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# Aptamer Structures: A Preview into Regulatory Pathways?

**The crystal structure of a streptomycin binding RNA aptamer displays a novel bipartite fold able to clamp the antibiotic. In view of the recent findings that metabolites directly control mRNA translation, we might expect that similar structures exist in natural RNAs.**

The notion that RNA molecules are able to fold and build binding pockets for small molecules first emerged when it was discovered that self-splicing group I introns have a cofactor [1]. Guanosine was the first of a list of metabolites that interact with high affinity and specificity with RNA. The same binding site located in the group I intron core can accommodate the amino acid arginine and many antibiotics, among them streptomycin, neomycin, and viomycin [2]. Today it is clear that RNA is a potent target for therapeutic drugs. In the past year, a plethora of high-resolution structures of antibiotic-ribosome complexes shed light into the binding mode and recognition principles of RNA-antibiotic interactions [3].

With the development of in vitro selection procedures, it became possible to isolate RNA aptamers for probably every water-soluble ligand, and the small size of these aptamers made them perfect tools to explore the rules that govern recognition of small molecules by RNA. High-resolution structures of several ligand-aptamer complexes have been determined, demonstrating the diversity of structural motifs RNA can fold into [4]. Both simple noncomposite folds that form tight binding pockets as well as complex composite modular shapes can be found. One important outcome of these studies will be a database with an extensive repertoire of RNA structural modules. The streptomycin binding aptamer presented by Tereshko et al. in this issue of *Chemistry & Biology* represents a novel RNA fold with a distinct way to encapsulate a small molecule [5].

To enhance the crystallization procedure, the original aptamer was split into two strands with dangling 5′ ends, a procedure that allowed crystals to develop a few minutes [6]. The streptomycin-aptamer complex adopts an unusually sophisticated structure characterized by a 90° kink between residues C106 and C107 at the bottom of the lower asymmetrical loop, giving the complex its L

Secondary Structure of the Streptomycin Aptamer

The secondary structure of the streptomycin aptamer with the 90° kink between bases C106 and C107 is shown. Solid black lines represent base pairing. Base triples are indicated with orange lines. Bases highlighted in dark blue interact directly with streptomycin, whereas the interaction of G6 and U112 (highlighted in light blue) with the antibiotic is mediated by a water molecule. Arrows indicate binding sites of the three metal ions (labeled M1 to M3).

shape (see Figure). Other important features of this loop are the base triple U16-C18-G110 (shown in orange) and the U-turn U16 to C18. The upper asymmetric internal loop forms a series of S-turns that span residues C5 to G13. Both of the internal loops zipper up and stack with the central stem, forming a tight structure surrounding the streptomycin binding pocket, which is located in the elbow of the L shape. The tight interlocking of both the upper and lower internal loops is stabilized by a magnesium ion interacting with residues U10-11 from the upper loop and residue C109 of the lower loop (indicated as M1 in the Figure). Residue C109 itself is involved in a noncanonical base pair with G12.

The antibiotic binding pocket is an elaborate structure in which walls are formed by bases from both interlocked loops. The streptose ring of streptomycin is buried deeply in the pocket and makes contacts with multiple residues, in particular residues at positions U11 and G12 from the upper loop and residues U16 and U17 from the lower loop. In contrast to the streptomycin-ribosome structure, most RNA-antibiotic contacts in the aptamer involve base edges and not backbone phosphates. Recognition between the antibiotic and the RNA is predominantly achieved through hydrogen bonds, one of which is mediated through a bridging water molecule. All of the $NH_2$, NH, and OH groups on the streptose ring are involved in intermolecular contacts, in contrast to the two other streptomycin rings, which are positioned outside the pocket and contribute to binding only through one hydrogen bond. The guanidinium group of the streptose ring is buried most deeply in the binding pocket and is involved in several hydrogen bonds. The substitution of this group by a carbamino group in bluensomycin is the reason for the tight aptamer discrimination between both antibiotics.

This structure demonstrates once more the diversity of RNA ligand interactions. While aromatic ligands like ATP, FMN, and theophylline stack between bases, streptomycin lies perpendicular to the base pair planes. Contrary to previously published aminoglycodise-aptamer structures, neomycin and tobramycin, where the antibiotics lie in the deep groove of a perturbed double helix, streptomycin is locked in place via the two intertwined asymmetric internal loops [4].

Many of the in vitro-selected aptamers adopt their final fold only after ligand binding, with the ligand being an essential part of the structure. In the absence of the ligand, the RNA is rather unstructured. This ligand-dependent structural stabilization prompted the design of a translation regulation system. Aptamers were inserted into the 5′ untranslated leader of messenger RNAs without affecting their expression. Only after addition of the ligand did the RNA fold, leading to repression of translation [7]. Since this discovery, many of us have wondered why nature did not make use of such a clever mechanism. Several years since researchers developed this regulatory concept, it was discovered that this mechanism is indeed used by nature. Metabolite binding domains in mRNAs, which refold after ligand binding, were recently found for cyanocobalamin, thiamine, and FMN [8]. These recent findings give us a taste of what is waiting to be discovered and clearly show that metabolite-RNA complexes will be used in the future for a yet unpredictable number of applications. We can now predict that many biosynthetic pathways will be regulated by metabolite binding “natural aptamers,” and we might even find a structure similar to the streptomycin aptamer in a bacterium producing streptomycin.

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# Resisting Bacterial Drug Resistance

**In this issue of *Chemistry & Biology*, Wright and colleagues report an elegant method for inhibiting enzymes critical for rendering bacteria drug resistant. By using cationic peptides as inhibitors, the authors have exploited two antibacterial mechanisms, making it doubly difficult for microbial retaliation.**

Aminoglycosides are one of the oldest classes of antibacterial natural products [1]. These compounds kill bacteria by binding tightly to the acceptor site (A site) on the 30S subunit of the ribosome and consequently inhibit bacterial protein synthesis. As is the case for all the other compounds classes of antibacterials, resistance to these drugs has increased rapidly with usage. In aminoglycoside-producing organisms, resistance to the compound results from the methylation of nucleotides at the A site in the ribosome, preventing the drug from binding due to steric and electrostatic interference. For the notorious pathogen *Mycobacterium tuberculo-*