Oligonucleotide aptamers that recognize small molecules

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Nucleic acid receptors ('aptamers'), which recognize a large variety of organic molecules of low molecular weight, have been isolated from combinatorial nucleic acid libraries by *in vitro* selection methods. Structural studies of nucleic acid–small molecule complexes provide insight into both the principles of molecular recognition by this class of biopolymers and the architecture of tertiary motifs in nucleic acid folding. Aptamers that recognize small molecules are increasingly applied as tools in molecular biology, from the detection of oxidative damage in DNA to conditional gene expression and from their use as modules for the engineering of allosteric ribozymes to biosensors.

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Abbreviations

8-oxo-dG	7,8-dihydro-8-hydroxy-2′-deoxyguanosine
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
NAD	nicotinamide adenine dinucleotide
PCR	polymerase chain reaction
ss	single-stranded

Introduction

A considerable number of aptamer sequences that bind to molecules of low molecular weight with high specificity and affinity have been described to date (see Table 1) and many of them have been thoroughly reviewed [1–4]. As summarized in Table 1, some small-molecule-binding aptamers have been structurally characterized (for excellent summaries of aptamer structures, see [5–11]). Specifically, the structural studies have established that aptamer–small molecule complexes provide a cornucopia for the study of tertiary motifs in RNA folding, which add to the many new motifs identified from the crystal structures of large, catalytic RNAs [12]. In addition to this, aptamers that complex molecules of low molecular weight are increasingly demonstrating their potential as useful tools in various interesting biological applications, some of which I will summarize in this review.

Novel fluorophore- and dye-binding aptamers

Holeman *et al.* [13] recently isolated RNA aptamers that recognize the fluorophore sulforhodamine B. The goal of this study was to find RNA motifs that can transfer a fluorescence label to RNA transcripts tagged with the aptamer. Such activity would allow the detection of RNAs *in vitro* and *in vivo*. One of the aptamers consisted of an unusually large motif of approximately 60 nucleotides that contained a three-way helical junction, with two highly conserved unpaired regions that directly contact the fluorophore ligand. One of the aptamers was

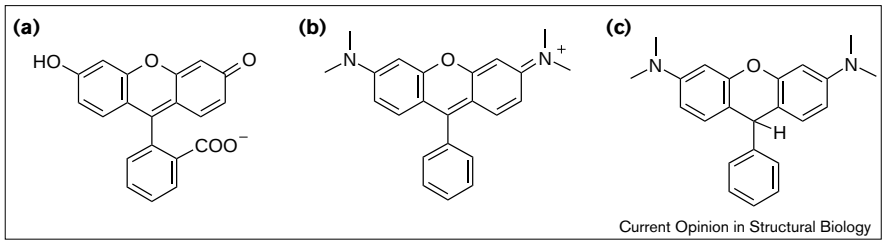
Table 1

Aptamers selected to bind small organic molecules.

Ligand	NMR structure reference	Protein Data Bank entry for structural data	Type of binding pocket	References
Miscellaneous small, organic ligands				
Organic dyes			RNA	[51]
Organic dyes			DNA	[52]
Theophyllin	[35]	1EHT	RNA	[53]
Dopamine			RNA	[54]
Hoechst 33258			RNA	[15••]
Sulforhodamine B			RNA	[13]
Sulforhodamine B			DNA	[14•]
Cellobiose			DNA	[16•]
Amino acids				
D-tryptophan			RNA	[55]
L-arginine			RNA	[56]
L-arginine	[36]	1koc	RNA	[31,57]
L-citrullin	[36]	1kod	RNA	[31,57]
L-argininamide	[58,59]	2arg	DNA	[60]
L-arginine			RNA	[32]
L-arginine			RNA	[33]
L-arginine			Mirror-image RNA	[61]
L-valine			RNA	[42]
L-isoleucine			RNA	[41]
Nucleotides and derivatives, biological cofactors				
AMP	[37,62,63]	1AMO,1RAW	RNA	[50,64,65]
AMP	[66]	1aw4	DNA	[67]
AMP			Mirror-image RNA	[68]
Guanosine			RNA	[34]
FMN	[38]	1FMN	RNA	[64]
FMN			RNA	[69]
NAD			RNA	[69]
Vitamin B_{12}			RNA	[70]
8-oxo-dG			DNA	[24•]
5′-cap			RNA	[22•]
Xanthene			RNA	[23]
Antibiotics				
Kanamycin A			RNA	[28]
Lividomycin			RNA	[28]
Tobramycin	[39,71]	1TOB	RNA	[17]
Neomycin B			RNA	[18,19]
Viomycin			RNA	[20•]
Chloramphenicol			RNA	[29]
Streptomycin			RNA	[21]
Others				
Rev peptide	[72]	1ULL	RNA	[73,74]
Vasopressin			Mirror-image RNA	[75•]

Figure 1

Chemical structures of **(a)** the fluorophore fluorescein, **(b)** sulforhodamine B and **(c)** dihydrotetramethylrosamine.



heavily mutagenized and reselected — this time for binding to the related fluorescein. The aptamers discriminate between sulforhodamine and fluorescein. When immobilized on beads, the aptamers specifically localize each fluorophore to the cognate matrix.

Wilson and Szostak [14•] also selected a guanosine-quartet-containing DNA aptamer with high affinity specifically for sulforhodamine B. This DNA aptamer was found to be catalytically active; it promoted the multiple turnover oxidation of the reduced form of a closely related molecule, dihydrotetramethyl rosamine, albeit with low efficiency (Figure 1).

Werstuck and Green [15••] recently showed that aptamers that bind to small organic molecules can potentially be used to control the expression of genes. They showed that aptamers that were selected to bind the aminoglycosides tobramycin and kanamycin A or certain dye molecules (Hoechst H33258) *in vitro* were also capable of binding these ligands *in vivo* (Figure 2).

The complementary DNA of these aptamers was inserted in tandem into the untranslated region of an expression plasmid with a β-galactosidase reporter gene. CHO cells were transfected with the expression plasmid and grown in the presence of Hoechst H33258. Quantification of β-galactosidase activities 24 h after transfection revealed that translation was prevented at 5–10 mM concentrations of Hoechst H33258 [15••].

Aptamers that recognize small biomolecules

As a first step towards the generation of high-affinity aptamers that bind to cell-surface oligosaccharides, Yang *et al.* [16•] selected DNA aptamers that recognize the disaccharide cellobiose, the repetitive unit in cellulose. The method of selection was simple, but quite effective; the DNA pool was incubated with cellulose, nonbinding sequences were eluted by a buffer wash and the remaining bound ssDNAs were recovered by elution with a cellobiose solution. Remarkably, the aptamers specifically recognize cellobiose both in solution and, also, in the context of the solid polymer cellulose, with apparent values of K_d between 10 and 0.1 μM, and can discriminate cellobiose from related disaccharides, such as lactose, maltose and gentiobiose. These DNA aptamers add to the several RNA sequences that have been isolated as binding to aminoglycoside saccharides [17–19,20•,21], the three-dimensional structures of many of which have been elucidated by NMR spectroscopy. The ability of the aptamers described by Yang *et al.* [16•] to differentiate among closely related, biologically important disaccharides, however, suggests that it may be possible to obtain a whole set of oligonucleotide receptors as recognition tools for arrays of surface antigens.

Haller and Sarnow [22•] isolated an aptamer that binds 1000-fold better to *N*-7-methylguanosine residues than to nonmethylated guanosines. As *N*-7-methylguanosine residues closely resemble the 5′ terminal cap structure of eukaryotic mRNAs, the cap-binding aptamer was revealed to specifically inhibit the translation of capped, but not

Figure 2

Chemical structures of **(a)** the antibiotics tobramycin and kanamycin A and **(b)** the dye molecule Hoechst 33258.

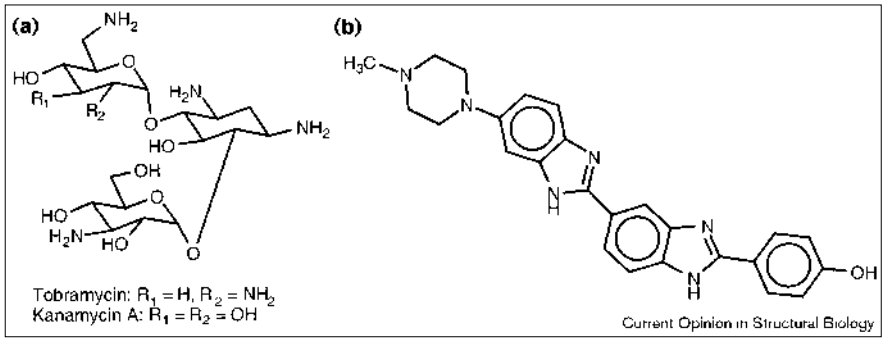
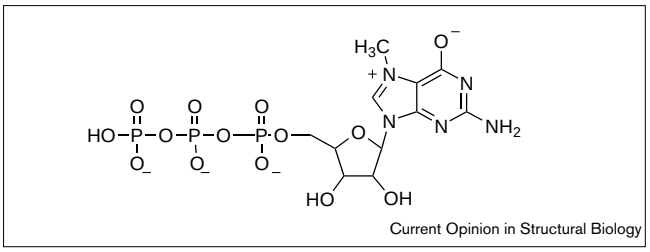
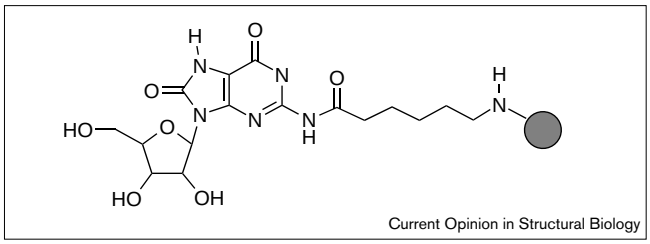


Figure 3



Chemical structure of the *N*-7-methylguanosine triphosphate used in the selection of cap-binding aptamers.

Figure 4



Chemical structure of 8-oxo-guanosine linked to the sepharose matrix (sphere) used in the selection of 8-oxo-guanosine-binding aptamers.

uncapped, mRNAs in cell-free lysates from either HeLa cells or yeast. These aptamers might thus be useful in studies of cap-dependent processes, such as pre-mRNA splicing and nucleocytoplasmic mRNA transport (Figure 3).

An RNA aptamer that binds to xanthine (2,6-dioxypurine) with a K_d of 3.3 μM was described by Kiga *et al.* [23]. This aptamer had slightly higher affinity to the closely related molecule guanine (K_d = 1.3 μM), but did not bind to less closely related nucleotides, such as adenine, cytosine or uracil.

Another guanine derivative that was a target in a RNA selection experiment was 7,8-dihydro-8-hydroxy-2′-deoxyguanosine (8-oxo-dG), a mutagenic lesion that can be found in cellular DNA as the result of oxidative damage at guanine bases [24•]. These lesions are the most common among all the oxidative DNA lesions that occur at a daily frequency of about 2×10^4 per cell (Figure 4). During DNA replication, they can convert guanosine bases to thymines. The selected RNA aptamer bound 8-oxo-dG with a K_d of 270 nM when the residue was located at the 3′ terminus of the DNA and with a K_d of 2800 nM when it was embedded within a ssDNA sequence. Competition experiments performed with DNA strands that did not contain the oxidative lesion revealed that the aptamer was able to detect a single 8-oxo-dG lesion within a background of greater than 10^4 deoxyguanosine residues. These results suggest that it may be possible to evaluate the 8-oxo-dG levels in cellular DNA or in a tissue sample using this aptamer.

Aptamers that recognize antibiotics

Many biologically active RNA molecules have been shown to interact with various chemically varied small molecules, including aminoglycoside, peptide and pseudodisaccharide antibiotics [25]. These interactions can lead to the inhibition of both ribosomal protein synthesis and the self-splicing or cleavage reactions of ribozymes. Most of these antibiotics not only inhibit RNA function, but can also, under certain conditions, stimulate RNA activity. Such activities were shown for the peptide antibiotic viomycin from the tuberactinomycin family [26] and also for the aminoglycoside antibiotic neomycin B [27•].

In vitro selections of antibiotic-binding RNA aptamers have previously been carried out in order to understand the principles underlying the binding and recognition of RNA by these molecules [17–19,28]; the three-dimensional structures of several of these aptamer–antibiotic complexes have been determined by NMR spectroscopy. Such studies provide an excellent means of facilitating our understanding of the principles of molecular recognition by RNA in general and may also help to better understand the function of the ribosome. During the past two years, some novel, interesting examples of antibiotic-binding RNA aptamers have added to these previous studies.

Wallis *et al.* [20•] isolated a variety of novel viomycin-binding RNA molecules by *in vitro* selection of an RNA library randomized at 74 nucleotide positions. The vast majority of the selected RNA molecules contained a continuous, highly conserved region of 14 nucleotides. Structural analyses, including enzymatic and chemical probing, Pb(II)-induced cleavage and mutational analyses, established that the conserved sequence forms a stem–loop structure, which is engaged in a long-range interaction forming a pseudoknot. The ability of the aptamer to form the pseudoknot is absolutely required for viomycin binding. A comparison of the selected viomycin-binding motif and the natural RNA target sites for viomycin showed that all these segments form a pseudoknot at the antibiotic-binding site.

Wallace *et al.* [21] extended these studies to streptomycin and Burke *et al.* [29] studied chloramphenicol. The first study resulted in aptamers that are able to discriminate among closely related streptomycin derivatives. Similar discriminatory effects are also obtained when these antibiotics are used in the inhibition of the group I ribozyme self-splicing reaction. The second study revealed chloramphenicol-binding RNA sequences. Chloramphenicol inhibits peptidyl transfer by binding to the central loop of domain V in 23S rRNA in the bacterial ribosome, the so-called peptidyl transferase loop. Indeed, one of the selected RNA motifs seems to superficially resemble some sequence characteristics of the chloramphenicol-binding site in the bacterial ribosome. It remains to be shown, however, whether these similarities truly reflect common

features of the ribosomal and aptameric chloramphenicol-binding sites on a secondary and tertiary structural level.

Aptamers that recognize amino acids

The fact that the genetic code associates individual amino acids with RNA sequences has tempted many scientists to inquire whether a connection, explainable through mechanisms of molecular recognition, exists between amino acids and their assigned codons. If the binding of amino acids by short RNA motifs led to codon assignment, then it may be interesting to analyze whether aptamers selected for amino acid binding reflect amino acid–codon interactions. In this context, Knight and Landweber [30] proposed, on the basis of statistical evidence, that such a connection does indeed exist. They tested whether the arginine-binding sites of five phylogenetically unrelated arginine aptamers, which were selected in three different laboratories under different conditions and for which structural data exist [31–34], contain a statistical excess of arginine codons or anticodons. A statistical association between the two arginine codon classes CGN and AGR, and the regions of the published aptamers that participate in arginine binding was observed. Arginine aptamers have a significant bias in favor of arginine codons at their binding sites; they contain far more arginine codons at their binding sites than expected by chance and more than was found for nonarginine-binding aptamers for which structural NMR data exist [35–39]. Thus, Knight and Landweber provide statistical evidence — at least for one amino acid — that supports the hypothesis [40] that amino acids can specifically interact with RNA sequences that contain their cognate codons.

It is not clear at present whether the observed statistical evidence can be extended to other amino acids or whether it is restricted to the special case of arginine. This is mainly due to the lack of sufficient structural data for other amino-acid-binding aptamers (see Table 1). In this context, however, it may certainly be important that an aptamer that binds the hydrophobic amino acid isoleucine does contain the isoleucine codon AUU at its isoleucine-binding site, as determined by chemical modification interference and by the analysis of truncated aptamers and aptamer mutants [41]. The isoleucine aptamer can discriminate between the hydrophobic sidechains of both the closely related amino acid valine and the related molecule norleucine. On the other hand, an aptamer selected for specific binding to L-valine does not contain conserved valine codons [42]. It will be interesting to apply statistical tests to other amino acid–RNA aptamer complexes once sufficient structural information has been gathered.

As Knight and Landweber [30] state in their paper, their data support this hypothesis only if the *in vitro* selection protocols applied to the amino acid/aptamer selections mimic an appropriate evolutionary environment and if the selections are influenced by the same chemical interactions as the codon assignments. Under the foregone conditions of the evolution of the genetic code, the selection constraints may have been very different from those acting during the *in vitro* selection of RNA aptamers for binding to a given amino acid, such as the amino acid frequency in the amino acid pool [43], the stability of the codon structure [44] or the available pool of unused codons.

Prospects

There is now a plethora of aptamers available that complex molecules of low molecular weight. Although detailed structural analyses of the first-generation aptamers have yielded insight both into RNA–ligand interactions and into the principles of the dynamics of molecular recognition by nucleic acids, there is still a lot to be learned about these aspects on a structural level. Structural data for as many RNA–aptamer complexes as possible will greatly facilitate the molecular modeling of biologically relevant nucleic acids in general. It will be exciting to compare structural data from crystallographic studies with those data obtained from solution studies by NMR spectroscopy.

There are also many new and exciting results that point to a possible diagnostic or therapeutic use for small-molecule-binding aptamers. Even applications in biotechnology, in which aptamers are used for conditional gene expression, are in sight. Aptamers will more and more be used as regulatory modules for engineering allosteric ribozymes by coupling catalytic domains with aptameric receptor domains [45•–48•]. For example, Robertson and Ellington [49•] recently described the *in vitro* selection of a novel ligase ribozyme that requires allosteric activation by an oligonucleotide effector molecule for activity. The allosteric ribozyme ligase is activated up to 10,000-fold by the oligonucleotide effectors. 'Rational' engineering of the ribozyme by incorporating the aptameric ATP-binding sequence [50] allowed its transformation into an enzyme that requires ATP, in addition to the oligonucleotide effector, for activity. This approach to effector-controlled ribozyme activity, when linked to reverse transcriptase/PCR technology, has the potential to be applied whenever the accurate diagnostic quantification of oligonucleotide or small-molecule analytes is desired. Such allosteric ribozymes or 'aptazymes' might possess a significant application potential in medicine, diagnostics and biotechnology as rate-controlled enzymes.

It may even be interesting to perform selections of aptamers for small molecules using libraries derived from genomic RNA, rather than from synthetic oligonucleotide libraries. Genomic nucleic acid libraries can be applied in the rapid identification of a potential natural binding sequence in small molecules that may interact with a nucleic acid, but for which a natural site of interaction is unknown. Genomic *in vitro* selection may also be useful, for example, to study the regulatory pathways that control gene expression or to test whether retroviral proteins, many of which bind to viral genomic RNA, also bind to

mRNAs from the host organism. In general, the *in vitro* selection of genomic libraries should be an extremely potent means of identifying natural RNA sequences with affinity to any given target molecule. It may well be that the RNAs in a cell could participate in monitoring biological processes that involve biological cofactors, such as ATP, GTP, FAD or NAD^+, through specific binding to these low molecular weight targets. Using genomic RNA libraries, such biologically relevant cellular RNA–cofactor interactions could easily be identified.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Ellington AD: **RNA selection. Aptamers achieve the desired recognition.** *Curr Biol* 1994, **4**:427-429.

2. Uphoff KW, Bell SD, Ellington AD: ***In vitro* selection of aptamers: the dearth of pure reason.** *Curr Opin Struct Biol* 1996, **6**:281-288.

3. Gold L, Polisky B, Uhlenbeck O, Yarus M: **Diversity of oligonucleotide functions.** *Annu Rev Biochem* 1995, **64**:763-797.

4. Osborne SE, Ellington AD: **Nucleic acid selection and the challenge of combinatorial chemistry.** *Chem Rev* 1997, **97**:349-370.

5. Cech TR, Szewczak AA: **Selecting apt RNAs for NMR.** *RNA* 1996, **2**:625-627.

6. Egli M: **Wie *in vitro* selektierte RNA-Aptamere funktionieren-die räumlichen Strukturen von Substrat-RNA-Aptamer-Komplexen.** *Angew Chem Int Ed* 1997, **36**:480-482. [Title translation: *In vitro* selected receptors rationalized: the first 3D structures of RNA aptamer/substrate complexes.]

7. Feigon J, Dieckmann T, Smith FW: **Aptamer structures from A to ζ.** *Chem Biol* 1996, **3**:611-617.

8. Heus HA: **RNA aptamers.** *Nat Struct Biol* 1997, **4**:597-600.

9. Patel DJ: **Structural analysis of nucleic acid aptamers.** *Curr Opin Chem Biol* 1997, **1**:32-46.

10. Patel DJ, Suri AK, Jiang F, Liang L, Fan P, Kumar RA, Nonin S: **Structure, recognition and adaptive binding in RNA aptamer complexes.** *J Mol Biol* 1997, **272**:645-664.

11. Westhof E, Masquida B, Jaeger L: **RNA tectonics: towards RNA design.** *Fold Des* 1996, **1**:R78-R88.

12. Batey RT, Rambo RP, Doudna JA: **Tertiary motifs in RNA structure and folding.** *Angew Chem Int Ed* 1999, **38**:in press.

13. Holeman LA, Robinson SL, Szostak JW, Wilson C: **Isolation and characterization of fluorophore-binding RNA aptamers.** *Fold Des* 1998, **3**:423-431.

14. • Wilson C, Szostak JW: **Isolation of a fluorophore-specific DNA aptamer with weak redox activity.** *Chem Biol* 1998, **5**:609-617.
This paper describes one of only a few examples in which an aptamer that was selected merely to bind a certain small molecule ligand was later shown to exhibit catalytic activity.

15. •• Werstuck G, Green MR: **Controlling gene expression in living cells through small molecule-RNA interactions.** *Science* 1998, **282**:296-298.
This was one of the first papers to show that aptamers exhibit their function even when integrated into a large mRNA inside a cellular context. In this case, an aptamer for a small dye or antibiotic molecule was inserted into the 5′ untranslated region of a reporter gene. In the presence of the small molecule, the expression of the reporter gene was drastically reduced in an aptamer/ligand-dependent fashion in higher eukaryotic cells.

16. • Yang Q, Goldstein IJ, Mei HY, Engelke DR: **DNA ligands that bind tightly and selectively to cellobiose.** *Proc Natl Acad Sci USA* 1998, **95**:5462-5467.
An interesting example of the isolation of an aptamer that binds to a small molecule in solution using an insoluble matrix composed of the polymerized ligand.

17. Wang Y, Killian J, Hamasaki K, Rando RR: **RNA molecules that specifically and stoichiometrically bind aminoglycoside antibiotics with high affinities.** *Biochemistry* 1996, **35**:12338-12346.

18. Wallis MG, Von Ahsen U, Schroeder R, Famulok M: **A novel RNA motif for neomycin recognition.** *Chem Biol* 1995, **2**:543-552.

19. Famulok M, Hüttenhofer A: ***In vitro* selection analysis of neomycin binding RNAs with a mutagenized pool of variants of the 16S rRNA decoding region.** *Biochemistry* 1996, **35**:4265-4270.

20. • Wallis MG, Streicher B, Wank H, von Ahsen U, Clodi E, Wallace ST, Famulok M, Schroeder R: ***In vitro* selection of a viomycin-binding RNA pseudoknot.** *Chem Biol* 1997, **4**:357-366.
The aptamer described is the second example of a pseudoknot structure for an *in vitro* selected RNA that binds to a small molecule (the first one was the aptamer for cyanocobalamine [70]). The characteristics of this structure led to the interesting observation that viomycin recognition by many biologically relevant natural RNAs may also occur via pseudoknot structures. This, in turn, may help to explain the mechanism by which viomycin modulates the dimerization of RNAs.

21. Wallace ST, Schroeder R: ***In vitro* selection and characterization of streptomycin-binding RNAs: recognition discrimination between antibiotics.** *RNA* 1998, **4**:112-123.

22. • Haller AA, Sarnow P: ***In vitro* selection of a 7-methyl-guanosine binding RNA that inhibits translation of capped mRNA molecules.** *Proc Natl Acad Sci USA* 1997, **94**:8521-8526.
An impressive example of a small-molecule-binding RNA aptamer that selectively blocks a biological process – the translation of capped mRNAs.

23. Kiga D, Futamura Y, Sakamoto K, Yokoyama S: **An RNA aptamer to the xanthine/guanine base with a distinctive mode of purine recognition.** *Nucleic Acids Res* 1998, **26**:1755-1760.

24. • Rink SM, Shen J-C, Loeb LA: **Creation of RNA molecules that recognize the oxidative lesion 7,8-dihydro-8-hydroxy-2′-deoxyguanosine (8-oxodG) in DNA.** *Proc Natl Acad Sci USA* 1998, **95**:11619-11624.
This paper shows that aptamers can potentially be used in examining very demanding problems of molecular recognition. Having been selected for its specific recognition of a monomeric guanosine derivative, this aptamer also is capable of recognizing the derivative with remarkable specificity and selectivity when embedded in single-stranded DNA. It will be interesting to see how this aptamer performs in 8-oxo-dG-derivatized double-stranded DNA.

25. Schroeder R, Von Ahsen U: **Interaction of aminoglycoside antibiotics with RNA.** In *Nucleic Acids and Molecular Biology*. Edited by Eckstein F, Lilley DMJ. Heidelberg: Springer-Verlag; 1996:53-74.

26. Olive JE, De Abreu DM, Rastogi T, Andersen AA, Mittermaier AK, Beattie TL, Collins RA: **Enhancement of Neurospora VS ribozyme cleavage by tuberactinomycin antibiotics.** *EMBO J* 1995, **14**:3247-3251.

27. • Earnshaw DJ, Gait MJ: **Hairpin ribozyme cleavage catalyzed by aminoglycoside antibiotics and the polyamine spermine in the absence of metal ions.** *Nucleic Acids Res* 1998, **26**:5551-5561.
In this paper, Earnshaw and Gait describe the interesting observation that neomycin B acts as an inhibitor of phosphodiester cleavage by the hairpin ribozyme, but only when divalent metal ions are present. In the absence of divalent metal ions, however, both neomycin B and spermidine stimulate the cleavage reaction. This phenomenon will be of great interest for understanding ribozymal cleavage mechanisms. This is one of very few examples of a nonmetal cofactor requirement for a ribozyme-catalyzed cleavage reaction.

28. Lato SM, Boles AR, Ellington AD: ***In vitro* selection of RNA lectins: using combinatorial chemistry to interpret ribozyme evolution.** *Chem Biol* 1995, **2**:291-303.

29. Burke DH, Hoffman DC, Brown A, Hansen M, Pardi A, Gold L: **RNA aptamers to the peptidyl transferase inhibitor chloramphenicol.** *Chem Biol* 1997, **4**:833-843.

30. Knight RD, Landweber LF: **Rhyme or reason: RNA-arginine interactions and the genetic code.** *Chem Biol* 1998, **5**:R215-R220.

31. Famulok M: **Molecular recognition of amino acids by RNA-aptamers: an L-citrulline binding RNA motif and its evolution into an L-arginine binder.** *J Am Chem Soc* 1994, **116**:1698-1706.

32. Geiger A, Burgstaller P, Von der Eltz H, Roeder A, Famulok M: **RNA aptamers that bind L-arginine with sub-micromolar dissociation constants and high enantioselectivity.** *Nucleic Acids Res* 1996, **24**:1029-1036.

33. Tao J, Frankel AD: **Arginine-binding RNAs resembling TAR identified by *in vitro* selection.** *Biochemistry* 1996, **35**:2229-2238.

34. Connell GJ, Yarus M: **RNAs with dual specificity and dual RNAs with similar specificity.** *Science* 1994, **264**:1137-1141.

35. Zimmerman GR, Jenison RD, Wick CL, Simorre J-P, Pardi A: **Interlocking structural motifs mediate molecular discrimination by a theophylline-binding RNA.** *Nat Struct Biol* 1997, **4**:644-649.

36. Yang Y, Kochoyan M, Burgstaller P, Westhof E, Famulok M: **Structural basis of ligand discrimination by two related RNA aptamers resolved by NMR spectroscopy.** *Science* 1996, **272**:1343-1347.

37. Jiang F, Kumar RA, Jones RA, Patel DJ: **Structural basis of RNA folding and recognition in an AMP-RNA complex.** *Nature* 1996, **382**:183-186.

38. Fan P, Suri AK, Fiala R, Live D, Patel DJ: **Molecular recognition in the FMN-RNA aptamer complex.** *J Mol Biol* 1996, **258**:480-500.

39. Jiang L, Suri AK, Fiala R, Patel DJ: **Saccharide-RNA recognition in an aminoglycoside antibiotic-RNA aptamer complex.** *Chem Biol* 1997, **4**:35-50.

40. Yarus M: **Amino acids as RNA ligands: a direct-RNA-template theory for the code's origin.** *J Mol Evol* 1998, **47**:109-117.

41. Majerfeld I, Yarus M: **Isoleucine:RNA sites with associated coding sequences.** *RNA* 1998, **4**:471-478.

42. Majerfeld I, Yarus M: **An RNA pocket for an aliphatic hydrophobe.** *Nat Struct Biol* 1994, **1**:287-292.

43. Eigen M, Lindemann BF, Tietze M, Winkler-Oswatitsch R, Dress A, von Haeseler A: **How old is the genetic code? Statistical geometry of tRNA provides an answer.** *Science* 1989, **244**:673-679.

44. Wong JT-F: **A co-evolution theory of the genetic code.** *Proc Natl Acad Sci USA* 1975, **72**:1909-1912.

45. • Tang J, Breaker RR: **Rational design of allosteric ribozymes.** *Chem Biol* 1997, **4**:453-459.
See annotation to [49•].

46. • Soukup GA, Breaker RR: **Engineering precision RNA molecular switches.** *Proc Natl Acad Sci USA* 1999, **96**:3584-3589.
See annotation to [49•].

47. • Tang J, Breaker RR: **Mechanism for allosteric inhibition of an ATP sensitive ribozyme.** *Nucleic Acids Res* 1998, **26**:4214-4221.
This study suggests a possible mechanism for the inhibition of an engineered ATP-aptamer-containing hammerhead ribozyme by ATP. (See, also, annotation to [49•].)

48. • Araki M, Okuno Y, Hara Y, Sugiura Y: **Allosteric regulation of a ribozyme activity through ligand-induced conformational change.** *Nucleic Acids Res* 1998, **26**:3379-3384.
See annotation to [49•].

49. • Robertson MP, Ellington AD: ***In vitro* selection of an allosteric ribozyme that transduces analytes to amplicons.** *Nat Biotechnol* 1999, **17**:62-66.
Papers [45•–49•] are, together, very impressive examples of rational ribozyme engineering. The incorporation of aptamer sequences into the hammerhead ribozyme [45•–48•] or into an *in vitro* selected ligase ribozyme [49•] led to ribozyme variants that performed catalytic activity as a function of the presence or absence of the aptamer ligand.

50. Sassanfar M, Szostak JW: **An RNA motif that binds ATP.** *Nature* 1993, **364**:550-553.

51. Ellington AD, Szostak JW: ***In vitro* selection of RNA molecules that bind specific ligands.** *Nature* 1990, **346**:818-822.

52. Ellington AD, Szostak JW: **Selection *in vitro* of single-stranded DNA molecules that fold into specific ligand-binding structures.** *Nature* 1992, **355**:850-852.

53. Jenison RD, Gill SC, Pardi A, Polisky B: **High-resolution molecular discrimination by RNA.** *Science* 1994, **263**:1425-1429.

54. Mannironi C, Di Nardo A, Fruscoloni P, Tocchini-Valentini GP: ***In vitro* selection of dopamine RNA ligands.** *Biochemistry* 1997, **36**:9726-9734.

55. Famulok M, Szostak JW: **Stereospecific recognition of tryptophan agarose by *in vitro* selected RNA.** *J Am Chem Soc* 1992, **114**:3990-3991.

56. Connell GJ, Illangesekare M, Yarus M: **Three small ribooligonucleotides with specific arginine sites.** *Biochemistry* 1993, **32**:5497-5502.

57. Burgstaller P, Kochoyan M, Famulok M: **Structural probing and damage selection of citrullin and arginine specific RNA aptamers identify base positions required for binding.** *Nucleic Acids Res* 1995, **23**:4769-4776.

58. Lin CH, Patel DJ: **Encapsulating an amino acid in a DNA fold.** *Nat Struct Biol* 1996, **3**:1046-1050.

59. Lin CH, Wang W, Jones RA, Patel DJ: **Formation of an amino-acid-binding pocket through adaptive zippering-up of a large DNA hairpin loop.** *Chem Biol* 1998, **5**:555-572.

60. Harada K, Frankel AD: **Identification of two novel arginine binding DNAs.** *EMBO J* 1995, **14**:5798-5811.

61. Nolte A, Klußmann S, Bald R, Erdmann VA, Fürste JP: **Mirror-design of L-oligonucleotide ligands binding to L-arginine.** *Nat Biotechnol* 1996, **14**:1116-1119.

62. Dieckmann T, Suzuki E, Nakamura GK, Feigon J: **Solution structure of an ATP-binding RNA aptamer reveals a novel fold.** *RNA* 1996, **2**:628-640.

63. Nonin S, Jiang F, Patel DJ: **Imino proton exchange and base pair kinetics in the AMP-RNA aptamer complex.** *J Mol Biol* 1997, **268**:359-374.

64. Burgstaller P, Famulok M: **Isolation of RNA aptamers for biological cofactors by *in vitro* selection.** *Angew Chem Int Ed* 1994, **33**:1084-1087.

65. Burke DH, Gold L: **RNA aptamers to the adenosine moiety of S-adenosyl methionine: structural inferences from variations on a theme and the reproducibility of SELEX.** *Nucleic Acids Res* 1997, **25**:2020-2024.

66. Lin CH, Patel DJ: **Structural basis of DNA folding and recognition in an AMP–DNA aptamer complex: distinct architectures but common recognition motifs for DNA and RNA aptamers complexed to AMP.** *Chem Biol* 1997, **4**:817-832.

67. Huizenga DE, Szostak JW: **A DNA aptamer that binds adenosine and ATP.** *Biochemistry* 1995, **34**:656-665.

68. Klußmann S, Nolte A, Bald R, Erdmann VA, Fürste JP: **Mirror-image RNA that binds D-adenosine.** *Nat Biotechnol* 1996, **14**:1112-1115.

69. Lauhon CT, Szostak JW: **RNA aptamers that bind flavin and nicotinamide redox cofactors.** *J Am Chem Soc* 1995, **117**:1246-1257.

70. Lorsch JR, Szostak JW: ***In vitro* selection of RNA aptamers specific for cyanocobalamin.** *Biochemistry* 1994, **33**:973-982.

71. Jiang L, Patel DJ: **Solution structure of the tobramycin-RNA aptamer complex.** *Nat Struct Biol* 1998, **5**:769-774.

72. Ye X, Gorin A, Ellington AD, Patel D: **Deep penetration of an α-helix into the widened RNA major groove in the HIV-1 Rev peptide-RNA aptamer complex.** *Nat Struct Biol* 1996, **3**:1026-1033.

73. Giver L, Bartel DP, Zapp ML, Green MR, Ellington AD: **Selective optimization of the Rev-binding element of HIV-1.** *Nucleic Acids Res* 1993, **21**:5509-5516.

74. Giver L, Bartel DP, Zapp ML, Green MR, Ellington AD: **Selection and design of high-affinity RNA ligands for HIV-1 Rev.** *Gene* 1993, **137**:19-24.

75. • Williams KP, Liu XH, Schumacher TN, Lin HY, Ausiello DA, Kim PS, Bartel DP: **Bioactive and nuclease-resistant L-DNA ligand of vasopressin.** *Proc Natl Acad Sci USA* 1997, **94**:11285-11290.
This paper, together with [61,68], shows that mirror-image RNAs of natural aptamers that were selected to bind to the mirror image of a certain target can stereospecifically recognize the target. The mirror-image RNAs are absolutely resistant to degradation by nucleases.