Ribosomal ambiguity made less ambiguous

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In PNAS, Fagan et al. (1) report a significant step forward in the quest to understand how the fidelity with which ribosomes translate the sequences of mRNAs into protein sequences is determined. The crystal structures discussed in this paper show that the B8 bridge between the two ribosomal subunits is critical in this regard. This work also brings into high relief a more general problem that remains poorly understood, namely the mechanism(s) responsible for the interactions observed between distant sites on the ribosome.

On the Contribution Ribosomes Make to the Fidelity of Translation

In all organisms, mRNA-directed protein synthesis is catalyzed by ribosomes. Their substrates are aminoacyl-tRNAs, of which there is at least one species for every kind of amino acid found in proteins. Ribosome-mediated Watson–Crick base pairing (mostly) between three-base sequences in mRNAs, i.e., codons, and 3-bp sequences in tRNAs, i.e., anticodons, determines the identity of the aminoacyl-tRNA selected from the pool at each step in the elongation of nascent peptide chains and hence the sequences of the proteins made.

In the 1960s, not long after mRNA was discovered, it was realized that mRNAs are translated far more accurately than can be explained by the small differences in stability that exist in solution between 3-bp RNA double helices that are cognate, i.e., correctly paired in a coding sense, and near-cognate helices, i.e., otherwise similar double helices that are not cognate at a single position. Enormous effort has been expended over the last 50 y to understand why this is so (2).

The first clear evidence that the ribosome plays a crucial role in determining the fidelity of translation emerged from studies of the mechanism of action of streptomycin. Streptomycin targets bacterial ribosomes, but unlike most antiribosomal antibiotics, it is not an inhibitor of protein synthesis per se. What it does instead is compromise the fidelity of translation. Not surprisingly, most of the errors induced by streptomycin reflect a reduction in the ability of the protein synthesizing system to discriminate

between cognate and near-cognate anticodons. Gorini and others isolated mutant strains of bacteria that are resistant to the drug (*SmR*), conditionally dependent on the drug (*SmD*), and finally, second-site revertants that reverse the *SmD* phenotype. They are called *ram* mutations for "ribosomal ambiguity." The *SmR* mutations all mapped to the gene for ribosomal protein S12, and in the absence of the drug, they cause

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an increase in the fidelity of protein synthesis. The *SmD* mutations alter the sequence of S7 and make translation hyperaccurate (3). The *ram* mutations found were mutations in S5 that reduce the fidelity of translation (4).

The reason rRNA mutations having similar phenotypes were not isolated in that era is not because they do not, or cannot, exist, but rather because they could not be selected for using the technology of the day. (In bacteria, every ribosomal protein is encoded by a single gene, but every rRNA is encoded by many.) This impediment has since been overcome, and a few years ago, Fredrick and colleagues (5) obtained a large number of 16S rRNA that have the *ram* phenotype. They fall into two classes: (*i*) mutations that alter bases in the S4/S5 region of the small ribosomal subunit and (*ii*) mutations that cluster around the B8 bridge, far from S5. The paper discussed here describes the crystal structures of ribosomes obtained from two of these mutant strains: G299A, which belongs to the S4/S5 class, and G347U, which belongs to the B8 class.

The fidelity with which ribosomes select aminoacyl-tRNAs is enhanced in two different ways. First, the decoding site of the

ribosome, where codons and anticodons interact, stabilizes cognate pairing relative to near-cognate pairing. Second, the mechanism of the selection process includes a proofreading step, which means that the appropriateness of the pairing between the anticodon of an aminoacyl-tRNA and a codon in the decoding center is tested twice before an aminoacyl-tRNA is used for protein synthesis.

The reason the active sites of polymerases like the ribosome can distinguish Watson–Crick base pairs from other base pairs, of which there are many, is that the geometries of the two Watson–Crick pairs are remarkably similar to each other but distinctly different from most of the rest. Their C1′ to C1′ distances are almost identical. In addition, the angles their glycosidic bonds make with a line drawn between their two C1s are almost the same. Furthermore, both pairs are so close to twofold symmetric that their left-right orientations, i.e., AU vs. UA, make no difference. Thus, if the active site of a polymerase includes groups that are placed so that they can hydrogen bond with backbone atoms presented on both sides of the minor groove face of Watson–Crick pairs, it will preferentially stabilize them. The discovery that the decoding center of the ribosome stabilizes the first 2 bp of any cognate codon–anticodon helix in exactly this way is one of the most important results to emerge thus far from ribosome crystallography (6).

Proofreading is easy to understand. If an enzyme selects its substrates in a two-stage process and the two stages are separated by a step that is effectively irreversible, e.g., the hydrolysis of a nucleoside triphosphate, the same interactions that enable its active site to distinguish proper substrates from near-proper substrates in the first stage can be used again in the second (7, 8). In the thermodynamic limit, the selectivity of processes of this sort will be the square of the selectivity of either one of them.

Ribosomes proofread. In the first stage, aminoacyl-tRNAs get delivered to the ribosome as part of a ternary complex that

includes elongation factor EF-Tu and GTP. The aminoacyl-tRNA in a ternary complex cannot be used for protein synthesis until after GTP hydrolysis. Once this has occurred, the consequent departure of EF-Tu·GDP from the ribosome permits a conformational change to occur that delivers the aminoacyl end of the aminoacyl-tRNA to the site on the large ribosomal subunit where peptide bonds are made. The probability that this conformational change, which is referred to as accommodation, will succeed is enhanced if the interaction between the anticodon of the aminoacyl-tRNA concerned with the codon presented to it in the decoding site is cognate.

The B8 Bridge and Action at a Distance in the Ribosome

More recently, a combination of kinetic measurements (9) and many new electron microscopic structures and crystal structures have added a lot to our understanding of the molecular mechanism of selection. The ribosomal nucleotides in the decoding center of the small ribosomal subunit that reinforce cognate pairing must change their locations to do so, and we now know that both the extra stability these reinforcing interactions confer on cognate codon–anticodon interactions and the associated conformational change contribute to the fidelity of translation. The local conformational change makes a difference because it somehow leads to an increase in the rate of GTP hydrolysis at a site on EF-Tu far from the decoding center. If that local conformational change does not occur, GTP hydrolysis is so slow that ternary complexes will fall off the ribosome before it takes place, but if it does happen, hydrolysis will occur before ternary complexes have time to dissociate, allowing their aminoacyl-tRNA moieties to enter the second stage of the selection process.

Accommodation is the key to the second stage of the selection process. Aminoacyl-tRNAs get delivered to the ribosome in a conformation that they would not adopt except for the constraints imposed on them by their interactions both with EF-Tu and the ribosome. After EF-Tu·GDP leaves the ribosome, a ribosome-bound aminoacyl-tRNA can return to its normal conformation, which will deliver its aminoacyl end to the

peptide bond–forming site on the large ribosomal subunit. While accommodation is going on, aminoacyl-tRNAs are bound to the ribosome only by the interactions their anticodon stems make with mRNA codons and the ribosome; they can dissociate. Because those interactions are stronger if they are cognate, one might expect that this alone would explain the contribution the second stage of the selection process makes to fidelity, but the kinetic data indicate that the truth is not so simple. Noncognate aminoacyl-tRNAs are indeed more likely to dissociate from the ribosome during accommodation than cognate aminoacyl-tRNAs, but the reason appears to be that the rate of accommodation is higher for cognate complexes than it is for near-cognate complexes. Thus, the local conformation change in the decoding center associated with cognate interactions appears to confer an advantage on those aminoacyl-tRNAs that are able to make them in both stages of the selection process.

The structures obtained by Fagan et al. (1) show that the structure of the B8 bridge, which links the two ribosomal subunits, is similar in both of the mutant ribosomes examined but different from what is seen in WT particles, and the authors' biochemical data show that both mutations affect the rate at which GTP is hydrolyzed by ribosome-bound ternary complexes. The effect is small if the ternary complex is cognate, but if the ternary complex is near-cognate, the rate of hydrolysis is abnormally high. Thus, the fraction of near-cognate complexes entering the second stage of the selection process will be unusually high in these mutant ribosomes, which is enough to explain the reduced fidelity with which they translate mRNAs. Interestingly, the conformation of

the B8 bridge in these mutant structures is similar to that seen in ribosome with cognate ternary complexes bound following GTP hydrolysis, which suggests that the B8 bridge somehow inhibits the GTPase activity of EF-Tu (10, 11).

It is comparatively easy to rationalize the phenotype of the G347U class of mutants. G347 is part of the B8 bridge, which abuts the EF-Tu component of ribosome-bound ternary complexes. It is much harder to understand why mutations of the G299A class have the same effects because G299 is far from G347, and for the same reason, it is every bit as challenging to explain why local conformational changes in the decoding center alter the GTPase activity of ribosome-bound EF-Tu. The authors use the phrase "long-distance conformational signaling" to describe these effects, but enzymologists are much more likely to use the word "allostery."

There are many reports of allosteric effects in the ribosome literature, as well as many discussion of sequences of coupled conformational changes linking distant sites on the ribosome that might explain their functional interactions. Such things may exist, but as the authors point out, there is no reason to think them necessary. The ribosome accesses many different conformational states during protein synthesis, and its progression from one conformation state to the next is driven by changes in relative free energies. Because modest changes in circumstances, i.e., the binding of a substrate to a site anywhere in the particle, can alter the relative free energies of its different conformational states, the coupling between the activities of its various sites need not depend on the molecular equivalent of pieces of telephone wire that connect its active sites.

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