Translational Termination Not Yet at Its End

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Our understanding of protein synthesis has progressed rapidly during the past year and the pace shows no sign of abating. In particular, the X-ray structures of the 30S (at about 3 Å resolution)[1, 2] and 50S (at 2.4 Å resolution)[3] subunits from *Thermus thermophilus* and *Haloarcula marismortui*, respectively, as well as a 70S – tRNA – mRNA complex (at 7.8 Å resolution)[4] from *Thermus thermophilus* are beginning to reveal the internal arrangement of the three ribosomal RNAs, over 50 ribosomal proteins, and the functional centers. The cryo-electron microscopy (cryo-EM) reconstructions of the 70S ribosome from the bacterial model organism *Escherichia coli* in complex with elongation factors, tRNA, and mRNA are providing answers about the dynamic nature of the ribosome as it progresses through the elongation cycle (for examples see refs. [5, 6]). The elongation cycle, however, is only one step in the synthesis of a protein, as the ribosome actually moves through three stages: 1) initiation, 2) elongation, and 3) termination, which includes recycling of the ribosomal subunits. Our understanding of the final step, termination and recycling, has seen many advancements in the last five years. Most importantly, the X-ray structures of eukaryotic release factor 1 (eRF1)[7] and of ribosomal recycling factor (RRF, sometimes also called RF4)[8] have lead to an understanding of the important reactions in the termination process, namely decoding of the stop codon, peptidyl-tRNA hydrolysis, and recycling of the subunits.

The ribosome consists of two unequally sized subunits and is considered one of the most complicated structures in the cell. The large subunit (50S in prokaryotes) consists of two rRNA molecules and 33 different proteins in the bacterial model organism *Escherichia coli*, and harbors the peptidyl-transferase center. This is the catalytic site where amino acids are linked together through peptide bonds (Figure 1, reaction 2). The smaller 30S subunit contains one rRNA chain and 21 proteins, and on this subunit decoding takes place at the A site, which is the initial tRNA-binding site for the incoming aminoacyl-tRNA. This aminoacyl-tRNA does not bind on its own to the A site, but rather the incoming tRNA is delivered as a ternary complex (Figure 1, reaction 1a) made of aminoacyl-tRNA (aa-tRNA), elongation factor Tu (EF-Tu), and GTP (aa-tRNA · EF-Tu · GTP). The tRNA-binding site adjacent to the A site is termed the P site (P for peptidyl-tRNA) since it is at this site that the tRNA with the nascent amino acid chain is bound before peptide bond formation. The third tRNA-binding site is

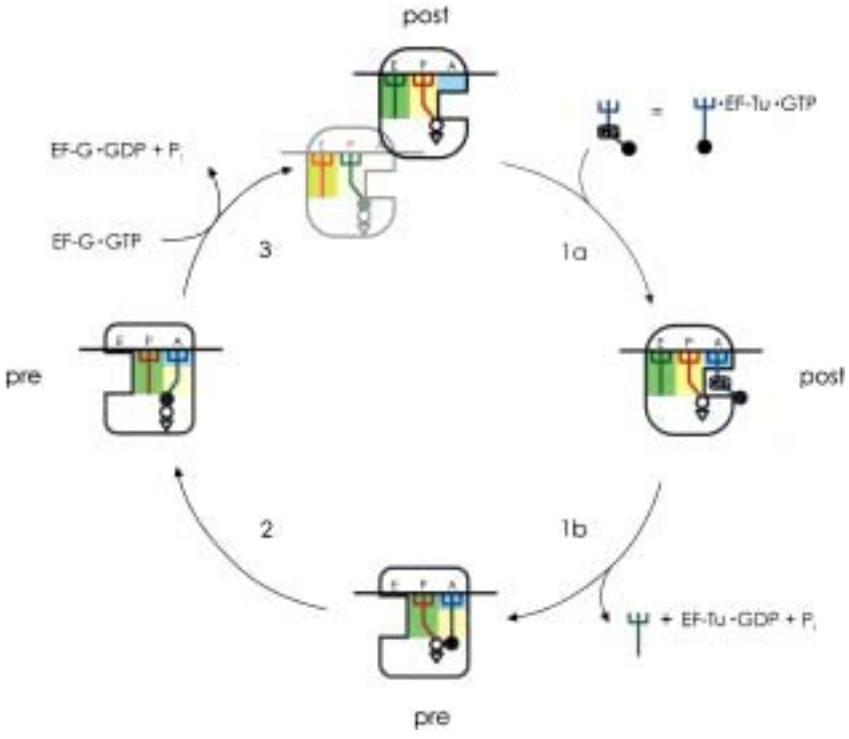

Figure 1. *The $\alpha-\varepsilon$ model for elongation. The essential feature of the $\alpha-\varepsilon$ model is the assumption of a movable domain of the ribosome that binds two tRNAs tightly and carries them between the A and P sites and the P and E sites, respectively. An elongation cycle starts with the binding of a new aminoacyl-tRNA to the A site according to the codon of the mRNA exposed at this site (reactions 1a and 1b, see ref. [21]). During A-site occupation the deacylated tRNA is released from the E site, since the movable domain jumps back to the A and P sites and leaves the E site without any tRNA-binding capacity (reaction 1b). Next, the peptidyl residue of the peptidyl-tRNA at the P site is cleaved and transferred to the α-amino group of the aminoacyl-tRNA at the A site (reaction 2). The result is that the A site carries now the peptidyl-tRNA prolonged by one amino acid. The next step is the simultaneous movement of the two tRNAs from the A and P sites to the P and E sites, respectively (reaction 3). This movement is called translocation and is promoted by another elongation factor, EF-G, in the presence of GTP. $P_i = PO_4^{3-}$.*

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termed the E site, and this site binds exclusively deacylated tRNAs as they exit the ribosome.

During the course of two elongation cycles the tRNA moves sequentially through the three tRNA binding sites (A, P, and E sites) as described by the $\alpha-\varepsilon$ model for elongation (Figure 1, see ref. [9] for a critical review of other models). The reactions of this cycle take place at the interface of the ribosomal subunits, and here both subunits form a space that neatly fits the shape of a tRNA molecule.[10] This therefore constrains any factor that has to function at the A site to adopt a tRNA-like shape. Indeed, one of the exciting findings in 1995 was the observation that the ternary complex aa-tRNA · EF-Tu · GTP has a shape very similar to that of elongation factor G (EF-G). In particular, the structural domains III, IV, and V of EF-G mimic a tRNA molecule. This was the first example of a protein mimicking a tRNA.[11]

The reactions of the elongation cycle are, in general, universally conserved, in contrast to those of the initiation phase, which are strikingly different in eukarya/archea on the one hand and in bacteria on the other hand. In addition, termination has apparently developed independently in eukarya/archea and bacteria after the separation of these evolutionary domains 2.7 billion years ago. In all three phylogenetic domains the reactions of the termination phase are particularly exciting in that they functionally mimic some of the steps in the elongation cycle (Figure 1) and therefore follow a similar reaction scheme, in spite of the differences between the bacteria domain and the eukarya/archea domains. For example, peptidyl transfer during elongation (Figure 1, reaction 2) is similar to peptidyl hydrolysis during termination, and a decoding step is found at both elongation and termination. Clearly, the differences between decoding at elongation and termination cannot be overlooked. During elongation the mechanism of decoding involves the complementarity of codon and anticodon on mRNA and tRNA, respectively, whereas during termination a stop codon is recognized by a protein. Furthermore, Tate et al. have shown that the stop signal does not consist solely of the stop codon, but of a tetraplet including the 3′ adjacent base.[12] This fourth base modulates the strength of the termination event, for example, uracil in the +4 position (UAA**U**) confers a more effective stop in *E. coli* than a cytosine in the +4 position (UAA**C**). The contacts of the release factors, unlike a tRNA, possibly even extend three bases downstream of the stop codon as indicated by crosslinking experiments.[12]

The release factors that promote the various steps of the termination reaction are divided into two classes; the class I release factors decode a stop codon and catalyze peptidyl hydrolysis while the class II release factor is a G protein that promotes the release of the class I release factor. Although the reactions of the termination phase are similar, the release factors in eukaryotes (eRF1) share little resemblance to the prokaryotic analogues (RF1/2) in terms of amino acid sequence,[13] and in fact secondary-structure prediction of the bacterial factors precludes a similar folding to that of eRF1.[7] Recently Karimi et al. determined the sequence of reactions in prokaryotic termination through a series of ingenious experiments (see ref. [14] and references therein). The authors suggest the following scheme for termination:

1) RF1 or RF2 recognize the stop signal in the decoding center and promote the release of the nascent chain through peptidyl-tRNA hydrolysis in the peptidyl-transferase center.
2) RF3 catalyzes the release of RF1 (RF2) in a GTP-dependent manner.
3) The simultaneous presence of EF-G and RRF dissociates the 70S ribosome into the 50S subunit and the mRNA · 30S · tRNA complex in a GTP-dependent manner.
4) Initiation factor 3 (IF3) then catalyzes the dissociation of tRNA, and subsequently the mRNA leaves the 30S subunit.

This model for bacterial termination nicely illustrates the cyclic nature of protein biosynthesis as the termination and initiation steps are functionally linked by the recycling of the subunits through the action of RRF and IF3. The role of EF-G in the recycling step is still not clear: Does it adopt a similar role as it has in the elongation cycle and translocate the RRF as discussed below, or does it assume a novel role, as proposed by Karimi et al.,[14] which results in the dissociation of the complex? This novel role of EF-G envisions the generation of a high-energy state resulting from the presence of both EF-G and RRF on the ribosome which is eventually dissipated by the dissociation of the ribosome complex.

The experimental results described above apply to prokaryotic termination and—with respect to the roles of the class I and II release factors—are more or less similar to the corresponding factors in eukaryotes. Some notable differences exist, for example, in eukaryotes one factor, eRF1, recognizes all three stop codons, whereas in prokaryotes two factors, RF1 and RF2, are involved. The stop codon UAA is recognized by both factors, UAG by RF1 alone, and UGA by RF2. The association of the class I and class II release factors with one another also differs between prokaryotes and eukaryotes. eRF1 and eRF3 can form a heterodimer outside the ribosome,[15] in contrast to the analogous factors in bacteria, which seem to contact each other on the ribosome.[16] Interestingly, eukaryotic eRF3 is essential for the life of a eukaryotic cell, whereas prokaryotic RF3 is not essential for the viability of a bacterial cell (for review see ref. [17]).

Despite these differences, information obtained from the crystal structure of the eukaryotic release factor 1[7] has applications to both the bacterial and eukaryotic worlds. The interaction with highly conserved functional domains on the ribosome, like the peptidyl-transferase center and the decoding center, would constrain both the prokaryotic and eukaryotic factors in a similar manner; as they also act in the A site, they should in some way resemble a tRNA. In fact, the structure of eRF1 displays a resemblance to tRNA.[7] The overall shape of eRF1 can be described as that of an asymmetric "Y" with a short and a long arm that has dimensions roughly comparable to that of tRNA, although the similarity to tRNA is not to the same extent as that seen with RRF (see below). Most interestingly, the GGQ sequence that has been implicated in the hydrolysis of peptidyl-tRNA by Frolova, Kisselev, and colleagues[18] is at the tip of the Y stem within a defined loop structure

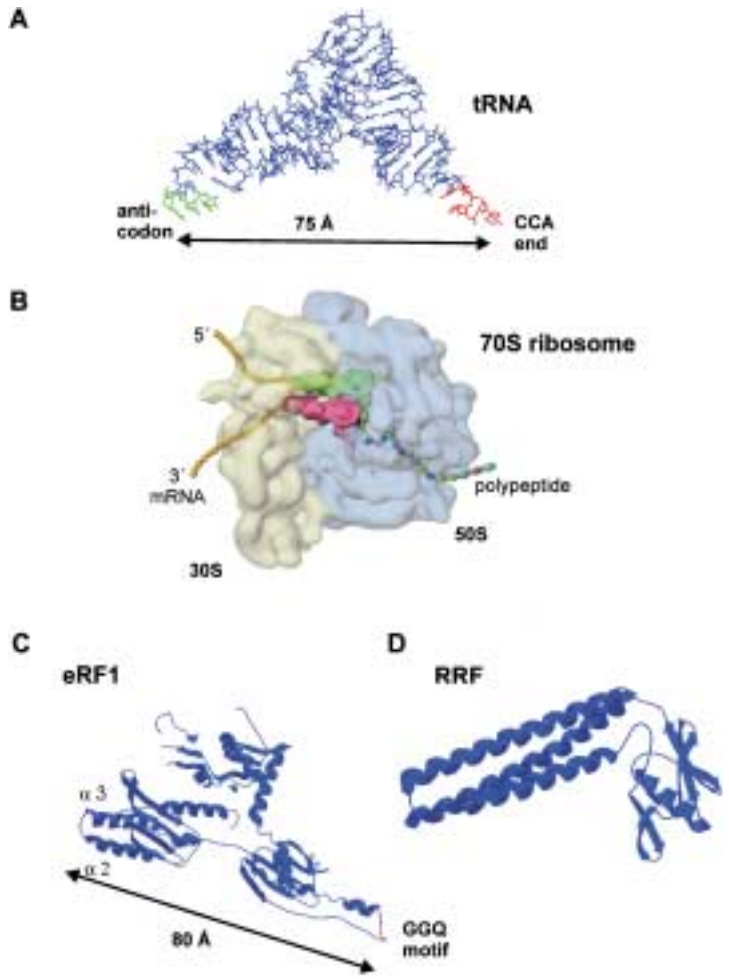

Figure 2. *eRF1 and RRF are protein factors that are presumed to interact with the A site in a manner similar to tRNA. A) A tRNA interacts with the peptidyl-transferase center at its acceptor stem (red) and with the decoding center at the anticodon (green). B) A small-scale figure showing a tRNA bound to the ribosomal P site (in green) and a tRNA bound to the A site (in pink; figure taken from ref. [22] with permission;* © American Scientist, *1998) C) eRF1 has the overall shape and dimensions of a tRNA. The GGQ motif (red) of eRF1 interacts with the peptidyl-transferase center and the groove formed by the $\alpha 2/\alpha 3$ helical hairpin, and the central β-sheet of domain 1 interacts with the stop codon. The GGQ motif and the anticodon-like site are separated by a distance similar to that between the acceptor stem and the anticodon in a tRNA molecule. D) The structure of RRF shows a strong resemblance to that of tRNA. The Protein Data Bank (PDB) accession codes for the structures presented here are: $tRNA^{Phe}$ (6tna), RRF (1dd5), and eRF1 (1dt9). The structural representations were created with programs RASMOL[23] and Swiss PDB-viewer.[24]*

(drawn in red in Figure 2C). This position corresponds to the CCA-3′ end of the tRNA, which interacts with the peptidyl-transferase center. The GGQ sequence is universally conserved, not only within the homologues of eRF2 in various organisms, but also in the heterologues RF1 and RF2 in bacteria.[18] Song et al. suggest that the glutamine residue of the conserved sequence transfers an H_2O molecule into the hydrophobic pocket of the peptidyl-transferase center. This transfer would allow a nucleophilic attack on the ester bond of the peptidyl-tRNA by the oxygen atom of a water molecule during termination, replacing the attack of the α-amino group of the newly selected aminoacyl-tRNA during an elongation cycle (Figure 3). The universal conservation of the GGQ motif implies that both the eukaryotic and prokaryotic factors interact with the peptidyl-transferase center and trigger peptidyl hydrolysis in a similar manner.

The tip of the long arm of the Y-shaped molecule opposite to the GGQ motif is conserved among the eRF1 molecules and forms a groove that probably recognizes the stop signal (Figure 2C).[7] This groove is separated by 80 Å from the GGQ sequence, roughly corresponding to the 75 Å distance between the CCA end and the anticodon of a tRNA molecule.[7] The eRF1 might undergo slight bending in order to bring the groove and the GGQ sequence to the correct distance of 75 Å. Finally, the short arm of the Y represents a domain that is responsible for the docking of the class II factor eRF3,[19] and this domain corresponds to the T stem of tRNA.

As mentioned above RRF is the second termination factor of which the structure has recently been solved. The structure reveals a possible mechanism for the 70S dissociation step described by Karimi et al.[14] (see above). The most striking feature of RRF is the astonishing structural similarity with tRNA, if one disregards the tRNA's CCA end (Figure 2D). This structural similarity, when taken together with the fact that the simultaneous presence of RRF and EF-G·GTP is obligatory for the recycling event,[14] prompted the authors to suggest the following elegant mechanism for the recycling reactions:[8] After the class I release factor has decoded the stop signal and triggered the hydrolysis of the synthesized polypeptide, its release is promoted by RF3 in a GTP-dependent manner. The result is a 70S ribosome carrying an mRNA and a deacylated tRNA at the P site. The tRNA and mRNA undergo codon – anticodon interaction and are thus tightly complexed with the ribosome. RRF binds to the empty A site independent of the codon and without contacts to the peptidyl-transferase center (as mentioned, the mimicry does not include the CCA end of a tRNA), then EF-G translocates RRF and the deacylated tRNA from the A and P sites to the P and E sites, respectively. A tRNA cannot bind to the E site in a stable fashion, if both P and E sites are not occupied by cognate tRNAs, that is, through codon – anticodon interactions.[20] It follows that the pseudo-POST state of the ribosome with an RRF factor at the P site and a deacylated tRNA at the E site is not stable and easily dissociates into the ribosomal subunits upon EF-G-dependent GTP hydrolysis. The beauty of this hypothesis lies in the fact that EF-G exploits its classical translocation function for the recycling process.

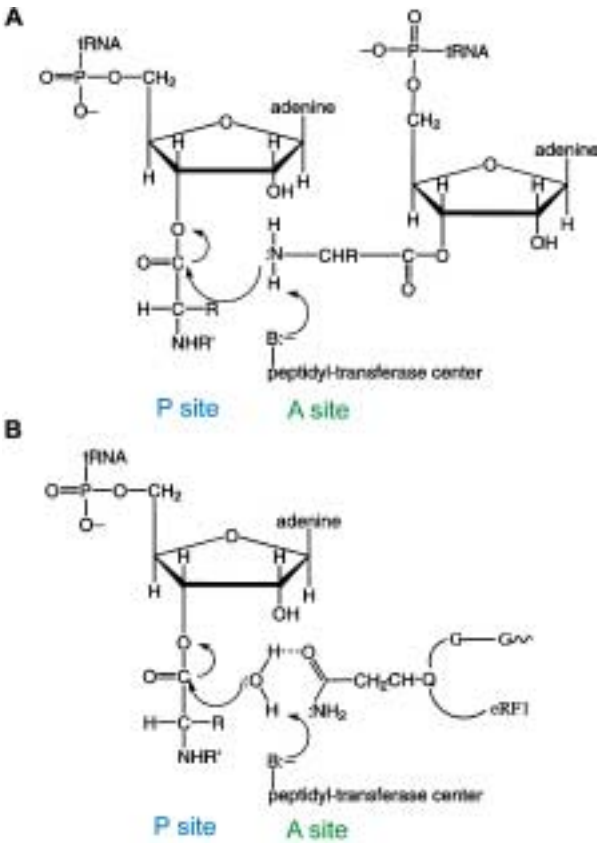

Figure 3. *A schematic drawing of the mechanism of peptidyl transfer and the proposed mechanism for peptidyl-tRNA hydrolysis (figure adapted from ref. [7]) A) Peptide bond formation during the elongation phase. A peptidyl-tRNA is at the P site and an aminoacyl-tRNA at the A site. The α-amino group of the latter initiates a nucleophilic attack on the ester bond of the peptidyl-tRNA. B) Hydrolysis of the polypeptide chain bound to the P-site tRNA during termination. The A-site-bound release factor eRF1 carries a water molecule that is coordinated by the glutamine residue of the universal GGQ motif and positions it into the peptidyl-transferase center, where it triggers a nucleophilic attack on the ester bond of the peptidyl-tRNA.*

We have seen that the crystal structures of the two termination factors, RRF and eRF1, have triggered a quantum leap in our understanding of the mechanisms of the termination process. Although the termination factors of eukaryotes and bacteria have evolved independently after separation of these evolutionary domains, equivalent solutions for the decoding of the stop signal and the hydrolysis of the peptidyl-tRNA have probably been exploited. However, it is as yet unclear whether or not the prokaryotic model for recycling the ribosomal subunits, which still has to be tested, is also valid for eukaryotic ribosomes.

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