Post-transcriptional regulation of CFTR protein expression by 5′untranslated region encoded regulatory elements

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Cystic fibrosis is a common, fatal genetic disease caused by mutations in the *cystic fibrosis transmembrane conductance regulator* gene (CFTR). CFTR encodes for a cAMP regulated chloride channel present in epithelial, cardiac and neuronal tissues, the loss of which impairs electrolyte transport across epithelial cells resulting in cystic fibrosis (CF) disease. CFTR expression is tightly controlled by a combination of transcriptional, post-transcriptional, translational and post-translational regulatory mechanisms, resulting in complex spatial, temporal, and pathological expression patterns. However, the regulatory mechanisms controlling CFTR expression *in vivo* are not well understood.

Despite the importance of the transcriptional regulation of CFTR, we have recently shown that CFTR is subject to post-transcriptional regulation through the action of upstream open reading frames (uORFs) encoded within the CFTR 5′untranslated region (5′UTR) (Davies *et al.*, 2004). We have investigated a highly conserved uORF present in the 5′UTR of the predominant epithelial of CFTR mRNA isoform. Evidence for uORF involvement in post- transcriptional regulation has been found in many eukaryotic genes, and has been attributed to disruption of ribosome scanning during translation, thereby modulating translation initiation at the main coding region.

We investigated the functional importance and mechanism of action of the 5′UTR in CFTR post-transcriptional regulation. We generated a series of expression constructs linking wildtype and mutant rabbit CFTR 5′UTR sequences to the firefly luciferase reporter gene. Following transfection of HT29 and CHO cells, measurement of luciferase activity indicated the effect of CFTR 5′UTR encoded regulatory elements on translation of the main coding region. For each construct, triplicate independent transfections were performed and co-transfection with renilla luciferase controlled for minor differences in transfection efficiency.

It was found that the wildtype CFTR 5′UTR (uORF present) supported translation of the main coding region at just 50% of the level produced by the positive control (beta-globin) 5′UTR, suggesting the presence of negative regulatory elements. Mutations that increase translation of the uORF result in a further 50-70% decrease in translation of the main coding region. In contrast, the elimination of the uORF translation, by truncation of the 5′UTR or direct mutation of the uORF, produces a 50% increase in translation of the main coding region compared to the wildtype CFTR 5′UTR. Overall the effect of each 5′UTR construct was very similar in both CFTR expressing (HT29) and non-expressing (CHO) cell lines. However, some small differences are indicative of tissue-specific *trans*-acting modulatory factors. These results identify a new mechanism of CFTR regulation, confirming the importance of 5′UTR regulatory elements in modulation of CFTR expression.

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