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Drugs targeting the ribosome

Thomas Hermann

Several classes of clinically important antibiotics target the bacterial ribosome, where they interfere with microbial protein synthesis. Structural studies of the interaction of antibiotics with the ribosome have revealed that these small molecules recognize predominantly the rRNA components. Over the past two years, three-dimensional structures of ribosome–antibiotic complexes have been determined, providing a detailed picture of the binding sites and mechanism of action of antibacterials, including 'blockbuster' drugs such as the macrolides. Structure-based approaches have come to fruition that comprise the design and crystal structure analysis of novel semi-synthetic antibiotics that target the ribosome decoding site.

Addresses
Department of Structural Chemistry, Anadys Pharmaceuticals Inc, 3115 Merryfield Row, San Diego, CA 92121, USA

Corresponding author: Hermann, Thomas (tchermann@sbcglobal.net)

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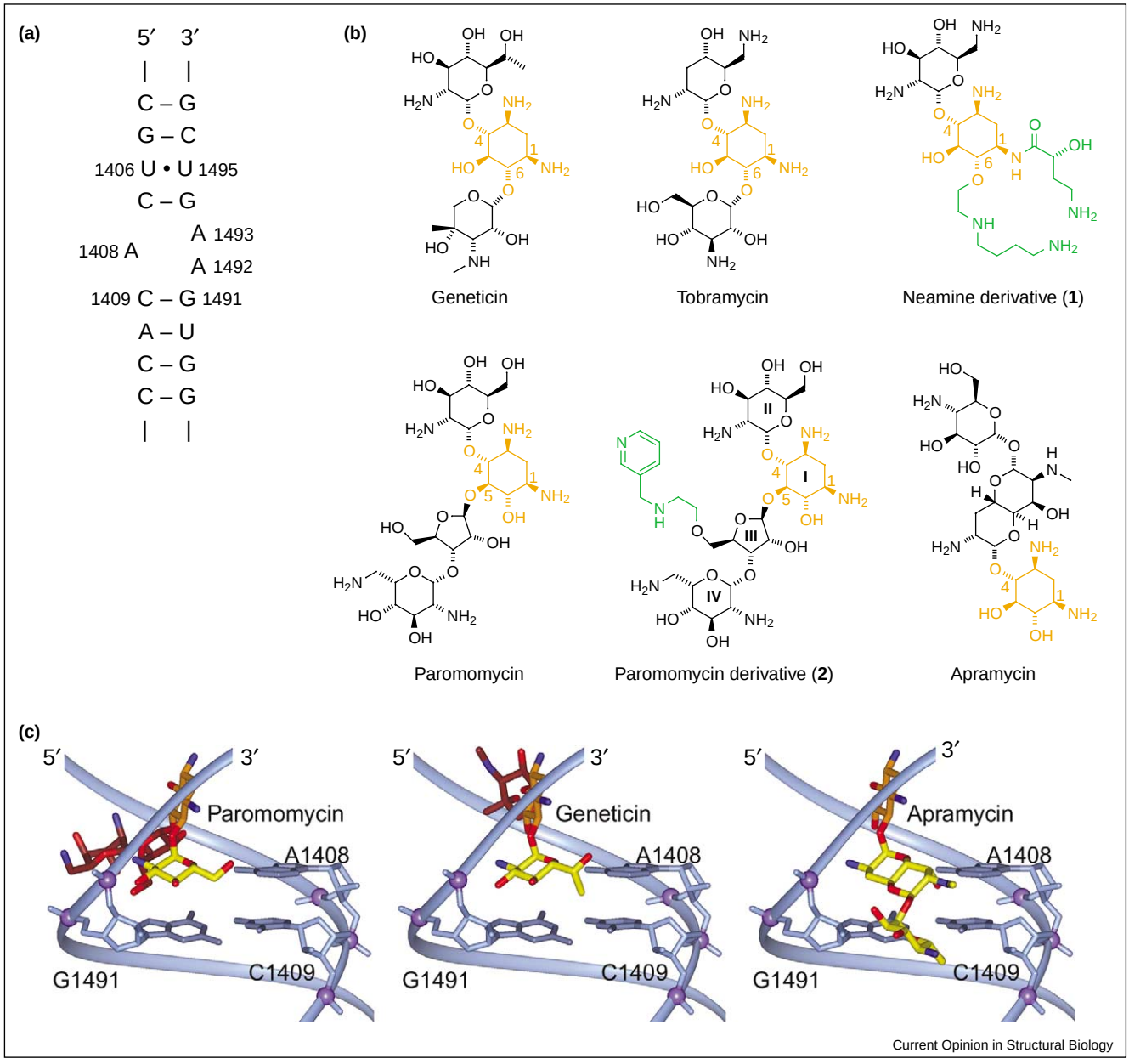
Introduction

The bacterial ribosome is the target of a chemically diverse group of natural secondary metabolites that bind to specific sites within the ribonucleoprotein complex, where they interfere with protein synthesis and ultimately inhibit microbial growth. Whereas the raison d'être of these natural products might be elusive [1], their discovery as antibiotics for the treatment of bacterial infections represents a major breakthrough of medicine in the 20th century. Ever since, a race began between the emergence of drug-resistant pathogens and the development of new antibiotics to combat them. Interest in the ribosome as a target for the discovery of new antibacterials has been spurred over the past few years by the structure determination of whole bacterial ribosomes, subunits, domains and antibiotic complexes thereof [2–5]. Three-dimensional structures of ribosome–antibiotic complexes have confirmed earlier biochemical work, revealing that the natural products interact predominantly with the RNA components of the ribosome (rRNA). Indeed, bacterial ribosomes contain the only validated RNA targets for which approved drugs are currently available. Therefore, ribosome-binding antibiotics provide an important paradigm for the study of RNA molecular recognition by small molecules. This review outlines advances in the structural analysis of ribosome–antibiotic complexes over the past two years. The first successes of structure-based approaches to the development of novel ribosome-targeted antibacterials will be discussed.

The decoding site as a target of aminoglycosides

The decoding site (or A-site) within bacterial 16S rRNA of the 30S subunit constitutes the 'reading head' of the ribosomal machinery that is involved in deciphering the mRNA code. The accurate match between the mRNA codon and the anticodon of the aminoacylated-tRNA is monitored by direct interaction with adenine bases of the decoding site structure, which, at its core, consists of a small internal rRNA loop. The decoding site loop is the target of many natural aminoglycoside antibiotics that, upon binding, interfere with translational fidelity by facilitating amino acid misincorporation (Figure 1). From 2000 onwards, structural data from X-ray crystallography have been emerging on the whole 30S ribosomal subunit complexed with several antibiotics, including the clinically relevant tetracyclins [6,7] and the decoding-site-binding aminoglycoside paromomycin [8]. Even before the spectacular successes of ribosome crystallography, an oligonucleotide that contained the decoding site RNA sequence was the first ribosomal fragment bound to an antibiotic for which a three-dimensional structure became available, when Puglisi and co-workers [9] determined the solution structure of the paromomycin complex. This early NMR work was later followed by analysis of crystal structures of decoding site oligonucleotide complexes with paromomycin [10], tobramycin [11] and, most recently, geneticin [12], performed in the Westhof laboratory. The availability of both NMR and high-resolution crystal structures of the paromomycin–decoding site complex allowed the comparison of the two techniques, and confirmed the validity of crystal structures for the investigation of conformationally flexible sites in RNA folds [13,14]. Importantly, the crystal structures of the aminoglycoside complexes with oligonucleotides reproduce accurately the natural state of the decoding site bound to aminoglycosides within the whole 30S subunit, and therefore provide authentic and readily accessible models of the molecular recognition of the ribosome *in vivo* [13]. This has been confirmed by fluorescence experiments using oligonucleotide model systems to investigate the

Figure 1



The ribosomal decoding site RNA **(a)** is the target of natural aminoglycosides and semi-synthetic derivatives **(b)**. Crystal structure analysis has been used to determine three-dimensional structures of decoding site RNA complexes with these antibiotics. The 2-DOS core, which is a shared determinant of RNA specificity among the aminoglycosides, is highlighted in orange. Synthetic modifications of the natural cores in derivatives **1** [20] and **2** [21] are in green. Bases that participate in interactions discussed in the text are numbered according to the *E. coli* 16S rRNA sequence. **(c)** Three-dimensional structures of paromomycin [10], geneticin [12] and apramycin [18•] bound to the decoding site RNA. Glycoside pseudo-base pairs form between A1408 and the terminal sugars (yellow) of paromomycin and geneticin, and the bicyclic sugar of apramycin. The apramycin complex also contains a pseudo-triple between the terminal sugar and G1491•C1409 [18•].

conformational flexibility of unpaired adenine residues 1492 and 1493, which are locked in one position upon binding aminoglycoside antibiotics [15,16].

A detailed analysis of the structures of the decoding site complexes with paromomycin, tobramycin and geneticin, in the context of genetic data, allowed the exploration of the molecular basis of various mutations that confer aminoglycoside resistance [17••] (see also Update). This study emphasizes that the U1406•U1495 pair and the A1408 residue of the decoding site RNA, along with the 2-deoxystreptamine (2-DOS) moiety of the aminoglyco-

sides, play key roles in the specific binding of the antibiotics to the bacterial decoding site. In the paromomycin, tobramycin and geneticin complexes, A1408 forms hydrogen bonds with the sugar substituent at the 4-position of the 2-DOS moiety (Figure 1), giving rise to a pseudo-base pair arrangement between the RNA base and the aminoglycoside sugar [13,17••]. Interestingly, a virtually identical geometry has been observed for the interaction of the bicyclic sugar of apramycin with A1408 [18•]. The apramycin complex additionally contains an unprecedented pseudo-base triple arrangement between the terminal sugar and the minor groove edge of the C1409•G1491 pair.

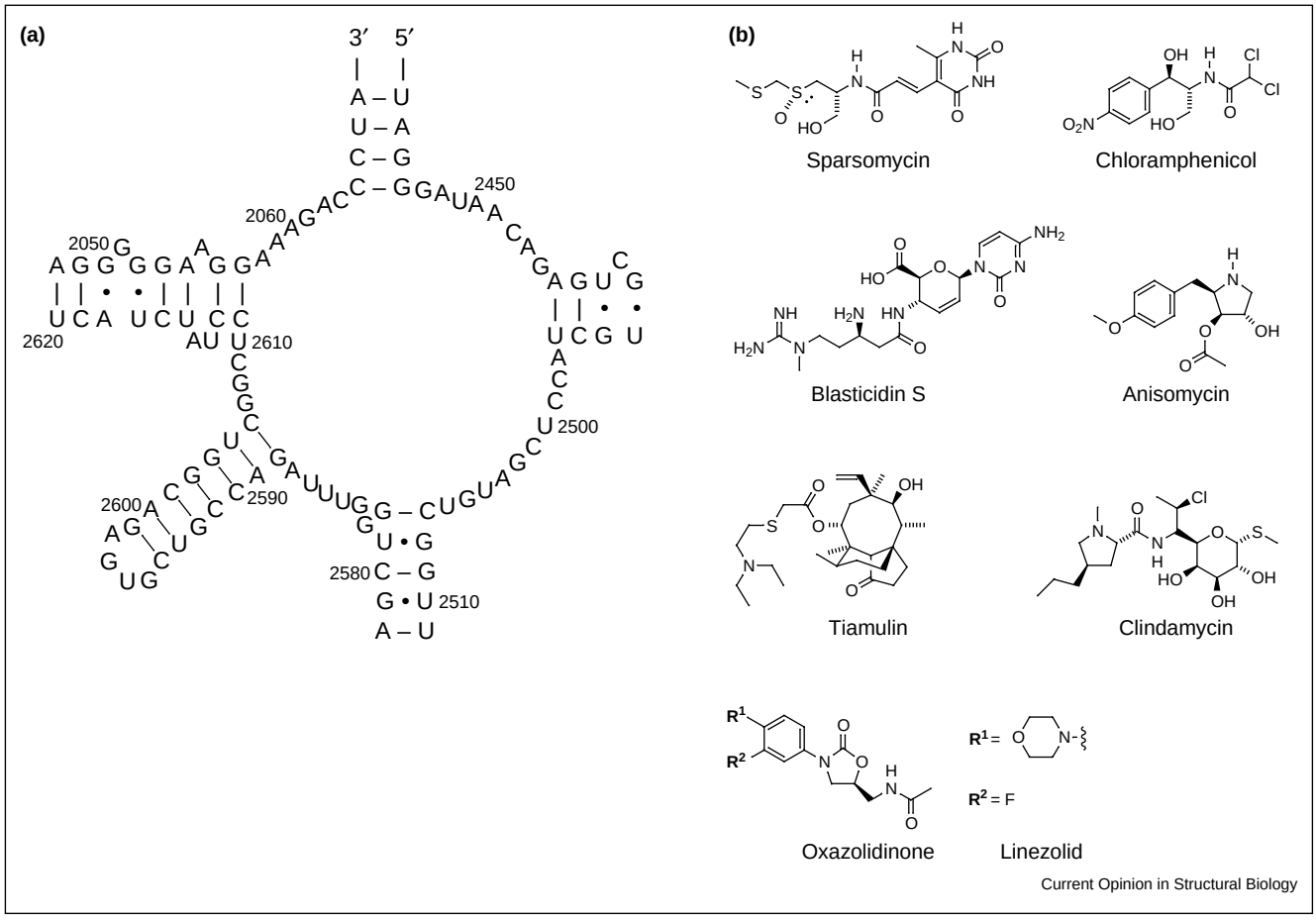
Because natural aminoglycosides suffer from low bioavailability and toxicity, both linked to their polycationic character, numerous attempts have been made to develop improved analogues and mimetics. Over the past few years, the availability of structural data on aminoglycoside complexes with the decoding site has spurred efforts to design and synthesize novel synthetic and semi-synthetic ligands of this RNA target [19]. Crystal structures were published of decoding site complexes with two semi-synthetic aminoglycosides, including a neamine derivative (**1**, Figure 1b) [20] and a modified paromomycin (**2**, Figure 1b) [21], both of which were active against drug-resistant bacteria. The structure of the neamine derivative (**1**) complex reveals that the N1-linked substituent of the ligand overlapped the binding site for hygromycin B, an aminoglycoside that recognizes an RNA structure immediately adjacent to the decoding site RNA. Structural data on the 30S ribosomal subunit have been used to specifically design artificial ligands that bridge the decoding site and the adjacent hygromycin-B-binding site [22]. In the crystal structure of the paromomycin derivative (**2**) complex, sugar rings III and IV adopt a unique binding conformation at the RNA target, whereas the positions of conserved 2-DOS ring I and sugar ring II are identical to those in the paromomycin complex [21], emphasizing the importance of the latter ring systems for specific decoding site recognition by aminoglycoside ligands.

The peptidyl-transferase center and peptide exit tunnel as targets of clinically important antibiotics

The peptidyl-transferase center (PTC) is the proper active site of the ribosome, providing the spatial and chemical environment that catalyzes peptide bond formation. Although the recently determined crystal structures of the 50S ribosomal subunit revealed that the environment of the PTC consists entirely of nucleotides of the 23S rRNA, it is still unclear whether the role of the active site is exclusively stereochemical in nature, by favorably pre-orienting the substrates, or whether the rRNA participates actively in the chemistry of peptide bond formation [23]. Given the key function of the PTC in protein synthesis, it should not be too surprising that numerous classes of naturally occurring translation inhibitors target this site on the ribosome. Indeed, several clinically important antibiotics, including the macrolides, streptogramins, chloramphenicol and the oxazolidinones, bind to rRNA at the PTC (Figures 2–4). So far, structural data are not available in the public domain from crystallography efforts on ribosomal complexes of the oxazolidinones. These antibiotics constitute the only fully synthetic class of inhibitor of the bacterial ribosome approved for use in humans (linezolid; Zyvox®) [24]. Biochemical, cross-linking and NMR studies indicate interaction of the oxazolidinones with 23S rRNA in the immediate vicinity of the PTC, in a region that overlaps the chloramphenicol-binding site [25,26].

Crystallography efforts have yielded three-dimensional structures of several PTC-binding ligands complexed with the 50S ribosomal subunit [3,27]. In most cases, the structural data not only are in excellent agreement with findings from earlier biochemical and genetic studies, but also provide a molecular basis for the mechanism of action of the translation inhibitors. Among the natural products for which co-crystals with the ribosomal subunit have been analyzed recently are sparsomycin, blasticidin S and anisomycin (Figure 2) [28•], all of which lack target specificity for the bacterial ribosome, thus ruling out their use as antibiotics. Interestingly, the cytosine derivative blasticidin S has been identified as binding to 23S rRNA at two non-overlapping sites, in each case forming a Watson–Crick-type interaction with a guanine residue [28•]. In the case of chloramphenicol (Figure 2), two different binding sites on the PTC have been observed in complexes with the 50S subunits from the eubacterium *Deinococcus radiodurans* [29] and the archaeon *Haloarcula marismortui* [28•]. As the validity of both sites is supported by a large body of biochemical and genetic data, including cross-linking experiments that confirm the presence of the *Haloarcula* site in the eubacterium *Escherichia coli* [30], they may be equally relevant to the antibiotic action of chloramphenicol [28•]. Binding of chloramphenicol to the site observed in the *Deinococcus* structure requires displacement of a magnesium ion present in the native 50S subunit and results in the creation of two new binding sites for intermediary metal ions, which might play a role in the inhibition mechanism of this antibiotic [29]. In antibiotic complexes with the *Deinococcus* 50S subunit, the chloramphenicol-binding site overlaps the binding regions for clindamycin, a lincosamide antibiotic [29], and tiamulin, a semi-synthetic pleuromutilin derivative that belongs to a somewhat exotic class of fungal diterpenoid metabolite (Figure 2) [31,32•]. A 50S ribosomal complex with tiamulin revealed the antibiotic bound in an RNA cavity formed mostly by bases of the PTC [32•]. In addition to hydrogen bonding, hydrophobic interactions of the base heterocycles contribute significantly to the stabilization of the tiamulin complex through van der Waals contacts with the tricyclic hydrocarbon core of the

Figure 2

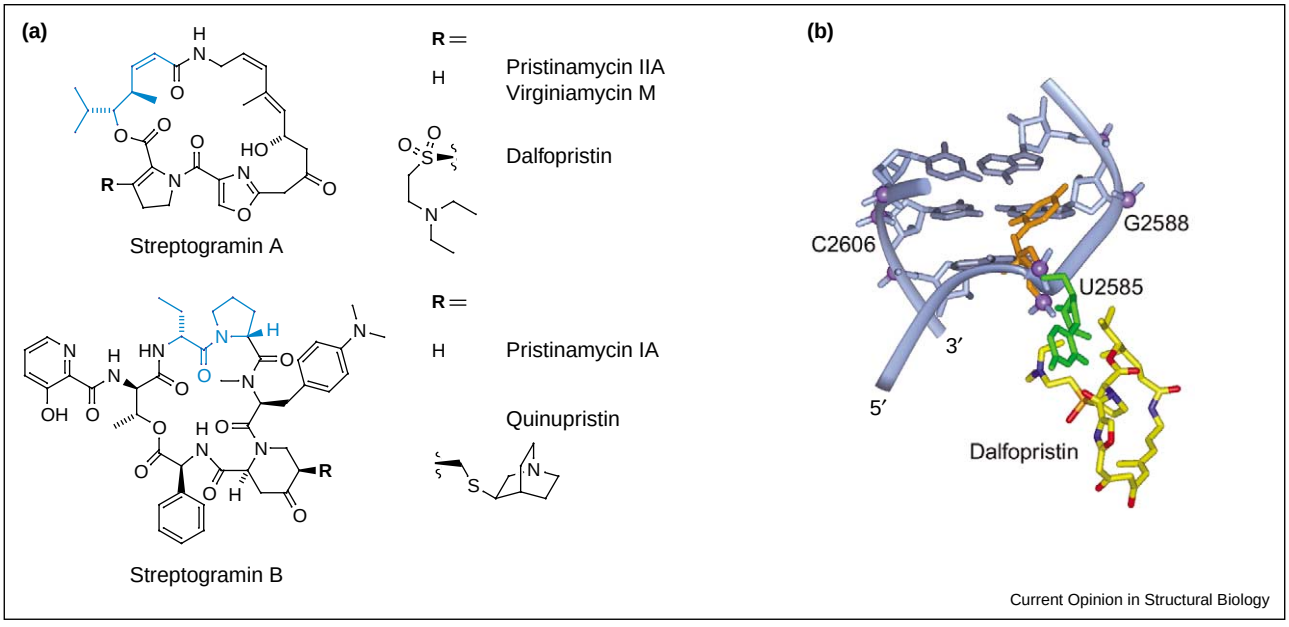


The ribosomal PTC **(a)** is targeted by a chemically diverse set of antibiotics **(b)**. Crystal structures have been published of 50S ribosomal subunits complexed with the natural products shown in (b) (although not the oxazolidinones). Oxazolidinones are so far the only class of totally synthetic antibiotics that bind to the ribosome.

diterpenoid. Interestingly, tiamulin binds at virtually the same position as the structurally unrelated macrolactone streptogramin A (Figure 3), which adopts a conformation that projects non-polar hydrocarbon fragments of the macrocycle towards the hydrophobic tiamulin-binding cavity [32•,33••].

The streptogramin antibiotics comprise two chemically distinct subfamilies of natural products, types A and B (Figure 3), which are co-synthesized by the same bacterial species in a ratio of approximately 7:3. Type A streptogramins, which are unsaturated macrolactones, and type B streptogramins, which are macrocyclic peptides of unusual amino acids, act synergistically on the ribosome by eliciting a bacteriocidal effect (although the individual components are bacteriostatic) [34]. A 7:3 mixture of streptogramin A and B derivatives (semi-synthetic dalfopristin and quinupristin, respectively) is marketed as Synercid® (Figure 3). The three-dimensional structure of the Synercid components complexed with the *Deinococcus* 50S subunit has been published [33••], together with an earlier structure of the streptogramin A compound virginiamycin M bound to the *Haloarcula* target [28•], revealing the binding sites of the antibiotics. The structural data yield insight into the observation that the presence of streptogramin A stimulates the binding of the B type compounds and provide a potential explanation for the post-antibiotic effect of the streptogramin A component [28•,33••]. The structures of the ribosomal complexes with the streptogramin A derivatives dalfopristin and virginiamycin M are in reasonable agreement, given the differences between the *Deinococcus* and *Haloarcula* species. The streptogramin A lactone ring forms hydrophobic interactions and three hydrogen bonds in a non-polar cavity shaped by residues of the PTC, overlapping with binding sites for chloramphenicol and tiamulin [33••]. The hydrocarbon fragment between the macrocyclic ester and amide linkages of streptogramin A,

Figure 3



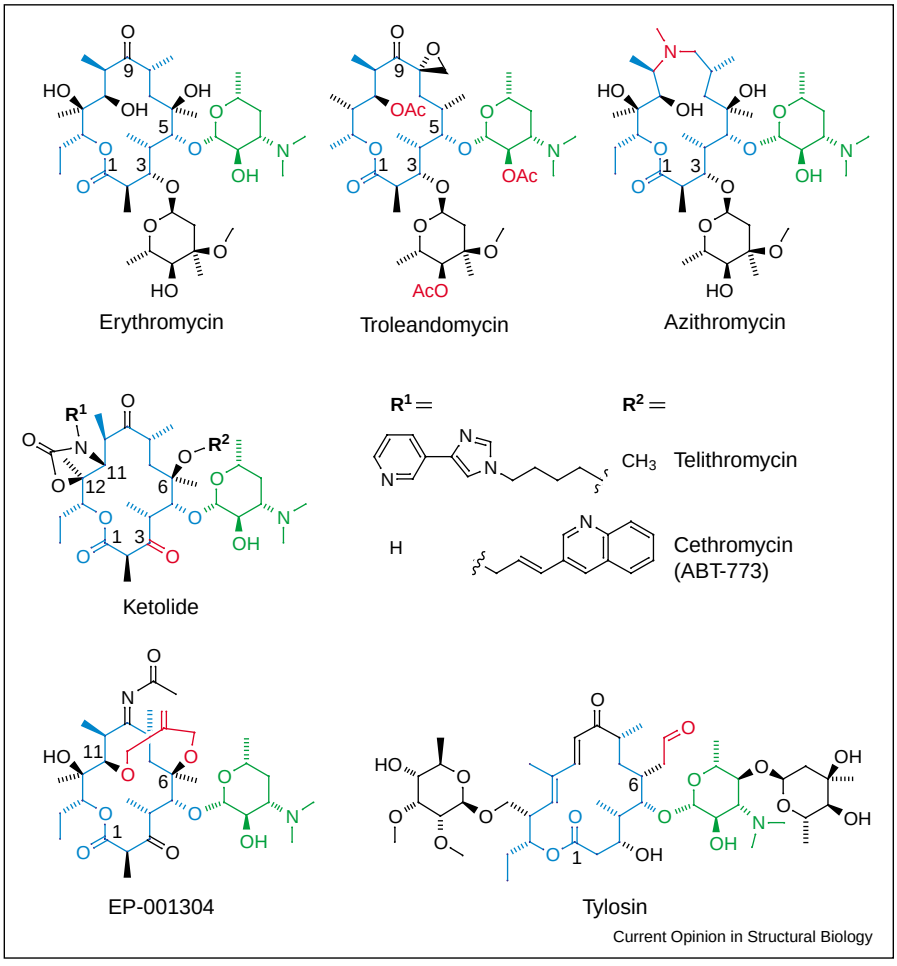
Streptogramin antibiotics and their mechanism of action. **(a)** Natural and semi-synthetic macrocyclic streptogramin antibiotics that target the ribosomal PTC. The presence of the A type macrolactones stimulates binding of the B class cyclic peptides to an adjacent site on the PTC. When bound to the ribosome, the antibiotics interact with each other via hydrophobic contacts of the fragments highlighted in blue. **(b)** Conformational change of U2585 induced by the binding of a streptogramin A antibiotic to the PTC. In the ligand-free state, U2585 points towards the drug-binding site (green), whereas in complex with dalfopristin, the nucleotide is flipped towards the G2588•C2606 base pair (orange) [33••].

including the exocyclic isopropyl group, provides a non-polar contact surface that stabilizes the binding of streptogramin B to the PTC, by allowing strong hydrophobic interactions between the two compounds. Streptogramin B is located at the entrance to the peptide exit tunnel, occupying space that coincides with the binding site for macrolide antibiotics. The streptogramin complex with the *Deinococcus* 50S subunit reveals a localized conformational change in the orientation of residue U2585, which has been hypothesized as the source of the bacteriocidal activity and the post-antibiotic effect of the Synercid® combination [33••,35]. In the antibiotic-bound state, U2585 forms alternative hydrogen bonds with the G2588•C2606 base pair, which might lock the PTC in a pseudo-stable conformation that returns to the native state only slowly after removal of the drug (Figure 3) [33••]. This phenomenon would be a rather spectacular example whereby the hysteresis of an RNA conformational switch causes a time-delayed biological function — here, the return of the ribosomal machinery to a productive state.

The spatial overlap of the ribosomal binding sites for chloramphenicol, clindamycin, tiamulin and the streptogramins has led to suggestions to use this structural information in the design of hybrid ligands by combining parts of these antibiotics [32•]. Although the chemical complexity of the resulting hybrid molecules might limit the scope of this approach for drug design, bifunctional ribosome ligands have recently been synthesized by linking the decoding site binder neomycin B with chloramphenicol or an oxazolidinone [36]. Results of biological testing of these bifunctional compounds have yet to be reported.

The macrolides are perhaps the clinically most important class of ribosome-targeted antibiotic (Figure 4) [37] and include the semi-synthetic ketolides, which were recently approved for use in humans (telithromycin, Ketek®). Crystal structures of macrolides complexed with the 50S ribosomal subunit have been emerging since 2001 [29,38,39••,40•,41••], illustrating the stunning molecular mechanism of action of these compounds (see also Update). Macrolides act as 'plugs' that block the progression of the nascent peptide through the ribosomal exit tunnel, which eventually leads to dissociation of the peptidyl-tRNA from the ribosome [42]. Three-dimensional structures are now available of 50S complexes with members of all important macrolide classes, including 14- and 16-membered lactones, such as erythromycin and tylosin, as well as the semi-synthetic 14-membered ketolides and 15-membered azalides (Figure 4). The crystal structures show the various macrolides binding adjacent to the PTC, about 10–15 Å into the entrance region of the

Figure 4



Natural and semi-synthetic macrolide antibiotics that target the ribosomal PTC at the peptide exit tunnel. Representatives are shown of the 14- and 16-membered lactones (erythromycin, troleandomycin, tylosin), as well as the 15-membered azalides (azithromycin) and the 14-membered ketolides (telithromycin, cethromycin, EP-001304). Crystal structures of these antibiotics bound to the 50S ribosomal subunit have been published for all but the 6,11-bridged ketolide EP-001304. The common lactone core is marked in blue; the shared desosamine moiety at the 5-position is in green. Unique structural features of the different macrolide classes are highlighted in red: troleandomycin carries peracetylated hydroxyl groups; azithromycin is a ring-expanded N-hetero-lactone; the ketolides have a carbonyl group at the 3-position that replaces the cladinose moiety of the natural macrolides; in EP-001304, an ether bridge connects the 6- and 11-positions of the macrocycle; like many 16-membered macrolides, tylosin contains an aldehyde functionality that has been implicated in the formation of a reversible covalent bond with rRNA [38].

polypeptide exit tunnel, where they sterically block the passage of the nascent peptide. Interestingly, Gram-negative bacteria such as *E. coli* produce a regulatory protein, ribosome modulation factor, that was suggested to inhibit protein synthesis under stress conditions by reversibly associating with the PTC and covering the peptide exit tunnel, similar to the macrolide antibiotics [43].

The crystal structures show the macrocycle bound within the tunnel and the saccharide substituent at the 5-position extended towards the PTC. With the exception of troleandomycin and azithromycin, which will be discussed below, direct interactions between the antibiotics and the ribosome form solely via residues of the 23S rRNA [29,38,39••,40•,41••]. Ribosomal proteins L3, L4, L22 and L34 contribute water-mediated contacts to the binding pocket. For the 50S complex with the 16-membered macrolide tylosin (Figure 4), crystal structure data suggest the formation of a reversible covalent bond between the aldehyde functionality at the 6-position and the exocyclic amino group of an adenine residue [38]. Electron density connecting these groups is consistent with the presence of a hemi-aminal functionality whose dehydration to the Schiff base might be prevented in the ribosome interior. For the ketolides, the loss of interactions with rRNA due to removal of the cladinose substituent at the 3-position is compensated by the addition of heteroaryl groups at either the 6-position

(cethromycin, formerly ABT-773) or the 11-position (telithromycin), or by modifications that bridge these positions (EP-001304 and bridged ketolides derived thereof [44]). Interestingly, even these synthetic modifications of the ketolide core participate exclusively in interactions with the RNA [39••,40•].

Azithromycin, a ring-expanded N-hetero-lactone (Figure 4), is the only macrolide that revealed a secondary binding site, immediately adjacent to and in direct contact with the primary binding site [39••]. Whereas the primary binding sites in both the *Haloarcula* and *Deinococcus* 50S subunits are positioned similarly to other macrolide-binding sites [38,39••], azithromycin bound to the secondary site in *Deinococcus* showed direct interactions with ribosomal proteins L4 and L22. It is not clear whether the second azithromycin-binding site observed in the crystal structure is of biological importance or rather is an artifact of the *Deinococcus* complex. It should be noted, however, that a careful genetic analysis of the effects of rRNA mutations on macrolide binding supports a binding mode for azithromycin that is very similar, if not identical, to that of 14-membered macrolides such as erythromycin [45••] (see also Update).

Troleandomycin is a semi-synthetic peracetylated macrolide that carries a potentially reactive spiro-oxirane functionality at the 8-position (Figure 4). Acetylation of the three hydroxyl groups of the macrolactone and the sugar substituents prevents the formation of hydrogen bonds in the 50S complex that are critical interactions in other macrolide complexes [41••]. As a consequence, troleandomycin binds at a position shifted deeper into the peptide exit tunnel and forms at least one direct contact with a ribosomal protein, L22. Importantly, troleandomycin triggers a significant conformational rearrangement of a domain of the L22 protein, leading to remodeling of the exit tunnel geometry. It has been suggested that L22 acts as a conformational switch that provides a gating mechanism for the control of nascent peptide elongation [41••].

The binding site for the macrolides is in good agreement among the different published structures. Some ambiguity remains concerning the conformation and precise orientation of the lactone ring, which adopts considerably different states in the crystal structures obtained for 50S complexes from *H. marismortui* and *D. radiodurans* [38,39••]. Conformational analyses of the macrolide core, together with the *Haloarcula* complex structures and genetic data, support the notion that a folded-out conformation of the lactone ring is preferred and required for effective binding to the ribosome, and thus for antibiotic efficacy of macrolides and ketolides [38,45••] (see also Update). In this conformation, most polar groups of the macrocycle are projected towards one face of the molecule, whereas the opposite side is hydrophobic. Semi-synthetic macrolides have been described, most prominently the 6,11-bridged ketolides [44], that aim to lock the lactone ring in the preferred conformation.

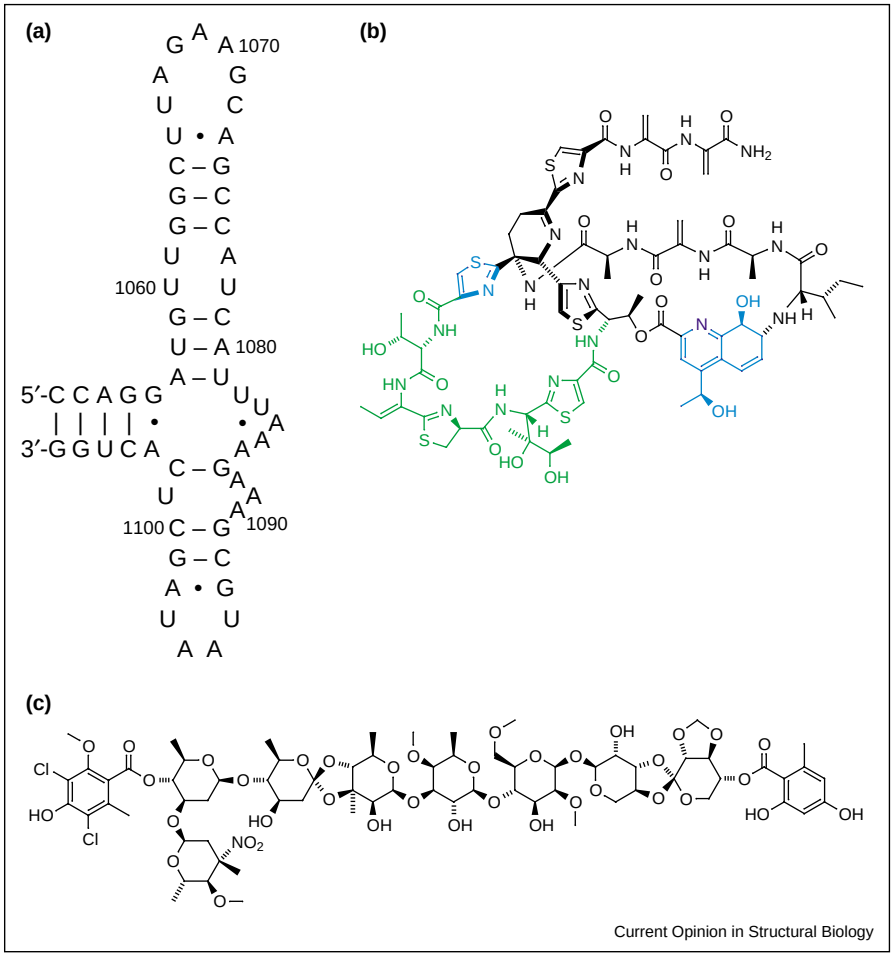
The uncertainty concerning the exact binding orientation of the macrolide core scaffold is a reminder of the ribosome's dynamic nature. The machinery of protein synthesis has evolved as a precision assembly line, with parts constantly in motion and pieces moving through its interior. Any static picture obtained by X-ray diffraction of ribosomes in the crystal will necessarily reflect a somehow artificial state of an immobilized machine whose complex workings can be only imperfectly deduced from the resting positions of its parts. Thus, the complete picture of macrolide binding and activity is likely to be more complex, perhaps involving transient interactions during the first steps of translation, and the formation of additional contacts between the antibiotics and the nascent peptide.

Other ribosome sites emerging as drug targets

In addition to the decoding site and the PTC, the ribosomal targets of thiostrepton and evernimicin (Figure 5) have come into the focus of antibiotic discovery efforts. In contrast to the antibiotics discussed above, binding sites have been identified for thiostrepton and evernimicin that are likely to involve both rRNA and proteins. Although crystal structures of ribosomal complexes are not available for either of these natural products, biochemical and NMR data have been used to map their target sites on the 50S subunit. Footprinting and mutation data suggest that evernimicin, a complex oligosaccharide antibiotic [46], binds to two hairpins (91 and 89) that are located on short extensions of 23S rRNA from the PTC [47]. Additional contacts might be formed with ribosomal protein L16 [47,48]. The evernimicin-binding site does not overlap with any other binding site for currently used antibiotics, which renders cross-resistance unlikely.

Thiostrepton, the most prominent member of the thiopeptide antibiotic family, which includes roughly 30 natural products from actinomycetes and *Streptomyces*, binds to 23S rRNA at the GTPase-associated domain of the 50S ribosomal subunit [49]. Although the water insolubility of thiostrepton has prevented crystallographic studies of these thiazole peptides in the past, NMR and biochemical studies have recently led to the construction of a three-dimensional model of thiostrepton docked to its RNA target [50]. The model was constructed using seven intermolecular NOEs (nuclear Overhauser enhancements) between the antibiotic and the RNA to dock the crystal structures of thiostrepton alone and of the RNA domain complexed with the L11 protein. The docked thiostrepton–RNA model suggests that a thiazole and a quinaldic acid fragment of the macrocycle might stack on unpaired adenine residues A1067 and A1095; this

Figure 5



The ribosomal GTPase site within 23S rRNA **(a)**, which is also the binding site for the L11 protein, is targeted by thiostrepton **(b)**, a cyclic peptide antibiotic. Thiostrepton fragments that were suggested to participate in the interaction with both the RNA and L11 protein are colored. The heterocycle scaffolds marked in blue (thiazole and quinaldic acid) might stack on A1067 and A1095 in the hairpin loops of the RNA (see text for discussion). **(c)** Evernimicin, an oligosaccharide antibiotic, binds to an RNA–protein interface between 23S rRNA and L16 protein.

is in agreement with biochemical, genetic and fluorescence data [50,51]. Additional contacts between thiostrepton and the L11 protein are likely to occur in the ribosome–antibiotic complex [50,52]. Insights from the thiostrepton–RNA model were used for structure-based design of peptide fragments as simplified mimetics of the macrocycle [53].

Conclusions

During the past five years, structure determination of the ribosome, its subunits and domains thereof has fueled an unprecedented expansion of insight into the cellular machinery of protein synthesis. Almost as a by-product, a wealth of structural data has emerged from this work that shed light on the molecular recognition of ribosomal components by small-molecule ligands. Structures of ribosomal subunits and their complexes with antibiotics have been published in short succession, attesting to the importance of small-molecule ligands as tools to explore and understand ribosome function. Antibiotics have a long history in ribosome research and it is now, with the availability of crystal structures, that we can fully appreciate the astounding level of detail to which the intricacies of the ribosomal machinery have been elucidated in the past using small-molecule ligands as instruments of investigation. Even now, with three-dimensional structures available of the ribosome and its components, small molecules remain irreplaceable tools for the study of ribosome function [54,55]. Many questions about the regulation of the translation machinery remain open, and the frontiers of ribosome research have shifted from the static structures to the challenge of unraveling the dynamics of the ribosome [35,56–59]. Investigations of the mechanism and dynamics of the

ribosome continue to rely heavily on small molecules for the preparation of uniform states of the translation machinery [60–62]. Structures of ribosome–antibiotic complexes illustrate the ability of small molecules to trigger conformational changes, as in the case of the streptogramins and troleandomycin, which has been implicated in interfering with a gating switch in the peptide exit tunnel. Prominent examples are the aminoglycosides, whose mechanism of action is based on the locking of one conformational state of the ribosomal decoding site [5,8].

Even more importantly than their role as tools for the study of the translation machinery, small-molecule ligands of the ribosome have proven to be invaluable drugs for the therapy of bacterial infections. All important ribosome-targeted antibiotics that are in use today were discovered and developed without explicitly exploiting knowledge of their binding sites and their interactions with ribosomal components. A recent survey of the patent literature shows that virtually all ribosome-directed antibiotics disclosed over the past two years seem to be the result of traditional medicinal chemistry, genetic engineering of producer organisms or natural product screening [19]. Even for the most recently emerging class of antibiotic, the synthetic oxazolidinones (which were discovered well into the age of advanced molecular biology), knowledge of their binding site and its three-dimensional structure is only beginning to be exploited for drug development [24,25]. However, the recent prolific expansion of structural information on antibiotics binding to the ribosome is expected to have a major impact on the development of novel antibiotics. Two areas of drug discovery will benefit most. First, and with successes expected within the next several years, the structure-guided improvement of established scaffolds that target the ribosomal PTC, specifically the macrolides, streptogramins, lincosamides and oxazolidinones. Both medicinal chemistry programs and the genetic manipulation of producer organisms will profit from structural data that allow the design of specific improvements to the complex natural products. Second, and perhaps lagging behind the former approaches by a few years, the design of new antibiotics based on novel chemical entities that target the bacterial ribosome. Given the time required for the development and disclosure of new drugs, we can expect to see the fruits of structure-based approaches emerging within the next five years. Overall, solving three-dimensional structures of the ribosome marks the beginning of a new era. Exciting times are ahead in ribosome research that will see, hand in hand, the unraveling of the mechanism of the ribosome and the application of this knowledge to the development of powerful new antibiotics.

Update

Recent work suggests that mutagenesis of the C1409•G1491 base pair differentiates between 6′-hydroxy- and 6′-amino-substituted aminoglycosides [63•]. Resistance effects of mutations at positions 1409 or 1491 are more pronounced for 6′-hydroxy-substituted aminoglycosides, although there is no direct interaction between the mutated RNA bases and the ligand 6′-position. The 6′-amino substituent is protonated under physiological conditions, imparting an additional strong electrostatic component to the interaction with the RNA and thus rendering binding of 6′-amino-substituted aminoglycosides less susceptible to mutations.

The recent publication of ribosomal co-crystal structures of some macrolides, ketolides and streptogramins [64••] has specifically addressed previously reported ambiguities concerning the conformation of macrolide antibiotics in 50S ribosomal complexes from the eubacterium *D. radiodurans* [29,39••,40•] and the archaeon *H. marismortui* [38]. The newly reported structures were obtained with G2099A (corresponding to A2058 in *E. coli*) mutant ribosomes from *Haloarcula*, which bind macrolides at affinities comparable to bacterial ribosomes. The *Haloarcula* complexes confirm that the macrolide erythromycin, the azalide azithromycin and the ketolide telithromycin all bind to the ribosome in almost exactly the same way, adopting a folded-out conformation of the lactone ring that projects a polar surface into the lumen of the peptide exit tunnel and a hydrophobic face towards the tunnel wall [64••]. Moreover, the ribosome-bound conformations of the antibiotics are very close to those of the free compounds.

The impact of rRNA sequence polymorphism on the A2058G macrolide resistance mutation has been investigated recently by Böttger and co-workers [65•]. The authors demonstrate that the degree of ketolide resistance conferred by the A2058G mutation is modulated by the identity of bases at the 2057•2611 base pair, explaining earlier findings for clinically relevant telithromycin resistance in pathogens. This work exploits crystal structures of ribosome–macrolide complexes to provide insight into the molecular basis of ketolide resistance and the interplay of the A2058G mutation with the polymorphic base pair.

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

- • of special interest
- •• of outstanding interest

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