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Selective Stabilization of Natively Folded RNA Structure by DNA Constraints

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The stabilization of folded RNA states has been widely studied. Experiments have investigated features intrinsic to RNA such as metal ion binding,[1] H-bonds,[2,3] and various tertiary structure motifs,[4] as well as RNA-protein contacts[5] and interactions with small-molecule ligands.[6] However, we lack a full understanding of how folded RNA conformations can be stabilized relative to RNA states that are unfolded, partially folded, or misfolded.[7]

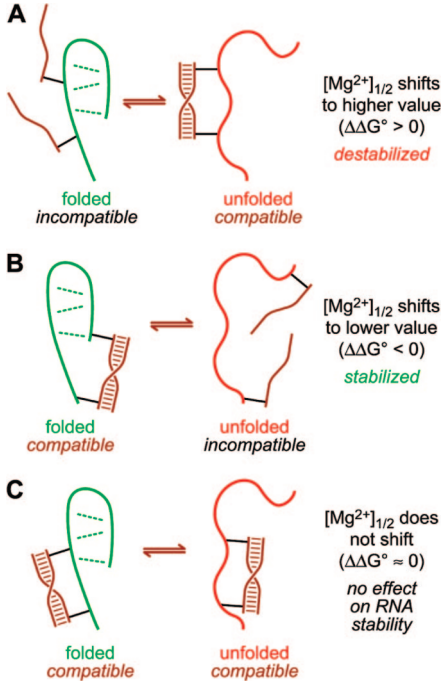

Figure 1. DNA constraints to influence RNA folding. (A) Destabilizing DNA constraint. (B) Stabilizing DNA constraint. (C) DNA constraint compatible with both the unfolded and folded RNA states. $\Delta\Delta G^\circ$ is defined as $(\Delta G^\circ_{+\mathrm{DNA}}) - (\Delta G^\circ_{-\mathrm{DNA}})$, where a negative value of $\Delta\Delta G^\circ$ means relative stabilization of the folded conformation.

Toward the goal of establishing rational control over RNA structure, we previously showed that a strategically attached double-stranded DNA constraint[8] can destabilize RNA folding[9] (or catalysis[10]) when the rigid DNA duplex is incompatible structurally with the native RNA conformation (Figure 1A). In such cases, the energetic cost of disrupting the DNA duplex must be paid in order for the RNA to fold properly, because the duplex is present only when the RNA is unfolded. This energetic cost is manifested experimentally in an increased Mg^{2+} requirement for RNA folding, as assayed by nondenaturing polyacrylamide gel electrophoresis (native PAGE).[2,11] These prior studies examined folding of the 160-nucleotide P4−P6 domain of the *Tetrahymena* group I intron RNA,[12,13] which adopts its characteristic 3D structure in the presence of sufficient Mg^{2+} (with $[Mg^{2+}]_{1/2} \approx 0.7$ mM at pH 8.3 and 35 °C). We[9,14] and several others[15,16] have analyzed the Mg^{2+} dependence of P4−P6 folding on native PAGE as a robust and consistent measure of the energetic consequences of various structural perturbations to this RNA.

A conceptual counterpart of our previous DNA constraint experiments to destabilize RNA folding[9] would be to achieve selective *stabilization* of the natively folded RNA upon attachment of a DNA duplex that is compatible only with the folded state (Figure 1B). However, if the DNA constraint is also compatible with the unfolded RNA structure, then the unfolded⇆folded equilibrium would not be perturbed by the DNA, and no net RNA stabilization or destabilization is expected (Figure 1C); this was confirmed in our earlier experiments.[9a] To achieve RNA stabilization with a DNA constraint, the constraint must be compatible only with the folded RNA state, which is challenging to design because little is known about the structures of unfolded RNAs. Here we demonstrate that selective and energetically significant stabilization of natively folded RNA structure is indeed possible using a strategically placed DNA constraint.

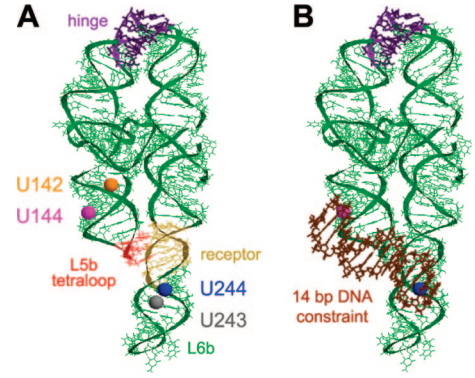

Figure 2. P4−P6 RNA and strategy for its selective stabilization by DNA constraints. (A) X-ray structure of native (folded) P4−P6.[13] Labeled are the nucleotide 2′-OH positions used as DNA attachment points in this study. Also labeled are the flexible "hinge" region and the tetraloop−receptor interaction that holds together the two helical domains of the RNA. (B) Representative computer modeling image illustrating the compatibility of the U144/U244 14 bp constraint with the native P4−P6 structure. As shown schematically in Figure 1B, this DNA constraint is expected to be incompatible with the unfolded state of the RNA, which should lead to relative stabilization of the compatible folded state. Modeling results were similar for the other three constraint combinations.

Due to the overall candy-cane shape of folded P4−P6 (Figure 2A), we anticipated that a suitable DNA duplex attached near the two remote stem-loop regions (i.e., L5b and L6b) should be structurally compatible with only the folded conformation of the RNA. In particular, because stiffening the flexible "hinge" of P4−P6 (marked in Figure 2A) by mutation firmly locks this RNA into its unfolded state,[12] we predicted that the unfolded state should be structurally incompatible with a short DNA constraint that is attached near L5b and L6b. Such a DNA constraint is expected to selectively stabilize the folded RNA relative to the unfolded RNA, which should induce a distinct decrease in the Mg^{2+} requirement for P4−P6 folding on native PAGE.

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We examined computer models of P4−P6 with various lengths of DNA duplex (10 to 20 base pairs) covalently attached at specific uridine 2′-positions (Figure 2B). Uridines were chosen because our previous chemical strategy for synthesis of RNA−DNA conjugates enables attachment of 5′-aldehyde-DNA by reductive amination with particular 2′-amino-2′-deoxyuridine nucleotides that are introduced synthetically into the RNA.[9] Here, the modeling identified several promising combinations of RNA attachment site and DNA duplex length. These combinations included both 14 bp and 12 bp DNA constraints attached at each of the U144/U244 and U142/U243 pairs of RNA positions.

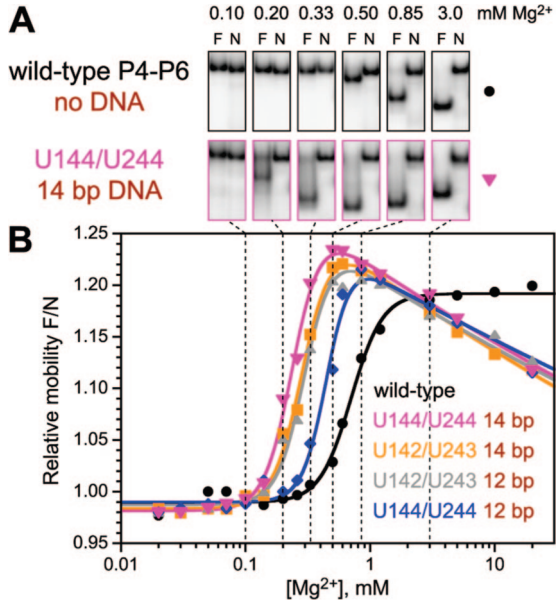

Figure 3. Native PAGE to determine the energetic effects of the designed DNA constraints on the P4−P6 RNA. (A) Representative native PAGE images for the U144/U244 14 bp constrained RNA along with unconstrained wild-type (wt) P4−P6. F denotes a foldable version of the RNA; N denotes a nonfoldable version for which base pairing in the hinge region (see Figure 2) prevents folding. (B) Relative mobility titration curves for the four DNA-constrained RNAs along with wt P4−P6 for comparison. The $\Delta\Delta G°$ values were computed from the Mg^{2+} midpoints ($[Mg^{2+}]_{1/2}$ values) as described previously.[2] The $[Mg^{2+}]_{1/2}$ for the U144/U244 14 bp RNA (pink, 0.24 mM) as compared with the value for wt P4−P6 (black, 0.70 mM) translates to a stabilizing $\Delta\Delta G°$ of −2.5 kcal/mol. The other three $\Delta\Delta G°$ values in kcal/mol are −2.1 (U142/U243 14 bp), −2.0 (U142/U243 12 bp), and −1.0 (U144/U244 12 bp). See the Supporting Information for details and a straightforward explanation of the downturn in the titration plots at high $[Mg^{2+}]$ for the DNA-constrained RNAs.

The requisite DNA-constrained P4−P6 RNAs were synthesized using the reductive amination approach described previously.[9a] Then, native PAGE was used to assay the Mg^{2+} dependence of RNA folding (Figure 3). In all four cases, a pronounced decrease in $[Mg^{2+}]_{1/2}$ was observed, with $\Delta\Delta G°$ relative to unconstrained P4−P6 as large as −2.5 kcal/mol (stabilizing) in the case of the U144/U244 14 bp constraint.[2] For comparison, the only modification of P4−P6 previously reported to stabilize this RNA is ΔC209 (i.e., deletion of nucleotide C209), and this deletion results in only 1.1 kcal/mol of stabilization.[15] The observed stabilization of 2.5 kcal/mol represents ∼14% of the estimated overall folding $\Delta G°$ for P4−P6 of ∼18 kcal/mol.[2]

Therefore, we have shown that an energetically substantial stabilization of the RNA tertiary structure can be achieved by strategic attachment of a DNA constraint that selectively stabilizes the folded conformation of the RNA. Although further work remains to explore the dependence of this stabilization on RNA identity and attachment sites as well as DNA constraint length, our data already demonstrate that significant stabilizations are observed when various attachment sites are used on the P4−P6 RNA. These results extend the observation that nature has not fully optimized P4−P6 folding.[15] These findings also demonstrate that an energetically nontrivial RNA stabilization can be introduced without requiring extensive contacts to the RNA itself. This may have implications for how nature uses other molecules such as proteins to stabilize specific folded RNA conformations.

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Supporting Information Available: Experimental details and explanation of Figure 3B curve fits. This material is available free of charge via the Internet at http://pubs.acs.org.

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