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An RNA Holliday Junction?
Structural and Dynamic Considerations of the Bacteriophage T4 Gene 60 Interruption

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A novel interrupted gene motif has been reported in which a 50-nucleotide insertion into bacteriophage T4 gene 60 appears to be present in the message at the time of translation, yet it is not translated. We present here a dynamic model for how translation may be occurring in the neighborhood of the interruption. The model involves formation of an RNA structure with similarities to a Holliday junction, followed by migration of the branch point in a strand exchange between message and interruption. The advantage of this model over previous ones is that at no time is a new tRNA required to pair with a discontinuous template.

Huang et al. (1988) have reported a novel gene interruption motif in gene 60 of bacteriophage T4, in which a 50-nucleotide intervening segment is neither removed nor translated. The sequence of the interruption suggests that it can be folded to bring the translated codons on either side of the interruption into close proximity (Fig. 1). The authors suggest that bridging the gap may enable the ribosome to skip across it. On the basis of both the structural and dynamic potentials of this RNA sequence, we are suggesting an alternative dynamic model for readthrough across the interruption involving branch migration of a Holliday-like junction between tRNA and mRNA.

One of the most striking features of the gene 60 interruption is the direct repeat of the sequence 5' UGGAU 3' at each end of the RNA dumbbell. This duplication is required in order for translation to continue beyond the interruption (Weiss et al. unpublished results). The duplication places the two strands containing the pentanucleotides in position to exchange strands with each other, as in the Holliday-like junction shown in Figure 2(a).

Branch migration of this junction would permit each succeeding tRNA in the vicinity of Gly46 to pair with a continuous template without the discontinuity of the gap in Figure 1. While the tRNA encoding Gly46 is paired with the message (Fig. 2(a)), the branch point in front of them can migrate to a position internal to the pentanucleotide repeat (Fig. 2(b)). This event may take place either before or after aminoacyl transfer and translocation; the order of events is not important to this model. More extensive branch migration is hindered by the lack of sequence homology between the message and the dumbbell outside these five bases. When tRNA Gly moves into the P site, the template 3' to Gly46 will now be in position to receive the next tRNA (Fig. 2(c)) without having to do so in the presence of the discontinuity of a Holliday-like branch point.

The structure shown in Figure 1 is related to a family of structures known as gapped dumbbells, in which two hairpins stack on one another. Not all gapped nucleic acid dumbbells are equally stable. Nuclear magnetic resonance studies of a DNA dumbbell similar to Figure 3(a) show that the exchange rate with solvent of the imino protons immediately adjacent to the gap is the same as the rate for bases more internal to the stem of either hairpin (L. Rinkel & I. Tinoco Jr, personal communication), implying: (1) that there is no fraying of the helix at the gap; and (2) that the two helices are stacked onto one another as though they were one long helix. Presumably, the same structure can form with equivalent stability in RNA.

The structure shown in Figure 3(b), in which an extra base-pair is added across the gap, cannot be made in molecular models without unpairing of the stacked helix due to bond length constraints. However, if the protruding bases are not required to pair, as in Figure 3(c), their presence may or may not lead to fraying at the center of the dumbbell. The protruding bases may even, in some cases,
fraying of the 5' stem–loop exposes the stop codon just inside the folded region, which may lead to premature termination of translation; and (2) the Gly46 codon in the translated portion of the message before the interruption would no longer be physically next to the Leu47 codon in the second half of the message.

The pairing of the anticodon loops on either side of the gap (Fig. 3(d)) could potentially act to tie together the free ends protruding from the dumbbell, forming a Holliday-like junction. Like the well-characterized DNA junctions, this one would consist of two helices linked by the crossing over of one strand from each side, forming a branch point capable of moving up or down the two helices. Figure 3(d) is unlike the normal Holliday junction, however, in that the fourth (tRNA) strand is not covalently continuous, allowing for greater conformational freedom. Although it is believed from nuclear magnetic resonance studies that the center four base-pairs of a DNA Holliday junction are

**Figure 3.** Relative stabilities of gapped nucleic acid dumbbells. (a) A simple gapped dumbbell. All bases are stacked as though it were not gapped. (b) This structure cannot form, because new base-pairs will not form across the gap without disrupting the stack. (c) If the protruding bases are not required to pair, it is not known whether their presence will cause fraying of the stack. (d) The protruding arms are stabilized by base-pairing with another strand. In this case, the bridging strand is discontinuous.

Invasion of the duplex to form a triple-stranded structure (Michel et al., 1989). This question awaits further study. Disruption of the analogous helices in Figure 1 or Figure 2 would have two effects: (1)
stacked onto their respective helices (Wemmer et al., 1985), it is not known whether a discontinuity in the mRNA template would inhibit the addition of the next tRNA.

It is not clear what the minimum structural requirements for long-range translational skipping might be, since this is the first such case to be reported. Consequently, there is no theoretical basis for predicting a translational skip from sequence information. The ability to branch-migrate removes the need for tRNA addition across such a discontinuity, while the requirement for the pentanucleotide duplication (Weiss et al. unpublished results) is consistent with a model involving strand exchange between the dumbbell and the message.

Other factors may also contribute to translation across the interruption. The very fact that the interruption can fold as a dumbbell rather than as a stem-loop, the size and shape of the dumbbell, or the presence of an unpaired third loop near the center of the dumbbell, may also play a role. This extra loop, for example, contains an eight-nucleotide sequence that is complementary to the ribosome binding sequence at the 5' end of the gene (Huang et al., 1988), and this overlaps a five-nucleotide sequence complementary to the message immediately 3' to the dumbbell. A pseudoknot formed by pairing these latter two regions could potentially serve to orient the dumbbell colinearly to the rest of the message in order to allow the message to pass through tight constrictions on the ribosome. The above model does not address the question of possible contributions of this loop.

RNA is dynamic, capable of forming multiple structures from the same sequence (e.g. attenuation (Kolter & Yanofsky, 1982), self splicing (Price & Cech, 1988), and ribosome conformational changes (Thompson & Hearst, 1983)). This is the first suggestion that RNA branch migration may be involved in an RNA-mediated activity. Whereas DNA branch migration is a spontaneous process that occurs rapidly in solution (Thompson et al., 1976), the environmental context of the ribosome could either facilitate or hinder migration of the mRNA–tRNA complex. It will be interesting to see the effects of altering the sequence of the interruption in order to delineate the respective roles of structure and dynamic motions of the message in facilitating the translational leap.

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References

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