

Searching for Molecular Solutions – Additional Material**CHAPTER 6**

These Files contain additional material relevant to **Chapter 6** of *Searching for Molecular Solutions*. The page numbers of the book pertaining to each section are shown in the Table below, the corresponding page number for this file, and the title of each relevant section.

Contents:

~ Book Reference Page Number	Page Number in this File	Section	
		No.	Title
212	2	A8	DNAzymes
227	8	A9	Altered Backbones

Section A8: **DNAzymes**

Relevant the section 'DNA Can Do It Too', of p. 212 of *Searching for Molecular Solutions*.

Enzymes of DNA

While folding of an aptamer into a conformation appropriate for ligand binding is one thing, mediating catalysis is another. Even after the notion of RNA enzymes no longer caused raising of eyebrows, the prospects for efficient DNA catalysis were not viewed as particularly favorable ¹. Although ribozyme nucleobases are now believed to be very significant for many catalytic events mediated by RNAs (as considered in Chapter 6 of *Searching for Molecular Solutions*), the 2'-hydroxyl group of RNA participates in the common mechanism of RNA hydrolysis ^{♥ 1}, and is strongly implicated in RNA self-cleavage and self-splicing reactions ^{2,3}. And this 2'-OH group of course is absent in DNA molecules. Indeed, replacement of key ribonucleotide residues of hammerhead ribozymes with corresponding 2'-deoxy residues ablates activity ⁴. By the same token, one cannot simply convert an active ribozyme into a corresponding DNA sequence and retain catalysis, as was demonstrated early in self-splicing RNA studies with the DNA strand corresponding to the Group I intron RNAs ¹.

[♥] This process involving the 2'-OH group is the reason that RNA is far more susceptible to base-mediated hydrolysis than DNA.

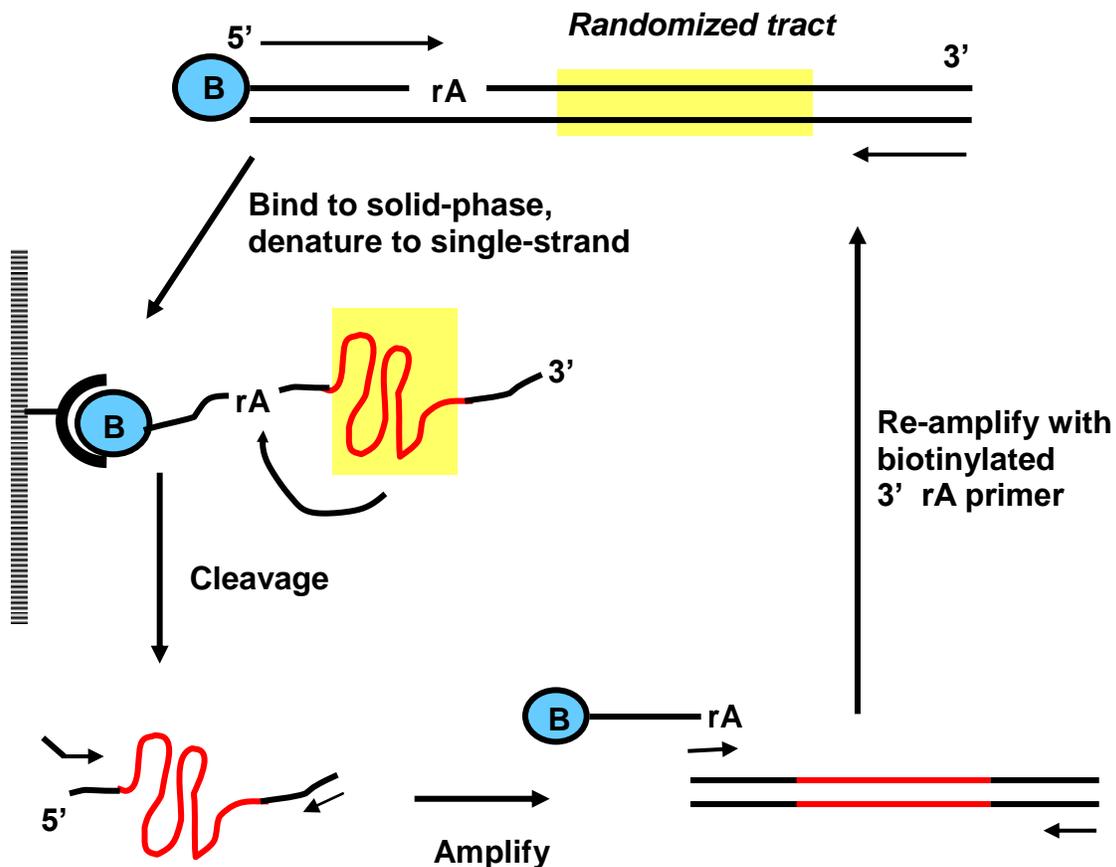


Fig. 6A8.1

Selection scheme for DNA enzyme cleaving a single 2'OH ribonucleotide linkage. A primer with a 5'-biotin and a 3' riboadenosine is used to amplify a randomized DNA sequence, which is then immobilized on a streptavidin solid-phase matrix and denatured to allow strand folding. DNA sequences capable of catalysis are self-cleaved from the solid-phase, amplified, and re-amplified with the special 5' biotin / 3' rA primer. (The first amplification generates the priming site for the latter primer). The cycle is then repeatable with the selected DNAs. Note that this does not directly select for ribonucleotide cleavage; the assumption is that the rA linkage is the most enzymatically facile for hydrolytic attack.

Initial *in vitro* evolution and selection experiments aimed at isolating DNA enzymes used RNA hydrolysis as the target catalysis, a theoretically 'easy' target in view of the facility of RNA in undergoing this reaction (While it is always important to distinguish the degree of acceleration of any reaction by a catalyst over the spontaneous background, it is particularly so for RNA hydrolysis with its relatively high background rate [♥]). Selection for DNA enzyme activity from a random library typically involves exploiting a self-cleavage effect in conjunction with solid-phase immobilization (Fig. 6A8.1). The first DNA enzyme ('deoxyribozyme' or 'DNAzyme') capable of RNA cleavage used divalent lead ions ⁶ (Pb⁺²), but magnesium-dependent DNAzymes were soon isolated ⁷, and by means of an alternative cofactor (the amino acid histidine) ⁸ or chemically modified nucleosides ^{9,10} metal ions can be dispensed with. Yet unmodified DNAzymes capable of metal-independent RNA cleavage at significant rates have also been identified ^{11,12}.

DNAzymes selected by the general procedure of Fig. 6A8.1 could not cleave all-RNA substrates ⁶, but modified selection approaches have yielded RNA-cleaving DNAzymes with near-general versatility ^{5,13}. As with ribozymes, though, RNA cleavage is not at all the end-point of DNA catalytic potential ¹⁴. DNA strands can also act as cleavage substrates for selected DNAzymes, and such strand scission can be directed in a sequence-specific manner ^{15,16}. Ligation of both RNA ^{*} ^{17,20} and DNA molecules ²¹ has been achieved using DNA catalysts.

[♥] The high rate of spontaneous RNA cleavage is also one of the reasons why cycling of selection is necessary to isolate RNA-cleaving DNA enzymes, since any RNA associated with an arbitrary (non-catalytic) DNA has a finite probability of spontaneous hydrolysis. Consequently, spurious candidate DNAzymes can be selected initially alongside *bona fide* catalysts, and this constitutes an additional source of background noise ⁵.

^{*} DNAzyme-mediated RNA ligation can result in 2'-5' or other completely unnatural phosphate linkages ¹⁷. In one strategy, a native RNA-cleaving DNAzyme was evolved into a ligase which only created 2'-5' linkages ¹⁸. Even so, DNAzyme RNA ligases creating natural 3'-5' linkages have also been achieved ^{19,20}.

DNAzyme kinases (catalyzing phosphate transfers) are known^{22,23}, and DNA-catalyzed carbon-carbon bond formation (in the form of the Diels-Alder reaction noted for ribozymes in Chapter 6) has been described²⁴. An interesting additional DNAzyme-catalyzed activity is the repair of thymidine dimers (which are one effect of ultraviolet irradiation), in a manner activated by light in the absence of organic cofactors^{25,26}.

This brief survey of DNA catalysis is not intended to be comprehensive, but rather to illustrate the contention that DNA can rival RNA as a catalytic agent^{24,27,28}. If this is so (as evidence suggests), then it follows in turn that 2'-hydroxyl groups are not essential for a diverse range of catalytic feats by nucleic acids^{19,28}. And if it is not chemistry which is limiting, then isolation of novel DNAzymes comes down to the ingenuity of experimental selection protocols²⁸. As already noted, there is no known natural precedent for a DNA role beyond an informational repository. This observation, in combination with the above recently-acquired knowledge of the range of DNA enzymes, suggests that the exclusivity of RNA in modern natural nucleic acid catalysis (and by the extension, the hypothetical RNA World) does not derive from any inherent chemical advantage of RNA itself, but is likely to be attributable to other factors⁶.

Yet the chemical differences between DNA and RNA make a difference in their respective folding pathways²⁹. We noted earlier that a ribozyme cannot be converted into a deoxyribozyme simply by rendering it into an equivalent DNA sequence⁴. Despite the fact that there is no one-to-one correspondence between ribozyme and DNAzyme sequences[▼], it has proven possible to use an *in vitro* evolutionary approach to create a transition between ribo- and deoxyribo-

▼ In the other direction (DNAzyme to ribozyme), it appears to be possible in at least in one case, where a short DNAzyme retained activity as a ribozyme of corresponding sequence³⁰. In this instance activity was associated with a G-quartet structure which may be maintained in both nucleic acids, and functional DNAzyme → ribozyme sequence correspondences are certainly not the rule³¹.

nucleic acid catalysts with the same ligase functions³¹. The evolved DNAzyme in turn could not be back-converted into a ribozyme with a matching sequence, showing that the acquisition of DNA catalysis in this case behaved as a discrete switch, rather than an expansion of activity³¹. Jumps (or switches) in sequence space where a new DNAzyme structural motif is acquired without gradual transition have been described, and show that a specific functional motif can act as a springboard towards novel functional structures otherwise hard to access directly from fully random sequences³². An interesting relationship between nucleic acid enzymes has been derived by engineering pairs of RNA-cleaving ribozymes and deoxyribozymes towards perfect complementarity, such that they can form RNA : DNA hybrid duplexes (Fig. 6A8.2). Of course, in this artificial situation, it is the ribozyme and deoxyribozyme complements of each other which are active, not the directly corresponding sequences³³.

For studies of both DNAzymes and DNA aptamers the issue of functional utility, as well as pure scientific interest, has often been a driving force. And a major issue here is the relatively increased stability of DNA over RNA, especially to base hydrolysis and RNases. An interesting observation in this regard is that not all RNA phosphodiester bonds are created equal, since the spontaneous cleavage of such linkages can vary by 4 orders of magnitude, subject to the effects of RNA secondary and tertiary structures to molecular chemical stability³⁴. DNAzymes may also be more robust in their tolerance for a wide range of different metal ion cofactors, and they also promise to have greater potential thermal resistance than their RNA counterparts. RNA-cleaving DNAzymes have thus been selected which retain activity at 90° C³⁵. *In vitro* selection has likewise yielded DNAzymes with elevated resistance to acidic conditions³⁶.

We have considered functional DNA and RNA molecules as separate entities, but in the future nucleic acid analogs with altered sugars, bases and backbones may supersede either option, a topic explored further in Chapter 6 of *Searching for Molecular Solutions*.

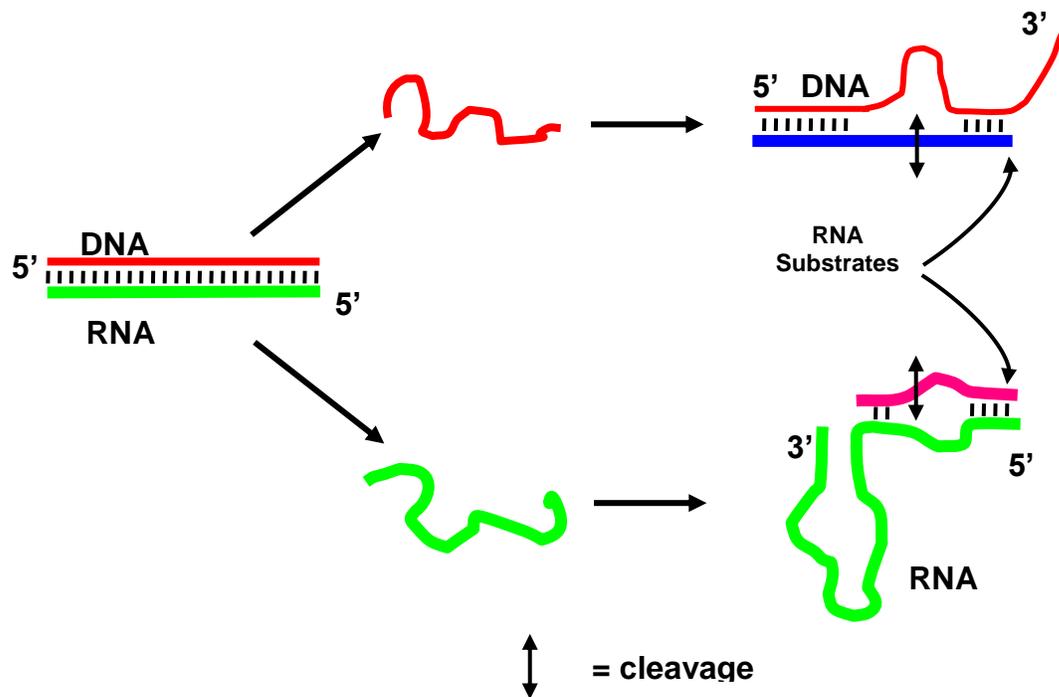


Fig. 6A8.2

Complementary ribozyme : deoxyribozyme arrangement ³³. When the RNA:DNA hybrid duplex is denatured, the single strands can independently cleave separate RNA substrates.

Section A9: ***Altered Backbones***

Relevant to p. 227 of *Searching for Molecular Solutions*.

RNA / TNA / PNA Structures

Nucleic acids with alternate backbones were described in Chapter 6 of *Searching for Molecular Solutions*, and this section provides a figure (Fig. 6A9.1 below) with structures for RNA in comparison with PNA (peptide nucleic acid) and TNA (threose nucleic acid).

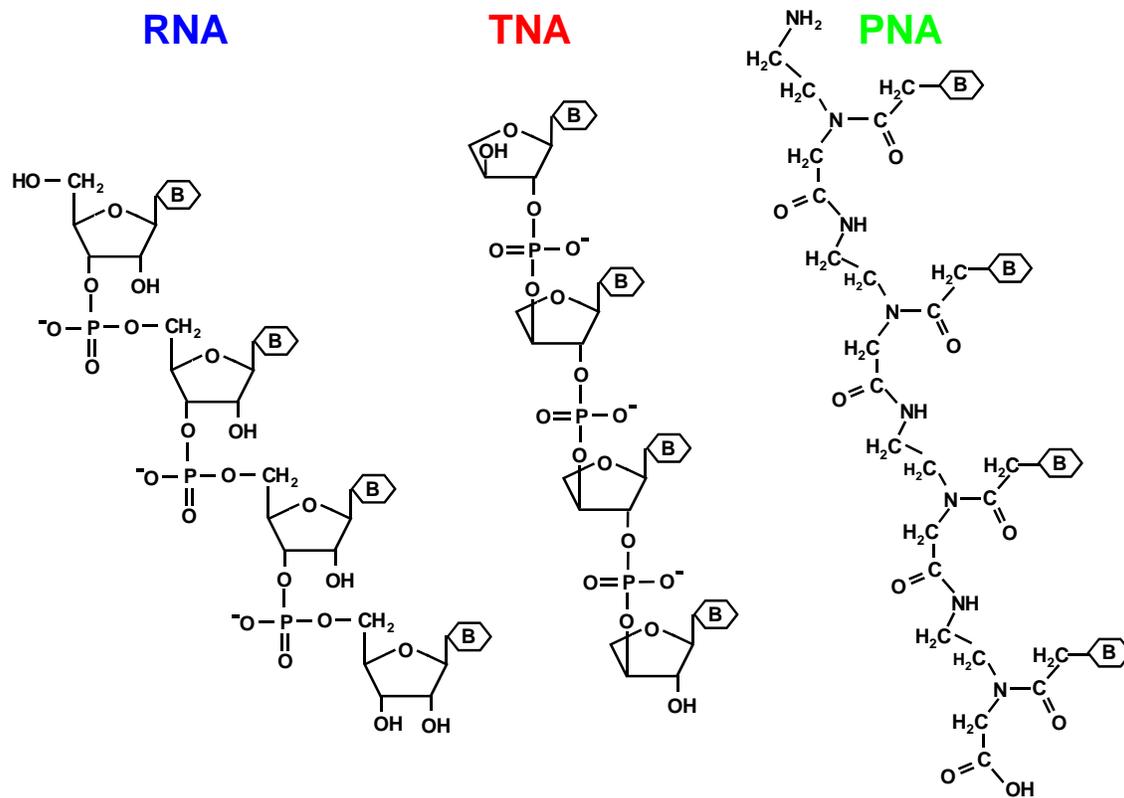


Fig. 6A9.1

Structures of single strands of RNA, TNA (α -L-threose nucleic acid) and PNA (peptide nucleic acid), each shown for oligomers of four bases (symbolized by B within hexagon).

References:

1. Cech, T. R. The chemistry of self-splicing RNA and RNA enzymes. *Science* **236**, 1532-9 (1987).
2. Doudna, J. A. & Lorsch, J. R. Ribozyme catalysis: not different, just worse. *Nat Struct Mol Biol* **12**, 395-402 (2005).
3. Fedor, M. J. & Williamson, J. R. The catalytic diversity of RNAs. *Nat Rev Mol Cell Biol* **6**, 399-412 (2005).
4. Joyce, G. F. Directed evolution of nucleic acid enzymes. *Annu Rev Biochem* **73**, 791-836 (2004).
5. Silverman, S. K. In vitro selection, characterization, and application of deoxyribozymes that cleave RNA. *Nucleic Acids Res* **33**, 6151-63 (2005).
6. Breaker, R. R. & Joyce, G. F. A DNA enzyme that cleaves RNA. *Chem Biol* **1**, 223-9 (1994).
7. Breaker, R. R. & Joyce, G. F. A DNA enzyme with Mg(2+)-dependent RNA phosphoesterase activity. *Chem Biol* **2**, 655-60 (1995).
8. Roth, A. & Breaker, R. R. An amino acid as a cofactor for a catalytic polynucleotide. *Proc Natl Acad Sci U S A* **95**, 6027-31 (1998).
9. Perrin, D. M., Garestier, T. & Helene, C. Bridging the gap between proteins and nucleic acids: a metal-independent RNaseA mimic with two protein-like functionalities. *J Am Chem Soc* **123**, 1556-63 (2001).
10. Ting, R., Thomas, J. M., Lermer, L. & Perrin, D. M. Substrate specificity and kinetic framework of a DNAzyme with an expanded chemical repertoire: a putative RNaseA mimic that catalyzes RNA hydrolysis independent of a divalent metal cation. *Nucleic Acids Res* **32**, 6660-72 (2004).
11. Faulhammer, D. & Famulok, M. Characterization and divalent metal-ion dependence of in vitro selected deoxyribozymes which cleave DNA/RNA chimeric oligonucleotides. *J Mol Biol* **269**, 188-202 (1997).
12. Geyer, C. R. & Sen, D. Evidence for the metal-cofactor independence of an RNA phosphodiester-cleaving DNA enzyme. *Chem Biol* **4**, 579-93 (1997).
13. Cruz, R. P., Withers, J. B. & Li, Y. Dinucleotide junction cleavage versatility of 8-17 deoxyribozyme. *Chem Biol* **11**, 57-67 (2004).
14. Hobartner, C. & Silverman, S. K. Recent advances in DNA catalysis. *Biopolymers* **87**, 279-92 (2007).
15. Carmi, N., Balkhi, S. R. & Breaker, R. R. Cleaving DNA with DNA. *Proc Natl Acad Sci U S A* **95**, 2233-7 (1998).

16. Carmi, N. & Breaker, R. R. Characterization of a DNA-cleaving deoxyribozyme. *Bioorg Med Chem* **9**, 2589-600 (2001).
17. Hoadley, K. A., Purtha, W. E., Wolf, A. C., Flynn-Charlebois, A. & Silverman, S. K. Zn²⁺-dependent deoxyribozymes that form natural and unnatural RNA linkages. *Biochemistry* **44**, 9217-31 (2005).
18. Flynn-Charlebois, A., Prior, T. K., Hoadley, K. A. & Silverman, S. K. In vitro evolution of an RNA-cleaving DNA enzyme into an RNA ligase switches the selectivity from 3'-5' to 2'-5'. *J Am Chem Soc* **125**, 5346-50 (2003).
19. Coppins, R. L. & Silverman, S. K. Rational modification of a selection strategy leads to deoxyribozymes that create native 3'-5' RNA linkages. *J Am Chem Soc* **126**, 16426-32 (2004).
20. Purtha, W. E., Coppins, R. L., Smalley, M. K. & Silverman, S. K. General deoxyribozyme-catalyzed synthesis of native 3'-5' RNA linkages. *J Am Chem Soc* **127**, 13124-5 (2005).
21. Sreedhara, A., Li, Y. & Breaker, R. R. Ligating DNA with DNA. *J Am Chem Soc* **126**, 3454-60 (2004).
22. Achenbach, J. C., Jeffries, G. A., McManus, S. A., Billen, L. P. & Li, Y. Secondary-structure characterization of two proficient kinase deoxyribozymes. *Biochemistry* **44**, 3765-74 (2005).
23. McManus, S. A. & Li, Y. Multiple occurrences of an efficient self-phosphorylating deoxyribozyme motif. *Biochemistry* **46**, 2198-204 (2007).
24. Chandra, M. & Silverman, S. K. DNA and RNA can be equally efficient catalysts for carbon-carbon bond formation. *J Am Chem Soc* **130**, 2936-7 (2008).
25. Chinnapen, D. J. & Sen, D. A deoxyribozyme that harnesses light to repair thymine dimers in DNA. *Proc Natl Acad Sci U S A* **101**, 65-9 (2004).
26. Chinnapen, D. J. & Sen, D. Towards elucidation of the mechanism of UV1C, a deoxyribozyme with photolyase activity. *J Mol Biol* **365**, 1326-36 (2007).
27. Breaker, R. R. DNA aptamers and DNA enzymes. *Curr Opin Chem Biol* **1**, 26-31 (1997).
28. Emilsson, G. M. & Breaker, R. R. Deoxyribozymes: new activities and new applications. *Cell Mol Life Sci* **59**, 596-607 (2002).
29. Siegfried, N. A., Metzger, S. L. & Bevilacqua, P. C. Folding cooperativity in RNA and DNA is dependent on position in the helix. *Biochemistry* **46**, 172-81 (2007).
30. Travascio, P., Bennet, A. J., Wang, D. Y. & Sen, D. A ribozyme and a catalytic DNA with peroxidase activity: active sites versus cofactor-binding sites. *Chem Biol* **6**, 779-87 (1999).
31. Paul, N., Springsteen, G. & Joyce, G. F. Conversion of a ribozyme to a deoxyribozyme through in vitro evolution. *Chem Biol* **13**, 329-38 (2006).

32. Chiuman, W. & Li, Y. Evolution of high-branching deoxyribozymes from a catalytic DNA with a three-way junction. *Chem Biol* **13**, 1061-9 (2006).
33. Kuhns, S. T. & Joyce, G. F. Perfectly complementary nucleic acid enzymes. *J Mol Evol* **56**, 711-7 (2003).
34. Soukup, G. A. & Breaker, R. R. Relationship between internucleotide linkage geometry and the stability of RNA. *Rna* **5**, 1308-25 (1999).
35. Nelson, K. E., Bruesehoff, P. J. & Lu, Y. In vitro selection of high temperature Zn(2+)-dependent DNAzymes. *J Mol Evol* **61**, 216-25 (2005).
36. Kandadai, S. A. & Li, Y. Characterization of a catalytically efficient acidic RNA-cleaving deoxyribozyme. *Nucleic Acids Res* **33**, 7164-75 (2005).