Ribosome-targeting antibiotics and mechanisms of bacterial resistance

Daniel N. Wilson[1,2]

Abstract | The ribosome is one of the main antibiotic targets in the bacterial cell. Crystal structures of naturally produced antibiotics and their semi-synthetic derivatives bound to ribosomal particles have provided unparalleled insight into their mechanisms of action, and they are also facilitating the design of more effective antibiotics for targeting multidrug-resistant bacteria. In this Review, I discuss the recent structural insights into the mechanism of action of ribosome-targeting antibiotics and the molecular mechanisms of bacterial resistance, in addition to the approaches that are being pursued for the production of improved drugs that inhibit bacterial protein synthesis.

Ribosomes are the protein-synthesizing factories of the cell. These large macromolecular machines provide the platform on which amino acids are polymerized in a template-dependent fashion to form polypeptide chains[1]. Given the fundamental nature of protein synthesis, it is not surprising that this process represents one of the main antibiotic targets in the bacterial cell[2–4].

The bacterial ribosome is composed of three ribosomal RNAs (rRNAs; 16S, 23S and 5S) and ~54 ribosomal proteins, with rRNA dominating the main functional sites. Protein synthesis can be divided into four main steps: initiation, elongation, termination and recycling (FIG. 1). Initiation involves the formation of a 70S ribosome from the constituent small (30S) and large (50S) subunits, and the accurate positioning of the mRNA start codon (usually AUG), together with the initiator tRNA (typically fMet-tRNA) at the ribosomal P-site. The efficiency and fidelity of this process is controlled by three specialized translation initiation factors: IF1, IF2 and IF3. The elongation phase involves a cycle of four major steps. First, an aminoacylated tRNA (aa-tRNA) is delivered to the A-site of the ribosome by elongation factor Tu (EF-Tu) in complex with GTP. During the process of decoding, the ribosome monitors the base-pairing interaction between the mRNA codon and the tRNA anticodon, ensuring that only tRNAs bearing the correct amino acid are accommodated. Peptide-bond formation occurs between the amino acids attached to the tRNAs in the A- and P-sites, resulting in transfer of the amino acid (or the polypeptide chain in later elongation cycles) from the P-site tRNA to the aa-tRNA in the A-site. In order to accommodate the next incoming aa-tRNA, the tRNAs are moved from the A- and P-sites to the P- and E-sites in a process known as translocation, which is facilitated by EF-G (BOX 1). As the polypeptide chain elongates, it passes through an exit tunnel in the 50S subunit before entering the cytoplasm, where protein folding occurs. The elongation cycle continues until a stop codon is encountered. Stop codons are recognized by termination release factors (RF1 and RF2) that hydrolyse the peptidyl-tRNA bond, releasing the polypeptide chain from the ribosome. The post-termination complex is then disassembled, thus allowing the components to be recycled for the next round of translation initiation.

Decades of biochemical studies, and more recent structural studies, have revealed the molecular basis by which a large and diverse array of antibacterial agents specifically inhibit protein synthesis[4,5] (FIG. 1; TABLE 1). The majority of known compounds target the elongation cycle, including all of the clinically important antibiotic classes: the aminoglycosides, chloramphenicols, fusidic acids, lincosamides, macrolides, oxazolidinones, streptogramins and tetracyclines[2]. Although a number of compounds specifically target the initiation phase, they are limited in terms of clinical application owing to problems with drug specificity (edeine and pactamycin), toxicity (evernimicin) and solubility (thiopeptides and thermorubin). Several antibiotics inhibit the termination and recycling phases of translation, but these compounds usually have a more pronounced effect during the elongation phase. However, fusidic acid and blasticidin S are exceptions because they inhibit recycling and termination, respectively, at substantially lower concentrations than the concentration needed to inhibit elongation[6,7].

[1]*Gene Center and Department for Biochemistry, Feodor-Lynenstr. 25, University of Munich, 81377 Munich, Germany.*
[2]*Center for integrated Protein Science Munich (CiPSM), Feodor-Lynenstr. 25, University of Munich, 81377 Munich, Germany.*
e-mail: Wilson@genzentrum.lmu.de
doi:10.1038/nrmicro3155

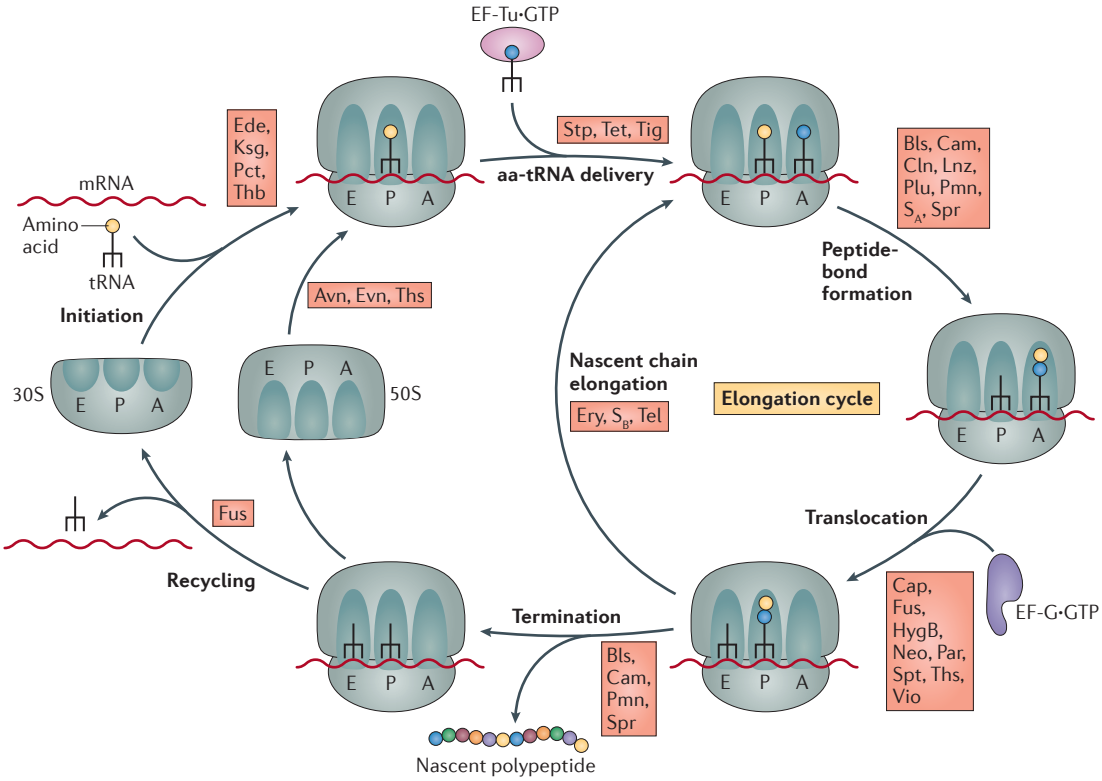


Figure 1 | **Antibiotic target sites during bacterial protein synthesis.** Initiation of protein synthesis involves the formation of a 70S ribosome (composed of a 30S and a 50S subunit) with the initiator tRNA and start codon of the mRNA positioned at the P-site. This process is inhibited by the antibiotics edeine (Ede), kasugamycin (Ksg), pactamycin (Pct) and thermorubin (Thb) on the 30S subunit, and by the orthosomycins avilamycin (Avn) and evernimicin (Evn), as well as thiostrepton (Ths) on the 50S subunit. The elongation cycle involves the delivery of the aminoacylated-tRNA (aa-tRNA) to the A-site of the ribosome by elongation factor Tu (EF-Tu), which is inhibited by streptomycin (Stp), tetracyclines (Tet) and glycylcyclines (tigecycline (Tig)). Peptide-bond formation between the A- and P-site tRNAs is inhibited by blasticidin S (Bls), chloramphenicol (Cam), lincosamides (clindamycin (Cln)), oxazolidinones (linezolid (Lnz)), pleuromutilins (Plu), puromycin (Pmn), streptogramin A (S_A) and sparsomycin (Spr). Translocation of the tRNAs is catalysed by EF-G and inhibited by the tuberactinomycins capreomycin (Cap) and viomycin (Vio), the aminoglycosides hygromycin B (HygB), neomycin (Neo) and paromomycin (Par), as well as fusidic acid (Fus), spectinomycin (Spt) and Ths. Elongation of the nascent chain is inhibited by the macrolides (erythromycin (Ery)), streptogramin B (S_B) and ketolides (telithromycin (Tel)). The final phases of termination and recycling lead to release of the polypeptide chain and subsequent dissociation of the 70S ribosome, followed by recycling of the components for the next round of initiation. Termination is inhibited by peptidyl-transferase inhibitors, such as Bls, Cam, Pmn and Spr, whereas recycling is inhibited by translocation inhibitors, especially Fus.

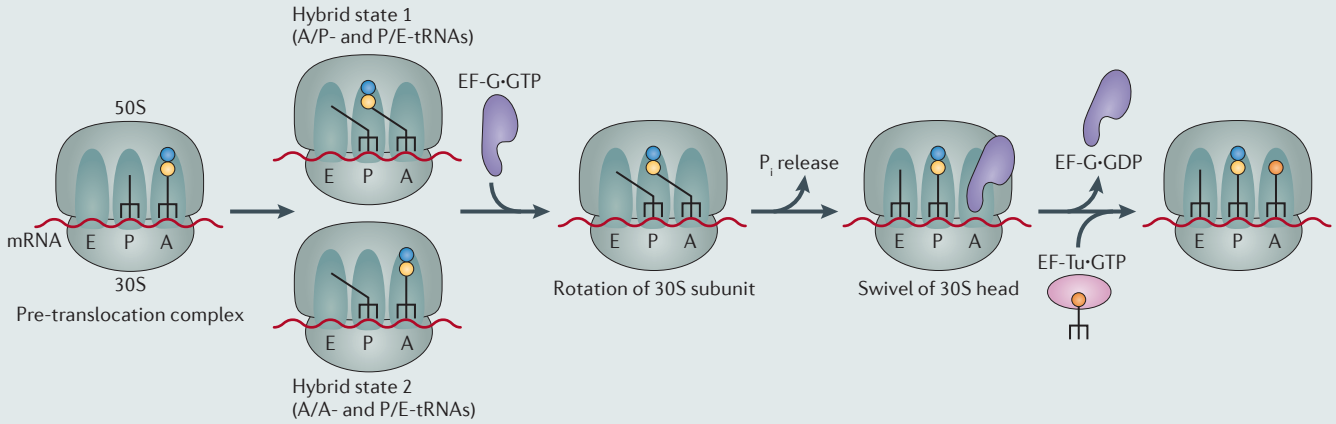
The initial structural insights into the mechanism of antibiotic action on the ribosome were obtained from crystal structures of antibiotics in complex with the 30S subunit of *Thermus thermophilus*, the 50S subunit of *Deinococcus radiodurans* and the 50S subunit of the archaeon *Haloarcula marismortui*[4]. However, the recent ability to determine the structures of the 70S ribosome from *T. thermophilus*[8] and *Escherichia coli*[9] at high resolution has led to a rapid increase in the number of structures of 70S–antibiotic complexes over the past 5–7 years. Crystal structures of almost all of the major ribosome-targeting antibiotic classes in complex with the ribosome have now been obtained. In this Review, I discuss the insights gained from these structures and highlight how this information is advancing our understanding of the molecular mechanisms underlying antibiotic action. In addition, an up-to-date overview of the main bacterial resistance mechanisms to ribosomal antibiotics is provided, as well as a discussion of the ongoing efforts to combat multidrug-resistant bacteria.

Structural basis of antibiotic action

Despite the large size of the ribosome, relatively few sites are targeted by our current arsenal of antibiotics. On the 30S subunit, the antibiotic binding sites are clustered along the path of the mRNA and tRNAs (FIG. 2a). Antibiotics that bind to the 30S subunit, such as edeine and kasugamycin, inhibit translation initiation by preventing a stable interaction between the initiator tRNA and the start codon at the P-site. The majority of other 30S-targeting antibiotics inhibit translation elongation by interfering with either the delivery of tRNAs to the A-site (for example, tetracyclines and streptomycins) or the subsequent translocation of the mRNA–tRNA

Box 1 | **Translocation of tRNAs through the bacterial ribosome**

Following peptide-bond formation, the nascent polypeptide chain is attached to the A-site tRNA and the P-site tRNA is deacylated. Both tRNAs must be moved through the ribosome to the P- and E-sites in order to vacate the A-site so that the incoming aminoacylated-tRNA (aa-tRNA) can become accommodated (see the figure). This complicated translocation reaction has been the subject of a number of excellent reviews[128–130]. Briefly, translocation can be simplified as a two-step process: an initial movement of the tRNAs with respect to the 50S subunit (which occurs spontaneously following peptide-bond formation), leading to the formation of so-called hybrid states, followed by the movement of the tRNAs with respect to the 30S subunit in a second reaction that is catalysed by elongation factor G (EF-G)[128]. Compared to the classical tRNA state, in the hybrid state, the anticodon and acceptor arms of the tRNAs occupy different sites on the 30S and 50S subunits, namely the A/P- and P/E-hybrid states[131]. At least two main ribosomal hybrid states are adopted directly following peptide-bond formation, hybrid state 1 and 2, which are intermediates for translocation[132]. Formation of both ribosomal hybrid states is coupled with an anticlockwise rotation of the 30S subunit relative to the 50S subunit[133,134], often referred to as the ratcheted state[135]. Whereas EF-G can sample both rotated ribosomal hybrid states[136], the binding of EF-G to the ribosome stabilizes the formation of the rotated hybrid state 2 (REF. 135). EF-G is a GTPase, which binds to the ribosome in the high-affinity GTP state. Hydrolysis of GTP to GDP + P_i (inorganic phosphate) by EF-G occurs rapidly on ribosome binding, but it is the slower release of P_i that correlates with the movement of the mRNA–$tRNA_2$ complex[137]. During translocation, the head of the 30S subunit swivels anticlockwise, delivering the tRNAs into the P- and E-sites[138]. Concomitantly, EF-G inserts domain IV into the A-site of the 30S subunit, thus acting as a doorstop to prevent back translocation of the translocated tRNAs. Release of P_i from EF-G leads to conformational changes in EF-G that eventually lead to the low-affinity GDP state, at which point EF-G dissociates from the ribosome[137]. At some point, the tRNAs and mRNA must disengage from the head of the 30S subunit, thereby allowing it to swivel back to a position that is observed in the classical post-translocated state. The ribosome is now ready for delivery of the next aa-tRNA to the A-site.



complex through the ribosome (such as the aminoglycosides hygromycin B, neomycin, pactamycin and spectinomycin; and the tuberactinomycins viomycin and capreomycin).

On the 50S subunit, most of the antibiotic binding sites cluster at or near the peptidyl-transferase centre (PTC), where peptide-bond formation occurs (FIG. 2b,c). Exceptions include the orthosomycins (evernimicin and avilamycin), which interact with 23S rRNA helices H89 and H91 (note that 'H' denotes helices of the large subunit and 'h' denotes helices of the small subunit) and interfere with the binding of IF2 (REFS 10,11); and the thiostrepton-like thiopeptides, which interact with H43 and H44 of the 23S rRNA and interfere with the binding of EF-G, EF-Tu and IF2 (REF. 4) (FIG. 2b). PTC-targeting antibiotics inhibit peptide-bond formation by perturbing or preventing the correct positioning of the aminoacylated ends of tRNAs in the PTC. The binding sites of PTC-targeting antibiotics overlap with the A-site tRNA (for example, chloramphenicol, lincosamides (clindamycin), oxazolidinones (linezolid), puromycin and sparsomycin) or the P-site tRNA (blasticidin S), or span both the A- and P-sites (for example, the pleuromutilin and streptogramin A (S_A) classes) (FIG. 2c). The binding sites of the macrolide and streptogramin B (S_B) classes are located adjacent to the PTC within the ribosomal exit tunnel. Most macrolide and S_B members do not inhibit peptide-bond formation per se but rather prevent elongation of most nascent chains, which leads to peptidyl-tRNA drop off and the abortion of translation. Below I discuss the structural basis for the inhibition of tRNA delivery, translocation and peptidyl transfer by ribosomal antibiotics.

Inhibition of tRNA delivery. Tetracyclines are broad-spectrum natural product antibiotics that were introduced into clinical practice in the 1940s[12]. The first two crystal structures of tetracycline–30S subunit complexes at 3.4–4.5 Å revealed that the drug binds to multiple sites on the small subunit; however, only one binding site was common to both structures[13,14]. This primary binding site overlaps with the position of the anticodon stem loop of the A-site tRNA, consistent with the wealth of biochemical evidence demonstrating that tetracyclines interfere with delivery of aa-tRNAs to the A-site[4] (FIG. 1). The widespread use of tetracyclines has led to an increase in resistance among clinically relevant pathogenic bacteria, thereby limiting their clinical efficacy[15]. To circumvent this problem, second-generation tetracycline derivatives (for example, doxycycline) and third-generation tetracycline derivatives (glycylcyclines) were developed. The glycylcycline tigecycline was approved

Peptidyl-transferase centre
(PTC). The site of peptide-bond formation on the large subunit, comprising universally conserved residues of domain V of the 23S ribosomal RNA.

Classical tRNA state
The tRNA binding state in which the anticodon and acceptor arms of the tRNA occupy the same sites on the 30S and 50S subunit, respectively.

Table 1 | **Mechanisms of action and resistance mechanisms of selected ribosome-targeting antibiotics**

Antibiotic	Molecular target	Inhibition mechanism	Resistance mechanisms*
Blasticidin S	50S	PTC, termination	DM, TM
Chloramphenicol	50S	PTC	DM, E, TA, TM
Clindamycin, lincomycin	50S	PTC	DM, E, TA, TM
Dalfopristin (S_A), quinupristin (S_B)	50S	PTC	E, TA, TM
Doxycycline, tigecycline	30S	tRNA delivery	DM, E, FP, TM
Edeine	30S	Initiation	DM, TM
Erythromycin, telithromycin	50S	Nascent chain elongation	DM, E, TA, TM
Evernimicin, avilamycin	50S	Initiation	TA, TM
Fusidic acid	EF-G	Elongation, recycling	E, FP, TM
Kasugamycin	30S	Initiation	DM, TA, TM
Kirromycin	EF-Tu	Elongation	TM
Linezolid	50S	PTC	E, TA, TM
Paromomycin, neomycin	30S	Translocation	DM, E, TA, TM
Puromycin	50S	PTC	DM
Sparsomycin	50S	PTC	E, TM
Spectinomycin	30S	Translocation	DM, E, TA, TM
Streptomycin	30S	Translocation	DM, E, TA, TM
Thermorubin	70S	Initiation	ND
Thiostrepton	50S	Factor binding	TA, TM
Tiamulin	50S	PTC	E, TA, TM
Viomycin, capreomycin	70S	Translocation	DM, TA, TM

DM, drug modification/degradation; E, efflux/membrane permeability; EF, elongation factor; FP, factor-assisted protection; ND, not determined; PTC, peptidyl-transferase centre; S_A, streptogramin A; S_B, streptogramin B; TA, target alteration via modification (or lack thereof); TM, target mutation. *Typical resistance mechanisms (although others might exist).

for clinical use in 2005 and has a higher affinity for the 30S subunit than the first- and second-generation tetracyclines, and also has enhanced antibacterial activity[16–18].

Recent crystal structures of tetracycline–70S and tigecycline–70S complexes at 3.1–3.3 Å (REF. 18) revealed that both drugs bind to the 70S exclusively at the primary binding site that was previously detected in the 30S subunit crystals[13] (FIG. 3a,b). Similarly to tetracycline, the polar face of tigecycline coordinates an Mg^{2+} ion to facilitate interactions with the phosphate backbone of h34. The higher resolution of the 70S structures revealed that tigecycline (and probably tetracycline) also coordinates a second Mg^{2+} ion to establish interactions with h31. The lack of sequence specificity in the interactions of these drugs with the 16S rRNA (the drugs interact with the rRNA phosphate-oxygens of the backbone rather than specific rRNA bases) is consistent with the broad-spectrum activity of the tetracycline and glycylcycline classes of antibiotics. The increased binding affinity of tigecycline[16–18] (compared with tetracycline) seems to result from a stacking interaction between the 9-t-butylglycylamido moiety of tigecycline and the π-orbital of nucleobase C1054 of the 16S rRNA (FIG. 3b). The 9-t-butylglycylamido moiety of tigecycline also increases the steric overlap of the drug with the A-site tRNA (FIG. 3b), which might contribute to the enhanced ability of tigecycline to block even early stages of tRNA entry into the A-site[18].

Targeting tRNA dynamics and translocation. One of the largest families of clinically used antibiotics is the 2-deoxystreptamine (2-DOS) aminoglycosides, which are chemically distinct from the non-DOS aminoglycoside antibiotics, such as streptomycin. The 2-DOS family comprises both natural products (such as neomycin and paromomycin) and semi-synthetic derivatives (such as dibekacin and amikacin). These drugs are potent inhibitors of the translocation reaction and also inhibit subunit splitting during ribosome recycling (FIG. 1). In addition, most members of this class induce translational misreading by stabilizing and promoting the binding of non-cognate tRNAs to the mRNA.

Early structural studies using RNA fragments[19] and subsequently 30S subunits[20] demonstrated that 2-DOS aminoglycosides interact with an internal loop of h44 at the decoding centre of the ribosome and stabilize a 'flipped-out' conformation of the rRNA nucleotides A1492 and A1493 (FIG. 3c). During decoding, A1492 and A1493 probe the minor groove of the duplex formed by the anticodon of the aa-tRNA and the mRNA codon, suggesting that this interaction is important for distinguishing between cognate and non-cognate tRNAs[21]. On the basis of these structures, the 2-DOS aminoglycosides (such as paromomycin and neomycin) were proposed to induce misreading by stabilizing the flipped-out conformation of A1492 and A1493 in the presence of non-cognate tRNAs[20,21]. However, recent experiments using *T. thermophilus* 70S

Stacking interaction
Non-covalent interaction established by parallel plate stacked orientation of two moieties, with at least one being aromatic.

Non-cognate tRNAs
tRNAs that have a mismatched base-pairing interaction with mRNA codons. Accommodation leads to misincorporation of an incorrect amino acid into the nascent polypeptide chain.

Decoding centre
The A-site region of the small ribosomal subunit that is responsible for discriminating cognate from non-cognate tRNAs based on anticodon–codon interactions. This site encompasses the universally conserved nucleotides A1492 and A1493 of helix 44 of the 16S rRNA.

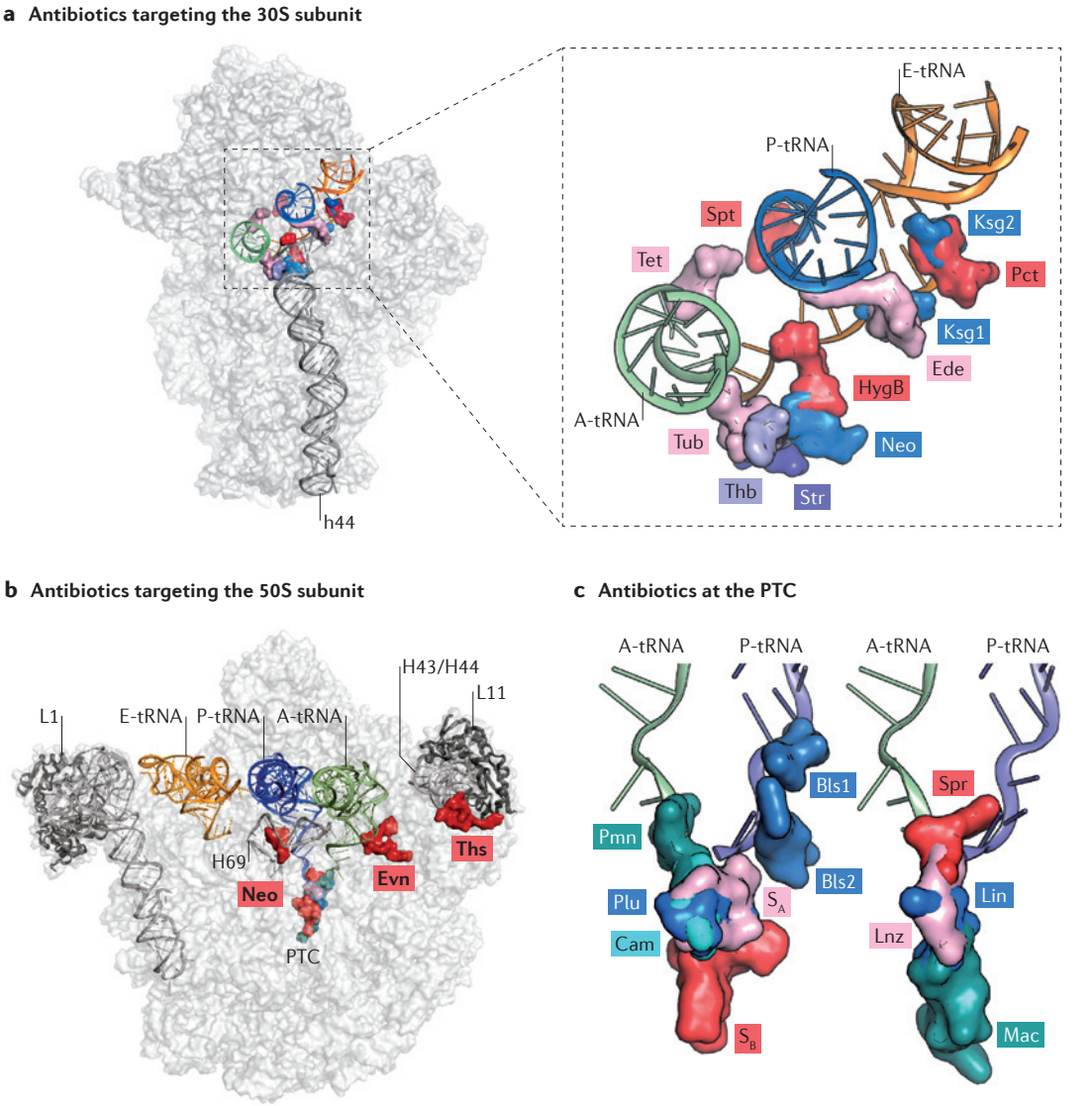


Figure 2 | **Antibiotic binding sites on the 30S and 50S ribosomal subunits. a** | Overview and enlargement of antibiotic binding sites along the mRNA binding channel of the 30S subunit, including tetracycline (Tet; Protein Data Bank (PDB) accession 4G5K–N)[18], spectinomycin (Spt; PDB accession 2QOU–X)[139], kasugamycin (Ksg1 and Ksg2; PDB accession 2HHH)[140], pactamycin (Pct; PDB accession 1HNX)[13], edeine (Ede; PDB accession 1I95)[14], hygromycin B (HygB; PDB accession 3DF1–4)[141], neomycin (Neo; PDB accession 4GAQ/R/S/U)[25], streptomycin (Str; PDB accession 1FJG)[142], thermorubin (Thb; PDB accession 3UXQ–T)[30] and tuberactinomycins (Tub; PDB accession 3KNH–K)[29]. The A-site tRNA (green), P-site tRNA (blue), E-site tRNA (orange) and h44 are highlighted for reference. **b** | Overview of the binding sites of neomycin (Neo; PDB accession 4GAQ/R/S/U)[25], evernimicin (Evn)[10] and thiostrepton (Ths; PDB accession 3CF5)[81] on the 50S subunit. The A-site tRNA (green), P-site tRNA (blue), E-site tRNA (orange), H43/H44, H69, peptidyl-transferase centre (PTC) and the L1 and L11 stalks are highlighted for reference. **c** | Enlargement of the binding sites of blasticidin S (Bls1 and Bls2; PDB accession 1KC8)[47], sparsomycin (Spr; PDB accession 1M90)[143], lincomycin (Lin; PDB accession 3OFX/Y/Z/0)[41], linezolid (Lnz; PDB accession 3DLL)[53], macrolides (Mac; PDB accession 1K9M)[43], puromycin (Pmn; PDB accession 1M90)[143], pleuromutilins (Plu; PDB accession 1XBP)[144], chloramphenicol (Cam; PDB accession 3OFA–D)[41] and streptogramins A and B (S_A and S_B; PDB accession 1SM1)[68] relative to the A-site and P-site tRNAs.

Cognate tRNAs
tRNAs that have a matched base-pairing interaction with mRNA codons. Accommodation leads to incorporation of the correct amino acid into the nascent polypeptide chain.

ribosomes demonstrate that non-cognate tRNAs can establish Watson–Crick-like interactions with the mRNA codon and, similarly to cognate tRNAs, stabilize the flipped-out conformation of A1492 and A1493 (REF. 22). Following these observations, it was suggested that destabilization and rejection of non-cognate tRNAs results from the increased energy expenditure required to adopt the unfavourable, non-canonical, Watson–Crick-like base pair[22]. In this model, rather than stabilizing the flipped-out conformation of A1492 and A1493 (REFS 20,21), paromomycin promotes misreading by inducing local changes in h44 and H69 that compensate for the energetically unfavourable, non-cognate tRNA–mRNA interaction[22]. The decoding process is dynamic, thus the stabilization of A1492 and A1493 in a flipped-out conformation and the compensation for the unfavourable, non-canonical,

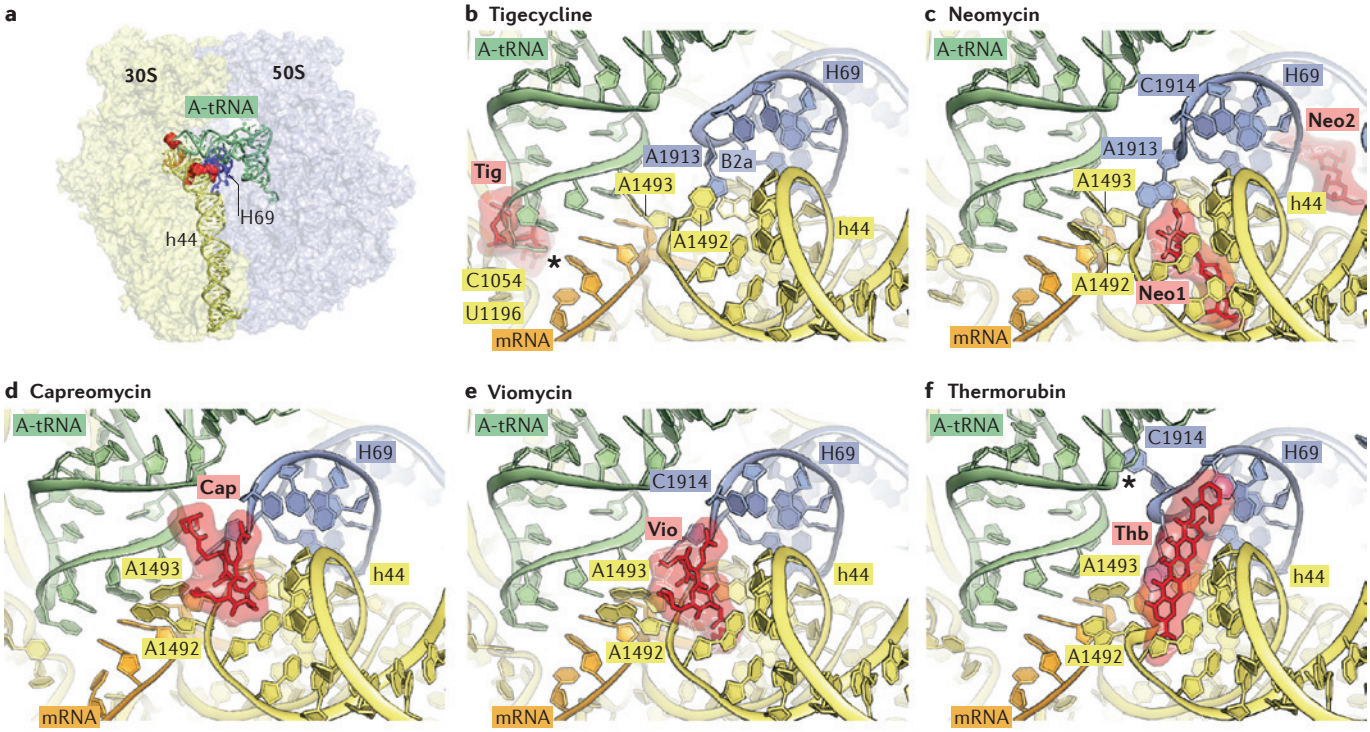


Figure 3 | **Antibiotic interactions at the decoding centre of the 30S subunit. a** | An overview of the antibiotic binding sites (red) on the ribosome (30S with h44 (yellow) and 50S with H69 (blue)) relative to the A-site tRNA (green). **b** | Tigecycline (Tig; Protein Data Bank (PDB) accession 4G5T–W)[18] inhibits translation by sterically clashing (asterisk) with and thereby preventing binding of the A-site tRNA (green) to the ribosome. **c** | Two molecules of neomycin (Neo1 and Neo2: PDB accession 4GAQ/R/S/U)[25] bind to the ribosome: Neo1 interacts with h44 and Neo2 with H69. Aminoglycosides, such as neomycin, inhibit translocation by stabilizing the A-site tRNA in the A-site. **d**,**e** | Tuberactinomycins, such as capreomycin (Cap; PDB accession 3KNL–O)[29] (**d**) and viomycin (Vio; PDB accession 3KNH–K)[29] (**e**), bind at h44 and prevent translocation by stabilizing a hybrid state of the ribosome (BOX 1). **f** | Thermorubin (Thb; PDB accession 3UXQ–T)[30] interacts with intersubunit bridge B2a, which connects the top of h44 of 16S ribosomal RNA (rRNA; yellow) to the tip of H69 of 23S rRNA (blue). Binding of this drug induces C1914 to flip out of H69 and adopt a position that sterically clashes with the A-site tRNA (asterisk).

Watson–Crick-like base pairs need not be mutually exclusive, and both might contribute to paromomycin-induced misreading. Further insights into 2-DOS aminoglycoside-induced misreading could come from the analysis of an apramycin–70S complex. Apramycin induces conformational changes in A1492 and A1493; however, unlike most other 2-DOS aminoglycosides, this drug does not induce translational misreading[23].

In addition to binding to h44 on the small subunit (Neo1), crystal structures of the 2-DOS aminoglycosides neomycin and gentamicin on the 70S ribosome have revealed a second binding site (Neo2) within H69 of the 23S rRNA[24,25] (FIG. 3c). H69 interacts with h44 of the 16S rRNA to form one of the major intersubunit bridges (B2a). Crystal structures show that the second neomycin binding site stabilizes an intermediate hybrid state by restricting movement of h44 relative to H69 within bridge B2a[25], possibly contributing to the impairment of translocation and ribosome recycling[24]. By contrast, kanamycin and gentamicin lack this second binding site, but this does not prevent these drugs from inhibiting translocation through stabilization of the classic pre-translocation state[26–28] (BOX 1).

The tuberactinomycins viomycin and capreomycin and the linear tetracycle thermorubin are all natural product antibiotics that bind at the interface of the 30S and 50S subunits in the 70S ribosome[29,30] (FIG. 3d–f). This observation explains why these antibiotics were not bound to either subunit alone when they were crystallized this way. The tuberactinomycins are cyclic peptide antibiotics that are particularly active against *Mycobacterium tuberculosis*, including multidrug-resistant strains[31]. Crystal structures of viomycin and capreomycin bound to the 70S ribosome reveal a single binding site that spans the ribosomal interface between h44 and H69 (REF. 29) (FIG. 3d,e). Viomycin inhibits translocation[32] by stabilizing the tRNAs in the intermediate hybrid state[33,34] (BOX 1), which is distinct from the intermediate hybrid state stabilized by neomycin[25,28]. Binding of the tuberactinomycins to h44 leads to a flipped-out conformation of A1492 and A1493 (REF. 29) (FIG. 3d,e), as observed with the 2-DOS aminoglycosides (FIG. 3c). However, unlike most 2-DOS aminoglycosides, the tuberactinomycins do not induce translational misreading[35].

Thermorubin, a natural product of the actinomycete *Thermoactinomyces antibioticus*, has excellent antimicrobial

Intersubunit bridges
Sites of interaction at the interface between the small and large ribosomal subunits.

Hybrid state
The tRNA binding state in which the anticodon and acceptor arms of the tRNA occupy different sites on the 30S and 50S subunit, respectively.

activity against a broad range of bacteria but is unsuitable for clinical use owing to its low solubility[30,36]. Similarly to the tuberactinomycins, the thermorubin binding site on the 70S ribosome spans the intersubunit space between H69 and h44 (REF. 30) (FIG. 3f), consistent with the 100-fold increased affinity of thermorubin for 70S ribosomes compared with individual subunits[37]. Thermorubin inhibits binding of fMet-tRNA to the P-site in an initiation factor-dependent manner[38]. Although the binding site of thermorubin does not overlap with the P-site tRNA, the drug induces conformational changes in the rRNA nucleotides A1913 and C1914, and this is proposed to perturb IF1 activity[30]. Furthermore, the conformation of C1914 in the presence of thermorubin is incompatible with A-site tRNA binding (FIG. 3f), suggesting that even if P-site tRNA binding occurs, subsequent accommodation of an A-site tRNA would be inhibited[30]. The distinct, yet overlapping, binding position of thermorubin with the aminoglycosides and tuberactinomycins suggests that this binding pocket might be a useful target for the development of new drugs[30].

Targeting the peptidyl-transferase centre. Early structural insights into the binding mode of PTC inhibitors came from crystal structures of antibiotics in complex with the 50S subunits of the bacterium *D. radiodurans* and the archaeon *H. marismortui*[3,4]. Despite the high sequence and structural conservation of the PTC between the two subunits, many differences were observed in the conformation, orientation and even the target site of the same antibiotic bound to these two ribosomal particles[4,39]. Many of these structures also showed differences in comparison to subsequent ribosome structures obtained from other bacteria, such as *E. coli* and *T. thermophilus*[40,41]. Although some differences might be explained by species specificity, a number of discrepancies seem to be the result of the limited resolution of the *D. radiodurans* structures[39,42], and in particular, the validity of the macrolide and chloramphenicol structures has been questioned[40,41,43–45].

On the bacterial 50S subunit, chloramphenicol binds at the PTC in a position that overlaps with the aminoacyl moiety of the A-site tRNA (Cam1)[40,41,46], consistent with chloramphenicol inhibition of peptide-bond formation by blocking aa-tRNA binding at the A-site. By contrast, the chloramphenicol binding site (Cam2) in the archaeal *H. marismortui* 50S subunit is located deeper in the exit tunnel and overlaps with the erythromycin binding site[47] (FIG. 4a). Chloramphenicol binding is not observed at the Cam1 binding site on the archaeal 50S subunit, which is consistent with the observation that higher concentrations of chloramphenicol are required to inhibit growth of archaea compared to bacteria[48]. Both chloramphenicol and erythromycin have also been reported to directly inhibit 50S subunit biogenesis[49]. However, recent studies indicate that these effects are indirect because the impairment in 50S subunit biogenesis results from an imbalance in the levels of ribosomal proteins and rRNA owing to specific inhibition of ribosomal protein translation by these drugs[50,51].

The A-site pocket is also the binding site for clindamycin[41,44,46] (FIG. 4b), sparsomycin[47] (FIG. 4c) and the oxazolidinone linezolid[52–54] (FIG. 4d). The positions occupied by the lincosamide clindamycin in the *H. marismortui* and *T. thermophilus* structures show a high degree of overlap with the A-site tRNA (FIG. 4b), which is consistent with the inhibition of peptide-bond formation by lincosamides[4]. The binding site of sparsomycin[47] also overlaps with the A-site tRNA and thus prevents peptide-bond formation[52–54] (FIG. 4c); however, unlike clindamycin, sparsomycin stabilizes the binding of tRNAs to the P-site, and by doing so induces translocation of an A-site tRNA to the P-site[55].

Blasticidin S also stabilizes tRNA in the P-site; however, the mechanism involved differs from that of sparsomycin[7]. The binding site for blasticidin S overlaps the canonical position of the CCA-end of the P-site tRNA, leading to the stabilization of a deformed P-site tRNA, in which C75 is flipped away from the drug and out towards the A-site[7] (FIG. 4d). The deformed P-site tRNA seems to be a poor substrate for peptide-bond formation and, in particular, peptide release, leading to the suggestion that blasticidin S might preferentially disrupt translation termination compared with peptidyl transfer[7].

Linezolid binds to the A-site (FIG. 4d) and seems to adopt similar orientations in *D. radiodurans*, *E. coli* and *H. marismortui* ribosomes[52–54]. Despite being located at the A-site, linezolid is thought to act as an initiation inhibitor by perturbing the position of initiator tRNA in the P-site[56]. The close proximity of linezolid and sparsomycin binding sites has led to the development of linezolid–sparsomycin conjugates that display improved antimicrobial activity compared to linezolid alone and are also active against linezolid-resistant strains[57]. Linezolid does not induce translocation, but linezolid–sparsomycin conjugates have this property[58]. The ability to induce translocation is not restricted to sparsomycin, having also been observed recently with other PTC inhibitors, such as chloramphenicol and clindamycin, albeit less efficiently than for sparsomycin[59].

Targeting the ribosomal exit tunnel. Macrolides are a large family of antibiotics that have been used clinically since the introduction of the naturally produced parent compound erythromycin in the early 1950s[4,12,60]. These drugs bind to a site that is adjacent to the PTC within the ribosomal exit tunnel, through which the nascent polypeptide chain egresses as it is synthesized[4,60] (FIG. 4e). Macrolides are composed of a macrolactone ring of varying size, to which several sugars and/or other side chains are attached. For example, the macrolide erythromycin consists of a ring of 14 atoms to which the C3-cladinose and C5-desosamine sugars are attached. The emergence and spread of macrolide resistance prompted the development of ketolides, such as telithromycin. Telithromycin has a C3-keto group rather than cladinose and a 11,12 cyclic carbamate with an extended alkyl–aryl side chain. Species-specific differences in the binding mode of some macrolides and ketolides are evident, as exemplified by the distinct positions adopted by the alkyl–aryl side chain of telithromycin in the

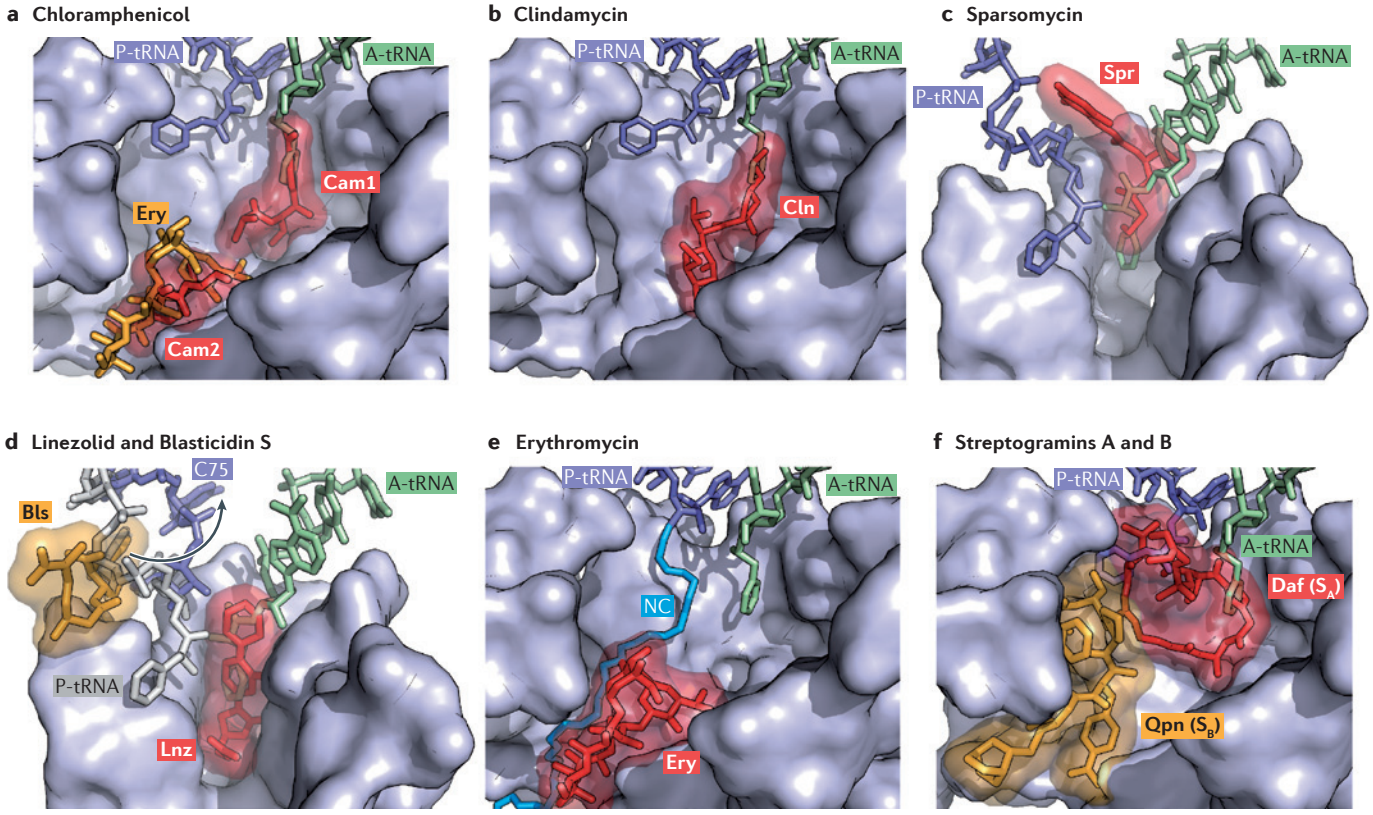


Figure 4 | **Antibiotic interactions at the peptidyl-transferase centre of the 50S subunit.** **a** | On the bacterial ribosome, chloramphenicol (Cam1; Protein Data Bank (PDB) accession 3OFA–D)[41] prevents peptide-bond formation by overlapping with the binding site of the A-site tRNA (green). By contrast, on the archaeal ribosome, chloramphenicol (Cam2; PDB accession 1NJJ)[47] binds deeper within the exit tunnel in a position that overlaps with the macrolide erythromycin (Ery; PDB accession 3OFO–R)[41]. **b** | The lincosamide clindamycin (Cln; PDB accession 3OFX/Y/Z/0)[41] prevents peptide-bond formation by sterically blocking A-site tRNA positioning at the peptidyl-transferase centre (PTC). **c** | Sparsomycin (Spr; PDB accession 1M90)[143] stabilizes P-site tRNA (purple) binding by promoting translocation of A-site tRNAs into the P-site and inhibiting peptidyl transfer. **d** | The oxazolidinone linezolid (Lnz; PDB accession 3DLL)[53] prevents peptide-bond formation by preventing A-site tRNA binding, but might also influence P-site tRNA positioning during initiation. By contrast, blasticidin S (Bls; PDB accession 4L6J–M)[7] binds to the P-site and induces the P-site tRNA to adopt a deformed conformation in which the C75 base is flipped (see arrow), thereby making it a poor substrate for peptide-bond formation and especially peptidyl-tRNA hydrolysis[7]. **e** | Ery (PDB accession 3OFO–R)[41] binds within the ribosomal exit tunnel and prevents elongation of the nascent polypeptide chain (NC)[145], thereby inducing peptidyl-tRNA drop-off. **f** | The streptogramin A (S_A) dalfopristin (Daf) and the streptogramin B (S_B) quinupristin (Qpn) (PDB accession 1SM1)[68] have adjacent binding sites at the PTC and within the ribosomal tunnel, respectively, enabling a synergistic interplay between the two drugs that inhibits binding of tRNAs at both the A- and P-sites.

H. marismortui 50S subunit[44] and the *D. radiodurans* 50S subunit[61,62] compared with the *E. coli* 70S ribosome[41] and the *T. thermophilus* 70S ribosome[40]; differences in the 23S rRNA nucleotides that interact with this side chain account for the different binding modes observed[39].

Macrolides with a C3-cladinose sugar, such as erythromycin, generally permit the synthesis of short polypeptide chains (oligopeptides) of 6–8 amino acids, whereas ketolides (which lack the bulky C3-cladinose sugar) allow for the polymerization of 9–10 amino acids before peptidyl-tRNA drop-off occurs and translation is aborted[63]. Translation of some specific oligopeptides causes macrolides to dissociate from the ribosome, thereby conferring macrolide resistance[64]. The current model proposes that the oligopeptides act as a 'bottle brush' to remove the macrolides as they pass through the ribosomal exit tunnel following their hydrolysis from the peptidyl-tRNA[64]. By contrast, another class of oligopeptides acts synergistically with the bound macrolide to induce translational arrest and regulate expression of downstream macrolide resistance genes[65] (see below). Recently, it was shown that distinct subsets of polypeptide chains are even able to completely bypass the drug-obstructed tunnel to become partially or fully synthesized[66]. Thus far, only a few bypass sequences have been identified, all of which are located in the amino terminus of the nascent polypeptide chain[66]. The structure of the drug was also found to influence the extent of protein synthesis: for example, the ketolide telithromycin permits more bypass synthesis than the macrolide erythromycin[66]. Ketolides lack the bulky C3-cladinose sugar of macrolides and therefore more space is available for bypass, providing a structural explanation for the differing levels of translation observed with each

Peptidyl-tRNA drop-off
Dissociation of tRNAs bearing the nascent polypeptide chain from the ribosome.

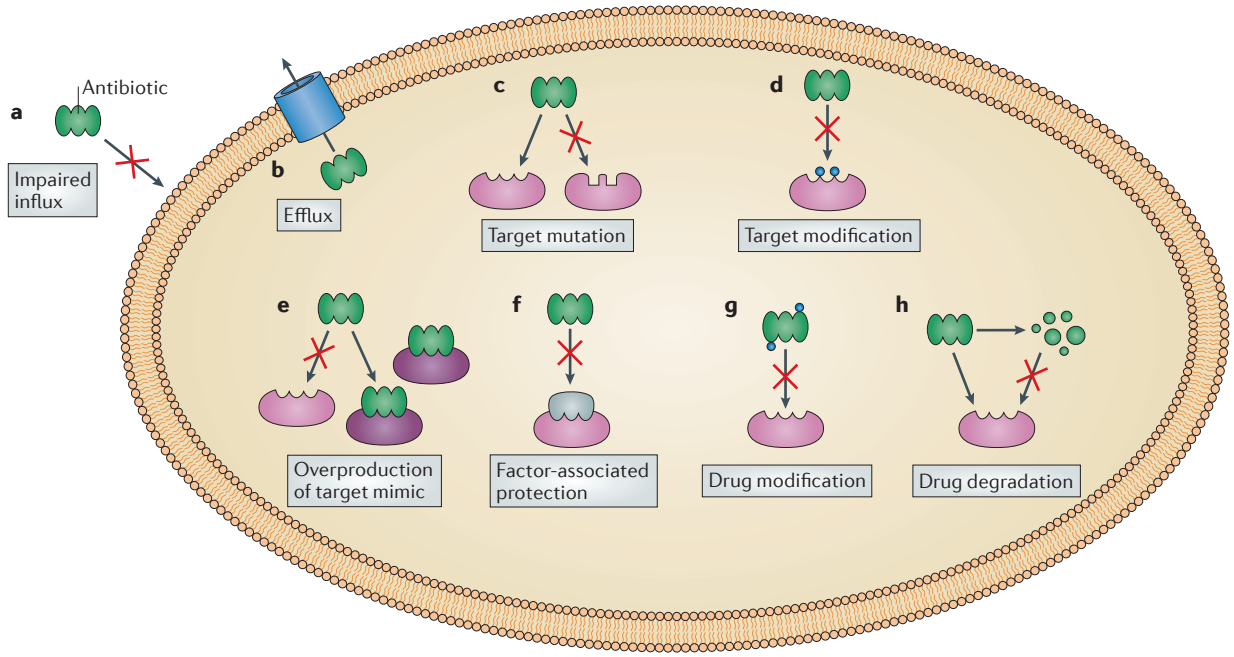


Figure 5 | **Antibacterial resistance mechanisms.** Resistance to ribosome-targeting antibiotics can be mediated by a range of mechanisms. These include impaired influx owing to low membrane permeability (**a**); active efflux of the drug from the cell (**b**); mutation (**c**) or modification (**d**) of the target (ribosomal RNA or ribosomal proteins), which lowers the affinity of the drug for the target; overproduction of a molecule that mimics the target (**e**), which lowers the effective drug concentration so that the target remains unbound; the recruitment of a specialized protein factor to actively remove the drug from the target (**f**); and modification or degradation of the drug (**g**,**h**).

of these drugs. These findings suggest that rather than inducing a global arrest of protein synthesis, macrolides and ketolides actually allow translation of only a small subset of proteins. Because telithromycin is bacteriocidal and allows more bypass than erythromycin (which is bacteriostatic), these data suggest that an imbalance in protein production is more detrimental to the cell than a global arrest of protein synthesis[66].

The ribosomal exit tunnel is also targeted by the streptogramin antibiotics. Streptogramins comprise a mixture of two distinct classes, S_A and S_B, which act synergistically on the ribosome[4,67]. The synergistic action lowers the minimal inhibitory concentration required for each compound, imparting activity against bacteria that carry macrolide resistance mutations[4,67]. The combination of dalfopristin (S_A) and quinupristin (S_B) (which is marketed as Synercid) is active against methicillin-resistant *Staphylococcus aureus* (MRSA). Crystal structures of streptogramins bound to the *D. radiodurans* as well as *H. marismortui* 50S subunits reveal that the S_A and S_B compounds bind to adjacent sites within the ribosomal exit tunnel[44,68] (FIG. 4f). S_B antibiotics occupy a similar binding site to the macrolides, whereas S_A antibiotics bind at the PTC and span the binding sites of the A-site and P-site tRNAs[44,47,68] (FIG. 4f). Binding of S_A antibiotics to the PTC induces a conformational change in the 23S rRNA nucleotide A2062 that promotes binding of S_B antibiotics, thus providing a structural explanation for their synergism[44,47,68]. Similarly to streptogramins, synergism has recently been reported for the antibiotics lankamycin and lankacidin[69]; lankacidin binds at the PTC and promotes structural rearrangements in the 23S rRNA that promote binding of the macrolide lankamycin[70].

Bacteriocidal
Bacteriocidal antibiotics induce cell death, as exemplified by the aminoglycoside class of ribosome-targeting antibiotics.

Bacteriostatic
Bacteriostatic antibiotics inhibit bacterial growth but do not induce cell death, such that removal of the antibiotic leads to restoration of growth. Most ribosome-targeting antibiotics are bacteriostatic.

MATE family of transporters
(Multidrug and toxic compound extrusion family of transporters). MATE transporters couple antibiotic efflux (against their prevailing concentration gradient) to the energetically favourable movement of sodium ions (Na^+) into the cell (along their electrochemical gradient).

Antibiotic resistance mechanisms

Naturally produced antibiotics are estimated to have originated >40 million years ago, suggesting that antibiotic resistance should be similarly old[71,72]. Many antibiotic-producing species, such as the soil-dwelling actinomycetes, are rich sources of antibiotic-resistance determinants and are suspected to be the origin of antibiotic resistance in many other bacterial species[72]. Indeed, evidence for exchange of antibiotic-resistance determinants between soil bacteria and clinical pathogens was recently reported, highlighting the significance of these environmental reservoirs for public health[73]. Similarly, the widespread use of antibiotics in agriculture has contributed to the resistance reservoir[74]. In addition to acquired resistance, some bacterial species have an intrinsic or innate resistance to different classes of antibiotics. A summary of ribosome-targeting antibiotics and their resistance mechanisms is presented in TABLE 1.

Membrane permeability. Gram-negative bacteria and mycobacteria are innately resistant to several antibiotics (such as macrolides) because their outer membrane restricts the influx of these drugs (FIG. 5a). Resistance to all major classes of ribosome-targeting antibiotics is also conferred to some extent by efflux in all bacteria (FIG. 5b). Structures of the MATE family of transporters[75–77] were recently solved, so now structures of all five families

of multidrug-resistance efflux pumps are represented[78], namely the major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, the resistance, nodulation and cell division (RND) family, the ATP-binding cassette (ABC) family and the MATE family. In Gram-negative bacteria, hydrophilic antibiotics usually permeate the cell via outer membrane porins, such as TolC. This porin uses different adaptor proteins to recruit the inner membrane pumps for efflux. For example, the adaptor AcrA recruits the AcrB pump and the MacA adaptor recruits the MacB pump. The MacAB–TolC complex exports macrolides specifically, whereas the ArcAB–TolC complex has a broad substrate profile, including antibiotics such as chloramphenicol, fusidic acid and tetracycline[79].

Mutation and modification of the target. Target mutation often results in reduced affinity for the drug, and this is a common mechanism of resistance to ribosome-targeting antibiotics[3,4] (FIG. 5c). Most ribosome-targeting antibiotics interact exclusively with rRNA; thus, mutation of rRNA nucleotides can directly (or indirectly) influence the conformation of the drug-binding site, leading to high-level resistance. Most bacteria have multiple copies of the rRNA operons (*E. coli* has seven), which means that high-level resistance requires mutation of most of these operons. Resistance is also conferred by mutations and/or deletions of ribosomal proteins, which are usually present as single gene copies. Because ribosomal proteins usually do not interact directly with the drug, resistance is thought to arise indirectly by inducing conformational changes in the rRNA. For example, mutation of proteins L3 and L22 confers tiamulin and macrolide resistance, respectively; however, these two proteins do not directly interact with these drugs[44,46,80]. L11 is an exception because it interacts directly with thiostrepton-like antibiotics, and mutation of this protein confers resistance to these antibiotics[81] (FIG. 2b). In many cases, the combination of both rRNA and ribosomal protein mutations results in increased resistance: for example, the rRNA mutation C2534U in combination with mutations in L3 and L4 confers linezolid resistance in *Staphylococcus aureus* isolates[82]. Because the majority of clinically used antibiotics target the conserved active sites on the ribosome (the decoding site on the 30S subunit and the PTC on the 50S subunit; FIG. 2a,b), mutation of the drug-binding site is accompanied by a fitness cost. However, compensatory mutations often restore fitness, although usually not to wild-type levels. The fitness cost of the C2576U mutation that confers linezolid resistance can be alleviated by alterations in ribosomal proteins L3 and L16 (REF. 83).

Modification of the drug-binding site can also reduce the affinity of the drug for its target (FIG. 5d), with methylation of rRNA by rRNA methyltransferases representing one of the most well-characterized and clinically relevant examples. The methyltransferase KgmA methylates the N7 atom of G1405 of the 16S rRNA, whereas the N1 atom of A1408 in h44 is methylated by KamA, and both modifications confer resistance to some aminoglycosides[84,85]. On the large subunit, Cfr monomethylates the C8 atom of the 23S rRNA nucleotide A2503 (located in the PTC)[86], conferring resistance to chloramphenicols, pleuromutilins, S_A antibiotics, lincosamides, oxazolidinones[87] and some macrolides[88]. The low fitness cost associated with this modification might account for the widespread dissemination of the *cfr* gene among pathogenic bacteria[89]. The Erm methyltransferases mono- or dimethylate 23S rRNA nucleotide A2058 within the exit tunnel, conferring resistance to macrolides, ketolides, lincosamides and S_B antibiotics[4]. In contrast to the Cfr modification, there is a fitness cost associated with methylation of A2058. Ribosomes with methylated A2058 have different translation profiles compared to wild-type ribosomes, suggesting that the fitness cost is associated with a deregulation of translation[90]. In a proof-of-principle example, *in vitro* translation of the *poxB* mRNA was shown to be less efficient with ribosomes containing a methylated A2058 residue compared to wild-type ribosomes. The associated fitness cost of this modification might explain why most *erm* genes are not constitutively expressed and are only induced when the antibiotic is present[65].

Loss of methylation can also confer resistance to certain antibiotics. A well-known example is the modest resistance to kasugamycin that occurs when the methyltransferase KsgA is deactivated. KsgA modifies the universally conserved nucleotides A1518 and A1519 located in h45 of the 16S rRNA[4]. Similarly, inactivation of TlyA, which methylates the 2′-hydroxyls of both C1409 in the 16S rRNA and C1920 in the 23S rRNA, confers resistance to the tuberactinomycins viomycin and capreomycin[91]. Furthermore, inactivation of RlmN, which methylates C2 of A2503 of the 23S rRNA, increases resistance to linezolid[92], and inactivation of RsmG (also known as GidB), which methylates the N7 of G527 of the 16S rRNA, confers low-level streptomycin resistance[93].

Overexpression and protection of the target. Resistance to some antibiotics arises as a result of increased expression of the target or of a mimic of the target because the mimic or target sequesters the drug, leaving some target molecules uninhibited (FIG. 5e). Although examples of target overexpression for ribosome-targeting antibiotics have not been found *in vivo*, the principle has been demonstrated *in vitro*. For example, overexpression of an rRNA fragment resembling h34 of the 16S rRNA confers resistance to the antibiotic spectinomycin[94]. Similarly, high levels of EF-Tu can restore translation in the presence of the EF-Tu inhibitor amythiamycin[95].

Another mechanism of resistance to ribosomal antibiotics involves factor-assisted protection of the drug target (FIG. 5f). Examples include the TetM and TetO proteins, which confer resistance to tetracycline[96], and the FusB and FusC proteins, which confer fusidic acid resistance[97]. TetO and TetM are paralogues of EF-G and bind to tetracycline-stalled ribosomes to sterically dislodge the drug from its binding site[98,99]. FusB and FusC bind to and promote dissociation of fusidic acid-bound EF-G from the ribosome, which allows translation and/or ribosome recycling to resume[100,101].

Porins
β-barrel proteins that span the cell membrane and act as pores through which molecules can diffuse.

Compensatory mutations
In the context of this Review, secondary mutations that arise to restore bacterial fitness following a decrease in fitness owing to antibiotic resistance mutations.

Translation profiles
An indication of the changes in the levels of synthesis of proteins by ribosomes.

Drug modification and degradation. Bacteria encode several enzymes that either modify or degrade antibiotics[102,103] (FIG. 5g,h). The modification mechanisms include phosphorylation (chloramphenicols[104,105], aminoglycosides (such as hygromycin A[106]) and macrolides), acetylation (aminoglycosides, chloramphenicols and S_A antibiotics), adenylation (aminoglycosides and lincosamides), glycosylation (macrolides) and hydroxylation (tetracycline[107] and tigecycline[108]), which prevents or impedes the binding of the corresponding drugs to their ribosomal targets (FIG. 5g). In terms of drug degradation, the esterases EreA and EreB cleave the macrocycle ester linkage, providing resistance to the macrolide class of antibiotics[102] (FIG. 5h). Furthermore, it was recently shown that tetracycline degradation not only reduces the competitive advantage of bacteria resistant to this drug but also, surprisingly, places a disadvantage on such cells as a result of the activities of the stable degradation products. Thus, degradation of this antibiotic might actually lead to selection against resistance[109].

Overcoming bacterial resistance

The global rise of multidrug-resistant bacteria, coupled with the paucity of novel antibiotics in the development pipeline, represents a major public health concern. Several strategies are underway to develop improved and novel antimicrobial agents.

Old targets, new drugs. The modification of scaffolds present in known and successfully used natural product antibiotics has led to successive generations of semi-synthetic derivatives with improved antimicrobial properties[12]. Currently, several ribosome-targeting antibiotics are in clinical trials, including the aminoglycoside plazomizin (Achaogen), the ketolides solithromycin (Cempra) and cethromycin (Advanced Life Sciences), the oxazolidinones PF-02341272 (Pfizer), AZD5847 (AstraZeneca), tedizolid (Trius) and radezolid (Rib-X), the pleuromutilin BC-3781 (Nabriva) and the glycylcyclines omadacycline (Paratek) and eravacycline (Tetraphase)[110,111]. Despite the drawback that these drugs target the same binding site as the parental compounds, the modified scaffolds have improved properties, such as solubility and drug uptake, and are less prone to efflux. Moreover, these derivatives typically have higher affinity for mutated or modified targets, and most also avoid drug modification and/or degradation.

The oxazolidinone linezolid was the first ribosome-targeting antibiotic with a unique scaffold (purely synthetic) to be approved for clinical use[12]. The binding site of linezolid overlaps with that of many other PTC inhibitors, such as chloramphenicol and clindamycin (FIG. 3). As such, linezolid use also selects for cross-resistance to these antibiotics[4]. Nevertheless, new scaffolds provide an opportunity to generate novel and improved derivatives and to explore the regions surrounding the drug-binding site. An alternative strategy is the use of dual-acting hybrid antibiotics that have distinct targets[112], such as the chimeric drugs linezolid–sparsomycin[57,58] and macrolide–chloramphenicol[113]. Major advances in the chemical and biological synthesis of antibiotics have increased the accessibility and diversity of lead scaffolds for the development of improved antimicrobial agents[114,115].

New targets, new drugs. A number of potent naturally produced antibiotics have been discovered with distinct target sites on the large subunit, such as the orthosomycins (evernimicin and avilamycin) and thiostrepton-like thiopeptides, which interact with 23S rRNA helices H89 and H91, and H43 and H44, respectively (FIG. 2a). Overcoming the solubility and toxicity problems associated with these antibiotics through chemical or biological synthesis could establish such antibiotics as attractive lead compounds for drug development[114,115]. In addition, several compounds that seem to target novel sites on the 30S subunit have been identified, including various peptide antibiotics[116–118] such as the synthetic nitrovinylfuran Furvina (2-bromo-5-(2-bromo-2-nitrovinyl)-furan)[119] and the naturally produced orthoformimycin[120]. In addition, the E-site on the large subunit of eukaryotic ribosomes is targeted by the eukaryotic-specific antibiotics 13-deoxytedanolide[121] and cycloheximide[122,123], suggesting that the bacterial E-site might also offer opportunities for the development of novel antimicrobials.

Outlook and future perspectives

The plethora of recent structures of antibiotics in complex with the ribosome has provided unparalleled insight into their mechanisms of action and has highlighted how resistance emerges through mutation or modification of the drug binding sites. Despite these insights, it should be realized that most antibiotics inhibit translation by trapping the ribosome in particular conformational states. As such, determining the structures of antibiotics bound to ribosomes in physiological translational states, rather than on empty ribosomes, will be one of the challenges for the future. Many antibiotics target the PTC and the exit tunnel; thus, future studies addressing the interaction of antibiotics with ribosomes carrying polypeptide chains will be important for the design of the next generation of drugs. Moreover, structures of additional antibiotic–ribosome complexes from diverse species, in particular those of pathogenic bacteria, will shed further light on the factors that govern species specificity, which should lead to the development of more selective or broader spectrum antimicrobials. Similarly, investigating how small molecules interact with eukaryotic ribosomes will be important for understanding the binding specificity of antimicrobial agents and for providing insights into drug resistance. The structures of yeast[124] and protozoan[123,125] ribosomes could potentially be used in the development of novel fungicides and antimalarial drugs, respectively. With the rapid improvements in cryo-electron microscopy, it is feasible that structures of human ribosomes[126] at atomic resolution could soon be obtained, and such structures could be used in the development of anticancer drugs and compounds that suppress nonsense mutations for the treatment of genetic disorders, such as cystic fibrosis and Duchenne

muscular dystrophy[127]. The success of such endeavours will require a concerted effort among academic researchers, small and large pharmaceutical companies and governmental agencies — only with common goals and strategies will society reap the benefits in the shortest possible time frame.

1. Voorhees, R. M. & Ramakrishnan, V. Structural basis of the translational elongation cycle. *Annu. Rev. Biochem.* **82**, 203–236 (2013).
2. Walsh, C. Where will new antibiotics come from? *Nature Rev. Microbiol.* **1**, 65–70 (2003).
3. Poehlsgaard, J. & Douthwaite, S. The bacterial ribosome as a target for antibiotics. *Nature Rev. Microbiol.* **3**, 870–881 (2005).
4. Wilson, D. N. The A-Z of bacterial translation inhibitors. *Crit. Rev. Biochem. Mol. Biol.* **44**, 393–433 (2009).
A comprehensive overview of the mechanism of action of antibiotics that target the bacterial translation apparatus.
5. Sohmen, D., Harms, J. M., Schlunzen, F. & Wilson, D. N. SnapShot: Antibiotic inhibition of protein synthesis I. *Cell* **138**, 1248 e1241 (2009).
6. Savelsbergh, A., Rodnina, M. V. & Wintermeyer, W. Distinct functions of elongation factor G in ribosome recycling and translocation. *RNA* **15**, 772–780 (2009).
7. Svidritskiy, E., Ling, C., Ermolenko, D. N. & Korostelev, A. A. Blasticidin S inhibits translation by trapping deformed tRNA on the ribosome. *Proc. Natl Acad. Sci. USA* **110**, 12283–12288 (2013).
8. Selmer, M. *et al.* Structure of the 70S ribosome complexed with mRNA and tRNA. *Science* **313**, 1935–1942 (2006).
The high-resolution crystal structure of the *T. thermophilus* 70S ribosome reported in this study opened the way to structures of *T. thermophilus* 70S–antibiotic complexes, such as those reported in references 7, 18, 29, 30 and 40.
9. Schuwirth, B. *et al.* Structures of the bacterial ribosome at 3.5 Å resolution. *Science* **310**, 827–834 (2005).
The high-resolution crystal structure of the *E. coli* 70S ribosome reported here opened the way to structures of *E. coli* 70S–antibiotic complexes, such as those reported in references 24, 25, 41, 139 and 141.
10. Belova, L., Tenson, T., Xiong, L. Q., McNicholas, P. M. & Mankin, A. S. A novel site of antibiotic action in the ribosome: interaction of evernimicin with the large ribosomal subunit. *Proc. Natl Acad. Sci. USA* **98**, 3726–3731 (2001).
11. Mikolajka, A. *et al.* Differential effects of thiopeptide and orthosomycin antibiotics on translational GTPases. *Chem. Biol.* **18**, 589–600 (2011).
12. Fischbach, M. A. & Walsh, C. T. Antibiotics for emerging pathogens. *Science* **325**, 1089–1093 (2009).
13. Brodersen, D. E. *et al.* The structural basis for the action of the antibiotics tetracycline, pactamycin, and hygromycin B on the 30S ribosomal subunit. *Cell* **103**, 1143–1154 (2000).
This study, together with references 14, 20 and 142, reports the first structures of antibiotics in complex with the 30S subunit.
14. Pioletti, M. *et al.* Crystal structures of complexes of the small ribosomal subunit with tetracycline, edeine and IF3. *EMBO J.* **20**, 1829–1839 (2001).
15. Roberts, M. C. Update on acquired tetracycline resistance genes. *FEMS Microbiol. Lett.* **245**, 195–203 (2005).
16. Olson, M. W. *et al.* Functional, biophysical, and structural bases for antibacterial activity of tigecycline. *Antimicrob. Agents Chemother.* **50**, 2156–2166 (2006).
17. Grossman, T. H. *et al.* Target- and resistance-based mechanistic studies with TP-434, a novel fluorocycline antibiotic. *Antimicrob. Agents Chemother.* **56**, 2559–2564 (2012).
18. Jenner, L. *et al.* Structural basis for potent inhibitory activity of the antibiotic tigecycline during protein synthesis. *Proc. Natl Acad. Sci. USA* **110**, 3812–3816 (2013).
19. Fourmy, D., Recht, M. I., Blanchard, S. C. & Puglisi, J. D. Structure of the A-site of *E. coli* 16S ribosomal RNA complexed with an aminoglycoside antibiotic. *Science* **274**, 1367–1371 (1996).
20. Ogle, J. M. *et al.* Recognition of cognate transfer RNA by the 30S ribosomal subunit. *Science* **292**, 897–902 (2001).
21. Ogle, J. M. & Ramakrishnan, V. Structural insights into translational fidelity. *Annu. Rev. Biochem.* **74**, 129–177 (2005).
22. Demeshkina, N., Jenner, L., Westhof, E., Yusupov, M. & Yusupova, G. A new understanding of the decoding principle on the ribosome. *Nature* **484**, 256–259 (2012).
23. Matt, T. *et al.* Dissociation of antibacterial activity and aminoglycoside ototoxicity in the 4-monosubstituted 2-deoxystreptamine apramycin. *Proc. Natl Acad. Sci. USA* **109**, 10984–10989 (2012).
24. Borovinskaya, M. A. *et al.* Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. *Nature Struct. Mol. Biol.* **14**, 727–732 (2007).
25. Wang, L. *et al.* Allosteric control of the ribosome by small-molecule antibiotics. *Nature Struct. Mol. Biol.* **19**, 957–963 (2012).
26. Cabanas, M. J., Vazquez, D. & Modolell, J. Inhibition of ribosomal translocation by aminoglycoside antibiotics. *Biochem. Biophys. Res. Commun.* **83**, 991–997 (1978).
27. Misumi, M., Nishimura, T., Komai, T. & Tanaka, N. Interaction of kanamycin and related antibiotics with the large subunit of ribosomes and the inhibition of translocation. *Biochem. Biophys. Res. Commun.* **84**, 358–365 (1978).
28. Feldman, M. B., Terry, D. S., Altman, R. B. & Blanchard, S. C. Aminoglycoside activity observed on single pre-translocation ribosome complexes. *Nature Chem. Biol.* **6**, 54–62 (2010).
29. Stanley, R. E., Blaha, G., Grodzicki, R. L., Strickler, M. D. & Steitz, T. A. The structures of the anti-tuberculosis antibiotics viomycin and capreomycin bound to the 70S ribosome. *Nature Struct. Mol. Biol.* **17**, 289–293 (2010).
30. Bulkley, D., Johnson, F. & Steitz, T. A. The antibiotic thermorubin inhibits protein synthesis by binding to inter-subunit bridge B2a of the ribosome. *J. Mol. Biol.* **416**, 571–578 (2012).
31. Jain, A. & Dixit, P. Multidrug-resistant to extensively drug resistant tuberculosis: what is next? *J. Biosci.* **33**, 605–616 (2008).
32. Modolell, J. & Vazquez, D. The inhibition of ribosomal translocation by viomycin. *Eur. J. Biochem.* **81**, 491–497 (1977).
33. Ermolenko, D. N. *et al.* The antibiotic viomycin traps the ribosome in an intermediate state of translocation. *Nature Struct. Mol. Biol.* **14**, 493–497 (2007).
34. Cornish, P. V., Ermolenko, D. N., Noller, H. F. & Ha, T. Spontaneous intersubunit rotation in single ribosomes. *Mol. Cell* **30**, 578–588 (2008).
35. Akbergenov, R. *et al.* Molecular basis for the selectivity of antituberculosis compounds capreomycin and viomycin. *Antimicrob. Agents Chemother.* **55**, 4712–4717 (2011).
36. Cavalleri, B., Turconi, M. & Pallanza, R. Synthesis and antibacterial activity of some derivatives of the antibiotic thermorubin. *J. Antibiot. (Tokyo)* **38**, 1752–1760 (1985).
37. Lin, F. & Wishnia, A. The protein synthesis inhibitor thermorubin. 1. Nature of the thermorubin-ribosome complex. *Biochemistry* **21**, 477–483 (1982).
38. Lin, F. & Wishnia, A. The protein synthesis inhibitor thermorubin. 2. Mechanism of inhibition of initiation on *Escherichia coli* ribosomes. *Biochemistry* **21**, 484–491 (1982).
39. Wilson, D. N. On the specificity of antibiotics targeting the large ribosomal subunit. *Ann. NY Acad. Sci.* **1241**, 1–16 (2011).
40. Bulkley, D., Innis, C. A., Blaha, G. & Steitz, T. A. Revisiting the structures of several antibiotics bound to the bacterial ribosome. *Proc. Natl Acad. Sci. USA* **107**, 17158–17163 (2010).
41. Dunkle, J. A., Xiong, L., Mankin, A. S. & Cate, J. H. Structures of the *Escherichia coli* ribosome with antibiotics bound near the peptidyl transferase center explain spectra of drug action. *Proc. Natl Acad. Sci. USA* **107**, 17152–17157 (2010).
References 40 and 41 reveal that macrolides bind to bacterial ribosomes, as reported previously for the archaeal 50S subunit. However, the data obtained question the validity of the conformation of macrolides and chloramphenicol reported in the earlier bacterial *D. radiodurans* structures (for example, reference 46).
42. Blaha, G. M., Polikanov, Y. S. & Steitz, T. A. Elements of ribosomal drug resistance and specificity. *Curr. Opin. Struct. Biol.* **22**, 750–758 (2013).
43. Hansen, J. L. *et al.* The structures of four macrolide antibiotics bound to the large ribosomal subunit. *Mol. Cell* **10**, 117–128 (2002).
Together with references 46 and 47, this study reports the first structures of antibiotics in complex with the 50S subunit.
44. Tu, D., Blaha, G., Moore, P. & Steitz, T. Structures of MLSBK antibiotics bound to mutated large ribosomal subunits provide a structural explanation for resistance. *Cell* **121**, 257–270 (2005).
45. Gurel, G., Blaha, G., Steitz, T. A. & Moore, P. B. Structures of triacetyloleandomycin and mycalamide A bind to the large ribosomal subunit of *Haloarcula marismortui*. *Antimicrob. Agents Chemother.* **53**, 5010–5014 (2009).
46. Schlünzen, F. *et al.* Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature* **413**, 814–821 (2001).
47. Hansen, J. L., Moore, P. B. & Steitz, T. A. Structures of five antibiotics bound at the peptidyl transferase center of the large ribosomal subunit. *J. Mol. Biol.* **330**, 1061–1075 (2003).
48. Mankin, A. S. & Garrett, R. A. Chloramphenicol resistance mutations in the single 23S rRNA gene of the archaeon *Halobacterium halobium*. *J. Bacteriol.* **173**, 3559–3563 (1991).
49. Champney, W. S. The other target for ribosomal antibiotics: inhibition of bacterial ribosomal subunit formation. *Infect. Disord. Drug Targets* **6**, 377–390 (2006).
50. Siibak, T. *et al.* Antibiotic-induced ribosomal assembly defects result from changes in the synthesis of ribosomal proteins. *Mol. Microbiol.* **80**, 54–67 (2011).
51. Siibak, T. *et al.* Erythromycin- and chloramphenicol-induced ribosomal assembly defects are secondary effects of protein synthesis inhibition. *Antimicrob. Agents Chemother.* **53**, 563–571 (2009).
52. Leach, K. L. *et al.* The site of action of oxazolidinone antibiotics in living bacteria and in human mitochondria. *Mol. Cell* **26**, 393–402 (2007).
53. Wilson, D. N. *et al.* The oxazolidinone antibiotics perturb the ribosomal peptidyl-transferase center and effect tRNA positioning. *Proc. Natl Acad. Sci. USA* **105**, 13339–13344 (2008).
54. Ippolito, J. A. *et al.* Crystal structure of the oxazolidinone antibiotic linezolid bound to the 50S ribosomal subunit. *J. Med. Chem.* **51**, 3353–3356 (2008).
References 52–54 show excellent agreement for the binding position of the oxazolidinone linezolid at the PTC of bacterial and archaeal ribosomes.
55. Fredrick, K. & Noller, H. F. Catalysis of ribosomal translocation by sparsomycin. *Science* **300**, 1159–1162 (2003).
56. Leach, K. L., Brickner, S. J., Noe, M. C. & Miller, P. F. Linezolid, the first oxazolidinone antibacterial agent. *Ann. NY Acad. Sci.* **1222**, 49–54 (2011).
57. Franceschi, F. & Duffy, E. M. Structure-based drug design meets the ribosome. *Biochem. Pharmacol.* **71**, 1016–1025 (2006).
58. Li, S., Cheng, X., Zhou, Y. & Xi, Z. Sparsomycin-linezolid conjugates can promote ribosomal translocation. *Chembiochem* **12**, 2801–2806 (2011).
59. Ermolenko, D. N., Cornish, P. V., Ha, T. & Noller, H. F. Antibiotics that bind to the A site of the large ribosomal subunit can induce mRNA translocation. *RNA* **19**, 158–166 (2013).
60. Kannan, K. & Mankin, A. S. Macrolide antibiotics in the ribosome exit tunnel: species-specific binding and action. *Ann. NY Acad. Sci.* **1241**, 33–47 (2012).
61. Berisio, R. *et al.* Structural insight into the antibiotic action of telithromycin on resistant mutants. *J. Bact.* **185**, 4276–4279 (2003).
62. Wilson, D. N., Harms, J. M., Nierhaus, K. H., Schlünzen, F. & Fucini, P. Species-specific antibiotic-ribosome interactions: Implications for drug development. *Biol. Chem.* **386**, 1239–1252 (2005).

63. Tenson, T., Lovmar, M. & Ehrenberg, M. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *J. Mol. Biol.* **330**, 1005–1014 (2003).
64. Tenson, T., Xiong, L. Q., Kloss, P. & Mankin, A. S. Erythromycin resistance peptides selected from random peptide libraries. *J. Biol. Chem.* **272**, 17425–17430 (1997).
65. Ramu, H., Mankin, A. & Vazquez-Laslop, N. Programmed drug-dependent ribosome stalling. *Mol. Microbiol.* **71**, 811–824 (2009).
66. Kannan, K., Vazquez-Laslop, N. & Mankin, A. S. Selective protein synthesis by ribosomes with a drug-obstructed exit tunnel. *Cell* **151**, 508–520 (2012). **This work demonstrates that macrolide antibiotics allow the synthesis of a small subset of proteins, thus conceptually changing our mechanistic understanding of the mechanism of action of this clinically important class of antibiotics.**
67. Vannuffel, P. & Cocito, C. Mechanism of action of streptogramins and macrolides. *Drugs* **51**, 20–30 (1996).
68. Harms, J., Schluenzen, F., Fucini, P., Bartels, H. & Yonath, A. Alterations at the peptidyl transferase centre of the ribosome induced by the synergistic action of the streptogramins dalfopristin and quinupristin. *BMC Biol.* **2**, 4 (2004). **Together with references 44 and 69–70, this study provides a structural basis for the synergistic activity of the streptogramin class of antibiotics.**
69. Auerbach, T. *et al.* The structure of ribosome-lankacidin complex reveals ribosomal sites for synergistic antibiotics. *Proc. Natl Acad. Sci. USA* **107**, 1983–1988 (2010).
70. Belousoff, M. J. *et al.* Crystal structure of the synergistic antibiotic pair, lankamycin and lankacidin, in complex with the large ribosomal subunit. *Proc. Natl Acad. Sci. USA* **108**, 2717–2122 (2011).
71. D'Costa, V. M. *et al.* Antibiotic resistance is ancient. *Nature* **477**, 457–461 (2011).
72. Davies, J. & Davies, D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* **74**, 417–433 (2010).
73. Forsberg, K. J. *et al.* The shared antibiotic resistome of soil bacteria and human pathogens. *Science* **337**, 1107–1111 (2012).
74. Allen, H. K. *et al.* Call of the wild: antibiotic resistance genes in natural environments. *Nature Rev. Microbiol.* **8**, 251–259 (2010).
75. He, X. *et al.* Structure of a cation-bound multidrug and toxic compound extrusion transporter. *Nature* **467**, 991–994 (2010).
76. Lu, M. *et al.* Structures of a Na^+-coupled, substrate-bound MATE multidrug transporter. *Proc. Natl Acad. Sci. USA* **110**, 2099–2104 (2013).
77. Tanaka, Y. *et al.* Structural basis for the drug extrusion mechanism by a MATE multidrug transporter. *Nature* **496**, 247–251 (2013).
78. van Veen, H. W. Structural biology: last of the multidrug transporters. *Nature* **467**, 926–927 (2010).
79. Piddock, L. J. Multidrug-resistance efflux pumps — not just for resistance. *Nature Rev. Microbiol.* **4**, 629–636 (2006).
80. Davidovich, C. *et al.* Induced-fit tightens pleuromutilins binding to ribosomes and remote interactions enable their selectivity. *Proc. Natl Acad. Sci. USA* **104**, 4291–4296 (2007).
81. Harms, J. M. *et al.* Translational regulation via L11: molecular switches on the ribosome turned on and off by thiostrepton and micrococcin. *Mol. Cell* **30**, 26–38 (2008).
82. LaMarre, J. *et al.* The genetic environment of the *cfr* gene and the presence of other mechanisms account for the very high linezolid resistance of *Staphylococcus epidermidis* isolate 426-3147L. *Antimicrob. Agents Chemother.* **57**, 1173–1179 (2013).
83. Billal, D. S., Feng, J., Leprohon, P., Legare, D. & Ouellette, M. Whole genome analysis of linezolid resistance in *Streptococcus pneumoniae* reveals resistance and compensatory mutations. *BMC Genomics* **12**, 512 (2011).
84. Beauclerk, A. A. D. & Cundliffe, E. Sites of action of two ribosomal RNA methylases responsible for resistance to aminoglycosides. *J. Mol. Biol.* **193**, 661–671 (1987).
85. Cundliffe, E. How antibiotic-producing organisms avoid suicide. *Ann. Rev. Microbiol.* **43**, 207–233 (1989).
86. Giessing, A. M. *et al.* Identification of 8-methyladenosine as the modification catalyzed by the radical SAM methyltransferase Cfr that confers antibiotic resistance in bacteria. *RNA* **15**, 327–336 (2009).
87. Long, K. S., Poehlsgaard, J., Kehrenberg, C., Schwarz, S. & Vester, B. The Cfr rRNA methyltransferase confers resistance to Phenicols, Lincosamides, Oxazolidinones, Pleuromutilins, and Streptogramin A antibiotics. *Antimicrob. Agents Chemother.* **50**, 2500–2505 (2006).
88. Smith, L. K. & Mankin, A. S. Transcriptional and translational control of the *mlr* operon, which confers resistance to seven classes of protein synthesis inhibitors. *Antimicrob. Agents Chemother.* **52**, 1703–1712 (2008).
89. LaMarre, J. M., Locke, J. B., Shaw, K. J. & Mankin, A. S. Low fitness cost of the multidrug resistance gene *cfr*. *Antimicrob. Agents Chemother.* **55**, 3714–3719 (2011).
90. Gupta, P., Sothiselvam, S., Vazquez-Laslop, N. & Mankin, A. S. Deregulation of translation due to post-transcriptional modification of rRNA explains why *erm* genes are inducible. *Nature Commun.* **4**, 1984 (2013).
91. Monshupanee, T., Johansen, S. K., Dahlberg, A. E. & Douthwaite, S. Capreomycin susceptibility is increased by TlyA-directed 2′-O-methylation on both ribosomal subunits. *Mol. Microbiol.* **85**, 1194–1203 (2012).
92. LaMarre, J. M., Howden, B. P. & Mankin, A. S. Inactivation of the indigenous methyltransferase RlmN in *Staphylococcus aureus* increases linezolid resistance. *Antimicrob. Agents Chemother.* **55**, 2989–2991 (2011).
93. Okamoto, S. *et al.* Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. *Mol. Microbiol.* **63**, 1096–1106 (2007).
94. Thom, G. & Prescott, C. D. The selection *in vivo* and characterization of an RNA recognition motif for spectinomycin. *Bioorgan. Med. Chem.* **5**, 1081–1086 (1997).
95. Gross, S. *et al.* Amythiamicin D and related thiopeptides as inhibitors of the bacterial elongation factor EF-Tu: modification of the amino acid at carbon atom C2 of ring C dramatically influences the activity. *Eur. J. Biochem.* http://dx.doi.org/10.1002/cmdc.201300323 (2013).
96. Connell, S. R., Tracz, D. M., Nierhaus, K. H. & Taylor, D. E. Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrob. Agents Chemother.* **47**, 3675–3681 (2003).
97. O'Neill, A. J. & Chopra, I. Molecular basis of fusB-mediated resistance to fusidic acid in *Staphylococcus aureus*. *Mol. Microbiol.* **59**, 664–676 (2006).
98. Dönhöfer, A. *et al.* Structural basis for TetM-mediated tetracycline resistance. *Proc. Natl Acad. Sci. USA* **109**, 16900–16905 (2012).
99. Li, W. *et al.* Mechanism of tetracycline resistance by ribosomal protection protein Tet(O). *Nature Commun.* **4**, 1477 (2013). **References 98 and 99 report structures of ribosome protection proteins on the ribosome, revealing that these factors confer tetracycline resistance by directly dislodging tetracycline from its ribosomal binding site rather than by an indirect mechanism as previously proposed.**
100. Cox, G. *et al.* Ribosome clearance by FusB-type proteins mediates resistance to the antibiotic fusidic acid. *Proc. Natl Acad. Sci. USA* **109**, 2102–2107 (2012).
101. Guo, X. *et al.* Structure and function of FusB: an elongation factor G-binding fusidic acid resistance protein active in ribosomal translocation and recycling. *Open Biol.* **2**, 120016 (2012).
102. Wright, G. D. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Adv. Drug Deliv. Rev.* **57**, 1451–1470 (2005).
103. Ramirez, M. S. & Tolmasky, M. E. Aminoglycoside modifying enzymes. *Drug Resist. Updat.* **13**, 151–171 (2010).
104. Mosher, R. H. *et al.* Inactivation of chloramphenicol by O-phosphorylation. A novel resistance mechanism in Streptomyces venezuelae ISP5230, a chloramphenicol producer. *J. Biol. Chem.* **270**, 27000–27006 (1995).
105. Rajesh, T. *et al.* Phosphorylation of chloramphenicol by a recombinant protein Yhr2 from *Streptomyces avermitilis* MA4680. *Bioorg. Med. Chem. Lett.* **23**, 3614–3619 (2013).
106. Dhote, V., Gupta, S. & Reynolds, K. A. An O-phosphotransferase catalyzes phosphorylation of hygromycin A in the antibiotic-producing organism *Streptomyces hygroscopicus*. *Antimicrob. Agents Chemother.* **52**, 3580–3588 (2008).
107. Volkers, G., Palm, G. J., Weiss, M. S., Wright, G. D. & Hinrichs, W. Structural basis for a new tetracycline resistance mechanism relying on the TetX monooxygenase. *FEBS Lett.* **585**, 1061–1066 (2011).
108. Moore, I. F., Hughes, D. W. & Wright, G. D. Tigecycline is modified by the flavin-dependent monooxygenase TetX. *Biochemistry* **44**, 11829–11835 (2005).
109. Palmer, A. C., Angelino, E. & Kishony, R. Chemical decay of an antibiotic inverts selection for resistance. *Nature Chem. Biol.* **6**, 105–107 (2010).
110. Butler, M. S. & Cooper, M. A. Antibiotics in the clinical pipeline in 2011. *J. Antibiot. (Tokyo)* **64**, 413–425 (2011).
111. Sutcliffe, J. A. Antibiotics in development targeting protein synthesis. *Ann. NY Acad. Sci.* **1241**, 122–152 (2011).
112. Pokrovskaya, V. & Baasov, T. Dual-acting hybrid antibiotics: a promising strategy to combat bacterial resistance. *Expert Opin. Drug Discov.* **5**, 883–902 (2010).
113. Wu, Y. J. & Su, W. G. Recent developments on ketolides and macrolides. *Curr. Med. Chem.* **8**, 1727–1758 (2001).
114. Nicolaou, K. C., Chen, J. S., Edmonds, D. J. & Estrada, A. A. Recent advances in the chemistry and biology of naturally occurring antibiotics. *Angew. Chem. Int. Ed Engl.* **48**, 660–719 (2009).
115. Walsh, C. T., Acker, M. G. & Bowers, A. A. Thiazolyl peptide antibiotic biosynthesis: a cascade of post-translational modifications on ribosomal nascent proteins. *J. Biol. Chem.* **285**, 27525–27531 (2010).
116. Brandi, L. *et al.* Specific, efficient, and selective inhibition of prokaryotic translation initiation by a novel peptide antibiotic. *Proc. Natl Acad. Sci. USA* **103**, 39–44 (2006).
117. Llano-Sotelo, B., Klepacki, D. & Mankin, A. S. Selection of small peptides, inhibitors of translation. *J. Mol. Biol.* **391**, 813–819 (2009).
118. Brandi, L. *et al.* Structural and functional characterization of the bacterial translocation inhibitor GE82832. *FEBS Lett.* **586**, 3373–3378 (2012).
119. Fabbretti, A. *et al.* The antibiotic Furvina® targets the P-site of 30S ribosomal subunits and inhibits translation initiation displaying start codon bias. *Nucleic Acids Res.* **40**, 10366–10374 (2012).
120. Maffioli, S. I. *et al.* Orthoformimycin, a selective inhibitor of bacterial translation elongation from *Streptomyces* containing an unusual orthoformate. *ACS Chem. Biol.* **8**, 1939–1946 (2013).
121. Schroeder, S. J., Blaha, G., Tirado-Rives, J., Steitz, T. A. & Moore, P. B. The structures of antibiotics bound to the E site region of the 50 S ribosomal subunit of *Haloarcula marismortui*: 13-deoxytedanolide and girodazole. *J. Mol. Biol.* **367**, 1471–1479 (2007).
122. Schneider-Poetsch, T. *et al.* Inhibition of eukaryotic translation elongation by cycloheximide and lactimidomycin. *Nature Chem. Biol.* **6**, 209–217 (2010).
123. Klinge, S., Voigts-Hoffmann, F., Leibundgut, M., Arpagaus, S. & Ban, N. Crystal structure of the eukaryotic 60S ribosomal subunit in complex with initiation factor 6. *Science* **334**, 941–948 (2011). **The structures of eukaryotic ribosomes reported in this work and in references 124–126 open the way to investigating the specificity of antibiotic interactions with the ribosome, as well as the development of anticancer drugs.**
124. Ben-Shem, A. *et al.* The structure of the eukaryotic ribosome at 3.0 Å resolution. *Science* **334**, 1524–1529 (2011).
125. Rabl, J., Leibundgut, M., Ataide, S. F., Haag, A. & Ban, N. Crystal structure of the eukaryotic 40S ribosomal subunit in complex with initiation factor 1. *Science* **331**, 730–736 (2011).
126. Anger, A. M. *et al.* Structures of the human and *Drosophila* 80S ribosome. *Nature* **497**, 80–85 (2013).
127. Keeling, K. M., Wang, D., Conard, S. E. & Bedwell, D. M. Suppression of premature termination codons as a therapeutic approach. *Crit. Rev. Biochem. Mol. Biol.* **47**, 444–463 (2012).
128. Noller, H. F., Yusupov, M. M., Yusupova, G. Z., Baucom, A. & Cate, J. H. D. Translocation of tRNA during protein synthesis. *FEBS Letter* **514**, 11–16 (2002).
129. Rodnina, M. V. & Wintermeyer, W. The ribosome as a molecular machine: the mechanism of tRNA-mRNA movement in translocation. *Biochem. Soc. Trans.* **39**, 658–662 (2011).

130. Chen, J., Tsai, A., O'Leary, S. E., Petrov, A. & Puglisi, J. D. Unraveling the dynamics of ribosome translocation. *Curr. Opin. Struct. Biol.* **22**, 804–814 (2013).
131. Moazed, D. & Noller, H. F. Intermediate states in the movement of transfer RNA in the ribosome. *Nature* **342**, 142–148 (1989).
132. Munro, J. B., Altman, R. B., O'Connor, N. & Blanchard, S. C. Identification of two distinct hybrid state intermediates on the ribosome. *Mol. Cell* **25**, 505–517 (2007).
133. Agirrezabala, X. *et al.* Visualization of the hybrid state of tRNA binding promoted by spontaneous ratcheting of the ribosome. *Mol. Cell* **32**, 190–197 (2008).
134. Julian, P. *et al.* Structure of ratcheted ribosomes with tRNAs in hybrid states. *Proc. Natl Acad. Sci. USA* **105**, 16924–16927 (2008).
135. Frank, J. & Agrawal, R. Ratchet-like movements between the two ribosomal subunits: their implications in elongation factor recognition and tRNA translocation. *Cold Spring Harb. Symp. Quant. Biol.* **66**, 67–75 (2001).
136. Chen, J., Petrov, A., Tsai, A., O'Leary, S. E. & Puglisi, J. D. Coordinated conformational and compositional dynamics drive ribosome translocation. *Nature Struct. Mol. Biol.* **20**, 718–727 (2013).
137. Savelsbergh, A. *et al.* An elongation factor G-induced ribosome rearrangement precedes tRNA-mRNA translocation. *Mol. Cell* **11**, 1517–1523 (2003).
138. Ratje, A. H. *et al.* Head swivel on the ribosome facilitates translocation by means of intra-subunit tRNA hybrid sites. *Nature* **468**, 713–716 (2010).
139. Borovinskaya, M. A., Shoji, S., Holton, J. M., Fredrick, K. & Cate, J. H. A steric block in translation caused by the antibiotic spectinomycin. *ACS Chem. Biol.* **2**, 545–552 (2007).
140. Schluenzen, F. *et al.* The antibiotic kasugamycin mimics mRNA nucleotides to destabilize tRNA binding and inhibit canonical translation initiation. *Nature Struct. Mol. Biol.* **13**, 871–878 (2006).
141. Borovinskaya, M. A., Shoji, S., Fredrick, K. & Cate, J. H. Structural basis for hygromycin B inhibition of protein biosynthesis. *RNA* **14**, 1590–1599 (2008).
142. Carter, A. P. *et al.* Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature* **407**, 340–348 (2000).
143. Hansen, J. L., Schmeing, T. M., Moore, P. B. & Steitz, T. A. Structural insights into peptide bond formation. *Proc. Natl Acad. Sci. USA* **99**, 11670–11675 (2002).
144. Schlunzen, F., Pyetan, E., Fucini, P., Yonath, A. & Harms, J. Inhibition of peptide bond formation by pleuromutilins: the structure of the 50S ribosomal subunit from *Deinococcus radiodurans* in complex with tiamulin. *Mol. Microbiol.* **54**, 1287–1294 (2004).
145. Seidelt, B. *et al.* Structural insight into nascent polypeptide chain-mediated translational stalling. *Science* **326**, 1412–1415 (2009).

Acknowledgements
I give special thanks to A. Mankin, B. Beatrix and members of my laboratory for critical reading of the manuscript. I also thank the Deutsche Forschungsgemeinschaft (WI3285/2-1) and the EMBO young investigator grant for funding research related to this topic.

Competing interests statement
The author declares no competing interests.

DATABASES
Protein Data Bank: http://www.rcsb.org/pdb/
1FJG | 1HNX | 1I95 | 1K9M | 1KC8 | 1M90 | 1M90 | 1NJI | 1SM1 | 1XBP | 2HHH | 2QOU–X | 3CF5 | 3DF1–4 | 3DLL | 3KNH–K | 3KNL–Q | 3OFA–D | 3OFO–R | 3OFX/Y/Z/0 | 3UXQ–T | 4G5K–N | 4G5T–W | 4GAQ/R/S/U | 4L6J–M

ALL LINKS ARE ACTIVE IN THE ONLINE PDF