Point of VIEW

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When Starting Over Makes More Sense

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ABSTRACT Proofreading mechanisms intrinsic to DNA and RNA polymers that contribute substantially to overall fidelity are lacking in the ribosome. New evidence, however, suggests that quality control in translation can occur after substrate incorporation by an abortive mechanism entailing premature release factor-catalyzed termination. These data shed new light on the importance and ubiquity of retrospective quality control mechanisms in ensuring the overall fidelity of nature's processive enzymes and demonstrate that competitive elongation reactions on the ribosome are kinetically sensitive to compositional features of the translating particle.

Enzymes responsible for DNA replication (DNA polymerase), messenger RNA (mRNA) synthesis (RNA polymerase) and the translation of mRNA into protein (the ribosome) share common mechanistic features that contribute to the faithful transmission and expression of genetic information. Although these multistep processes are biochemically distinct, fidelity in each system is determined collectively by (i) proper localization to the start site of synthesis forming the initiation complex; (ii) processive polymerization of a full-length, template-encoded substrate; and (iii) efficient termination of synthesis. In the case of RNA polymerase and the ribosome, product release must occur only at programmed sites within the template reading frame. The synthesis (elongation) phases of all three processive enzymes operate with extraordinary precision ($<$1 error in 10^4 substrate incorporation events). Mechanistically, fidelity during polymer elongation can partially be attributed to one or more induced-fit processes wherein correct substrates precipitate conformational changes in the enzyme necessary for the polymerization reaction. In order to ensure the faithful transmission of genetic information to progeny cells, DNA polymerase must operate with extraordinary fidelity ($<$1 error in 10^7 incorporation events). To achieve this feat, DNA polymerase also possesses an intrinsic 3′−5′ exonuclease activity, enabling it to reverse track and edit misincorporated substrates out of the nascent polymer through a hydrolytic mechanism (*1*). RNA polymerase, although it operates with lower fidelity, presumably because the cost of mistakes carries less impact as it is not transmitted and mRNA transcripts are transient in nature, also possesses an intrinsic 3′−5′ exonuclease activity allowing mistakes to be edited retrospectively following misincorporation events (*2*). By contrast, the ribosome lacks similar retrospective editing capabilities. Fidelity during translation elongation has therefore been widely understood to rely principally on an induced-fit mechanism by which correctly charged, cognate aminoacyl-transfer RNA (aa-tRNA) substrates are preferentially selected by the translating particle (*3*). Correspondingly, translation occurs with lower precision (~1 error in 10^4 incorporation events). Here, the argument is similar: the cost of making errors in protein synthesis is low given that proteins are generally transient in nature, robust to amino acid changes and safeguards are in place for assisting or degrading misfolded proteins.

Through a careful series of *in vitro* biochemical experiments, Zaher *et al.* (*4*) now show that high-fidelity translation may also be ensured by a retrospective "editing" mechanism wherein misincorporated aa-tRNA substrates increase the probability of subsequent translational errors, ultimately leading to the premature termination of protein synthesis (Figure 1). Using purified translational components and an established miscoding translation system, the authors show that the presence of misincorporated tRNAs, within both the peptidyl (P) and exit (E) sites, increased the rates of further aa-tRNA misincorporation events

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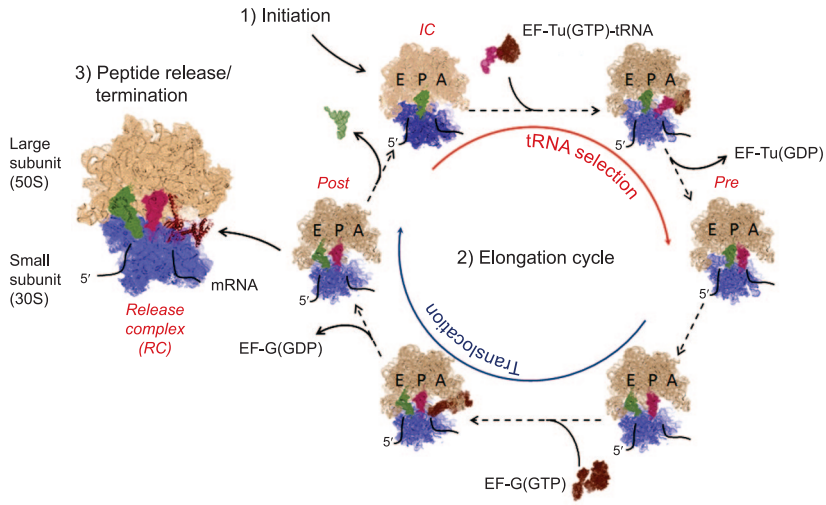

Figure 1. Bacterial translation elongation cycle. The conversion of genetic information in the form of mRNA into protein begins with the assembly of both intact ribosomal subunits (30S (blue) and 50S (tan) in bacteria) into the functional (70S) particle at the start site of protein synthesis. The ribosome's directional transit through the mRNA open reading frame (ORF), elongation, proceeds *via* sequential aa-tRNA selection and translocation processes catalyzed by the GTPases elongation factors (EF-Tu and -G, respectively). EF-Tu and -G (brown) bind to overlapping sites at the A site, located at the leading edge of the ribosome, and hydrolyze GTP while bound to the particle. EF-Tu- and EF-G-mediated reactions occur in rapid succession to drive amino acid polymerization according to the mRNA codon sequence. After translocation, E-site tRNA release and subsequent aa-tRNA selection events may be physically coupled through a yet-unknown mechanism. Release factors (RF-1/RF-2) also bind the ribosome in the same region as EF-Tu and EF-G. Canonically, RF-1 or RF-2 bind with appreciable affinity to the ribosome to facilitate termination of protein synthesis only when the A site is occupied by specific nonsense, stop, codons. The work of Zaher and Green now show that competitive biochemical reactions at the A site are dictated in part by conformational processes in the ribosome influenced by mRNA codon–tRNA anticodon interactions in the E and P sites. When the ribosome misincorporates aa-tRNA, pathways leading to promiscuous aa-tRNA selection and termination become kinetically more favorable.

and unprogrammed, release factor-catalyzed peptide termination at sense codons. Thus, misincorporation events change the nature of competing substrate interaction processes at the aminoacyl (A) site of the ribosome in favor of termination. This mechanism reflects the intrinsically competitive nature of elongation reactions in translation. This retrospective editing process is distinct from DNA and RNA polymerase, but the message is clear: the ribosome can recognize mistakes after substrate misincorporation as a means of quality control to increase the apparent, overall fidelity of protein synthesis.

An intriguing question is why mechanistically and evolutionarily such an editing process during translation would be preferred over a retrospective exopeptidase mechanism. After all, premature termination of protein synthesis, like spurious drop-off events resulting from poor processivity, may potentially lead to the accumulation of truncated protein products that may be detrimental to the cell. Previous work has shown that such products, when incorporated into the membrane, can result in catastrophic porosity (*5*). Here, unique physical and biochemical distinctions of the ribosome mechanism must be considered. Although all processive enzymes must faithfully select and incorporate only cognate substrates from much larger excesses of those near- and noncognate and remain tethered to the reaction products during synthesis, structural distinction of these complex enzymes specifies disparities in how these challenges must be met. In nucleic acid polymerases, the incorporation of ~500–700 Da DNA or RNA nucleotide triphosphates (NTPs) are mediated by canonical base pairing interactions with the template. Substrate incorporation follows an induced-fit-type mechanism and leads to the formation of short DNA–DNA or DNA–RNA helical duplexes within the active site. The byproduct of the reaction, pyrophosphate, quickly dissociates from the particle. On the ribosome, the ~25 kDa, L-shaped, aa-tRNA substrate incorporates into the A site of the translating particle through both canonical and noncanonical pairing interactions with the mRNA codon at the small ribosomal subunit decoding center. The induced-fit mechanism, entailing stimulated GTP hydrolysis by elongation factor-Tu, commits cognate aa-tRNA to peptide bond formation by allowing its 3′-aminoacyl acceptor stem to enter into the RNA-rich, large ribosomal subunit peptidyltransferase center (PTC) (*3*). Near- and noncognate aa-tRNAs must be preferentially rejected during this process. A substrate-assisted nucleophilic addition–elimination reaction (*6*) transfers the nascent chain to the newly incorporated tRNA upon peptide bond formation (Figure 1, tRNA selection). Here, two important distinctions from DNA and RNA polymerase are immediately apparent. First, the distinct size of the aa-tRNA substrate specifies that the PTC be ~70 Å distal to the decoding center, spatially separating these enzymatic centers. Second, the product of the polymerization reaction, deacylated P-site tRNA, remains tightly bound to both ribosomal subunits through direct interactions. Mechanistic peculiarities of the ribosome may therefore be attributed to the nature of its substrates and how

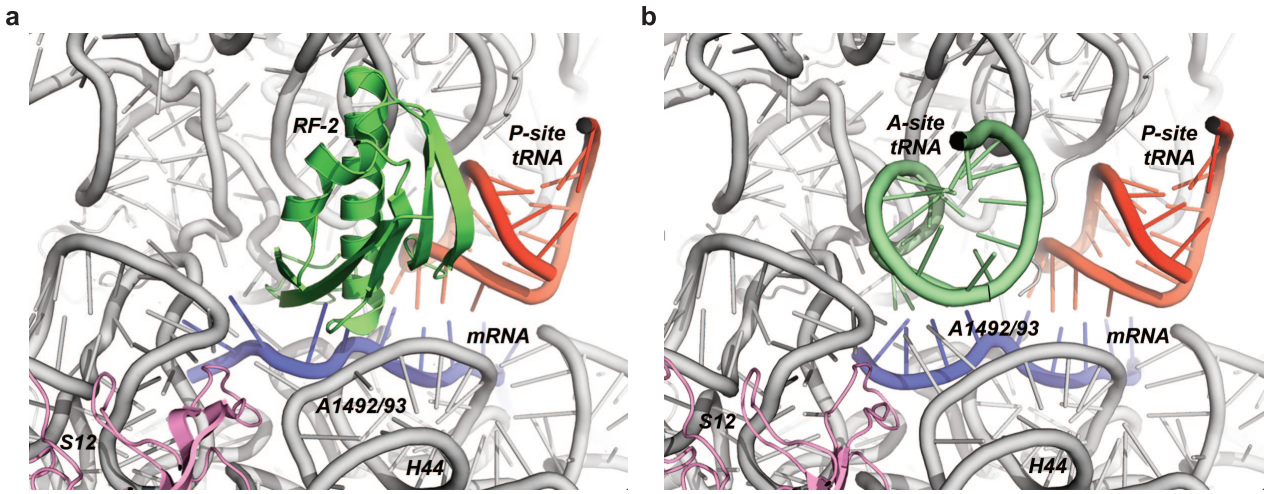

Figure 2. Structural data reveal that aa-tRNA and release factors uniquely engage the small subunit decoding center immediately proximal to the P-site codon–anticodon interaction. Close-up view of the small (30S) subunit highlighting tRNA and release factor interactions in the decoding center proximal to ribosomal protein S12 (pink). Ribosomal RNA (gray) engages the P-site tRNA (red), mRNA (blue), and A-site ligands (green) through intimate packing interactions. Strikingly, high-resolution structures show that RF-1 (left panel; PDB ID 3d5a) and the anticodon stem loop (right panel; PDB ID 2j00) differentially engage mRNA and the universally conserved A1492 and A1493 residues of helix-44 (H44) of the 30S subunit. Conformational flexibility in this region is thought to play a key role in the decoding and release factor mechanisms.

they interact with this processive enzyme. The function of tRNAs as adapter molecules in translation specifies that any communication between the decoding center and PTC must occur at a distance through the flexible tRNA structure.

Following synthesis reactions, subsequent substrate incorporation events require the newly occupied active site to be vacated. In the case of nucleotide polymerases, the product base pair has to move only a short distance (~3 Å) downstream of the active site in order to place the terminal 3′-hydroxyl in good position for a subsequent NTP reaction. On the ribosome, peptidyl-tRNA in the A site and deacylated P-site tRNA macromolecules must translocate by ~10 Å to bring the next codon into the decoding center. (Figure 1, Translocation). Disparities in both the molecular weight and distance displacement of reaction products suggest that the energetics of translocation must be markedly different. In the limit where reverse translocation is prerequisite to editing, exopeptidase activity would be energetically costly. More critical perhaps may be the peptide bond formation reaction mechanism itself and the kinetic stability of the peptide bond in the PTC relative to the phosphodiester linkage. On the ribosome, the newly incorporated amino acid is covalently tethered to both the tRNA and the peptide. A retrospective, exopeptidase editing mechanism would therefore need to excise the misincorporated element and religate the nascent peptide to the 3′-end A-site tRNA. Such a reaction mechanism may be exceedingly complex for a reaction center composed principally of RNA.

Through careful analysis, Zaher and Green revealed that mismatched codon–anticodon interactions in the P site alter the mechanism of downstream aa-tRNA and release factors selection. Somehow these unusual molecular determinants function to stimulate downstream misincorporation events and promiscuous termination on sense codons. Logically, these perturbations may be attributed to proximity effects (Figure 2). Indeed, an intimate physical relationship between these functional centers has been demonstrated: mutations in ribosomal protein S12 that increase A-site tRNA affinity lower P-site tRNA affinity and *vice versa* (*7*). With the benefit of recent structural (*8–10*) and computational (*11*) data, such effects may be understood to arise from changes in local conformational dynamics of critical residues within helix-44 of the small subunit rRNA (including residues A1492/93). By extension, the fidelity mechanism observed by Zaher and Green may be explained if mismatched codon–anticodon pairs in the P site broadly, and nonspecifically, increase A-site ligand affinities. However, the authors note that when more than one aa-tRNA selection error occurs and mismatched codon–anticodon pairs are present in both the E and P sites, a further enhanced release factor activity and acceleration of promiscuous termination rates are observed. In this case, the byproduct of the first misincorporation event, located in the E site, somehow affects the competitive reactions of aa-tRNA and re-

lease factor selection over long distance. This finding validates previous models proposing that codon−anticodon interactions are sensed by the ribosome in the E site and suggests that a more complex interplay of components and conformational changes contribute to the mechanisms of aa-tRNA selection and release (*12*). Observation of allosteric control of the ribosome mechanism originating from the E site is also in line with a growing body of evidence suggesting that the ribosome is an intrinsically dynamic molecular machine (*13, 14*). If the ribosome is indeed metastable in nature, functional distinctions in ribosome activities may arise from even subtle compositional variations. The work of Zaher and Green demonstrates that single base pair disruptions between the P- and E-site tRNA and the mRNA codon alter the kinetic determinants of tRNA selection and release within the ~3 MDa translating particle. Such perturbations are apparently significant enough to convert release factor-catalyzed termination on sense codons to a substantially weighted kinetic pathway.

In light of the many variations in rRNA and protein sequence among organisms, compositional distinctions in the ribosome that occur during translation in the cell, and post-translational modifications of the ribosome's protein components, further experiments are warranted to explore whether these alterations affect the ribosome mechanism (*14, 15*). The work of Zaher and Green anticipates that such distinctions may be central to translational control in the cell.

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