T62, D51, D80 thru H_2O
Transducin α	T44, R174	G41, K42, S43, T177	E39, K42, R174, T177, G199	S43, T177, S43, T177 D196 thro H_2O
Rec A	T73	K72, T73	K72, E96, Q194	T73, E96, D144
CFTR NBD1	G463, R516	G463, K464, T465, S466	K464, R487, S549, G551, Q552, R516, S519	S485, T547, S549, E543, D572
CFTR NBD2	G1249	G1249, K1250, S1251, T1252	K1250, S1347, G1349	D1270, S1347, D1370

lead to prolonged opening of the channel. However, they could not establish from their data whether ATP hydrolysis is required for channel opening because analogs and polyphosphate compounds do not appear to compete with ATP for the opening transition. Hwang et al. [25] studied the channel activity of CFTR and came to the conclusion that ATP hydrolysis at NBD1 controls channel opening and ATP hydrolysis at NBD2 regulates channel closing.

The proposed regulatory model for NBD2 is analogous to the switching mechanism proposed for GTP binding proteins. By analogy with the mechanism of GTP hydrolysis of ras p21, EF-Tu and G-proteins, the ATP bound form in NBD2 might be the active conformation that turns on the transmission signal (and/or keeps the channel open) and the hydrolysis of ATP to bound ADP may shift the conformation to an inactive form, i.e. to turn off the signal transduction or close the channel.

Alternatively, the overall mechanism could be, as with GTP-binding proteins, that NBD1 is the primary activation switch and NBD2 acts to exchange out the ADP to recycle the switch for ATP activation. Subsequent exchange of ADP off of NBD2 could require competitive binding by the high, physiological levels of ATP [26] often necessary for CFTR channel activation.

8. Conclusions

The structural similarities between G-proteins and the NBDs of CFTR addressed in this review may prove to be useful in understanding the regulatory role of NBDs in opening and closing the CFTR chloride channel. It is clear from the sequence homology and structure–activity data that the conformational changes in switch I and switch II regions are critical for regulating the ON/OFF switching mechanism. Structure–activity data also indicates that the functional roles of the NBDs are different, a difference that our structural modeling suggests may relate to hydrolysis-dependent molecular switching versus nucleotide exchange recycling and perhaps ATP/ADP ratio sensing. Though ADP bound to NBD2 appears to close the CFTR channel, more data is necessary to confirm that the bound ADP actually comes from the hydrolysis of ATP at NBD2.

It will also be interesting to investigate by specific mutations whether the NBDs of CFTR can be locked in a conformation that would favor the opening state of the CFTR channel. Since the natural, CF related mutations in L1 and L4 regions of CFTR have only been found to have decreased or no channel activity, their impact on this mechanism should only be negative in contrast to the well-known mutations of GTP-binding proteins which favor the active or open state of the switch, e.g. the oncogenic mutation of Gly-12 to Val or Asp in ras p21 [9]. Specific mutations in the functional regions outlined here and subsequent structure-activity analysis may lead to a better understanding of the relationship between ATP binding/hydrolysis and CFTR channel activity.

References

[1] Riordan, J.R., Rommens, J.M., Kerem, B.-S., Alon, N., Rozmahel, Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J., Drum, M.L., Jannuzzi, M.C., Collins, F.S. and Tsui, L.-C. (1989) Science 245, 1066–1073.

[2] Welsh, M.J., Anderson, M.P., Rich, D.P., Berger, H.A., Denning, G.M., Ostedgaard, L.S.,Sheppartd, D.N., Cheng, S.H., Gregory, R.J. and Smith, A.E. (1992) Neuron 8, 821–829.

[3] Higgins, C.F. (1993) Cystic Fibrosis – Current Topics (J.A. Dodge, D.J.H. Brock and J.H. Widdicombe eds.) vol. 1, pp. 139–153, Wiley, New York.

[4] Hyde, S.C., Emsley, P., Hartshorn, M.J., Mimmack, M.M., Gileadi, U., Pearce, R., Gallagher, M.P., Gill, D.R., Hubbard and Higgins, C.F. (1990) Nature 346, 362–365.

[5] Dreusicke, D., Karplus, P.A. and Schulz, G.E. (1988) J. Mol. Biol. 199, 359–371.

[6] Mimura, C.S., Holbrook, S.R. and Ames, G.F.-L. (1991) Proc. Natl. Acad. Sci. USA 88, 84–88.

[7] Carson, M.R. and Welsh, M.J. (1993) Am. J. Physiol. 265 (Lung Cell. Mol. Physiol. 9), L27–L32.

[8] Ames, G.F.-L. and Lecar H. (1992) FASEB J. 6, 2660–2666.

[9] Milburn, M.V., Tong, L., DeVos, A.M., Brunger, A., Yamaizumi, Nishimura, S. and Kim, S.-H. (1990) Science 247, 939–945.

[10] Gilman, A.G. (1987) Annu. Rev. Biochem. 56, 615–649.

[11] Bourne, H.R., Sanders, D.A. and McCormick, F. (1991) Nature 349, 117–127.

[12] Lambrignt, D.G., Noel, J.P., Hamm, H.E. and Sigler, P.B. (1994) Nature 369, 621–628.

[13] Tsui, L.-C.(1992) Trends Genet. 8, 392–398.

[14] Gregory, R.J., Rich, D.P., Cheng, S.H., Souza, D W., Paul, S., Manavalan, P., Anderson, M .P., Welsh, M.J. and Smith, A.E. (1991) Mol. Cell. Biol. 11, 3886–3893.

[15] Wittinghofer, A. and Pai, E.F. (1991) Trends Biochem. Sci. 16, 282–287.

[16] Berkower, C. and Michaelis, S. (1991) EMBO J. 10, 3777–3785.

[17] Tsui, L.-C., Markiewicz, D., Zielenski, J., Corey, M. and Durie, P. (1993) Cystic Fibrosis – Current Topics (J.A. Dodge, D.J.H. Brock and J.H. Widdicomb eds.) vol. 1, pp. 27–44, Wiley, New York.

[18] Noel, J.P., Hamm, H.E. and Sigler, P.B. (1993) Nature 366, 654–662.

[19] Coleman, D.E., Berghuis, A.M., Lee, E., Linder, M.E., Gilman, A.G. and Sprang, S.R. (1994) Science 265, 1405–1412.

[20] Pai, E.F.P., Krengel, U., Petsko, G.A., Goody, R.S., Kabsch and Wittinghofer, A. (1990) EMBO J. 9, 2351–2359.

[21] Story, R.M. and Steitz, T.A. (1992) Nature 355, 374–376.

[22] Anderson, M.P. and Welsh, M. (1992) Science 257, 1701–1704.

[23] Smit, L.S., Wilkinson, D.J., Mansoura, M.K., Collins, F.S. and Dawson, D.C. (1993) Proc. Natl. Acad. Sci. USA 90, 9963–9967.

[24] Gunderson, K.L. and Kopito, R.R. (1994) J. Biol. Chem. 269, 19349–19353.

[25] Hwang, T.-C., Nagel, G., Nairn, A.C. and Gadsby, D.C. (1994) Proc. Natl. Acad. Sci. USA 91, 4698–4702.

[26] Quinton, P.M. and Reddy, M.M. (1992) Nature 360, 79–81.

[27] Manavalan, P., Smith, A.E. and McPherson, J.M. (1993) J. Prot. Chem. 12, 279–290.

[28] Clark, J., Moore, L., Krasinskas, A., Way, J., Battey, J., Tamkun, J. and Kahn, R.A. (1993) Proc. Natl. Acad. Sci. USA 90, 8952–8956.