FUNCTIONAL SHARK AND HUMAN CFTR PROTEINS ASSOCIATE STRONGLY WHEN EXPRESSED IN INSECT SF9 CELLS

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The quaternary structure of the CFTR chloride channel is unknown. Thus while there is reasonable evidence that CFTR polypeptides alone are sufficient to generate fully regulated low conductance anion channels (Bear et al, Cell 68:809-818, 1992), there are somewhat conflicting reports of the number of these polypeptides which may associate and form a channel (Marshall et al, J. Biol. Chem 269:2987-2995, 1994; Eskandari et al, Proc. Natl. Acad. Sci. USA 95:11235-11240, 1998; Zerhusen et al, J. Biol. Chem. 274:7627-7630, 1999). Indeed the oligomeric states of most ABC protein have not been rigorously defined. The exceptions are the K_{ATP} channels which each contain four SUR (sulfonyl urea receptor) subunits (Clement et al, Neuron 18:827-838, 1997). However, this may reflect the tetrameric assembly of the associated inwardly rectifying K^+ channel-forming polypeptides more than the inherent tendency of the ABC protein subunits to oligomerize.

We have been studying the structure, function and biosynthesis of human and shark CFTR as they are endogenously expressed in the apical membranes of epithelial cells and after heterologous expression in a variety of hosts. Shark and human CFTR sequences are highly conserved but sufficiently divergent to enable the generation of monoclonal antibodies (mAbs) which do not cross react between the two species. We have raised panels of mAbs against CFTR from both species, some of which cross react and some which do not. Among the latter set, L12B4 detects human CFTR with high sensitivity but does not recognize shark CFTR at all whereas 76.1.2 has the inverse capability. This enabled us to test whether oligomeric structures containing one or more of the homologous polypeptides from the two species were formed when they were coexpressed in the same cell. To do this, Sf9 insect cells were coinfected with two different recombinant baculoviruses, one containing the human CFTR cDNA sequence and the other the shark. Control infections with each separately resulted in CFTR channel activity in the Sf9 membranes.

Sequential immunoprecipitation and immunoblotting of NP40 detergent extracts of these coinfected cells using mAbs, L12B4 and 76.1.2 in either order revealed strong associations between shark and human CFTR polypeptides, consistent with the possibility that more than one CFTR molecule may contribute to the formation of an active chloride channel. There are, however, several caveats to this interpretation at this stage. Expression levels in baculovirus-infected insect cells are much higher than in the epithelial cells to which CFTR is native; complex oligosaccharide chains are not added in insect cells as they are in mammals and elasmobranchs; the associations detected by coimmunoprecipitation may not reflect authentic subunit interactions. Thus far we have not detected sufficient differences in the biophysical parameters of shark and human CFTR channels to use the presence or absence of intermediate values as evidence for or against heteroligomeric channel structure (Supported by the NIDDK of the NIH, DK51619).