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Topical Review

Structural Insights into the CFTR-NHERF Interaction

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Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) is a low-conductance chloride channel located at the apical membrane of epithelial cells where it mediates cAMP-dependent chloride secretion (reviewed in [2, 23, 71]). Abnormal CFTR function is associated with the pathogenesis of human diseases, including cystic fibrosis, secretory diarrhea, and pancreatitis [2, 20]. Cystic fibrosis is caused by mutations in the *CFTR* gene that decrease the cell surface expression and/or activity of the CFTR protein and is the most common lethal genetic disease in Caucasians. By contrast, secretory diarrhea is caused by overstimulation of CFTR in intestinal epithelial cells by bacterial enterotoxins and is the second largest cause of infant mortality in the developing world. Therefore, the elucidation of the molecular mechanisms underlying CFTR regulation will not only provide a deep understanding of transepithelial electrolyte transport but it will also facilitate the development of novel clinical treatments for CFTR-associated diseases.

CFTR belongs to the ABC (ATP-Binding Cassette) membrane transporter superfamily (subfamily C, member ABCC7) (reviewed in [7, 13, 30, 33, 66]). ABC transporters use the chemical energy of ATP to move diverse sets of solutes across the membrane, including amino acids, peptides, large proteins, lipids, sugars, pigments, and anions. These transporters represent the largest gene superfamily in many sequenced microbial genomes and share a common architectural organization comprising two cytoplasmic ABC domains with ATPase activity and two membrane-spanning domains each consisting of six or more transmembrane segments (TM). These four domains may be expressed as separate polypeptides or half-transporters, such as the putative lipid A transporter MsbA from *Escherichia coli* [8], or fused together in a single polypeptide, as in the CFTR protein. The energy of ATP binding and hydrolysis is used to select and transport the substrates through the lipid bilayer by unknown mechanisms. The current draft of the human genome sequence contains 48 ABC genes, 16 of which encode transporters with known function, including the multidrug resistance protein, the transporter for antigen presentation, and the sulfonylurea receptor (a compilation of the known human ABC transporters can be found at *http://www.nutrigene.4t.com/humanabc.htm*). Because of the central role of ABC transporters in bacterial virulence and serious human disorders, including cystic fibrosis, multidrug resistance, hypercholesterolemia, Stargardt disease, and adrenoleukodystrophy [7, 13, 33, 66], a thorough understanding of the molecular mechanisms underlying their function is clinically important.

Here I will discuss briefly recent developments in the structural analysis of ABC transporters that have provided mechanistic insights into the CFTR regulation, and will examine in more depth the structural determinants of the interaction between the Na^+/H^+ exchanger regulatory factor (NHERF) and the C-terminal tail of CFTR, as revealed by recent crystallographic studies. Because the structural analysis of CFTR is an underdeveloped field, I will also discuss future research directions that are urgently needed to elucidate the molecular basis of CFTR function and its regulation by other proteins.

Molecular Architecture of CFTR and ABC Transporters

CFTR is a unique member of the ABC superfamily in that it is an ATP-regulated chloride channel and not a transporter. The 1480-amino-acid CFTR protein consists of two homologous halves, each containing

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six TMs connected with extracellular and cytoplasmic "loops" and a nucleotide-binding domain (NBD) (another name for the ABC used in the CFTR literature). The two halves are linked by a cytoplasmic regulatory domain (*R*) that contains many consensus sites for phosphorylation by protein kinase A (PKA), C (PKC), and cGMP-dependent protein kinase II [2, 23, 71]. Remarkably, when the two CFTR halves are expressed as separate proteins in the same cells, they assemble into functional channels, indicating that covalent linkage of the two halves is not required for channel assembly and function [2, 71].

To date, high-resolution structures of the entire CFTR channel or its domains do not exist. This lack of structural information has hampered the elucidation of mechanisms underlying CFTR function at the molecular level since the cloning of the *CFTR* gene in 1989 [63]. In the absence of a three-dimensional atomic model of CFTR, the proposed channel topology and interdomain relationships are inferred from structure-function analyses. The CFTR channel is thought to have a large extracellular vestibule that extends into the membrane, whereas the selectivity filter is located in the cytoplasmic part of the channel where the pore becomes narrow. Although the location of the gate that regulates ion conduction through the channel is currently unknown, it is well established that the channel gating is controlled by conformational changes in the cytoplasmic domains [2, 71].

Notwithstanding the lack of information on the CFTR structure, a major breakthrough in the three-dimensional organization of ABC transporters was recently achieved by the crystal structure determination of the MsbA from *E. coli* at 4.5 Å resolution [8]. The MsbA structure provided the first model for the transmembrane domain and cytoplasmic loops of a complete ABC transporter, as well as the topological relationship of these regions to the ABC domains. This structure established the mode of interaction of the α-helical TM segments with each other and revealed that the cytoplasmic "loops" are actually extensions of the TM α-helices into the cytoplasm. Surprisingly, the MsbA ABC domains are positioned remotely from each other and thus are unable to associate upon ATP binding. Although this arrangement could reflect an active conformation of the transporter, it has raised the possibility that the MsbA structure does not represent a physiologic dimer [31, 75]. In this context, it is important to note that the molecular envelope of the MsbA crystal structure differs substantially from those observed for the multidrug resistance protein 1 and the transporter for antigen presentation obtained by electron microscopy of single particles [64, 77]. Despite the limitations of the MsbA structure to represent transporters that translocate hydrophilic substrates [8], it nonetheless provides a starting structural framework that will guide future experiments toward a better understanding of the mechanochemistry of ABC transporters.

The 4.5 Å electron density map of MsbA did not reveal the ABC structure at high resolution. However, insights into the ABC fold were provided by crystal structures of several ABC domains, including the histidine periplasmic permease (HisP) from *Salmonella typhimurium* [36], the trehalose/maltose transporter (MalK) from *Thermococcus litoralis* [14], the human transporter for antigen presentation TAP1 [24], as well as the MJ1267 [37] and MJ0796 [82] ABCs from *Methanococcus jannaschii*. In addition, the crystal structure of the ATPase domain of the DNA repair enzyme Rad50 from *Pyrococcus furiosus* revealed a homodimer induced upon binding to ATP [34]. Rad50 is distantly related to ABC transporters but its ATPase domain is structurally similar to those of ABC members, primarily at the ATP-binding site. Interestingly, a recent crystal structure of the vitamin B_{12} transporter BtuCD from *E. coli* at 3.2 Å resolution [45] revealed that its ABC domains contact each other in an arrangement similar to the Rad50 ATPase dimer but different from that observed for the MsbA protein. These studies established the conserved tertiary structure of the NBD/ABC fold, which comprises a core α/β subdomain containing the consensus nucleotide-binding motifs Walker A (GX_4GKS/T) (X denoting any amino acid) and Walker B ($RX_{6-8}\Phi_4D$) (Φ representing a hydrophobic residue), an antiparallel β subdomain that interacts with the base of the nucleotide, and an α subdomain that contains the ABC transporter signature sequence LSGGQ. The Walker A motif follows a β-strand and it forms a loop (P-loop) that wraps around the α- and β-phosphates of the nucleotide, followed by an α-helix. The Walker B motif forms a β-strand followed usually by a glutamate and it may help coordinate the Mg^{2+} ion possibly through a water molecule [34] or it may polarize the attacking water molecule [36]. The function of the signature motif LSGGQ has not been determined unambiguously and it may act as a γ-phosphate sensor in the opposing molecule of the ABC dimer [34] and/or may signal to the membrane-spanning domains [33]. Although these structures provided important insights into the molecular basis of ABC transporter function, more studies are needed to define the ATP-dependent conformational changes of the ABC domains that underlie the functional cycles of these transporters and the gating of the CFTR channel. For example, there is controversy concerning the structure of the ABC dimer, which is thought to be a conserved feature of ABC transporters. The aforementioned crystal structures of isolated ABCs revealed several potential, albeit mutually exclusive, dimeric arrangements of these domains and failed to resolve unequivocally their oligomeric organization.

Regulation of CFTR Gating

The CFTR channel gating is thought to be controlled by three distinct processes: i) phosphorylation of the *R* domain; ii) binding and hydrolysis of ATP by the NBDs; and iii) interactions of CFTR domains among themselves and with other proteins (reviewed in [2, 23, 42, 71]). Phosphorylation of the *R* domain is a prerequisite for channel activation, and the channel open probability is directly related to the extent of phosphorylation. Deactivation of CFTR is brought about by protein phosphatases, including PP2A and PP2C [46]. However, phosphorylation is not sufficient for CFTR activation. A second mechanism for the control of channel gating involves the binding and hydrolysis of ATP by the two NBDs. Numerous studies have provided evidence that the CFTR NBDs play different but cooperative roles in controlling channel gating [2, 23, 71]. These domains share limited overall sequence similarity (less than 30% amino-acid identity) and exhibit sequence variations even in the Walker and signature motifs. For example, a conserved glutamate at the end of the Walker B motif that activates the hydrolytic water for attack on the γ-phosphate of ATP is replaced by a serine in the CFTR NBD1, suggesting that NBD1 may not hydrolyze ATP efficiently. Indeed, it was recently shown that ATP binds stably and dissociates slowly from the CFTR NBD1, while it is rapidly hydrolyzed by the NBD2 [3], demonstrating the non-equivalency of these NBDs. In addition, the signature motif LSHGH of CFTR NBD2 deviates from the consensus LSGGQ. This asymmetry of sequence conservation in the ATP-binding and active sites of the CFTR NBDs may reflect the different roles of these domains in channel gating, as have been demonstrated by many biochemical studies [2, 23, 71]. Elucidation of the structural basis of the NBD1-NBD2 interactions, their functional asymmetry and cyclic conformational changes that control CFTR gating awaits crystallographic analysis of these domains in the apo form, as well as complexed with nucleotides.

In addition to phosphorylation and ATP hydrolysis, recent studies have revealed a third mechanism of CFTR regulation operating through interactions of the CFTR domains among themselves and with other proteins. Specifically, it has been demonstrated that the N-terminal domain (NTD) of CFTR interacts directly with the *R* domain and functions as a positive regulator of the channel activity [42, 55]. At least part of this regulatory function has been mapped to a cluster of acidic residues in the CFTR NTD, whose sequential removal results in a graded inhibition of CFTR activity [21, 55]. Furthermore, syntaxin 1A, a membrane protein that plays a central role in neurotransmitter release, binds to the CFTR NTD and inhibits channel activity [42, 56–58]. In fact, it appears that the CFTR channel is regulated through binding of its NTD to a protein complex composed of syntaxin 1A and the synaptosome-associated protein SNAP-23 [12]. Remarkably, two recent studies have shown that the channel gating is also modulated through association of the CFTR C-terminal domain (CTD) with NHERF [61] and the CFTR-associated protein-70 (CAP70) [80] also known as PDZK1 [43]. These proteins interact with the CFTR C-terminal tail through a pair of PDZ (PSD-95/Discs-large/ZO-1) domains and it is thought that they activate the channel probably by inducing and/or stabilizing its dimerization [61, 80]. However, the oligomeric state of the functional CFTR channel is currently unknown. Early studies failed to co-immunoprecipitate biochemically different CFTR proteins expressed in the same cells, suggesting that CFTR is a monomer [48]. By contrast, functional analyses of coexpressed CFTR molecules with distinct properties, as well as electron micrographs of CFTR particles led to the conclusion that CFTR is a homodimer [18, 83]. The monomer hypothesis was reinforced by recent biochemical and electrophysiological experiments [10] although these studies could not exclude the possibility that CFTR channels are transiently tethered together by other proteins to form larger macromolecular complexes. Therefore, this issue remains controversial and definitive proof of the CFTR quaternary structure, as well as elucidation of the molecular mechanisms underlying CFTR activation by NHERF and CAP70 await future structural and functional studies.

Role of PDZ-containing Proteins in CFTR Apical Localization and Function

PDZ domains are protein interaction modules that play fundamental roles in the assembly of membrane receptors, ion channels, and other signaling molecules into specific signal transduction complexes [19, 28, 35, 68]. Such macromolecular complexes organized by PDZ-containing proteins have been termed transducisomes [19, 76] and are thought to increase the speed and specificity of signal transmission from membrane receptors to physically coupled downstream signaling molecules. The PDZ fold comprises a six-stranded antiparallel β-barrel capped by two α-helices. C-terminal peptides interact with PDZ domains by a β-sheet augmentation process, in which the peptide forms an additional antiparallel β-strand in the PDZ β-sheet. Early studies categorized PDZ domains based on their target sequence specificity into class I domains that bind to peptides with the consensus X-(S/T)-X-Φ and class II domains that recognize the motif X-Φ-X-Φ [28, 35, 68, 73]. Those studies pointed to the importance of peptide residues at positions 0 and −2 for the specificity and affinity (position 0 referring to the C-terminal residue),

whereas the residue −1 was thought to play no role in the interaction. This conclusion was corroborated by initial structural analyses, which showed that the side chain of the penultimate peptidic residue was facing towards the solution and did not bind to PDZ [15]. However, it became clear from subsequent studies that the structural determinants of the PDZ-ligand interaction are more complex than initially thought. For example, several PDZ domains have specificities that do not fall into these classes, implying the existence of more PDZ categories [4, 28], whereas others bind both class I and II ligands, suggesting an intrinsic flexibility in these modules to accommodate both polar and nonpolar side chains at position −2 [4, 35]. Furthermore, certain PDZ domains can also interact with internal protein sequences that adopt a β-hairpin structure [32]. Although the structural basis for ligand selection by PDZ domains is not well understood and reclassification of PDZs based on structural and affinity studies seems likely in the future, in this discussion I will use the current classification scheme of these domains.

NHERF is a cytoplasmic protein that was originally cloned as an essential cofactor for the PKA-mediated inhibition of the Na^+/H^+ exchanger 3 from the renal brush border [54, 81]. NHERF, also known as EBP50 (ezrin/radixin/moesin-binding phosphoprotein-50) [62], contains two tandem class I PDZ domains that interact differentially with numerous target proteins and a C-terminal ezrin/radixin/moesin-binding module that associates with the cortical actin cytoskeleton. The PDZ domains of NHERF and its related protein NHERF2 (also known as E3KARP) promote homo- and heterotypic protein-protein interactions, thereby orchestrating the clustering of ion channels and membrane receptors into transducisomes at the apical plasma membrane [70, 78]. The N-terminal PDZ domain of NHERF (designated PDZ1) spans residues 11–94 and binds to the C-terminal tails of several membrane receptors and ion channels, including the sequences QDTRL, NDSLL, and EDSFL, of CFTR, β_2 adrenergic receptor (β_2AR), and platelet-derived growth factor receptor (PDGFR), respectively [26, 27, 49]. The NHERF PDZ2 domain (residues 150–235) recognizes different sequence motifs than PDZ1 [79] and very few PDZ2 targets have been identified so far, including the c-Yes-associated protein YAP-65 [51] and the chloride channel ClC-3B [60].

The PDZ-binding motif DTRL of the CFTR C-terminal tail is essential for anchoring this chloride channel to the apical membrane because its deletion results in mislocalization of CFTR in airway and kidney epithelial cells [52, 53, 72]. The importance of the last four residues for the normal function of CFTR is also demonstrated by the occurrence of a stop mutation at Gln1476 in a patient with cystic fibrosis [*http://genet.sickkids.on.ca*]. Nevertheless, additional sequences within the CFTR regions spanning residues 1370–1394 and 1404–1425 are also required for the apical localization of this channel [50]. Moreover, recent studies provided evidence that the PDZ-interacting sequence of CFTR is not an apical membrane-sorting motif but it controls the endocytic recycling and apical retention of CFTR [74]. Since several PDZ-containing proteins interact with this motif, it is difficult to dissect the contribution of each of these proteins in the endocytic recycling, apical localization/retention and activity of CFTR because of potential functional redundancy. For example, the lack of a phenotype associated with CFTR dysfunction in a targeted disruption of the *NHERF* gene in mice [69] can be attributed to functional compensation by NHERF2, CAP70 and/or other PDZ-containing proteins that interact with CFTR.

In addition to the apical membrane localization/retention, the bivalent binding of the NHERF PDZ domains to the CFTR C-terminal region was shown to activate the channel [61]. A similar effect on the CFTR activity was observed upon binding of the CAP70 region harboring the third and fourth PDZ domains of this protein to the CFTR C-terminal tail [80]. Since both PDZ domains of NHERF were required for the regulation of CFTR gating, it was proposed that these domains interact with distinct CFTR molecules, promoting channel dimerization and increasing the open probability of this channel [5, 61, 80]. Although the precise mechanism behind the channel activation brought about by dimerization is unknown, the demonstration that the NHERF and CAP70 binding to CFTR directly affected channel gating provided the first evidence that PDZ-mediated interactions may have regulatory functions, in addition to assembling transducisomes.

Structural Determinants of the CFTR Interaction with the NHERF PDZ1 Domain

A first glimpse at the molecular recognition of CFTR by NHERF was provided by the crystal structure of the NHERF PDZ1 domain complexed with the CFTR C-terminal sequence QDTRL determined at 1.7 Å resolution [39]. The overall topology of NHERF PDZ1 is similar to other PDZ structures, consisting of six β-strands (β1–β6) and two α-helices (α1 and α2) (Fig. 1). The strands comprise an antiparallel β-sandwich with one β-sheet formed by β1, β6, β4, and β5, and the second β-sheet formed by β2, β3, and β4 strands. The fold is stabilized by hydrophobic interactions involving the conserved residues Leu17, Phe26, Leu28, Ile39, Leu53, Leu59, Val76, Ile79, Val86, and Leu88, which form the core of the molecule. The CFTR peptide inserts into the PDZ1 binding pocket antiparallel to the β2 strand

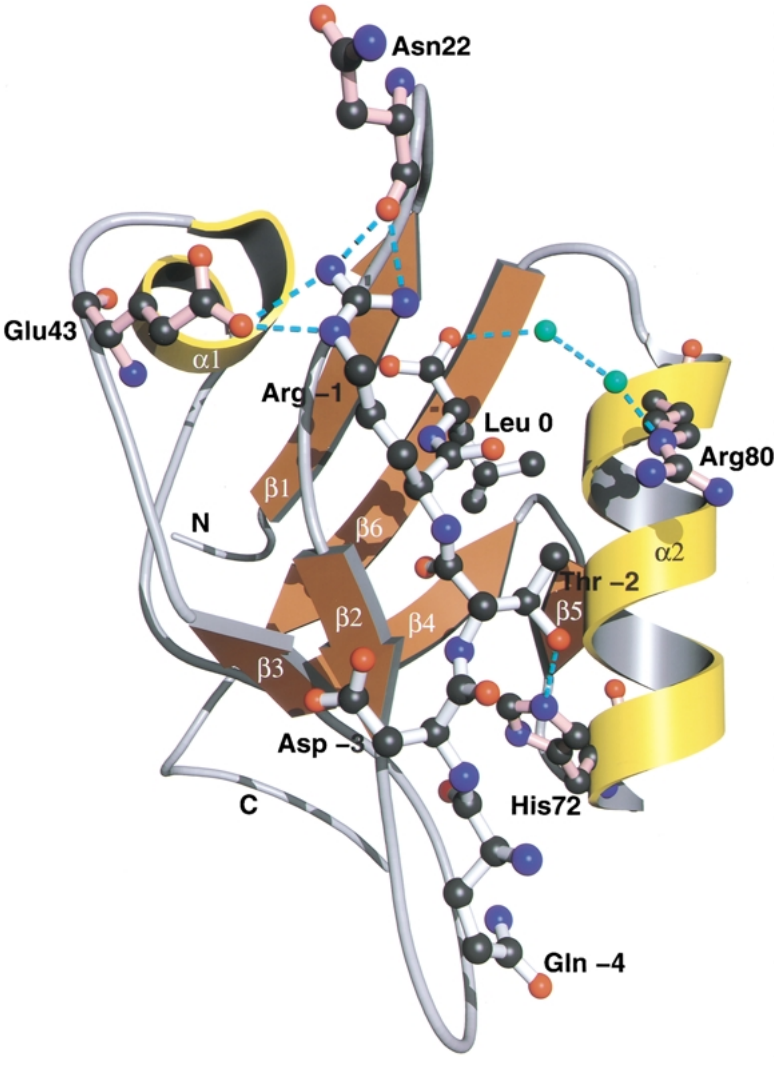

Fig. 1. Ribbon diagram of the human NHERF PDZ1 domain bound to the CFTR C-terminal sequence QDTRL. The β-strands are colored *brown* and the α-helices *yellow*. The peptide ligand is shown as a *white* ball-and-stick model. The PDZ residues Asn22, Glu43, His72, and Arg80 that participate in hydrogen bonding with peptidic side chains are shown as *pink* ball-and-stick models. Carbon, oxygen, and nitrogen atoms are shown in *black*, *red*, and *blue*, respectively. Water molecules are shown as *green spheres* and hydrogen bonds as *cyan dashed lines*. The figure was made using the published atomic coordinates (Protein Data Bank code 1i92).

and extends the β-sheet of PDZ1. In this arrangement, the invading pentapeptide is highly ordered, as indicated by low temperature factors. The side chain of the peptidic Gln −4 does not make contacts with PDZ1 residues and only the carbonyl oxygen forms a hydrogen bond with the amide nitrogen of Gly30, indicating that Gln −4 does not contribute to the specificity of the interaction. By contrast, Asp −3, Thr −2, and Leu 0 are engaged in numerous interactions with PDZ1, consistent with biochemical evidence on the important roles of these residues in the specificity and affinity of the NHERF PDZ1-CFTR interaction. Specifically, the side chain of Asp −3 forms a hydrogen bond with His27 and a salt bridge with Arg40. Similarly, the amide nitrogen and carbonyl oxygen of Thr −2 hydrogen bond with the carbonyl oxygen and amide nitrogen of Leu28, respectively, while the side chain of Thr −2 hydrogen bonds with the imidazole group of His72 (Fig. 1). The latter interaction corroborates the critical role of a threonine or serine residue at position −2 of the ligand and a conserved histidine at the beginning of the α2 helix for the specificity of class I PDZ-peptide interactions [26, 28, 35, 68, 73, 79].

The side chain and carboxylate group of Leu 0 enter into a hydrophobic pocket formed by the NHERF residues Tyr24, Gly25, Phe26, Leu28, Val76, and Ile79. The isobutyl group of Leu 0 makes hydrophobic contacts with Phe26 and Ile79. In addition, the carboxyl oxygen of Leu 0 is engaged in hydrogen bonding with the amide nitrogen atoms of Gly25 and Phe26, whereas the carbonyl oxygen of Leu 0 hydrogen bonds directly with the amide nitrogen of Tyr24 and indirectly with the guanido group of Arg80 in the α2 helix through two ordered water molecules (Fig. 1). The involvement of Arg80 in carboxylate binding through ordered water molecules differs from other PDZ structures where this function is mediated by an arginine residue in the β1-β2 loop [15, 28], corresponding to NHERF PDZ1 Lys19. Interestingly, the NHERF Lys19 does not participate in hydrogen bonding with the terminal carboxylate group, indicating that there are signifi-

cant variations in the atomic structural determinants of the PDZ-ligand interactions.

Previous biochemical studies demonstrated that substitution of the C-terminal leucine with valine in peptide ligands markedly reduced binding to the NHERF PDZ1 domain [26, 27, 79]. Likewise, replacement of the highly conserved Leu1480 in CFTR by alanine abrogated the apical localization of this channel due to abolishment of the CFTR-PDZ interaction [53]. How does the NHERF PDZl domain discriminate between the side chain of a C-terminal leucine and smaller side chains like those of valine or alanine? The NHERF PDZ1-CFTR structure showed that the isobutyl group of Leu 0 makes several hydrophobic contacts in the PDZ1 carboxylate-binding pocket [39], suggesting that the hydrophobic character of this cavity would likely exclude polar and charged side chains. Furthermore, comparison of the NHERF PDZ1 and PSD-95 PDZ3 crystal structures complexed with peptide ligands having C-terminal leucine and valine residues, respectively, revealed that their hydrophobic pockets have different sizes and shapes [38]. The NHERF PDZ1 cavity is large and the isobutyl group of Leu 0 fits snugly in this pocket, whereas the smaller side chains of valine and alanine would leave vacated spaces within this cavity that would be energetically unfavorable [17]. Thus, it appears that the tight fit of the leucine side chain in the hydrophobic cavity provides an explanation for the strict requirement for C-terminal leucine in all the high-affinity ligands of NHERF PDZ1, and the poor affinity of this domain for C-terminal valine and alanine residues. By contrast, the smaller cavity of PSD-95 PDZ3 interacts tightly with the isopropyl group of valine, making the accommodation of the larger isobutyl group of leucine stereochemically challenging. Therefore, the sequence variation among different PDZ domains generates hydrophobic cavities with distinct volumes and shapes, providing a selectivity mechanism for ligand recognition based on the stereochemical complementarity of the peptidic C-terminal residue and the volume/shape of the cavity. In this context, it is important to note that both NHERF PDZ1 and PSD-95 PDZ3 are currently considered as class I domains despite their fundamental differences in discriminating between the C-terminal residues of their cognate ligands. This underscores the problem with the current classification of PDZ domains and provides a compelling argument for a more elaborate reclassification scheme that would take into account the exquisite ligand selectivity of these modules.

The Importance of Arg −1 for the NHERF PDZ1-CFTR Interaction

The NHERF PDZ1-CFTR crystal structure also revealed a novel multivalent interaction of the arginine at position −1 of the CFTR peptide with two PDZ1 residues [39]. As mentioned above, early PDZ-peptide selection studies concluded that the residue at position −1 of the peptide ligand makes no contribution to the specificity and affinity of the PDZ-peptide interaction. Nevertheless, subsequent biochemical studies demonstrated that arginine is the preferred residue at position −1 for optimal binding to NHERF PDZ1. For example, affinity selection experiments showed that NHERF PDZ1 selected almost exclusively ligands with arginine at position −1 [79]. In addition, point mutagenesis of the penultimate arginine to alanine, phenylalanine, leucine, or glutamic acid, decreased the affinity of the PDZl-ligand interaction [26]. However, the structural basis for the NHERF PDZl ability to discriminate between different side chains at the −1 position of the peptide remained obscure until the structural analysis of the NHERF PDZ1-CFTR complex. This crystal structure revealed that the guanido group of Arg −1 forms two salt bridges with the side chain of Glu43 and two hydrogen bonds with the carbonyl oxygen of Asn22 (Fig. 1). These interactions provided the first structural explanation for the remarkable preference for a penultimate arginine by NHERF PDZ1 and consolidated previous biochemical results on the importance of this amino acid in the affinity of the interaction. Importantly, involvement of the penultimate residue in the PDZ-ligand interaction is not exclusive to the NHERF-CFTR complex but it seems to represent a more general theme in the selectivity mechanisms of other PDZ domains. For example, the PDZ2 domain of the membrane-associated guanylate kinase MAGI3 also binds preferentially to ligands having a tryptophan at position −1 [22], and the PDZ1 domain of the scaffolding protein INAD forms a disulfide bond with the penultimate cysteine of the peptide ligand [41]. Therefore, it appears that PDZ domains have a preference for specific side chains at position −1 and interact optimally with peptide ligands having the corresponding penultimate residues. The NHERF-CFTR structure also allows the prediction that the penultimate arginine of other ligands that interact with NHERF PDZ1, such as the C-terminal sequence TRL of the Na/P_i-cotransporter IIa [25, 29], is involved in similar networks of salt bridges and hydrogen bonds with the Glu43 and Asn22 residues of NHERF.

Similarities and Differences between the NHERF PDZ1 Interaction with CFTR and Membrane Receptors

Two recent crystal structures of the NHERF PDZ1 domain complexed with the C-terminal regions of β_2AR and PDGFR provided new structural insights into the contribution of the penultimate peptidic res-

idue to the affinity of the PDZ-ligand interaction [40]. In these structures the isobutyl group of Leu −1 and the phenolic ring of Phe −1 of the β_2AR and PDGFR ligands, respectively, engage in hydrophobic interactions with several PDZ1 residues. The side chains of Phe −1 and Leu −1 follow a path similar to that of the aliphatic portion of the Arg −1 side chain in the CFTR-PDZ1 structure, facing towards the PDZ residues Asn22 and Glu43. These two PDZ residues exhibit large conformational changes and they seem to play a critical role in the ability of NHERF PDZ1 to accommodate ligands with penultimate side chains of different hydrophobicity and polarity [40]. It remains to be seen whether the corresponding residues of other PDZ domains have similar roles in ligand recognition.

The three crystal structures of NHERF PDZ1 bound to CFTR, β_2AR, and PDGFR C-terminal tails represent the first structural analysis of a PDZ domain bound to three different ligands and provide an opportunity to identify significant differences in the PDZ-ligand interactions [39, 40]. One important difference was observed in the PDZ1-β_2AR structure, where the side chain of Asn −4 makes two hydrogen bonds with the amide nitrogen and carbonyl oxygen of Gly30, respectively, that contribute to the affinity of this interaction. By contrast, the side chains of the residues at position −4 of the CFTR and PDGFR ligands do not interact with PDZ1 amino acids. Another difference among these three structures is that the carbonyl oxygen atoms of the penultimate residues of both β_2AR and PDGFR ligands make direct hydrogen bonds with the guanido group of Arg80, whereas in the PDZ1-CFTR complex the carbonyl oxygen of Arg −1 does not hydrogen bond with Arg80. In addition, the carbonyl oxygen of Leu 0 interacts indirectly with Arg80 through two ordered water molecules in the PDZ1-β_2AR and PDZ1-CFTR but not in the PDZ1-PDGFR complex. Thus, the structural analysis of NHERF PDZ1 bound to CFTR, β_2AR, and PDGFR C-terminal sequences demonstrated that the ordered water molecules and hydrogen bond networks stabilizing the PDZ-ligand interaction differ even for slightly different ligands bound to the same PDZ domain.

Future Directions

The structural analysis of CFTR is in its infancy. Although remarkable progress has been made in our understanding of CFTR function during the past decade, structural studies of this channel and its complexes with regulatory proteins will undoubtedly revolutionize the field. Because crystallization of the full-length CFTR is a daunting task, crystallographic analyses of individual domains and domain complexes with regulatory and scaffolding proteins, such as syntaxin 1A, NHERF, NHERF2, and CAP70, is an alternative approach that will yield extremely useful information about the function and regulation of this channel. Importantly, high-resolution atomic models of CFTR domains will be instrumental in determining the structures of full-length CFTR crystals, when they become available.

The crystal structure of the NHERF PDZ1 domain bound to the C-terminal region of CFTR has provided important insights into the molecular determinants of an interaction interface that is critical for the apical localization/retention and gating of the CFTR channel. At the same time however, it underscores the urgent need for more structural studies of protein complexes involving larger CFTR and NHERF fragments. Arguably, among the most pressing questions to be addressed is the structural basis of the mechanism behind the regulation of CFTR gating by the two PDZ domains of NHERF [61]. At present, the spatial arrangement of the NHERF PDZ domains in relation to each other and the mode of their interaction with two distinct CFTR molecules to induce channel dimerization are unknown. Furthermore, the somewhat controversial issue of the CFTR C-terminal recognition by the NHERF PDZ2 domain also needs to be resolved structurally. While early studies suggested that NHERF PDZ2 has a selectivity for sequence motifs different than that present in the CFTR C-terminal tail [79], a protein fragment spanning residues 132–299 of NHERF and encompassing the PDZ2 domain was shown to interact with the CFTR CTD [61]. The latter finding raises the intriguing possibility that sequences outside the NHERF PDZ2 borders may participate in interactions with CFTR residues located upstream of the C-terminal four amino acids. Therefore, structures of NHERF protein fragments containing both PDZ1 and PDZ2 domains complexed with the entire CFTR CTD are required to elucidate the molecular mechanism of CFTR gating by NHERF. Of course, similar structural studies of the CFTR CTD bound to other PDZ-containing proteins, including NHERF2 and CAP70, will provide equally important mechanistic insights into the CFTR regulation.

In addition to promoting dimerization of CFTR molecules, the multi-PDZ scaffolding proteins NHERF, NHERF2, and CAP70 may also link this chloride channel to a wide variety of transporters, ion channels, kinases, phosphatases, and cytoskeletal elements. It is well established that CFTR regulates the activity of a growing list of transporters and ion channels, including Na^+/H^+ exchangers, Cl^-/HCO_3^- exchangers, epithelial Na^+ channels, renal K^+ channels, outwardly rectifying Cl^- channels, and Ca^+-activated Cl^- channels (reviewed in [44, 67]). Although it is not known how CFTR regulates the activities of so many ion channels and transporters, an attractive possibility emanating from the multi-

plicity of PDZ-containing proteins that associate with CFTR is that multi-PDZ proteins orchestrate combinatorial interactions of CFTR with other channels to modulate their function. In support of this scenario, the regulatory interaction between CFTR and Na^+/H^+ exchanger 3 requires the C-terminal PDZ-binding motif of CFTR, suggesting that these proteins are organized into supramolecular complexes by NHERF or other PDZ-containing proteins [1]. Furthermore, the recent identification of the chloride channel ClC-3B as a target of NHERF PDZ2 [60] supports the hypothesis that NHERF may organize the assembly of a ternary complex containing CFTR and ClC-3B through its two PDZ domains. Interestingly, coexpression of CFTR with NHERF and ClC-3B in epithelial cells resulted in ClC-3B-dependent outwardly rectifying chloride channel activity regulated by CFTR [60], providing evidence for a critical role of NHERF in this interchannel regulation. Similar macromolecular complexes assembled by multi-PDZ proteins have been described in other systems, as exemplified by the coupling of the ionic channels TRP and TRPL to multiple signaling molecules by the five PDZ-containing scaffolding protein INAD in *Drosophila* retinal cells [19, 28, 68, 76]. Therefore, defining the structural basis of the physical interactions among CFTR, NHERF, and other proteins within these multi-component complexes will reveal their molecular relationships and elucidate the mechanisms underlying CFTR function at the atomic level.

From a clinical perspective, structural information on the CFTR channel and its complexes with regulatory proteins may have important implications for the development of molecular medical approaches for treating CFTR-associated diseases. For cystic fibrosis it would be desirable to develop CFTR agonists that would enhance the activity of mutant CFTR proteins, in particular the CFTR-ΔF508, which harbors a deletion of Phe508 and represents the most common mutation in cystic fibrosis accounting for about 70% of all disease-causing alleles [6]. The CFTR-ΔF508 protein does not fold correctly and is retained in the endoplasmic reticulum where it is targeted for degradation, leading to low channel density and reduced chloride transport in the apical membrane of epithelial cells [2]. Because CFTR-ΔF508 can function as a chloride channel when expressed in the plasma membrane [16, 47, 65], it is conceivable, at least in theory, that development of CFTR agonists acting through NHERF to promote apical localization/retention and dimerization of CFTR-ΔF508 might augment channel activity in these patients. A similar approach could be also used in other cystic fibrosis-associated mutations that affect the membrane localization of the CFTR channel. In practice however, it may prove difficult to develop agonists that would increase the affinity of NHERF for CFTR because the sequence DTRL is considered to be the optimum ligand for NHERF PDZ1 [26]. Nonetheless, since it is possible that additional residues in the CFTR CTD also interact with the NHERF PDZ1-PDZ2 region, it could be envisioned that these interaction interfaces might provide more amenable targets for developing strategies to increase the affinity of the NHERF-CFTR interaction. Importantly, recent studies have identified a PDZ-containing Golgi-associated protein designated CAL [11], also known as PIST [59] or FIG [9], which modulates the membrane expression of CFTR. The CAL PDZ binds to the C-terminal tail of CFTR and promotes retention of this channel within the cell [11], suggesting that inhibitors of the CAL-CFTR interaction may increase the CFTR traffic to the apical membrane. Therefore, structural studies of the CFTR CTD bound to the PDZ domains of NHERF and other CFTR-associated proteins such as CAL, will reveal the three-dimensional interaction interfaces of these proteins and will provide structural frameworks for developing novel approaches aiming at enhancing CFTR activity. Likewise, structural analysis of the CFTR NTD/syntaxin 1A/SNAP-23 complex would guide the design of small-molecule compounds that block this interaction and enhance the chloride channel activity in cystic fibrosis patients carrying partial-loss-of-function mutations in the *CFTR* gene.

Structural studies of CFTR are also crucial for the development of new treatments for secretory diarrhea. Bacterial toxins that induce cyclic nucleotide production in the intestine promote CFTR phosphorylation and channel hyperactivation, which in turn results in massive secretion of salt and water [2, 20]. For example, activation of the PKA and cGMP-dependent protein kinase II by the cholera toxin and heat-stable enterotoxin from *E. coli*, respectively, results in overstimulation of CFTR, which subsequently leads to intestinal fluid and electrolyte secretion (secretory diarrhea) and dehydration. The role of NHERF and other PDZ-containing proteins in the apical membrane localization/retention and function of CFTR could be exploited for the development of novel CFTR inhibitors, which would act by blocking these interactions. Towards this goal, the atomic structures of the NHERF PDZ domains complexed with the CFTR CTD would guide the structure-based design of CFTR-PDZ blockers, and hopefully lead to the development of new therapeutics against secretory diarrhea.

A recent flurry of publications describing the crystallographic analysis of ion channels and ABC transporters leaves no doubt that we are entering a new era of high-resolution structural characterization of these membrane proteins. The unique properties of CFTR, being a chloride channel, a regulator of other channels, and a member of the ABC superfamily, in combination with its central role in the pathogenesis

of serious human diseases, make it a highly privileged target for structural studies. It is hoped that the wealth of structural and functional information to be discovered in the coming years on the regulation of CFTR trafficking, apical localization, and gating will lead to the development of novel ways to modulate channel function that may have clinical applications in treating CFTR-associated diseases.

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