REVIEW: BIOCHEMISTRY

Adaptive Recognition by Nucleic Acid Aptamers

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Nucleic acid molecules play crucial roles in diverse biological processes including the storage, transport, processing, and expression of the genetic information. Nucleic acid aptamers are selected in vitro from libraries containing random sequences of up to a few hundred nucleotides. Selection is based on the ability to bind ligand molecules with high affinity and specificity. Three-dimensional structures have been determined at high resolution for a number of aptamers in complex with their cognate ligands. Structures of aptamer complexes reveal the key molecular interactions conferring specificity to the aptamer-ligand association, including the precise stacking of flat moieties, specific hydrogen bonding, and molecular shape complementarity. These basic principles of discriminatory molecular interactions in aptamer complexes parallel recognition events central to many cellular processes involving nucleic acids.

Aptamers are RNAs and DNAs originating from in vitro selection experiments (termed SELEX: systematic evolution of ligands by exponential enrichment), which, starting from random sequence libraries, optimize the nucleic acids for high-affinity binding to given ligands (*1, 2*). Predominantly unstructured in solution, aptamers fold upon associating with their ligands into molecular architectures in which the ligand becomes an intrinsic part of the nucleic acid structure. Because the evolutionary pressure on aptamer sequences during selection is directed primarily toward the binding of the ligands, the three-dimensional structures of aptamer complexes reflect highly optimized scaffolds for specific ligand recognition (Table 1). Unlike nucleic acids originating from biological sources, which are optimized with respect to multiple aspects of their cellular functions, aptamers do not trade off specificity in ligand binding for additional functions. Nevertheless, the architectures of aptamer complexes are valuable for the study of molecular recognition processes and yield a diversity of three-dimensional motifs, which recur in biologically relevant nucleic acid folds (*3*). This review outlines structural approaches to understanding the molecular principles of ligand–nucleic acid interactions that govern the specific recognition of and discrimination between different ligand classes in aptamer complexes.

Aromatic Ligands

In contrast to intercalation of aromatic ligands into double-stranded nucleic acids, which is a relatively unspecific process that is promiscuous with regard to both binding site selection and alterations of the ligand structure, the association of aromatic ligands with their aptamers can be highly specific. Three-dimensional solution structures of aptamer complexes with flat ligands (Fig. 1A) reveal that specificity and increased binding affinity are achieved by a combination of stacking and hydrogen-bonding interactions.

The theophylline-binding RNA aptamer displays an affinity for its cognate ligand 10,000 times that of caffeine, which differs from theophylline by only a single methyl group (Fig. 1, A and B) (*4*). In addition to stacking interactions, which stabilize the theophylline ligand within the aptamer fold and are characterized by interlocking of a base zipper, a 1-3-2 stacking motif, and an S-turn, intermolecular hydrogen bonding contacts contribute to the binding affinity and provide ligand selectivity (*5*). By stacking above a platform of two base-paired nucleotides consecutive within one strand, theophylline is oriented in a coplanar fashion and facing the Watson-Crick edge of an adjacent cytosine base (Fig. 1B). Hydrogen bonding between the cytosine and the purine-like theophylline gives rise to a pseudo–base pair with one partner provided by the aptamer ligand. This pairing alignment would be disrupted by the additional bulky methyl group in the caffeine ligand (Fig. 1A), accounting for discriminatory recognition by the RNA aptamer. A similar ligand-base recognition arrangement is observed at the ligand-binding site of the flavin mononucleotide (FMN)–RNA aptamer complex (*6*), with the flat isoalloxazine moiety of the FMN ligand stacked above a base triple platform (*7*) (Fig. 1C). An adenine coplanar with the ligand recognizes, through hydrogen bonding, polar groups along the edge of the isoalloxazine ring, which penetrates deeply into the binding pocket.

The combination of a non–Watson-Crick base interaction motif as a stacking surface and an adjacent single base providing a docking site for specific hydrogen bonding is a common theme also observed in the ligand-binding pockets of the adenosine monophosphate (AMP)–RNA (*8*) and AMP-DNA (*9*) aptamer complexes. Despite the distinct sequences, secondary structure alignments, and overall tertiary folds of the AMP-RNA (*10, 11*) and AMP-DNA (*12*) aptamers, molecular details of ligand binding attest to strikingly

Table 1. Nucleic acid aptamers for which three-dimensional structures have been determined. ND, not determined.

Ligand	Nucleic acid*	Affinity K_d [μM]	3D structure†
Theophylline	RNA (*4*)	~0.3	NMR, 1EHT (*5*)
FMN	RNA (*6*)	~0.5	NMR, 1FMN (*7*)
AMP	DNA (*9*)	~6	NMR, 1AW4 (*12*)
	RNA (*8*)	~10	NMR, 1AM0, 1RAW (*10*, *11*)
Arginine	2 DNA (*15*)	~125	NMR, 1OLD, 2ARG (*18*, *20*)
	RNA (*16*)	~60	NMR, 1KOC (*19*)
Citrulline	RNA (*16*)	~65	NMR, 1KOD (*19*)
Tobramycin	2 RNA (*25*)	~ 0.009	NMR, 1TOB (*32*)
		~ 0.012	NMR, 2TOB (*33*)
Neomycin B	RNA (*26*)	~ 0.115	NMR, 1NEM (*34*)
HIV-1 Rev peptide	2 RNA (*40*)	~ 0.004	NMR, 1ULL, 484D (*41*, *42*)
HTLV-1 Rex peptide	RNA (*43*)	~ 0.025	NMR, 1C4J (*44*)
MS2 coat protein	3 RNA (*45*)	ND	X-ray, 5-7MSF (*45*, *46*)
Thrombin	DNA (*47*)	~ 0.025	NMR, 148D (*38*); x-ray, 1HAO (*39*)

*The number of different sequences that have been studied is indicated. †The structure determination method (e.g., NMR, nuclear magnetic resonance) and the Protein Data Bank entry for the atomic coordinates are given.

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convergent recognition strategies in both ligand–nucleic acid complexes. In the AMP-DNA aptamer complex, two molecules of AMP are recognized by hydrogen bonding between their Watson-Crick edges and the minor groove edge of guanine bases (*12*) (Fig. 1D). Each AMP·G pseudo–base pair stacks between a reversed Hoogsteen G·G pair and an adenine. Precisely the same molecular motif, namely, a G·G pair as a stacking platform and hydrogen bonding between the AMP ligand and a guanine, determines the ligand-binding site in the AMP-RNA aptamer (Fig. 1E), which, however, associates with only a single ligand molecule (*10, 11*). The distinct hydrogen bonding scheme in the AMP·G pseudo-base accounts for discrimination against the three other nucleotide bases by the AMP-binding aptamers. The AMP·G pseudo–base pair in the RNA-aptamer complex is part of a GNRA-like motif (where N is any nucleotide and R is a purine), an extremely stable structural element of many RNAs (*13*). The participation of the AMP molecule, substituting for an adenosine residue in a common motif of the RNA three-dimensional structure, emphasizes the role of the ligand as an intrinsic part of aptamer architecture.

Amino Acids

The prominent role played by arginine, with its long, flexible side chain carrying a cationic guanidinium group (Fig. 2A), in the nucleic acid-binding motifs of many proteins has rendered this basic amino acid a prime ligand for raising nucleic acid aptamers by in vitro selection experiments (*14–16*). Structural studies of three different arginine-aptamer complexes in solution have revealed differences in the determinants for specific recognition of the arginine side chain by these aptamers as compared with known protein–nucleic acid complexes. The guanidinium group interacts exclusively with the bases in both RNA and DNA aptamer complexes. By contrast, additional contacts between the cationic guanidinium side chain of conserved arginine and phosphate groups of the nucleic acids are found in protein–nucleic acid complexes (*17*).

In both a DNA (*18*) and an RNA (*19*) arginine-binding aptamer (Fig. 2, B and D), the guanidinium group of arginine is aligned coplanar with the Watson-Crick edge of a cytosine base, which forms two hydrogen bonds with the ligand. In another DNA aptamer complex (*20*) (Fig. 2C), the arginine side chain is buttressed between the Hoogsteen face of a coplanar guanine and a tilted cytosine. All three arginine-aptamer complexes show the characteristic embedding of the amino acid side chain within a cluster of bases. The interacting arginine and cytosine are sandwiched by stacking base pairs in one of the DNA complexes (Fig. 2B), whereas stacking and tilted bases enclose the ligand in the second DNA aptamer (Fig. 2C) and the RNA-aptamer (Fig. 2D) complexes. The tight encapsulation of the ligand within base-lined pockets maximizes the specificity of ligand recognition by excluding promiscuous contacts with the phosphate backbone and interactions mediated by solvent molecules.

Similar structural features characterize the ligand-binding site of an RNA aptamer specific for citrulline (*19*) (Fig. 2E), which differs from arginine by a carbonyl oxygen replacing an imino nitrogen atom of the guanidinium group (Fig. 2A). The citrulline-binding RNA has been used as a starting sequence for the in vitro selection of the arginine-binding aptamer (*16*). Some similarity in the overall fold of the two aptamers differing by three nucleotides within the ligand-binding region was, thus, expected. Comparison of the ligand-binding pockets reveals a three-dimensional arrangement of the bases with only minimal, but critical, differences between the two aptamers (Fig. 2, D and E). In both complexes, a cytidine residue is proximal to the amino acid, which is further enclosed by a stacking non–Watson-Crick G·G pair and two perpendicular bases (*19*). The precise discrimination between the two amino acids by the aptamers originates from the distinct shapes and orientation of polar functional groups between the ligand-binding pockets. In the citrulline aptamer, the terminal urea group of the ligand is forced to rotate by 90° as compared with the guanidinium group of arginine. As a consequence, it was proposed that citrulline forms hydrogen bonds with the tilted guanine and packs against the cytosine (Fig. 2E), whereas the role of these two bases is reversed in the arginine aptamer (Fig. 2D).

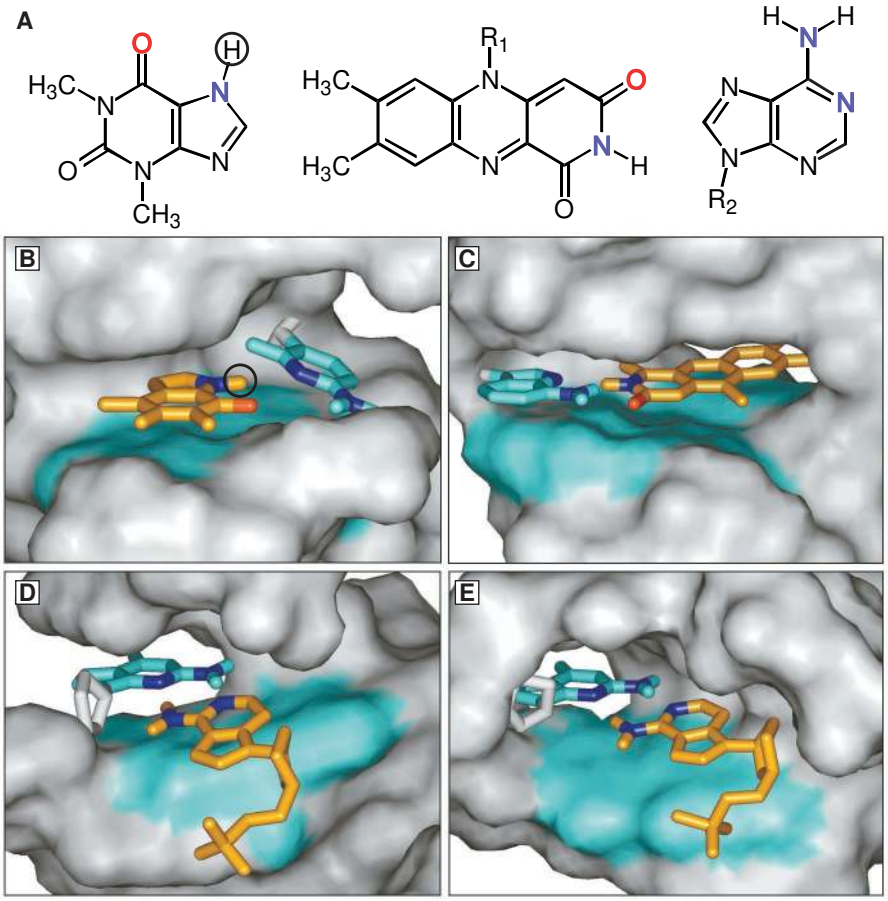

Fig. 1. Molecular recognition of flat aromatic ligands by nucleic acid aptamers. (**A**) (left to right) Theophylline (in caffeine, the encircled hydrogen is replaced by a methyl group); flavin mononucleotide (FMN), an isoalloxazine derivative; and adenosine monophosphate (AMP). The ligand-binding pockets are shown for the complexes of (**B**) a theophylline-RNA aptamer (*5*), (**C**) an FMN-RNA aptamer (*7*), (**D**) an AMP-DNA aptamer (*12*), and (**E**) an AMP-RNA aptamer (*10*). In all four complexes, selective ligand binding involves a planar surface (cyan) above which the ligand (orange) stacks coplanar with an adjacent base (cyan sticks), which forms specific intermolecular hydrogen bonds. The stacking surface is constituted by pairs or triples of coplanar bases interacting in non–Watson-Crick arrangements. Polar nitrogen (blue) and oxygen (red) atoms participating in hydrogen bonds are marked.

Oligosaccharides

The aminoglycoside antibiotics, oligosaccharide molecules carrying several positively charged ammonium groups, exert their bio-

logical activity by specific binding to the ribosomal RNA of bacteria (*21*). Some catalytic RNAs, such as the hammerhead ribozyme (*22*), hepatitis delta virus (HDV) ribozymes (*23*), and self-splicing group I introns (*24*), are also inhibited by aminoglycosides. The capacity of aminoglycosides for specific binding to certain RNAs has been exploited to obtain high-affinity aptamer molecules selective for different aminoglycosides (*25–27*). Aminoglycoside aptamers have been shown to be functional in vivo, controlling gene expression as drug-inducible translational switches in the 5′ untranslated regions of eucaryotic messenger RNAs (*28*). The hallmarks of molecular recognition between aminoglycosides and nucleic acids (*29*) have been revealed by the three-dimensional solution structures of ribosomal 16*S* A-site RNA constructs bound to paromomycin (*30*) and gentamicin (*31*), and of RNA aptamers (*25, 26*) in complex with tobramycin (*32, 33*) (Fig. 3A) and neomycin B (*34*).

Despite the differences in the sequences and secondary structures of the aminoglycoside aptamer RNAs, many key features of the ligand-RNA interaction are conserved. The hydrophobic face of the alicyclic ring in both tobramycin and neomycin packs against the floor of the deep (i.e., major) groove, aligned by non–Watson-Crick pairs and flanked by a single-stranded loop, which folds over the ligand in all three complexes. To allow accommodation of the ligand, the deep groove is widened by either a bulged nucleotide (*32*) or non–Watson-Crick base pairs (*33, 34*) on complex formation. The RNAs tightly encapsulate the alicyclic ring and one amino sugar (Fig. 3B), in part by a single bulged base, which acts as a flap closing the groove. The remaining amino sugar, closest to the attachment site on the solid support during the in vitro selection procedure, is directed outward into the solvent.

Shape complementarity between the aminoglycosides and the RNA folds and distinct hydrogen bonds involving ammonium groups of the antibiotics explain, in part, the high specificity by which RNA aptamers exclusively recognize their cognate ligands. Other factors that enhance binding specificity and affinity include structural electrostatic complementarity (*35*) between the negatively charged RNA and the cationic ligands. The RNA binding pocket is lined by negative charges creating a binding surface that is complementary to the three-dimensional arrangement of positively charged ammonium groups in the oligosaccharide scaffold of the aminoglycosides (Fig. 3C). Thus, the potential disruption of a key interaction involving a cationic ammonium group permits a tobramycin-binding RNA aptamer to discriminate against gentamicin (*33*). Structural electrostatic complementarity between cationic antibiotics and negatively charged pockets in RNA folds, frequently occupied by metal ions, has also been discovered for natural RNA molecules (*35, 36*).

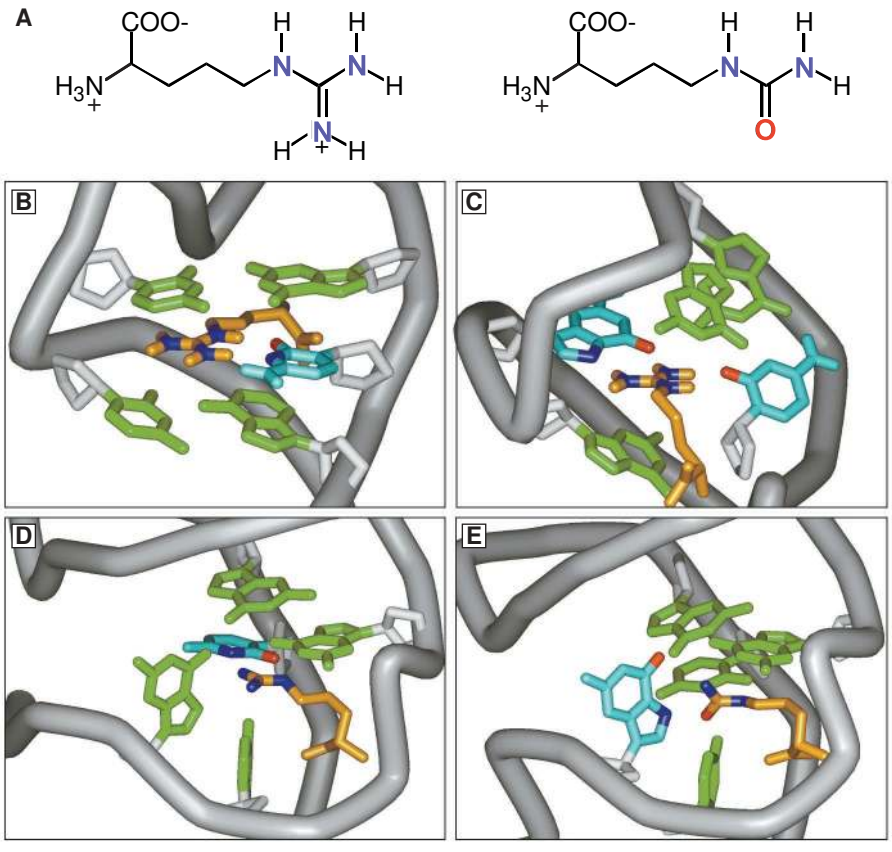

Fig. 2. Molecular recognition of the basic amino acids (**A**) arginine (left) and citrulline (right) by nucleic acid aptamers. The ligand-binding pockets are shown for arginine in complex with two different DNA aptamers (**B** and **C**) (*18, 20*) and RNA-aptamer complexes (*19*) of arginine (**D**), and citrulline (**E**). In all four complexes, the positively charged amino acid side chain (orange) penetrates deeply into the nucleic acid fold where intermolecular hydrogen bonds are formed exclusively with bases (cyan). The ligand-binding pockets are lined by clusters of bases (green) excluding both the negatively charged phosphate backbone and solvent water. Polar nitrogen (blue) and oxygen (red) atoms participating in hydrogen bonds are marked.

Peptides and Proteins

The structural data on RNA-aptamer complexes of peptides and proteins provide valuable insights into highly specific molecular recognition processes important for viral and cellular RNAs, which usually work in concert with protein partners. Three-dimensional structures have been solved for a DNA aptamer (*37*) both free in solution (*38*) and bound to human thrombin (*39*), two different RNA aptamers (*40*) bound to a 17-residue peptide derived from human immunodeficiency virus type 1 (HIV-1) Rev protein (*41, 42*) (Fig. 4A), an RNA aptamer (*43*) bound to a 16-nucleotide oligomer peptide from human T cell leukemia virus (HTLV-1) Rex protein (*44*), and three sequence-related RNA aptamers in complex with the 14-kD bacteriophage MS2 coat protein (*45, 46*). The thrombin DNA aptamer is unusual in that it already adopts a defined quadruplex structure in solution in the absence of the ligand (*38*). The crystal structure of the DNA-thrombin complex, however, did not reveal conclusive details about specificity of ligand discrimination (*47*).

Comparison of the RNA complexes of the Rev and Rex peptides versus the MS2 coat protein bound to their cognate RNA aptamers reveals striking differences in the molecular adaptation processes upon complex formation. Whereas the structure of the MS2 coat protein is unaffected by the binding of aptamers (*45, 46*), the conformation of the bound Rev peptide is dictated by the RNA architecture (*42*). In the complexes with stem-loop II B derived from the HIV Rev-response element (RRE) (*48*) and a related aptamer (designated family I Rev-binding aptamer) (*41*) (Fig. 4B), the Rev peptide, which is predominantly unstructured in solution (*49*), binds in an α-helical conformation to the RNAs. The same peptide adopts an extended conformation in complex with a different aptamer RNA (designated family II Rev-binding

aptamer) (*42*) (Fig. 4C). The peptide inserts into the RNA deep groove, widened through adaptive formation of non–Watson-Crick purine·purine pairs and a U·A:U base triple, in both aptamer complexes. In addition, the complexes are stabilized by nonspecific intermolecular contacts between the arginine guanidinium groups of the peptide and the phosphate groups of the RNAs. Specific hydrogen bonds between the deep groove edges of guanines and guanidinium groups on pairs of arginine residues mediate the precise recognition of the Rev peptide in both RNA-aptamer complexes. Additional motifs for ligand discrimination involve a non–Watson-Crick purine·purine base pair interacting with an asparagine side chain in the RRE (*48*) and the family I aptamer (*41*) complexes, and stacking of a tryptophan moiety on a pyrimidine base of the family II RNA aptamer complex (*42*). In many protein-RNA complexes, non–Watson-Crick base pairs and triples also play key roles for protein recognition (*50*), namely by distorting the RNA deep groove for ligand docking and by providing unique sets of hydrogen bonding sites.

The available structural data on RNA binding peptides has established that widened RNA deep grooves can accommodate minimal elements of protein secondary structure (*51*), such as isolated α helices (*41, 48, 52*), β sheets (*53*), and extended conformations (*42, 44*). By contrast, DNA binding proteins that target the major groove require two or more secondary structure elements to form stable nucleic acid complexes. This difference may reflect the increased depth of the RNA "major" groove and its distinct irregular architecture associated with bulges, non–Watson-Crick base pairs, and triple and junctional alignments.

Distinct from the conformational adaptability of the Rev peptide, the MS2 coat protein retains the three-dimensional fold observed for the free protein (*54*) on binding to either natural viral RNA (*55*) or RNA aptamers, which target the "native" binding site (*45, 46*) (Fig. 4D). Indeed, it is the RNAs that change their conformation on complex formation with the MS2 coat protein. Both the natural RNA and the aptamers contain a critical unpaired adenine residue, which stacks between the flanking helices in the free RNAs (*56*) but is looped out in the protein-bound complexes (*45, 46, 55*). This bulged adenine is one of three unpaired bases that mediate the specific recognition of the RNAs by the MS2 coat protein. Two looped-out adenines form intermolecular hydrogen bonds within hydrophobic pockets on the protein surface, along with an unpaired cytosine, which stacks precisely on a tyrosine side chain (Fig. 4E). These intermolecular interactions with the protein stabilize the looped-out conformation of the unpaired bases and, in the case of the critical adenine, drive the rearrangement, which leads to unstacking of the base.

Characteristics of Ligand-Binding Pockets in Aptamers

Three-dimensional structural analyses have provided insights into key questions concerning molecular recognition by nucleic acid–aptamer complexes, namely, what is the structural basis of highly specific ligand discrimination by aptamers, and what are the differences between ligand-binding sites in aptamers versus either natural nucleic acids or proteins?

The enclosure of large parts of the ligand by the nucleic acid is the basis for specific recognition of the cognate ligand in aptamer complexes. The folding of the nucleic acid around the ligand provides numerous discriminatory intermolecular contacts, which have been outlined in the sections above. Depending on the ligands, discrimination is based on different effects. Steric hindrance due to a methyl group prevents binding of caffeine to the theophylline aptamer (*5*). A specific hydrogen bonding scheme, which is required for the formation of a pseudo–base pair involving the ligand, is responsible for the selection of adenine as a ligand in the AMP aptamers (*10–12*). Hydrogen bonding is also the key for the discrimination in the aptamers for arginine and citrulline (*19*). Aminoglycoside ligands are recognized by their aptamers through a combination of electrostatic and shape complementarity along with distinct hydrogen bonds involving polar groups in the antibiotics (*32–34*). In the peptide and protein aptamer complexes, the ligands are structurally more complex, and so is the interplay of the various discriminatory contacts, including stacking, shape complementarity, electrostatic interactions, and hydrogen bonding (*41, 42, 44–46*).

The distinctions in ligand recognition between aptamers and proteins (*57*) are obviously rooted in the different nature of the building blocks of these macromolecular architectures. In proteins, the diversity of the 20 amino acids allows for a multitude of interactions and precise shape complementarity in open substrate-binding sites. In nucleic acids, including aptamers, the structurally more uniform four nucleotides are limited in possible alternative ways to pack around arbitrary ligands. Therefore, the fit of ligands into binding sites in aptamer folds displays a less-than-perfect shape complementarity, which can be compensated for through deep encapsulation of the ligand. The planarity of the nucleotide bases favors stacking interactions in aptamer complexes, whereas intermolecular hydrogen bonds and general acid-base interactions are preferred for substrate-binding by proteins. Stacking plays a pivotal role in most of the aptamer complexes, not only in the cases of overall flat ligands such as FMN, theophylline, and AMP, but also for peptides, which participate in interdigitative stacking involving the planar guanidinium groups of arginine side chains (*41, 42, 44*). Albeit to a lesser extent, intermolecular hydrogen bonds also contribute to ligand binding in aptamer complexes. Thus, in their ligand-binding pockets, aptamers and proteins share in common the network character of multiple interactions.

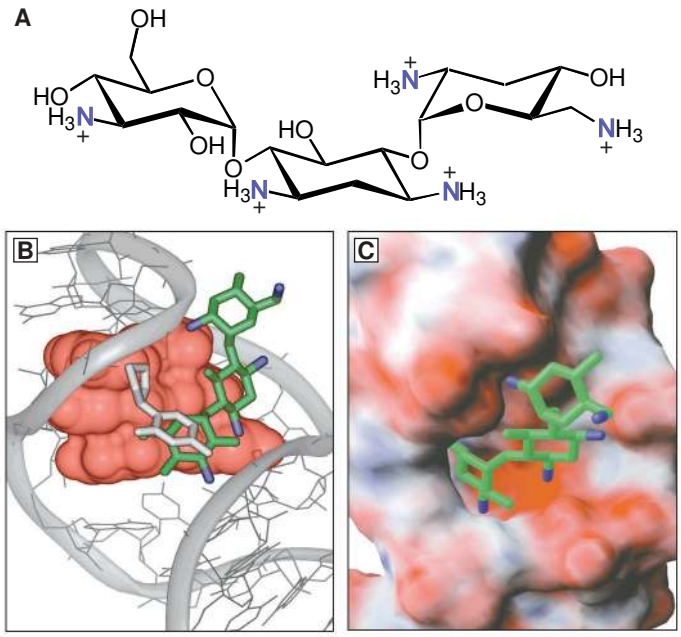

Fig. 3. Molecular recognition of the aminoglycoside antibiotic tobramycin (**A**) by an RNA aptamer. (**B**) In the aptamer complex (*33*), the RNA encapsulates the tobramycin ligand (green), which packs against the base edges (red) within the deep groove. A base flap (gray sticks) closes the groove above the bound drug. (**C**) The ligand-binding pocket provides a negatively charged environment displaying shape complementarity between electronegative sites (red) in the cavity and the positions of the cationic ammonium groups (blue) in the aminoglycoside. The RNA surface is colored according to the electrostatic potential, with red indicating negative charge and blue indicating positive charge (*73*).

A comparison of ligand-binding sites in artificial aptamers and natural nucleic acids reveals unique structural features attesting the different characteristics of evolutionary pressure acting on these two families of macromolecules. The functions of natural nucleic acids as parts of an intricate network of biological processes require a biased cooptimization of different structural motifs, among them ligand-binding sites. In contrast, the single function of aptamers is the binding of a given ligand. As a consequence, aptamers have higher affinities (Table 1) for their cognate ligands, as compared with ligand-binding sites in natural nucleic acids. The molecular basis of the high binding affinities of aptamers is associated with an intricate encapsulation of the ligand, which becomes part of the nucleic acid architecture. Interlocking of RNA and ligand structures is impressively demonstrated by the AMP-RNA aptamer, in which the adenine ligand participates in a conserved G·A base pair as a structural element of a GNRA tetraloop (see above) (*10, 11*). Similarly, the theophylline-binding pocket comprises three stacked base triples, one of which involves theophylline as a hydrogen bonding partner (*5*). Aptamers often comprise unpaired loop regions, which are disordered in the free nucleic acid and acquire a defined conformation by adaptive folding around the ligand. In some aptamer complexes, such as the aminoglycoside-binding RNAs (*32–34*) and peptide-binding RNAs (*42, 44*), single unpaired bases are conformationally immobilized as flaps over the ligand-binding sites. In summary, the differences in ligand binding between aptamer and natural nucleic acids boil down to the most decisive distinction concerning the conformational changes occurring in the association process. In recognition processes involving both natural RNA targets and substrates, it is the ligands that have evolved to adapt to the nucleic acid architectures. By contrast, aptamers bind their ligands by adaptive recognition, which involves different conformational ordering processes as outlined above.

Perspectives

Structural data on small molecule–RNA complexes will be especially helpful for the rational exploration of RNA as a drug target. The key roles that RNAs play in all steps of gene expression, transport, catalysis, and other cellular processes render them prime targets for therapeutic intervention (*58*). Drug design approaches for cellular RNA targets that combine structural data on RNA complexes with modeling techniques are especially promising, given the extraordinary success of molecular modeling of higher-order RNA architectures (*59*).

Beyond the answers they contribute to questions of three-dimensional structure, nucleic acid aptamers will provide unique tools in medicinal diagnosis and biotechnology (*60*) and might even serve as potential therapeutics. As biosensors, aptamers have been identified that recognize specific surface components of pathogens, such as anthrax spores (*61*) and African trypanosomes (*62*). Aptamers raised against specific targets including cellular proteins can be linked to fluorescent labels and used as superior and inexpensive substitutes for antibodies (*63*). Many applications of antibodies can be realized using aptamers, which display even higher ligand affinities. The approach to raise antibodies against transition states of chemical reactions in order to obtain specific catalysts (*64*) has been used to obtain aptamers that promote the isomerization of biphenyls (*65*) and accelerate the reaction rate of Diels-Alder cycloadditions (*66*).

In engineered nucleic acid constructs, aptamers can serve as molecular switches based on the conformational ordering they undergo upon ligand binding (*67, 68*). In combination with catalytic nucleic acid motifs, aptamer switches allow the construction of allosteric ribozymes and DNAzymes, which can be regulated by small molecule cofactors. For example, an AMP-activated nucleic acid ligase has been selected from a sequence library obtained from joining an AMP aptamer domain to a random sequence (*69*). Such nucleic acid constructs, termed "aptazymes" (*68*), may be useful as extremely sensitive molecular sensors, with the aptamer domain recognizing the presence of a ligand and the catalytic domain amplifying the signal.

Although nucleic acid aptamers are obtained by in vitro selection, they can retain high affinity and specificity for their cognate ligands when expressed in living cells. Their in vivo stability can be enhanced through modifications of the sugar phosphate backbone (*70*) or through use of mirror-image analogs (*71*). RNA aptamers have been used in vivo as protein-targeted inhibitors, which bind to a cellular protein thereby interfering with the function of the target (*72*). Insertion of aptamers into the 5′ untranslated region of messenger RNAs provides a handle to control the expression of specific genes in living cells (*28*). Translation of such aptamer-mRNA constructs can be regulated by the reversible ligand-dependent conformational change of the aptamer domain.

Since the early studies on the feasibility of in vitro selection for obtaining nucleic acid molecules with high affinity for a given li-

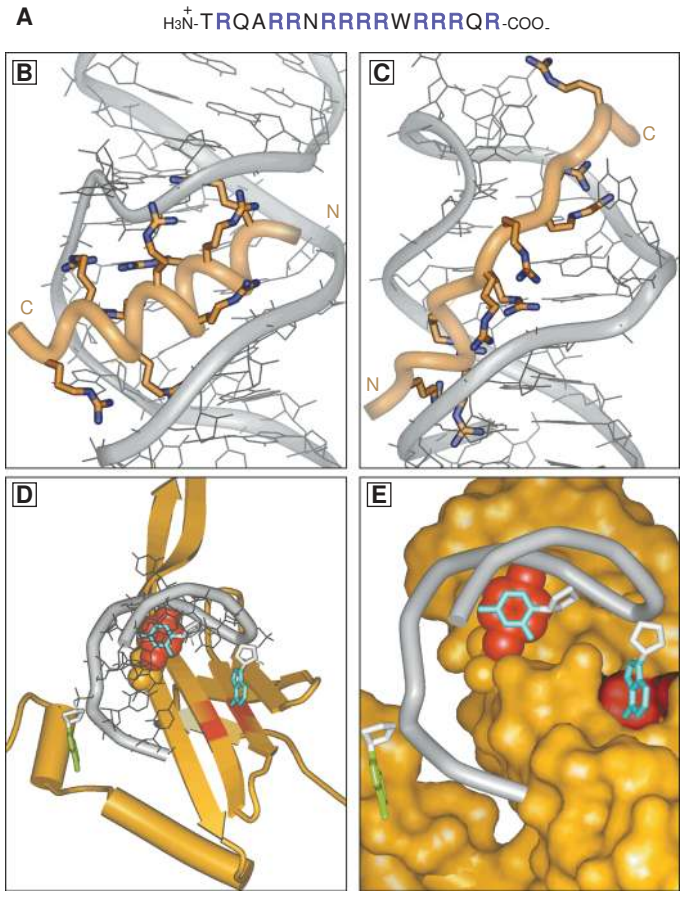

Fig. 4. Molecular recognition of peptides and proteins by RNA aptamers. The bound conformation of an arginine-rich peptide (**A**) from the human immunodeficiency virus (HIV-1) Rev protein is dictated by the nature of the RNA aptamer. (**B**) In one type of Rev aptamers, the bound peptide (orange) folds into an α-helical conformation within the widened deep groove of the RNA (*41*). (**C**) In a different aptamer, the peptide binds also within the widened deep groove, yet in an extended conformation (*42*). (**D** and **E**) An RNA aptamer (gray) recognizing the bacteriophage MS2 coat protein (orange) binds to the surface of antiparallel β sheets (*45*). Specific recognition of the protein by the RNA aptamer is provided by looped-out bases (cyan and green), which are inserted into cavities and involved in stacking interactions (red).

gand (*1*), aptamers and their complexes with ligands have proved extremely useful for the understanding of molecular evolution and intermolecular recognition. Their first successful applications as molecular sensors and switches suggest that aptamers will be similarly useful as molecular tools.

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