

P013 Artificial IRESs based on a novel target for PTB
Keith A. Spriggs, Sally-Anne Mitchell, John Le Quesne
and Anne Willis

School of Pharmacy, University of Nottingham, UK

It is becoming increasingly evident that the 5' UTRs of many cellular genes contain internal ribosome entry segments (IRESs). Natural IRESes are comprised of regions of structured RNA, although conservation of specific structural elements between cellular IRESes has yet to be demonstrated. These structured regions can interact with a number of trans-acting factors which act to modulate IRES function. We have demonstrated a rôle for polypyrimidine tract binding protein (PTB) in the function of numerous cellular IRESs, and have identified a novel PTB binding motif.

We have used this knowledge to construct artificial IRESs (AIREs) based on concatenated PTB binding sites. These artificial IRESs are smaller than naturally occurring IRESes, and were initially made by cloning synthetic DNA oligonucleotides into the intercistronic region of a dicistronic luciferase reporter plasmid. Importantly, these AIREs show good activity *in vitro* and *in vivo*. The most active artificial element is comprised of two tandem repeats of an extended PTB binding sequence followed by two inverted repeats of the same sequence, and we have demonstrated that PTB binds to this double stranded RNA structure. RNAi PTB knockdowns significantly reduce the activity of these AIREs in HeLa cells.