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RNA Tertiary Structure Mediation by Adenosine Platforms

Jamie H. Cate, Anne R. Gooding, Elaine Podell, Kaihong Zhou, Barbara L. Golden, Alexander A. Szewczak, Craig E. Kundrot, Thomas R. Cech, Jennifer A. Doudna*

The crystal structure of a group I intron domain reveals an unexpected motif that mediates both intra- and intermolecular interactions. At three separate locations in the 160-nucleotide domain, adjacent adenosines in the sequence lie side-by-side and form a pseudo–base pair within a helix. This adenosine platform opens the minor groove for base stacking or base pairing with nucleotides from a noncontiguous RNA strand. The platform motif has a distinctive chemical modification signature that may enable its detection in other structured RNAs. The ability of this motif to facilitate higher order folding provides one explanation for the abundance of adenosine residues in internal loops of many RNAs.

Ribozymes and large RNA components of spliceosomes and ribosomes fold into complex three-dimensional architectures. To form these biologically active structures, helical regions must pack together specifically. Comparative sequence analysis (*1*, *2*), biochemical experiments (*2*–*5*), and modeling based on intermolecular contacts in crystals of small RNAs (*6*) have identified some elements responsible for long-range tertiary interactions in large RNAs, but their molecular details are largely unknown. The crystal structure of the 160-nucleotide P4-P6 domain of the *Tetrahymena thermophila* self-splicing intron (*7*) has revealed several new types of long-range contacts, including three examples of the adenosine platform motif described below.

The secondary structure of the P4-P6 domain, like that of many other large RNAs, contains base-paired regions interspersed with internal loops (Fig. 1A). As in other RNAs, many of the loops contain a

J. H. Cate, K. Zhou, J. A. Doudna, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520.
A. R. Gooding, E. Podell, B. L. Golden, A. A. Szewczak, T. R. Cech, Department of Chemistry and Biochemistry and Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80309.
C. E. Kundrot, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309.
*To whom correspondence should be addressed.

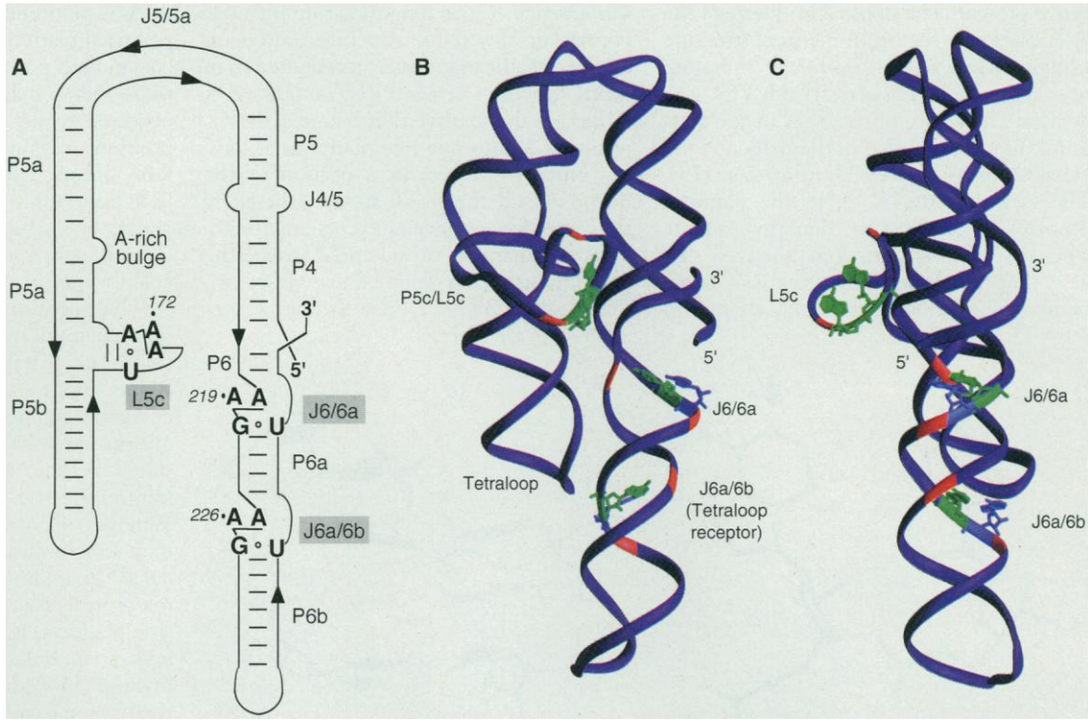

Fig. 1. (**A**) Schematic secondary structure of the P4-P6 domain, with adenosine platforms in boldface. The noncanonical base pair below each platform is also shown. (**B**) Location of adenosine platforms in the crystal structure. The structure is shown in the same orientation as that of (A); adenosines of the platforms are highlighted in blue and green, while the backbone positions of the wobble base pairs below the platforms are indicated in red. Blue adenosines are accessible to dimethyl sulfate in the presence and absence of the docking partner associated with the platform; green adenosines are protected from dimethyl sulfate modification when the docking partner is present (see text and Fig. 3 for details). (**C**) Another view of the crystal structure, rotated 90° about the long axis of the molecule. The conserved core region is to the right in (A) and (B) and the core faces the reader in (C). This figure was prepared by means of the molecular graphics program RIBBONS (*24*).

high proportion of adenosines (*8*). At three different sites in the crystal structure of the domain, two such adenosines assume a side-by-side configuration which we call an adenosine platform, or A-A platform (Fig. 1, B and C). The A-A platform motif consists of adjacent adenosines in a helical strand that form a pseudo base pair within the helix (Fig. 2A). The 3′ A continues the stacking of the A-form helix below it, and the 5′ A stacks on the opposite strand of the helix (Fig. 2, B and C). Although the two adenosines may share one hydrogen bond (*9*, *10*), the driving force for the platform appears to be base stacking. A non–Watson-Crick base pair below each platform (G·U in two cases, a non–Watson-Crick A·U in the third) shifts the base positions to increase stacking with the two adenosines of the platform. In addition, the ribose of the 5′ A has a noncanonical pucker (*11*), facilitating a kink in the phosphate backbone that broadens the minor groove in the dimension parallel to the helix axis. The overall geometries of the platforms are similar (rmsd, 0.5 to 1.2 Å), although we observe differences in the coordination of water and metal ions in the major grooves of the motifs (*12*).

Each adenosine platform mediates a long-range contact in the RNA (Fig. 3). One of the contacts is intramolecular, and the other two occur between adjacent molecules in the crystal lattice. The intramolecular contact contributes to a key component of the domain tertiary structure. Contained within the tetraloop receptor motif found in group I introns and other RNAs (*2*), the platform in J6a/6b opens the minor groove to enable the GAAA tetraloop of the same molecule to dock above the platform. This interaction helps hold together the two helical halves of the molecule (*7*). The other two platforms, in L5c and J6/6a, are involved in packing the two molecules into the asymmetric unit of the crystal, perhaps mimicking contacts between different domains in the intact intron. In the crystal, two G·C base pairs connect J6/6a of molecule A to L5c of molecule B and vice versa (Fig. 3). The platform in J6/6a stacks in the helix as if it were a base pair. This allows nucleotides in the strand opposite the platform to flip away from the helix and base pair with nucleotides from L5c in the neighboring molecule. The helix and stacking in J6/6a continue above the platform with a C·G base pair formed between the strand of the platform and a G at the 5′ end of the molecule (Fig. 3). The platform in L5c reciprocates the base-pairing interaction with J6/6a. In this case, however, the platform caps a helix, allowing the intermolecular base pairs to stack on top; the helix is effectively extended two base pairs by the long-range contact.

Mutational analysis supports the functional role of two of the A-A platform motifs. Activity of P4-P6 variants can be assessed in vitro in a three-component ribozyme in which the P4-P6 domain assembles with the rest of the intron via tertiary interactions (*13*). Mutation of the A-A platform in J6a/6b (the tetraloop receptor), which prevents its interaction with the tetraloop and thereby destabilizes the P4-P6 domain (*5*), reduced activity 70-fold (*14*). Removal of the A-A platform motif in J6/6a has no discernible effect on the overall structure of the P4-P6 domain (*5*), yet ribozyme activity dropped 25-fold (*14*). These results suggest that the A-A platform in J6/6a contributes to interdomain association, while that in J6a/6b contributes to P4-P6 structure and perhaps additionally to its association with other regions of the ribozyme. While the three-component functional assay was performed in 80 mM $MgCl_2$, a condition that suppresses many ribozyme mutations (*15*, *16*), the two A-A platform mutants still showed significantly reduced activity.

A consistent pattern of dimethyl sulfate modification of the RNA is observed at each A-A platform. When the intact *Tetrahymena* intron is probed with dimethyl sulfate, the 5′ adenosine of each platform is protected from methylation at its N1 position (*17*). In the P4-P6 domain by itself, the 5′ As of the platforms in J6/6a and L5c are susceptible to methylation (*17*). The 5′ A of the platform in J6a/6b (the tetraloop receptor) also becomes susceptible to N1-methylation by dimethyl sulfate when the tetraloop-receptor interaction is disrupted by mutation (*17*, *18*). Thus in each case, the 5′ A is protected from dimethyl sulfate only when the associated long-range con-

tact is present. The unusual geometry of the A-A platforms seen in the crystal structure explains these results. Although there are no apparent hydrogen bonds to the Watson-Crick face of the 5′ A in the platform, the base is buried in the helix and the N1 position is inaccessible (Fig. 2A) (*19*). The fact that the 5′ A in the platform becomes accessible to dimethyl sulfate when its docking partner is removed suggests that the platform structure is dynamic or undergoes a conformational change in the absence of the long-range interaction. Formation of a stable structure only upon binding of a ligand is well precedented from NMR structures of small RNA motifs (*20*). Although the methylation data clearly implicate the J6/6a and L5c platforms as having binding partners in a portion of the intron outside the P4-P6 domain, these tertiary interactions have not yet been located.

A comparison of potential adenosine platforms in similar group I introns [subclasses IC1 and IC3 (*2*, *21*)] reveals that, whereas A-A platforms are most common (83/93 potential platforms surveyed), variation occurs in both positions. The stacking interactions that stabilize the platform would be expected to favor purines at the platform positions, yet only one potential G-G platform occurs. Pyrimidines occur at one or both positions in eight cases. Whether these variations are functionally significant is unclear in that no activity information is available for the introns in question (*22*).

The adenosine platform motif provides one explanation for the abundance of adenosines in internal loops of many large RNAs. Since stacking may be the primary energetic component of the platform, the adenine base is perhaps particularly well suited because it stacks efficiently while minimizing the potential for steric clash. Whether adenosine platforms are a common structural stepping stone to higher order RNA folding is uncertain, but the tetraloop receptor, which contains an adenosine platform, is widespread in the group I and group II families of introns (*2*). The invariant A-A dinucleotide at the A site in the ribosome (nucleotides 1492 and 1493 in *Escherichia coli* 16S ribosomal RNA) exhibits a dimethyl sulfate protection pattern characteristic of the platform motif when probed in the presence and absence of tRNA (*23*). Use of an adenosine platform to mediate tRNA binding at a fundamental step of translation would suggest that adenosine platforms arose early in evolution as an efficient mechanism for building complex, functional RNA architectures.

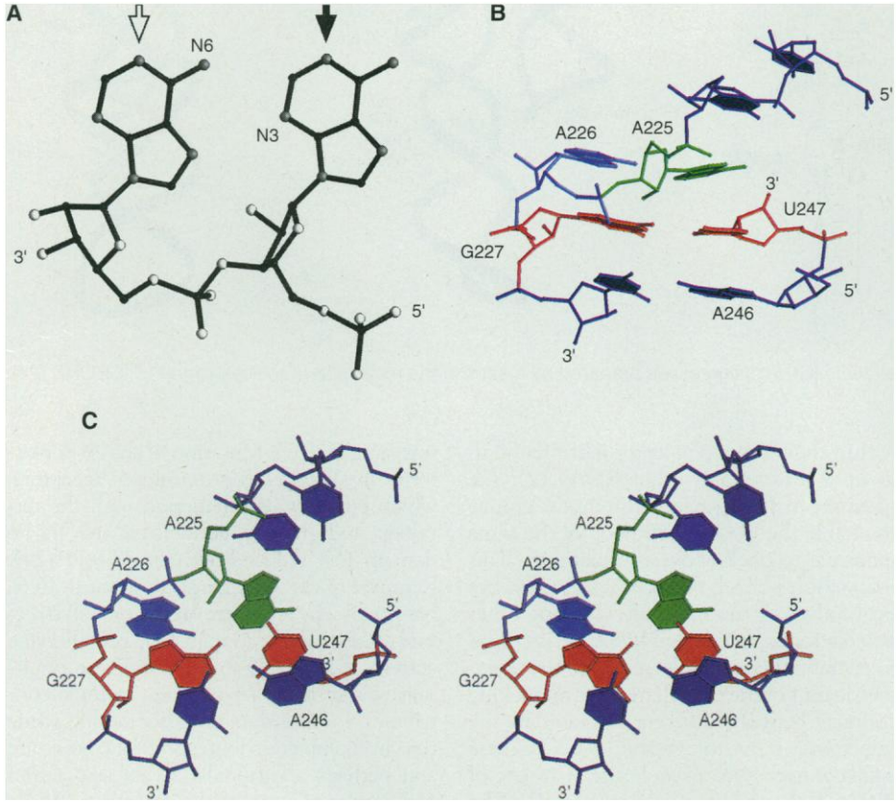

Fig. 2. (**A**) View of an adenosine platform looking down the helix axis. N3 of the 5′ A and N6 of the 3′ A are within hydrogen bonding distance (2.8 to 3.4 Å). Closed arrow, N1 position of the 5′ A, which is protected from dimethyl sulfate when the long-range contact is formed; open arrow, N1 position of the 3′ A which shows variable dimethyl sulfate protection (see *18*). (**B**) The adenosine platform in the tetraloop receptor; color scheme as in Fig. 1. (**C**) Stereo view from underneath the platform, looking up the helix axis. Figure prepared with RIBBONS (*24*).

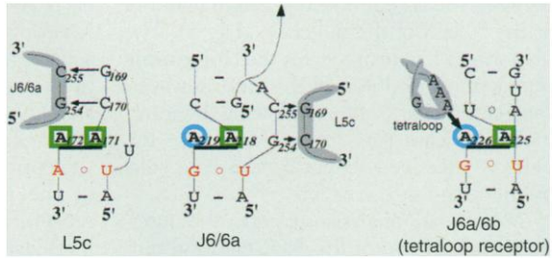

Fig. 3. The different kinds of long-range interactions that occur near the adenosine platforms. At left and in the center, reciprocal interactions occur between L5c and J6/6a in the two molecules in the asymmetric unit of the crystal; at right, the tetraloop docks above the platform in the tetraloop receptor. The docking partner for each platform is shaded. Blue adenosines (circles) are accessible to dimethyl sulfate in the presence and absence of the docking partner associated with the platform. Green adenosines (squares) are protected from dimethyl sulfate modification when the docking partner is present. The noncanonical base pair below each platform is highlighted in red.

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11. In the two platforms in internal loops (J6/6a and J6a/6b), the 5′-A pucker is O4′-endo, C1′-exo; in L5c the 5′-A of the platform has C3′-exo sugar pucker. Canonical A-form helices have C3′-endo ribose puckers; *W. Saenger, Principles of Nucleic Acid Structure* (Springer-Verlag, New York, 1984), pp. 55–68.
12. Comparisons between platforms in the internal loops (J6/6a and J6a/6b) involved superposition of all atoms in the A-A and the G-U wobble below. Comparisons between the L5c platforms in the two molecules in the asymmetric unit included the analogous four residues; comparisons between the L5c and internal bulge platforms included only the adenosines of the platform and the 3′ purine in each case.

In both intrahelical platforms (J6/6a and J6a/6b), strong density in the $F_o - F_c$ electron density map is consistent with a metal ion or water molecule in the major groove coordinated to the ribose and phosphate of the 5′ A and 3′ A of the platform, respectively, and to N7 and O6 of the G in the G·U wobble below the motif. In the L5c loop platform, however, there is density for a putative magnesium ion coordinated to phosphates of the 3′ A of the platform and the adjacent A of the A·U noncanonical pair. Both molecules in the asymmetric unit reflect these differences between the platforms.
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Three Cognitive Markers of Unconscious Semantic Activation

Anthony G. Greenwald,* Sean C. Draine, Richard L. Abrams

A "response window" technique is described and used to reliably demonstrate unconscious activation of meaning by subliminal (visually masked) words. Visually masked prime words were shown to influence judged meaning of following target words. This priming-effect marker was used to identify two additional markers of unconscious semantic activation: (i) the activation is very short-lived (the target word must occur within about 100 milliseconds of the subliminal prime); and (ii) unlike supraliminal prime-target pairs, a subliminal pair leaves no memory trace that can be observed in response to the next prime-target pair. Thus, unconscious semantic activation is shown to be a readily reproducible phenomenon but also very limited in the duration of its effect.

Demonstrations of judgments or actions being influenced by unperceived stimuli (*1*) have both interesting and uninteresting possibilities for interpretation. The uninteresting possibility is that perceptual measurements have been insensitive—the critical stimuli may have been perceived, but the research apparatus or procedure failed to register that perception. The more interesting—but also controversial—possibility is that stimulus-triggered cognition has indeed occurred without conscious perception of the initiating stimulus. Tests of the hypothesis of unaware perception date from the late 19th century (*2*). When claims of analysis of semantic information from unperceived stimuli were strongly pressed in the second half of the 20th century (*3*), methodological critiques (*4*) of the adequacy of evidence for such claims resulted in widespread skepticism about those claims.

In this controversial domain, experimental work of the past two decades has focused on claimed findings of subliminal semantic activation (*5*)—the claim that word meanings are analyzed when words are presented so as to evade conscious perception. Subliminal semantic activation is most often investigated with priming procedures. Subjects perform a two-choice categorization task that is supplemented by the presentation of a subliminal prime word shortly before each to-be-judged target stimulus word. The two categorization tasks that have been used most often for tests of subliminal priming have the subject decide whether or not a target letter string forms a word (*6*) or whether a target word is pleasant or unpleasant in meaning (*7*). Priming is said to occur when the meaning of the prime affects the speed or accuracy of response to the target. Priming is given the controversial label "subliminal" if it occurs when the prime is visually masked to reduce or eliminate conscious perception (*8*).

Despite numerous empirical demonstrations, subliminal priming has remained a controversial phenomenon because (i) reported findings have been statistically weak, (ii) it has been difficult to provide convincing evidence that visually masked prime words are indeed not consciously perceived, (iii) published replications are rare, and (iv) many active investigators have accumulated multiple unpublished and unsuccessful attempts to replicate their own or others' published findings. Against this background of empirical difficulty, Draine and Greenwald (*9*) recently described a "response window" procedure that, in combination with visual masking procedures that can be implemented easily on standard computer displays, reliably produces statistically strong subliminal priming effects. Here we use the response window procedure to establish a few empirical properties of subliminal semantic priming.

Subjects (*10*) performed a categorization task either for affectively polarized words (to be categorized as pleasant or unpleasant in meaning) or for common first names (to be categorized as male or female). In different conditions within each experiment, the interval between start of prime and start of target stimulus—an interval referred to as the prime-target stimulus onset asynchrony (SOA)—was varied through values that ranged from 67 to 400 ms. Subjects were assigned to either subliminal or supraliminal priming according to a counterbalancing scheme that also systematically varied both the order in which SOA values appeared and which of the two item sets (male or female names, pleasant or unpleasant words) was used in the priming task. Each subject provided indirect measure (priming) data for two or three 50-trial blocks at each SOA

Department of Psychology, University of Washington, Seattle, WA 98195, USA.

*To whom correspondence should be addressed at Department of Psychology, University of Washington, Box 351525, Seattle, WA 98195–1525, USA. E-mail: agg@u.washington.edu