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Introduction to Section IV: Biophysical Methods to Approach CFTR Structure

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Abstract

Inefficient folding of CFTR into a functional three-dimensional structure is the basic pathophysiologic mechanism leading to most cases of cystic fibrosis. Knowledge of the structure of CFTR and placement of these mutations into a structural context would provide information key for developing targeted therapeutic approaches for cystic fibrosis. As a large polytopic membrane protein containing disordered regions, intact CFTR has been refractory to efforts to solve a high-resolution structure using X-ray crystallography. The following chapters summarize current efforts to circumvent these obstacles by utilizing NMR, electron microscopy, and computational methodologies and by development of experimental models of the relevant domains of CFTR.

Keywords

CFTR structure; NMR; EM; crystallography; spectroscopy

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) lead to the development of cystic fibrosis (CF), one of the most common, fatal autosomal-recessive diseases (1, 2). CFTR is a large membrane protein composed of at least five individual domains: two nucleotide-binding domains (NBDs), two transmembrane domains (TMDs), and a regulatory domain (RD) (Fig. 21.1). The NBDs and TMDs are common to the ABC transporter supergene family, of which CFTR is a member of the C subfamily (ABCC7). These domains (3, 4) allow CFTR to mediate chloride conductance (5–7) and regulate the activity of several other critical transport systems in the apical membrane of epithelial cells (8–11). Detailed structural information at a variety of resolutions is critical for understanding how the protein performs these functions. The presence of disease-causing mutations in the CFTR gene leads to an absence of one or more of these activities. Such loss-of-function effects can arise from one or more of several distinct mechanisms including direct effects on channel function (12–14), effects on regulation (15), or effects that cause aberrant folding (16, 17) and trafficking (18) or a combination of these. Whereas the primary sequence of a protein contains the information required for achieving a functional, native conformation, it is not surprising that defective CFTR folding is the most common of the mechanisms leading to dysfunction (16, 17). Again, structural information can provide critical insight into the molecular pathogenesis of these mutations and the means to counter the aberrant steps.

Information about the structure of the CFTR molecule can be generated at several different levels of detail. First, biochemical and immunochemical methods can reveal the presence of post-translational modifications that reflect the enzymes CFTR has come in contact with and, thus, its history in a cell. The cell has its own intricate methods for distinguishing and sorting aberrant protein molecules from those that continue on to a functional native structure, which are reflected in these changes (19–21). This information indicates, at an extremely reliable, albeit gross level, whether or not CFTR has folded into a native structure. These approaches are covered in great detail in a number of other chapters within **Section**

III of this book. At the next level of detail, studies of full-length CFTR can provide information about the state of individual domains by combining limited proteolysis and electrophoretic separation with identification of domains using antibodies with known CFTR epitopes. In this manner, the effects of specific mutations on domains can be assessed by comparisons to the wild-type protein (22–24). Low-resolution approaches have greatly informed our understanding of the structure and function of CFTR and the molecular pathology of many of the disease-causing mutations. However, a desire to understand function and dysfunction at a structural level motivates the difficult goal of determining higher resolution structures of CFTR and its domains that are summarized in the next four chapters.

The structure of full-length CFTR at a resolution that allows for determining the relative apposition of the domains can be elucidated experimentally using incisive biophysical approaches such as electron microscopy and electron crystallography as described in **Chapter 22** by Ford et al. (22, 25, 26). These approaches require significant amounts of purified full-length CFTR and are sample preparation, data collection, and computationally intensive. The structures produced to date provide information about the identity of domains in the structure and changes in their relationship that correlate with alterations in nucleotide content and phosphorylation state, two parameters known to regulate the activity of CFTR. These methods hold great promise for the future in that resolution in the single digit Ångstrom level can be achieved and the presence of disordered regions in CFTR does not interfere as much as they might in the case of X-ray crystallography.

While full-length CFTR has proven refractory to X-ray crystallography to date, structures of other members of the ABC transporter family have been solved to high resolution utilizing this approach (27–29). While these homologues lack some important features of CFTR, for example, the R-domain and regulatory insertion (RI) and regulatory extension (RE) within NBD1, they share the four fundamental domains and, can thus, act as a starting point for producing structural models of CFTR. This approach, described in **Chapter 23** by Serohijos et al., can provide molecular models useful for formulating specific hypotheses for experimental testing (3, 30–33). More sophisticated computational approaches that calculate the folding trajectories of CFTRNBD1 have been developed and utilized to identify differences between wild-type and F508del and residues important in these differences (33). This work points the way to novel therapeutic interventions by small molecules to circumvent the differences in the mutant and wild-type folding.

An ultimate goal remains, however, the solution of high-resolution structures of the intact CFTR and mutants thereof in a variety of states that define the functional and pathogenic mechanisms. In particular, structures in the low Ångstrom resolution range will be required to reveal the conformation of the side chains as would be required for defining the binding mode of CFTR ligands. Also, information about the structural dynamics is also likely to be key and will come from NMR, as discussed in **Chapter 25** by Kanelis et al., and related experiments. A dissect and build approach has been utilized in the absence of technology to produce and analyze the full-length CFTR at this resolution at the present time. The assumption underlying this operation is that the domains of CFTR can be isolated as independent units that retain structure and at least partial function. This dissection has proven valid for the case of the NBDs and the R-domain. The second half of the operation is to build by recombining the high-resolution information garnered from the component domains, perhaps as directed by electron microscopy results.

To date, high-resolution structures have been produced for the NBD1 domains from murine and human CFTR (34–37) and human NBD2 (in fusion with a portion of the related bacterial NBD, MalK) (*pdb*: 3GD7). These structures have provided important insight into

the fold of the CFTR NBDs, the details of how they interact with nucleotide ligands, and the position of the critical F508 residue and other positions mutated in CF (35). The B-factors for these structures point to increased dynamics of particular regions when specific mutations or phosphorylations are introduced (36). Methods for producing and characterizing these domains are presented in **Chapters 23, 24, and 25**. **Chapter 24** focuses on the history of development of systems for producing CFTR-NBD1 as an illustrative example.

Dynamical changes in the conformation of CFTR are important at a variety of levels. Changes in conformation and in the relative orientation of the domains define the mechano-chemical reaction cycle of gating. Order–disorder transitions and conformational rearrangements underlie control of these domain rearrangements and, thus, the mechanisms by which nucleotide binding and hydrolysis and CFTR phosphorylation regulate the activity of the channel (38, 39). Folding is by definition the dynamic changes in conformation that occur as the native state is assembled. Where static structural information is difficult, dynamic structural information requires temporal information about the structure over many different timescales. **Chapter 25**, by Kanelis et al., summarizes the use of NMR spectroscopy to address many of the critical questions about the regulation of CFTR and the effects of mutations on the dynamics of the domains.

Structural information relevant to the function and dysfunction of CFTR forms the basis of a rational approach to therapeutic discovery and development. However, CFTR is an integral membrane protein that is produced in cells in low abundance creating significant obstacles to structural studies. Moreover, it contains significant regions of disorder even when it is in its native state – disorder that is central to its function, but that impedes many structural experiments – obviating a need for information about the dynamics of these structures. Finally, in several cases, including the prevalent F508del mutation, the mutant protein does not fold efficiently into the final structure. Since many of these disease-causing folding mutants retain at least partial function when they are induced to fold in vitro or in cell culture systems (40, 41), a critical challenge is to produce structural information relevant to these partially folded or misfolded states. Computational methods have a key advantage for directing work in this difficult area. The following chapters provide a primer on current experimental and computational methods for structural work on full-length CFTR, its domains, and the dynamical states central to folding and function. Understanding the structural details of function and the folding process may not only provide fundamental knowledge but also have practical application in the development of future treatments for the disease.

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Fig. 21.1.

Three views of a model of CFTR sans the R domain, which is largely disordered. The model was produced using the crystal structures of CFTR-NBD1 and the structures of related ABC transporters as previously described (3). In all views, NBD1 is shown with a *gray surface*, ICL1 as a *gray cartoon ribbon*, ICL4 as a *charcoal cartoon ribbon*, F508 as a *black surface*, ATP as a *black space-fill*, and NBD2 and the rest of the TMDs as a *cartoon line*. The views are all from the perspective of the plane of the membrane (*dotted line* indicates approximate position of the interfacial portion of the membrane) and are rotated by 90° relative to each other on the indicated axis. The image, produced with pymol, highlights the position of F508 on the surface of NBD1 at the interface with ICL4, the two ATP molecules sandwiched between NBD1 and NBD2 that correspond to the open state of the channel, and predicted domain swap between the TMDs where ICL1 and ICL4 form a unit that interacts with NBD1.