

P033 Unwinding the mechanism of frameshifting using antisense oligonucleotides

Zhaoru Lin and Ian Brierley

Division of Virology, Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK

Programmed -1 ribosomal frameshifting during elongation regulates gene expression in many RNA viruses and certain cellular genes. Frameshifting is stimulated by two *cis*-acting signals, a heptameric slippery sequence and a downstream mRNA secondary structure, usually an RNA pseudoknot. The pseudoknot is believed to compromise the ribosomal helicase and resist unwinding, leading to frameshifting at the slippery sequence.

While effort has focused on the role of pseudoknots in frameshifting, recent work has revealed that a *trans*-acting signal, an antisense RNA oligonucleotide (AON) complementary to a region downstream of the slippery sequence, is also able to stimulate frameshifting in the absence of an inherent mRNA secondary structure. We are probing the mechanism of oligonucleotide-stimulated frameshifting using two AONs identical in sequence but differing in base chemistries. While both AONs stimulate -1 frameshifting, differences in the extent of ribosomal pausing and accessibility of the slippery sequence to probes suggest that this occurs by contrasting mechanisms. Preliminary data also hint at a possible stimulation of $+1$ frameshifting within the same construct. Ultimately, we wish to purify ribosomes stalled by AONs for cryo-EM. By comparing these structures with complexes stalled at a pseudoknot, we hope to gain insight into the mechanisms of frameshifting.