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Protein tRNA Mimicry in Translation Termination

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Summary

Recent advances in the structural and molecular biology uncovered that a set of translation factors resembles a tRNA shape and, in one case, even mimics a tRNA function for deciphering the genetic code. Nature must have evolved this 'art' of molecular mimicry between protein and ribonucleic acid using different protein architectures to fulfill the requirement of a ribosome 'machine'.

Termination of protein synthesis takes place on the ribosomes as a response to a stop, rather than a sense, codon in the 'decoding' site (A site). Translation termination requires two classes of polypeptide release factors (RFs): a class-I factor, codon-specific RFs (RF1 and RF2 in prokaryotes; eRF1 in eukaryotes), and a class-II factor, non-specific RFs (RF3 in prokaryotes; eRF3 in eukaryotes) that bind guanine nucleotides and stimulate class-I RF activity. The underlying mechanism for translation termination represents a long-standing coding problem of considerable interest since it entails protein-RNA recognition instead of the well-understood codon-anticodon pairing during the mRNA-tRNA interaction.

Molecular mimicry between protein and nucleic acid is a novel concept in biology, proposed in 1995 from three crystallographic discoveries, one, on protein-RNA mimicry, and the other two, on protein-DNA mimicry. Nyborg, Clark and colleagues have first described this concept when they solved the crystal structure of elongation factor EF-Tu:GTP:aminoacyl-tRNA ternary complex and found its overall structural similarity with another elongation factor EF-G including the resemblance of part of EF-G to the anticodon stem of tRNA (Nissen et al. 1995). Protein mimicry of DNA has been shown in the crystal structure of the uracil-DNA glycosylase-uracil glycosylase inhibitor protein complex (Mol et al. 1995; Savva and Pear 1995) as well as in the NMR structure of transcription factor TBP-$TAF_{II}230$ complex (Liu et al. 1998). Consistent with this discovery, functional mimicry of a major autoantigenic epitope of the human insulin receptor by RNA has been suggested (Doudna et al. 1995) but its nature of mimic is still largely unknown. The milestone of functional mimicry between protein and nucleic acid has been achieved by the discovery of 'peptide anticodon' that deciphers stop codons in mRNA (Ito et al. 2000). It is surprising that it took 4 decades since the discovery of the genetic code to figure out the basic mechanisms behind the deciphering of its 64 codons.

tRNA mimic by elongation factor EF-G

In 1994, two crystallography groups have solved the three-dimensional (3D) structure of *Thermus thermophilus* EF-G, a translocase protein that forwards peptidyl tRNA from the A site to the P site on the ribosome (Earsson et al. 1994; Czworkowski et al. 1994). They proposed 5 subdomains of EF-G, i.e., G, G', II-V, whose C-terminal part, domains III-V, appears to mimic the shapes of the acceptor stem, the anticodon helix and the T stem of tRNA, respectively. This resemblance between part of EF-

G and tRNA was first described when the 3D structure of the ternary complex of Phe-tRNA, *Thermus aquaticus* elongation factor EF-Tu, and the non-hydrolyzable GTP analog, GDPNP, has been solved (Nissen et al. 1995). The 3D structure of the ternary complex is almost completely superimposable with EF-G:GDP complex, and domain IV of EF-G forms a protruding 'rod' conformation, which is similar to the shape of the anticodon arm of tRNA. This mimic was also noticed independently by Ito et al. (1996); when the Phe-tRNA structure per se was aligned with the C-terminal part of EF-G, using the C-alpha coordinates from domains III-V, the two structures were superimposable except for minor differences (see Nakamura et al. 2000).

Another structural test of 'EF-G tRNA mimicry' is to map the position of EF-G in the ribosome by directed hydroxyl radical probing. Wilson and Noller (1998) have mapped the location and orientation of EF-G in the ribosome using Fe(II) tethered to 18 different (single cysteine) positions on the surface of EF-G bound to the ribosome. The data provide convincing evidence for the ribosomal A-site occupation by domain 4 of EF-G as well as the proximity of the tip of domain 4 to the 30S decoding site. Not only tethered radical footprinting but cryo-electron microscopy analysis of the EF-G ribosome complex clearly points out the ribosomal A-site occupation by domain IV of EF-G as well as the proximity of the tip of domain IV to the 30S decoding site (Agrawal et al. 1998). Thus, the structural mimicry of domain IV, inferred from the crystallographic comparison, extends to its position in or near the tRNA-binding region of the ribosome, suggesting common requirements for the structure and function on the ribosome.

tRNA mimic by release factors

A tRNA-like property has been speculated for RFs because they are involved in reading stop codons, instead of tRNA, during the termination of protein synthesis (Nakamura et al. 1996; Tate et al. 1996). The fact that two RFs from prokaryotes exhibit codon specificity led many researchers to think that they interact directly with their codons. However, evidence has been lacking for such direct contact until recently. A path that could lead to the solution of this puzzle was created when it was realized that there are universally conserved structures in RFs. This insight led to a seven domain model of RF, designating domains A through G, and to a novel hypothesis of 'molecular mimicry' between RF and tRNA (Ito et al. 1996; Nakamura et al. 1996). Domains D and E of RFs appear to share primary (and secondary) sequence homology with the C-terminal portion, domain IV, of EF-G that mimics the shape of the anticodon helix of tRNA. Therefore, it seemed that domains D/E of RF constitute a 'tRNA-mimicry' domain necessary for RF binding to the ribosomal A site, and encode an anticodon-mimicry element to recognize the stop codons.

This prediction was confirmed recently by the discovery of a peptide determinant in RFs equivalent to the anticodon of tRNA (Ito et al., 2000). Genetic selection combined with biochemical studies showed that the tripeptides Pro-Ala-Thr in RF1 and Ser-Pro-Phe in RF2 determine the RF identity and that the first and third amino acids independently discriminate between the second and third purine bases, respectively. Thus, at the first position, Pro is restrictive to A (RF1), while Ser is permissive to both A and G (RF2). At the third position, Thr is permissive to A and G (RF1), while Phe is restrictive to A (RF2). These two discrimination switches operate separately since the Pro-Pro-Phe variant recognizes only UAA while the Ser-Pro-Thr variant recognizes three stop codons and UGG as well. Hence, they were referred to as a tripeptide 'anticodon' that deciphers stop codons in mRNA (for a review, see Nakamura et al. 2000).

Functional sites of interaction between RF1 and the ribosome have been assigned by directed hydroxyl radical probing (Wilson et al. 2000). Interestingly, the site-directed radical cleavages from positions 187 and 192 of RF1 in 16S rRNA are very similar to the corresponding positions on EF-G, when bound to the ribosome in the post-translocational state. In particular, Fe(II) tethered to EF-G at the tip of its anticodon-mimicking domain IV results in identical rRNA cleavages in the head and platform of the small subunit surrounding the decoding site. RF1 positions 187 and 192 flank the tripeptide anticodon (188-190). Thus, the anticodon mimic inferred from the functional study, extends to its position in or near the tRNA-binding region of the ribosome, providing a strong support for the RF-tRNA mimicry model.

The crystal structure of human eRF1 to 2.8 Å has been published by Barford and colleagues (Song et al. 2000). They pointed out that the overall shape and dimensions of eRF1 resemble a tRNA molecule with domains 1, 2, and 3 of eRF1 corresponding to the anticodon stem, aminoacyl acceptor stem, and T stem of a tRNA molecule. This domain assignment relies on the assumptions that the universal GGQ motif (Frolova et al. 1999) located at the tip of domain 2 is assumed to be a structural counterpart of the tRNA aminoacyl group on the CCA-3' acceptor stem and that domain 1, in which a codon-specific discrimination defect can be created (Bertram et al. 2000), may be equivalent to the anticodon of tRNA (Song et al. 2000). Nevertheless, if we extend the bacterial tripeptide 'anticodon' analogy to eRF1s, it can be speculated that a 'Thr-Ala-Ser' tripeptide adjacent to the helical hairpin might play the role of an omnipotent discriminator tripeptide (Nakamura et al. 2000). This speculation gets some support from the strong conservation of Thr-Ala-Ser in eukaryotes with *Tetrahymena* as the exception with Lys-Ala-Ser in this position (Karamyshev et al. 1999). Interestingly, *Tetrahymena* has UGA as sole stop codon with UAA and UAG reassigned to glutamine codons, which is consistent with its exceptional tripeptide. A simple omnipotent discriminator tripeptide of *E. coli* type, however, could not account for the exclusive recognition of all three stop codons, since it would recognize UGG as well (Ito et al. 2000). Therefore, eRF1 must have a different discrimination mechanism.

tRNA mimic by ribosome recycling factor

After release of nascent polypeptides by RFs, the posttermination complex composed of the ribosome, deacylated tRNA (in P-site), a class-I RF (in A-site) and mRNA needs to be dissociated for the next round of protein synthesis. As first step, in bacteria, RF3 accelerates the dissociation of RF1 and RF2 from the ribosome in a GTP-dependent manner, thereby playing as a class-I-RF-recycling factor in vitro (Freistroffer et al. 1997). Upon GTP hydrolysis, RF3 is also released from the ribosome, thereby leaving behind the posttermination complex with mRNA, deacylated tRNA in the P-site, and the empty A-site, which is believed to be a substrate for another factor, RRF for ribosome recycling factor. Early studies by Kaji and colleagues in 1970's have unraveled that RRF is required for the dissociation of the posttermination ribosomal complex in bacteria in concert with EF-G (reviewed by Janosi et al. 1996). More recent studies by Ehrenberg and colleagues have found that fast recycling of ribosomes requires both RF3 and RRF in vitro (Freistroffer et al. 1997; Pavlov et al. 1997). Nevertheless, how RRF dissociates the posttermination complex has long been puzzling.

The crystal structure of RRF has recently been solved to 2.55, 2.3 and 2.6 Å resolution by three groups using RRF proteins from *Thermotoga maritima* (Selmer et al. 1999), *E. coli* (Kim et al. 2000) and *T. thermophilus* (Toyoda et al. 2000). These three molecules are composed of two domains, a long three-helix bundle (domain 1) and a three-layer β/α/β sandwich (domain 2), and superimpose almost

perfectly with $tRNA^{Phe}$ except for the amino acid-binding 3' end. Selmer et al. (1999) have proposed that RRF is a near perfect tRNA mimic to explain the mechanistic disassembly of the posttermination ribosomal complex. They speculate that RRF binds to the A-site of the ribosome and that EF-G translocates RRF from the A- to the P-site and deacylated tRNA from the P- to the E-site of the ribosome in a GTP-dependent manner, where it would dissociate rapidly ('tRNA-mimic translocation' model). However, the model by Selmer et al. (1999) is not consistent with the biochemical findings of Karimi et al. (1999), which show, first, that RRF and EF-G split the ribosome into subunits in a reaction that requires GTP hydrolysis ('subunit disassembly' model) and, second, that the initiation factor IF3 is required for the removal of deacylated tRNA from the P-site of the 30S particle. We assume that nature may not have created such protein of a tRNA mimic to simply substitute for tRNA unless protein is required to pursue some function(s) that tRNA cannot do.

Regardless of the resemblance between the shapes of RRF and tRNA, very little is known about the structure-and-function relationship of RRF. RRF is a tRNA-like L shape molecule consisting of two domains that are bridged by two loops which function as a flexible hinge. Although the individual domain structures are similar, the interdomain angle is potentially variable and the hinge flexibility is vital for the function of RRF (Toyoda et al. 2000). The truncation of C-terminal 9 amino acids, which are in direct contact with the hinge, impairs the active conformation of the hinge and/or the three-helix bundle in domain 1, so that RRF no longer binds to the ribosome (Fujiwara et al. 2001). This RRF variant defective in ribosome binding regains the binding capacity through multiple secondary changes occurring in three topologically distinct regions of RRF, two of which are equivalent to the tip of the anticodon stem and the upper surface of the acceptor stem of tRNA (Fujiwara et al. 2001). These findings suggest that RRF interacts with the ribosome in a way similar to tRNA, spanning 30S and 50S subunits, for exerting its action using the flexible gooseneck. If RRF binds to the A-site of the ribosome in a manner similar to tRNA and splits the ribosome into subunits, RRF must exert its action within the A-site in concert with EF-G. EF-G can generate a post- to pre-peptidyltransfer transition state of the ribosome coupled with GTP hydrolysis. This energy-driven transition may involve distortion of the interface between 30S and 50S ribosome particles. Therefore, it is tempting to speculate that either of the domains connected by the flexible gooseneck of RRF may penetrate into a distorted interface and interfere with post- to pre-peptidyltransfer transition, shifting the equilibrium toward a direct uncoupling of 30S and 50S.

Recently, Ishino et al. (2000) have monitored the formation of ribosome complexes on the surface-coupled RRF of *E. coli* in real time with a BIACORE 2000 instrument based on the surface plasmon resonance technique. They have found that RRF tends to interact more efficiently with 50S subunits, which seems to be of biological significance through mutational and antibiotic analysis (Ishino et al. 2000). This might be interpreted as indicating that part of the RRF-binding site in the 50S subunit is sequestered in the 70S ribosome state and exposed in the free 50S subunit state. Hence, it is tempting to speculate, or more likely, that such RRF-binding site(s) in 50S subunits are located at or near the interface between 50S and 30S subunits, which are hardly accessible in the 70S ribosome. Of two models proposed for RRF, the preferential and stable binding of RRF to 50S subunits cannot be easily accounted for by the 'tRNA-mimic translocation' model, but rather favors the 'subunit disassembly' model. The polysome-to-monosome breakdown assay has been used as a conventional assay for the RRF activity (Hirashima and Kaji 1972) but this does not necessarily reflect the primary action mechanism of RRF because the assay uses crude polysome fractions under the condition which favors

the re-assembly of 30S and 50S subunits to 70S ribosomes. Further mechanistic analysis will be needed.

Molecular mimicry as a key to understand evolution

What's most remarkable in molecular mimicry is the fact that the three proteins, EF-G, RRF and eRF1, structurally known as a tRNA mimic possess completely different protein folds with unrelated primary and secondary structures of protein. How these unrelated protein architectures evolved to mimic a tRNA shape? These distinct protein folds are interpreted as indicating that a mimic of the shape of a tRNA works as an entrance pass to sit in the cockpit (A-site) in a ribosome 'machine', however, the action once sitting there is diverse by different translation factors, which should inquire the different protein folds for the action. This is, in some sense, equivalent to the animal or plant 'mimicry' where the mimic itself is not the purpose but it is to cheat objects by imitating a shape or a color for diverse purposes such as to prey, evade, lure, pollinate, threaten, etc. Nature must have evolved this 'art' of molecular mimicry using different protein architectures for the diverse actions, still keeping a similar shape to fulfill the requirement of the ribosome.

Given the RNA world hypothesis, one might speculate that most or many proteins might have evolved to substitute for the RNA ancestors during the evolution. What RNA ancestors could have been replaced by protein and what could not during the process of the world transition? Are there any ancestor(s) for tRNA mimic? How different architectures for tRNA mimic evolved independently? In this modern DNA/protein world, most such RNA ancestors might have disappeared and we are probably looking at a few molecular fossils in the translational apparatus, such EF-G, eRF1 and RRF. Extending this scenario one step further, we would assume that molecular mimicry should be the one principle for reviving the disappeared world of RNA when combined with experimental evolutionary methods such SELEX for Systematic Evolution of Ligands by EXponential enrichment (Tuerk and Gold 1990). The future study on tRNA mimic associated with the translational apparatus will provide us with a clue to this fundamental problem of molecular evolution of life.

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