RIBOSOME-TARGETING ANTIBIOTIC MECHANISMS

Putting the antibiotics chloramphenicol and linezolid into context

Growing evidence suggests that many ribosome-targeting antibiotics inhibit protein synthesis context specifically, which has important implications for drug development. New work reveals the structural basis of context-specific action of the classic translation inhibitor chloramphenicol and the oxazolidinones linezolid and radezolid.

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Peptide bond formation on the ribosome occurs at the peptidyltransferase center of the large ribosomal subunit and entails nucleophilic attack by the aminoacyl-tRNA located at the A-site (A-tRNA) on the peptidyl-tRNA positioned at the P-site (P-tRNA) (Fig. 1a). Previous structures of antibiotics such as chloramphenicol and linezolid bound to non-translating ribosomes show that these compounds bind within the A-site and overlap with the aminoacyl moiety of the A-tRNA (Fig. 1b,c)[1]. Based on this, one could envisage that these molecules would interfere with each and every round of peptide bond formation by preventing full A-tRNA accommodation during translation elongation. However, this turns out not to be the case. In 2016, ribosome profiling of bacterial cells treated with chloramphenicol or linezolid instead revealed that translation does not become arrested uniformly over the open reading frame, but rather does so at distinct sites[2]. Specifically, translation arrest is predominantly influenced by the amino acid in the –1 position of the nascent polypeptide—that is, the penultimate amino

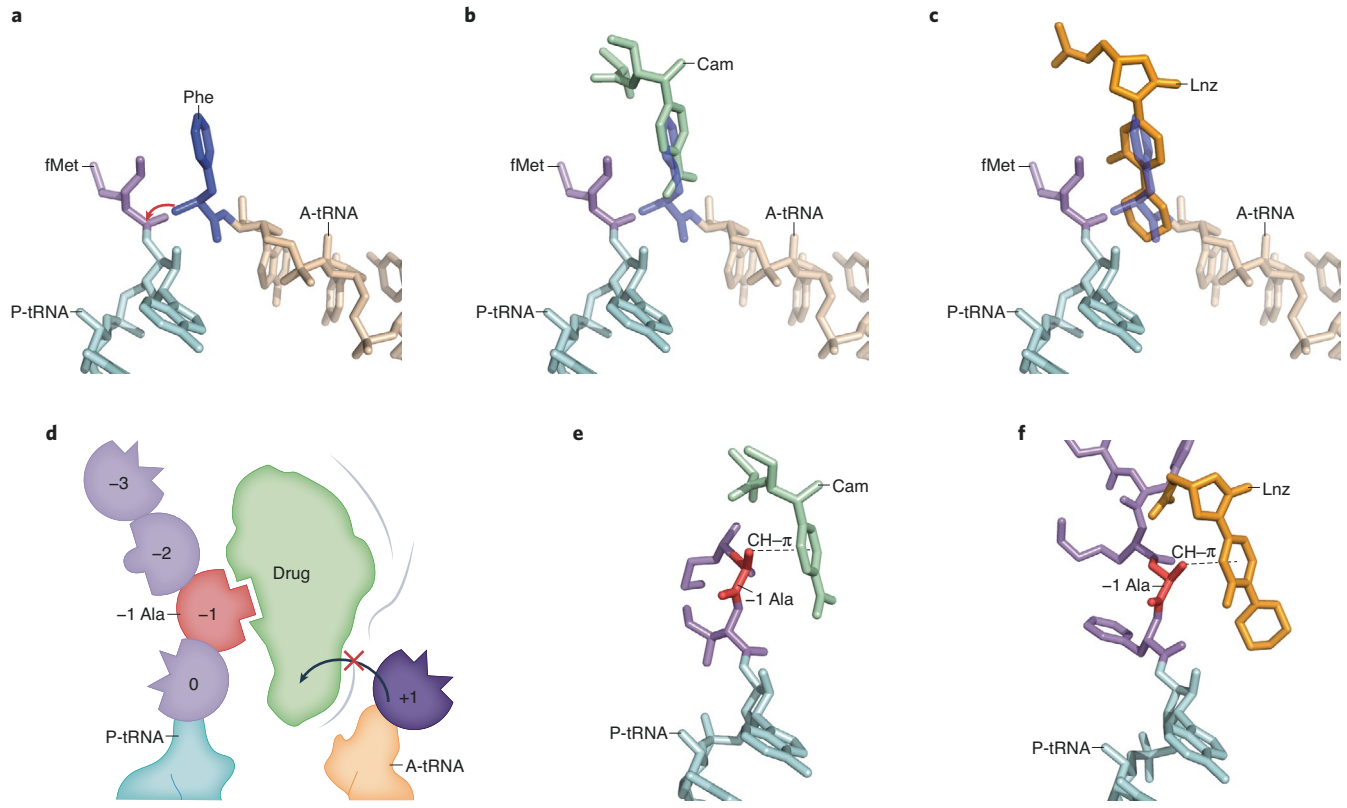

Fig. 1 | Mechanism of action of chloramphenicol and linezolid during protein synthesis. a, Canonical positions of A- and P-tRNAs, showing peptidyl transfer of an incoming phenylalanine to an initiator methionine (PDB ID 1VY4)[17]. **b**, As in **a** but with transparent A-site tRNA and a superimposed chloramphenicol (Cam) molecule, showing a steric occlusion of A-tRNA entry in the presence of Cam (PDB ID 6ND5)[18]. **c**, As in **a** but with transparent A-site tRNA and a superimposed linezolid (Lnz), showing a steric occlusion of A-tRNA entry in the presence of Lnz (PDB ID 3DLL)[19]. **d**, Schematic of the effect of the –1 position of the nascent chain (P-tRNA) on the action of chloramphenicol and linezolid. **e**, Chloramphenicol and P-tRNA with alanine in –1 position, highlighting the CH–π interaction explaining the enhanced affinity and inhibitory action of Cam in the presence of alanine in the –1 position[5]. **f**, Linezolid and P-tRNA with alanine in –1 position, highlighting the CH–π interaction explaining the enhanced affinity and inhibitory action of Lnz in the presence of alanine in the –1 position[6].

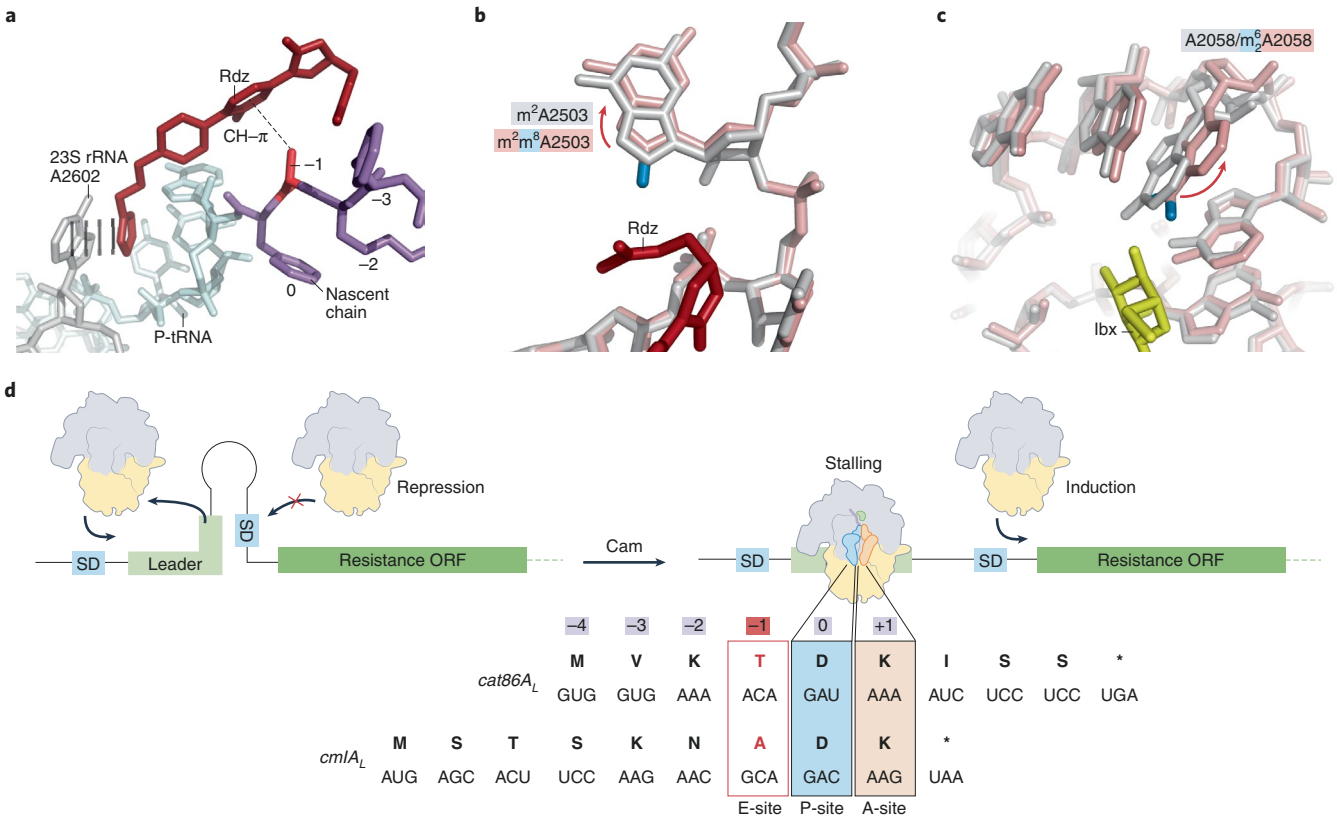

Fig. 2 | Insights into radezolid and chloramphenicol resistance mechanisms. a, Interaction of radezolid (Rdz) with 23S rRNA A2602 and nascent chain on the ribosome. The –1 alanine side chain is shown in red. The Rdz D-ring stacks with rRNA A2602 (grey lines)[6]. **b**, Superimposition of radezolid (Rdz) on the A2503-modified (modified by Cfr methyltransferase; pink) and wild-type (grey) ribosome[6]. The shift of the modified A2503 is indicated by a red arrow. **c**, Superimposition of iboxamycin (Ibx) on the A2058-modified (pink) and unmodified (grey) ribosome, showing the shift in dimethylated A2058 compared to the unmodified structure[9]. **d**, Schematic for chloramphenicol (Cam)-dependent stalling on the uORFs *cat86*$_L$ and *cmlA*$_L$ to induce expression of the Cat86 and CmlA antibiotic-resistance determinants (modified from ref. [20]; reproduced with permission from Elsevier). Sequences of the stalling leader peptides Cat86A$_L$ (GenBank K00544.1 nucleotides 83–112) and CmlA$_L$ (GenBank U12338.3 nucleotides 6814–6843), with stalling positions indicated below. Asterisks indicate stop codons.

acid that is attached to the peptidyl-tRNA (Fig. 1d). For both drugs, the strongest arrest was observed when alanine was in the –1 position, with minor arrests observed with serine and threonine[2]. Together with related findings[3,4], this has raised the questions: How can the nascent polypeptide chain influence the ability of antibiotics such as linezolid and chloramphenicol to arrest translation elongation? And can such information be used to guide development of future generations of improved antibiotics?

Syroegin et al.[5] employed X-ray crystallography to determine structures of chloramphenicol bound to ribosomes bearing different peptidyl-tRNA mimics with alanine, threonine or phenylalanine at the –1 position. The structures revealed that the binding of chloramphenicol to the ribosomal A-site is directly stabilized by CH–π interactions between the alanine (or threonine) side chain and the nitrophenyl ring of chloramphenicol[5] (Fig. 1e). By contrast, chloramphenicol was not observed when phenylalanine was in the –1 position, presumably because of steric overlap between its bulky side chain and the drug[5]. Similar findings were observed by Tsai et al.[6], who determined cryo-electron microscopy structures of the oxazolidinone linezolid in complex with vacant ribosomes as well as linezolid-arrested ribosomes bearing a nascent polypeptide chain with an alanine in the –1 position. These structures revealed that, as with chloramphenicol, binding of linezolid to the ribosomal A-site is directly stabilized by a CH–π interaction between the alanine side chain and the B-ring of linezolid[6] (Fig. 1f). Taking these observations together, a model emerges in which the binding of chloramphenicol or linezolid to the ribosome is favored by the presence of optimally sized amino acids in the –1 position that create a 'snug fit' between the drug, the ribosome and the nascent polypeptide chain[5,6]. The presence of smaller amino acids (glycine) in the –1 position is disfavorable to drug binding because of loss of the CH–π interaction, whereas the presence of amino acids with larger side chains appear to sterically occlude drug binding[5,6].

In addition to providing a structural basis for the context-specific action of these inhibitors, these studies also provide detailed molecular insights that will be important for future development of improved antimicrobials to combat the ever-rising threat of multidrug-resistant pathogenic bacteria. In addition to linezolid, Tsai et al.[6] analyzed radezolid, a second-generation oxazolidinone, revealing that it exhibits a context-specific inhibition analogous to that of linezolid. Structures of radezolid on the ribosome indicated that, as with linezolid, the B-ring forms a CH–π interaction with the alanine side chain. Furthermore, the D-ring of radezolid, which is absent in linezolid, establishes additional stacking interactions with nucleotide A2602 of 23S rRNA[6]

(Fig. 2a), presumably explaining radezolid's enhanced potency compared to linezolid and its potency against linezolid-resistant strains[6,7]. A prevalent resistance mechanism against oxazolidinones (as well as chloramphenicol) involves methylation of 23S rRNA nucleotide A2503 by the Cfr methyltransferase[8]. Here the modification is expected to protrude into the drug-binding site, generating a steric clash and reducing the drug's affinity for the ribosome. Whereas Cfr confers high-level linezolid resistance, radezolid retains some activity against Cfr-modified ribosomes, albeit not in a context-specific fashion[6]. To investigate this, Tsai et al.[6] determined a cryo-EM structure of radezolid bound to Cfr-modified ribosomes, revealing that radezolid retains the CH–π interaction with alanine in the –1 position and can even displace the modified A2503 from its canonical position observed in the unmodified structure (Fig. 2b). Presumably, radezolid, but not linezolid, can displace the modified A2503 as a result of the higher affinity arising from the additional stacking interaction with A2602[6]. This scenario is reminiscent of that reported recently whereby iboxamycin, a semi-synthetic clindamycin derivative, retains activity against ribosomes dimethylated at 23S rRNA nucleotide A2058 by Erm methyltransferases[9] (Fig. 2c). This has important implications for antibiotic development, suggesting that introducing functional groups that establish additional interactions with the ribosome may increase affinity sufficiently that the drug can displace modified nucleotides. It will be interesting to see whether this is also true for nucleotide mutations that give rise to antibiotic resistance.

The expression of many antibiotic-resistance determinants, including some resistance methyltransferases, is regulated to occur only in the presence of the drug[10,11]. In these systems, drug-dependent translational stalling within an upstream open reading frame (uORF), or so-called leader peptide, causes a rearrangement of mRNA secondary structure that induces the expression of a downstream resistance gene[10,11]. Two well-characterized examples are the chloramphenicol resistance genes *cat86A* and *cmlA*, which are regulated by chloramphenicol-dependent stalling in the $cat86A_L$ and $cmlA_L$ uORFs, respectively[10,11] (Fig. 2d). In the presence of chloramphenicol, ribosomes arrest on these uORFs with threonine and alanine in the –1 position of the leader peptide, respectively[2,10,11] (Fig. 2d). Thus, the structures reported by Syroegin et al.[5] also provide insight into the context-specific arrest mechanism used by specific bacteria to regulate the expression of resistance determinants. The similarity in the mechanisms of inhibition suggests that linezolid, a purely synthetic compound, will also induce expression of chloramphenicol-resistance genes for leader peptides with alanine in the –1 position. Thus, efforts to develop linezolid derivatives with expanded stalling profiles should also consider potential side effects related to the induction of antibiotic-resistance genes. The Erm methyltransferases, which dimethylate A2058 to confer macrolide resistance, are also regulated by context-specific arrest within upstream leader peptides[11,12]. However, for the structurally studied Erm leader peptides, it appears that the nascent chain does not stabilize macrolide binding—as observed by Syroegin et al.[5] and Tsai et al.[6] for chloramphenicol and linezolid—but rather the macrolide promotes defined conformations of the nascent polypeptide chain, which in turn arrest translation using a variety of diverse mechanisms[13–15]. The chloramphenicol- and linezolid-arrest mechanism is distinct from those described previously for macrolides.

Although the role of the –1 position in context-specific inhibition has now become clearer, ribosome profiling experiments revealed that an alanine in the –1 position of the nascent chain is not, by itself, sufficient for translational arrest—there are many encoded alanines where no translational arrest occurs[2], suggesting that additional nascent chain context is likely to also contribute to stalling efficiency. Similar observations have been made for macrolide stalling at arginine–leucine–arginine motifs[12,13,16]. It will be interesting in the future to determine to what extent context-specific inhibition is relevant to other classes of ribosome-targeting inhibitors, even those that do not target the peptidyltransferase center. ❐

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Competing interests

The authors declare no competing interests.