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The termination of translation

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Recent results from cryoelectron microscopy, crystallography, and biochemical experiments have shed considerable light on the process by which protein synthesis is terminated when a stop codon is reached. However, a detailed understanding of the underlying mechanisms will require higher-resolution structures of the various states involved.

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Introduction

The process of protein synthesis is terminated by one of the three stop codons which, unlike sense codons, are not recognized by a tRNA, but by proteins called class I release factors (RF) [1,2]. In prokaryotes, RF1 recognizes UAG, whereas RF2 is specific for UGA. Both factors recognize UAA. In eukaryotes, a single release factor, eRF1, recognizes all the three stop codons. Upon stop-codon recognition, class I RFs catalyze hydrolysis of the ester bond that links the nascent polypeptide chain to P-site tRNA, and thereby induce peptide release (Figure 1). Subsequently, class II RFs (RF3 in bacteria, eRF3 in eukaryotes) remove the class I RF from the ribosome. Finally, the ribosome recycling factor (RRF) together with elongation factor G (EF-G) recycles ribosomes for the next round of translation in a process that involves splitting apart of the two ribosomal subunits. Here we focus on recent progress toward a structural understanding of the mechanisms underlying these processes.

Many states of the termination of translation have been visualized by cryoelectron microscopy (cryoEM), typically at resolutions of 10–20 Å [3–6,7•,8]. Recently, crystal structures were determined of the ribosome with RF1 and RF2 at resolutions of 5.9 and 6.7 Å, respectively, allowing a more accurate and detailed interpretation of release factors in the ribosome [9••]. Crystal structures at 3.5–4 Å have also been obtained of domain 1 of RRF with the 50S subunit [10•] and, more recently, of RRF with the entire ribosome [11••,12••].

How do class I RFs recognize stop codons?

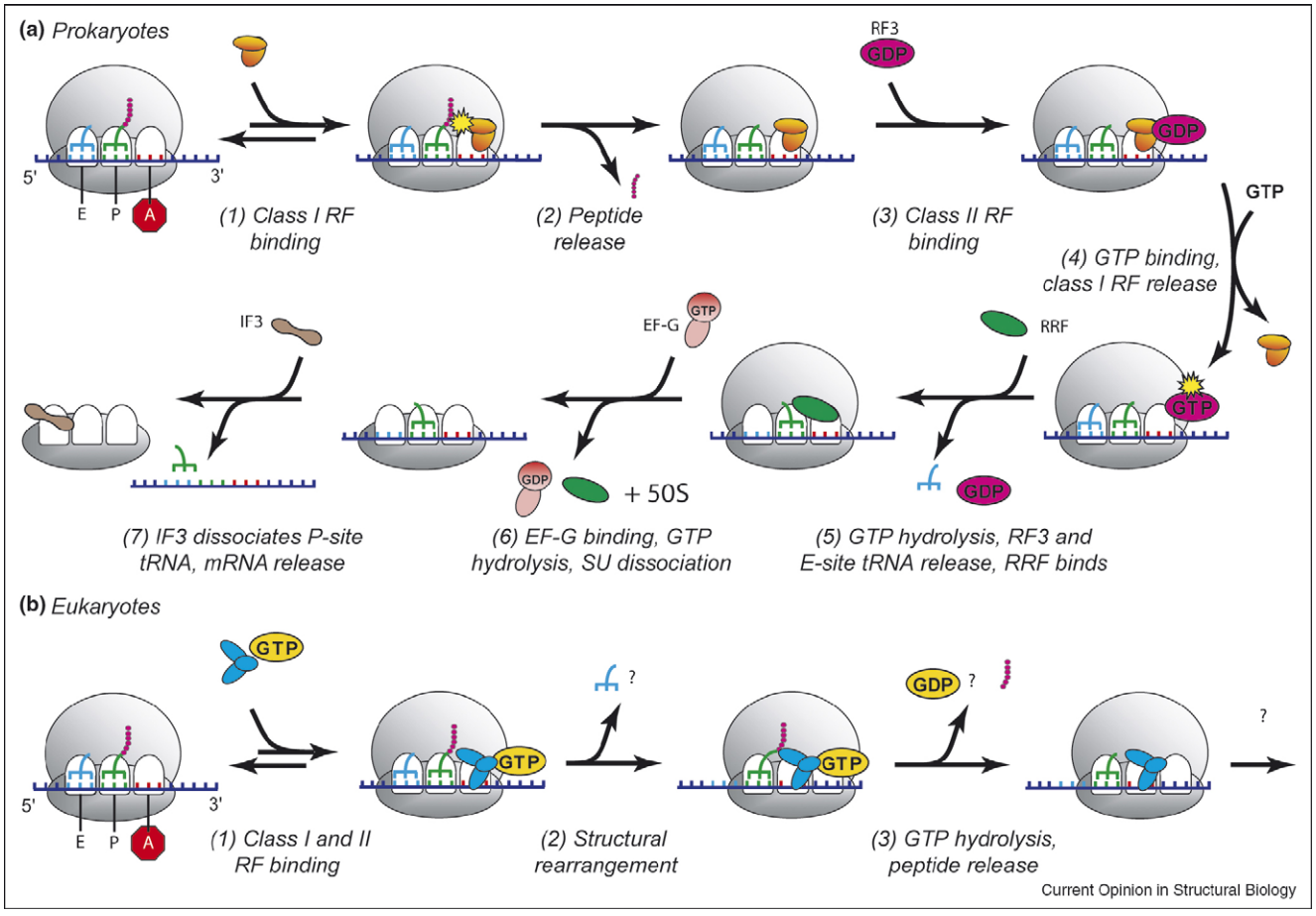
Genetic selections led to the identification of a putative 'tripeptide anticodon' motif, P(A/V)T in RF1 and SPF in RF2 [13]. The crystal structures of release factors RF1 and RF2 in the ribosome (designated RC1 and RC2, respectively) show that the entire loop region containing the tripeptide motif surrounds the stop codon particularly in the second and third codon positions (Figure 2a,b) [9••]. In addition, the tip of helix α5 approaches the first codon base and may be involved in recognizing uridine, which is common to all the three stop codons. In agreement with the influence of the downstream bases on the termination efficiency of class I RFs [14], electron density extends from the tip of the anticodon loop to the base 3′ of the stop codon in the RC crystal structures. The previously annotated human mitochondrial mtRF1 [15] contains four extra amino acids following the PXT motif and may mediate recognition of the mitochondria-specific stop codons AGG and AGA, whereas the recently identified mtRF1a is more similar to RF1 [16].

Stop-codon recognition by RFs occurs without proofreading, but surprisingly is more accurate than tRNA decoding. It also allows the degeneracy of A or G at the second codon base for RF2. The effect of mutations of the 16S rRNA bases directly at the decoding site (A1492, A1493, and G530) is different for tRNA and RF1/2 [17•]. Nucleotides from other 16S rRNA regions (U1196, C1054, G530, and C518) approach the RF loop containing the tripeptide anticodon and their mutation adversely affects RF-specific stop-codon recognition but does not affect tRNA binding [18,19,20•]. Thus, stop-codon recognition by RF1 and RF2 is direct, intricate, and significantly differs from tRNA decoding of sense codons.

How do class I RFs stimulate peptidyl-tRNA hydrolysis?

Sequence comparison and mutational studies revealed a universally conserved GGQ motif located in domain 3 of RF1 and RF2 which was shown to mediate ester bond hydrolysis [21]. The GGQ loop, which is disordered in the isolated crystal structures except when constrained by crystal contacts [22], is ordered in the ribosome (Figure 2b,c) [9••]. The tip of this loop reaches into the peptidyl transferase center (PTC) where it faces A76 of P-site tRNA. The closest 23S RNA nucleotides are U2585 and A2602, which are most essential for RF-mediated hydrolysis based on mutational data [23]. The only long side chain which can span the distance to the

Figure 1



Schematic overview of the termination of translation. **(a)** Prokaryotes: the stop codon in the A site signals the end of the coding sequence of a particular gene transcript. Upon stop-codon recognition (1), RF1 or RF2 hydrolyze the ester bond of peptidyl-tRNA (2), which leads to peptide release from the ribosome. The class I release factor is removed from the ribosome by the action of the class II release factor RF3 and GTP. Only after peptide release does the translating ribosome act as a guanine nucleotide exchange factor for RF3 [52] (3). The exchange of GTP for GDP leads to a conformational change in RF3, which causes the dissociation of RF1 or RF2 from the ribosome (4). GTP hydrolysis on RF3 leads to dissociation of the factor itself, followed by RRF binding (5). EF-G binding and GTP hydrolysis results in transient dissociation of the ribosomal subunits (6). The 30S subunit is accessible to IF3 which results in release of P-site tRNA and mRNA [39,40••] (7). **(b)** Eukaryotes: the eukaryotic class II RF, eRF3, acts cooperatively with eRF1 in release of the polypeptide chain from the ribosome [46•]. After binding of eRF1, eRF3, and GTP (1), a translocation-like structural rearrangement occurs (2). GTP hydrolysis by eRF3 precedes peptidyl-tRNA hydrolysis by eRF1 and peptide release (3). It is not clear whether eRF3 leaves the ribosome after GTP hydrolysis or after peptide release and how eukaryotic termination complexes are recycled for a new round of translation.

ribose of A76 is the universally conserved glutamine, but surprisingly its mutation does not affect RF activity [24]. However, it is crucial for specifically selecting water as a nucleophile [25•]. This amino acid is methylated at its N5 position in prokaryotes and eukaryotes [26,27].

Crystallographic studies reveal that the binding of tRNA analogs containing at least the three terminal nucleotides CCA to the A site of the *Haloarcula marismortui* 50S ribosomal subunit causes the movement of several 23S rRNA nucleotides (2583–2585 and 2506) [28••]. In this induced state, the ester group of peptidyl-tRNA becomes exposed for nucleophilic attack by the α-amino group of the incoming amino acid. The authors proposed that class I RFs, similar to tRNAs, induce a conformation of the PTC which allows the chemically more demanding nucleophilic attack of an activated water molecule. A similar hydrogen-bonding pattern as for the A-site analog was observed in molecular dynamics (MD) simulations using an 11-residue loop containing the GGQ motif [29•].

The use of a single PTC for two distinct reactions, peptide bond formation and peptidyl-tRNA hydrolysis, may have required the evolution of two different classes of molecules, namely tRNA and protein release factors, to allow optimization of both reactions without interference.