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Abstract

C-terminal tailing is an ancient and conserved form of peptide synthesis that protects cells from incomplete and potentially toxic translation products. Filbeck et al. (2020) and Crowe-McAuliffe et al. (2020) use structural, genetic, and biochemical approaches to elucidate the mechanisms driving C-terminal tailing.

Cells have evolved specialized quality control mechanisms to detect and handle stalled mRNA translation. The best understood mechanism involves the tmRNA system, in which a unique "error handling" mRNA replaces a problematic mRNA mid-translation (Moore and Sauer, 2007; Rae, Gordiyenko, and Ramakrishnan, 2019). tmRNA is only found in certain bacteria, however, not in archaea or eukarya. There also exists a more recently discovered and less well-understood pathway, first found in eukaryotes and later in bacteria, called ribosome-associated quality control (RQC) (Sitron and Brandman, 2020). RQC targets stalled nascent chains for degradation. In eukaryotic RQC, the large ribosomal subunit of a stalled ribosome is separated from the small ribosomal subunit and mRNA while retaining the stalled nascent chain and covalently bound P-site tRNA. This large subunit-nascent chain complex is then targeted by quality control factors that extract and rout the nascent chain to the proteasome. As part of RQC, the large ribosomal subunit can elongate stalled nascent chains in the absence of mRNA and the small ribosomal subunit (Shen et al., 2015; Lytvynenko et al., 2019). These C-terminal "tails" promote degradation of stalled nascent chains (Sitron and Brandman, 2020). In this issue of *Molecular Cell*, Filbeck et al. (2020) and Crowe-McAuliffe et al. (2020) report on the basis for RQC and C-terminal tailing of stalled nascent chains in a gram-positive bacterium.

Prior studies of C-terminal tailing revealed that amino acid-bearing tRNAs are recruited to the large ribosomal subunit by Rqc2-family proteins and appended to the nascent chain via the peptidyl transferase center (PTC) (Shen et al., 2015) with no need for GTP or canonical elongation factors (Osuna et al., 2017). These and other studies in eukaryotes showed that these "CAT tails" are C-terminal extensions composed of alanine, threonine, and potentially other amino acids (Shen et al., 2015; Wu et al., 2019), while in bacteria, C-terminal tails are composed of alanine (Lytvynenko et al., 2019). Yet many mechanistic questions have remained unanswered. How do the ribosome and Rqc2-family proteins initiate C-terminal

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tailing? How does C-terminal tailing processively elongate nascent chains? Are additional factors or energy sources required? How have C-terminal tailing reactions evolved in the different kingdoms of life? To explore these questions, Filbeck et al. and Crowe-McAuliffe et al. employ a combination of cryo-EM, tRNA profiling, C-terminal tailing activity assays, and genetic interactions with tmRNA in *B. subtilis* strains challenged with translation-stalling antibiotics.

Excitingly, different biochemical approaches led each group to capture different structural states of the C-terminal tailing reaction. Synthesizing the new insights from each group thus leads to a remarkably complete picture of C-terminal tailing in this bacterium, including the recognition of stalled ribosomes harboring P-site tRNA, the decoding step that determines tRNA^{Ala}-specific A-site loading, an A/P hybrid state intermediate consistent with peptide bond-formation, and, finally and remarkably, an E-site-like exit state (Figure 1). Analysis of these states reveals a degree of molecular mimicry between the steps of canonical protein synthesis and the seemingly simpler C-terminal tailing reaction. The Rqc2-family protein RqcH reads the anticodon loop and positions the A-site tRNA^{Ala} in a structurally analogous way to canonical elongation, with the amino terminus of the alanine inserted into the canonical A-site position. In addition, RqcH positions peptidyl-tRNA in the P-site and the aminoacyl-tRNA in the A-site such that peptide bond formation occurs within the PTC. This enables the nascent chain to transfer via an A/P hybrid-like state, through a "scissor" movement of Rqc2, and displacement of the now-deacylated tRNA to an E-site-like state (Figure 1).

In addition to the structural explanation of tRNA decoding by RqcH and the state model inferred from the suite of structures, each group shared another discovery about the evolutionary history of RQC. In reconstructions of RqcH-bound 50S ribosome subunits, a new protein was intimately associated with RqcH and the peptidyl-tRNA when bound in the "classical" P-site-like conformation. Inspection of the density, mass spectrometry, and comparison with a homologous crystal structure confirmed the identity of this protein as YabO, a homolog of the *E. coli* protein, Hsp15. Structurally, YabO's role may be to stabilize the P-site conformation during RqcH dynamics. The simultaneous loss of genes encoding YabO and tmRNA phenocopied the synthetic sickness of ssrA rqcH double deletions reported previously (Lytvynenko et al., 2019), consistent with a role for YabO in RQC. Furthermore, Crowe-McAuliffe et al. show phylogenetically that bacteria that have RqcH can be distinguished from those that lack RqcH by their sequence of YabO. Specifically, if Hsp15-family proteins contain an extension found in Gram-negative bacteria, like *E. coli*, the organism lacks RqcH and the RQC system more broadly. Consistent with co-evolution between RqcH and YabO/Hsp15, heterologous expression of *E. coli* Hsp15 could not rescue the defects of ssrA yabO cells. Accordingly, Filbeck et al. and Crowe-McAuliffe et al. propose to rename Hsp15 to RqcP in *B. subtilis* and potentially other gram-positive organisms in recognition of its relationship with the P-site tRNA.

The authors' discoveries about bacterial RQC inform a host of long-standing and new questions. Does eukaryotic C-terminal tailing involve a YabO equivalent, and do additional factors remain to be discovered? Do extended Hsp15-family proteins in gram negative bacteria play a divergent role in a ribosome rescue reaction? What is the function of the

domains found in the eukaryotic Rqc2 family of proteins but not found in RqcH? How do eukaryotic Rqc2 proteins recruit non-alanine tRNAs, such as threonine and potentially others, and what cellular role does this play? The authors' works are significant steps in uncovering an ancient and intriguing form of protein synthesis with emerging roles in health and disease (Wu et al., 2019; Martin et al., 2020; Sitron and Brandman, 2020).

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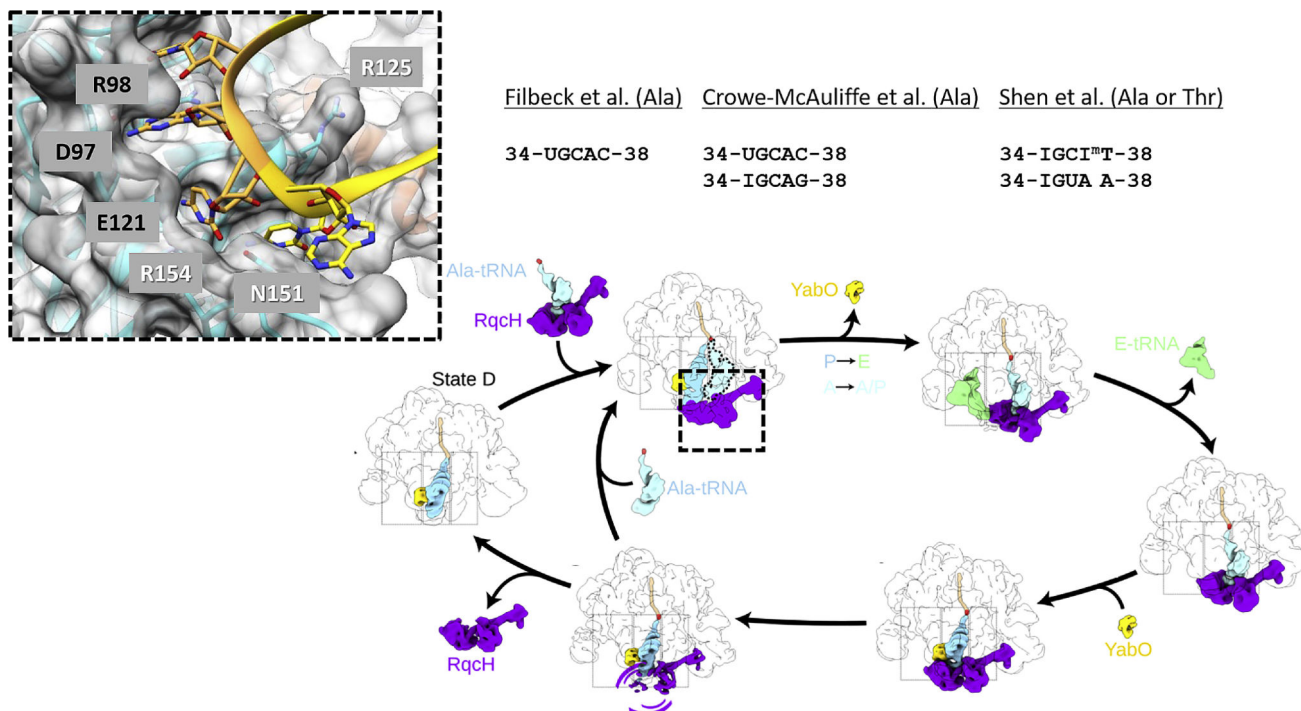
Figure 1. C-Terminal Tailing Mimics Aspects of Canonical Translation

Reproduced from Fig. 2C of Filbeck et al., highlighting the structural basis of tRNA decoding by RqcH. Universally conserved (black) versus variable (white) residues of RqcH and their spatial relationship with the anticodon loop sequences observed in different organisms. Model figure reproduced from Fig. 6 of Crowe-McAuliffe et al., illustrating the cycle of C-terminal tailing, similar to canonical elongation cycles, on the 50S mediated by RqcH and YabO/RqcP.