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Mechanism of inhibition of translation termination by blasticidin S

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Abstract

Understanding the mechanisms of inhibitors of translation termination may inform development of new antibacterials and therapeutics for premature-termination diseases. We report the crystal structure of the potent termination inhibitor BlaS bound to the ribosomal 70S•RF1 termination complex. BlaS shifts the catalytic domain 3 of RF1 and restructures the peptidyl transferase center. Universally conserved uridine 2585 in the peptidyl transferase center occludes the catalytic backbone of the GGQ motif of RF1, explaining the structural mechanism of inhibition. Rearrangement of domain 3 relative to the codon-recognition domain 2 provides insight into the dynamics of RF1 implicated in termination accuracy.

Graphical abstract

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Graphics Abstract

Revealing the structural mechanisms of translation termination inhibitors may inform development of new antibacterials [1] or drugs to treat genetic diseases caused by mutations that introduce premature termination codons [2]. Recent studies uncovered molecules that inhibit peptide release [3] or trap a post-release ribosome state [4]. We have shown that the nucleoside analog blasticidin S (BlaS; Fig. 1a), which binds the peptidyl transferase center of bacterial 70S [3] and eukaryotic 80S [5] ribosomes, inhibits a release stage of translation termination [3]. The structural explanation for termination inhibition by BlaS, however, remains unsolved because ribosome•BlaS structures lacked release factors. Release factors (RF1 and RF2 in bacteria) recognize a stop codon in the ribosomal A site and catalyze the hydrolysis of peptidyl-tRNA bound in the P site, resulting in peptide release (reviewed in ref [6]). In this work, we report a 3.4-Å resolution crystal structure of a bacterial 70S ribosome bound to release factor 1 (RF1) and BlaS (Fig. 1b-c, see also Supplementary Data).

BlaS induces conformational changes in the peptidyl transferase center that explain how it inhibits termination by RF1. As in the structure without RF1 [3], BlaS intercalates between C74 and A76 at the $^{74}\text{CCA}^{76}$ 3' end of the P-site tRNA, displacing C75 by ~ 7 Å toward the A site. The displacement of P-site tRNA nucleotide C75 shifts the position of RF1's universally conserved $^{233}\text{GGQ}^{235}$ catalytic motif by ~ 2 Å (more than twice the coordinate error; see Fig. S1, Table S1 in Supplementary Data), compared to its position in 70S•RF1 structures [7]. A2602 of 23S rRNA, which is also critical for termination, normally packs in a pocket within domain 3 of RF1 to stabilize the GGQ motif [8]. In the presence of BlaS, however, A2602 shifts by ~ 10 Å (measured at the N6 atom) away from its position in the 70S•RF1 complex and thus disengages from the GGQ loop of RF1 (Fig. S2a-b). Moreover, the displacement of C75 by BlaS shifts the phosphate backbone of A76. U2585, which

normally packs on A76 backbone, moves toward BlaS by ~ 3 Å (measured at the O4 atom; Fig. 1d-e; Fig. S3). As a result, the O4 atom of U2585 is placed within hydrogen-bonding distance of the backbone NH group of Gln 235 from RF1's GGQ motif (Fig. 1d, Fig. S4).

The backbone NH of Gln 235 is essential for peptidyl-tRNA hydrolysis. In crystal structures of RF1- and RF2-bound 70S termination complexes without BlaS, the backbone NH group of Gln 235 hydrogen bonds with the substrate [9] and leaving group (i.e., the A76 ribose of the P-site tRNA), and may stabilize the transition-state intermediate [7]. Furthermore, mutation of Gln 235 to proline—but not other amino acids—eliminates peptide release activity without disrupting the conformation of release factor on the ribosome [10]. Our structure therefore suggests that BlaS inhibits peptidyl-tRNA hydrolysis by shifting the GGQ motif and preventing the catalytic group from optimally interacting with the substrate ([9], Fig. S5), product, or reaction intermediates, and/or by disrupting the coordination of the nucleophile. Translation inhibitors that induce a similar P-site tRNA distortion (e.g., bactobolin A [11]) may have a similar mechanism of action in termination and ribosome rescue pathways [12–15].

Our structure also provides new insight into termination accuracy. BlaS distorts domain 3 of RF1, which carries the catalytic GGQ motif. Helix $\alpha 7$ is curved (Fig. 1c), with His 279 at its center shifted by ~ 3 Å relative to its position in the normally straight helix (Fig. S1). The position of RF1's codon-recognition domain 2 and its interactions with the stop codon, however, are nearly identical to those in termination complexes without BlaS [7,8]. Thus, our structure demonstrates that accommodation of the codon-recognition domain on the stop codon can occur independently of full accommodation of the catalytic domain. Inter-domain flexibility has been proposed to underlie termination accuracy, in which peptide release is strictly coordinated with stop-codon recognition (reviewed in [6]). A stop-codon in the ribosomal A site is thought to induce a large-scale rearrangement of RF1, from a compact to an extended conformation, that inserts the catalytic domain into the peptidyl transferase center [16]. Recent cryo-EM structures of ArfA•RF2-bound ribosome rescue complexes revealed that RF2 can adopt a compact state when bound to the rescue factor ArfA in the absence of an A-site codon [17, 18]. In every structure of a termination complex bound to a stop codon, however, release factor is fully extended, with domain 3 in the catalytically engaged conformation. Although the conformation of RF1 in our structure could represent an “off-pathway” intermediate caused by BlaS, it might also coincide with an intermediate state of RF1 opening. Regardless, displacement of the catalytic domain relative to the codon-recognition domain in this structure is consistent with the mechanistic model of termination accuracy due to inter-domain movement.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Wilson DN. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. *Nat Rev Microbiol.* 2014; 12:35–48. [PubMed: 24336183]
2. Keeling KM, Xue X, Gunn G, Bedwell DM. Therapeutics based on stop codon readthrough. *Annu Rev Genomics Hum Genet.* 2014; 15:371–94. [PubMed: 24773318]
3. Svidritskiy E, Ling C, Ermolenko DN, Korostelev AA. Blastcidin S inhibits translation by trapping deformed tRNA on the ribosome. *Proc Natl Acad Sci U S A.* 2013; 110:12283–8. [PubMed: 23824292]
4. Florin T, Maracci C, Graf M, Karki P, Klepacki D, Berninghausen O, et al. An antimicrobial peptide that inhibits translation by trapping release factors on the ribosome. *Nat Struct Mol Biol.* 2017
5. Garreau de Loubresse N, Prokhorova I, Holtkamp W, Rodnina MV, Yusupova G, Yusupov M. Structural basis for the inhibition of the eukaryotic ribosome. *Nature.* 2014; 513:517–22. [PubMed: 25209664]
6. Korostelev AA. Structural aspects of translation termination on the ribosome. *RNA.* 2011; 17:1409–21. [PubMed: 21700725]
7. Laurberg M, Asahara H, Korostelev A, Zhu J, Trakhanov S, Noller HF. Structural basis for translation termination on the 70S ribosome. *Nature.* 2008; 454:852–7. [PubMed: 18596689]
8. Svidritskiy E, Madireddy R, Korostelev AA. Structural Basis for Translation Termination on a Pseudouridylated Stop Codon. *J Mol Biol.* 2016; 428:2228–36. [PubMed: 27107638]
9. Jin H, Kelley AC, Loakes D, Ramakrishnan V. Structure of the 70S ribosome bound to release factor 2 and a substrate analog provides insights into catalysis of peptide release. *Proc Natl Acad Sci U S A.* 2010; 107:8593–8. [PubMed: 20421507]
10. Santos N, Zhu J, Donohue JP, Korostelev AA, Noller HF. Crystal structure of the 70S ribosome bound with the Q253P mutant form of release factor RF2. *Structure.* 2013; 21:1258–63. [PubMed: 23769667]
11. Amunts A, Fiedorczuk K, Truong TT, Chandler J, Peter Greenberg E, Ramakrishnan V. Bactobolin A binds to a site on the 70S ribosome distinct from previously seen antibiotics. *J Mol Biol.* 2015; 427:753–5. [PubMed: 25562208]
12. Gagnon MG, Seetharaman SV, Bulkley D, Steitz TA. Structural basis for the rescue of stalled ribosomes: structure of YaeJ bound to the ribosome. *Science.* 2012; 335:1370–2. [PubMed: 22422986]
13. Huter P, Muller C, Beckert B, Arenz S, Berninghausen O, Beckmann R, et al. Structural basis for ArfA-RF2-mediated translation termination on mRNAs lacking stop codons. *Nature.* 2017; 541:546–9. [PubMed: 27906161]
14. Ma C, Kurita D, Li N, Chen Y, Himeno H, Gao N. Mechanistic insights into the alternative translation termination by ArfA and RF2. *Nature.* 2017; 541:550–3. [PubMed: 27906160]
15. Zeng F, Chen Y, Remis J, Shekhar M, Phillips JC, Tajkhorshid E, et al. Structural basis of co-translational quality control by ArfA and RF2 bound to ribosome. *Nature.* 2017; 541:554–7. [PubMed: 28077875]
16. Trappl K, Joseph S. Ribosome Induces a Closed to Open Conformational Change in Release Factor 1. *J Mol Biol.* 2016; 428:1333–44. [PubMed: 26827724]
17. Demo G, Svidritskiy E, Madireddy R, Diaz-Avalos R, Grant T, Grigorieff N, et al. Mechanism of ribosome rescue by ArfA and RF2. *Elife.* 2017; 6
18. James NR, Brown A, Gordiyenko Y, Ramakrishnan V. Translational termination without a stop codon. *Science.* 2016; 354:1437–40. [PubMed: 27934701]

Highlights

- 70S crystal structure shows that blasticidin S distorts the catalytic domain of RF1
- U2585 occludes catalytic moiety of GGQ
- Blasticidin S reveals interdomain flexibility of RF1

Figure 1. Crystal structure of RF1 bound to the 70S ribosome in the presence of blasticidin S (a) Chemical structure of blasticidin S. (b) Structure of RF1 bound to the 70S ribosome in the presence of BlaS. (c) Structure and unbiased F_o-F_c density for BlaS and RF1 with bent domain 3 (see also Fig. S1). (d, e) BlaS induces rearrangement of the peptidyl transferase center. Movement of U2585 toward the catalytic backbone NH group of Gln 235 in response to BlaS is shown by arrow (d). Structure of the 70S•RF1 complex in the absence of BlaS [8] (PDB 5J4D) is shown in panel (e). See also Figures S2-S5.