Curse of the Hairpin Loop

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Robert T. Batey
University of Colorado Boulder
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Curse of the Hairpin Loop

A recent crystal structure of a protein-RNA complex between the ribosomal protein L7Ae and a hairpin loop from a Box H/ACA sRNA illustrates how binding may occur in a minimal kink-turn motif.

One of the banes of structural analysis of RNA and its complexes is its maddening tendency to crystallize as dimers and in alternative conformations. This property is exemplified in studies of RNA tetraloops, which are observed as hairpin loops by NMR spectroscopy (Cheong et al., 1990) but crystallize as duplexes with mismatched base pairs at the loop nucleotides (Holbrook et al., 1991). Alternative conformations in RNA provide a rich source of further complications, as in the hammerhead ribozyme, whose structure is still being debated. When this occurs, the challenge is to relate the structure back to genetic and biochemical studies to ascertain whether it provides insights into the biologically relevant form of the RNA. Such is the case of the crystal structure of a protein-RNA complex found with the Box H/ACA sRNP, an enzyme responsible for modification of RNA, as described by Hamma and Ferré-D'Amaré in this issue of *Structure* (Hamma and Ferré-D'Amaré, 2004).

Among the many exciting aspects of RNA architecture revealed by the crystal structure of the ribosome, a new recurring motif called a kink-turn (K-turn) or GA motif was identified (Klein et al., 2001; Vidovic et al., 2000). This motif was found to occur six times in the 23S RNA of *H. marismortui* and was often associated with proteins. After their initial characterization within the ribosome, K-turns were found in a diverse set of protein-RNA complexes, including the spliceosomal U4 snRNP (Vidovic et al., 2000), the Box H/ACA pseudouridine synthases (Rozhdestvensky et al., 2003), the Box C/D methylases (Kuhn et al., 2002), and an mRNA autoregulatory element (Mao et al, 1999), underscoring their fundamental importance as a foundation upon which ribonucleoprotein enzymes are built.

While the K-turns were found to differ from one another with regard to their exact sequences, a consensus K-turn sequence was readily deduced (Klein et al., 2001). This sequence consists of two helices joined by an asymmetric internal loop. One of the helices is Watson-Crick paired (typically two G-C pairs are found nearest to the loop), while the other helix contains two consecutive noncanonical sheared G•A pairs (Figures 1A and 1B). The loop is comprised of three unpaired nucleotides on one strand that are organized to create a 120° kink in the RNA. The kinked structure is stabilized through stacking interactions between two of the three unpaired nucleotides of the loop with the two helices. One nucleotide stacks on the canonical stem, one stacks on the noncanonical stem, and one extrudes into solution and is often an important recognition element for proteins. The type I A-minor interaction (Nissen et al, 2001) occurring between the adenosine of the second G•A pair in the noncanonical stem and the first base pair of the Watson-Crick stem also appears to be important to overall kink-turn stability.

A variant on the kink-turn motif has recently been identified in both the archaeal Box C/D and H/ACA sRNPs in which the Watson-Crick stem is eliminated, creating a hairpin loop. As archaeal L7Ae is able to recognize these minimal K-turn motifs almost as well as the classic form (10 nM versus 50–100 nM binding affinities [Rozhdestvensky et al, 2003]), they are likely to be important players in the assembly of ribonucleoprotein particles. To address whether the same protein can specifically bind the hairpin loop form of the K-turn, Hamma and Ferré-D'Amaré have solved the crystal structure of L7Ae bound to this RNA element. Unfortunately, once again rather than crystallizing as the desired hairpin loop, the protein-RNA complex contains a duplex. On the surface, this may seem to preclude an understanding of how L7Ae recognizes loop sequences; however, a careful analysis of the structure reveals otherwise.

This complex consists of an RNA duplex containing

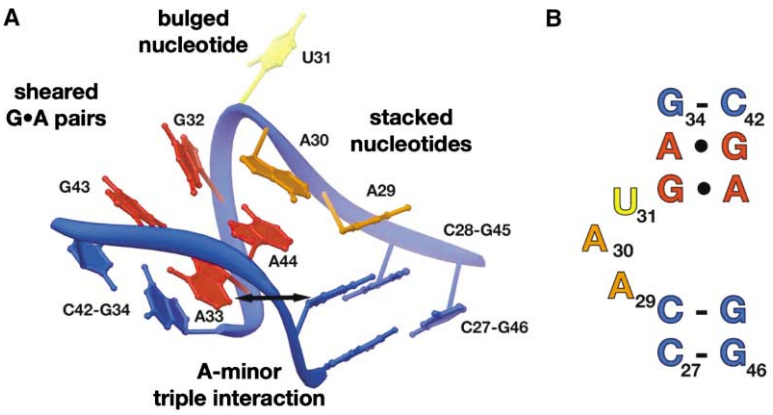

Figure 1. Anatomy of the Classic RNA Kink-Turn Motif as Exemplified by the U4 snRNA (PDB ID, 1E7K)

(A) Tertiary structure of the kink-turn motif highlighting the conserved features including two tandem sheared G•A base pairs (red), an internal bulge containing two bases that stack upon each of the two flanking helices (orange), and a single extruded base (yellow). The two helical regions (G•A stem on the left and the Watson-Crick stem on the right) interact through an A minor triple interaction (depicted by double arrow).

(B) Secondary structural representation of the RNA kink-turn. Nucleotide numbering is consistent with that of the U4 snRNA.

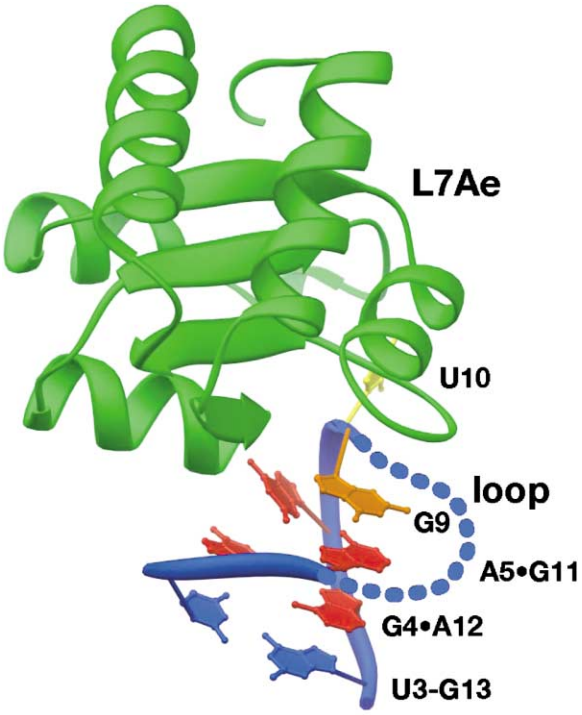

Figure 2. Global Architecture of the Minimum Kink-Turn Motif, as Observed in the L7ae-Box H/ACA Hairpin Loop Structure (PDB ID, 1SDS)

Only those elements of the RNA that are structurally homologous to the classic kink-turn (Figure 1) are displayed, along with the *M. jannaschii* L7Ae protein for reference. The dashed line depicts the connection between the two RNA strands as would occur in the hairpin loop, but whose exact conformation is unknown due to the dimerization of the RNA in the crystals. Nucleotide numbering is consistent with that in Hanna and Ferré-D'Amaré.

two non-Watson-Crick G•A stems, each independently binding an L7Ae protein, and a central internal loop bound by four calcium ions (Hamma and Ferré-D'Amaré, 2004). Despite the fact that the structure obtained was not that of a hairpin loop, similarities between the current structure and the classic kink-turn motif support the idea that we can nevertheless learn something about the terminal loop from this structure. L7Ae, as in the *H. marismortii* ribosomal 50S subunit, makes key contacts to the G•A stem through residues E33 which simultaneously hydrogen bonds to the base of G11 (of the G•A pair nearest to the internal loop) and the 2′ hydroxyl of G4 (of the second G•A pair). Further recognition is mediated through N32 and K36 that contact the noncanonical stem helix and a set of hydrogen bonding interactions between lysine residues and the key protruded uracil (U10) (global arrangement is shown in Figure 2). Clearly, a similar pattern of L7Ae recognition can be established between the two forms of the kink-turn motif.

Along with the lack of the Watson-Crick stem, the minimal kink-turn (Figure 2) also lacks the type I A-minor interaction between the two stems surrounding the asymmetric internal loop. This feature, which directly connects the first G-C pair of the Watson-Crick stem to the second G•A pair of the G•A stem is presumably an important stabilizing feature of the kink-turn motif, consistent with its importance in establishing the tertiary structure of many RNAs. Superposition of the G•A stems and loop nucleotides of the two kink-turn RNAs reveals an almost identical arrangement of the backbone and bases, providing credence to the idea that this structure reflects how the hairpin loop is organized. Furthermore, along with recent biochemical studies of the kink-turn motif (Goody et al, 2004), this structure suggests the kink-turn is primarily organized through its interactions with proteins, rather than being an autonomous folding unit. The lack of architectural elements in the RNA that serve to stabilize the classic kink-turn in the minimal form is most likely overcome by the expenditure of L7Ae's binding energy, as suggested by the characteristically lower affinity of the protein for the hairpin loop.

While the crystal structure has not revealed L7Ae bound to an RNA hairpin loop proper, the authors have nonetheless revealed a structural basis for the minimal form of a fundamental RNA motif. Although the curse of the stem loop was not avoided completely, they have dealt with the problem admirably.

John W. Hardin and Robert T. Batey
Department of Chemistry and Biochemistry
University of Colorado, Boulder
Boulder, Colorado 80309

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