RNA as a Drug Target: The Case of Aminoglycosides

Quentin Vicens[a, b] and Eric Westhof*[a]

1. Introduction

Proteins are essential for the viability of any living cell. Therefore, it is no surprise that the ribosome—a synthesis machine of ≈2.3 MDa molecular weight formed by 3 long RNA chains and about 55 proteins—is the target of half of the antibiotics characterized thus far.[1, 2] For 40 years, microbiological, pharmacological, and biochemical data have helped to decipher the mechanisms of action of various antibiotics, by providing clues about their binding sites (that is, through footprinting experiments[3] and identification of mutations[4]) and their mechanisms of action (that is, by kinetic measurements[5]). A critical advance in the understanding of these mechanisms has recently been made with the resolutions of the crystal structures of bacterial ribosomal particles complexed to several classes of protein synthesis inhibitors.[6–11] These structures definitively show that antibiotics predominantly target ribosomal RNA molecules, rather than ribosomal proteins. Each crystal structure of the same particle bound to various antibiotics, partners, and cofactors implicated in the protein synthesis provides a snapshot of the inhibitory process at the molecular level. When assembled, these individual pictures help to reconstruct the whole course of action and indirectly improve our understanding of the protein synthesis process. Crystal structures have also been solved for an isolated domain of the ribosome in complex with several antibiotics from the aminoglycoside family.[12–14] The comparative analyses of these high-resolution structures aided in deciphering the contribution of each antibiotic functional group to the binding and offered a molecular basis to some resistance and toxicity mechanisms.[15]

The present Minireview will focus on the interactions between aminoglycosides and the 16S ribosomal RNA (rRNA). It will (1) summarize the challenges small molecules must face to target RNA, (2) describe the molecular recognition between rRNA and aminoglycosides as well as the mechanism of action of aminoglycoside antibiotics, (3) discuss how strategies aimed at overcoming resistance due to RNA modifications or mutations need to cope with toxicity, and (4) stress the advantages of targeting RNA molecular switches, whose recurrence is now revealed in various RNA molecules.

2. RNA as a Drug Target

On the basis of its chemical structure, RNA does not appear to be a very promising drug target: It is made of only four different, planar bases and every nucleotide is negatively charged.[16] On the other hand, one can argue that the intricate architectures that RNA molecules can adopt lead to the formation of pockets and cavities where shape-specific rather than sequence-specific binding could be achieved.[17] Several observations then come to mind:

1) The formation of RNA cavities necessitates a close proximity of phosphate groups, which leads to a heightened importance of electrostatic forces and the roles of tightly bound water molecules and ions, especially the divalent magnesium ions, which can be partly dehydrated.

2) The formation of pockets or enlarged grooves requires the presence of non-Watson–Crick pairs and bulged residues. The optimist will stress the fact that function arises through the assembly of existing RNA motifs, which are clearly diverse in architecture.[18] Furthermore, although most of the energy content of a given folded RNA is contained in the secondary structure consisting of regular double-stranded helices, the free energy content of a three-dimensional RNA fold is, as in proteins, between -5 and -10 kcal mol^{-1}. Thus, a binding constant in the nanomolar range—which can be achieved for a small molecule—is able to compete with the final steps of RNA folding. However, the pessimist will remark that it is becoming apparent that the number of ways of embedding non-Watson–Crick pairs within helices is limited, which leads to a rather restricted number of RNA motifs that could be chosen as potential targets.[19] Strikingly, RNA motifs appear to be like Russian dolls, with smaller motifs associated into larger motifs.[20]

Structurally, the antibiotics target different regions of RNA molecules in the ribosome. They bind in the shallow groove (spectinomycin)[7] or the deep groove (hygromycin B)[6] of a helix, at a three-adenine bulge (aminoglycosides),[7, 12] or in the exit tunnel of the nascent polypeptide chain (macrolides).[9, 11] Additionally, they interact in many ways with RNA, as shown in Figure 1: (1) only with phosphate groups (streptomycin),[7]

[a] *Prof. E. Westhof, Dr. Q. Vicens*
Institut de Biologie Moléculaire et Cellulaire du CNRS
Modélisation et simulations des Acides Nucléiques, UPR 9002
Université Louis Pasteur
15 rue René Descartes
67084 Strasbourg Cedex (France)
Fax: (+33) 3-88-60-22-18
E-mail: E.Westhof@ibmc.u-strasbg.fr

[b] *Dr. Q. Vicens*
Current address
Howard Hughes Medical Institute
University of Colorado:
Department of Chemistry and Biochemistry
Boulder, CO 80309-0215 (USA)

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim DOI: 10.1002/cbic.200300684

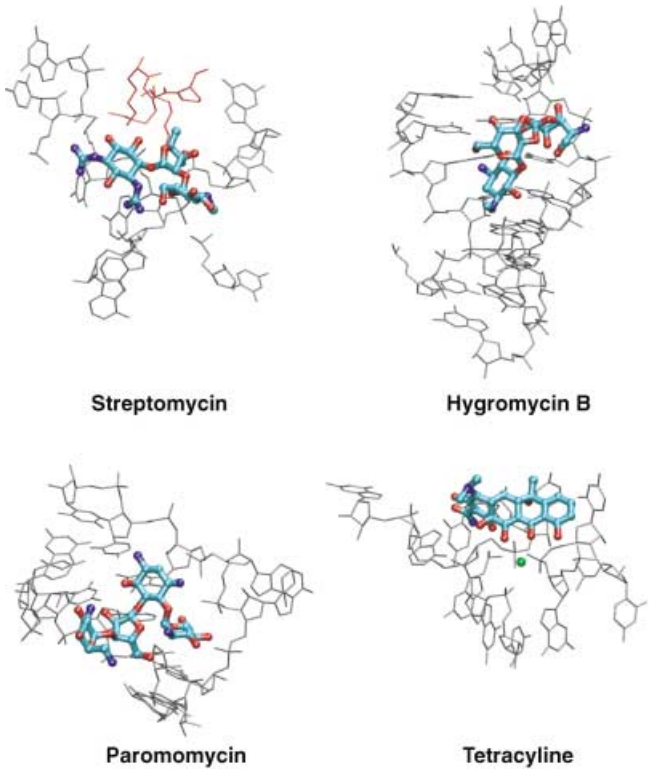
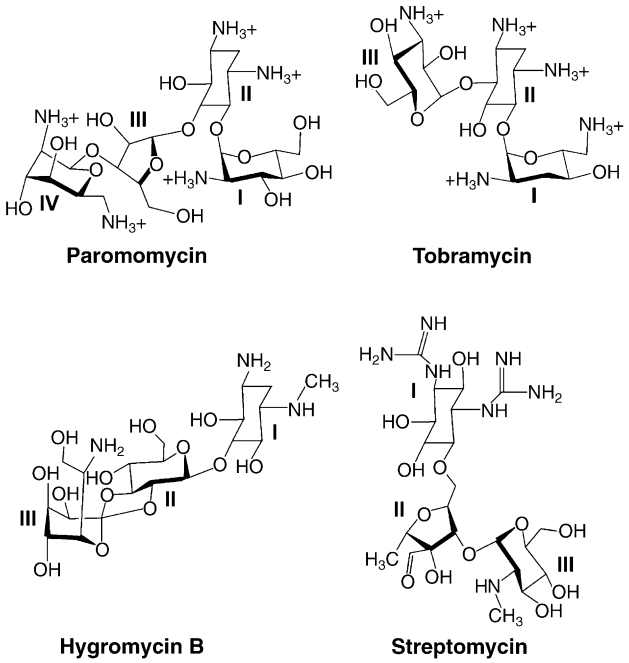


Figure 1. Diversity of antibiotic binding modes to the ribosome. The drugs displayed are streptomycin (Protein Databank (PDB) no.: 1FJG), hygromycin B (PDB no.: 1HNZ), paromomycin (PDB no.:1FJG), and tetracycline (PDB no.: 1l97). Antibiotics are shown as ball-and-stick representations colored by atom type (C = cyan, O = red, N = dark blue); nucleotides, amino acids, and magnesium ions are shown in grey, red, and green, respectively.



***Scheme 1.** Chemical structures of representatives of four aminoglycoside subclasses.*

(2) mainly with bases (hygromycin B, spectinomycin),[6, 7] (3) with a mixture of both (paromomycin, tobramycin),[7, 12, 13] (4) through magnesium ions (tetracycline, chloramphenicol, sparsomycin)[10, 11, 21] or a protein side chain (streptomycin).[7] The antibiotics can mimic base stacking (pactamycin)[6] or form pseudo base-pairing interactions with ribosomal bases (blasticidin S, paromomycin, and related aminoglycosides).[7 12, 13, 21]

Consequently, in addition to the fact that the protein synthesis offers several functional steps that could be altered, the variety of antibiotic binding sites and modes of action indicates that the ribosome still constitutes a promising target for future inhibitors.[2] The discovery in the late 1990s of a new class of antibiotics exhibiting a unique mechanism of action—the oxazolidinones[22]—reinforced the confidence scientists have in that perspective. In addition, many other RNA molecules have been proposed to be potential targets for known antibiotics like aminoglycosides and/or new semisynthetic drugs:[23–25] HIV RNA,[26] RNase P,[27] group I introns,[28] and tmRNA.[29]

3. Mechanisms of Action of Aminoglycosides

Aminoglycoside antibiotics are oligosaccharides containing several ammonium groups.[23, 30] Different subclasses are distinguished on the basis of their chemical structure and their mechanism of action (Scheme 1).[31] Aminoglycosides belonging to the paromomycin and tobramycin subclasses interfere with translation[32] by binding to the aminoacyl-transfer RNA (tRNA) decoding site (A site) on the 16S rRNA.[3] Kinetic analyses showed that, during decoding, a correct tRNA – messenger RNA (mRNA) interaction induces a conformational change of the A site to permit translation.[33] Aminoglycosides disturb the fidelity of this selection step by stabilizing a similar conformation for near-cognate complexes.[5, 34, 35] Additionally, aminoglycosides were shown to inhibit translocation,[36] although the nature and the relative importance of this effect remain to be understood.[37, 38]

Since aminoglycosides have been shown to bind in vitro to various RNA molecules (see Section 2), it can be expected that they interfere with various RNA-dependent regulation pathways in vivo.[23–25] Many studies revealed that in order to be taken up into the cells, aminoglycosides also interact with non-RNA molecules, like the acidic phospholipids of the human epithelia membrane,[39] and perturb the activities of many transmembrane proteins, that is, the protein megalin,[40] several phospholipases,[39] the Na^+/K^+ ATPase,[41] and the oligopeptide binding protein.[42] Consequently, depending on the cell type, these various effects may contribute to the antibacterial action of aminoglycosides, but might also stimulate toxicity in humans. The detailed mechanism of action of aminoglycosides is apparently a complex and delicate balance between several processes.[25, 30, 43] However, binding of aminoglycosides to the A site constitutes the key in their mode of action, since rRNA point mutations that preclude aminoglycoside – A site interactions confer high-level resistance to aminoglycosides (see Section 5).[44, 45]

Crystallographic structures of various 30S particle complexes revealed the mechanism of action of aminoglycosides at the ribosomal level with atomic details. During decoding, the A site

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

changes its conformation from an "off" conformation (A1492 and A1493 are folded in the shallow groove of the A site) to an "on" conformation (A1492 and A1493 fully bulge out from the A site).[34, 46, 47] This conformational change is necessary to allow A1492 and A1493 to specifically interact with the first two of the three base pairs formed by the cognate codon:anticodon interaction.[47] It also provokes the transition of the ribosome from an open form to a closed form that is stabilized by contacts involving the cognate tRNA and the ribosome.[34, 35] Aminoglycosides lock the A site in the open conformation (Figure 1)[7] and by doing so they also pay for a part of the energetic cost associated with the tRNA-dependent ribosome closure.[34, 35] As a consequence, the ribosome has lost its ability to discriminate cognate versus noncognate tRNA – mRNA associations.[34, 47]

Small RNA oligonucleotides containing the A site isolated from its natural environment were shown to bind aminoglycosides similarly to the full ribosome.[48] Crystal structures of an RNA double helix containing two A sites were solved in complex with paromomycin, tobramycin, and geneticin.[12–14] They characterized the binding mode of aminoglycosides at high resolution: The puckered sugar ring I is inserted into the A-site helix by stacking against a guanine residue and by forming two hydrogen bonds to the Watson – Crick sites of the universally conserved A1408 (Figure 2). As was observed in the 30S particle,

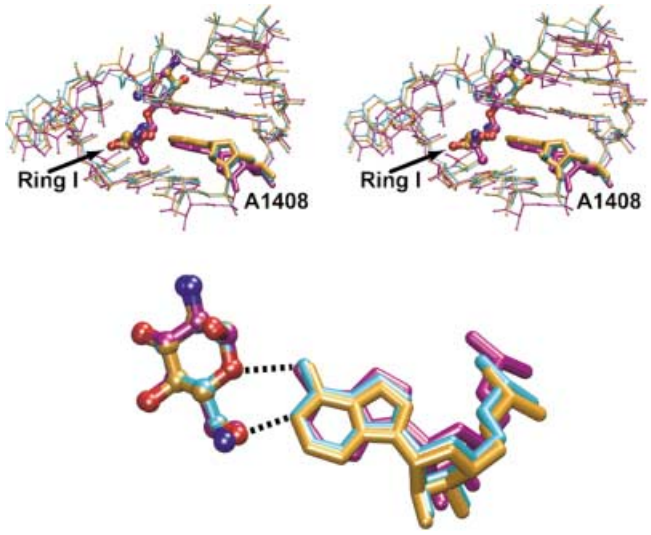


Figure 2. Insertion of aminoglycosides into the rRNA. Above: Stereoview from the shallow groove of the A site bound to paromomycin (gold; PDB no.: 1J7T), tobramycin (cyan; PDB no.: 1LC4), and geneticin (magenta; PDB no.: 1MWL). The superimposition is based on the common rings I and II. Adenine residue 1408 is shown in bold. Solvent molecules and rings III and IV of the antibiotics are omitted for clarity. Below: Detailed depiction of the hydrogen bonds involving ring I and A1408.

this particular interaction helps to maintain residues A1492 and A1493 in the bulged-out conformation that induces misreading.[47] The conserved 2-deoxystreptamine ring (ring II) forms similar hydrogen bonds in the three complexes, and its binding is made possible by the adaptability created by the universally conserved U1406:U1495 pair.[45] The additional rings contact different nucleotides of the A site according to the substitution type of ring II. One third of the total RNA – aminoglycoside contacts were shown to be mediated by water molecules.[12, 15]

4. Roles of Positive Charges in the Driving Force of Binding and of Water Molecules in Accommodating Molecular Diversity

Early theoretical work showed that the driving force of binding of the positively charged aminoglycosides to the negatively charged RNA molecules is dominated by electrostatic interactions and the displacement of cations from the RNA. It was then suggested that some of the positively charged ammonium groups would occupy magnesium-ion positions, located at favorable sites in the global electrostatic environment.[49] The recent crystal structures of several antibiotics bound to small RNA oligonucleotides containing the A site[12–14] gave some support to that suggestion. In all crystal structures, a conserved ammonium group (N3) is fully dehydrated and makes similar hydrogen bonds to the RNA. The pK_a value of ammonium group N3 was shown to be 5.7 in solution, the lowest value of all ammonium groups in the structure.[50] Aminoglycoside binding to the RNA increases this pK_a value to 6.1 – 6.4 in the complex.[51] A comparison of those structures with the structure of the 30S particle in the absence of any drug indicates that a magnesium ion usually occupies the location adopted by ammonium group N3 in the antibiotic – RNA complexes. However, the position of the magnesium ion is not fully occupied (occupancy not equal to one), and additional structural examples are missing to rule out the possibility that this situation is a coincidence.

Solvent molecules were explicitly shown to play a key role in the molecular recognition and the binding of aminoglycosides to the A site. For example, as can be seen in Figure 3, the

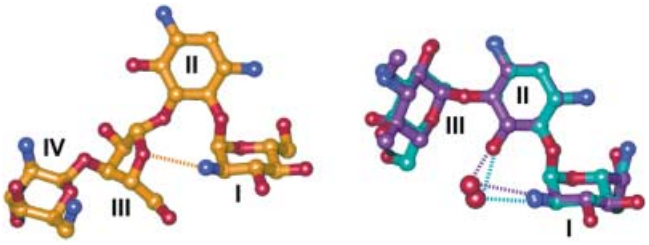


Figure 3. Example of a direct intramolecular hydrogen bond in the paromomycin complex (left) replaced by an intramolecular water-bridged hydrogen bond in the tobramycin and geneticin complexes (right; the superimposition is based on the atoms of rings I and II; the color code is as in Figure 2).

intramolecular hydrogen bond involving ammonium group N2′ on ring I and O4′′ on ring III of paromomycin[12] is replaced in tobramycin[13] and geneticin[14] by a water-bridged hydrogen bond from the conserved ammonium group N2′ on ring I to O5 on ring II, which suggests that this water molecule helps to maintain the correct orientation of rings I and II in these structures. Several of the water molecules participating in the water-mediated hydrogen bonds between the RNA and the aminoglycosides reside in sites that are equivalent to those found around naked RNA helices in crystal structures[52] or in

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

models resulting from molecular dynamics simulations.[53] Such exploitation of the RNA hydration shell reduces the degree of dehydration each compound has to undergo in order to accomplish tight and specific binding. Remarkably, the differences in the chemical structures of the compounds belonging to the paromomycin and the tobramycin families (Scheme 1) are compensated for by water molecules that make hydrogen bonds from the conserved parts of the aminoglycosides to the RNA.[15] These various aspects exemplify the crucial participation of water molecules in the aminoglycoside binding strategy.

5. Conservation of the A Site and Drug – RNA Direct Contacts: Is the Search for New Antibiotics a Fight against Past and Future Molecular Evolution?

Soil bacteria and fungi developed aminoglycosides in order to combat bacterial enemies.[1] However, bacteria have struck back and built up an armada of resistance mechanisms that have spread extensively since the introduction of antibiotics in clinical practices; this has forced physicians to reconsider their prescriptions.[2, 54] In particular, microbiological experiments have discovered that the single A1408G mutation in the A site is sufficient to produce high-level resistance against aminoglycosides.[4, 44, 55] Molecular modeling based on the crystal structures of the wild-type A site bound to aminoglycosides have showed that the mutation disrupts a set of invariant hydrogen bonds between the antibiotic and the RNA, which prevents any active binding of the aminoglycoside to the A site.[45]

One way to overcome this type of resistance could be to synthesize more versatile molecules that would still bind to mutated A sites. However, binding to the human cytoplasmic[56] and mitochondrial[57] A sites, whose sequences are very close to the bacterial one (Figure 4), is known to be an origin of toxicity,

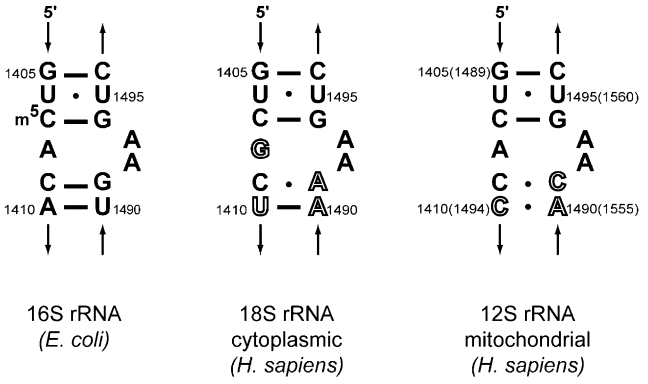


Figure 4. *Sequences of the A site in various organisms. The nucleotides different in the bacterial (left) and eukaryotic cytoplasmic (middle) or mitochondrial (right) A sites are outlined. The* Escherichia coli *numbering is specified; the 12S mitochondrial numbering is indicated in brackets.*

especially in the case of the A1555G mutation of the mitochondrial A site, which has been found to enhance ear toxicity in patients treated with aminoglycosides.[58] Drugs designed to ignore the A1408G mutation might also bind to the eukaryotic A sites and therefore be severely toxic.[44] Knowledge of structure, together with comparative genomic analysis, should facilitate the development of new broad-spectrum antibiotics with higher selectivity for bacterial ribosomes and less toxicity to eukaryotic ribosomes.

6. The Importance of Hitting a Molecular Switch

The binding of aminoglycosides to the A site, by mimicking the conformation adopted in presence of cognate tRNA – mRNA codon association, shunts a molecular switch and results in a loss in translation fidelity. Similarly, recent results indicate that sparsomycin catalyzes ribosomal translocation by binding to the peptidyl-tRNA and the 23S rRNA,[21, 59] a process normally performed by elongation factor G and guanosine triphosphate.[38] The effects of sparsomycin in the translocation process can be understood if sparsomycin binds preferentially to the translocated state, thereby shunting another type of control point in the translation process. Interestingly, the aminoglycoside hygromycin B, which binds mainly at the level of the conserved U1406:U1495 pair and, thus, between the A and P sites, blocks ribosomal translocation without inducing misreading.[60] Since the aminoglycosides binding to the A site (paromomycin, tobramycin, etc.) also contact the U1406:U1495 pair, one would expect that they also hinder translocation (as shown by Fredrick and Noller[38]). The study of aminoglycoside variants which do not contact the U1406:U1495 pair would be helpful in the further understanding of these connected processes.

In short, although a strong specificity might be difficult to achieve with oppositely charged molecules, targeting motifs that undergo dynamic exchange between alternative conformations should improve the biological activity of antibacterial compounds. By analogy to the aminoglycoside and sparsomycin cases, RNA targets ought to be chosen in regions switching between alternative states where each state—referred to as an "on" or "off" state—leads to a different biological action. Only one of the two states would then exist in the presence of the small molecule (Figure 5). Such a situation was found for the inhibition of the yeast $tRNA^{Asp}$ aminoacylation reaction by aminoglycosides: The binding of tobramycin alters the native

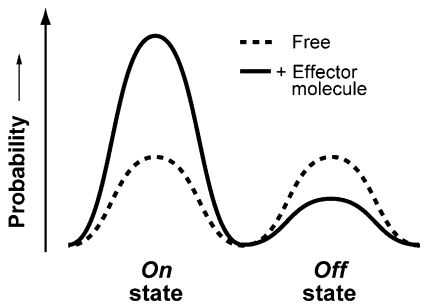


Figure 5. *General scheme for the inhibition of RNA molecular switches by small molecules. The effector is shown here to stabilize the "on" state.*

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

conformation of the tRNA so that binding of the synthetase becomes less favorable.[61]

This approach is likely to gain in favor now that it is becoming apparent that several important biological mechanisms are similarly regulated by molecular switches involving distinct conformational states of RNA molecules.[62, 63] For example, small effectors promote up- or down-regulation of a particular protein expression by binding to the 5′-UTR regions of the corresponding mRNA, thereby fastening a particular "on" or "off" conformation.[62, 64] Therefore, in addition to the bacterial ribosome, which still constitutes a gold mine of potential drug targets, the understanding and the identification of such control mechanisms should ultimately help to identify new potential viral or bacterial targets for therapeutic intervention.

7. Summary and Outlook

Whereas the standard approaches of designing drugs to target proteins have benefitted from crystal structures of complexes between inhibitors and proteins for the last 20 years, the first crystal structures of small molecules bound to RNA pockets were solved only a few years ago. The crystal structures of the ribosomal subunits from various organisms bound to antibiotics used for decades gave a strong impetus to the antibiotic research field by revealing that most of the drugs bind to defined RNA motifs. Further understanding of the translation steps should help to identify new targets. In addition, RNA molecules offer significant advantages as drug targets when compared to proteins, one of them being the possibility of modulating the expression level of any protein by targeting its mRNA instead of directly inhibiting the protein. This approach will probably come to the fore now that it is known that small molecules can bind RNA and act as effectors in vivo.

However, the very fact that RNA self-assembly and recognition motifs are modular and recurrent—a fantastic structural advantage for RNA compared to proteins—constitutes a major drawback for future drug designers, due to the difficulties that might need to be faced in order to achieve specificity. But, in the end, whether a protein or an RNA is the target, the fight against bacteria will always be hindered by the evolution of resistant molecules. Consequently, the developments of future therapies will require the concerted efforts of microbiologists, structural biologists, bioinformaticians, and chemists.

Acknowledgements

We thank Erik Böttger for numerous discussions, as well as Art Zaug and David Zappulla for careful reading of the manuscript.

Keywords: aminoglycosides · antibiotics · ribosomes · RNA · RNA recognition · RNA structure

[1] E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond, M. J. Waring, *The molecular basis of antibiotic action*, 2nd ed., John Wiley and Sons, London, **1981**.
[2] C. Walsh, *Nature* **2000**, *406*, 775 – 781.
[3] D. Moazed, H. F. Noller, *Nature* **1987**, *327*, 389 – 394.
[4] E. De Stasio, D. Moazed, H. F. Noller, A. E. Dahlberg, *EMBO J.* **1989**, *8*, 1213 – 1216.
[5] T. Pape, W. Wintermeyer, M. V. Rodnina, *Nat. Struct. Biol.* **2000**, *7*, 104 – 107.
[6] D. E. Brodersen, W. M. Clemons, Jr., A. P. Carter, R. J. Morgan-Warren, B. T. Wimberly, V. Ramakrishnan, *Cell* **2000**, *103*, 1143 – 1154.
[7] A. P. Carter, W. M. Clemons, D. E. Brodersen, R. J. Morgan-Warren, B. T. Wimberly, V. Ramakrishnan, *Nature* **2000**, *407*, 340 – 348.
[8] T. Auerbach, A. Bashan, J. Harms, F. Schluenzen, R. Zarivach, H. Bartels, I. Agmon, M. Kessler, M. Pioletti, F. Franceschi, A. Yonath, *Curr. Drug Targets Infect. Disord.* **2002**, *2*, 169 – 186.
[9] J. L. Hansen, J. A. Ippolito, N. Ban, P. Nissen, P. B. Moore, T. A. Steitz, *Mol. Cell.* **2002**, *10*, 117 – 128.
[10] M. Pioletti, F. Schlünzen, J. Harms, R. Zarivach, M. Glühmann, H. Avila, A. Bashan, H. Bartels, T. Auerbach, C. Jacobi, T. Hartsch, A. Yonath, F. Franceschi, *EMBO J.* **2001**, *20*, 1829 – 1839.
[11] F. Schlünzen, R. Zarivach, J. Harms, A. Bashan, A. Tocilj, R. Albrecht, A. Yonath, F. Franceschi, *Nature* **2001**, *413*, 814 – 821.
[12] Q. Vicens, E. Westhof, *Structure* **2001**, *9*, 647 – 658.
[13] Q. Vicens, E. Westhof, *Chem. Biol.* **2002**, *9*, 747 – 755.
[14] Q. Vicens, E. Westhof, *J. Mol. Biol.* **2003**, *326*, 1175 – 1188.
[15] Q. Vicens, E. Westhof, *Biopolymers* **2003**, *70*, 42 – 57.
[16] E. Westhof, P. Auffinger in *Encyclopedia of analytical chemistry* (Ed.: R. A. Meyers), John Wiley and Sons, Chichester, **2000**, pp. 5222 – 5232.
[17] T. Hermann, E. Westhof, *Comb. Chem. High Throughput Screen.* **2000**, *3*, 219 – 234.
[18] R. T. Batey, R. P. Rambo, J. A. Doudna, *Angew. Chem.* **1999**, *111*, 2472 – 2491; *Angew. Chem. Int. Ed.* **1999**, *38*, 2327 – 2343.
[19] N. B. Leontis, E. Westhof, *Curr. Opin. Struct. Biol.* **2003**, *13*, 300 – 308.
[20] N. B. Leontis, E. Westhof, *J. Mol. Biol.* **1998**, *283*, 571 – 583; E. Westhof, B. Masquida, L. Jaeger, *Fold. Des.* **1996**, *1*, R78-R88.
[21] J. L. Hansen, P. B. Moore, T. A. Steitz, *J. Mol. Biol.* **2003**, *330*, 1061 – 1075.
[22] S. M. Swaney, H. Aoki, M. C. Ganoza, D. L. Shinabarger, *Antimicrob. Agents Chemother.* **1998**, *42*, 3251 – 3255; M. R. Barbachyn, C. W. Ford, *Angew. Chem.* **2003**, *115*, 2056 – 2070; *Angew. Chem. Int. Ed.* **2003**, *42*, 2010 – 2023.
[23] F. Walter, Q. Vicens, E. Westhof, *Curr. Opin. Chem. Biol.* **1999**, *3*, 694 – 704.
[24] R. Schroeder, C. Waldsich, H. Wank, *EMBO J.* **2000**, *19*, 1 – 9; Y. Tor, *Angew. Chem.* **1999**, *111*, 1681 – 1685; *Angew. Chem. Int. Ed.* **1999**, *38*, 1579 – 1582; K. Michael, Y. Tor, *Chem. Eur. J.* **1998**, *4*, 2091 – 2098.
[25] G. J. Zaman, P. J. Michiels, C. A. van Boeckel, *Drug Discov. Today* **2003**, *8*, 297 – 306.
[26] E. Ennifar, J. C. Paillart, R. Marquet, B. Ehresmann, C. Ehresmann, P. Dumas, P. Walter, *J. Biol. Chem.* **2003**, *278*, 2723 – 2730; C. Cabrera, A. Gutiérrez, J. Barretina, J. Blanco, A. Litovchick, A. Lapidot, B. Clotet, J. A. Esté, *Antiviral Res.* **2002**, *53*, 1 – 8; C. Faber, H. Sticht, K. Schweimer, P. Rösch, *J. Biol. Chem.* **2000**, *275*, 20 660 – 20 666; H.-Y. Mei, A. A. Galan, N. S. Halim, D. P. Mack, D. W. Moreland, K. B. Sanders, H. N. Truong, A. W. Czarnik, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2755 – 2760; J. B.-H. Tok, L. J. Dunn, R. C. Des Jean, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1127 – 1131.
[27] T. D. Eubank, R. Biswas, M. Jovanovic, A. Litovchick, A. Lapidot, V. Gopalan, *FEBS Lett.* **2002**, *511*, 107 – 112
[28] U. von Ahsen, H. F. Noller, *Science* **1993**, *260*, 1500 – 1503.
[29] S. Corvaisier, V. Bordeau, B. Felden, *J. Biol. Chem.* **2003**, *278*, 14 788 – 14 797.
[30] G. D. Wright, A. M. Berghuis, S. Mobashery in *Resolving the antibiotic paradox: progress in understanding drug resistance and development of new antibiotics* (Eds.: B. P. Rosen, S. Mobashery), Plenum Press, New York, **1998**, pp. 27 – 69.
[31] T. K. Ritter, C.-H. Wong, *Angew. Chem.* **2001**, *113*, 3616 – 3641; *Angew. Chem. Int. Ed.* **2001**, *40*, 3508 – 3533.
[32] J. Davies, L. Gorini, B. D. Davis, *Mol. Pharmacol.* **1965**, *1*, 93 – 106.
[33] T. Pape, W. Wintermeyer, M. Rodnina, *EMBO J.* **1999**, *18*, 3800 – 3807.
[34] J. M. Ogle, F. V. Murphy, M. J. Tarry, V. Ramakrishnan, *Cell* **2002**, *111*, 721 – 732.
[35] J. M. Ogle, A. P. Carter, V. Ramakrishnan, *Trends Biochem. Sci.* **2003**, *28*, 259 – 266.
[36] M. J. Cabanas, D. Vazquez, J. Modolell, *Biochem. Biophys. Res. Commun.* **1978**, *83*, 991 – 997; J. Davies, B. D. Davis, *J. Biol. Chem.* **1968**, *243*, 3312 – 3316.
[37] S. S. Phelps, O. Jerinic, S. Joseph, *Mol. Cell.* **2002**, *10*, 799 – 807.

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[38] K. Fredrick, H. F. Noller, *Science* **2003**, *300*, 1159 – 1162.
[39] M. P. Mingeot-Leclercq, P. M. Tulkens, *Antimicrob. Agents Chemother.* **1999**, *43*, 1003 – 1012.
[40] S. K. Moestrup, S. Cui, H. Vorum, C. Bregengard, S. E. Bjorn, K. Norris, J. Gliemann, E. I. Christensen, *J. Clin. Invest.* **1995**, *96*, 1404 – 1413.
[41] X. H. Du, C. L. Yang, *Nephrol. Dial. Transplant.* **1994**, *9*, *Suppl. 4*, 135 – 140.
[42] M. B. Acosta, R. C. Ferreira, G. Padilla, L. C. Ferreira, S. O. Costa, *J. Med. Microbiol.* **2000**, *49*, 409 – 413.
[43] B. D. Davis, *Microbiol. Rev.* **1987**, *51*, 341 – 350.
[44] E. C. Böttger, B. Springer, T. Prammananan, Y. Kidan, P. Sander, *EMBO Rep.* **2001**, *2*, 318 – 323.
[45] P. Pfister, S. Hobbie, Q. Vicens, E. C. Böttger, E. Westhof, *ChemBioChem* **2003**, *4*, 1078 – 1088.
[46] B. T. Wimberly, D. E. Brodersen, W. M. Clemons, Jr., R. J. Morgan-Warren, A. P. Carter, C. Vonrhein, T. Hartsch, V. Ramakrishnan, *Nature* **2000**, *407*, 327 – 339.
[47] J. M. Ogle, D. E. Brodersen, W. M. Clemons, Jr., M. J. Tarry, A. P. Carter, V. Ramakrishnan, *Science* **2001**, *292*, 897 – 902.
[48] P. Purohit, S. Stern, *Nature* **1994**, *370*, 659 – 662; M. I. Recht, D. Fourmy, S. C. Blanchard, K. D. Dahlquist, J. D. Puglisi, *J. Mol. Biol.* **1996**, *262*, 421 – 436; H. Miyaguchi, H. Narita, K. Sakamoto, S. Yokoyama, *Nucleic Acids Res.* **1996**, *24*, 3700 – 3706.
[49] Y. Tor, T. Hermann, E. Westhof, *Chem. Biol.* **1998**, *5*, R277 – 283; T. Hermann, E. Westhof, *J. Mol. Biol.* **1998**, *276*, 903 – 912.
[50] R. E. Botto, B. Coxon, *J. Am. Chem. Soc.* **1983**, *105*, 1021 – 1028.
[51] M. Kaul, D. S. Pilch, *Biochemistry* **2002**, *41*, 7695 – 7706.
[52] P. Auffinger, E. Westhof, *J. Biomol. Struct. Dyn.* **1998**, *16*, 693 – 707; B. Masquida, C. Sauter, E. Westhof, *RNA* **1999**, *5*, 1384 – 1395.
[53] P. Auffinger, E. Westhof, *Biophys. Chem.* **2002**, *95*, 203 – 210.
[54] M. M. Neuhauser, R. A. Weinstein, R. Rydman, L. H. Danziger, G. Karam, J. P. Quinn, *JAMA* **2003**, *289*, 885 – 888.
[55] T. Prammananan, P. Sander, B. A. Brown, K. Frischkorn, G. O. Onyi, Y. Zhang, E. C. Böttger, R. J. Wallace, Jr., *J. Infect. Dis.* **1998**, *177*, 1573 – 1581; M. Recht, S. Douthwaite, K. D. Dahlquist, J. D. Puglisi, *J. Mol. Biol.* **1999**, *286*, 33 – 43; P. Sander, T. Prammananan, E. C. Böttger, *Mol. Microbiol.* **1996**, *22*, 841 – 848.
[56] S. R. Lynch, J. D. Puglisi, *J. Mol. Biol.* **2001**, *306*, 1037 – 1058; S. Bar-Nun, Y. Shneyour, J. S. Beckmann, *Biochim. Biophys. Acta* **1983**, *741*, 123 – 127.
[57] B. H. Ali, *Gen. Pharmacol.* **1995**, *26*, 1477 – 1487; D. I. Kurtz, *Biochemistry* **1974**, *13*, 572 – 577.
[58] T. Hutchin, G. Cortopassi, *Antimicrob. Agents Chemother.* **1994**, *38*, 2517 – 2520; K. Hamasaki, R. R. Rando, *Biochemistry* **1997**, *36*, 12323 – 12328.
[59] J. L. Hansen, T. M. Schmeing, P. B. Moore, T. A. Steitz, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11670 – 11675.
[60] P. Pfister, M. Risch, D. E. Brodersen, E. C. Böttger, *Antimicrob. Agents Chemother.* **2003**, *47*, 1496 – 1502.
[61] F. Walter, J. Pütz, R. Giegé, E. Westhof, *EMBO J.* **2002**, *21*, 760 – 768.
[62] A. Nahvi, N. Sudarsan, M. S. Ebert, X. Zou, K. L. Brown, R. R. Breaker, *Chem. Biol.* **2002**, *9*, 1043.
[63] A. Bashan, I. Agmon, R. Zarivach, F. Schluenzen, J. Harms, R. Berisio, H. Bartels, F. Franceschi, T. Auerbach, H. A. Hansen, E. Kossoy, M. Kessler, A. Yonath, *Mol. Cell.* **2003**, *11*, 91 – 102; M. Mandal, J. Boese, J. E. Barrick, W. C. Winkler, R. R. Breaker, *Cell* **2003**, *113*, 577 – 586.
[64] W. Winkler, A. Nahvi, R. R. Breaker, *Nature* **2002**, *419*, 952 – 956.

Received: June 11, 2003 [M684]

 © 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim