Translational fidelity: error-prone versus hyper-accurate ribosomes

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Decoding mRNA is a multistep process involving the RNA and protein components of the ribosome, and external factors; little is known about the mechanism, however. New evidence suggests that a central region in small ribosomal RNA switches between two helices in translation to maintain translational fidelity.

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Translation is carried out by the ribosome, a multifunctional RNA–protein complex composed of two subunits. It is now generally accepted that ribosomal RNA (rRNA), rather than ribosomal proteins, is the major player in many aspects of translation [1]. Besides maintaining the structural integrity of the ribosome, rRNA has also been shown to be involved in other functions such as transfer RNA (tRNA) binding, peptidyl transferase catalysis and antibiotic binding. A central aspect of translation is the decoding of messenger RNA (mRNA), a process involving ribosomal proteins (in particular S4, S5 and S12), tRNA, mRNA and external protein factors. Although it was originally viewed that the protein component of the ribosome was the important factor in mRNA decoding, recent research has indicated that the RNA component has a central role. In a recent publication by Lodmell and Dahlberg [2], mutational, footprinting and functional analyses have revealed a conformational change in the 16S rRNA during decoding and/or translocation. The authors suggest a central role for a particular 16S rRNA substructure in these processes, thus extending our view of the importance of the RNA component of the ribosome.

The decoding process

The task of decoding is to convert the information provided by the codons on a mRNA into their corresponding amino acids. Only discrimination between the cognate and noncognate tRNAs that deliver the amino acids to the ribosomal acceptor site (A site) ensures the correct peptide sequence. The discrimination reaction is accelerated by the ribosome, as the tRNA–mRNA interaction *per se* is too weak to allow discrimination. However, nonspecific interactions between tRNA and ribosome should not be too strong; that is, the release of noncognate tRNAs should be fast because it would otherwise impede the speed of translation. Accuracy of decoding and speed of translation have therefore evolved to be in the right balance for effective and accurate translation. The amino acids are delivered to the ribosome by tRNAs in a ternary complex consisting of a charged tRNA and the elongation factor Tu (EF-Tu) with GTP bound to it. The structures of several elongation factors complexed with ligands have been determined in the past year (see [3] for review). Recognition of the bound tRNA occurs at least twice: the first time in a process termed initial selection. The ternary complex is bound via the tRNA anticodon region to the mRNA in the ribosomal A site only in the 30S subunit. Selection occurs by the faster dissociation of noncognate tRNAs than cognate tRNAs from the A site and is independent of GTP consumption. Only subsequently, a conformational change in EF-Tu is induced and GTP bound to EF-Tu is hydrolysed [4], allowing the aminoacyl end of the tRNA, which was previously caged by EF-Tu, to also enter the ribosomal A site in the 50S subunit. Recently, the state prior to tRNA release by EF-Tu was nicely visualised by electron cryomicroscopy [5]. Before the amino acid bound to the tRNA can actually be donated to the growing peptide chain by the peptidyl transferase reaction, however, tRNA selection occurs a second time. The rate constant of ribosome-induced GTP hydrolysis was shown to be four orders of magnitude higher with cognate tRNA than with noncognate tRNA. A kinetic proof-reading mechanism that couples EF-Tu-dependent GTP hydrolysis to tRNA selection has been suggested [6]. The whole process has remained enigmatic, however. Of central importance is the question of where the structural components that accelerate the tRNA selection are located within the ribosome and, following this, how the actual signal of correct codon–anticodon interaction from one end of the tRNA (the anticodon) is delivered to the GTPase centre of EF-Tu at the other end of the tRNA (the aminoacyl end).

Factors influencing translational fidelity

It was discovered a long time ago that certain antibiotics (i.e. streptomycin and the neomycin-like aminoglycosides) specifically interfere with the process of tRNA selection. Their presence results in the incorporation of the wrong amino acids into a growing peptide chain and the suppression of stop codons. The sites of interaction of such antibiotics with the 16S rRNA have been identified by footprinting analysis and localisation of mutations that confer resistance to antibiotics. These sites are located in the so-called decoding region (around nucleotides 1400–1500), around position 530 (the 530 loop) and around position 912 [7]. Earlier, mutations in the ribosomal protein S12 were shown to result in resistance to (and also dependence on) streptomycin [8]. In the absence of antibiotics,

Table 1

Comparison of factors that cause error-prone or hyper-accurate translation.

	Translation	
	Error-prone	Hyper-accurate
Nonspecific tRNA affinity for the A site	Higher	Lower
Ribosomal proteins	S4, S5 mutants	S12 mutants
Antibiotics	Streptomycin, neomycin-like	
16S rRNA 912 region	Stable 912/885 helix	Stable 912/888 helix

Not included is the long list of mutations at sites in 16S or 23S rRNA that cause only one of the two phenotypes in translation.

many of the protein mutations caused an increased accuracy of translation (hyper-accurate or restrictive) both *in vivo* and *in vitro*. Second-site mutations in ribosomal proteins S4 or S5 suppressed this hyper-accurate phenotype and, when investigated in the absence of the primary mutation, conferred a decreased accuracy or error-prone translation called ram (for ribosomal ambiguity). From these findings, it was concluded that S4, S5 and S12 modulate the balance between fast, error-prone translation and slow, hyper-accurate translation. Miscoding-inducing antibiotics or ram mutations are believed to increase the nonspecific, codon-independent affinity of tRNA for the ribosomal A site [9,10] (Table 1).

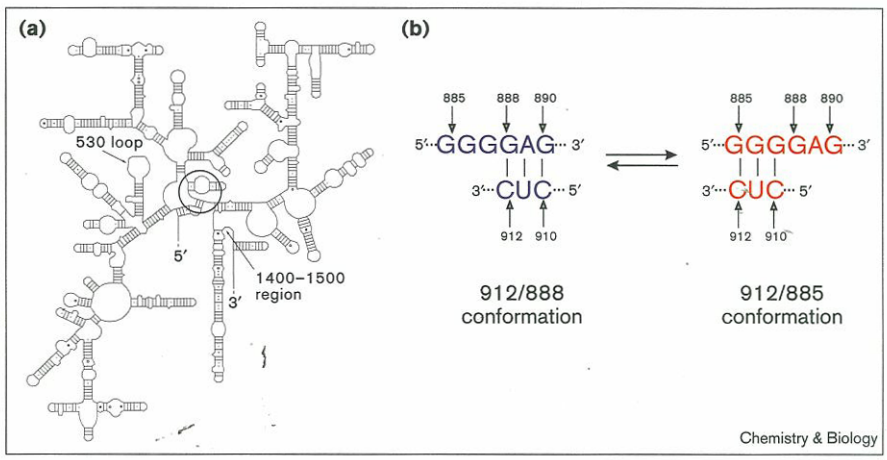
Not only mutations in ribosomal proteins lead to changes in decoding accuracy, however. For example, a mutation at position 1469 in 16S rRNA from *Escherichia coli* was shown to suppress the streptomycin dependence conferred by a mutant S12 protein and also resulted in a ram phenotype when segregated from the streptomycin-dependence allele [11]. A variety of other mutations in 16S rRNA were found to affect the decoding process and most of them were located in the sites of interaction with antibiotics that induce miscoding. To make matters even more complicated, mutations in the 23S rRNA were also found to impair translational fidelity (e.g. [12,13]), although this could be explained by the transduction of the tRNA–mRNA interaction signal to the GTPase centre of EF-Tu, since the majority of the EF-Tu protein is bound to the 50S subunit [5]. A comprehensive list of the effects of mutations in 16S and 23S rRNA can be found at the web site of the ribosomal mutation database (http://www.fandm.edu/Departments/Biology/Databases/RNA.html).

All these data gave quite a heterogeneous picture of how the ribosome controls decoding. Depending on the point of view, mutations in rRNA could be explained as causing the more important protein machinery to misfunction or *vice versa*. This point may have been clarified by the new work of Lodmell and Dahlberg [2] who, on the basis of previous work [14], investigated the role of a central region in the *E. coli* 16S rRNA around position 912 (Figure 1).

Stabilising helices in the 16S rRNA 912 region

The trinucleotide stretch at positions 910–912 in the 16S rRNA has the propensity to base pair to regions 885–887 and 888–890 with almost equal stability (Figure 1) and this propensity has been shown previously to be conserved among all organisms, despite sequence variation in the these regions, by comparative sequence analysis [14]. Using site-directed mutagenesis, mutants were generated that shifted the equilibrium towards either one of the two conformations and the behaviour of the conformations in translation was characterised [2]. Although most of the experiments, including the ones described here, were performed in a eubacterial system (*E. coli*), all available evidence suggests that the homologous ribosomal structures are also involved in the decoding process in eukaryotic systems [15].

Figure 1



The 16S RNA. **(a)** The secondary structure of the 16S rRNA from *Escherichia coli*; the 912 region is circled. **(b)** The proposed helix rearrangement of the 912 region during the process of decoding.

Stabilisation of the 912/885 helix conformation resulted in the increased read-through of stop codons *in vivo* whereas stabilisation of the alternate helix (912/888) resulted in a hyper-accurate phenotype [2]. When the mutant ribosomes bearing the stable 912/885 helix were combined with the S5 ram mutation the resulting cells were nonviable, possibly because of an additive effect on the synthesis rate of incorrect proteins. Combining this rRNA mutant with the S12 hyper-accurate mutation resulted in healthy cells, indicating that the mutations compensated for each other. In contrast, cells bearing ribosomes with the 912/888 helix were not viable with the S12 hyper-accurate mutation but were healthy when combined with the S5 ram mutation (compare with Table 1). The affinity of tRNA binding to the ribosomal A site was increased in the conformation leading to a ram phenotype and decreased in the conformation leading to a restrictive phenotype, again consistent with the picture described above.

Footprinting experiments confirmed the existence of the intended helix stabilisation in the rRNA mutants. The same technique was then used to search for conformational changes in the 16S rRNA. At several positions, a stabilised helix conformation induced an increased reactivity of a base towards chemical attack whereas stabilising the alternate helix led to a decreased chemical reactivity of the same base. Strikingly, bases G1491 and G1494, previously assigned to be involved in tRNA A site interaction, showed an increased reactivity in mutant ribosomes that had a restrictive phenotype. As this coincides with a decreased nonspecific binding of tRNA, it emphasises the importance of these nucleotide for codon-dependent tRNA binding to the ribosomal A site. Bases previously assigned to be involved in tRNA binding to the ribosomal P site (peptidyl site; G1401 and G926), however, had a decreased chemical reactivity in mutants that had a restrictive phenotype, in agreement with the proposal that there is an inverse relationship between the stability of tRNAs bound to the ribosomal A site and those bound to the P site [16]. The two extreme phenotypes of decoding, highly accurate or error-prone translation, can therefore now be mimicked by mutations in only one central region of the small ribosomal RNA. This is in contrast with all other mutations described previously which conferred only one of the phenotypes and suggests that the region plays an important role in the process of decoding.

A simple model for the actual decoding process drawn by Lodmell and Dahlberg [2] from these results is that the 912/888 conformation is adopted only transiently to perform tRNA selection, possibly by inducing a conformational change that more or less restricts tRNA binding to the mRNA, reducing or abolishing codon-independent interactions between tRNA and ribosome. A noncognate tRNA would then be released easily. Subsequently, the nonspecific interactions with a cognate tRNA are restored to proceed in translation.

It remains to be determined if this region is involved in initial selection or proof-reading or both. To my knowledge, most of the described ribosomal mutations affect the initial selection step. Interestingly, agents that induce miscoding (antibiotics, ethanol, etc.) were found to affect both selection steps [17], and it was suggested that this may point to the possibility that both steps of tRNA selection involve the same or overlapping pathways or structures within the ribosome [18].

The physical tRNA anticodon–mRNA interaction was shown to occur in the cleft of the 30S ribosomal subunit, the 1400–1500 region of the 16S rRNA. The 530 loop, however, is proposed to be involved in the signalling pathway of a correct codon–anticodon combination by interaction with EF-Tu [18]. The recent visualisation of the ternary complex–ribosome association, stalled by kirromycin, localises a domain of EF-Tu in the vicinity of the ribosomal proteins S4, S5 and S12 [5]. As these proteins were shown to bind to the 530 loop [19], this rRNA region might communicate with EF-Tu either directly or assisted by proteins. Stabilising one of the two possible helices within the 912 region causes a conformational change in specific regions of the 16S rRNA (530 loop, 1200 region and 1400–1500 region). These results therefore support the idea that the 912 region is a centrally located link required for communication or signal transduction between the specific 16S rRNA regions, as emphasised by the recent analysis of mutations in these regions. Introducing mutations in the 530 loop or the 1400–1500 region did not lead to conformational changes in one of these functionally linked regions [20], indicating that only the 912 region has the intrinsic capability to induce the conformational changes required for decoding. In terms of signal transmission, a search for possible conformational changes in the 23S rRNA caused by a stabilised conformation in the 912 region will certainly be very informative. As also pointed out by Lodmell and Dahlberg [2], the observed conformational change in the 1200 region suggests an involvement of the 912 region in translocation and this possibility should not be completely dismissed.

Two other positions in the 16S rRNA (G530 and A908) were previously proposed to be indicator nucleotides for conformational changes during the decoding process, comparable to the 912 region, based on their reactivity towards chemical probes in error-prone or hyper-accurate ribosomes [18,21]. In the mutant ribosomes, the G530 position did not show any altered chemical reactivity and the change in chemical reactivity at position 908 was the opposite of what was found earlier (the restrictive rRNA mutation caused an enhanced chemical reactivity of A908; J.S. Lodmell and A.E. Dahlberg, unpublished observations).

The chemical probing experiments, however, were performed under somewhat different conditions. The finding that combining one mutationally stabilised conformation in the 912 region with a mutation in a ribosomal protein has an additive effect, or in a different combination can be compensating each other, argues against the idea that the 912 region is the sole structure responsible for decoding. Rather, it suggests that several (independent) steps or structures are involved.

Alternatively, one can imagine that in the combination of a mutant ribosomal protein and a rRNA mutant that compensate each other, the mutant protein could help resolve the mutationally stabilised helix, thereby restoring translational fidelity or, in the nonviable combination, a mutant ribosomal protein could drive a stabilised helix that is allowed some 'breathing' (i.e. the thermodynamic opening and closing of the helix) further towards the base-paired conformation.

These questions now can be addressed directly, however, by combining the different mutants and the experimental tools at hand. So far, we cannot accommodate all the accumulated data in a model for decoding. But we may end up with a single responsible structural component organising the decoding process.

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