48 Insights into the transmission interfaces of human CFTR from molecular modeling

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Models of the 3D structure of full-length human CFTR described during the last years have provided new insights into the molecular basis of CFTR functioning. They have in particular shed light on the interdomain interfaces which are critical for signal transmission and can thus be considered for *in silico* screening of drugs (correctors and modulators) capable of filling the cavities displayed at these interfaces in the wild type or mutated protein.
However, the modeling methodology does largely influence the characteristics of those interdomain interfaces. Here, relying on all the available experimental data on ABC transporters (full length and isolated domains), we show that there is a considerable structural plasticity in these regions which allows to adapt the partner domains. The careful consideration of such a structural plasticity in our modeling strategy leads us to propose at present four models of human CFTR, two (based on the Sav1866 and MsbA structures) in a similar open channel conformation and two (based on the MsbA and P-gp structures) in notably different closed channel conformations. We interestingly show that the global features of the transmission interfaces [between the membrane-spanning domains (MSDs) and the nucleotide binding domains (NBDs)] are remarkably conserved in these models between the open and closed forms. Further investigation of the details of the MSDs/NBDs interface also suggests that subtle differences may participate in the general mechanisms by which the signal is transmitted between MSDs and NBDs.
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49* Probing the oligomeric state of CFTR

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The CFTR functions as a Cl^- channel and controls electrolyte transport in epithelia. The defective synthesis and/or regulation of CFTR are implicated in two important human diseases: Cystic Fibrosis (CF) and secretory diarrhea. CF is one the most common lethal inherited disorders and is due to loss of function mutations in CFTR while a disproportionately excessive response of CFTR, usually to bacterial or viral toxins, leads to a secretory diarrhea, killing millions of kids under the age of five throughout the world. Despite understanding that CFTR activity is responsible for major pathology, the exact nature of the CFTR conducting pore remains controversial, with findings pointing both to monomeric and multimeric assembly of the CFTR. We have utilized CFTR tagged with cyan (ECFP) or yellow (EYFP) fluorescent proteins on the C or N termini. Using FRET we found no appreciable increase in CFP fluorescence after selective photobleaching of YFP, indicative of FRET not occurring. These findings show that the cytoplasmic tails of CFTR are not in sufficient proximity for the occurrence of FRET, suggestive of a monomeric organization of CFTR. However, upon phosphorylation and activation of the CFTR, FRET was observed with C-terminal tags suggesting a more complex interaction. These observations indicate that the state of the CFTR oligomerization might not be static; rather that it might depend upon the biochemical state of the channel or associated proteins.
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50* Low temperature and chemical rescue affect the molecular proximity of DF508-CFTR and ENaC

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In patients with CF, the genetic errors in the *cftr* gene lead to a defective epithelial Cl^- transport which is proposed to be the culprit for the clinical manifestation of CF. However, some of the transport abnormalities, Na^+ hyperabsorption for example, is hard to explain by defective Cl^- transport only. Hence, in addition to an abnormal Cl^- transport as a result of a defective CFTR, understanding other functions of the CFTR such as its regulatory role is important for the treatment of CF airway disease. In this study we have examined the association of ENaC subunits with mutated DF508-CFTR, the most common disease causing mutation in CF. Deletion of phenylalanine at position 508 prevents proper processing and targeting of CFTR to the plasma membrane. We found that ENaC subunits could be co-immunoprecipitated with DF508-CFTR. Additionally, we evaluated the DF508-CFTR and ENaC association by FRET. FRET efficiencies were not significantly different from negative controls suggesting that DF508-CFTR and ENaC are not in close proximity to each other under basal conditions. However, after partial correction of DF508-CFTR misprocessing by low temperature and chemical rescue we observed a positive FRET signal, especially between DF508-CFTR and b-ENaC. Our findings suggest that the mutated version of CFTR reduces the close association of DF508-CFTR and ENaC, suggesting that rescue of Cl^- transport alone by allowing for the trafficking of DF508-CFTR may not ameliorate the Na+ hyperabsorption seen in CF airway disease. This further underscores the importance of ENaC in the pathogenesis of CF.
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52 Protein analysis of mutant CFTR in human tissues

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Only few studies have yet described immunoblot analysis of CFTR in human tissue. Literature data on the major cystic fibrosis mutation F508del with regard to maturation and function in human tissue are inconsistent. In the context of the upcoming studies on correctors and potentiators of mutant CFTR it is important to enlarge our knowledge about mutant CFTR protein biochemistry. We initiated studies to clarify the distribution and abundance of isoforms of normal and mutant CFTR performed both with intestine tissue and with lung tissue. Freshly excised rectal biopsies or snap-frozen tissues from freshly explanted non-CF and CF subjects were used for protein analysis. The outcome of both studies differed: In rectal biopsies of homozygous F508del CF-subjects the mature complex-glycosylated C-band of F508del CFTR was mostly detectable, albeit in different amounts, but not in mutant lung tissue (CFTR genotypes: F508del/F508del, F508del/R553X, F508del/deletion, homozygous 3849+10kb C-T, unknown) at this stage of global respiratory insufficiency. The mannose-rich B-band was detectable in 6 of 8 lungs and in all rectal biopsies. In conclusion, CFTR immunoblot analyses are feasible with CF lung and rectal tissues. Both should be used to test the influence of potentiators or correctors on the abundance and processing of mutant CFTR.