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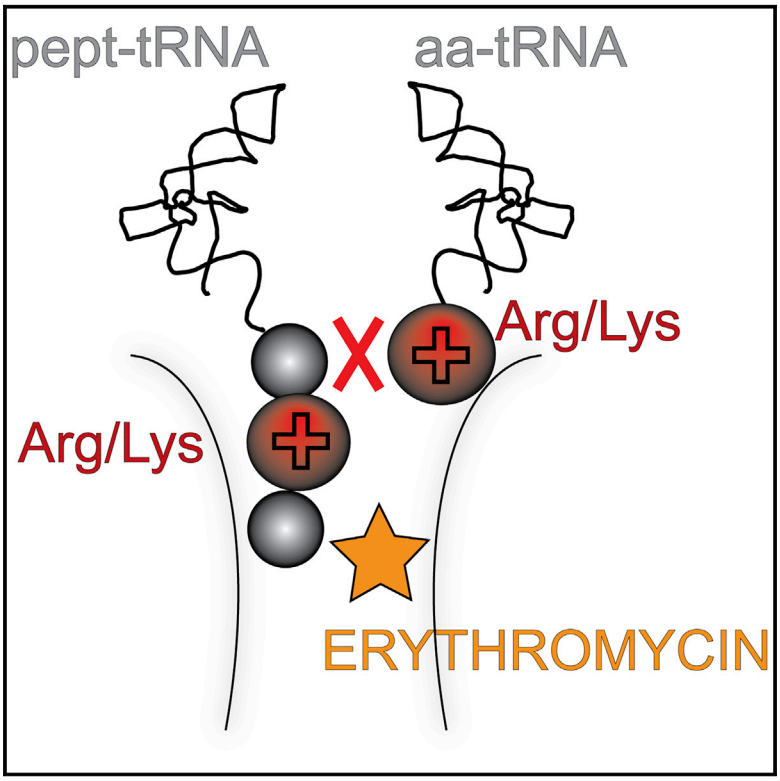
Report

Binding of Macrolide Antibiotics Leads to Ribosomal Selection against Specific Substrates Based on Their Charge and Size

Graphical Abstract



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In Brief

Macrolide antibiotics stall ribosomes at the Arg/Lys-X-Arg/Lys motif. Sothiselvam et al. find that the charge and size of key amino acid side chains in this motif make peptide bond formation inefficient. Antibiotics greatly magnify the problem of these intrinsically difficult donor-acceptor pairs.

Highlights

- Macrolide antibiotics induce ribosome stalling at Arg/Lys-X-Arg/Lys motifs
- The size and charge of key residue side chains hinder peptide bond formation
- Properties of the nascent protein affect the catalytic capacity of the ribosome

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Binding of Macrolide Antibiotics Leads to Ribosomal Selection against Specific Substrates Based on Their Charge and Size

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SUMMARY

Macrolide antibiotic binding to the ribosome inhibits catalysis of peptide bond formation between specific donor and acceptor substrates. Why particular reactions are problematic for the macrolide-bound ribosome remains unclear. Using comprehensive mutational analysis and biochemical experiments with synthetic substrate analogs, we find that the positive charge of these specific residues and the length of their side chains underlie inefficient peptide bond formation in the macrolide-bound ribosome. Even in the absence of antibiotic, peptide bond formation between these particular donors and acceptors is rather inefficient, suggesting that macrolides magnify a problem present for intrinsically difficult substrates. Our findings emphasize the existence of functional interactions between the nascent protein and the catalytic site of the ribosomal peptidyl transferase center.

INTRODUCTION

The ribosome is an incredibly complex and sophisticated protein synthesis machine that represents one of the major antibiotic targets in bacterial cells (Wilson, 2009). Although many ribosome-targeting inhibitors completely abolish protein synthesis, some of them interfere with translation in a context-specific manner. Macrolides, such as erythromycin (ERY) and its derivatives, represent the best-studied examples of inhibitors whose action critically depends on the amino acid sequence of the synthesized protein (Davis et al., 2014; Hardesty et al., 1990; Kannan et al., 2012, 2014; Starosta et al., 2010). Depending on the nature of the nascent chain and the structure of the drug, synthesis of the polypeptide can be arrested during very early rounds (Otaka and Kaji, 1975; Tenson et al., 2003), at the later stages of translation elongation (Davis et al., 2014; Kannan et al., 2014), or not at all (Kannan et al., 2012; Starosta et al., 2010). Although optimizing clinical outcomes of antibiotic therapy hinges on achieving an understanding of the mode of drug action, a mechanistic explanation for the context specificity of macrolides is lacking.

Macrolide antibiotics target the large ribosomal subunit. The drugs bind in the nascent peptide exit tunnel (NPET) at a short distance from the peptidyl transferase center (PTC). Binding of the antibiotic obstructs the NPET and restricts placement of the nascent chain in the tunnel and its progression from the PTC to the tunnel exit (Bulkley et al., 2010; Dunkle et al., 2010; Schlünzen et al., 2001; Tu et al., 2005). When the newly synthesized peptide chain grows to around four amino acids, it reaches the site of antibiotic binding (Arenz et al., 2014a, 2014b). Synthesis of some proteins by the drug-bound ribosome is interrupted at this stage, especially when the macrolide, like ERY, contains a C3-bound cladinose sugar that protrudes into the lumen of the tunnel (Bulkley et al., 2010; Dunkle et al., 2010; Schlünzen et al., 2001). However, some nascent peptides are able to bypass the antibiotic, allowing their continued synthesis in spite of the presence of a bulky drug molecule in the NPET (Hardesty et al., 1990; Kannan et al., 2012; Starosta et al., 2010). Ketolide drugs, which lack a C3 cladinose, rarely inhibit translation at the early stages, and synthesis of many proteins continues past the initial rounds of protein elongation (Kannan et al., 2012, 2014).

Although macrolides do not inhibit synthesis of many proteins at the early rounds, translation of the majority of polypeptides by the drug-bound ribosome is eventually interrupted at the later stages of elongation (Davis et al., 2014; Kannan et al., 2014). Strikingly, abrogation of translation does not occur randomly, but, rather, drug-bound ribosomes become arrested at specific, well defined mRNA sites. Ribosome profiling analysis carried out in Gram-positive and Gram-negative bacteria treated with macrolides helped identify the major sites of late translation arrest and allowed for initial classification of problematic sequences (Davis et al., 2014; Kannan et al., 2014). Several amino acid motifs conducive to macrolide-induced arrest emerged from these studies. One of the most prevalent motifs conforms to the consensus R/K-X-R/K, where R and K represent arginine and lysine, respectively, and X represents any amino acid. In vitro biochemical testing supported the conclusion drawn from the profiling analysis that the ribosome stalls when the codon specifying the middle amino acid (X) of the motif enters the P site. Accordingly, the first residue of the consensus (R or K)