MUTANTS OF tRNA, RIBOSOMES AND mRNA AFFECTING FRAMESHIFTING, HOPPING OR STOP CODON READ-THROUGH

J.F. Atkins[1], K. Herbst[1,2], M. O'Connor[1,3], T.M.F. Tuohy[1], R.B. Weiss[1], N.M. Wills[1,2] and R.F. Gesteland[1,2].

[1]Department of Human Genetics and [2]Howard Hughes Medical Institute, 6160 Eccles Genetics Bldg., University of Utah, Salt Lake City, Utah 84112
[3]Section of Biochemistry, Brown University, Providence, Rhode Island 02912

INTRODUCTION

Programmed non-standard translation elongation events, frameshifting, hopping and read-through, are important in the decoding of a minority of mRNAs from diverse sources. In this chapter we provide an overview of some of the approaches we are taking to investigate this "recoding" (Gesteland *et al.*, 1992). Also included is an overview of some current studies on mutants which cause frameshifting and read-through, at sites where it does not normally occur *i.e.*, non-programmed events.

RIBOSOMAL PROTEIN MUTANT WHICH RESTORES HIGH-LEVEL HOPPING

Fifty nucleotides separate the first 46 codons from the last 114 codons in the mature mRNA of bacteriophage T4 gene *60* (Huang *et al.*, 1988; Weiss *et al.*, 1990). Ribosomes hop over this 50-nucleotide coding gap at high efficiency (Weiss *et al.*, 1990; Dayhuff *et al.*, 1992). One of the several necessary requirements for this hop is an mRNA stem-loop structure which contains the 5' end of the coding gap (Weiss *et al.*, 1990). Herbst *et al.* (unpublished) have now isolated a mutant of *Escherichia coli* which restores high efficiency hopping to variants of gene *60* mRNA in which the stem-loop is altered. The mutation mapped to *rplI*, the gene coding for protein L9 of the large ribosomal subunit. In the mutant the codon for serine$_{93}$ was altered (Herbst *et al.*, unpublished). At least some non-programmed hopping events were also significantly affected by the mutant.

The Translational Apparatus, Edited by K.H. Nierhaus *et al.*, Plenum Press, New York, 1993

MUTANTS OF THE mRNA CONTEXT AND STRUCTURE FOR READ-THROUGH OF THE MURINE LEUKEMIA VIRUS *gag* GENE TERMINATOR

The *gag* and *pol* genes of Mo-MuLV and a minority of other retroviruses are separated by an in-frame UAG codon. Approximately 5% of ribosomes read through this stop codon and synthesize a gag-pol fusion polypeptide that is the sole source of *pol* gene products. N-terminal sequencing of the protease product (Yoshinaka *et al.*, 1985) showed that its coding region spanned the UAG codon and that glutamine is encoded by the UAG. The "leakiness" of this UAG, in contrast to most UAG codons, is due to a signal programmed in the mRNA that acts as a "stimulator" for read-through. The stimulator in this case is a pseudoknot located 8 nucleotides 3' to the UAG codon (Wills *et al.*, 1991; Feng *et al.*, 1992; see also Felsenstein, K.M. and Goff, S.P., 1992). Disruption of base-pairing in either of the two stems of the pseudoknot greatly diminishes or abolishes read-through. Regeneration of the structure via compensatory mutations restores read-through.

Although the pseudoknot is essential for read-through, the 8-nucleotide purine-rich sequence "spacer" between the UAG and the start of the pseudoknot is also important (Honigman *et al.*, 1991; Feng *et al.*, 1992). Alteration of several spacer nucleotides has a deleterious effect on read-through (Wills *et al.*, unpublished). The length of the spacer region is also important. Decreasing the spacer by 3 nucleotides or increasing the spacer by 3 nucleotides reduces read-through, though caution is required in distinguishing a spacing requirement from sequence requirements.

Unlike MuLV, in the majority of retroviruses the *pol* gene is in the -1 frame in relation to the *gag* gene and frameshifting is required to generate the gag-pol fusion protein. The stimulator for this frameshifting, at least in Mouse Mammary Tumor Virus (MMTV), is a pseudoknot (Chamorro *et al.*, 1992), as had been first found for frameshifting in the Coronavirus, Infectious Bronchitis Virus (Brierley *et al.*, 1989). The spacing between pseudoknots which promote frameshifting and their shifty sites fall within a narrow range of 3-9 nucleotides. Interestingly, a similar spacing is found between pseudoknots which stimulate read-through and their stop-codons.

As the pseudoknot required for MuLV stop codon read-through appears grossly similar to its counterpart in MMTV required for frameshifting, we replaced the MuLV pseudoknot with the pseudoknot sequence from the *gag-pro* shift site of MMTV. The MuLV-MMTV hybrid showed 10% of the MuLV read-thorough activity (Wills *et al.*, 1991), indicating that stem structures alone are not sufficient. We have focused our attention on the 18 nucleotides in the second loop of the MuLV pseudoknot.

A series of 3-nucleotide deletions has defined a region of importance at the 5' end of loop 2. In contrast, any, or all, of the remaining nucleotides in loop 2 can be deleted with no effect (Wills *et al.*, unpublished). Single nucleotide replacements within the critical codons implicate particular bases in those codons as specific determinants for read-through. These results are perhaps surprising in light of the absence of sequence requirements in

loop 2 of the IBV pseudoknot (Brierley *et al.*, 1991). Perhaps this indicates a specific interaction of these nucleotides with rRNA, ribosomal protein(s) or release factor to effect read-through or some additional RNA structure.

MUTANTS OF THE 5'C OF THE CONSERVED CCA AT THE 3' END OF tRNAs

It has been proposed that the movement of tRNA from the A to P to E sites during translation involves the dissolution and formation of pairing between the CCA tRNA tail of tRNA and successive series of nucleotides in the large subunit rRNA (Moazed and Noller, 1989). In a selection for mutants of translation components which cause frameshifting at discrete places where it is not programmed to occur O'Connor *et al.*, (unpublished) have isolated mutants of *E. coli* $tRNA^{Val}_{1}$ which have an altered the CCA terminus. These mutants also cause stop codon read-through. The available data can be interpreted by a model in which a non-standard interaction between the 3' three nucleotides of the tRNA and 23S rRNA results in an altered conformation of 23S rRNA in a neighboring ribosomal site, the A site, with consequences for decreased discrimination.

FRAMESHIFTING CAUSED BY A tRNA WITH 10 EXTRA BASES

In collaboration with Z. Li and M. Deutscher, we are investigating the processing of an unusual mutant $tRNA^{Arg}_{2}$. This mutant is a frameshift mutant suppressor which reads the 4-base sequence CCGU as a single codon (Tuohy *et al.*, 1992). It is unusual in that in addition to a predicted 9-nucleotide anticodon loop, the DNA sequence of the cloned tRNA gene predicts a 10-nucleotide duplication of the 3' part of the TFC arm. The sequence of the 10 bases is $^{5'}$AATCCTCCCG$^{3'}$. There are a number of possibilities for the structure of the mature tRNA; it may be that the 10-nucleotide duplication is precisely excised from the precursor tRNA. It is also possible that the precursor tRNA is processed in a different way resulting in the deletion of the last 10 nucleotides of the mature tRNA requiring the mature tRNA to refold in such a way as to approximate the structure of a conventional tRNA, but with two mismatches in the acceptor stem, and a possible turnover of CCC$^{3'}$ to CCA$^{3'}$. The third possibility under consideration is that the extra 10 nucleotides are not processed out of the mature form, but that they remain, in a unconventionally folded tRNA. This tRNA may not only function in normal translation (if it does), but can function as a frameshift suppressor.

ACKNOWLEDGEMENTS

We thank Linda Nichols for dedicated technical assistance. This work was supported by the Howard Hughes Medical Institute and by NIH grant #GM48152.

REFERENCES

Brierley, I., Digard, P., and Inglis, S.C., 1989, Characterization of an efficient coronavirus ribosomal frameshifting signal: requirement for an RNA pseudoknot, *Cell* 57:537.

Brierley, I., Rolley, N.J., Jenner, A.J., and Inglis, S.C., 1991, Mutational analysis of the RNA pseudoknot component of a coronavirus ribosomal frameshifting signal, *J. Mol. Biol.* 220:889.

Chamorro, M., Parkin, N., and Varmus, H.E., 1992, An RNA pseudoknot and an optimal heptameric shift site are required for highly efficient ribosomal frameshifting on a retroviral messenger RNA, *Proc. Natl. Acad. Sci. USA* 89:713.

Dayhuff, T.J., Gesteland, R.F., and Atkins, J.F., 1992, Electrophoresis, autoradiography and electroblotting of peptides: T4 gene *60* hopping, *BioTechniques* 13:499.

Felsenstein, K.M., and Goff, S.P., 1992, Mutational analysis of the *gag-pol* junction of Moloney murine leukemia virus: requirements for expression of the *gag-pol* fusion protein, *J. Virol.* 66:6601.

Feng, Y.-X., Yuan, H., and Rein, A., and Levin, J.G., 1992, Bipartite signal for readthrough suppression in murine leukemia virus mRNA: an eight-nucleotide purine-rich sequence immediately downstream of the *gag* termination codon followed by an RNA pseudoknot, *J. Virol.* 66:5127.

Gesteland, R.F., Weiss, R.B., and Atkins, J.F., 1992, Recoding: Reprogrammed genetic decoding, *Science* 257:1640.

Huang, W.M., Ao, S.Z., Casjens, S., Orlandi, R., Zeikus, R., Weiss, R., Winge, D., and Fang, M., 1988, A persistent untranslated sequence within bacteriophage T4 DNA topoisomerase gene *60*, *Science* 239:1005.

Honigman, A., Wolf, D., Yash, S., Falk, H., and Panet, A., 1991, *cis* Acting RNA sequences control the *gag-pol* translation readthrough in murine leukemia virus, *Virol.* 183:313.

Moazed, D., and Noller, H.F., 1989, Intermediate states in the movement of transfer RNA in the ribosome, *Nature* 342:142.

Tuohy, T.M.F., Thompson, S., Gesteland, R.F., and Atkins, J.F., 1992, Seven, eight and nine-membered anticodon loop mutants of $tRNA_2^{Arg}$ which cause +1 frameshifting. Tolerance of DHU arm and other secondary mutations, *J. Mol. Biol.* **228**:1042.

Weiss, R.B., Huang, W.-M., and Dunn, D.M., 1990, A nascent peptide is required for ribosomal bypass of the coding gap in bacteriophage T4 gene *60*, *Cell* 62:117.

Wills, N.M., Gesteland, R.F., and Atkins, J.F., 1991, Evidence that a downstream pseudoknot is required for translational read-through of the Moloney murine leukemia virus *gag* stop codon, *Proc. Natl. Acad. Sci. USA* 88:6991.

Yoshinaka, Y., Katoh, I., Copeland, T.D., and Oroszlan, S., 1985, Murine leukemia virus protease is encoded by the *gag-pol* gene and is synthesized through suppression of an amber termination codon, *Proc. Natl. Acad. Sci. USA* 82:1618.