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Modulation of RNA function by aminoglycoside antibiotics

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One of the most important families of antibiotics are the aminoglycosides, including drugs such as neomycin B, paromomycin, gentamicin and streptomycin. With the discovery of the catalytic potential of RNA, these antibiotics became very popular due to their RNA-binding capacity. They serve for the analysis of RNA function as well as for the study of RNA as a potential therapeutic target. Improvements in RNA structure determination recently provided first insights into the decoding site of the ribosome at high resolution and how aminoglycosides might induce misreading of the genetic code. In addition to inhibiting prokaryotic translation, aminoglycosides inhibit several catalytic RNAs such as self-splicing group I introns, RNase P and small ribozymes *in vitro*. Furthermore, these antibiotics interfere with human immunodeficiency virus (HIV) replication by disrupting essential RNA–protein contacts. Most exciting is the potential of many RNA-binding antibiotics to stimulate RNA activities, conceiving small-molecule partners for the hypothesis of an ancient RNA world. SELEX (systematic evolution of ligands by exponential enrichment) has been used in this evolutionary game leading to small synthetic RNAs, whose NMR structures gave valuable information on how aminoglycosides interact with RNA, which could possibly be used in applied science.

Keywords: aminoglycosides/aptamers/decoding/NMR structure/ribozymes

Introduction

The golden age of antibiotic research was the 1960s, when they were discovered and their mode of action deciphered. Due to their miraculous therapeutic effects, it was generally believed that the problem of bacterial infection was solved. Now, 40 years later, antibiotic discovery is back on the agenda, because most of the successful antibiotics are losing their potential since bacteria have acquired resistance genes (Davies, 1994). The emergence and dissemination of antibiotic resistance in pathogens is a major problem for human health and requires the identification of new antibiotics. To design novel and more effective antibiotics, against which pathogens have not yet established resistance factors, a detailed functional and structural understanding of the interactions between the drug and its target site is essential.

One group of antibiotics is especially difficult to study, namely the inhibitors of prokaryotic translation. Their target site is, in many cases, rRNA. The complexity of the ribosome makes high resolution structure determination extremely challenging, and the role of rRNA is still not well understood. In addition to inhibiting the ribosome, many of the translation inhibitors also target other RNAs, mainly ribozymes and two functional domains in human immunodeficiency virus (HIV) RNA (reviewed in Schroeder and von Ahsen, 1996; Wallis and Schroeder, 1997). In the past few years, the information obtained on the structures and functions of RNA has increased rapidly, and a more detailed understanding of the mode of action of RNA-binding antibiotics has been gained.

Aminoglycoside antibiotics are multiply charged compounds of high flexibility. The positive charges are attracted to the negatively charged RNA backbone. The flexibility of the aminoglycosides facilitates accommodation into a binding pocket within internal loops of RNA helices or into ribozyme cores for making specific contacts. The majority of these antibiotics are composed of amino sugars linked to a 2-deoxystreptamine ring (Figure 1; ring II). The conserved elements among aminoglycosides are rings I and II and, within ring II, the amino groups at positions 1 and 3. These elements are essential for binding to the decoding site of the 16S rRNA. The 2-deoxystreptamine ring is substituted, most commonly, at positions 4 and 5, as in the neomycin class, or at positions 4 and 6, as in the kanamycin and gentamicin classes (Figure 1).

The peptide antibiotic viomycin, a member of the tuberactinomycin family widely used against tuberculosis, behaves in many respects like the aminoglycosides: it binds to the decoding site of 16S rRNA, thereby inducing miscoding, and inhibits several ribozymes. Therefore, we include viomycin in this review. This compound is a cyclic peptide containing non-coded amino acids in addition to the amino acids arginine, lysine and serine, which often occur in RNA-binding domains of proteins (Figure 1).

Aminoglycoside antibiotics cause misreading of the genetic code

Translation of mRNAs into proteins requires decoding of the genetic information, a complex process taking place at ribosomes. The evidence that RNA plays the key role in this decoding process is overwhelming (Green and Noller, 1997). The fidelity of aminoacyl-tRNA selection involves an initial selection and proofreading by the ribosome (Pape *et al*., 1999). Antibiotics, which disturb the decoding process and induce misreading of the genetic code, interact with distinct sites in the 16S rRNA (Moazed and Noller, 1987; Woodcock *et al*., 1991). During decoding, the anticodon triplet pairs with its cognate codon on

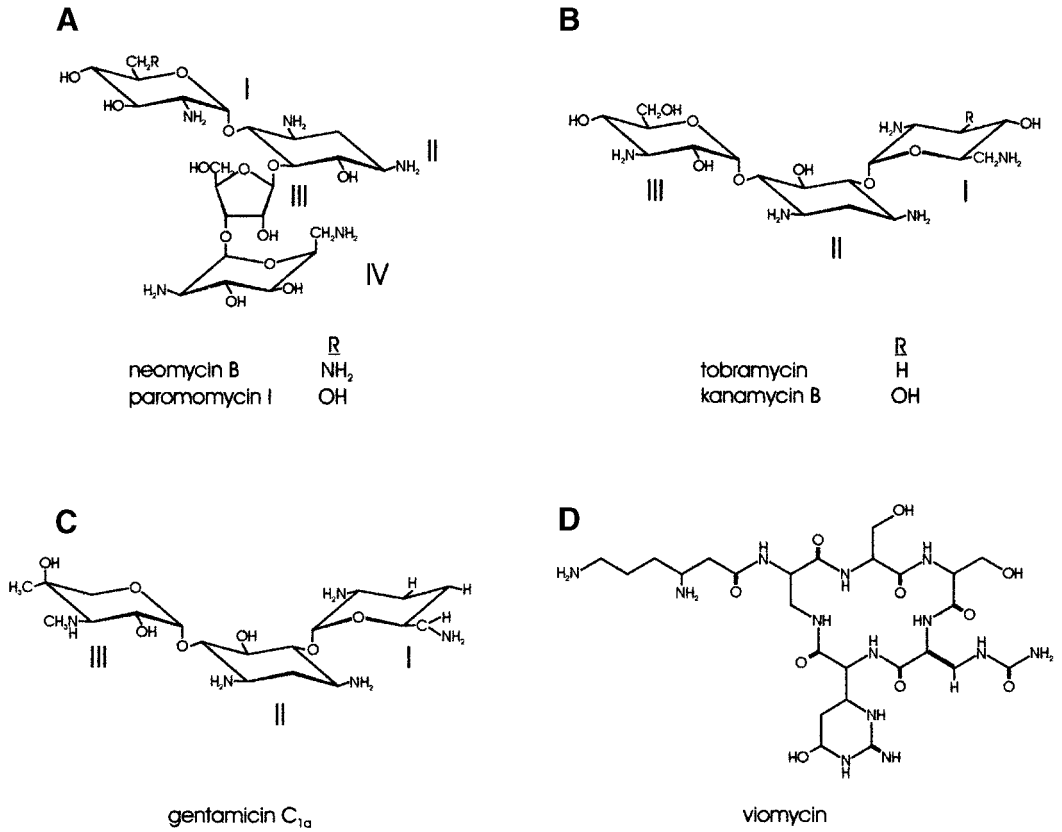

Fig. 1. Antibiotics that interfere with the decoding process and with ribozyme catalysis. (**A**) Neomycin-class antibiotics with a 2-deoxystreptamine (ring II), disubstituted at positions 4 (ring I) and 5 (rings III and IV). (**B**) Kanamycin-class antibiotics and (**C**) gentamicin C_{1a} with substitutions at positions 4 (ring I) and 6 (ring III). (**D**) The peptide antibiotic viomycin.

the mRNA and protects bases on the 16S rRNA from chemical modification in the same region as the aminoglycosides (Moazed and Noller, 1986). The decoding site is composed of nucleotides 1400–1410 and 1490–1500, which form a stem–loop–stem structure also known as the A-site of the *Escherichia coli* 16S rRNA (Figure 2D). Decoding is achieved by the interaction of the A-site with the backbone of the codon–anticodon helix, thereby protecting positions N1 of A1492 and A1493 from chemical modification (Moazed and Noller, 1986). Mutating these positions to G is deleterious to tRNA binding, but can be compensated by 2′ fluorine substitutions in the mRNA codon (Yoshizawa *et al*., 1999).

The size of the ribosome seemed an insurmountable hurdle to obtaining a high resolution structure of the decoding site bound with aminoglycoside antibiotics. Recent X-ray structures of the 30S and 70S complexes of *Thermus thermophilus* ribosomes give a three-dimensional picture, visualizing the decoding site in the ribosome architecture (Cate *et al*., 1999; Clemons *et al*., 1999), although the resolution of these structures is not high enough to see the contacts between an antibiotic and the decoding site. An alternative approach was the determination of the solution structure of a subdomain of the *E.coli* 16S rRNA complexed with aminoglycoside antibiotics using nuclear magnetic resonance (NMR). Large RNAs are often composed of small modules that have the ability to fold into autonomous domains enabling the dissection into small RNAs, as demonstrated by Purohit and Stern for the decoding site of 16S rRNA (Murphy and Cech, 1993; Purohit and Stern, 1994; Schroeder, 1994). The determination of the NMR structure of a model A-site complexed with the aminoglycoside antibiotics paromomycin, neomycin B and gentamicin was therefore possible (Fourmy *et al*., 1996, 1998a; Yoshizawa *et al*., 1998).

Binding of aminoglycosides to a 27mer A-site RNA containing their target site was found to result in the same footprint pattern as in the ribosome context (Recht *et al*., 1996). The NMR solution structures of this RNA alone or complexed either with paromomycin or with gentamicin are shown in Figure 2A–C. Figure 2D shows the secondary structure of the A-site RNA, with the bases that are protected from chemical modification by neomycin-class antibiotics indicated by triangles. The asymmetric internal loop, which is the antibiotic-binding site (shown in blue in Figure 2A), is closed by the Watson–Crick base pair C1407–G1494 and by the non-canonical base pairs U1406–U1495 and A1408–A1493. The upper and lower stems are thus connected by a continuous but disturbed helix, which displays a widened major groove facilitating docking of the antibiotics. The antibiotic-binding pocket is created by the base pair A1408–A1493 and by the single bulged adenine A1492. Rings I and II of the antibiotics establish specific contacts with the RNA. In the complexes with paromomycin (Figure 2B) and gentamicin (Figure 2C), ring I is stacked upon G1491 and ring II spans the base pairs U1406–U1495 and C1407–G1494. Rings III of these two antibiotics are positioned differently. Binding of the antibiotics to the RNA induces a conformational change in the RNA, displacing the two universally

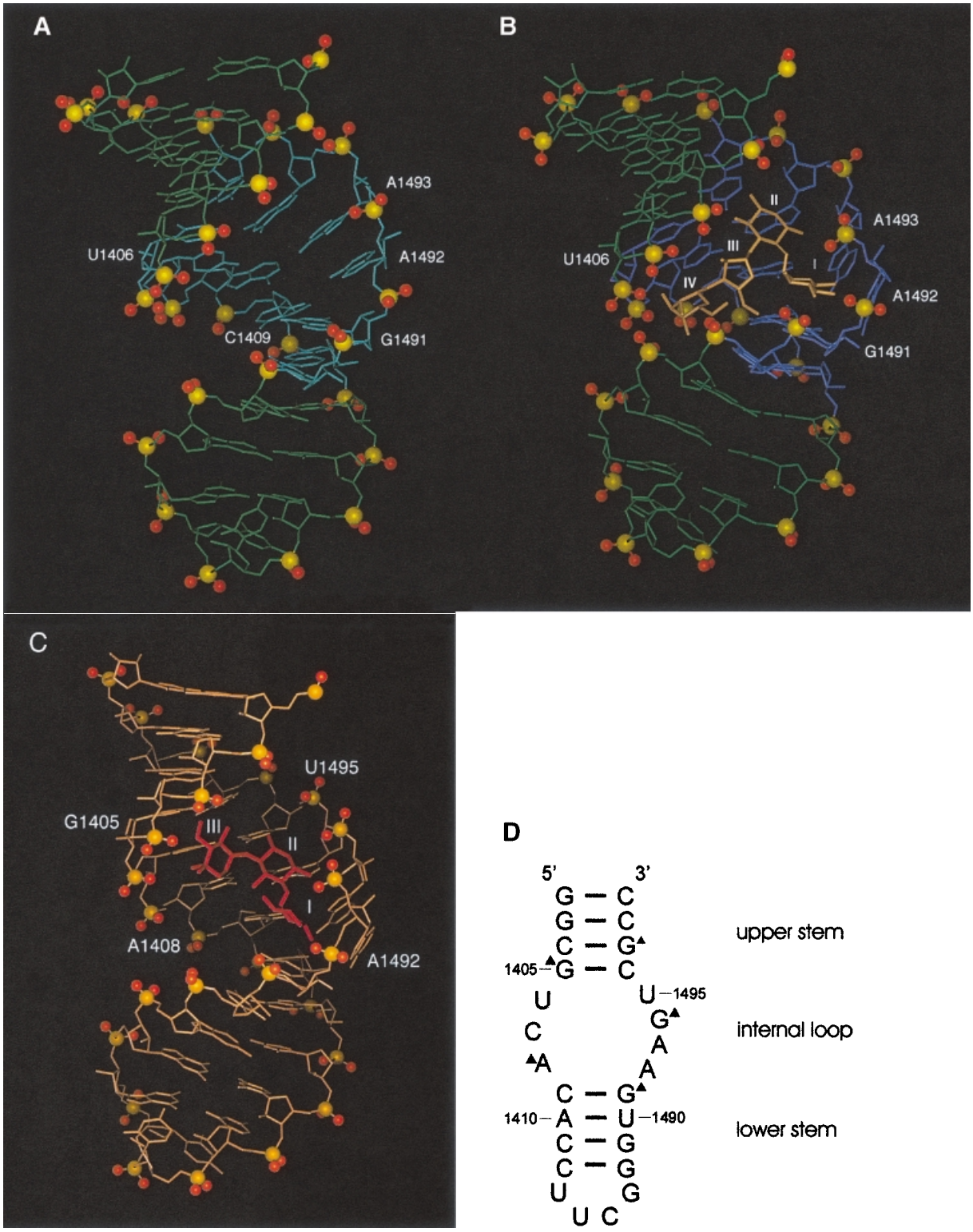

Fig. 2. Single representative NMR solution structures of the decoding site of the 16S rRNA from *E.coli*. All heavy atoms are highlighted and the core (nucleotides U1406–A1410 and U1490–U1495) of the aminoglycoside-binding site is always shown in a specific colour. (**A**) The free A-site RNA (Fourmy *et al*., 1998a). (**B**) The A-site RNA complexed with paromomycin (in gold, Fourmy *et al*., 1996, 1998b). (**C**) Gentamicin C_{1a} (in red) bound to the A-site RNA (Yoshizawa *et al*., 1998). (A–C) were kindly provided by Dominique Fourmy and Joseph D.Puglisi. (**D**) Secondary structure of the A-site RNA oligonucleotide showing the DMS footprints observed in the presence of aminoglycosides and marked with ▲.

conserved residues A1492 and A1493 towards the minor groove, thereby probably switching the A-site into a high affinity state for mRNA–tRNA recognition and reducing the rejection rate of near-cognate tRNAs (Karimi and Ehrenberg, 1994; Fourmy *et al*., 1998b). The two conserved adenines seem to be pre-positioned by the antibiotics to contact the backbone of the codon–anticodon complex. This increased affinity of the A-site for tRNAs

in the presence of antibiotics might result in misreading of the genetic code.

The NMR structures of this A-site RNA provided a first structural rationale for the functioning of the decoding site. Puglisi and co-authors suggest that the decoding site is an irregular helix that binds antibiotics via its major groove and might contact the codon–anticodon complex via its minor groove (Fourmy *et al*., 1996). The first evidence that the backbone of the codon–anticodon complex is contacted via the 2′ hydroxyl groups of the codon was the indispensability of 2′ hydroxyls in the mRNA for tRNA binding (Potapov *et al*., 1995). This contact was demonstrated elegantly by the rescue of mutations in the A-site by 2′ modification of the codon, suggesting a minor groove–minor groove interaction between the decoding site and the codon–anticodon duplex (Yoshizawa *et al*., 1999). A well characterized minor groove–minor groove contact between two RNA helices exists in group I self-splicing introns (Strobel and Cech, 1995; Strobel *et al*., 1998).

Neomycin B inhibits ribozymes by displacement of essential metal ions

In addition to interfering with the translation of the genetic code, aminoglycoside antibiotics inhibit the activity of several ribozymes *in vitro*. Group I intron self-splicing (von Ahsen *et al*., 1991), self-cleavage of the hammerhead (Stage *et al*., 1995) and the human hepatitis delta virus (HDV) ribozymes (Rogers *et al*., 1996; Chia *et al*., 1997), the magnesium-induced self-cleavage reaction of the hairpin ribozyme (Earnshaw and Gait, 1998) and the tRNA processing activity of RNase P RNA (Mikkelsen *et al*., 1999) are all inhibited by the same aminoglycosides, most prominently by neomycin B and tobramycin. Concerning the inhibition of the hammerhead ribozyme, it was first reported that neomycin B competes with at least one magnesium ion and that the inhibitory efficiency is pH dependent, indicating that the protonated amino groups of the antibiotic are essential for its inhibitory activity (Clouet-d'Orval *et al*., 1995). The existence of the three-dimensional structure of the hammerhead ribozyme (Scott *et al*., 1996) facilitated molecular dynamic simulations with aminoglycoside–hammerhead ribozyme complexes. These simulations revealed an intriguing complementarity between the positively charged amino groups of the antibiotics and the magnesium ion-binding sites in the ribozyme core, suggesting that a single bound neomycin B molecule displaces several essential magnesium ions from the catalytic core (Hermann and Westhof, 1998a).

A structure–function study showed that, similarly to the interference with the hammerhead cleavage reaction, the amino groups of neomycin B are important for the inhibition of group I *td* intron splicing. For example, paromomycin, which differs from neomycin B only by the change of one amino group to a hydroxyl group, is 100-fold less efficient in inhibiting splicing. The same effect is true for several other almost identical antibiotics (von Ahsen *et al*., 1992). Structural probing of the *sunY* group I intron in the presence of neomycin B suggested that more than one antibiotic molecule is bound to the RNA (von Ahsen and Noller, 1993). Since the molecular dynamic simulations had suggested displacement of magnesium ions for the hammerhead ribozyme, we probed for metal ion displacement by neomycin B in the group I intron. Therefore, a recently developed method using Fe^{2+}-generated hydroxyl radicals to probe for metal ions within RNA molecules was applied (Berens *et al*., 1998). A prominent protection from backbone cleavage was observed in the presence of neomycin B at the bulged nucleotide of the P7 stem in the core of the *td* intron. Mutation at this position resulted in resistance to neomycin B, revealing an essential contact site between the antibiotic and the intron RNA (Hoch *et al*., 1998). These observations allowed docking of the neomycin B molecule into the core of the intron. Since no high resolution structure with divalent metal ions exists for the core of group I introns, docking was performed on structural models that are based on experimental data (Michel and Westhof, 1990; Lehnert *et al*., 1996). Brownian dynamics simulations were used to identify sites in the RNA folds that are potential metal ion-binding sites (Hermann and Westhof, 1998b). Figure 3 shows the three-dimensional model of the *td* intron with highlighted charged regions and two proposed metal ions (Streicher *et al*., 1996; T.Hermann and E.Westhof, personal communication). Neomycin B, in yellow, is docked into the ribozyme core, demonstrating the overlap between the amino groups of neomycin B and the proposed metal ions (in green).

Two types of aminoglycoside-binding sites

When aminoglycoside antibiotics were found to inhibit self-splicing introns, we searched for features common in group I introns and in rRNA in order to explain their common inhibitors (Schroeder *et al*., 1993). A functional similarity was observed between the decoding process and the binding of the splice site to the intron core. Both processes involve docking of an RNA helix into a conserved internal asymmetric RNA loop. After detailed analyses of both processes, it became evident that the inhibitory mechanisms of aminoglycosides on decoding and self-splicing are significantly different. While the antibiotic-binding site in the decoding site consists of a helical domain with a disturbed widened major groove, the binding site of aminoglycosides in ribozymes is complex metal ion-containing pockets surrounded by more than two RNA strands. Therefore, we propose that there are two types of aminoglycoside-binding sites: the first consists of asymmetric internal loops as in the decoding site (Fourmy *et al*., 1996), in the RRE and Tar domains of HIV (Zapp *et al*., 1993; Mei *et al*., 1995) and in several *in vitro* selected small RNAs (Jiang and Patel, 1998). The second type of binding site for aminoglycosides are the central metal ion-binding pockets in the catalytic cores of ribozymes (Hermann and Westhof, 1998a). While in the type I binding site, the mode of action is probably through a slight distortion of the RNA structure and interference with the binding of the functional substrate, in the type II binding sites the aminoglycosides act through displacement of essential divalent metal ions. So far, there is no evidence that magnesium ions are involved in the decoding process, but this cannot be excluded.

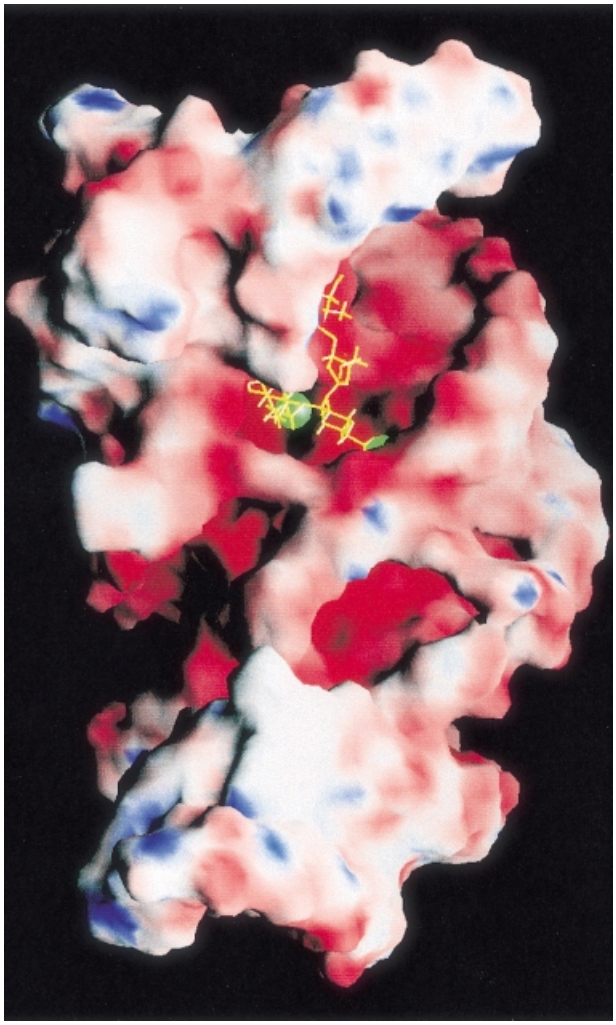

Fig. 3. The electrostatic field around the three-dimensional fold of the *td* group I intron was formed by spatial alignment of charged atoms (Hermann and Westhof, 1998b). Negative surface charge is coloured in red, positive charge in blue. Proposed metal ions in the core of the ribozyme are indicated in green. Neomycin B docked into the core is shown in yellow. This figure was kindly provided by Thomas Hermann and Eric Westhof.

Aminoglycosides indirectly interfere with splicing of the td intron *in vivo*

All studies on the inhibition of ribozymes by aminoglycosides until recently had been performed *in vitro*. To assess whether these antibiotics act similarly within the cell, splicing of the T4 phage *td* intron was studied in the presence of various aminoglycoside antibiotics in antibiotic-sensitive and -resistant strains (Waldsich *et al*., 1998). In aminoglycoside-sensitive strains, splicing was severely inhibited and mis-splicing was induced. Since splicing of the *td* intron is dependent on ribosomal function *in vivo* (Semrad and Schroeder, 1998; Clodi *et al*., 1999), this effect might be indirect. Consequently, splicing activity was tested in a background of resistant ribosomes using resistance genes coding for proteins that methylate the rRNA without altering the antibiotics (Holmes and Cundliffe, 1991; Holmes *et al*., 1991). In this context, splicing was no longer sensitive to the aminoglycosides tested. Furthermore, splicing of the *Tetrahymena* group I intron, which is translation independent, was not inhibited by neomycin B *in vivo*—conclusive evidence that neomycin B inhibits splicing of the *td* intron indirectly by interfering with translation. This clearly implies that the RNA-binding properties of aminoglycosides differ significantly *in vitro* and *in vivo*.

The ribozyme derived from human HDV was also found to be insensitive to aminoglycosides *in vivo*, although this ribozyme is sensitive *in vitro* (Chia *et al*., 1997). Why do neomycin B and related antibiotics not inhibit ribozymes *in vivo*? *In vitro*, group I intron splicing is inhibited via displacement of the magnesium ions essential for catalysis by the antibiotic's protonated amino groups (Hoch *et al*., 1998). On the one hand, the intron core might not be accessible to the aminoglycosides, because it is protected by proteins. On the other hand, the essential metal ions might be bound to the intron more tightly *in vivo* than *in vitro*, thereby withstanding displacement. Alternatively, the local pH *in vivo* might not be adequate for the protonation of the amino groups. *In vivo*, neomycin B and related compounds were found to display their inhibitory activity solely in the context of type I binding sites (Zapp *et al*., 1993; Werstruck and Green, 1998). These observations should be a caution against *in vitro* approaches to drug screening (Waldsich *et al*., 1998).

Antibiotics as 'actibiotics'

A decade ago, Julian Davies suggested that antibiotics, like other secondary metabolites, were not invented purely for the purpose of killing bacteria, but rather to act as effector molecules in the regulation of many biological activities (Davies, 1990). Antibiotics should not be viewed merely as inhibitors, but rather as modulators. A first and very good example for such an effector activity are streptomycin-dependent ribosomes. Streptomycin, like many aminoglycosides, influences translation accuracy by increasing translational errors (Davies *et al*., 1965). Mutations in ribosomal proteins and in rRNA that show altered translation accuracy leading to hyperaccurate ribosomes can confer resistance and sometimes dependence on streptomycin (reviewed in von Ahsen, 1998). In this streptomycin-dependent background, the antibiotic is a positive effector molecule, because it is absolutely required for viability.

Aminoglycoside antibiotics also promote the cleavage reaction of the hairpin ribozyme in the absence of metal ions. This ribozyme does not depend on divalent metal ions for catalysis, but its folding is greatly stimulated by magnesium (Nesbitt *et al*., 1997). Neomycin B can replace magnesium ions required for folding and thus strongly stimulates the activity of the ribozyme (Earnshaw and Gait, 1998). This is in contrast to the action of neomycin B on the hammerhead ribozyme, which is inhibited by the antibiotic by displacement of essential Mg^{2+} ions from the catalytic core.

The most exciting antibiotic with stimulatory effects is the peptide viomycin. It shows inhibitory effects on translation similar to aminoglycosides (Marrero *et al*., 1980; Wurmbach and Nierhaus, 1983) and it is a competitive inhibitor of group I intron splicing (Wank *et al*., 1994) and of the HDV ribozyme (Rogers *et al*., 1996). At sub-

inhibitory concentrations, it switches the group I intron from a *cis*-acting to a *trans*-acting ribozyme. In the presence of viomycin, the excised intron forms covalently linked intron oligomers instead of circular molecules (Wank and Schroeder, 1996). Furthermore, viomycin enhances self-cleavage of the *Neurospora crassa* VS ribozyme and greatly decreases the magnesium concentration required for activity. More significantly, viomycin stimulates the *trans*-cleavage reaction by inducing interactions between RNA molecules (Olive *et al*., 1995). This *trans*-activation potential of viomycin led us to postulate that small peptides such as viomycin might have played an active role in an early RNA world scenario. To test this hypothesis, we performed an *in vitro* selection experiment with a group I intron, which we incubated either with a pool of random sequence RNAs or with a pool of viomycin-binding RNA molecules. In the presence of viomycin, the intron specifically selected viomycin-binding RNAs from the pool, forming novel recombinant molecules consisting of covalently linked intron and pool molecules (Wank *et al*., 1999). These results led to the assumption that small peptides such as the antibiotic viomycin are able to enlarge the catalytic potential of ribozymes by stimulating their assembly and thus increasing the number of possible reaction partners. Small molecular weight effectors with properties like the peptide viomycin might have played an important role in the transition from a putative RNA world to an RNA–protein world.

In vitro selected antibiotic-binding RNA aptamers

To circumvent the size problem of natural RNA targets complexed with their respective antibiotics, a second approach, alternative to dissection, was undertaken: SELEX (systematic evolution of ligands by exponential enrichment) is a method that enables the identification of small RNA molecules with desired properties (Ellington and Szostak, 1990). A series of such small RNAs, called aptamers, which bind small ligands with high affinity (Ellington, 1994) including several translation inhibitors, was isolated (Lato *et al*., 1995; Wallis *et al*., 1995, 1997; Wang and Rando, 1995; Burke *et al*., 1997; Wallace and Schroeder, 1998). These aptamers are small enough to be structurally characterized by NMR. First high resolution insights into the interaction mode of aminoglycosides and RNA have been provided recently by the NMR structures of two aptamers complexed with tobramycin and another aptamer complexed with neomycin B (Jiang *et al*., 1997, 1999; Jiang and Patel, 1998). Although the tobramycin and neomycin B aptamers were isolated in different laboratories, starting from independently synthesized pools, the features that these aptamers have in common are striking.

In all three structurally characterized aptamers, as well as in the 16S rRNA decoding site, the ligands lie in the major groove site of an irregular helix. Aminoglycosides cannot bind to the major groove of a regular A-type RNA helix, because this groove is too narrow and deep (Ellington, 1993). All binding sites of aminoglycosides that are in helical domains display irregularities in the helix, which open up and widen the major groove. The

Fig. 4. (**A**) Secondary structures of *in vitro* selected tobramycin and neomycin B RNA aptamers (Wallis *et al*., 1995; Wang and Rando, 1995). Dashed lines indicate non-Watson–Crick and the full line Watson–Crick interactions which zipper up the loops for the formation of the respective antibiotic-binding pockets. (**B**) Stick view of a representative neomycin–RNA aptamer structure (Jiang *et al*., 1999). The RNA segment shown extends from nucleotide A6 to U24. The Watson–Crick base pairs are coloured in cyan, whereas the mismatches are given in green. The flap base A16 is highlighted in magenta. The neomycin molecule bound to the aptamer RNA is presented in yellow. This figure was kindly provided by Dinshaw Patel.

secondary structures of a tobramycin aptamer and a neomycin B aptamer, which have been characterized by NMR, are shown in Figure 4A. They both contain a simple stem–loop fold closed by a large loop containing 14 or 13 residues. Upon complex formation with the antibiotics, the loops adopt their respective structure by zippering up through formation of non-Watson–Crick base pairs, such as G–U or G–A mismatches. As an example of such an aptamer complexed with its respective drug, the neomycin B aptamer is shown in Figure 4B (Jiang *et al*., 1999). The loop is closed by three consecutive

G–U mismatches, a Watson–Crick G–C pair and a sheared G–A base pair. Residue A14 and G15 stack upon each other and A16 loops out into solution. Neomycin B (in yellow) binds to the floor of the widened major groove (in green) and is partially encapsulated by the flap base A16 (in magenta). Neomycin B rings I and II are encapsulated; ring IV is directed outwards from the binding pocket. The antibiotic is anchored in place through potential hydrogen bonds involving the amino groups of the antibiotic and the base edges as well as the backbone phosphates of the RNA. The final loop structure of the neomycin B aptamer has all the features of a five membered GNRA loop, which is closed by a sheared G–A pair (Jiang *et al*., 1999). GNRA loops recently have been found to manifest a quite high morphological variability (Abramovitz and Pyle, 1997; Legault *et al*., 1998).

After the independent isolation and NMR structure determination of three aptamers complexed with their respective aminoglycoside antibiotics, it became apparent that there are many solutions to how RNA can form a binding pocket for aminoglycosides, but that there are general features that all these solutions have in common. The structurally characterized *in vitro* selected aptamers that bind aminoglycosides display type I binding sites, since they contain widened major grooves and are able to bind the drug *in vivo* (Werstuck and Green, 1998). Furthermore, the *in vitro* selected viomycin-binding aptamers, which were selected out of pools of total random sequence RNAs, all form pseudoknots like the three natural target sites of viomycin (Wallis *et al*., 1997).

Development of novel drugs that target RNA

RNA recently has been recognized as a target site for therapeutic intervention because small molecules inhibit RNA functions involved in prokaryotic translation and viral replication (Pearson and Prescott, 1997). Additionally, the insertion of an *in vitro* selected aptamer into the 5′-untranslated leader of an mRNA in mammalian cells resulted in translation inhibition upon addition of the ligand (Werstuck and Green, 1998). Thus, small molecules that bind to RNAs can be used in many aspects of biology and medicine.

The obstacles for the isolation of novel drugs with enhanced specificity for a distinct RNA motif and with reduced toxicity to the cell have been approached in different ways, including combinatorial synthesis, high-throughput screens and chemical modification of existing aminoglycosides. A series of novel techniques have been developed to measure specificity and affinity of small molecules for RNA (Hendrix *et al*., 1997; Hamasaki and Rando, 1998; Wong *et al*., 1998). A library of neomycin B ‘mimetics’ was constructed through combinatorial synthesis using the conserved neamine core (rings I and II) as the basic building block. Novel neomycin derivatives that bind to the RRE domain of HIV RNA with micromolar affinity were obtained (Park *et al*., 1996). A rapid high-throughput screening approach led to the identification of novel splicing inhibitors, which are not structurally related to any of the known antibiotics which bind RNA (Mei *et al*., 1997). Chemical modification of the aminoglycosides through enhancement of their charges and reduction of their basicity resulted in an increased affinity for their target RNAs (Wang and Tor, 1997b). Dimerization of aminoglycosides also led to a strong increase in the binding affinity for the hammerhead ribozyme (Wang and Tor, 1997a). An *in silico* strategy for the docking of cationic drugs to RNA targets was developed by using molecular dynamic simulations (Hermann and Westhof, 1999). All these novel techniques will finally lead to a better understanding of antibiotic–target interactions and help in the fight against infectious deseases.

The aminoglycosides are only one class of RNA-binding antibiotics. We are looking forward to the detailed characterization of the mode of action of many prominent RNA-binding antibiotics, such as the peptidyl transferase inhibitors, the tetracyclines and the translocation inhibitor thiostrepton, to mention just a few.

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References

Abramovitz,D.L. and Pyle,A.M. (1997) Remarkable morphological variability of a common RNA folding motif: the GNRA tetraloop–receptor interaction. *J. Mol. Biol.*, **266**, 493–506.

Berens,C., Streicher,B., Schroeder,R. and Hillen,W. (1998) Visualizing metal-ion-binding sites in group I introns by iron (II)-mediated Fenton reactions. *Chem. Biol.*, **5**, 163–175.

Burke,D.H., Hoffman,D.C., Brown,A., Hansen,M., Pardi,A. and Gold,L. (1997) RNA aptamers to the peptidyl transferase inhibitor chloramphenicol. *Chem. Biol.*, **4**, 833–843.

Cate,J.H., Yusupov,M.M., Yusupova,G.Z., Earnest,T.N. and Noller,H.F. (1999) X-ray crystal structure of 70S ribosome functional complexes. *Science*, **285**, 2095–2104.

Chia,J.S., Wu,H.L., Wang,H.W., Chen,D.S. and Chen,P.J. (1997) Inhibition of hepatitis delta virus genomic ribozyme self-cleavage by aminoglycosides. *J. Biomed. Sci*., **4**, 208–216.

Clemons,W.C.J., May,J.L.C., Wimberly,B.T., McCutcheon,J.P., Capel,M.S. and Ramakrishnan,V. (1999) Structure of a bacterial 30S ribosomal subunit at 5.5 Å resolution. *Nature*, **400**, 833–840.

Clodi,E., Semrad,K. and Schroeder,R. (1999) Assaying RNA chaperone activity *in vivo* using a novel RNA folding trap. *EMBO J.*, **18**, 3776–3782.

Clouet-d’Orval,B., Stage,T.K. and Uhlenbeck,O.C. (1995) Neomycin inhibition of the hammerhead ribozyme involves ionic interactions. *Biochemistry*, **34**, 11186–11190.

Davies,J. (1990) What are antibiotics? Archaic functions for modern activities? *Mol. Microbiol*., **4**, 1227–1232.

Davies,J. (1994) Inactivation of antibiotics and the dissemination of resistance genes. *Science*, **264**, 375–382.

Davies,J., Gorini,L. and Davis,B.D. (1965) Misreading of RNA codewords induced by aminoglycoside antibiotics. *Mol. Pharmacol.*, **1**, 93–106.

Earnshaw,D.J. and Gait,M.J. (1998) Hairpin ribozyme cleavage catalysed by aminoglycoside antibiotics and the polyamine spermine in the absence of metal ions. *Nucleic Acids Res.*, **26**, 5551–5561.

Ellington,A.D. (1993) Out of shape but fit for recognition. *Curr. Biol.*, **3**, 375–377.

Ellington,A.D. (1994) RNA selection. Aptamers achieve the desired recognition. *Curr. Biol.*, **4**, 427–429.

Ellington,A.D. and Szostak,J.W. (1990) *In vitro* selection of RNA molecules that bind specific ligands. *Nature*, **346**, 818–822.

Fourmy,D., Recht,M.I., Blanchard,S.C. and Puglisi,J.D. (1996) Structure of the A-site of *Escherichia coli* 16S ribosomal RNA complexed with an aminoglycoside antibiotic. *Science*, **274**, 1367–1371.

Fourmy,D., Recht,M.I. and Puglisi,J.D. (1998a) Binding of neomycin-class aminoglycoside antibiotics to the A-site of 16S rRNA. *J. Mol. Biol.*, **277**, 347–362.

Fourmy,D., Yoshizawa,S. and Puglisi,J.D. (1998b) Paromomycin binding induces a local conformational change in the A-site of 16S rRNA. *J. Mol. Biol.*, **277**, 333–345.

Green,R. and Noller,H.F. (1997) Ribosomes and translation. *Annu. Rev. Biochem*., **66**, 679–716.

Hamasaki,K. and Rando,R.R. (1998) A high-throughput fluorescence screen to monitor the specific binding of antagonists to RNA targets. *Anal. Biochem*., **261**, 183–190.

Hendrix,M., Priestley,E.S. and Joyce,J.F. (1997) Direct observation of aminoglycoside–RNA interactions by surface plasmon resonance. *J. Am. Chem. Soc.*, **119**, 3641–3648.

Hermann,T. and Westhof,E. (1998a) Aminoglycoside binding to the hammerhead ribozyme: a general model for the interaction of cationic antibiotics with RNA. *J. Mol. Biol.*, **276**, 903–912.

Hermann,T. and Westhof,E. (1998b) Exploration of metal ion binding sites in RNA folds by Brownian-dynamics simulations. *Structure*, **6**, 1303–1314.

Hermann,T. and Westhof,E. (1999) New strategies for docking cationic drugs to RNA targets. In Schroeder,R. and Wallis,M.G. (eds), *RNA-binding Antibiotics*. R.G.Landes Company, Austin, TX, in press.

Hoch,I., Berens,C., Westhof,E. and Schroeder,R. (1998) Antibiotic inhibition of RNA catalysis: neomycin B binds to the catalytic core of the *td* group I intron displacing essential metal ions. *J. Mol. Biol.*, **282**, 557–569.

Holmes,D.J. and Cundliffe,E. (1991) Analysis of a ribosomal RNA methylase gene from *Streptomyces tenebrarius* which confers resistance to gentamicin. *Mol. Gen. Genet.*, **229**, 229–237.

Holmes,D.J., Drocourt,D., Tiraby,G. and Cundliffe,E. (1991) Cloning of an aminoglycoside-resistance-encoding gene, *kam*C, from *Saccharopolyspora hirsuta*: comparison with *kam*B from *Streptomyces tenebrarius*. *Gene*, **102**, 19–26.

Jiang,L. and Patel,D.J. (1998) Solution structure of the tobramycin–RNA aptamer complex. *Nature Struct. Biol.*, **5**, 769–774.

Jiang,L., Suri,A.K., Fiala,R. and Patel,D.J. (1997) Saccharide–RNA recognition in an aminoglycoside antibiotic–RNA aptamer complex. *Chem. Biol.*, **4**, 35–50.

Jiang,L., Majumdar,A., Hu,W., Jaishree,T.J., Xu,W. and Patel,D.J. (1999) Saccharide–RNA recognition in a complex formed between neomycin B and an RNA aptamer. *Structure Fold Des*., **7**, 817–827.

Karimi,R. and Ehrenberg,M. (1994) Dissociation rate of cognate peptidyl-tRNA from the A-site of hyper-accurate and error-prone ribosomes. *Eur. J. Biochem*., **226**, 355–360.

Lato,S.M., Boles,A.R. and Ellington,A.D. (1995) *In vitro* selection of RNA lectins: using combinatorial chemistry to interpret ribozyme evolution. *Chem. Biol.*, **2**, 291–303.

Legault,P., Li,J., Mogridge,J., Kay,L.E. and Greenblatt,J. (1998) NMR structure of the bacteriophage lambda-N peptide/boxB RNA complex: recognition of a GNRA fold by an arginine-rich motif. *Cell*, **93**, 289–299.

Lehnert,V., Jaeger,L., Michel,F. and Westhof,E. (1996) New loop–loop tertiary interactions in self-splicing introns of subgroup IC and ID: a complete 3D model of the *Tetrahymena thermophila* ribozyme. *Chem. Biol.*, **3**, 993–1009.

Marrero,P., Cabanas,M.J. and Modolell,J. (1980) Induction of translational errors (misreading) by tuberactinomycins and capreomycins. *Biochem. Biophys. Res. Commun*., **97**, 1047–1052.

Mei,H.Y., Galan,A.A. and Halim,N.S. (1995) Inhibition of an HIV-1 Tat-derived peptide binding to TAR RNA by aminoglycoside antibiotics. *Bioorg. Med. Chem. Lett*., **5**, 2755–2760.

Mei,H.Y., Cui,M., Lemrow,S.M. and Czarnik,A.W. (1997) Discovery of selective, small-molecule inhibitors of RNA complexes—II. Self-splicing group I intron ribozyme. *Bioorg. Med. Chem*., **5**, 1185–1195.

Michel,F. and Westhof,E. (1990) Modelling of the three-dimensional architecture of group I catalytic introns based on comparative sequence analysis. *J. Mol. Biol.*, **216**, 585–610.

Mikkelsen,N.E., Brannvall,M., Virtanen,A. and Kirsebom,L.A. (1999) Inhibition of RNase P RNA cleavage by aminoglycosides. *Proc. Natl Acad. Sci. USA*, **96**, 6155–6160.

Moazed,D. and Noller,H.F. (1986) Transfer RNA shields specific nucleotides in 16S ribosomal RNA from attack by chemical probes. *Cell*, **47**, 985–994.

Moazed,D. and Noller,H.F. (1987) Interaction of antibiotics with functional sites in 16S ribosomal RNA. *Nature*, **327**, 389–394.

Murphy,F.L. and Cech,T.R. (1993) An independently folding domain of RNA tertiary structure within the *Tetrahymena* ribozyme. *Biochemistry*, **32**, 5291–5300.

Nesbitt,S., Hegg,L.A. and Fedor,M.J. (1997) An unusual pH-independent and metal-ion-independent mechanism for hairpin ribozyme catalysis. *Chem. Biol.*, **4**, 619–630.

Olive,J.E., De Abreu,D.M., Rastogi,T., Andersen,A.A., Mittermaier,A.K., Beattie,T.L. and Collins,R.A. (1995) Enhancement of *Neurospora* VS ribozyme cleavage by tuberactinomycin antibiotics. *EMBO J.*, **14**, 3247–3251.

Pape,T., Wintermeyer,W. and Rodnina,M. (1999) Induced fit in initial selection and proofreading of aminoacyl-tRNA on the ribosome. *EMBO J.*, **18**, 3800–3807.

Park,W.K.C., Auer,M., Jaksce,H. and Wong,C.-H. (1996) Rapid combinatorial synthesis of aminoglycoside antibiotic mimetics: use of a polyethylene glycol-linked amine and a neamine-derived aldehyde in multiple component condensation as strategy for discovery of new inhibitors of the HIV RNA Rev responsive element. *J. Am. Chem. Soc.*, **118**, 10150–10155.

Pearson,N.D. and Prescott,C.D. (1997) RNA as a drug target. *Chem. Biol.*, **4**, 409–414.

Potapov,A.P., Triana-Alonso,F.J. and Nierhaus,K.H. (1995) Ribosomal decoding processes at codons in the A or P sites depend differently on 2′-OH groups. *J. Biol. Chem.*, **270**, 17680–17684.

Purohit,P. and Stern,S. (1994) Interactions of a small RNA with antibiotic and RNA ligands of the 30S subunit. *Nature*, **370**, 659–662.

Recht,M.I., Fourmy,D., Blanchard,S.C., Dahlquist,K.D. and Puglisi,J.D. (1996) RNA sequence determinants for aminoglycoside binding to an A-site rRNA model oligonucleotide. *J. Mol. Biol.*, **262**, 421–436.

Rogers,J., Chang,A.H., von Ahsen,U., Schroeder,R. and Davies,J. (1996) Inhibition of the self-cleavage reaction of the human hepatitis delta virus ribozyme by antibiotics. *J. Mol. Biol.*, **259**, 916–925.

Schroeder,R. (1994) Translation. Dissecting RNA function. *Nature*, **370**, 597–598.

Schroeder,R. and von Ahsen,U. (1996) Interaction of aminoglycoside antibiotics with RNA. In Eckstein,F. and Lilley,D.M.J. (eds), *Nucleic Acids and Molecular Biology*. Springer-Verlag, Berlin, Germany, Vol. 10, pp. 53–74.

Schroeder,R., Streicher,B. and Wank,H. (1993) Splice-site selection and decoding: are they related? *Science*, **260**, 1443–1444.

Scott,W.G., Murray,J.B., Arnold,J.R.P., Stoddard,B.L. and Klug,A. (1996) Capturing the structure of a catalytic RNA intermediate: the hammerhead ribozyme. *Science*, **274**, 2065–2069.

Semrad,K. and Schroeder,R. (1998) A ribosomal function is necessary for efficient splicing of the T4 phage thymidylate synthase intron *in vivo*. *Genes Dev.*, **12**, 1327–1337.

Stage,T.K., Hertel,K.J. and Uhlenbeck,O.C. (1995) Inhibition of the hammerhead ribozyme by neomycin. *RNA*, **1**, 95–101.

Streicher,B., Westhof,E. and Schroeder,R. (1996) The environment of two metals ions surrounding the splice site of a group I intron. *EMBO J.*, **15**, 2556–2564.

Strobel,S.A. and Cech,T.R. (1995) Minor groove recognition of the conserved G–U pair at the *Tetrahymena* ribozyme reaction site. *Science*, **267**, 675–679.

Strobel,S.A., Ortoleva-Donnelly,L., Ryder,S.P., Cate,J.H. and Moncoeur,E. (1998) Complementary sets of noncanonical base pairs mediate RNA helix packing in the group I intron active site. *Nature Struct. Biol.*, **5**, 60–66.

von Ahsen,U. (1998) Translation fidelity: error-prone versus hyper-accurate ribosomes. *Chem. Biol.*, **5**, R3–R6.

von Ahsen,U. and Noller,H.F. (1993) Footprinting the sites of interaction of antibiotics with catalytic group I intron RNA. *Science*, **260**, 1500–1503.

von Ahsen,U., Davies,J. and Schroeder,R. (1991) Antibiotic inhibition of group I ribozyme function. *Nature*, **353**, 368–370.

von Ahsen,U., Davies,J. and Schroeder,R. (1992) Non-competitive inhibition of group I intron RNA self-splicing by aminoglycoside antibiotics. *J. Mol. Biol.*, **226**, 935–941.

Waldsich,C., Semrad,K. and Schroeder,R. (1998) Neomycin B inhibits splicing of the *td* intron indirectly by interfering with translation and enhances missplicing *in vivo*. *RNA*, **4**, 1653–1663.

Wallace,S.T. and Schroeder,R. (1998) *In vitro* selection and characterization of streptomycin-binding RNAs: recognition discrimination between antibiotics. *RNA*, **4**, 112–123.

Wallis,M.G. and Schroeder,R. (1997) The binding of antibiotics to RNA. *Prog. Biophys Mol. Biol*., **67**, 141–154.
Wallis,M.G., von Ahsen,U., Schroeder,R. and Famulok,M. (1995) A novel RNA motif for neomycin recognition. *Chem. Biol.*, **2**, 543–552.
Wallis,M.G., Streicher,B., Wank,H., von Ahsen,U., Clodi,E., Wallace,S.T., Famulok,M. and Schroeder,R. (1997) *In vitro* selection of a viomycin-binding RNA pseudoknot. *Chem. Biol.*, **4**, 357–366.
Wang,H. and Tor,Y. (1997a) Dimeric aminoglycosides: design, synthesis and RNA binding. *Bioorg. Med. Chem. Lett*., **7**, 1951–1956.
Wang,H. and Tor,Y. (1997b) Electrostatic interactions in RNA–aminoglycosides binding. *J. Am. Chem. Soc.*, **119**, 8734–8735.
Wang,Y. and Rando,R.R. (1995) Specific binding of aminoglycoside antibiotics to RNA. *Chem. Biol.*, **2**, 281–290.
Wank,H. and Schroeder,R. (1996) Antibiotic-induced oligomerisation of group I intron RNA. *J. Mol. Biol.*, **258**, 53–61.
Wank,H., Rogers,J., Davies,J. and Schroeder,R. (1994) Peptide antibiotics of the tuberactinomycin family as inhibitors of group I intron RNA splicing. *J. Mol. Biol.*, **236**, 1001–1010.
Wank,H., Clodi,E., Wallis,M. and Schroeder,R. (1999) The antibiotic viomycin as a model peptide for the origin of the co-evolution of RNA and proteins. *Orig. Life Evol. Biosphere*, **29**, 391–404.
Werstuck,G. and Green,M.R. (1998) Controlling gene expression in living cells through small molecule–RNA interactions. *Science*, **282**, 296–298.
Wong,C.H., Hendrix,M., Priestley,E.S. and Greenberg,W.A. (1998) Specificity of aminoglycoside antibiotics for the A-site of the decoding region of ribosomal RNA. *Chem. Biol*., **5**, 397–406.
Woodcock,J., Moazed,D., Cannon,M., Davies,J. and Noller,H.F. (1991) Interaction of antibiotics with A- and P-site-specific bases in 16S ribosomal RNA. *EMBO J.*, **10**, 3099–3103.
Wurmbach,P. and Nierhaus,K.H. (1983) The inhibition pattern of antibiotics on the extent and accuracy of tRNA binding to the ribosome and their effect on the subsequent steps in chain elongation. *Eur. J. Biochem*., **130**, 9–12.
Yoshizawa,S., Fourmy,D. and Puglisi,J.D. (1998) Structural origins of gentamicin antibiotic action. *EMBO J.*, **17**, 6437–6448.
Yoshizawa,S., Fourmy,D. and Puglisi,J.D. (1999) Recognition of the codon-anticodon helix by ribosomal RNA. *Science*, **285**, 1722–1725.
Zapp,M.L., Stern,S. and Green,M.R. (1993) Small molecules that selectively block RNA binding of HIV-1 Rev protein inhibit Rev function and viral production. *Cell*, **74**, 969–978.

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