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Ribosomes Left in the Dust: Diverse Strategies for Peptide-Mediated Translation Stalling

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Title: Ribosomes left in the dust: diverse strategies for peptide-mediated translation stalling

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Summary

In two recent papers, Arenz et al. (2014) and Bischoff et al. (2014) provide structural insights into drug-induced, peptide-mediated stalling of the ribosome.

Main Text

Ribosomes are fast, accurate, and highly processive enzymes. While the ribosome translates the vast majority of the proteome efficiently, certain polypeptide sequences stall the ribosome either on their own or in combination with a small-molecule (Cruz-Vera et al., 2011). Moreover, a number of biological processes have evolved to rely on these peptide-mediated stalling events for their functionality, including the induction of erythromycin/macrolide resistance (ErmC), regulation of intracellular tryptophan levels (TnaC), and modulation of the SecA secretory system (SecM). Over the years, a number of studies have identified many of the key residues in both the peptides and ribosome exit tunnel that contribute to pausing. However, what has remained unclear is how these interactions affect the structure of the ribosome and in particular the peptidyl-transferase center (PTC) to prevent further elongation or termination. Two recent papers in Molecular Cell (Arenz et al 2014) and Cell Reports (Bischoff et al 2014) harness the power of recent advances in Cryo-EM to address these issues. Both groups report high-resolution cryo-EM map of peptide-stalled ribosomes that help to shed light on the interactions and mechanisms that nascent peptides utilize to mediate these events.

Building on their previous Cryo-EM studies of the erythromycin-resistance leader sequence ErmBL, Arenz and colleauges now address how the related ErmCL, which contains the well-conserved IFVI stalling sequence found in many erythromycin-resistance genes, is able to

modulate interactions between the nascent peptide and exit tunnel residue and how these interactions lead to inactivation of the PTC (Arenz et al 2014b) (Arenz et al., 2014). They find that in contrast to ErmBL, the ErmCL peptide follows a distinct path in the exit tunnel, bringing it much closer to, and even contacting, the bound erythromycin at the bulky cladinose ring of erythromycin and F7 of the ErmCL peptide (Fig. 1, top left) (Arenz et al 2014b). This agreed well with previous results demonstrating that macrolides without cladinose rings abrogate stalling (Vazquez-Laslop et al., 2008). In addition to contacting erythromycin, the C-terminal fragment of the peptide interacts with rRNA residues U2506 and U2586 via the conserved IFVI motif. They also observed interactions between the N-terminus of the peptide and residues in the exit tunnel previously identified by mutagenesis, although the density is too poor to definitively assign. With regard to how ErmCL is able to modulate the PTC to prevent further peptide elongation, Arenz et al find that a number of residues important for A-site tRNA binding and accommodation including U2585, A2602, and U2506 are stabilized by the ErmCL peptide in conformations that are distinct from those of the accommodated, non-accommodated, or ErmBL structures (Fig. 1, top right). These stabilized conformations appear to be sufficient to preclude proper positioning of the incoming A-site tRNA and explain why no bound A-site tRNA is observed in their structure.

In contrast to ErmCL, the TnaC peptide has evolved an entirely different mechanism to stall ribosomes. Previous mutagenesis and structural studies had shed light on many of the key residues involved in peptide-mediated stalling (Cruz-Vera et al., 2006; Seidelt et al., 2009). However, it remained unclear precisely where the tryptophan (Trp) molecule bound to the ribosome and how this contributed to inactivation of the PTC and translation blockade (Martinez et al., 2014). To decipher this, Bischoff et al, utilized an *in vivo* purification scheme to isolate a

large quantity of nearly homogenous TnaC-stalled ribosomes (Bischoff et al 2014). Unexpectedly, the resulting Cryo-EM structure revealed not one, but two Trp molecules bound in the exit tunnel. Both Trp molecules bind to pockets created by interactions between the nascent TnaC peptide and the rRNA (Fig. 1, left middle panel). The first Trp binds between V20 and I19 of the peptide and U2586 of the ribosome. The second Trp binds in a kink of the peptide at N17 and K18, and U2609 and A752 in the 23S rRNA. The second Trp also makes contact with A2058 and A2059, residues important for organizing the PTC. Together these interactions are sufficient to stabilize A2058, A2059, and A2602 in non-productive positions within the PTC and prevent RF2 binding and peptide release (Fig. 1 right middle panel). Yet precisely how these interactions conspire to stop translation remains enigmatic. Previous experiments hint at the importance of the slowly reacting codon pair (Pro-Stop), however, the precise ordering of the conformational changes and tryptophan binding requires future analysis (Cruz-Vera et al., 2006; Martinez et al., 2014).

Two long-standing questions of peptide-mediated stalling have been how the peptides specifically interact with the exit tunnel and/or bound small molecules and how these interactions lead to a non-functional PTC. Here, Arenz, Bischoff, and colleagues demonstrate that both exit tunnel binding and PTC inactivation can occur through a variety of mechanisms. For ErmCL, erythromycin binds first, which likely provides the necessary contact between rRNA residues in the exit tunnel and the nascent peptide to halt translation. In contrast, the TnaC peptide slows translation on its own, potentially allowing sufficient time for the two Trp molecules to bind the exit tunnel and completely stall the ribosome (Bischoff et al 2014) (Cruz-Vera et al., 2006; Martinez et al., 2014). Interestingly, these peptides represent just two examples from among a wide variety of known ribosome-stalling peptides. For instance, ErmBL, does not

rely on the structural contacts between the bound erythromycin and also stabilizes a unique conformation of the PTC that allows the A-site tRNA to bind but prevents peptide bond formation (Arenz et al., 2014). And while there is not yet a definitive high-resolution structure of SecM-mediated stalling, previous Cryo-EM studies suggest that the elongating peptide first interacts with the early exit tunnel and then with the constriction point imposed by large subunit proteins L4 and L22 (Fig. 1, bottom left and right) (Bhushan et al., 2011). Upon continued translation, the peptide gradually becomes more compacted, stabilized, and ultimately stalled. Thus, it seems that no two peptides stall the ribosome in the same manner. Moreover, biochemical and ribosomal profiling studies have identified a number of peptide motifs that slow translation to varying degrees both *in vitro* and *in vivo* (Elgamal et al., 2014; Woolstenhulme et al., 2013). Whether these effects are mediated solely by interactions between the exit tunnel and nascent peptide or in combination with differences in tRNA abundance and reactivity remains unclear. However, it will be exciting to explore how proteins have evolved to utilize this continuum of translational pausing as a means of modulating gene expression and biological regulation.

Figure Legends

Figure 1. Schematic of the interactions between the indicated nascent peptide, ligands and the exit tunnel of the ribosome (left). Resulting conformational changes to the peptidyl transferase center from these interactions (right).

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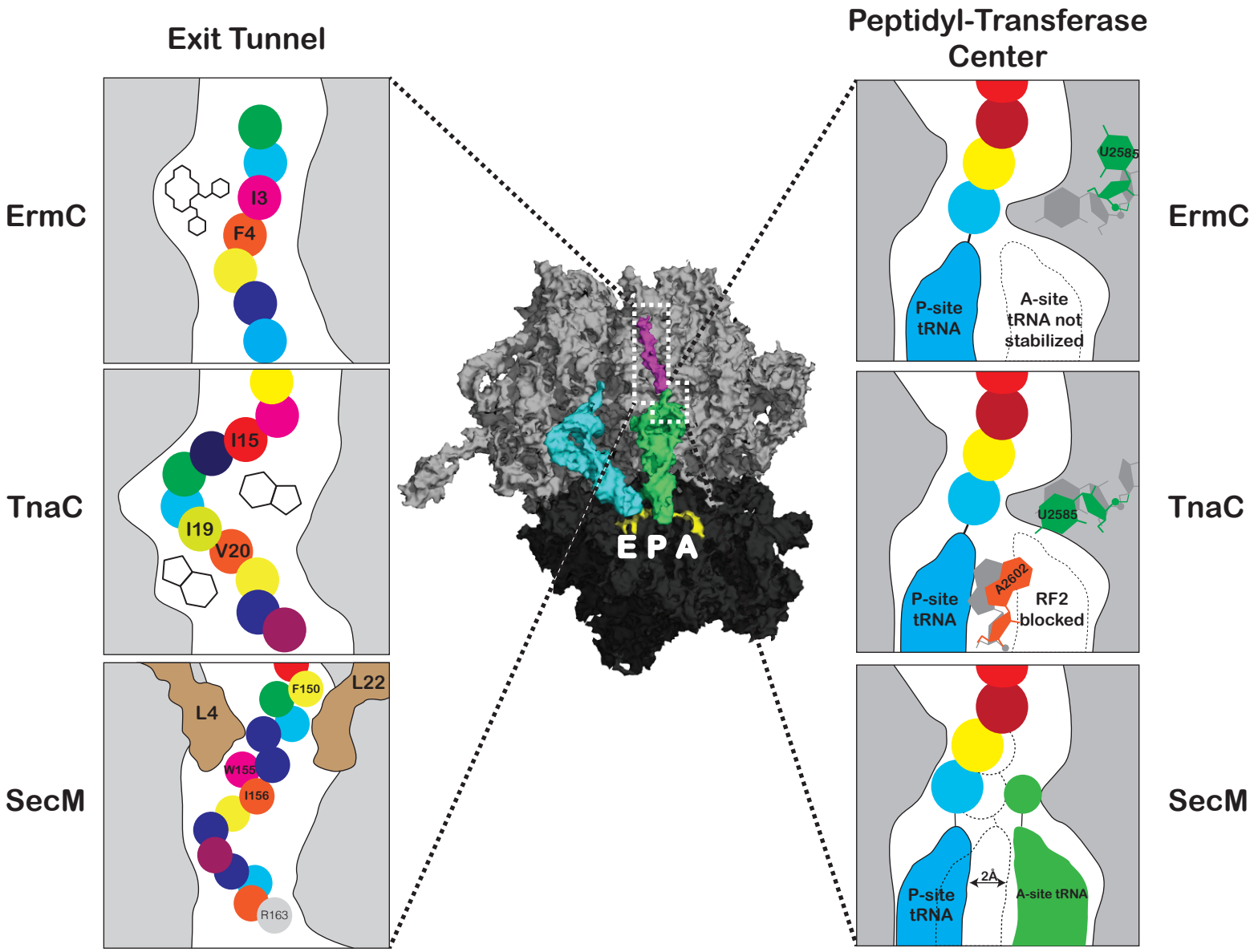
Exit Tunnel
Peptidyl-Transferase Center
ErmC
TnaC
SecM
I3
F4
I15
I19
V20
L4
L22
F150
W155
I156
R163
E P A
U2585
P-site tRNA
A-site tRNA not stabilized
A2602
RF2 blocked
2Å
A-site tRNA