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CFTR, a channel with the structure of a transporter

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The cystic fibrosis transmembrane conductance regulator (CFTR) belongs to a superfamily of active transport molecules. However, when expressed in a wide variety of heterologous cell systems and when purified to homogeneity and reconstituted in planar lipid bilayers, it exhibits low conductance chloride channel activity. We postulate that the active transport capability of the molecule has been adapted to provide very stringent metabolic control of this channel which is responsible for chloride secretion and hydration of wet epithelial surfaces.

The most consistent phenotypic feature of cystic fibrosis is an altered electrolyte concentration in exocrine secretions [1]. During the 1980's evidence accumulated suggesting that a defective epithelial chloride conductance pathway might be responsible [2]. At the end of the decade the gene which when mutated, causes cystic fibrosis (CF) was identified by genetic means and cloned [3–5]. The primary structure of the gene product, CFTR indicated that it belonged to a superfamily of membrane transporters responsible for the active transport of a diverse range of substrates in both prokaryotes and eukaryotes [4–6]. The most closely related eukaryotic members are the P-glycoprotein multidrug transporters which export from cells many hydrophobic compounds including some cancer drugs [7] and the product of the STE6 gene of yeast which exports the 'a' mating factor peptide [8,9]. The hallmark of this class of molecules is the juxtaposition of a membrane associated domain containing six putative bilayer spanning α-helices (TM6) and a large nucleotide binding domain (NBF). The binding and hydrolysis of ATP at the latter is believed to provide the energy for transport of substrate presumably via a route constituted by elements of the membrane associated domain. These transporters contain two of each of these domains, superficially appearing to represent duplications but there is evidence that the sets are non-equivalent [10,11].

In the case of CFTR the question is how does a molecule with this structure contribute to the chloride conductance pathway which is apparently defective in CF. Extensive heterologous expression studies have strongly suggested that CFTR itself is a highly regulated low conductance chloride channel [12–19] and the recent purification and reconstitution into planar lipid bilayers has confirmed this beyond reasonable doubt [20]. The most obvious distinction of CFTR from other members of this transporter super family is the presence of an 'extra' highly charged domain in the centre of the molecule separating the two TM6-NBF motifs [4]. We have postulated that this R domain, so named because it contains the phosphorylation sites involved in channel gating, may be a tethered substrate for the active export function of CFTR. Such a model is depicted schematically in Fig. 1. The large arrows are intended to indicate this abortive active export of the large spherical R domain up against the charged inner mouth of the putative pore constituted by some as yet undefined packing of the transmembrane helices. In this position changes in the phosphorylation state of protein kinase A substrate sites (solid triangles) may control gating. In such a model neither the action of ATP at the NBFs nor the action of PKA on the R-domain is energetically coupled to chloride movement which depends only on the electrochemical gradient of the ion and occurs at rates compatible with passage through a channel. Regulation of the activity

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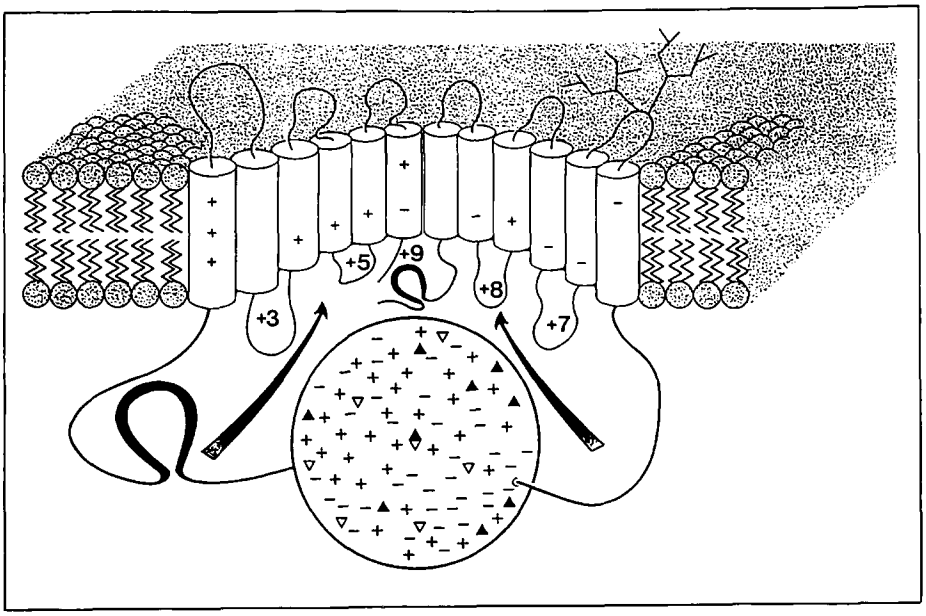

Fig. 1. Schematic representation of CFTR as both a transporter (an exporter) and a chloride channel. Cylinders denote putative transmembrane helices, omega-like shapes the nucleotide-binding folds and the large sphere, the R domain. Regulation of channel activity depends on both an active maintenance of R domain in correct configuration at the inner mouth of the pore and phosphorylation and dephosphorylation of the R domain at sites depicted by solid triangles.

of this channel is extremely tightly regulated by the two independent catalytic activities described.

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References

1 Welsh, M.J. (1990) FASEB J. 4, 2718–2725.
2 Quinton, P.M. (1990) FASEB J. 4, 2709–2717.
3 Rommens, J.M., Iannuzzi, M.C., Kerem, B.-S., Drumm, M.L., Melmer, G., Dean, M., Rozmahel, R., Cole, J.L., Kennedy, D., Hidaka, N., Zsiga, M., Buchwald, M., Riordan, J.R., Tsui, L.-C. and Collins, F. (1989) Science 245, 1059–1065.
4 Riordan, J.R., Rommens, J.M., Kerem, B.-S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M.L., Iannuzzi, M.C., Collins, F.S. and Tsui, L.-C. (1989) Science 245, 1066–1073.
5 Kerem, B.-S., Rommens, J.M., Buchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Buchwald, M. and Tsui, L.-C. (1989) Science 245, 1073–1080.
6 Riordan, J.R., Alon, N., Grzelczak, Z., Dubel, S. and Sun, S. (1991) Adv. Exp. Med. Biol. 290, 19–29.
7 Endicott, J.A. and Ling, V. (1989) Annu. Rev. Biochem. 58, 137–171.
8 McGrath, S.P. and Varsharsky, A. (1989) Nature, 340, 400–404.
9 Kuchler, K., Sterme, R.E. and Thorner, J. (1989) EMBO J. 8, 3973–3984.
10 Chin, J.E., Soffir, R., Noonan, K.E., Choi, K. and Roninson, I.R. (1989) Mol. Cell Biol. 9, 3808–3820.
11 Rich, D.P., Gregory, R.J., Anderson, M.P., Manavalan, P., Smith, A.E. and Welsh, M.J. (1991) Science, 253, 205–207.
12 Rich, D.P., Gregory, R.J., Anderson, M.P., Manavalan, P., Smith, A.E. and Welsh, M.J. (1991) Science 253, 205–207.
13 Drumm, M.L., Pope, H.A., Cliff, W.H., Rommens, J.M., Marvin, S.A., Tsui, L.-C., Collins, F.S., Frizzell, R.A. and Wilson, J.M. (1990) Cell 62, 1227–1233.
14 Anderson, M.P., Rich, D.P., Gregory, R.J., Smith, A.E. and Welsh, M.J. (1991) Science 251, 679–682.
15 Kartner, N., Hanrahan, J.W., Jensen, T.J., Naismith, A.L., Sun, S., Ackerley, C.A., Reyes, E.F., Tsui, L.-C., Rommens, J.M., Bear, C.E. and Riordan, J.R. (1991) Cell 64, 681–691.
16 Rich, D.P., Gregory, R.J., Anderson, M.P., Manavalan, P., Smith, A.E. and Welsh, M.J. (1991) Science 253, 205–207.
17 Bear, C., Duguay, F., Naismith, A.L., Kartner, N., Hanrahan, J.W. and Riordan, J.R. (1991) J. Biol. Chem. 266, 19142–19145.
18 Dalemans, W., Barbry, P., Champigny, G., Jallat, S., Dott, K., Dreyer, D., Crystal, R.G., Pavarani, A., Lecocq, J.-P. and Lazdunski, M. (1991) Nature 354, 526–528.
19 Drumm, M.L., Wilkinson, D.S., Smit, L.S., Worrell, R.T., Strong, T.V., Frizzell, R.A., Dawson, D.C. and Collins, F.S. (1991) Science 254, 1797–1799.
20 Bear, C.E., Li, C., Kartner, N., Bridges, R.J., Jensen, T.J., Ramjeesingh, M. and Riordan, J.R. (1992) Cell 68, 809–818.