Trends in Genetics

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Spotlight

Decoding a ribosome uncertainty

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The ribosome is among the most ancient macromolecular complexes. Throughout evolution, the function of the ribosome has remained essential and conserved: the decoding of an mRNA template with tRNA-linked amino acids, to synthesize a protein. In a recent study, Holm *et al.* capture evolutionary distinctions in the structure and kinetics of 'mRNA decoding' by the human ribosome.

The ribosome decodes mRNA into polypeptides in all domains of life. Capturing the structure and kinetic movements of the ribosome during mRNA decoding might be aptly described as a ribosome 'uncertainty principle': the structure and kinetics cannot both be measured exactly at the same time (at least not yet). Holm *et al.* recently met this challenge by combining distinct experiments, leveraging the strengths of each while compensating for their weaknesses [1]. Cryogenic electron microscopy (cryoEM) captured the human ribosome literally frozen in time, an ultra-high resolution still snapshot of its structure. In tandem, single-molecule Förster resonance energy transfer (smFRET) tracked the real-time movements of tR NAs and the ribosome, with the associated structures unseen. Weaving these orthogonal methods together into a '4D' concept in space and time led this team to detail how mRNA decoding evolved from bacteria to humans.

Two intriguing prior observations led to the motivation for this study. First, mRNA translation is slower and higher fidelity in eukaryotes, such as humans, compared with bacteria, as originally described in biochemical experiments [2,3]. Second, it was known that the global structures of bacteria and human ribosomes differ, with new ribosomal proteins and rRNA domains expanding during evolution (Figure 1A). Despite many molecular features of mRNA decoding and peptide elongation being highly conserved, there were hints that

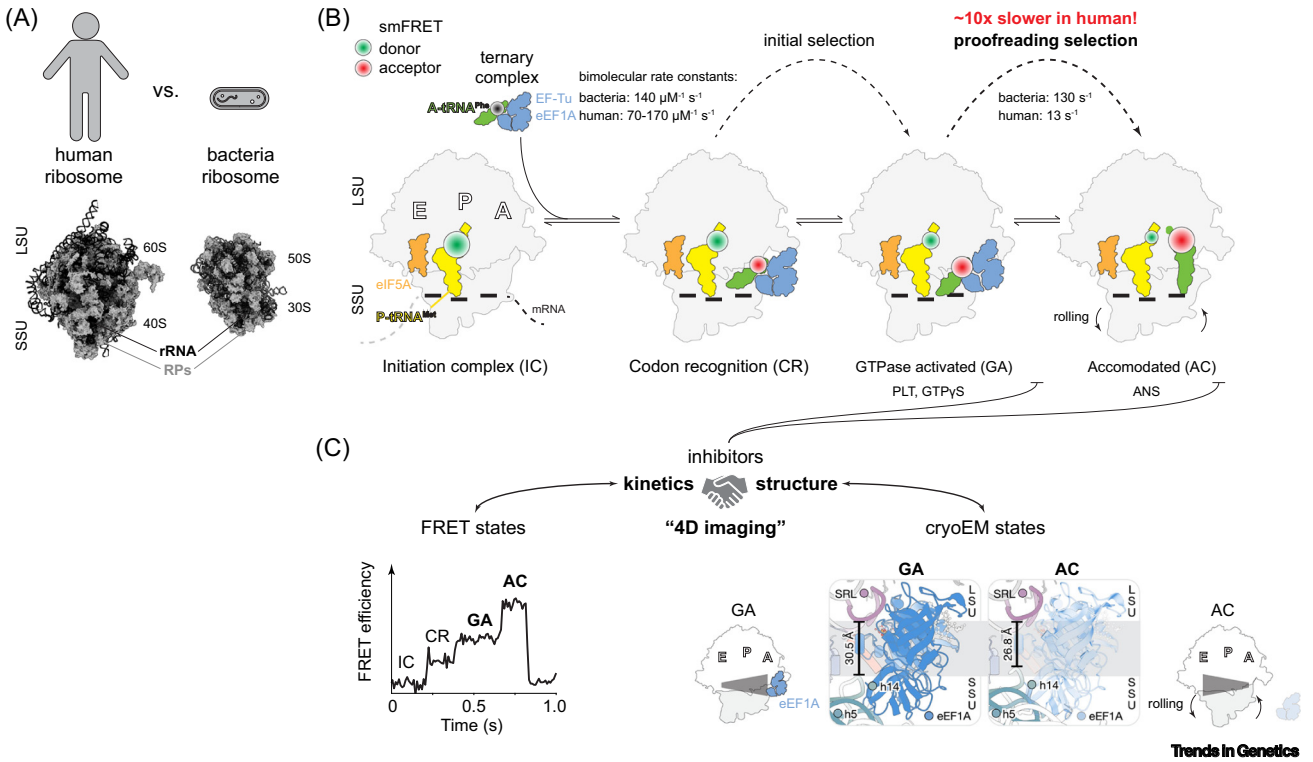

Figure 1. Weaving together ribosome kinetics and structure during mRNA decoding. (A) Ribosomes in humans and bacteria differ, with the evolutionary expansion of rRNA and ribosomal proteins (RPs). (B) The actions of human ribosomes were tracked during mRNA decoding. The proximity of P-site and delivered A-site tRNAs were visualized by single-molecule Förster resonance energy transfer (smFRET) labels across a high-resolution time course. (C) The detected FRET transition states were blocked with inhibitors, which were then likewise applied to stall ribosomes for cryogenic electron microscopy (cryoEM) analysis of the corresponding structural states. Cartoons in (A) and (C) were created with BioRender (biorender.com). Human and bacteria ribosomes in (A) were derived from Protein Data Bank models 4V6X and 7K00, respectively. Cartoons in (B) and data in (C) were derived from [1], under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Abbreviations: ANS, anisomycin; h5, h14, helices 5 and 14; LSU, large subunit; PLT, plitidepsin; SRL, sarcin/ricin loop; SSU, small subunit.

key structural rearrangements are also evolutionarily specific. For example, cryoEM analysis of human ribosomes revealed a distinct 'rolling' motion of the small subunit during translation [4]. The extent to which such dynamic movements are evolutionarily distinct remains a matter of debate [5], and how such distinctions impact the speed and fidelity of mRNA decoding was unresolved.

The decoding of mRNA into a polypeptide is a universally conserved two-step process, which maximizes decoding fidelity (Figure 1B). In the initial selection step, the mRNA codon positioned in the ribosome A-site is sampled by aminoacyl-tRNAs (aa-tRNA) in a ternary complex, comprising GTP, a GTPase (EF-Tu in bacteria, eEF1A in eukaryotes), and an aa-tRNA. After a cognate (matching) aa-tRNA binds, large conformational changes trigger GTP hydrolysis. This initiates the second step, proofreading selection, in which the 3′-end of the cognate aa-tRNA is transferred to the peptidyl transferase center for peptide bond formation. While in bacteria the mechanism of decoding is well understood, it is less clear what eukaryotic-specific elements are responsible for the higher fidelity and slower rate of mRNA decoding in eukaryotes.

Holm *et al.* built upon their well-established smFRET systems in bacteria [6] and humans [7]. The beauty of real-time single-molecule experiments is that they track complicated multistep processes in a single experiment, yielding reaction rates between each step (Figure 1B,C). The authors first immobilized ribosome initiation complexes (IC) containing a donor-labeled P-site initiator tRNA (fMet-tRNA^{fMet}) to a glass surface for single molecule imaging. Then they delivered the ternary complex, in which the Phe-tRNA^{Phe} cargo was labeled with an acceptor dye to monitor interactions at the ribosome A-site. This setup monitored how the ternary complex initially binds to the IC, and then how the ribosome transitions stochastically and reversibly between the different states during decoding. As in previous studies, they could visualize codon recognition (CR), followed by GTPase activation (GA), and the fully accommodated (AC) state by tracking the movements of the aa-tRNA through each of these steps. The authors determined the apparent bimolecular rate of ternary complex association with human ribosomes, and found rates similar to those in bacteria [6], concordant with a previous human study [7]. However, in contrast to bacteria, the final proofreading selection step after GTPase hydrolysis was tenfold slower, and most cognate aa-tRNA binding events were productive.

A critical experimental thread was necessary to weave these kinetic data into structural states: inhibitors of decoding (Figure 1C). The authors utilized an array of ribosome-binding inhibitors and a nonhydrolyzable GTP analog (GTPγS) to pinpoint which FRET state transitions were blocked. Then, focusing on those acting during proofreading selection, these same inhibitors were applied to stall ribosomes before plunge freezing for cryoEM. With this link between the FRET states and structural states in hand, their high-resolution atomic models could rationalize the human distinctions in proofreading selection.

The evolution of one proofreading feature stands out: the tandem movements of eukaryote-specific eEF1A and the rolling of the human small ribosomal subunit (Figure 1C). Before proofreading selection, the α2 helix in eEF1A is engaged with structural bridge B8 between the large and small ribosomal subunits (an 'intersubunit bridge'). Proofreading selection requires breaking of this contact, followed by small subunit rolling. This rolling motion generates a seesaw or 'allosteric' relationship between the A- and E-sites. Before proofreading selection in the GA state, the A-site is relatively open and the E-site relatively closed; after, in the AC state, the A-site closes and the E-site opens. The concept of allostery emerged over 20 years ago [8], with some controversy, but the findings of Holm *et al.* provide evidence in support of this concept. In essence, with more molecular contacts that are made and broken, decoding slows down and achieves higher fidelity in eukaryotes.

Why might have human ribosomes evolved toward higher fidelity, slower decoding activity? Multicellular eukaryotes in particular may be less tolerant to errors in translation that yield unfinished, dysfunctional, or toxic proteins, where compromised cellular fitness or signaling can impact the whole complex tissue. Some cell types are particularly sensitive to abnormal translation, such as neurons. Future studies are required to clarify the extent of functional evolutionary distinction built into human ribosomes.

For example, while rolling was proposed to be mammalian specific, it was similarly described in structural analysis of human mitochondrial ribosomes [9], which likely share bacterial origins. Furthermore, the study by Holm *et al.* was designed to capture mRNA decoding in a limited context: the +1 A-site codon after the P-site initiator methionine (i.e., the very first round of decoding). Downstream in the coding sequence, the ribosome encounters diverse mRNA–tRNA sequence contexts, and eIF5a is absent, which may engage different structural features and movements.

Besides the expansion of rRNAs and RPs, their post-transcriptional/translational modifications are further functionalizations gained in evolution [10]. Moving forward, linking the vast array of ribosome variations to functional mechanisms will be important *in vivo*, validating these *in vitro* data. Cryo-electron tomography (cryoET) is already shedding light on ribosome states *in*

situ [11]. While this study and others demonstrate that combining smFRET with cryoEM is very powerful, the linking of kinetics with structure is indirect. Future method advancements may allow for the recording of smFRET followed by cryoET of the same ribosome complex, to more tightly weave together ribosome kinetics and structures.

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Declaration of interests

No interests are declared.

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