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Deoxyribozymes That Synthesize Branched and Lariat RNA

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Branched RNA molecules are formed naturally by the spliceosome and by group II introns.¹ In a typical 2',5'-branched RNA, the 2'-hydroxyl of an internal nucleotide and the 5'-hydroxyl of another nucleotide are linked by a phosphodiester bond. In a lariat RNA, the strand extending from the branch point 2'-OH wraps around to become the same RNA that contains the branch site (Figure 1A). Studies of intron processing and other biochemical reactions of branched RNA² (e.g., those involving lariat debranching enzyme Dbr³) would be greatly facilitated by simple synthetic access to branched and lariat RNA. This is currently a significant experimental challenge by either solution-phase or solid-phase techniques.⁴ As part of comprehensive efforts to identify deoxyribozymes (DNA enzymes) that ligate RNA,⁵ we report here new metal-dependent deoxyribozymes with significant practical potential for synthesizing branched and lariat RNA.

We previously reported an *in vitro* selection strategy to identify deoxyribozymes that ligate RNA.⁵ Our initial deoxyribozymes join a 2',3'-cyclic phosphate with a 5'-hydroxyl to form solely non-native 2'-5' phosphodiester linkages. Here, we sought linear RNA by attack of a terminal 2'(3')-hydroxyl on a 5'-triphosphate (Figure 1B).⁶ We began with random 40-nt (N₄₀) DNA pools and used the reported selection strategy.⁵ Four parallel selections were performed in which the 3'-terminal ribonucleotide that donates the 2'(3')-hydroxyl was either A, C, dA, or dC (d = 2'-deoxy; the dA and dC substrates were intended to favor formation of a native 3'-5' RNA linkage). After nine selection rounds, the Mg²⁺-dependent ligation activity of each selection pool had increased to ~20%, comparable to that observed in our earlier selections.⁵ Assay of each pool's product surprisingly indicated that the RNA products were not linear but 2',5'-branched (evidence is given below). The ligation reaction was presumed to occur as in Figure 1C. Because each pool gave similar products, one pool (dC) was chosen for further study.

Individual deoxyribozymes were cloned and characterized.⁵ Results with the 9F7, 9F21, 9F13, and 9F18 deoxyribozymes are representative (Figure 2). Ligation rates k_{obs} were determined in a trimolecular assay format (Figure 2A).⁵ Both Mn²⁺ and Mg²⁺ supported ligation activity; complete analysis of the metal dependence will be reported elsewhere. At 37 °C and pH 7.5 with 20 mM Mn²⁺, k_{obs} ranges from 0.1 to 2.2 min⁻¹ with >85% yield. Yields are comparable with 80 mM Mg²⁺, although k_{obs} is lower (0.1 to 0.9 h⁻¹; see Supporting Information). For comparison, *in vitro* branch formation by Mg²⁺-dependent self-splicing group II introns occurs with $k_{\text{obs}} \approx 10^{-4}$ h⁻¹,⁷ and the spliceosome *in vitro* has $k_{\text{obs}} \approx 1$ h⁻¹.⁸ The background ligation rate with an exactly complementary DNA splint and 20 mM Mn²⁺ is $k_{\text{bgd}} \approx 4 \times 10^{-7}$ min⁻¹ (see Supporting Information for details). This leads to a calculated rate enhancement $k_{\text{obs}}/k_{\text{bgd}}$ of up to ca. 5×10^6 -fold with Mn²⁺.

The formation of 2',5'-branched RNA linkages was confirmed using lariat debranching enzyme Dbr (Figure 3A). The site of branching was mapped by partial alkaline hydrolysis, which

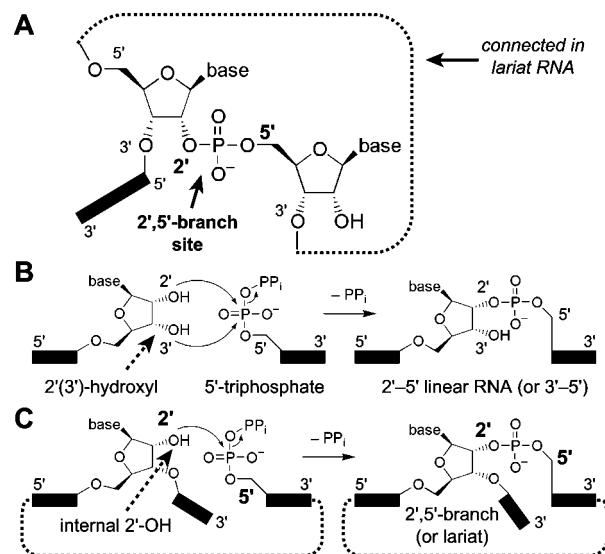


Figure 1. Branched and lariat RNA. (A) Structure of 2',5'-branched and lariat RNA. (B) Formation of linear RNA by attack of a terminal 2'(3')-hydroxyl on a 5'-triphosphate (PP_i = pyrophosphate). (C) Formation of 2',5'-branched RNA by attack of an internal 2'-OH on a 5'-triphosphate. Note that when the 3'-end of the right-hand substrate is joined to the 5'-end of the left-hand substrate (dotted line), the product is a lariat RNA.

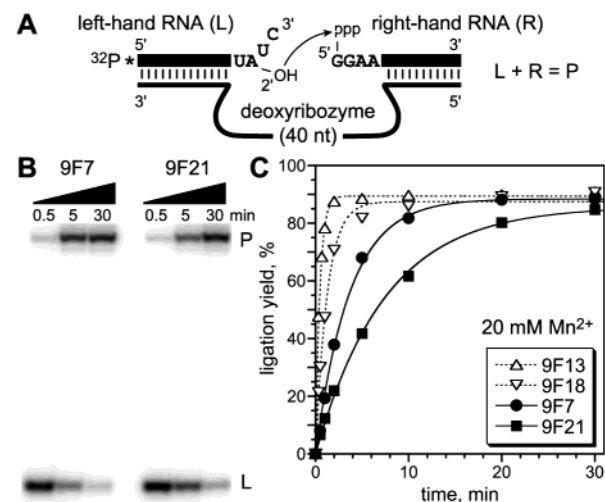


Figure 2. Deoxyribozymes that create branched and lariat RNA. (A) Trimolecular reaction format showing the RNA sequence of the ligation junction region. (B) 20% PAGE reveals efficient ligation to form branched RNA by the new deoxyribozymes (37 °C, 50 mM HEPES, pH 7.5, 20 mM MnCl₂; see Supporting Information for complete data sets). (C) Time course of branched RNA formation.

generates an unbroken "ladder" from linear RNA and a gap-containing ladder for branched RNA (Figure 3B). The gap location and size correlates directly with the branch site. 9F7 and 9F21 create a branch at the particular A indicated in Figure 2A, whereas 9F13

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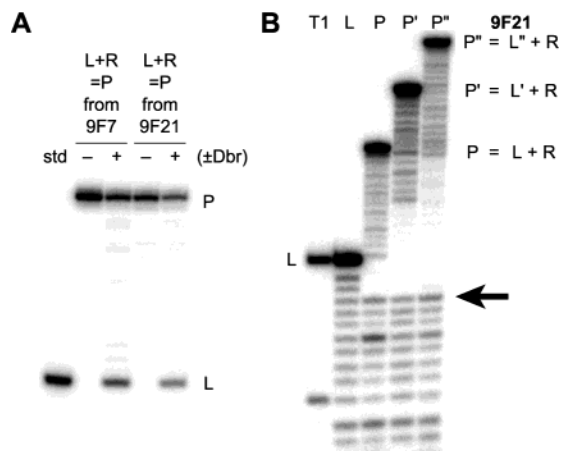


Figure 3. Demonstrating 2',5'-branching in the ligated RNA products from the 9F7 and 9F21 deoxyribozymes. (A) Treatment of the L + R product P with debranching enzyme Dbr (–, incubation without Dbr; +, 4.5-h incubation with Dbr; partial conversion is to avoid degradation). (B) Partial alkaline hydrolysis of the 9F21 ligated product; 9F7 is similar. Lanes: T1, digestion of L with RNase T1 (cleavage 3' of G, for ladder calibration); L, left-hand substrate RNA; P, ligated product L + R; P', L' + R where L' has six additional nt in its 3'-tail; P'', L'' + R where L'' has 18 additional nt. In all cases, the branch site (arrowhead) maps unambiguously to the adenosine indicated in Figure 2A.

and 9F18 induce branching at the U one nt to its 5'-side. Ligation assays using RNA substrates with single 2'-deoxy substitutions at each of the last four nt of the left-hand substrate were consistent with these branch-point assignments (data not shown). Alkaline hydrolysis and Dbr assays were also used to demonstrate that the 9F7 and 9F21 deoxyribozymes can create branched RNA with a longer 3'-tail originating from the left-hand substrate. Rates are lower, but good yields are maintained (data not shown; 9F13 and 9F18 have not yet been tested). The branch site is the same regardless of the tail length (Figure 3B).

Because natural 2',5'-branches are typically found at A residues, the 9F7 and 9F21 deoxyribozymes (which branch at A) are of particular interest. Outside of the ligation junction region, all nucleotides in the binding arms of 9F21's substrate RNAs may be changed with little reduction in ligation rate or yield, as long as the DNA sequence is changed to the Watson–Crick complement (data not shown). This suggests that the new deoxyribozymes can be developed into general branched RNA-forming reagents.

Naturally occurring 2',5'-branched RNA is usually observed during RNA splicing, where the branch is incorporated into a lariat structure (Figure 1A).^{1a} Synthesis of lariat RNA without using a natural intron or spliceosome is virtually unknown. RNA minilariats have been prepared, but these and other approaches are severely restricted in scope.⁹ We therefore explored the use of our new deoxyribozymes to create lariat RNA. When the right- and left-hand RNA substrates were connected by an unstructured 100-nt loop, 9F21 successfully created lariat RNA (Figure 4). Therefore, deoxyribozymes are therefore capable of synthesizing lariat RNA as well as simpler 2',5'-branched RNA.

In summary, we have demonstrated that branched and lariat RNA can be synthesized by deoxyribozymes that have significant potential for convenient and practical generalization. We are currently working to expand the scope of branched and lariat RNA formation by the new deoxyribozymes, including variation of loop size and composition for the lariats. There is little precedent for creation of branched RNA by any non-natural nucleic acid enzyme.¹⁰ Because nature's spliceosome is likely a ribozyme,¹¹ the new deoxyribozymes are of considerable mechanistic interest as well as practical importance for their catalytic abilities.

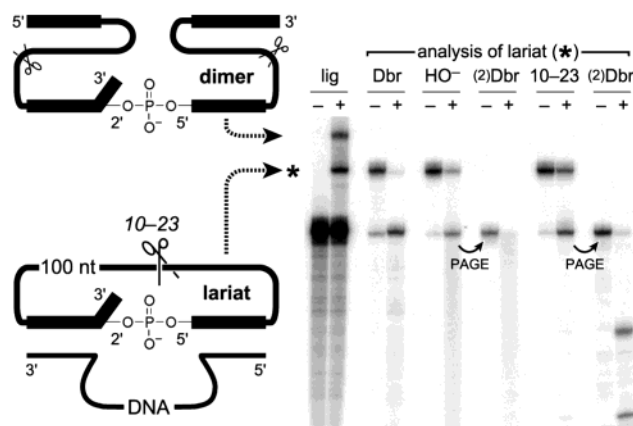


Figure 4. Lariat RNA is synthesized by the 9F21 deoxyribozyme. The PAGE image shows formation of lariat RNA (37 °C, pH 7.5, 10 mM MnCl₂, 1.5 h; ~15% yield; substrate prepared by transcription with α -³²P-CTP). A second product is the dimer (top, ~10% yield) formed from two RNA substrates annealed to one deoxyribozyme. Two minor side products (~1%) migrate higher on the gel (not shown; see Supporting Information). The lariat structure was confirmed by partial digestions with Dbr, alkaline hydrolysis, the 10–23 deoxyribozyme (which cleaves within the lariat loop), and combinations as indicated. The label “(2)” indicates a reaction performed on the PAGE-purified product from the preceding assay. See Supporting Information for detailed analysis of this gel image as well as analogous experiments on the dimer.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Burge, C. B.; Tuschl, T.; Sharp, P. A. In *The RNA World*, 2nd ed.; Gesteland, R. F., Cech, T. R., Atkins, J. F., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1999; pp 525–560. (b) Michel, F.; Ferat, J. L. *Annu. Rev. Biochem.* **1995**, *64*, 435–461. (c) Jacquier, A. *Biochimie* **1996**, *78*, 474–487. (d) Bonen, L.; Vogel, J. *Trends Genet.* **2001**, *17*, 322–331.
- (2) Carriero, S.; Braich, R. S.; Hudson, R. H.; Anglin, D.; Friesen, J. D.; Damha, M. J. *Nucleosides Nucleotides, Nucleic Acids* **2001**, *20*, 873–877.
- (3) (a) Nam, K.; Hudson, R. H.; Chapman, K. B.; Ganesan, K.; Damha, M. J.; Boeke, J. D. *J. Biol. Chem.* **1994**, *269*, 20613–20621. (b) Ooi, S. L.; Dann, C., 3rd; Nam, K.; Leahy, D. J.; Damha, M. J.; Boeke, J. D. *Methods Enzymol.* **2001**, *342*, 233–248.
- (4) For leading references, see: (a) Damha, M. J.; Ganesan, K.; Hudson, R. H.; Zabarylo, S. V. *Nucleic Acids Res.* **1992**, *20*, 6565–6573. (b) Sproat, B. S.; Beijer, B.; Gröthli, M.; Ryder, U.; Morand, K. L.; Lamond, A. I. *J. Chem. Soc., Perkin Trans. 1* **1994**, 419–431. (c) von Büren, M.; Petersen, G. V.; Rasmussen, K.; Brandenburg, G.; Wengel, J. *Tetrahedron* **1995**, *51*, 8491–8506. (d) Gröthli, M.; Eritja, R.; Sproat, B. *Tetrahedron* **1997**, *53*, 11317–11346.
- (5) (a) Flynn-Charlebois, A.; Wang, Y.; Prior, T. K.; Rashid, I.; Hoadley, K. A.; Coppins, R. L.; Wolf, A. C.; Silverman, S. K. *J. Am. Chem. Soc.* **2003**, *125*, 2444–2454. (b) Flynn-Charlebois, A.; Prior, T. K.; Hoadley, K. A.; Silverman, S. K. *J. Am. Chem. Soc.* **2003**, *125*, 5346–5350.
- (6) Bartel, D. P.; Szostak, J. W. *Science* **1993**, *261*, 1411–1418.
- (7) Daniels, D. L.; Michels, W. J., Jr.; Pyle, A. M. *J. Mol. Biol.* **1996**, *256*, 31–49.
- (8) Grabowski, P. J.; Padgett, R. A.; Sharp, P. A. *Cell* **1984**, *37*, 415–427.
- (9) (a) Sund, C.; Agback, P.; Chattopadhyaya, J. *Tetrahedron* **1991**, *47*, 9659–9676. (b) Sund, C.; Agback, P.; Chattopadhyaya, J. *Tetrahedron* **1993**, *49*, 649–668. (c) Reese, C. B.; Song, Q. *Nucleic Acids Res.* **1999**, *27*, 2672–2681. (d) Carriero, S.; Damha, M. J. *J. Org. Lett.* **2003**, *5*, 273–276.
- (10) Tuschl, T.; Sharp, P. A.; Bartel, D. P. *EMBO J.* **1998**, *17*, 2637–2650.
- (11) (a) Collins, C. A.; Guthrie, C. *Nat. Struct. Biol.* **2000**, *7*, 850–854. (b) Villa, T.; Pleiss, J. A.; Guthrie, C. *Cell* **2002**, *109*, 149–152.

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