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ANTIMICROBIALS

Putting antibiotic action into context

Ribosomes are the site of protein synthesis and one of the main targets of antimicrobials in the bacterial cell. The ribosomal peptidyl transferase center (PTC), which resides in the large ribosomal subunit, catalyses peptide-bond formation and peptide release. PTC-targeting antibiotics, such as the natural compound chloramphenicol or the synthetic inhibitor linezolid, bind to the A-site in the PTC, and it was suggested that this interaction prevents the correct positioning of the amino-acylated ends of tRNAs (aa-tRNA) and thus peptide-bond formation. As binding of aa-tRNAs at the A-site is a key step during protein synthesis, it was suggested that chloramphenicol and linezolid function as universal inhibitors of translation. However, the model that chloramphenicol and linezolid indiscriminately inhibit the formation of any peptide bond was in conflict with several observations, including the differential inhibition of translation of specific mRNA templates and the induction of chloramphenicol resistance genes. In this study, Marks *et al.* showed that chloramphenicol and linezolid preferentially stall ribosomes at specific mRNA locations rather than function as global inhibitors of peptide-bond formation.

The authors used toeprinting analysis and ribosome-profiling experiments to determine the position at which ribosomes were stalled on various mRNAs. If chloramphenicol and linezolid function as universal inhibitors, the ribosome preincubated with those compounds should stall at the initiator codon of any mRNA; however, they found that ribosomes were stalled at specific downstream sites, with varying efficiencies. They went on to analyse drug-induced translational arrest at selected codons and searched for specific sequence signatures among amino acids encoded within nine codons preceding the arrest site (positions −1 to −9 in the peptide), the arrest codons (the P-site; position 0 in the peptide) and the following codon (the A-site; position +1). Interestingly, the arrest sites for both drugs showed a strong enrichment for alanine and, to a lesser extent, for serine and threonine at the −1 position. However, there was no preference for a specific codon, which suggests that the penultimate amino acid in the nascent chain rather than the mRNA sequence or the tRNA structure is important for drug action. Drug-induced ribosome stalling at the Leu5 codon in the *hns* gene was among the strongest arrest sites, and *in vitro* mutagenesis experiments revealed that replacing the penultimate alanine residue at position −1 with any other amino acid greatly reduced ribosome stalling.

Moreover, the authors showed that the presence of glycine codons in either the P-site or A-site negatively correlated with ribosome stalling. These data suggest that chloramphenicol and linezolid inhibit translation in a site-specific manner, and that inhibition efficiency depends on the amino acid context in the nascent chain.

> “the efficiency of chloramphenicol and linezolid to inhibit translation is site-specific and depends on the amino acid context in the nascent chain”

In the absence of the antibiotic, expression of the chloramphenicol-resistance gene *cmlAL* is repressed because its ribosome-binding site is sequestered in the mRNA secondary structure; in the presence of chloramphenicol, ribosomes are arrested at a specific codon in the leader ORF of the inducible resistance gene, which triggers a conformational change in the mRNA that releases the ribosome-binding site and activates the expression of the resistance gene. The authors showed that chloramphenicol stalls translation of the *cmlAL* leader ORF when alanine is present at the penultimate position of the peptide, which suggests that the site-specific arrest of ribosomes at the regulatory ORF of resistance genes is governed by sequence specificity of chloramphenicol action. In addition, linezolid also arrested ribosomes at the same site, which suggests that not only natural antibiotics but also synthetic drugs can induce natural antibiotic resistance genes.

In summary, this study reveals that PTC-targeting ribosomal antibiotics function in a context-specific manner, dependent on the penultimate amino acid in the nascent chain and the amino acids in the P-site and A-site of the PTC. These findings highlight that insights into the mechanism of action of ribosomal antibiotics are crucial for the development of novel and more effective antimicrobials to tackle bacterial resistance.

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ORIGINAL ARTICLE Marks, J. *et al.* Context-specific inhibition of translation by ribosomal antibiotics targeting the peptidyl transferase center. *Proc. Natl Acad. Sci.* http://dx.doi.org/10.1073/pnas.1613055113 (2016)
FURTHER READING Wilson, D. L. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nat. Rev. Microbiol.* **12**, 35–48 (2014)