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Binding of an HIV Rev Peptide to Rev Responsive Element RNA Induces Formation of Purine–Purine Base Pairs†

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ABSTRACT: The Rev responsive element (RRE) is an RNA secondary structural element within the *env* gene of HIV and is the binding site for the viral Rev protein. Formation of the Rev–RRE complex is involved in regulation of splicing and transport of mRNA from the nucleus. To understand the structural basis for the specific recognition of RRE by Rev, we have studied a model system for this interaction using NMR. We have obtained a specific 1:1 complex between an RNA derived from stem IIB of RRE, which contains the highest affinity Rev binding site, and a modified Rev_{34-50} peptide, which binds the RRE as an α-helix [Tan, R., et al. (1993) *Cell 73*, 1031–1040]. Binding of the peptide was accompanied by a conformational change in the RNA, which resulted in the formation of additional base pairs not present in the free RNA. Two of these induced base pairs are purine–purine pairs within the internal loop of RRE, which had been previously proposed on the basis of biochemical experiments [Bartel, D. P., et al. (1991) *Cell 67*, 529–536]. The formation of non-Watson–Crick base pairs, interactions in the major groove, and protein-induced conformational changes may prove to be common characteristics of RNA recognition of proteins.

Phylogenetic studies have shown that conserved regions of biologically important RNAs often reside in loops, bulges, and internal loops (Gutell et al., 1985). Structural studies on a variety of RNAs have revealed that these regions are often stabilized by non-Watson–Crick (mismatch) base pairs. Some examples include the GNRA and UUCG tetraloops prevalent in ribosomal RNA, both of which include base pairs between the first and last nucleotides of the loop (Cheong et al., 1990; Heus & Pardi, 1991). The sarcin/ricin and E loops of ribosomal RNA were also shown by NMR to have extensive mismatch base pairing (Szewczak et al., 1993; Wimberly et al., 1993). In addition to providing a role in RNA structure, non-Watson–Crick base pairs may also be important for RNA–protein recognition. Biochemical and genetic identification of protein binding sites in RNAs has shown that they often include important non-Watson–Crick regions (Cook et al., 1991; Gutell et al., 1985; Roy et al., 1990).

Another prevalent feature of RNA–protein interactions seems to be the conformational flexibility of the RNA. For example, in HIV TAR,[1] the essential base triple is not present in the free RNA but only forms as TAR changes its conformation upon binding of arginine or arginine-containing peptides (Puglisi et al., 1992). Comparison of the crystal structures of $tRNA^{Gln}$ free and bound to its cognate synthetase

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[1] Abbreviations: RRE, Rev responsive element; NMR, nuclear magnetic resonance; RNA, ribonucleic acid; HIV, human immunodeficiency virus; EDTA, ethylenediaminetetraacetic acid; TAR, transactivating region; NTP, nucleoside triphosphate; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; DQF-COSY, double-quantum-filtered correlation spectroscopy; TOCSY, total correlation spectroscopy; HSQC, heteronuclear single-quantum coherence.

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