Immunodetection of activation state of human and shark CFTR

April Mengos, J.D. Campbell, Tamas Hegedus, Tim Jensen and John R. Riordan
Mayo Clinic College of Medicine, Scottsdale, AZ 85268

CFTR plays a primary role in the regulation of chloride secretion in epithelial tissues of marine and terrestrial vertebrates by responding to protein kinase A (PKA) mediated phosphorylation of its R-domain. The PKA activation is due to cyclic AMP elevation on binding on extracellular secretory agonists. In the absence of these secretory stimuli CFTR is maintained in a quiescent non-gating state by phosphatases that very efficiently remove phosphoryl groups added by PKA. The mechanism by which the phosphorylation of multiple (~ 10) R-domain sites permit channel gating on binding of the ATP ligand to the nucleotide binding domains (NBDs) is not known but does involve conformation changes at the level of the R-domain[1] and the whole protein[2]. This conformation change can be readily detected by a significant retardation of the mobility of the mature shark or human proteins in SDS-PAGE. This mobility shift provides facile monitoring of the activation state of CFTR in isolated membranes, whole cells or intact tissue such as shark rectal gland. In the case of the human protein we have developed phosphorylation-sensitive monoclonal antibodies (mAbs) that can detect three of the major PKA phosphorylation sites in the R-domain individually (Figure 1). The use of these reagents in combination with the gel mobility shift enables detection of the reversible activation (phosphorylation) and inactivation (dephosphorylation) of the CFTR channel. In addition to this simple diagnostic test of activation state these methods begin to provide mechanistic insights. The kinetics of phosphorylation of the three separate sites, that we can follow, reveal that they are occupied in an ordered fashion rather than simultaneously and provide the basis for distinguishing between a processive and a distributive mechanism. (Supported by the NIH).

1. **Dulhanty AM and Riordan JR.** Phosphorylation by cAMP-dependent protein kinase causes a conformational change in the R domain of the cystic fibrosis transmembrane conductance regulator. Biochemistry 33: 4072-4079, 1994.

2. **Grimard V, Li C, Ramjeesingh M, Bear CE, Goormaghtigh E, and Ruysschaert JM.** Phosphorylation-induced conformational changes of cystic fibrosis transmembrane conductance regulator monitored by attenuated total reflection-Fourier transform IR spectroscopy and fluorescence spectroscopy. J Biol Chem 279: 5528-5536, 2004.

Detection of CFTR with phosphorylation-sensitive antibodies