Review

Panagiotis Poulis, Frank Peske and Marina V. Rodnina*

The many faces of ribosome translocation along the mRNA: reading frame maintenance, ribosome frameshifting and translational bypassing

https://doi.org/10.1515/hsz-2023-0142
Received February 21, 2023; accepted March 22, 2023;
published online April 21, 2023

Abstract: In each round of translation elongation, the ribosome translocates along the mRNA by precisely one codon. Translocation is promoted by elongation factor G (EF-G) in bacteria (eEF2 in eukaryotes) and entails a number of precisely-timed large-scale structural rearrangements. As a rule, the movements of the ribosome, tRNAs, mRNA and EF-G are orchestrated to maintain the exact codon-wise step size. However, signals in the mRNA, as well as environmental cues, can change the timing and dynamics of the key rearrangements leading to recoding of the mRNA into production of trans-frame peptides from the same mRNA. In this review, we discuss recent advances on the mechanics of translocation and reading frame maintenance. Furthermore, we describe the mechanisms and biological relevance of non-canonical translocation pathways, such as hungry and programmed frameshifting and translational bypassing, and their link to disease and infection.

Keywords: mRNA; recoding; ribosome; translation fidelity; translocation; tRNA.

1 Introduction

The ribosome is the molecular machine that synthesizes proteins using mRNAs as templates. During initiation, the small (SSU) and large (LSU) ribosomal subunits assemble on the mRNA start codon, which defines the open reading frame (ORF) for the codon-by-codon progression of the ribosome along the mRNA. During translation elongation, the mRNA codons are read by aminoacyl-tRNAs (aa-tRNAs) that deliver the amino acids to the ribosome, thereby ensuring the correct translation of the mRNA sequence into the sequence of the protein. After decoding, the aa-tRNA accommodates in the peptidyl transferase center where the ribosome catalyzes peptide bond formation, thereby adding the next amino acid to the growing nascent peptide chain. Then, the ribosome moves by one codon along the mRNA in a process called translocation, which is catalyzed by elongation factor G (EF-G, Figure 1A). The machinery for translation elongation has evolved to maintain the collinearity of the mRNA sequence of the ORF and the sequence of the protein synthesized. However, the stochastic nature of translocation, stimulatory elements in the mRNA and the availability of tRNAs and translation factors can drive recoding events that produce trans-frame peptides. This review discusses recent advances on the mechanics of translocation along the mRNA. We focus on how EF-G facilitates tRNA–mRNA movement and how the translational reading frame is maintained during translocation. Furthermore, we describe the mechanism of non-canonical translocation pathways that produce trans-frame peptides and their role in disease and infection in humans.

2 The dynamics of ribosome complex at the start of translocation

At the starting point of translocation, the highly dynamic pre-translocation (PRE) complex contains a peptidyl-tRNA in the A site and a deacylated tRNA in the P site, formed as a result of the peptidyl transferase reaction on the ribosome (Figure 1B) (Blanchard et al. 2004; Cornish et al. 2009; Fei et al. 2008; Frank and Agrawal 2000; Julian et al. 2008; Moazed and Noller 1989; Munro et al. 2007; Ratje et al. 2010; Sharma et al. 2016). The tRNAs fluctuate between their classical (C) and hybrid (H) states. In the C state, the acceptor

***Corresponding author: Marina V. Rodnina**, Department of Physical Biochemistry, Max Planck Institute for Multidisciplinary Sciences, Am Fassberg 11, D-37077 Göttingen, Germany,
E-mail: rodnina@mpinat.mpg.de. https://orcid.org/0000-0003-0105-3879
Panagiotis Poulis and Frank Peske, Department of Physical Biochemistry, Max Planck Institute for Multidisciplinary Sciences, Am Fassberg 11, D-37077 Göttingen, Germany. https://orcid.org/0009-0004-4117-5904 (P. Poulis)

Open Access. © 2023 the author(s), published by De Gruyter. This work is licensed under the Creative Commons Attribution 4.0 International License.

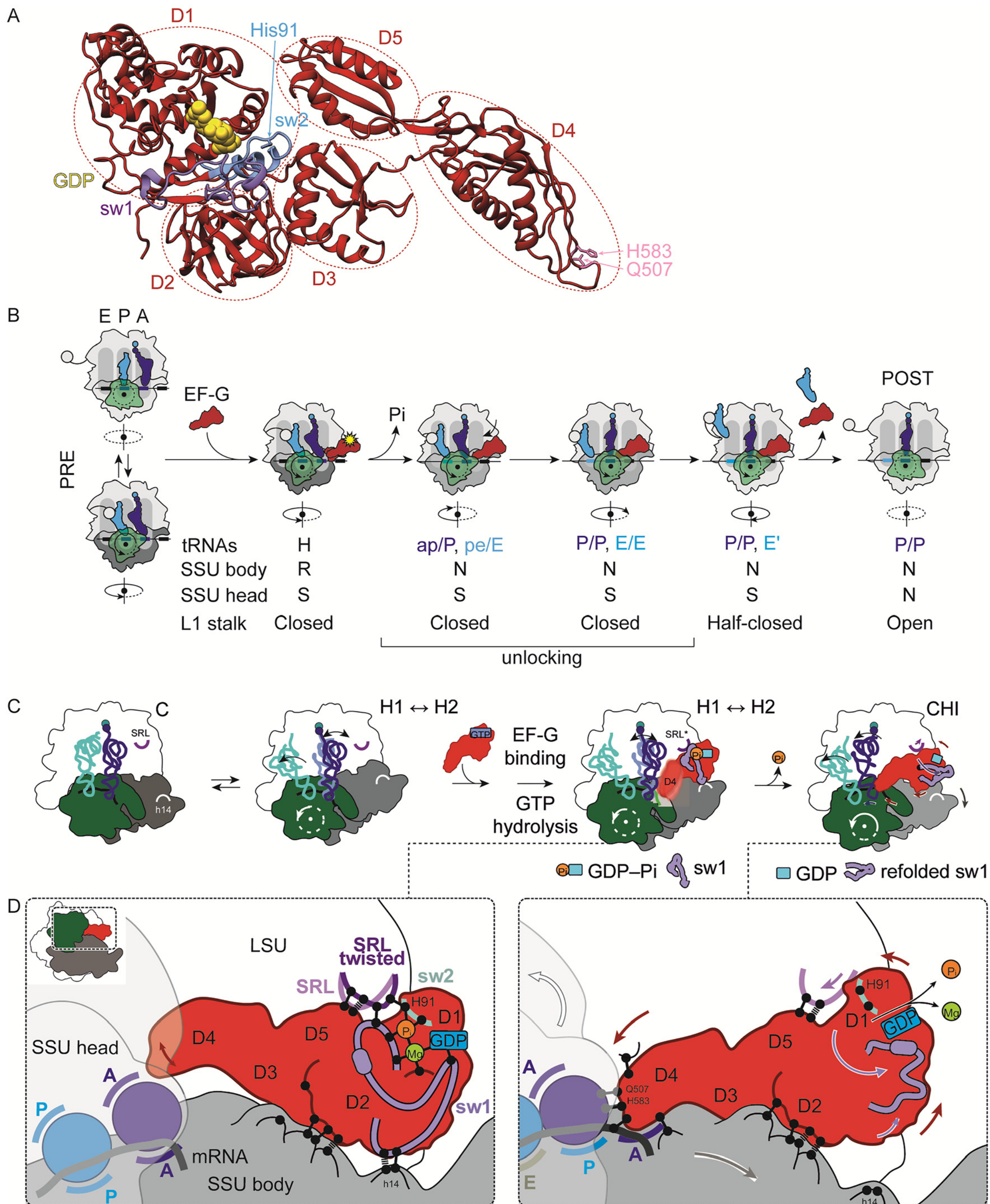

Figure 1: Molecular mechanics of translocation. (A) Structure of *E. coli* EF-G. Domains (D) 1–5 are shown in dotted circles. Switch regions 1 (sw1) and 2 (sw2) are shown in purple and light blue, respectively. The catalytic His91 is shown in light blue as sticks. The key residues H583 and Q507 are shown in pink. GDP is shown as yellow spheres. Adapted from PDB 7PJW. (B) Translocation steps. EF-G binding stabilizes the R/H state of the ribosome. GTP hydrolysis and P_i release drive unlocking of the ribosome and the tRNAs move to the CHI states. When the tRNAs reach the POST state, the deacylated tRNA is released from the ribosome, EF-G–GDP dissociates and the SSU head adopts the non-swiveled conformation. The ribosome is shown in grey, with the SSU body domain in dark gray and the SSU head domain in green. EF-G is depicted in red, peptidyl-tRNA in blue, deacylated tRNA in cyan, L1 stalk as light grey circle, mRNA in black solid line. Codons (thick solid lines) and amino acids (circles) are shown in same colors as respective tRNAs. GTP hydrolysis by EF-G is shown as a yellow flare. R, rotated state of the SSU body; H, hybrid tRNA state; S, swiveled state of the SSU head; N, non-rotated/non-swiveled state for detailed description, see text. (C) Structural rearrangements that lead to ribosome unlocking and CHI formation. Sarcin-ricin loop (SRL) is shown as purple solid line, helix 14 (h14) as white solid line, switch 1 (sw1) in purple, GDP as blue box, P_i as orange sphere. (D) Details on CHI formation and ribosome unlocking upon P_i release. Prior to P_i release (left), EF-G sw1 contacts the SRL, GDP–P_i, Mg^{2+} and 16S rRNA helices h5, h14 and h15 of the SSU

stems and anticodon loops of the tRNAs are located in the A and P sites on both subunits, which is denoted as A/A and P/P for the peptidyl-tRNA and deacylated tRNA, respectively. In the H state, the acceptor stems and the tRNA elbow regions move towards the P and E site on the LSU, whereas the anticodon loops remain in the A and P sites on the SSU (Figure 1B). The deacylated tRNA adopts a single hybrid conformation (P/E), while the peptidyl-tRNA can fluctuate between two distinct hybrid conformations denoted as A/P (H1) and A/P* (H2) that differ only in the position of the tRNA elbow (Adio et al. 2015; Agirrezabala et al. 2008; Blanchard et al. 2004; Carbone et al. 2021; Chen et al. 2011a; Dunkle et al. 2011; Kim et al. 2007; Moazed and Noller 1989; Munro et al. 2007; Petrychenko et al. 2021; Rundlet et al. 2021; Zhang et al. 2009). The relative arrangement of SSU and LSU in the C state is called a non-rotated (N) state of the ribosome. Upon H state formation, the SSU moves relative to the LSU by ~8° in counterclockwise direction looking from the SSU side (Figure 1B) adopting the rotated (R) state (Agirrezabala et al. 2008; Altuntop et al. 2010; Cornish et al. 2008; Ermolenko et al. 2007; Ermolenko and Noller 2011; Fischer et al. 2010; Frank and Agrawal 2000; Julian et al. 2008; Sharma et al. 2016; Zhang et al. 2009). This is accompanied by swiveling of the SSU head domain by ~6° in the direction of tRNA movement across the SSU head-body axis (swiveled, S, state; Figure 1B) (Belardinelli et al. 2016; Guo and Noller 2012; Mohan et al. 2014; Petrychenko et al. 2021; Ratje et al. 2010; Schuwirth et al. 2005; Wasserman et al. 2016; Zhang et al. 2009). Finally, the L1 stalk, a hinge-like structure that helps the deacylated tRNA out of the E site, changes its conformation from open in the C state to closed in the H state, where it moves towards the E site and is in contact with the elbow region of the P/E tRNA (Figure 1B) (Bock et al. 2013; Cornish et al. 2009; Fei et al. 2008, 2009; Mohan and Noller 2017). The PRE complex spontaneously fluctuates between the C/N and H/R states with rates of about 1–10 s^{-1} (Adio et al. 2015; Cornish et al. 2008; Fei et al. 2008; Munro et al. 2007; Poulis et al. 2022; Sharma et al. 2016). These motions are stochastic and reversible (Gavrilova and Spirin 1971; Katunin et al. 2002; Konevega et al. 2007; Shoji et al. 2006), but the translocation of the tRNA anticodons on the SSU is inefficient, as the ribosome remains in a locked global conformation. Rapid translocation requires ribosome unlocking, i.e., coordinated global movements in the ribosome complex with both tRNAs moving simultaneously in the direction of translocation (Bock et al. 2013; Fischer et al. 2010; Holtkamp et al. 2014; Munro et al. 2010). EF-G induces and orchestrates those movements and accelerates translocation to physiologically relevant rates at the cost of GTP hydrolysis.

3 EF-G and the mechanism of tRNA–mRNA translocation

EF-G (eEF2 in eukaryotes) is a translational GTPase that promotes translocation. EF-G consists of five domains (Figure 1A) (Czworkowski et al. 1994; Evarsson et al. 1994). Domain 1 (D1) contains the GTP binding pocket and bears the key histidine residue (His91 in *Escherichia coli* EF-G) required for GTP hydrolysis (Figure 1A) (Cunha et al. 2013). As in all members of the GTPase superfamily, the switch 1 (sw1) and switch 2 (sw2) regions in D1 are flexible elements that sense GTP binding and hydrolysis and link the nucleotide-binding state of EF-G to its binding state on the ribosome (Figure 1A) (Carbone et al. 2021; Petrychenko et al. 2021; Rodnina et al. 2019). The initial recruitment of EF-G D1 to the ribosome occurs through interactions with the L12 stalk, the only multicopy protein structure of the ribosome containing four to eight copies of the bL12 protein (Davydov et al. 2013; Diaconu et al. 2005). The L12 interaction orients EF-G across the SSU-LSU interface with EF-G domain 4 (D4) pointing towards the codon-anticodon complex in the A site of the SSU (Figure 1B) (Brilot et al. 2013; Lin et al. 2015; Salsi et al. 2014, 2015; Stark et al. 2000). In the GTP-bound form, D1 and D2 of EF-G contact the so-called sarcin-ricin loop of the 23S rRNA on the LSU (SRL, helix H95) and the SSU body (helices h5, h14 and h15) respectively, thereby acting as a pawl that stabilizes the R state of the SSU body, the S state of the SSU head domain, the H states (A/P*, P/E) of tRNAs, and the closed state of the L1 stalk (Figure 1B) (Adio et al. 2015; Belardinelli et al. 2016; Carbone et al. 2021; Chen et al. 2011a; Fei et al. 2008; Holtkamp et al. 2014; Munro et al. 2010; Petrychenko et al. 2021; Sharma et al. 2016). Docking of EF-G D1 on the SRL of the LSU brings together all the components of the GTPase center, and positions His91 of EF-G in a catalytically active orientation, leading to GTP hydrolysis (Figure 1B and C) (Maracci and Rodnina 2016; Rodnina et al. 2019). After GTP hydrolysis but before P_i release, D4 of EF-G remains flexible, while sw1 contacts GDP-P_i, SRL and the SSU body (Figure 1C and D) (Carbone et al. 2021; Petrychenko et al. 2021).

Evidence from ensemble kinetics measurements indicated that P_i release from EF-G facilitates a rearrangement

body, while EF-G D4 is highly flexible. Upon P_i release (right), contacts of sw1 with GDP, Mg^{2+}, as well as h14 of the SSU body are lost, while new contacts with the SSU body are established. This leads to the back rotation of the SSU body and an upward movement of EF-G domains 1–3 (D1-3) that positions domain 4 (D4) in contact with the tRNA-mRNA duplex, which now adopts the CHI state. Adapted from (Petrychenko et al. 2021).

that promotes the tRNA movement on the SSU and this step coincides with a change in relative orientation of the SSU head and body domains and a backward rotation of the SSU relative to the LSU (Belardinelli et al. 2016; Savelsbergh et al. 2000). Recent time-resolved cryo-EM studies demonstrate how conformational changes of the ribosome complex upon P_i release facilitate the tRNA movement on the SSU (Carbone et al. 2021; Petrychenko et al. 2021). P_i release acts as a trigger for a loaded spring to promote tRNA movement (Figure 1D). The loss of P_i coordination unleashes sw1 from the SRL, which leads to an upward rotational motion of D1-3 of EF-G on the LSU and moves D4 deeper into the A site of the SSU where it contacts the tRNA-mRNA duplex (Figure 1D) (Carbone et al. 2021; Petrychenko et al. 2021). These rearrangements lead to ribosome unlocking: the SSU body rotates back, while the SSU head domain swivels by ~18–21° (Figure 1B–D) (Belardinelli et al. 2016; Carbone et al. 2021; Chen et al. 2016; Guo and Noller 2012; Petrychenko et al. 2021; Ramrath et al. 2013; Sharma et al. 2016; Zhou et al. 2014). Ribosome unlocking results in forward movement of the peptidyl- and the deacylated tRNA into an EF-G–induced chimeric state (CHI), where their anticodon stem loops are located between the A and P (ap/P) and P and E sites (pe/E) on the SSU, respectively, following the movement of the SSU head (Figure 1B and D) (Adio et al. 2015; Belardinelli et al. 2016; Bock et al. 2013; Carbone et al. 2021; Fischer et al. 2010; Petrychenko et al. 2021; Ramrath et al. 2013; Schuwirth et al. 2005; Zhou et al. 2014).

The back swiveling of the SSU head domain brings the tRNAs into the P/P and E/E states and lock the peptidyl-tRNA in the P site (Figure 1B) (Belardinelli et al. 2016; Gao et al. 2009; Wasserman et al. 2016). Shortly after, the deacylated tRNA is released from the ribosome with the help of the L1 stalk (Adio et al. 2015; Belardinelli et al. 2016; Bock et al. 2013; Chen et al. 2011b, 2013b; Choi and Puglisi 2017; Fei et al. 2008, 2009; Lill et al. 1986; Munro et al. 2010; Petropoulos and Green 2012; Semenkov et al. 1996; Uemura et al. 2010; Wasserman et al. 2016) and EF-G-GDP dissociates from the ribosome (Belardinelli et al. 2016; Carbone et al. 2021). The ribosome adopts the posttranslocation state (POST), which is static and where the A site is available to accommodate the next aa-tRNA (Figure 1B) (Adio et al. 2015; Carbone et al. 2021; Poulis et al. 2022; Rundlet et al. 2021; Sharma et al. 2016; Wasserman et al. 2016).

Local movements of elements involved in translocation are very rapid (on the µs and sub-µs scale (Bock et al. 2013)) and only loosely coupled with one another (Fischer et al. 2010), creating a flat landscape of interconnecting PRE states separated by a steep energetic barrier from the ensemble of the POST states (Munro et al. 2009). The rugged translocation landscape accounts for the existence of multiple (~500,000,000) alternative translocation pathways (Bock et al. 2013). EF-G and GTP hydrolysis favor a particular, well-defined translocation pathway with a low energy barrier by orchestrating the fluctuations of individual components into a global movement (Belardinelli et al. 2016; Holtkamp et al. 2014), which explains the acceleration of translocation by EF-G. In the absence of GTP hydrolysis, translocation is up to 50 times slower (Katunin et al. 2002; Munro et al. 2010; Pan et al. 2007; Rexroad et al. 2022; Rodnina et al. 1997) and follows a different pathway than the GTPase-controlled reaction (Belardinelli et al. 2016). Blocking GTP hydrolysis slows forward and abolishes backward SSU head motions (Belardinelli et al. 2016; Rexroad et al. 2022), slows down the backward rotation of the SSU body and the dissociation of the deacylated tRNA and abolishes EF-G release (Belardinelli et al. 2016). Although EF-G binding alone can promote translocation by 10,000-fold (Katunin et al. 2002), which appears to be a large effect compared to that of GTP hydrolysis (50-fold), translocation in the absence of GTP hydrolysis is too slow (in the range of 0.1–0.5 s^{-1}) to support efficient and rapid (~10–20 aa/s) protein synthesis in the cell. Interestingly, the requirement for GTP hydrolysis is abolished under nutrient-fluctuating conditions. Gut commensal bacteria replace EF-G with EF-G2, a GTPase-deficient paralog that promotes translocation without GTP hydrolysis, although at a slower rate, in order to sustain translation at conditions of carbon starvation (Han et al. 2023). Other potential translocation pathways are favored in the presence of antimicrobial inhibitors that block the rapid translocation route (Belardinelli et al. 2021). Changes in the translocation pathway can also induce recoding events via non-canonical translocation, leading to the synthesis of more than one polypeptide from the same mRNA (Rodnina et al. 2020). Two remarkable recoding examples are ribosome frameshifting and translational bypassing, which are discussed below in more detail.

4 Reading frame maintenance

Maintenance of the reading frame is crucial for correct translation, however, occasionally the ribosome can slip by one or more nucleotides towards the 5′ end (in – direction) or 3′ end (in + direction) of the mRNA. The prevalent –1 frameshifting occurs on slippery mRNA sequences of the type X XXY YYZ, where the same tRNA can base pair with both the 0-frame and the trans-frame overlapping codons XXY and XXX or YYZ and YYY (Figure 2A). At equilibrium, the frameshifting efficiency depends on the free energy difference, ΔΔG, of base pairing in the 0 frame ($\Delta G_{0\ \mathrm{frame}}$)

and –1 frame ($\Delta G_{-1\ frame}$) (Bock et al. 2019). Lower ΔG in –1 frame favors frameshifting, while lower ΔG in the 0 frame renders frameshifting thermodynamically unfavorable (Figure 2A). Ribosomes that resume translation in the –1 frame produce peptides that differ in sequence from those of the 0-frame ORF and terminate at a trans-frame stop codon, thus leading to the synthesis of peptides of different amino acid sequence and length. Yet, despite the risks of frameshifting, slippery sequences are relatively abundant in the coding genome, for example the slippery sequence A AAA AAG is only mildly underrepresented in the coding genome of *E. coli* (Gurvich et al. 2003). Overall, ~10% of human mRNAs are predicted to contain a slippery sequence (Belew et al. 2008). If the frameshifting efficiency was solely thermodynamically controlled, close to 50% of these sequences would result in frameshifting and thus about 5% of cellular proteins would end up as trans-frame junk. We actually do not know whether this does not occur in the cell, as there are many unidentified peptides in every proteomic database, and such truncated peptides could be removed by the quality control machineries. However, experiments with frameshifting reporters suggest that the occurrence of spontaneous frameshifting on slippery sequences is quite low (Caliskan et al. 2014; Gurvich et al. 2003), suggesting that translating ribosome can ensure correct translocation step size even on slippery sequences.

Recent studies revealed that EF-G is a key player in reading frame maintenance (Peng et al. 2019; Zhou et al. 2019). In the structure of the PRE complex that moved spontaneously (i.e., in the absence of EF-G) into the CHI state (i.e., with an unlocked conformational state of the ribosomal subunits and the SSU head swiveled by 21° relative to the SSU body), base pairing between the tRNA and the P-site codon is partially disrupted and the 3′ nucleotide of the P-site codon is flipped towards the A-site tRNA, resulting in a shift by –1 nucleotide (Zhou et al. 2019). Also the A-site tRNA makes partial contacts with the –1 mRNA nucleotide, suggesting a propensity for frameshifting. In contrast, in the presence of EF-G, the 0 frame is maintained, because the A-site tRNA is held in place by the interactions with D4 of EF-G, in particular with residues Q507 and H583 (Figure 1A and D) (Carbone et al. 2021; Fischer et al. 2010; Petrychenko et al. 2021; Rundlet et al. 2021; Zhang et al. 2009). Mutational analysis shows that Q507 and H583 indeed prevents spontaneous frameshifting, as well as reduced the rate of translocation (Peng et al. 2019). Analysis of different amino acid substitutions of Q507 indicates that slower translocation correlated with higher frameshifting efficiency, suggesting that opening a time window for the tRNAs to equilibrate between frames promotes slippage (Peng et al. 2019). Kinetic analysis provided an estimate for the rate of the slippage, which reflects the energy barrier due to

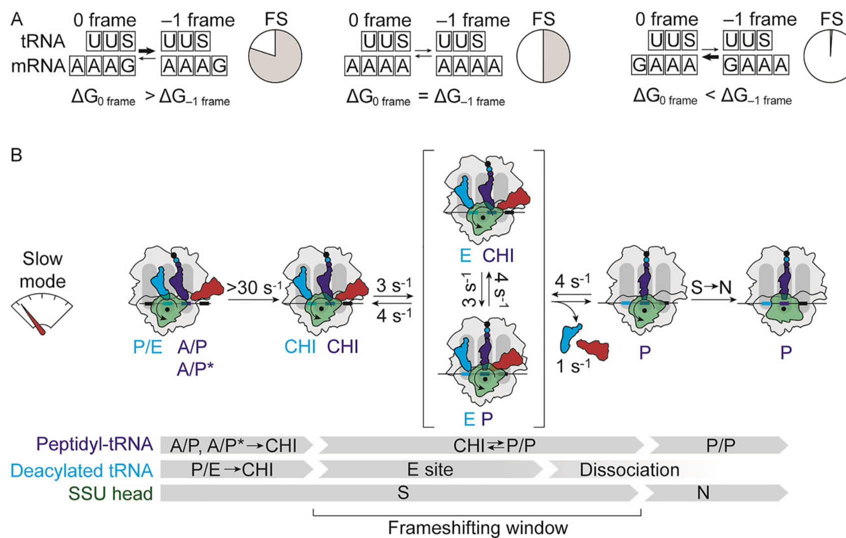

Figure 2: Mechanism of spontaneous ribosome frameshifting. (A) Free energy difference as the driving force for frameshifting. Thick arrows show the thermodynamically favorable direction. Pie charts show the experimentally calculated frameshifting efficiency (FS, grey) for each sequence (Bock et al. 2019). AAA and AAG code for Lys; S is 5-methylaminomethyl-2-thio uridine 34 in the $tRNA^{Lys}$. (B) Kinetic model of translocation on slippery mRNA. A fraction of ribosomes translocates in slow mode, where peptidyl-tRNA fluctuates between CHI and P/P, whereas locking of the P/P state is impaired. Deacylated tRNA translocates rapidly and dissociates from the ribosome, thus allowing pept-tRNA to sample 0- and –1-frame codons. Back swiveling of the SSU head is delayed as a result of peptidyl-tRNA fluctuations. Rates of the elemental reactions are indicated (Poulis et al. 2022).

breaking codon-anticodon interactions in the 0 frame and re-establishing them in the –1 frame). At the rate of 3–10 s^{-1}, slippage into the trans frame is slower than translocation in the 0 frame, which explains why frameshifting is upregulated by the elements that slow down ribosome progression, such as regulatory mRNA elements. However, ribosome stalling is not the only reason for frameshifting. Single-molecule FRET studies reveal that translocation on a slippery mRNA can follow two modes: a fast, accurate and a slow, frameshifting-prone mode (Poulis et al. 2022). In the fast mode, timely ribosome unlocking drives fast and synchronized tRNA translocation and locking of the tRNAs in POST states by inducing rapid SSU head back swiveling (Peng et al. 2019; Poulis et al. 2022). In the frameshifting-prone mode, tRNA movements are uncoupled: while the deacylated tRNA moves rapidly from the P to the E site and is released from the ribosome, the translocation of the peptidyl-tRNA from the A to the P site is slow and stalled between CHI (ap/P) and POST (P/P) states (Figure 2B) (Peng et al. 2019; Poulis et al. 2022). During slow translocation of the peptidyl-tRNA, the SSU head domain remains in a long-lived swiveled conformation (Peng et al. 2019; Poulis et al. 2022). This allows sampling between the 0- and –1-frame codons by the peptidyl-tRNA, which creates the time window for the tRNA to shift the reading frame. EF-G, via the residues Q507 and H583 at the tip of D4, suppresses the frameshifting-prone pathway, thus allowing the ribosome to maintain the ORF (Peng et al. 2019; Poulis et al. 2022). A recent study suggested that also GTP hydrolysis by EF-G facilitates reading frame maintenance by preventing EF-G drop-off during translocation (Rexroad et al. 2022). However, replacement of GTP by a non-hydrolysable analog does not facilitate frameshifting, because translocation is stalled at an early stage of translocation prior to the formation of the CHI state (Poulis et al. 2022). In summary, these results show that EF-G-orchestrated translocation is important not only for rapid ribosome movement along the mRNA, but also for the reading frame maintenance on slippery sequences.

5 “Hungry” frameshifting and the production of neopeptides

Although EF-G usually prevents spontaneous frameshifting, limited availability of charged cognate aa-tRNAs can cause depletion-triggered, or “hungry”, frameshifting. Hungry frameshifting occurs in “idling” ribosome complexes residing on slippery sequences when the cognate tRNA reading the next A-site codon is lacking (Figure 3A and B) (Caliskan et al. 2017; Riegger and Caliskan 2022). This can occur when nutrient starvation depletes the pool of a particular amino acid, or when a tRNA is not available, either because it is naturally rare or because its production is altered at stress conditions (Figure 3A). In the time window where the A site is unoccupied by aa-tRNA, the interactions of the P-site peptidyl-tRNA with the 0-frame codon can be disrupted and the tRNA can re-pair with a trans-frame codon (Figure 3B) (Caliskan et al. 2017). Hungry frameshifting can move the ribosome in both –1 and +1 direction. For example, a structure of the POST complexes on the slippery sequence CCC U (CCC and CCU are read by the same $tRNA^{Pro}$ isoacceptor) shows that the anticodon stem loop of the P-site $tRNA^{Pro}$ moved into an intermediate position between the P and E sites on the SSU (denoted as e*/E, clearly distinct from the canonical P/P conformation), accompanied by an mRNA compaction that brings the +1 base closer to the tRNA anticodon thus conferring +1 frameshifting (Hoffer et al. 2020). In some cases, hungry frameshifting acts to regulate gene expression. A well-studied example is the autoregulation of the synthesis of release factor 2 (RF2) that mediates peptide release during translation termination (Baranov et al. 2002; Craigen and Caskey 1986; Donly and Tate 1991). The availability of RF2 in the cell regulates its production by fine tuning termination on its own *prfB* mRNA. When RF2 is abundant, translation is efficiently terminated on a 0-frame stop codon leading to synthesis of a short peptide of RF2. In contrast, when RF2 is limiting, termination on the 0-frame stop codon is impaired, resulting in an idle ribosome complex that carries peptidyl-tRNA in the P site. The peptidyl-tRNA shifts into the +1 frame and resumes translation that produces the active form of RF2.

Hungry frameshifting is increasingly linked to different human diseases. For example, it appears to plays an important role in maintaining viral protein production during human immunodeficiency virus (HIV) infection (Korniy et al. 2019a). A hallmark of the HIV genome is a frameshifting site, where the second slippery codon (UUA) of the slippery sequence in the 0 frame is decoded by a $tRNA^{Leu}$ isoacceptor with an anticodon UAA (Figure 3C) (Korniy et al. 2019a). Under conditions where $tRNA^{Leu(UAA)}$ is depleted, an alternative –1-frame product is formed as a result of hungry frameshifting (Korniy et al. 2019a). Notably, $tRNA^{Leu(UAA)}$ is significantly less abundant in human T lymphocytes and macrophages, the cell types mainly targeted by HIV, than in other cell types (Korniy et al. 2019a). Furthermore, hungry frameshifting has recently been implied in neurodegeneration and cancer. The pathological forms of the *ATXN3* and *HTT* genes, the causative factors of spinocerebellar ataxia type 3 and Huntington’s disease, respectively, contain expanded CAG repeats on the genomic level

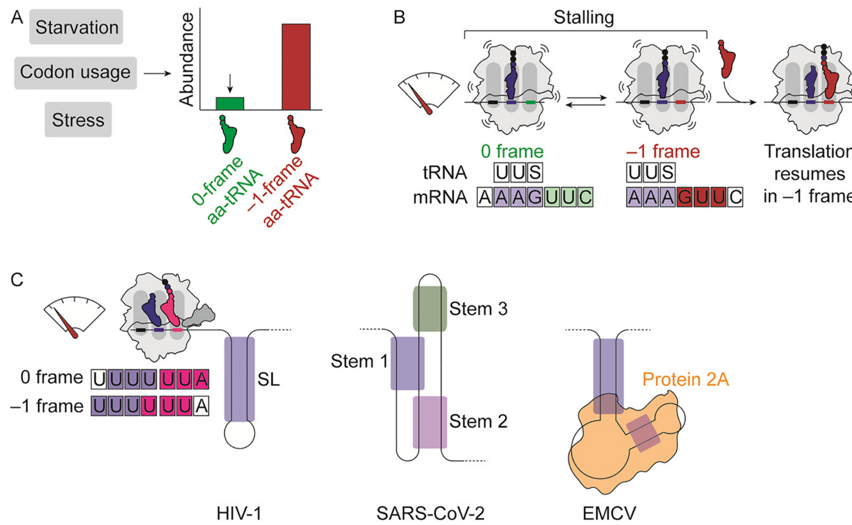

Figure 3: Special cases of ribosome frameshifting. (A) Factors that affect tRNA abundance in the cell. (B) Molecular mechanism of hungry frameshifting. The ribosome is stalled on slippery sequences due to limited availability of 0-frame aa-tRNAs. The peptidyl-tRNA spontaneously shifts the frame and the ribosome resumes translation in −1 frame. (C) *cis* and *trans* stimulatory elements of viral programmed frameshifting sites. Helical regions are shown in violet, green and purple shades. Protein 2A of EMCV is shown in orange.

that are translated into poly-glutamine stretches (Girstmair et al. 2013; McLoughlin et al. 2020; Stochmanski et al. 2012; Tabrizi et al. 2020; Toulouse et al. 2005). However, poly-alanine and poly-serine variants have also been detected in postmortem brain samples from patients (Ayhan et al. 2018; Davies and Rubinsztein 2006; Gaspar et al. 2000; Stochmanski et al. 2012; Toulouse et al. 2005; Wojciechowska et al. 2014). Previous studies suggested that the pathologically long CAG repeats on the mRNA exhaust the cellular pool of $tRNA^{Gln}$ (Girstmair et al. 2013). This leads to ribosome stalling on CAG stretches and hungry frameshifting, thus allowing incorporation of alternative tRNAs, such as $tRNA^{Ala}$ in −1 frame or $tRNA^{Ser}$ in +1 frame (Caliskan et al. 2017; Gallant and Lindsley 1993; Temperley et al. 2010). Poly-Gln, poly-Ala and poly-Ser variants are highly prone to protein aggregation, which can result in neuron death and neurodegeneration (Ayhan et al. 2018; Davies and Rubinsztein 2006; Girstmair et al. 2013). In cancer, it has recently been shown that interferon-γ-based antitumor therapy causes upregulation of tryptophan catabolism enzymes, which leads to intracellular Trp depletion, thereby decreasing the availability of charged Trp-$tRNA^{Trp}$ (Bartok et al. 2021; Champagne et al. 2021). This triggers cancer-specific hungry frameshifting and the production of frameshifted neopeptides, i.e., unique cancer-specific peptides that are targeted with high selectivity by the immune system. The neopeptides are then processed by the immunoproteasome generating neo-antigens presented at the cancer cell surface and recognized by T cells, which in turn mediate cancer cell clearance (Bartok et al. 2021; Champagne et al. 2021).

6 Programmed ribosome frameshifting

Frameshifting can be promoted by *cis* mRNA elements that hinder ribosome progression across the mRNA, which is usually described as programmed ribosome frameshifting (PRF, Figure 3C) (Caliskan et al. 2014; Chung et al. 2010; Giedroc and Cornish 2009; Namy et al. 2006). PRF is prevalent in mobile genetic elements such as viruses, transposons and retroviral insertion remnants. In viruses, −1PRF regulates the synthesis of polyproteins that are essential for virus propagation and particle assembly (Atkins et al. 2021; Kelly et al. 2020; Kendra et al. 2017, 2018; Moomau et al. 2016; Napthine et al. 2019, 2021; Riegger and Caliskan 2022; Wang et al. 2019; Zimmer et al. 2021). A well-studied example is the production of the Gag-Pol polyprotein of HIV (Jacks et al. 1988). The synthesis of the Gag-Pol polypeptide chain depends on a −1PRF event at the 3′ end of the *gag* gene, where a stem loop is formed downstream of the slippery sequence (Figure 3C). After frameshifting, the 0-frame stop codon that would terminate translation of the *gag* ORF is omitted and, instead, translation continues in −1 frame into the *pol* ORF (Jacks et al. 1988). The resulting Gag-Pol polyprotein is then cleaved by viral proteases to generate the Gag and Pol viral proteins. Similar strategy to produce proteins are found in coronaviruses, such as SARS-CoV-2, the causative agent of the Covid-19 pandemic, where a pseudoknot structure (Figure 3C) downstream of a slippery sequence stimulates frameshifting in the border of *ORF1a*

and *ORF1b* (Bhatt et al. 2021; Riegger and Caliskan 2022). Due to the essential role of PRF in virus propagation, there is a growing interest in the design of small molecules that bind the stimulatory elements and antiviral strategies that target PRF (Anokhina and Miller 2021; Kelly et al. 2021; Korniy et al. 2019b; Matsumoto et al. 2018; Ritchie et al. 2014).

The mechanism of –1PRF shows similarities but also differences to spontaneous frameshifting. In both cases, the overall EF-G dwell time on the ribosome is increased, while translocation of the peptidyl-tRNA from the A to the P site and the SSU head back swiveling are delayed (Caliskan et al. 2014; Chen et al. 2014; Choi et al. 2020). The main difference is that, during –1PRF, the dissociation of the deacylated tRNA from the E site is also delayed (Caliskan et al. 2014; Chen et al. 2013a), in contrast to the undisturbed and rapid tRNA release during spontaneous frameshifting (Peng et al. 2019; Poulis et al. 2022). The completion of translocation during –1PRF occurs when the secondary structure unwinds, most likely by proteins uS3, uS4 and uS5 that possess a helicase activity (Desai et al. 2019; Takyar et al. 2005). Notably, unwinding of mRNA secondary structure can by itself shift to the slow ribosome gear, although – in contrast to spontaneous frameshifting – the translocation appears to be stalled at an early stage, prior to CHI formation (Desai et al. 2019). Previous studies identified that the stimulatory mRNA secondary structures can adopt a wide spectrum of conformers (Halma et al. 2019, 2021; Neupane et al. 2021; Ritchie et al. 2017). Such structural plasticity, in combination with the stability of the RNA secondary structures, modulates the timing of ribosome stalling and the efficiency of frameshifting (Bao et al. 2022; Choi et al. 2020; Ritchie et al. 2012). Additionally, EF-G may perform multiple rounds of binding and dissociation before translocation is complete and the extent of this sampling is affected by the stability of the mRNA structure (Chen et al. 2014; Choi et al. 2020). Dissociation of EF-G from ribosome complexes that did not complete translocation alleviates the EF-G-dependent reading frame control and may allow the tRNAs to move into their thermodynamically favored reading frame, thereby promoting frameshifting.

In addition to the *cis* elements encoded in the mRNA, also *trans* factors such as viral or host proteins can modulate viral PRF. In contrast to *cis* elements, which appear to define a constant frameshifting efficiency by stalling the ribosome in the CHI state, *trans* factors can provide a temporal regulation of PRF efficiency. An example of temporal modulation of PRF by viral protein 2A is described in cardioviruses (Hill et al. 2021; Napthine et al. 2017). During infection by the encephalomyocarditis virus (EMCV), protein 2A gradually accumulates reaching higher concentrations at late stage of infection. Protein 2A interacts with the secondary structure element of the frameshifting site and stabilizes the structure (Figure 3C) (Hill et al. 2021). Therefore, frameshifting is increased and protein synthesis is directed towards the production of structural viral proteins. Interferon-induced host antiviral factors also act *in-trans* to abolish viral frameshifting. The short isoform of the zinc-finger antiviral protein (ZAP-S) recognizes the viral stimulatory element in the frameshifting site and destabilizes its structure, causing inhibition of frameshifting (Zimmer et al. 2021). The interferon-induced protein Shiftless binds to ribosomes at the frameshifting site and recruits the termination and mRNA degradation machinery that targets the viral genome (Jager et al. 2022; Napthine et al. 2021; Wang et al. 2019).

PRF has also been described in cellular genes. In bacteria, it is implicated in the fine tuning of protein stoichiometry (Blinkowa and Walker 1990). The most prominent example is the *dnaX* mRNA of *E. coli*, which encodes for the γ and τ subunits of the DNA polymerase holoenzyme (Blinkowa and Walker 1990; Caliskan et al. 2017). Translation in 0 frame produces the τ subunit chain and terminates on a 0-frame stop codon. However, a frameshifting site at the 3′ end of the τ subunit ORF leads to slippage to the –1 frame and translation continues towards the synthesis of the τ subunit. Another interesting example reports the role of PRF in copper tolerance in *E. coli* (Meydan et al. 2017). Translation of the copA mRNA in 0 frame produces a copper transporter, while the shift to the –1 frame synthesizes a copper chaperone that facilitates the trafficking of copper ions to the transporter.

7 Translational bypassing

Another very interesting example of an unconventional EF-G function is translational bypassing, i.e., the skipping of an mRNA sequence by the translating ribosome to produce a protein from two discontinuous ORFs (Rodnina et al. 2020). Gene *60* of bacteriophage T4 is a well-studied example of ribosome bypassing. The bypassing site of the mRNA is highly structured containing a 5′ stem loop, a take-off stem loop, the 50-nt non-coding sequence and a downstream 3′ stem loop (Figure 4A) (Agirrezabala et al. 2017; Klimova et al. 2019). The ribosome translates the first 46 codons of *ORF1* and pauses when the GGA take-off codon is in the P site (Figure 4B). During pausing, the take-off stem loop forms a secondary structure in the A site that mimics a tRNA anticodon stem loop, prevents accommodation of aa-tRNAs and accessory factors and induces a hyper-rotated state of the SSU body (Agirrezabala et al. 2017; Klimova et al. 2019). In this

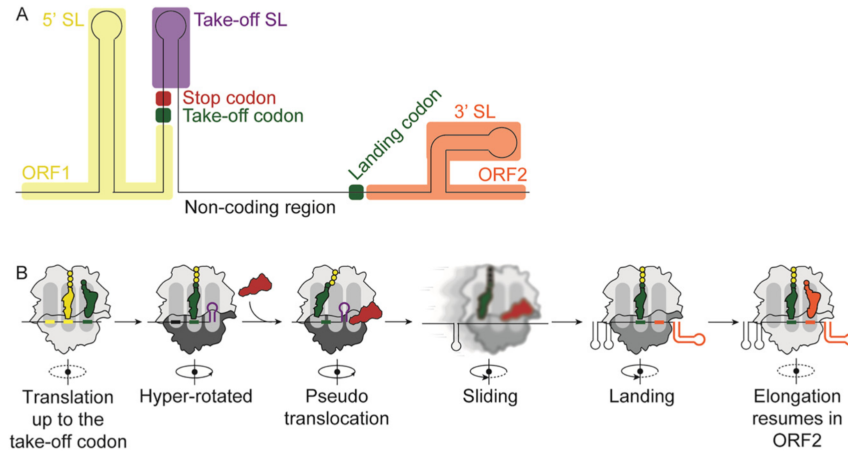

Figure 4: The mechanism of ribosome bypassing. (A) Schematic of the bypassing site on the phage T4 gene *60*. *ORF1* is shown in green, take-off stem loop (SL) in yellow, matching take-off and landing codons in deep green, stop codon in red, *ORF2* in orange. (B) Molecular mechanics of ribosome bypassing. The ribosome stalls on the take-off codon of *ORF1*. EF-G (red) binds to a hyper-rotated ribosome and induces pseudo-translocation and sliding. Finally, the ribosome lands on the landing codon and resumes translation in *ORF2*.

non-canonical conformation, EF-G drives a round of pseudo translocation that dislocates the peptidyl-tRNA from the P-site codon and initiates sliding. During sliding, EF-G hydrolyzes GTP to a greater extent compared to canonical translocation, while refolding of the 5′ and take-off stem loops prevent backward movement. At the same time, retention of the peptidyl-$tRNA^{Gly}$ on the ribosome is supported by extensive interactions between the nascent peptide chain and the peptide exit tunnel, thus preventing tRNA drop-off or termination (Agirrezabala et al. 2017). In the end, the 3′ stem loop guides the landing of the peptidyl-$tRNA^{Gly}$ on an identical GGA codon. The ribosome adopts a canonical conformation and resumes translation in the *ORF2* (Figure 4B). Further examples of translational bypassing are found in the mitochondria of yeast *Magnusiomycetes capitatus* (Lang et al. 2014). This non-canonical function of EF-G may be involved not only in bypassing, but also in ribosome movement along the 3′ UTRs (Guydosh and Green 2014; Miettinen and Bjorklund 2015), although the functional role of this movement remains unclear.

8 Conclusions and perspectives

Recent advances in understanding the action of EF-G revealed how this translational GTPase facilitates movement in a large macromolecular complex. While the key role of GTP hydrolysis and P_i release has been discovered a while ago, recent structures show the exact mechanism by which P_i release alters the interactions of EF-G with the ribosome and results in the stabilization of the CHI state, the key intermediate of tRNA translocation. However, we know surprisingly little about the subsequent steps of translocation, e.g., the timing and order of steps upon tRNA movement from the CHI to the P/P-E/E state, dissociation of the E-site tRNA, the concomitant back swiveling of the SSU head and dissociation of EF-G. Understanding these late steps in translocation is important, because uncoupling of tRNA movements and EF-G dissociation facilitates frameshifting, leading to synthesis of neopeptides linked to different medical conditions. Mutations in EF-G are often associated with antibiotic resistance, however, except for well-studied resistances against the antibiotic fusidic acid, little is known about how mutations confer the resistance phenotypes. Switching between the fast and slow modes of translocation is a new and exciting finding raising many questions about the role of EF-G in the switching, and prevalence of this mechanism in regulating translation. Understanding the multitude of frameshifting mechanisms – spontaneous, hungry, and programmed by *cis* and *trans* elements – allows to predict the sequence of potential neopeptides, which can be validated by the analysis of proteomics data, and provide new insights into mechanisms by which cells increase their proteomic repertoire and respond to environmental cues. Finally, the non-canonical role of EF-G in facilitating ribosome sliding along the mRNA shows how fundamental mechanisms of molecular movement can be repurposed and raises the question of how the propensity for sliding is used in the cell. The role of EF-G in maintaining the reading frame and in recoding events shows how a translational GTPase connects protein synthesis by the ribosome to maintenance of the cellular proteostasis and regulates the cellular response in health and disease.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: The work on EF-G mechanism in translocation and recoding was funded by the Deutsche Forschungsgemeinschaft (DFG, Project number 105286809) in the framework of the Collaborative Research Center 860 (SFB 860). M.V.R. also acknowledges the support of the DFG Leibniz Prize and the Max Planck Society.
Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Adio, S., Senyushkina, T., Peske, F., Fischer, N., Wintermeyer, W., and Rodnina, M.V. (2015). Fluctuations between multiple EF-G-induced chimeric tRNA states during translocation on the ribosome. Nat. Commun. 6: 7442.

Agirrezabala, X., Lei, J., Brunelle, J.L., Ortiz-Meoz, R.F., Green, R., and Frank, J. (2008). Visualization of the hybrid state of tRNA binding promoted by spontaneous ratcheting of the ribosome. Mol. Cell 32: 190–197.

Agirrezabala, X., Samatova, E., Klimova, M., Zamora, M., Gil-Carton, D., Rodnina, M.V., and Valle, M. (2017). Ribosome rearrangements at the onset of translational bypassing. Sci. Adv. 3: e1700147.

Altuntop, M.E., Ly, C.T., and Wang, Y. (2010). Single-molecule study of ribosome hierarchic dynamics at the peptidyl transferase center. Biophys. J. 99: 3002–3009.

Anokhina, V.S. and Miller, B.L. (2021). Targeting ribosomal frameshifting as an antiviral strategy: from HIV-1 to SARS-CoV-2. Acc. Chem. Res. 54: 3349–3361.

Atkins, J.F., O'Connor, K.M., Bhatt, P.R., and Loughran, G. (2021). From recoding to peptides for MHC class I immune display: enriching viral expression, virus vulnerability and virus evasion. Viruses 13: 1–29.

Ayhan, F., Perez, B.A., Shorrock, H.K., Zu, T., Banez-Coronel, M., Reid, T., Furuya, H., Clark, H.B., Troncoso, J.C., Ross, C.A., et al. (2018). SCA8 RAN polySer protein preferentially accumulates in white matter regions and is regulated by eIF3F. EMBO J. 37: 1–15.

Bao, C., Zhu, M., Nykonchuk, I., Wakabayashi, H., Mathews, D.H., and Ermolenko, D.N. (2022). Specific length and structure rather than high thermodynamic stability enable regulatory mRNA stem-loops to pause translation. Nat. Commun. 13: 988.

Baranov, P.V., Gesteland, R.F., and Atkins, J.F. (2002). Release factor 2 frameshifting sites in different bacteria. EMBO Rep. 3: 373–377.

Bartok, O., Pataskar, A., Nagel, R., Laos, M., Goldfarb, E., Hayoun, D., Levy, R., Korner, P.R., Kreuger, I.Z.M., Champagne, J., et al. (2021). Anti-tumour immunity induces aberrant peptide presentation in melanoma. Nature 590: 332–337.

Belardinelli, R., Sharma, H., Caliskan, N., Cunha, C.E., Peske, F., Wintermeyer, W., and Rodnina, M.V. (2016). Choreography of molecular movements during ribosome progression along mRNA. Nat. Struct. Mol. Biol. 23: 342–348.

Belardinelli, R., Sharma, H., Peske, F., and Rodnina, M.V. (2021). Perturbation of ribosomal subunit dynamics by inhibitors of tRNA translocation. RNA 27: 981–990.

Belew, A.T., Hepler, N.L., Jacobs, J.L., and Dinman, J.D. (2008). PRFdb: a database of computationally predicted eukaryotic programmed -1 ribosomal frameshift signals. BMC Genom. 9: 339.

Bhatt, P.R., Scaiola, A., Loughran, G., Leibundgut, M., Kratzel, A., Meurs, R., Dreos, R., O'Connor, K.M., McMillan, A., Bode, J.W., et al. (2021). Structural basis of ribosomal frameshifting during translation of the SARS-CoV-2 RNA genome. Science 372: 1306–1313.

Blanchard, S.C., Kim, H.D., Gonzalez, R.L., Puglisi, J.D., and Chu, S. (2004). tRNA dynamics on the ribosome during translation. Proc. Natl. Acad. Sci. USA. 101: 12893–12898.

Blinkowa, A.L. and Walker, J.R. (1990). Programmed ribosomal frameshifting generates the Escherichia coli DNA polymerase III gamma subunit from within the tau subunit reading frame. Nucleic Acids Res. 18: 1725–1729.

Bock, L.V., Blau, C., Schroder, G.F., Davydov, I.I., Fischer, N., Stark, H., Rodnina, M.V., Vaiana, A.C., and Grubmuller, H. (2013). Energy barriers and driving forces in tRNA translocation through the ribosome. Nat. Struct. Mol. Biol. 20: 1390–1396.

Bock, L.V., Caliskan, N., Korniy, N., Peske, F., Rodnina, M.V., and Grubmuller, H. (2019). Thermodynamic control of -1 programmed ribosomal frameshifting. Nat. Commun. 10: 4598.

Brilot, A.F., Korostelev, A.A., Ermolenko, D.N., and Grigorieff, N. (2013). Structure of the ribosome with elongation factor G trapped in the pretranslocation state. Proc. Natl. Acad. Sci. USA. 110: 20994–20999.

Caliskan, N., Katunin, V.I., Belardinelli, R., Peske, F., and Rodnina, M.V. (2014). Programmed -1 frameshifting by kinetic partitioning during impeded translocation. Cell 157: 1619–1631.

Caliskan, N., Wohlgemuth, I., Korniy, N., Pearson, M., Peske, F., and Rodnina, M.V. (2017). Conditional switch between frameshifting regimes upon translation of dnaX mRNA. Mol. Cell 66: 558–567.e554.

Carbone, C.E., Loveland, A.B., Gamper, H.B., Hou, Y.M., Demo, G., and Korostelev, A.A. (2021). Time-resolved cryo-EM visualizes ribosomal translocation with EF-G and GTP. Nat. Commun. 12: 7236.

Champagne, J., Pataskar, A., Blommaert, N., Nagel, R., Wernaart, D., Ramalho, S., Kenski, J., Bleijerveld, O.B., Zaal, E.A., Berkers, C.R., et al. (2021). Oncogene-dependent sloppiness in mRNA translation. Mol. Cell 81: 4709–4721.e4709.

Chen, C., Cui, X., Beausang, J.F., Zhang, H., Farrell, I., Cooperman, B.S., and Goldman, Y.E. (2016). Elongation factor G initiates translocation through a power stroke. Proc. Natl. Acad. Sci. USA. 113: 7515–7520.

Chen, C., Stevens, B., Kaur, J., Cabral, D., Liu, H., Wang, Y., Zhang, H., Rosenblum, G., Smilansky, Z., Goldman, Y.E., et al. (2011a). Single-molecule fluorescence measurements of ribosomal translocation dynamics. Mol. Cell 42: 367–377.

Chen, C., Stevens, B., Kaur, J., Smilansky, Z., Cooperman, B.S., and Goldman, Y.E. (2011b). Allosteric vs. spontaneous exit-site (E-site) tRNA dissociation early in protein synthesis. Proc. Natl. Acad. Sci. USA. 108: 16980–16985.

Chen, C., Zhang, H., Broitman, S.L., Reiche, M., Farrell, I., Cooperman, B.S., and Goldman, Y.E. (2013a). Dynamics of translation by single ribosomes through mRNA secondary structures. Nat. Struct. Mol. Biol. 20: 582–588.

Chen, J., Petrov, A., Johansson, M., Tsai, A., O'Leary, S.E., and Puglisi, J.D. (2014). Dynamic pathways of -1 translational frameshifting. Nature 512: 328–332.

Chen, J., Petrov, A., Tsai, A., O'Leary, S.E., and Puglisi, J.D. (2013b). Coordinated conformational and compositional dynamics drive ribosome translocation. Nat. Struct. Mol. Biol. 20: 718–727.

Choi, J., O'Loughlin, S., Atkins, J.F., and Puglisi, J.D. (2020). The energy landscape of -1 ribosomal frameshifting. Sci. Adv. 6: eaax6969.

Choi, J. and Puglisi, J.D. (2017). Three tRNAs on the ribosome slow translation elongation. Proc. Natl. Acad. Sci. USA. 114: 13691–13696.

Chung, B.Y., Firth, A.E., and Atkins, J.F. (2010). Frameshifting in alphaviruses: a diversity of 3′ stimulatory structures. J. Mol. Biol. 397: 448–456.

Cornish, P.V., Ermolenko, D.N., Noller, H.F., and Ha, T. (2008). Spontaneous intersubunit rotation in single ribosomes. Mol. Cell 30: 578–588.

Cornish, P.V., Ermolenko, D.N., Staple, D.W., Hoang, L., Hickerson, R.P., Noller, H.F., and Ha, T. (2009). Following movement of the L1 stalk between three functional states in single ribosomes. Proc. Natl. Acad. Sci. USA. 106: 2571–2576.

Craigen, W.J. and Caskey, C.T. (1986). Expression of peptide chain release factor 2 requires high-efficiency frameshift. Nature 322: 273–275.

Cunha, C.E., Belardinelli, R., Peske, F., Holtkamp, W., Wintermeyer, W., and Rodnina, M.V. (2013). Dual use of GTP hydrolysis by elongation factor G on the ribosome. Translation (Austin) 1: e24315.

Czworkowski, J., Wang, J., Steitz, T.A., and Moore, P.B. (1994). The crystal structure of elongation factor G complexed with GDP, at 2.7 A resolution. EMBO J. 13: 3661–3668.

Davies, J.E. and Rubinsztein, D.C. (2006). Polyalanine and polyserine frameshift products in Huntington's disease. J. Med. Genet. 43: 893–896.

Davydov, I.I., Wohlgemuth, I., Artamonova, I.I., Urlaub, H., Tonevitsky, A.G., and Rodnina, M.V. (2013). Evolution of the protein stoichiometry in the L12 stalk of bacterial and organellar ribosomes. Nat. Commun. 4: 1387.

Desai, V.P., Frank, F., Lee, A., Righini, M., Lancaster, L., Noller, H.F., Tinoco, I., and Bustamante, C. (2019). Co-Temporal force and fluorescence measurements reveal a ribosomal gear shift mechanism of translation regulation by structured mRNAs. Mol. Cell 75: 1007–1019.e1005.

Diaconu, M., Kothe, U., Schlunzen, F., Fischer, N., Harms, J.M., Tonevitsky, A.G., Stark, H., Rodnina, M.V., and Wahl, M.C. (2005). Structural basis for the function of the ribosomal L7/12 stalk in factor binding and GTPase activation. Cell 121: 991–1004.

Donly, B.C. and Tate, W.P. (1991). Frameshifting by eukaryotic ribosomes during expression of Escherichia coli release factor 2. Proc. Biol. Sci. 244: 207–210.

Dunkle, J.A., Wang, L., Feldman, M.B., Pulk, A., Chen, V.B., Kapral, G.J., Noeske, J., Richardson, J.S., Blanchard, S.C., and Cate, J.H. (2011). Structures of the bacterial ribosome in classical and hybrid states of tRNA binding. Science 332: 981–984.

Ermolenko, D.N., Majumdar, Z.K., Hickerson, R.P., Spiegel, P.C., Clegg, R.M., and Noller, H.F. (2007). Observation of intersubunit movement of the ribosome in solution using FRET. J. Mol. Biol. 370: 530–540.

Ermolenko, D.N. and Noller, H.F. (2011). mRNA translocation occurs during the second step of ribosomal intersubunit rotation. Nat. Struct. Mol. Biol. 18: 457–462.

Evarsson, A., Brazhnikov, E., Garber, M., Zheltonosova, J., Chirgadze, Y., al-Karadaghi, S., Svensson, L.A., and Liljas, A. (1994). Three-dimensional structure of the ribosomal translocase: elongation factor G from *Thermus thermophilus*. EMBO J. 13: 3669–3677.

Fei, J., Bronson, J.E., Hofman, J.M., Srinivas, R.L., Wiggins, C.H., and Gonzalez, R.L. (2009). Allosteric collaboration between elongation factor G and the ribosomal L1 stalk directs tRNA movements during translation. Proc. Natl. Acad. Sci. USA. 106: 15702–15707.

Fei, J., Kosuri, P., MacDougall, D.D., and Gonzalez, R.L. (2008). Coupling of ribosomal L1 stalk and tRNA dynamics during translation elongation. Mol. Cell 30: 348–359.

Fischer, N., Konevega, A.L., Wintermeyer, W., Rodnina, M.V., and Stark, H. (2010). Ribosome dynamics and tRNA movement by time-resolved electron cryomicroscopy. Nature 466: 329–333.

Frank, J. and Agrawal, R.K. (2000). A ratchet-like inter-subunit reorganization of the ribosome during translocation. Nature 406: 318–322.

Gallant, J. and Lindsley, D. (1993). Ribosome frameshifting at hungry codons: sequence rules, directional specificity and possible relationship to mobile element behaviour. Biochem. Soc. Trans. 21: 817–821.

Gao, Y.G., Selmer, M., Dunham, C.M., Weixlbaumer, A., Kelley, A.C., and Ramakrishnan, V. (2009). The structure of the ribosome with elongation factor G trapped in the posttranslocational state. Science 326: 694–699.

Gaspar, C., Jannatipour, M., Dion, P., Laganiere, J., Sequeiros, J., Brais, B., and Rouleau, G.A. (2000). CAG tract of MJD-1 may be prone to frameshifts causing polyalanine accumulation. Hum. Mol. Genet. 9: 1957–1966.

Gavrilova, L.P. and Spirin, A.S. (1971). Stimulation of "non-enzymic" translocation in ribosomes by p-chloromercuribenzoate. FEBS Lett. 17: 324–326.

Giedroc, D.P. and Cornish, P.V. (2009). Frameshifting RNA pseudoknots: structure and mechanism. Virus Res. 139: 193–208.

Girstmair, H., Saffert, P., Rode, S., Czech, A., Holland, G., Bannert, N., and Ignatova, Z. (2013). Depletion of cognate charged transfer RNA causes translational frameshifting within the expanded CAG stretch in huntingtin. Cell Rep. 3: 148–159.

Guo, Z. and Noller, H.F. (2012). Rotation of the head of the 30S ribosomal subunit during mRNA translocation. Proc. Natl. Acad. Sci. USA. 109: 20391–20394.

Gurvich, O.L., Baranov, P.V., Zhou, J., Hammer, A.W., Gesteland, R.F., and Atkins, J.F. (2003). Sequences that direct significant levels of frameshifting are frequent in coding regions of *Escherichia coli*. EMBO J. 22: 5941–5950.

Guydosh, N.R. and Green, R. (2014). Dom34 rescues ribosomes in 3′ untranslated regions. Cell 156: 950–962.

Halma, M.T.J., Ritchie, D.B., Cappellano, T.R., Neupane, K., and Woodside, M.T. (2019). Complex dynamics under tension in a high-efficiency frameshift stimulatory structure. Proc. Natl. Acad. Sci. USA. 116: 19500–19505.

Halma, M.T.J., Ritchie, D.B., and Woodside, M.T. (2021). Conformational Shannon entropy of mRNA structures from force Spectroscopy measurements predicts the efficiency of -1 programmed ribosomal frameshift stimulation. Phys. Rev. Lett. 126: 038102.

Han, W., Peng, B.Z., Wang, C., Townsend, G.E., Barry, N.A., Peske, F., Goodman, A.L., Liu, J., Rodnina, M.V., and Groisman, E.A. (2023). Gut colonization by Bacteroides requires translation by an EF-G paralog lacking GTPase activity. EMBO J. 42: e112372.

Hill, C.H., Pekarek, L., Napthine, S., Kibe, A., Firth, A.E., Graham, S.C., Caliskan, N., and Brierley, I. (2021). Structural and molecular basis for Cardiovirus 2A protein as a viral gene expression switch. Nat. Commun. 12: 7166.

Hoffer, E.D., Hong, S., Sunita, S., Maehigashi, T., Gonzalez, R.L.J., Whitford, P.C., and Dunham, C.M. (2020). Structural insights into mRNA reading frame regulation by tRNA modification and slippery codon-anticodon pairing. eLife 9: 1–20.

Holtkamp, W., Cunha, C.E., Peske, F., Konevega, A.L., Wintermeyer, W., and Rodnina, M.V. (2014). GTP hydrolysis by EF-G synchronizes tRNA movement on small and large ribosomal subunits. EMBO J. 33: 1073–1085.

Jacks, T., Power, M.D., Masiarz, F.R., Luciw, P.A., Barr, P.J., and Varmus, H.E. (1988). Characterization of ribosomal frameshifting in HIV-1 gag-pol expression. Nature 331: 280–283.

Jager, N., Ayyub, S.A., Korniy, N., Peske, F., Hoffmann, M., Rodnina, M.V., and Pohlmann, S. (2022). Mutagenic analysis of the HIV restriction factor Shiftless. Viruses 14: 1–19.

Julian, P., Konevega, A.L., Scheres, S.H., Lazaro, M., Gil, D., Wintermeyer, W., Rodnina, M.V., and Valle, M. (2008). Structure of ratcheted ribosomes with tRNAs in hybrid states. Proc. Natl. Acad. Sci. USA 105: 16924–16927.

Katunin, V.I., Savelsbergh, A., Rodnina, M.V., and Wintermeyer, W. (2002). Coupling of GTP hydrolysis by elongation factor G to translocation and factor recycling on the ribosome. Biochemistry 41: 12806–12812.

Kelly, J.A., Olson, A.N., Neupane, K., Munshi, S., San Emeterio, J., Pollack, L., Woodside, M.T., and Dinman, J.D. (2020). Structural and functional conservation of the programmed -1 ribosomal frameshift signal of SARS coronavirus 2 (SARS-CoV-2). J. Biol. Chem. 295: 10741–10748.

Kelly, J.A., Woodside, M.T., and Dinman, J.D. (2021). Programmed -1 ribosomal frameshifting in coronaviruses: a therapeutic target. Virology 554: 75–82.

Kendra, J.A., Advani, V.M., Chen, B., Briggs, J.W., Zhu, J., Bress, H.J., Pathy, S.M., and Dinman, J.D. (2018). Functional and structural characterization of the Chikungunya virus translational recoding signals. J. Biol. Chem. 293: 17536–17545.

Kendra, J.A., de la Fuente, C., Brahms, A., Woodson, C., Bell, T.M., Chen, B., Khan, Y.A., Jacobs, J.L., Kehn-Hall, K., and Dinman, J.D. (2017). Ablation of programmed -1 ribosomal frameshifting in Venezuelan equine encephalitis virus results in attenuated neuropathogenicity. J. Virol. 91: 1–13.

Kim, H.D., Puglisi, J.D., and Chu, S. (2007). Fluctuations of transfer RNAs between classical and hybrid states. Biophys. J. 93: 3575–3582.

Klimova, M., Senyushkina, T., Samatova, E., Peng, B.Z., Pearson, M., Peske, F., and Rodnina, M.V. (2019). EF-G-induced ribosome sliding along the noncoding mRNA. Sci. Adv. 5: eaaw9049.

Konevega, A.L., Fischer, N., Semenkov, Y.P., Stark, H., Wintermeyer, W., and Rodnina, M.V. (2007). Spontaneous reverse movement of mRNA-bound tRNA through the ribosome. Nat. Struct. Mol. Biol. 14: 318–324.

Korniy, N., Goyal, A., Hoffmann, M., Samatova, E., Peske, F., Pohlmann, S., and Rodnina, M.V. (2019a). Modulation of HIV-1 Gag/Gag-Pol frameshifting by tRNA abundance. Nucleic Acids Res. 47: 5210–5222.

Korniy, N., Samatova, E., Anokhina, M.M., Peske, F., and Rodnina, M.V. (2019b). Mechanisms and biomedical implications of -1 programmed ribosome frameshifting on viral and bacterial mRNAs. FEBS Lett. 593: 1468–1482.

Lang, B.F., Jakubkova, M., Hegedusova, E., Daoud, R., Forget, L., Brejova, B., Vinar, T., Kosa, P., Fricova, D., Nebohacova, M., et al. (2014). Massive programmed translational jumping in mitochondria. Proc. Natl. Acad. Sci. USA. 111: 5926–5931.

Lill, R., Robertson, J.M., and Wintermeyer, W. (1986). Affinities of tRNA binding sites of ribosomes from *Escherichia coli*. Biochemistry 25: 3245–3255.

Lin, J., Gagnon, M.G., Bulkley, D., and Steitz, T.A. (2015). Conformational changes of elongation factor G on the ribosome during tRNA translocation. Cell 160: 219–227.

Maracci, C. and Rodnina, M.V. (2016). Review: translational GTPases. Biopolymers 105: 463–475.

Matsumoto, S., Caliskan, N., Rodnina, M.V., Murata, A., and Nakatani, K. (2018). Small synthetic molecule-stabilized RNA pseudoknot as an activator for -1 ribosomal frameshifting. Nucleic Acids Res. 46: 8079–8089.

McLoughlin, H.S., Moore, L.R., and Paulson, H.L. (2020). Pathogenesis of SCA3 and implications for other polyglutamine diseases. Neurobiol. Dis. 134: 104635.

Meydan, S., Klepacki, D., Karthikeyan, S., Margus, T., Thomas, P., Jones, J.E., Khan, Y., Briggs, J., Dinman, J.D., Vazquez-Laslop, N., et al. (2017). Programmed ribosomal frameshifting generates a copper transporter and a copper chaperone from the same gene. Mol. Cell 65: 207–219.

Miettinen, T.P. and Bjorklund, M. (2015). Modified ribosome profiling reveals high abundance of ribosome protected mRNA fragments derived from 3′ untranslated regions. Nucleic Acids Res. 43: 1019–1034.

Moazed, D. and Noller, H.F. (1989). Intermediate states in the movement of transfer RNA in the ribosome. Nature 342: 142–148.

Mohan, S., Donohue, J.P., and Noller, H.F. (2014). Molecular mechanics of 30S subunit head rotation. Proc. Natl. Acad. Sci. USA. 111: 13325–13330.

Mohan, S. and Noller, H.F. (2017). Recurring RNA structural motifs underlie the mechanics of L1 stalk movement. Nat. Commun. 8: 14285.

Moomau, C., Musalgaonkar, S., Khan, Y.A., Jones, J.E., and Dinman, J.D. (2016). Structural and functional characterization of programmed ribosomal frameshift signals in west nile virus Strains reveals high structural plasticity among cis-acting RNA elements. J. Biol. Chem. 291: 15788–15795.

Munro, J.B., Altman, R.B., O'Connor, N., and Blanchard, S.C. (2007). Identification of two distinct hybrid state intermediates on the ribosome. Mol. Cell 25: 505–517.

Munro, J.B., Sanbonmatsu, K.Y., Spahn, C.M., and Blanchard, S.C. (2009). Navigating the ribosome's metastable energy landscape. Trends Biochem. Sci. 34: 390–400.

Munro, J.B., Wasserman, M.R., Altman, R.B., Wang, L., and Blanchard, S.C. (2010). Correlated conformational events in EF-G and the ribosome regulate translocation. Nat. Struct. Mol. Biol. 17: 1470–1477.

Namy, O., Moran, S.J., Stuart, D.I., Gilbert, R.J., and Brierley, I. (2006). A mechanical explanation of RNA pseudoknot function in programmed ribosomal frameshifting. Nature 441: 244–247.

Napthine, S., Bell, S., Hill, C.H., Brierley, I., and Firth, A.E. (2019). Characterization of the stimulators of protein-directed ribosomal frameshifting in Theiler's murine encephalomyelitis virus. Nucleic Acids Res. 47: 8207–8223.

Napthine, S., Hill, C.H., Nugent, H.C.M., and Brierley, I. (2021). Modulation of viral programmed ribosomal frameshifting and stop codon readthrough by the host restriction factor Shiftless. Viruses 13: 1–14.

Napthine, S., Ling, R., Finch, L.K., Jones, J.D., Bell, S., Brierley, I., and Firth, A.E. (2017). Protein-directed ribosomal frameshifting temporally regulates gene expression. Nat. Commun. 8: 15582.

Neupane, K., Zhao, M., Lyons, A., Munshi, S., Ileperuma, S.M., Ritchie, D.B., Hoffer, N.Q., Narayan, A., and Woodside, M.T. (2021). Structural dynamics of single SARS-CoV-2 pseudoknot molecules reveal topologically distinct conformers. Nat. Commun. 12: 4749.

Pan, D., Kirillov, S.V., and Cooperman, B.S. (2007). Kinetically competent intermediates in the translocation step of protein synthesis. Mol. Cell 25: 519–529.

Peng, B.Z., Bock, L.V., Belardinelli, R., Peske, F., Grubmuller, H., and Rodnina, M.V. (2019). Active role of elongation factor G in maintaining the mRNA reading frame during translation. Sci. Adv. 5: eaax8030.

Petropoulos, A.D. and Green, R. (2012). Further in vitro exploration fails to support the allosteric three-site model. J. Biol. Chem. 287: 11642–11648.

Petrychenko, V., Peng, B.Z., de AP, Schwarzer, A.C., Peske, F., Rodnina, M.V., and Fischer, N. (2021). Structural mechanism of GTPase-powered ribosome-tRNA movement. Nat. Commun. 12: 5933.

Poulis, P., Patel, A., Rodnina, M.V., and Adio, S. (2022). Altered tRNA dynamics during translocation on slippery mRNA as determinant of spontaneous ribosome frameshifting. Nat. Commun. 13: 4231.

Ramrath, D.J., Lancaster, L., Sprink, T., Mielke, T., Loerke, J., Noller, H.F., and Spahn, C.M. (2013). Visualization of two transfer RNAs trapped in transit during elongation factor G-mediated translocation. Proc. Natl. Acad. Sci. USA. 110: 20964–20969.

Ratje, A.H., Loerke, J., Mikolajka, A., Brunner, M., Hildebrand, P.W., Starosta, A.L., Donhofer, A., Connell, S.R., Fucini, P., Mielke, T., et al. (2010). Head swivel on the ribosome facilitates translocation by means of intra-subunit tRNA hybrid sites. Nature 468: 713–716.

Rexroad, G., Donohue, J.P., Lancaster, L., and Noller, H.F. (2022). The role of GTP hydrolysis by EF-G in ribosomal translocation. Proc. Natl. Acad. Sci. USA. 119: e2212502119.

Riegger, R.J. and Caliskan, N. (2022). Thinking outside the frame: impacting genomes capacity by programmed ribosomal frameshifting. Front. Mol. Biosci. 9: 842261.

Ritchie, D.B., Cappellano, T.R., Tittle, C., Rezajooei, N., Rouleau, L., Sikkema, W.K.A., and Woodside, M.T. (2017). Conformational dynamics of the frameshift stimulatory structure in HIV-1. RNA 23: 1376–1384.

Ritchie, D.B., Foster, D.A., and Woodside, M.T. (2012). Programmed -1 frameshifting efficiency correlates with RNA pseudoknot conformational plasticity, not resistance to mechanical unfolding. Proc. Natl. Acad. Sci. USA. 109: 16167–16172.

Ritchie, D.B., Soong, J., Sikkema, W.K., and Woodside, M.T. (2014). Anti-frameshifting ligand reduces the conformational plasticity of the SARS virus pseudoknot. J. Am. Chem. Soc. 136: 2196–2199.

Rodnina, M.V., Korniy, N., Klimova, M., Karki, P., Peng, B.Z., Senyushkina, T., Belardinelli, R., Maracci, C., Wohlgemuth, I., Samatova, E., et al. (2020). Translational recoding: canonical translation mechanisms reinterpreted. Nucleic Acids Res. 48: 1056–1067.

Rodnina, M.V., Peske, F., Peng, B.Z., Belardinelli, R., and Wintermeyer, W. (2019). Converting GTP hydrolysis into motion: versatile translational elongation factor G. Biol. Chem. 401: 131–142.

Rodnina, M.V., Savelsbergh, A., Katunin, V.I., and Wintermeyer, W. (1997). Hydrolysis of GTP by elongation factor G drives tRNA movement on the ribosome. Nature 385: 37–41.

Rundlet, E.J., Holm, M., Schacherl, M., Natchiar, S.K., Altman, R.B., Spahn, C.M.T., Myasnikov, A.G., and Blanchard, S.C. (2021). Structural basis of early translocation events on the ribosome. Nature 595: 741–745.

Salsi, E., Farah, E., Dann, J., and Ermolenko, D.N. (2014). Following movement of domain IV of elongation factor G during ribosomal translocation. Proc. Natl. Acad. Sci. USA. 111: 15060–15065.

Salsi, E., Farah, E., Netter, Z., Dann, J., and Ermolenko, D.N. (2015). Movement of elongation factor G between compact and extended conformations. J. Mol. Biol. 427: 454–467.

Savelsbergh, A., Matassova, N.B., Rodnina, M.V., and Wintermeyer, W. (2000). Role of domains 4 and 5 in elongation factor G functions on the ribosome. J. Mol. Biol. 300: 951–961.

Schuwirth, B.S., Borovinskaya, M.A., Hau, C.W., Zhang, W., Vila-Sanjurjo, A., Holton, J.M., and Cate, J.H. (2005). Structures of the bacterial ribosome at 3.5 Å resolution. Science 310: 827–834.

Semenkov, Y.P., Rodnina, M.V., and Wintermeyer, W. (1996). The “allosteric three-site model” of elongation cannot be confirmed in a well-defined ribosome system from Escherichia coli. Proc. Natl. Acad. Sci. USA. 93: 12183–12188.

Sharma, H., Adio, S., Senyushkina, T., Belardinelli, R., Peske, F., and Rodnina, M.V. (2016). Kinetics of spontaneous and EF-G-accelerated rotation of ribosomal subunits. Cell Rep. 16: 2187–2196.

Shoji, S., Walker, S.E., and Fredrick, K. (2006). Reverse translocation of tRNA in the ribosome. Mol. Cell 24: 931–942.

Stark, H., Rodnina, M.V., Wieden, H.J., van Heel, M., and Wintermeyer, W. (2000). Large-scale movement of elongation factor G and extensive conformational change of the ribosome during translocation. Cell 100: 301–309.

Stochmanski, S.J., Therrien, M., Laganiere, J., Rochefort, D., Laurent, S., Karemera, L., Gaudet, R., Vyboh, K., Van Meyel, D.J., Di Cristo, G., et al. (2012). Expanded ATXN3 frameshifting events are toxic in Drosophila and mammalian neuron models. Hum. Mol. Genet. 21: 2211–2218.

Tabrizi, S.J., Flower, M.D., Ross, C.A., and Wild, E.J. (2020). Huntington disease: new insights into molecular pathogenesis and therapeutic opportunities. Nat. Rev. Neurol. 16: 529–546.

Takyar, S., Hickerson, R.P., and Noller, H.F. (2005). mRNA helicase activity of the ribosome. Cell 120: 49–58.

Temperley, R., Richter, R., Dennerlein, S., Lightowlers, R.N., and Chrzanowska-Lightowlers, Z.M. (2010). Hungry codons promote frameshifting in human mitochondrial ribosomes. Science 327: 301.

Toulouse, A., Au-Yeung, F., Gaspar, C., Roussel, J., Dion, P., and Rouleau, G.A. (2005). Ribosomal frameshifting on MJD-1 transcripts with long CAG tracts. Hum. Mol. Genet. 14: 2649–2660.

Uemura, S., Aitken, C.E., Korlach, J., Flusberg, B.A., Turner, S.W., and Puglisi, J.D. (2010). Real-time tRNA transit on single translating ribosomes at codon resolution. Nature 464: 1012–1017.

Wang, X., Xuan, Y., Han, Y., Ding, X., Ye, K., Yang, F., Gao, P., Goff, S.P., and Gao, G. (2019). Regulation of HIV-1 gag-pol expression by Shiftless, an inhibitor of programmed -1 ribosomal frameshifting. Cell 176: 625–635.e614.

Wasserman, M.R., Alejo, J.L., Altman, R.B., and Blanchard, S.C. (2016). Multiperspective smFRET reveals rate-determining late intermediates of ribosomal translocation. Nat. Struct. Mol. Biol. 23: 333–341.

Wojciechowska, M., Olejniczak, M., Galka-Marciniak, P., Jazurek, M., and Krzyzosiak, W.J. (2014). RAN translation and frameshifting as translational challenges at simple repeats of human neurodegenerative disorders. Nucleic Acids Res. 42: 11849–11864.

Zhang, W., Dunkle, J.A., and Cate, J.H. (2009). Structures of the ribosome in intermediate states of ratcheting. Science 325: 1014–1017.

Zhou, J., Lancaster, L., Donohue, J.P., and Noller, H.F. (2014). How the ribosome hands the A-site tRNA to the P site during EF-G-catalyzed translocation. Science 345: 1188–1191.

Zhou, J., Lancaster, L., Donohue, J.P., and Noller, H.F. (2019). Spontaneous ribosomal translocation of mRNA and tRNAs into a chimeric hybrid state. Proc. Natl. Acad. Sci. USA. 116: 7813–7818.

Zimmer, M.M., Kibe, A., Rand, U., Pekarek, L., Ye, L., Buck, S., Smyth, R.P., Cicin-Sain, L., and Caliskan, N. (2021). The short isoform of the host antiviral protein ZAP acts as an inhibitor of SARS-CoV-2 programmed ribosomal frameshifting. Nat. Commun. 12: 7193.