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Protein-tRNA mimicry in translation

Yoshikazu Nakamura
IMSUT Institute of Medical Science, University of Tokyo, Tokyo, Japan

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.

Polypeptide Release Factors

The genes encoding bacterial RF1 (*prfA*) and RF2 (*prfB*) have been identified. Mutations in these genes often cause misreading of stop signals, increased frameshifting, or temperature-sensitive growth of the cells. The decrease in the cellular level and efficiency of RFs, therefore, leads to increased translational readthrough as the result of an abnormally long pausing of ribosomes at the stop signal. Unlike RF1 and RF2, a third factor, RF3, has received little attention since its initial characterization in the 1970's; its biological significance in protein synthesis has been a long-standing puzzle. After two decades of silence, the gene for RF3 was discovered. The existence of a protein with RF activity in eukaryotes was demonstrated some twenty years ago in rabbit reticulocytes. After two decades of investigation, a eukaryotic protein family with the properties of RFs, designated eRF1, was discovered recently. It includes a Sup45 protein of *S. cerevisiae* that is involved in omnipotent suppression of three nonsense codons during translation. Eukaryotic counterparts of bacterial RF3 were identified recently and are referred to as eRF3. The eRF3 family includes a Sup35 protein of *S. cerevisiae* that carries G-domain motifs and is involved in omnipotent suppression of nonsense codons.

RF-tRNA Molecular Mimicry

Upon accumulation of RF sequences from different organisms, the

conservation of protein motifs has emerged in prokaryotic and eukaryotic RFs, as well as in the C-terminal portion of elongation factor EF-G, a translocase protein that forwards peptidyl tRNA from the A site to the P site on the ribosome. The three-dimensional structure of *T. thermophilus* EF-G comprises five subdomains; the C-terminal part, domains III-V, appears to mimic a tRNA shape. Furthermore, it appears that an RF region shares homology with domain IV of EF-G, thus constituting a 'tRNA-mimicry' domain necessary for RF binding to the ribosomal A site. The model of tRNA-mimicry by RF could explain how RFs have the ability to recognize the stop codon. There are now several lines of genetic and biochemical evidence for RF-tRNA mimicry including protein anticodon mimic. We assume that at least two sites of RF are involved in correct reading of the stop codon. Moreover, a single amino acid substitution (E167K) in RF2 permits it to terminate translation at all three stop codons through inactivation of the putative third-base discriminator function of RF2. This omnipotent RF2* restores the viability of a chromosomal RF1/RF2 double knockout. Therefore, prokaryotic and eukaryotic RFs should share the same anticodon moiety and only one omnipotent RF is sufficient for bacterial growth, similar to the eukaryotic single omnipotent factor.

Class-II Release Factors

Analogous to the initiation and elongation steps of translation, the termination step involves hydrolysis of GTP to GDP by RF3 or eRF3. The basic function of the G domain is to switch the protein conformation between two alternative states, a GTP-bound ON-state and a GDP-bound OFF-state. The model of RF-tRNA mimicry predicts that class-II GTP/GDP-binding proteins, RF3 and eRF3, may be an 'EF-Tu-like' vehicle protein to bring class-I proteins to the A site of the ribosome or an 'EF-G-like' translocase protein. Since there are some apparent differences between bacterial RF3 and eukaryotic eRF3, we assume that eRF3 is functionally closer to EF-Tu than EF-G in eukaryotes, while RF3 is closer to EF-G than EF-Tu in prokaryotes.

Ribosomal Recycling

Most textbooks end the description of protein synthesis with the RF-mediated release of the completed polypeptide chain from the peptidyl tRNA. This is a gross oversight because there is an additional crucial step in protein synthesis; recycling of ribosomes through decomposition of the termination complex. In bacteria, this process requires RRF and is fundamental because the gene for RRF is essential for cell growth and the living cell must re-use the ribosome, RF and tRNA for the next round of

protein synthesis. Upon release of the polypeptide chain, the ribosomal P and A sites remain occupied with a deacylated tRNA and a tRNA-mimicking RF protein, respectively. The final step for recycling may be a direct decomposition of this complex. RF3 accelerates the dissociation of RF1 and RF2 from the ribosome in a GTP-dependent manner and that fast recycling of ribosomes requires both RF3 and RRF. RF3 can also substitute for EF-G in RRF-dependent ribosome recycling reactions *in vitro*.

Evolution of Release Factors

Why do prokaryotes have two RFs, while eukaryotes have only one? To date, three archaebacteria whose genome sequences have been completed have single eukaryotic RFs. Therefore, prokaryotic RF1 and RF2 should have diverged from the progenitor before eukaryotes and archaebacteria diverged. In view of the finding that RF2 can revert (easily) to an omnipotent factor, there should have been some evolutionary bias, rather than a simple 'frozen accident', to force prokaryotes to maintain two RFs.

eRF3 - Yeast Prion

The yeast eRF3 (Sup35) is a non-Mendelian prion-like element called [*PSI*+] that was discovered some thirty years ago as a modifier of tRNA-mediated nonsense suppression in *S. cerevisiae*. Sup35 has several N-terminal tandem peptide repeats analogous to other prion proteins of mammals. Over-expression of the wild-type Sup35 gene results in the de novo appearance of the corresponding [*PSI*+] phenotype, revealing that the [*PSI*+] factor is a self-modified protein analogous to mammalian prions. Moreover, chaperone proteins such as Hsp104 affect the [*PSI*+] phenotype. Sup35 is now known to assume two functionally distinct conformations which differentially influence the efficiency of translation termination.

Recoding - Alternate Reading of the Genetic Code

Another aspect of translation termination that attracts scientists is the fact that the stop codon is often recognized as alternate genetic codes for frameshifting, readthrough or selenocysteine incorporation (referred to as 'recoding' for reprogrammed genetic decoding). Recoding of the stop codon requires several regulatory elements to subvert the normal stop signal recognition in competition with RFs. Therefore, translational termination can now be thought of in two ways: either as the general and fundamental event resulting in the release of the protein product, or as a pause or "yield" for more specialized events beyond the constraints of the genetic code. Understanding 'recoding' requires a thorough understanding of the mechanism of polypeptide termination as well as the mechanisms specific to 'recoding'.

Perspectives - Molecular Mimicry

The molecular mimicry between EF-G, the EF-Tu:GTP: aminoacyl-tRNA ternary and perhaps the RF1/2:RF3:GTP complex makes it likely that these complexes fit into a similar pocket in the ribosome during decoding. Similarly, the initiation factors IF1 and IF2 may fulfill a function similar to that of EF-G during elongation. In other words, the shape of tRNA functions as a master key to unlock the ribosome and activate the initiation, elongation and termination steps of protein synthesis. This resemblance between part of the translation factor and tRNA represents a novel concept of molecular mimicry between nucleic acid and protein. Consistent with this discovery, functional mimicry of a major autoantigenic epitope of the human insulin receptor by RNA has been described and protein mimicry of DNA has been shown in the crystal structure of the uracil-DNA glycosylase-uracil glycosylase inhibitor protein complex. Nature must have evolved this 'art' of molecular mimicry between protein and tRNA to satisfy common requirements for structure and function during protein synthesis on the ribosome.

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Yoshikuzu Nakamura

1972	Kyoto Univ.	(B.S.)
1977	"	(Ph.D.)
1977-1981	Kyoto Univ./ Univ. Utah	(Post-Doc.)
1978-1986	IMSUT Univ. Tokyo	(Research Associate)
1986-present	"	(Associate Professor)

Major research field - Translation termination in eukaryotic gene expression