Chemistry & Biology
Preview

Defining the Defect in F508 del CFTR: A Soluble Problem?

Charles M. Deber,[1,2,*] Joanne C. Cheung,[1,2] and Arianna Rath[1,2]
[1]Division of Molecular Structure & Function, Research Institute, Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada
[2]Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada
*Correspondence: deber@sickkids.ca
DOI 10.1016/j.chembiol.2008.01.001

Previously reported crystal structures of CFTR F508 del-NBD1 were determined in the presence of solubilizing mutations. In this issue of *Chemistry & Biology*, Pissarra et al. (2008) show that partial rescue of the trafficking and gating defects of full-length CFTR occurs in vivo upon recapitulation of the solubilizing F494N/Q637R or F428S/F494N/Q637R substitutions in *cis* with F508 del.

Cystic fibrosis (CF) disease is caused by mutations in the 1480-residue cystic fibrosis transmembrane conductance regulator (CFTR) (Riordan et al., 1989). CFTR is one of a group of over 45 active transporters termed the ABC transporter family. Typical family members are organized into two cytosolic nucleotide-binding domains and two transmembrane domains. Unique to CFTR is an additional cytosolic regulatory (R) domain with phosphorylation sites for cAMP-dependent protein kinase. In healthy individuals, CFTR localizes to the apical membrane of epithelial cells, and functions as a chloride channel. Disease-causing mutations in the CFTR gene can result in defects in synthesis, trafficking, stability, function, and/or regulation of the CFTR protein (Welsh and Smith, 1993).

The CFTR variant where phenylalanine 508 is deleted (F508 del) is the primary CF-associated mutation, affecting approximately two-thirds of alleles (Kerem et al., 1989). The deletion occurs in the first N-terminal nucleotide-binding domain (NBD1), and causes misfolding and biosynthetic arrest of CFTR (Amaral, 2006; Lukacs and Durie, 2003). At the cellular level, F508 del is retained in the endoplasmic reticulum (ER) by the quality control (QC) system and is rapidly degraded (Yang et al., 1993). Lowering the incubation temperature of cells expressing F508 del CFTR allows the protein to traffic to the cell surface, suggesting the defect of F508 del lies in "kinetic trap(s)" in the folding pathway (Cyr, 2005). As well, the isolated wt NBD1 and F508 del NBD1 domains do not differ significantly in terms of thermodynamic stability (Thibodeau et al., 2005), an indication that other domains of CFTR likely participate in the overall folding process.

Electron microscopy has provided overall CFTR topology (Rosenberg et al., 2004), but atomic resolution data remain unavailable for the full-length molecule. Meanwhile, considerable efforts have been directed toward determining—and comparing—the structures of normal versus F508 del forms of NBD1. Initially, protein appropriate for structural studies could be obtained only for mouse NBD1 out of 10 organisms tested, including human (Lewis et al., 2004). Recovery of human wild-type and F508 del NBD1 for crystallization ultimately required that certain amino acid changes be introduced to the domains in *cis* (Lewis et al., 2005). The first human F508 del-NBD1 structure obtained carried seven additional mutations, three of which (suppressor mutations G550E, R553Q, and R555K) were known to rescue the F508 del-CFTR defect (Chang et al., 1999; DeCarvalho et al., 2002; Teem et al., 1996). Two F508 del-NBD1 structures lacking the suppressor mutations but retaining certain other alterations (F494N/Q637R or F428S/F494N/Q637R) necessary for protein solubility subsequently became available (Lewis et al., 2005, http://www.pdb.org). The latter structures were found to be nearly identical to F508 del-NBD1 with the suppressors present (Figure 1A), and all indicated that F508 del promotes little change in the NBD1 fold overall but does alter local surface conformation at the F508 del site (Figure 1B; Lewis et al., 2004; Lewis, 2005). Small surface changes that impair interdomain interactions of F508 del-NBD1, rather than large conformational changes or an altered fold, were therefore concluded to be the fundamental defect associated with the F508 del mutation.

In view of this significant number of background mutations, the question arises as to whether the solved crystallographic structure may actually represent a "corrected" F508 del NBD1. To address this question, Pissarra et al. (2008) report in this issue on the trafficking in mammalian cell lines of full-length CFTR proteins carrying wt, F508 del, or F508 del with the F494N/Q637R or F429S/F494N/Q637R replacements. In agreement with previous studies, wt CFTR was processed to the mature Band C form, indicating trafficking out of the ER, whereas only a Band B form was seen for F508 del CFTR, suggesting the protein did not traffic out of the ER (Amaral, 2006; Lukacs and Durie, 2003). Strikingly, the solubilizing mutations, notably the triple mutant F429S/F494N/Q637R, appeared to promote some N-glycan processing in F508 del CFTR, to produce Band C, along with bands of MW intermediate between those of Band B and Band C, suggesting that these replacements partially rescue the trafficking defect. Cell surface biotinylation assays further indicated that proteins containing the solubilizing mutations were present at the plasma membrane. As well, iodide efflux assays showed that at 37°C, F508 del CFTR containing the solubilizing mutations had an iodide efflux time course like that of wt CFTR, albeit with lower magnitudes that were comparable to F508 del CFTR rescued by incubation at 26°C. At the latter temperature, the magnitudes of iodide efflux were similar to wt CFTR.

Pissarra et al., (2008) also showed that CFTR molecules carrying solubilizing

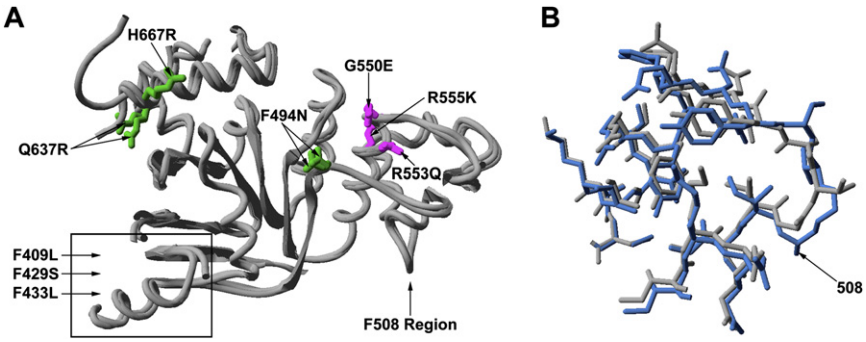

Figure 1. Solved NBD1 Structures of CFTR

(A) Comparison of F508 del-NBD1 structures. Protein backbone residues are shown as ribbon diagrams. F508 del-NBD1 structures obtained with solubilizing mutations in *cis* (F494N/Q637R and F429S/ F494N/ Q637R; Lewis, 2005) are superimposed with the structure of F508 del-NBD1 obtained with solubilizing mutations and suppressor mutants in *cis* (F409L/F429S/F433L/H667R and G550E/R553Q/R555K, respectively; Lewis et al., 2004). Suppressor mutant side chains are colored pink and solubilizing mutant side chains colored green where they are visible in the respective crystal structures. Portions of the NBD1 chains corresponding to the regulatory insert (boxed) were invisible in each of the three crystal structures. The F409L, F429S, and F433L solubilizing substitutions localize to this region of missing density.

(B) Local structure in the F508 region of wild-type (undeleted, blue) and F508 del (gray) NBD1 crystal structures. The undeleted and F508 del-NBD1 structures carry the solubilizing mutations F429S/ F508A/H667R or F494N/Q637R, respectively. Residues 502–524 of each structure are shown. Figure produced with Swiss-PdbViewer (Guex and Peitsch, 1997).

mutations behaved similarly to wt CFTR with respect to current amplitude, open probability (P_o), mean burst duration (MBD), and interburst interval (IBI), when examined at the single channel level by patch clamp techniques. Whereas F508 del CFTR rescued to the cell surface by low temperature incubation had a much different channel gating than wt CFTR, the presence of the solubilizing mutations increased the P_o, restored the MBD to wt value, and shortened the prolonged IBI, particularly the triple mutant which decreased the IBI from 10-fold longer than wt CFTR to only 3-fold.

Given the degree of rescued CFTR function observed with solubilizing mutations present, Pissarra et al., (2008) suggest that available structures of F508 del-NBD1 represent a partially corrected conformation rather than a truly perturbed NBD1 fold. This may prove to be the case. However, until a ''naked'' F508 del-NBD1 structure becomes available the specific effects of the solubilizing substitutions on the overall NBD1 fold remain unclear, nor do we know whether the observed relatively minor perturbations (compare wild-type- and F508 del-NBD1 local structure in the 508 region; Figure 1B) are sufficient to evoke the structural and functional damage displayed by the full-length molecule. Furthermore, it is not known at what stage chaperones intervene to assist CFTR folding, nor how well developed are the structures they sense.

Crystal structures provide a wealth of atomic detail, yet present rigidified ''snapshots'' of the end-point of protein folding rather than a motion picture of protein conformation along its folding pathway or at its destination structure. At the same time, trafficking and electrophysiological experiments on full-length CFTR guide us to conclusions that cannot be provided by crystallization of a single domain. As such, a synergistic arsenal of cell biological and biophysical techniques provides the optimum route to fully understand the disruptive effects of F508 del.

REFERENCES

Amaral, M.D. (2006). J. Inherit. Metab. Dis. *29*, 477–487.

Chang, X.B., Cui, L., Hou, Y.X., Jensen, T.J., Aleksandrov, A.A., Mengos, A., and Riordan, J.R. (1999). Mol. Cell *4*, 137–142.

Cyr, D.M. (2005). Nat. Struct. Mol. Biol. *12*, 2–3.

DeCarvalho, A.C., Gansheroff, L.J., and Teem, J.L. (2002). J. Biol. Chem. *277*, 35896–35905.

Guex, N., and Peitsch, M.C. (1997). Electrophoresis *18*, 2714–2723.

Kerem, B., Rommens, J.M., Buchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Buchwald, M., and Tsui, L.-C. (1989). Science *245*, 1073–1080.

Lewis, H.A. (2005). Pediatr. Pulmonol. *40*, 190–191.

Lewis, H.A., Buchanan, S.G., Burley, S.K., Conners, K., Dickey, M., Dorwart, M., Fowler, R., Gao, X., Guggino, W.B., Hendrickson, W.A., et al. (2004). EMBO J. *23*, 282–293.

Lewis, H.A., Zhao, X., Wang, C., Sauder, J.M., Rooney, I., Noland, B.W., Lorimer, D., Kearins, M.C., Conners, K., Condon, B., et al. (2005). J. Biol. Chem. *280*, 1346–1353.

Lukacs, G.L., and Durie, P.R. (2003). N. Engl. J. Med. *349*, 1401–1404.

Pissarra, L.S., Farinha, C.M., Xu, Z., Schmidt, A., Thibodeau, P.H., Cai, Z., Thomas, P.J., Sheppard, D.N., and Amaral, M.D. (2008). Chem. Biol. *15*, this issue, 62–69.

Riordan, J.R., Rommens, J.M., Kerem, B., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., et al. (1989). Science *245*, 1066–1072.

Rosenberg, M.F., Kamis, A.B., Aleksandrov, L.A., and Ford, R.C. (2004). J. Biol. Chem. *279*, 39051–39057.

Teem, J.L., Carson, M.R., and Welsh, M.J. (1996). Receptors Channels *4*, 63–72.

Thibodeau, P.H., Brautigam, C.A., Machius, M., and Thomas, P.J. (2005). Nat. Struct. Mol. Biol. *12*, 10–16.

Welsh, M.J., and Smith, A.E. (1993). Cell *73*, 1251–1254.

Yang, Y., Janich, S., Cohn, J.A., and Wilson, J.M. (1993). Proc. Natl. Acad. Sci. USA *90*, 9480–9484.

 ©2008 Elsevier Ltd All rights reserved