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Original Article

CFTR structure

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Abstract

Structural studies of the cystic fibrosis transmembrane conductance regulator (CFTR) protein are critical to understand molecular mechanisms involved in gating of the apical anion channel as well as the way in which the gating is regulated, especially by the regulatory region (R region). They are also instrumental for understanding the root cause of cystic fibrosis (CF) and supporting the development of therapeutic strategies. In this short review, we summarize recent progress in the knowledge of the CFTR 3D structure and briefly discuss implications for CF drug development.
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Structural biology of the CFTR protein already has a longstanding history. Structural and dynamic studies of isolated, cytosolic domains have been conducted using X-ray crystallography and nuclear magnetic resonance spectroscopy (NMR), among other approaches. One of the major challenges for biophysical measurements was to produce minimally mutated stable constructs of the two nucleotide-binding domains, NBD1 and the even more difficult NBD2 (reviewed in [1]). Recently, significant progress for NBD2 has been made by identifying stabilizing mutations in a comprehensive way, using a bioinformatics analysis [2]. These methodological investigations open avenues for studying the defects in NBD-NBD interactions caused by NBD2 mutants such as N1303K (Thibodeau et al., unpublished results). On the other hand, the poor solubility and stability of the CFTR protein and its dynamic fluctuations, including significant regions of intrinsic disorder, have hampered progress towards high resolution structures of the full-length protein for a long time, although 2D crystals and single particle analysis using EM provided interesting data at medium resolution [3]. Studies of full-length CFTR were thus first undertaken using comparative modeling, including molecular dynamics (MD) simulations, using homologues in the ATP-binding cassette (ABC) transporter family of proteins to which CFTR belongs (reviewed in [4]), before medium-high-resolution models of the 3D structures of the full-length CFTR were obtained using cryo-electron microscopy [5,6]. Altogether, the structural studies, combining experimental and theoretical approaches at the domain and whole protein levels, offer new perspectives for understanding the behavior of wild-type and mutant CFTR, including responses to drug binding. The following review further develops the recent insights gained into the 3D structure of the full-length CFTR protein, as well as into interactions of the disordered regulatory region or R region.

1. 3D structures of full-length CFTR protein: combining theoretical and experimental data

A major breakthrough was achieved in the past year, with the publication of medium-to-high-resolution 3D structures of the full-length CFTR, first from zebrafish [5] and then human [6], obtained using single-particle cryo-electron microscopy (cryo-EM). These structures provide insights about a non-phosphorylated, apo

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form of the channel, representing a closed and inactive channel state. In these structures, the nucleotide-binding domains (NBDs) are fully dissociated and observed density for a short helix of the R region was suggested to lock the structure in an inward-facing conformation, with the NBDs splayed apart (Fig. 1A). The medium-resolution structures of two other proteins from the ABCC subfamily, MRP1 [7] and SUR1 [8,9], were also solved in a similar inward-facing conformation, sharing with CFTR a common core of membrane spanning domains (MSDs), and a vestibule penetrating halfway into the lipid bilayer. In the three structures, a newly-described N-terminal "lasso", located N-terminal to MSD1, packs against the transmembrane helices. Notwithstanding this progress, there are still many unanswered questions in the field. Despite the excellent resolution for the MSD regions observed in the cryo-EM 3D structure, the resolution obtained for the cytosolic domains (NBDs, but also intracellular loops (ICLs) connecting NBDs to the MSDs) was low and very little information was obtained for the critical regulatory R region. In addition and most importantly, these first experimental 3D structures correspond to inactive forms of the channel and information about further conformational states, representing relevant stages in the channel-gating cycle, was thus still missing. Finally, these experimental structures give only limited access to the dynamical behavior of the protein, which plays a critical role in the channel function and regulation.

Well before these cryo-EM 3D structures, several 3D models of the assembly of CFTR MSDs/NBDs, in different forms relevant to the gating cycle, were built by comparative modeling, based on crystal structures of ABC exporters ([4] for a review). Starting from outward-facing, Sav1866-based 3D models (in which the two NBDs are tightly associated, with ATP bound at the interface), short MD simulations or other approaches enabled development

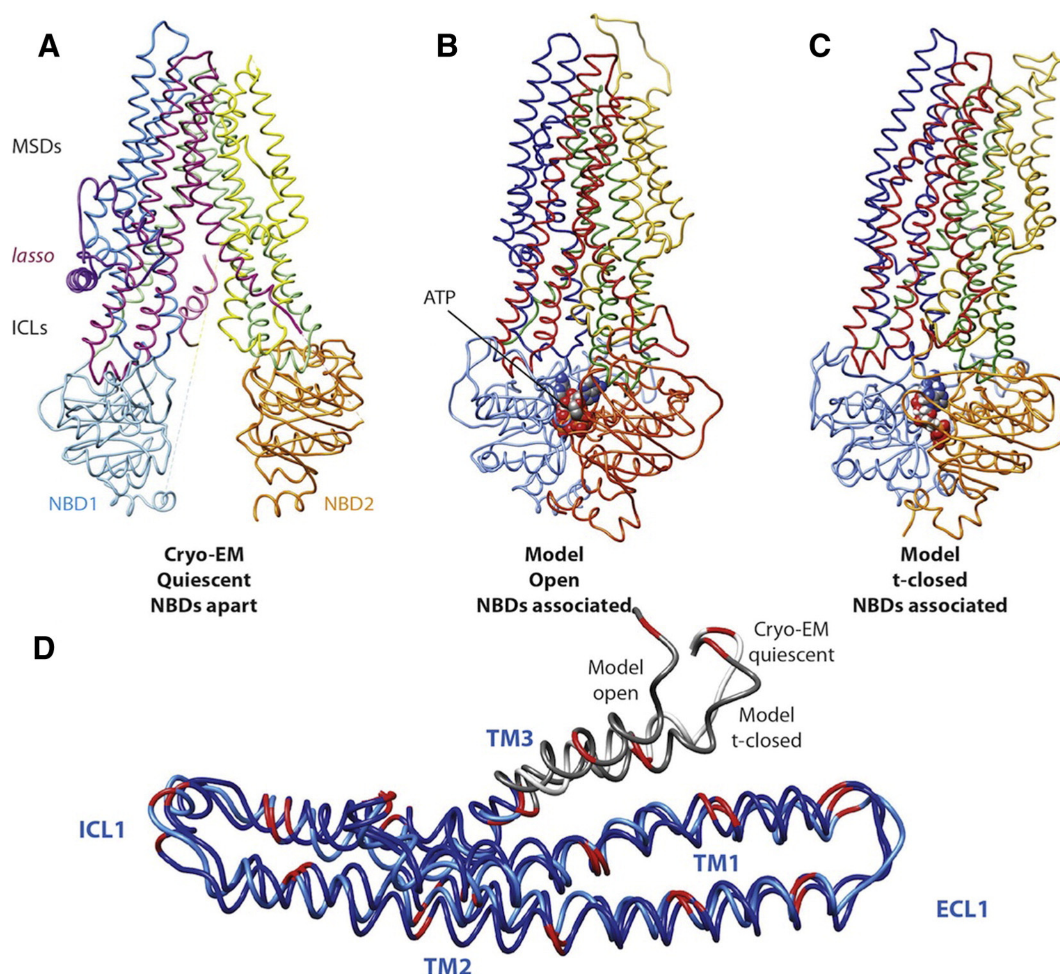

Fig. 1. (A, B, C) Ribbon representation of the cryo-EM 3D structure of human CFTR in a quiescent (apo) form (A, PDB ID: 5uak [6]), compared to models of the open (B) and t-closed (C) forms [10], obtained after short MD simulations of a Sav1866-based 3D model. The stability of the t-closed model is now further supported by advanced MD simulations (Elbahnsi A, Hoffmann B, Mornon JP, Callebaut I and Pietrucci F, unpublished data). Different colors are used to highlight the different domains (intracellular loops, ICLs; lasso region; and nucleotide-binding domains, NBDs) and blocks of three consecutive TM helices of the membrane-spanning domains (MSDs) of the CFTR protein. ATP molecules are represented in a space-filling mode at the interface of the NBDs in models of the open and closed forms of CFTR. (D) The first block of three consecutive TM helices in these 3D structures are superimposed (TM1, TM2 and TM3 in blue), highlighting a good correspondence between amino acid positions (every ten residues shown in red) and thus the good match between experimental and theoretical data, with the intracellular and extracellular loops 1 are labeled ICL1 and ECL1, respectively. A similar match is observed for the three other blocks of consecutive helices. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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