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Translation Elongation and Recoding in Eukaryotes

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In this review, we highlight the current understanding of translation elongation and recoding in eukaryotes. In addition to providing an overview of the process, recent advances in our understanding of the role of the factor eIF5A in both translation elongation and termination are discussed. We also highlight mechanisms of translation recoding with a focus on ribosomal frameshifting during elongation. We see that the balance between the basic steps in elongation and the less common recoding events is determined by the kinetics of the different processes as well as by specific sequence determinants.

OVERVIEW OF TRANSLATION ELONGATION

The mechanism of translation elongation is conserved in all kingdoms of life. Whereas most of the mechanistic details of the process have been elucidated in studies of bacterial translation (see Rodnina 2018), the key steps are shared between eukaryotes and bacteria. In eukaryotes, translation initiation culminates with formation of an 80S initiation complex in which Met-tRNA$_i^{Met}$ is bound in the P (peptidyl) site of the ribosome. The anticodon of the Met-tRNA$_i^{Met}$ is base-paired with the start codon of the messenger RNA (mRNA), and the second codon of the open reading frame (ORF) is in the A (aminoacyl) site of the ribosome. Elongation commences with delivery of the cognate elongating aminoacyl-tRNA (transfer RNA) to the A site of the ribosome (Fig. 1). The eukaryotic translation elongation factor eEF1A, like its bacterial ortholog EF-Tu, is activated upon binding guanosine triphosphate (GTP) and forms a ternary complex upon binding an aminoacyl-tRNA. The eEF1A•GTP•aminoacyl-tRNA complex binds in the A site. Base-pairing interactions between the anticodon of the aminoacyl-tRNA and the A-site codon trigger GTP hydrolysis by eEF1A. The eEF1A•GDP complex is released and the aminoacyl-tRNA is accommodated into the A site.

High-resolution cryo-electron microscopy (EM) structures of decoding complexes have provided insights into how the bacterial and eukaryotic ribosomes sense proper decoding (Jobe et al. 2018). The 18S ribosomal RNA (rRNA)

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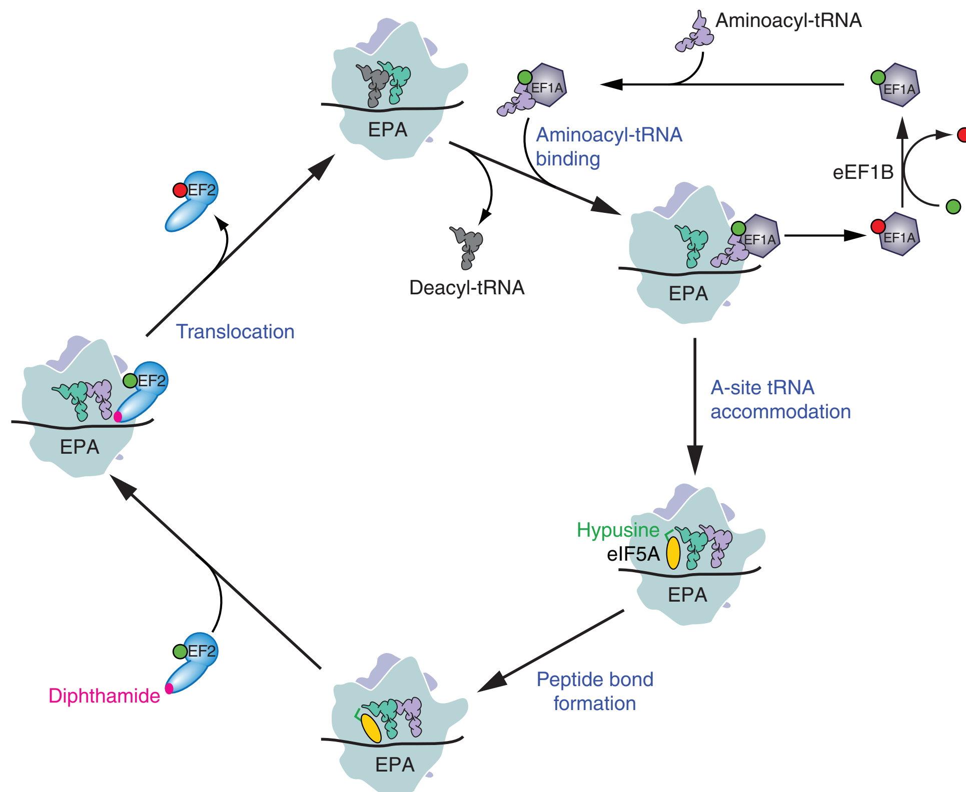

Figure 1. Model of the eukaryotic translation elongation pathway. At the *top*, an eEF1A•GTP•aminoacyl-tRNA (transfer RNA) ternary complex binds to the A (aminoacyl) site of an 80S ribosome with the anticodon loop of the tRNA in contact with the messenger RNA (mRNA). Following GTP hydrolysis and release of an eEF1A•GDP binary complex, the aminoacyl-tRNA is accommodated into the A site, and the eEF1A•GDP is recycled to eEF1A•GTP by the exchange factor eEF1B. During catalysis of peptide bond formation, the A- and P (peptidyl)-site tRNAs shift into hybrid states with the acceptor ends of the tRNAs moving to the P and E sites, respectively. Substrate positioning for peptide bond formation is aided by binding of the factor eIF5A and its hypusine modification (green) in the E site. Following peptide bond formation, the factor eEF2•GTP with its diphthamide modification (magenta) binds in the A site and promotes translocation of the tRNAs into the canonical P and E sites. Following release of the deacylated tRNA from the E site, the next cycle of elongation commences with binding of the appropriate eEF1A•GTP•aminoacyl-tRNA to the A site. Throughout, GTP is depicted as a green ball and GDP as a red ball; also, the large ribosomal subunit (light blue) is displayed transparently to enable visualization of the tRNAs, factors, and mRNA bound to the decoding center at the interface between the large and small subunits and of tRNAs, interacting with the peptidyl transferase center in the large subunit. Note, however, that the positions of the mRNA, tRNAs, and factors are drawn for clarity and are not meant to specify their exact places on the ribosome.

helix h44 residues A1824 and A1825 in mammalian (rabbit) ribosomes (A1755 and A1756 in *Saccharomyces cerevisiae* and A1492 and A1493 in *Escherichia coli*, respectively) as well as the residue G626 in rabbit ribosomes (G577 in *S. cerevisiae* and G530 in *E. coli*) interact with the minor groove of the codon–anticodon helix and stabilize A-site tRNA binding by hydrogen bonding (Ogle et al. 2001; Shao et al. 2016; Loveland et al. 2017). Interestingly, when flipped out of helix 44 (h44), the residues A1824 and A1825 (or A1492 and A1493 in bacteria) interact with the first two codon pairs in the codon–anticodon duplex, enabling the +3 position to participate in wobble interactions related to the degeneracy of the genetic code (Loveland et al. 2017).

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As the h44 residues also flip out to interact with mispaired codon–anticodon helices formed with near-cognate tRNAs in the A site (Demeshkina et al. 2012), it has been proposed that the interaction of G626 (G530 in bacteria) may perform a more crucial function as the latching nucleotide that fixes the codon–anticodon helix in the decoding center of the ribosome (Loveland et al. 2017). In addition to providing insights into decoding, the recent structures of eukaryotic ribosomal complexes have provided insights into GTPase activation of eEF1A as well as of eRF3 in termination complexes and of Hbs1 in ribosome rescue complexes (Shao et al. 2016). In these structures, interactions between the sarcin-ricin loop of the large ribosomal subunit and the Switch 2 loop of the GTPase domains helps position the catalytic His residue to promote GTP hydrolysis (Shao et al. 2016). Moreover, the amino terminus of the eukaryote-specific ribosomal protein eS30 becomes ordered upon cognate codon–anticodon interaction in the A site, and a conserved His residue inserts into the decoding center to form potentially stabilizing contacts (Shao et al. 2016). These novel interactions may contribute to the reported enhanced accuracy of eukaryotic versus bacterial elongation (Kramer et al. 2010). Finally, the structural studies of the eukaryotic elongation complex revealed a conserved binding site for inhibitors of eEF1A and EF-Tu. The translational inhibitor didemnin B, which specifically impairs eukaryotic elongation, was found to bind in a cleft between the G domain and domain III of eEF1A in a position that overlaps with the binding site of the structurally unrelated antibiotic kirromycin on EF-Tu (Shao et al. 2016). Like kirromycin, didemnin B and the functionally related eEF1A inhibitor ternatin (Carelli et al. 2015) are thought to prevent the structural rotations in eEF1A required for release of the factor from the ribosome following GTP hydrolysis.

The heart of protein synthesis is peptide bond formation, and the conservation of the ribosome active site structure suggests that the mechanism of peptide bond formation is universally conserved. Following release of eEF1A and accommodation of the aminoacyl-tRNA into the A site, peptide bond formation with the peptidyl-tRNA in the P site occurs rapidly. Composed of conserved rRNA elements in the large ribosomal subunit, the peptidyl transferase center (PTC) of the ribosome is well conserved between bacteria and eukaryotes (Ben-Shem et al. 2010, 2011; Klinge et al. 2011), and principally functions by positioning the peptidyl- and aminoacyl-tRNAs for catalysis. The factor eIF5A, the ortholog of bacterial elongation factor P (EF-P), binds in the E site, interacts with the acceptor arm of the peptidyl-tRNA, and is thought, like EF-P (Doerfel et al. 2015), to promote peptide bond formation by inducing a favorable positioning of the substrates (Gutierrez et al. 2013; Melnikov et al. 2016a,b; Schmidt et al. 2016; Shin et al. 2017).

During peptide bond formation, the nascent peptide is transferred from the peptidyl-tRNA in the P site to the amino group of the A-site aminoacyl-tRNA (aa-tRNA) to form a new extended peptidyl-tRNA. Peptide bond formation is accompanied by repositioning of the tRNAs into hybrid states (Moazed and Noller 1989) and by subunit rotation. Cryo-EM imaging of eukaryotic elongation complexes has revealed three states of tRNA binding and subunit rotation upon peptide bond formation: in unrotated complexes, the newly formed peptidyl-tRNA is in the A site and deacylated tRNA is in the P site; in rotated-1 complexes, the deacylated tRNA adopts a hybrid P/E state with the anticodon paired with the mRNA in the P site and the acceptor arm of the tRNA in the E site, while the peptidyl-tRNA remains in the classic A site; and in the rotated-2 state, the deacylated tRNA is in the hybrid P/E state and the peptidyl-tRNA is repositioned into a hybrid A/P state with the anticodon paired with mRNA in the A site and the peptide attached to the acceptor arm in the P site (Budkevich et al. 2011; Behrmann et al. 2015). Translocation of the tRNAs to the canonical E and P sites is promoted by the elongation factor eEF2, the eukaryotic ortholog of the bacterial factor EF-G. Structural studies have revealed that eEF2 binds in the A site where it is thought to "unlock" the decoding interaction of the helix h44 nucleotides (A1755 and A1756 in *S. cerevisiae*) with the

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codon–anticodon duplex in the A site (Spahn et al. 2004; Taylor et al. 2007; Abeyrathne et al. 2016; Murray et al. 2016). Whereas no structures of canonical eukaryotic translocation intermediates (ribosomes with eEF2 and two translocating tRNAs) have been reported, based on similarity with the bacterial system swiveling of the small subunit head and reverse rotation of the small subunit relative to the large subunit are thought to accompany movement of the tRNAs into the canonical P and E sites (P/P and E/E states) (Ratje et al. 2010; Ermolenko and Noller 2011; Ramrath et al. 2013) and to allow for release of eEF2•GDP from the posttranslocation ribosome.

Following translocation, a deacylated tRNA occupies the E site and peptidyl-tRNA is positioned in the P site. It was previously proposed that release of the E-site tRNA from eukaryotic ribosomes is coupled to binding of the eEF1A•GTP•aminoacyl-tRNA ternary complex in the A site (Triana-Alonso et al. 1995; Anand et al. 2003) as also proposed for the bacterial system (Burkhardt et al. 1998). At odds with this proposal, various kinetic analyses revealed that release of the E-site tRNA and binding of the A-site tRNA are not strictly coupled in bacterial systems (Semenkov et al. 1996; Uemura et al. 2010; Chen et al. 2011; Petropoulos and Green 2012). Indeed, in one single molecule kinetic study, it was seen that deacylated tRNA is released slowly from the E site of human ribosomes following translocation, but independent of the binding of A-site aa-tRNA (Ferguson et al. 2015). Higher affinity binding of deacylated tRNA to the E site may be a feature that distinguishes eukaryotic from bacterial translation, and could impose novel or additional requirements on the translation factors that function at the E site.

As stated above, the basic mechanism of translation elongation is conserved between bacteria and eukaryotes. Whereas many of the studies elucidating the ribosomal and translation factor contributions to translation elongation have focused on the bacterial system, we focus this review on eukaryotic translation elongation and the features that distinguish eukaryotic from bacterial translation elongation.

THE EUKARYOTIC TRANSLATION ELONGATION FACTORS

In contrast to the complex factor requirements in translation initiation, elongation is assisted by a minimal set of factors. In addition to the canonical factors eEF1A/EF-Tu and eEF2/EF-G, the elongation factor eIF5A/EF-P is also conserved between eukaryotes and bacteria. In contrast, the ATPase eEF3 appears to be restricted to fungi and perhaps some other single-cell eukaryotes. In this section, we will highlight properties of the eukaryotic translation elongation factors with a focus on the unique features that distinguish the eukaryotic factors from their bacterial counterparts.

eEF1A–eEF1B

The GTPase eEF1A binds aminoacyl-tRNA in a ternary complex with GTP. Following GTP hydrolysis on the ribosome, the eIF1A is released in a binary complex with GDP. As with EF-Tu, the spontaneous rate of GDP dissociation from eEF1A is slow and the guanine nucleotide exchange factor eEF1B is required to recycle inactive eEF1A•GDP to active eEF1A•GTP (Gromadski et al. 2007). Whereas the complementary factor EF-Ts in bacteria is a single polypeptide, eEF1B is composed of two or three subunits (depending on the organism) and destabilizes GDP binding to eEF1A by a mechanism that is distinct from that employed by EF-Ts (Andersen et al. 2001; Rodnina and Wintermeyer 2009). The catalytic eEF1Bα subunit forms a dimeric complex with eEF1Bγ in yeast and forms trimeric complexes in mammals and plants consisting of eEF1Bα, eEF1Bγ, and either eIF1Bβ (mammals) or eIF1Bδ (plants). Overexpression of eIF1A or mutations in eEF1A that lower guanine nucleotide binding affinity bypass the essential requirement for eEF1Bα in yeast (Kinzy and Woolford 1995; Carr-Schmid et al. 1999); however, the suppression is incomplete as the eEF1A mutations increase nonsense suppression and show increased sensitivity to translation elongation inhibitors (Carr-Schmid et al. 1999).

In humans, eEF1A is encoded by two genes: *EEF1A1* and *EEF1A2*. Mutations in *EEF1A2*

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have been linked to a novel intellectual disability and epilepsy syndrome (Nakajima et al. 2015; Inui et al. 2016; Lam et al. 2016), and overexpression of eEF1A2 has been reported in a variety of cancers (Lee and Surh 2009). eEF1A in mammals and yeast is posttranslationally modified on several residues (Dever et al. 1989; Cavallius et al. 1993), most notably by methylation of lysines. Several eEF1A lysine methyltransferases have recently been identified (Lipson et al. 2010; Jakobsson et al. 2015, 2017; Hamey et al. 2016; Malecki et al. 2017), and loss of methylation has been linked to altered translation (Jakobsson et al. 2017; Malecki et al. 2017). These connections to human health are consistent with the expectation that gene expression is quite precisely tuned at the level of translation.

eEF2

Translation elongation factor eEF2, like EF-G, is a structural mimic of the eEF1A•GTP•aminoacyl-tRNA ternary complex (Jorgensen et al. 2003). Domain IV of eEF2, like the anticodon loop of tRNA, binds deep in the A-site decoding center to promote translocation of the tRNAs and mRNA on the ribosome following peptide bond formation. A conserved His residue at the tip of domain IV of eEF2 is posttranslationally modified to diphthamide (2-[3-carboxyamido-3-(trimethylammonio)propyl]histidine); this diphthamide modification is conserved in eukaryotes and archaea (Su et al. 2013; Schaffrath et al. 2014) but is not present on bacterial EF-G. The diphthamide residue is ADP-ribosylated by diphtheria toxin produced by *Corynbacterium diphtheriae*, as well as by exotoxin A from *Pseudomonas aeruginosa* and cholix toxin from *Vibrio cholera* (Schaffrath et al. 2014). The ADP-ribosylation of eEF2 inactivates the factor, blocks protein synthesis, and impairs cell growth; however, the molecular basis for how ADP-ribosylation impairs eEF2 function has not been fully resolved (Davydova and Ovchinnikov 1990; Nygard and Nilsson 1990; Jorgensen et al. 2004; Taylor et al. 2007; Mateyak and Kinzy 2013).

Synthesis of diphthamide requires a four-step pathway and the action of seven gene products (DPH1-7). Lack of the diphthamide modification is lethal in mice as a result of significant developmental defects (Chen and Behringer 2004; Nobukuni et al. 2005; Liu et al. 2006; Webb et al. 2008; Thakur et al. 2012; Yu et al. 2014). Moreover, mutations in DPH1/OVCA1 have been linked to ovarian cancer (Chen and Behringer 2004). Surprisingly, despite its deep conservation, diphthamide is not essential in yeast. Yeast lacking the first enzyme required for diphthamide synthesis and, thus, presenting an unmodified His residue at the tip of domain IV, grow normally, suggesting perhaps that diphthamide plays a role in translational fidelity rather than the fundamental mechanism of protein synthesis. Mice lacking the diphthamide biosynthetic enzymes DPH1, DPH3, or DPH4 exhibit severe developmental defects or embryonic lethality (Chen and Behringer 2004; Liu et al. 2006; Webb et al. 2008), indicating that diphthamide synthesis or perhaps another function of these enzymes is required during development. Despite this critical role of diphthamide in development, mammalian CHO and MCF7 cells lacking the ability to synthesize diphthamide are viable (Liu et al. 2004; Stahl et al. 2015), and the only clear phenotypes in these mutants are their insensitivity to diphtheria toxin and altered nuclear factor (NF)-κB and tumor necrosis factor pathways. However, both yeast (Ortiz et al. 2006) and mammalian (Liu et al. 2012) cells lacking diphthamide show increased levels of programmed −1 ribosomal frameshifting, revealing a positive impact of diphthamide on translational fidelity and suggesting that diphthamide may augment the function of eEF2 in promoting precise ribosomal translocation.

Recent biochemical studies examining eEF2 function in a novel translocation reaction required for translation initiation on the internal ribosome entry sites (IRESs) from the cricket paralysis virus (CrPV) and the Taura syndrome virus (TSV) have provided additional insights into the function of the diphthamide modification on eEF2. Pseudoknot I (PKI) of the IRES binds in the A site and mimics a tRNA bound to its mRNA codon. To enable translation, PKI must be translocated to the P site (reviewed in Butcher and Jan 2016). Whereas eEF2 with or

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without the diphthamide modification functions equivalently on canonically initiated elongation complexes analyzed in an in vitro peptide synthesis assay, peptide synthesis initiated on the CrPV IRES is impaired when eEF2 lacks the diphthamide modification (Murray et al. 2016). These results likely reflect a heightened requirement for diphthamide to promote high-fidelity translocation by the IRES and are supported by recent cryo-EM structures of translocation complexes that reveal interactions between diphthamide and PKI (Abeyrathne et al. 2016; Murray et al. 2016). Domain IV of eEF2 is inserted into the A site where it stabilizes PKI in a conformation that resembles a hybrid state with the pseudocodon–anticodon interaction in the A site. The diphthamide residue directly interacts with the pseudocodon–anticodon helix of PKI (Abeyrathne et al. 2016) and appears to disrupt the interaction of the ribosome decoding center h44 residues A1753 and A1754 (*Kluyveromyces lactis* 18S rRNA) with the PKI (Abeyrathne et al. 2016; Murray et al. 2016), and thus may directly facilitate translocation. It is tempting to speculate that loss of the interaction between diphthamide and the codon–anticodon helix during canonical elongation might contribute to the increased ribosomal frameshifting observed in cells lacking diphthamide. Taken together, these structural and biochemical studies of the IRES-dependent translation, together with the in vivo experiments revealing heightened ribosomal frameshifting in cells lacking diphthamide, suggest that diphthamide functions to optimize the efficiency and fidelity of ribosomal translocation during translation elongation.

In addition to the diphthamide modification, eEF2 is also modified by phosphorylation. The Ca^{2+}-activated kinase eEF2K phosphorylates eEF2 in metazoans on Thr56 and blocks translation by impairing eEF2 binding to the ribosome (Carlberg et al. 1990). As covered in other reviews (see Proud 2018), the activity of eEF2K is regulated by nutrients via mammalian target of rapamycin complex 1 (mTORC1) and/or AMP-activated protein kinase (AMPK) (Kenney et al. 2014) and during neuronal signaling (Taha et al. 2013).

eEF3

The translation elongation factor eEF3 is restricted to fungi and appears to be specifically required for protein synthesis with yeast ribosomes. Whereas yeast eEF1 and eEF2 will functionally substitute for their mammalian counterparts to promote translation with mammalian ribosomes in vitro, the mammalian factors eEF1 and eEF2 will only work with yeast ribosomes when eEF3 is added as well (Skogerson and Engelhardt 1977). eEF3 contains two ATP-binding cassettes (ABCs) and possesses ribosome-stimulated ATPase activity. Mutations in a chromodomain insert in the second ABC domain impair general translation and ribosome-stimulated ATPase activity (Sasikumar and Kinzy 2014). A low-resolution cryo-EM structure of eEF3 bound to the ribosome revealed that eEF3 contacts both the central protuberance of the 60S subunit and the head of the 40S subunit (Andersen et al. 2006). In this structure, the chromodomain of eEF3 is located near the E site of the ribosome, consistent with the model that eEF3 may promote release of deacylated tRNA from the E site following translocation (Triana-Alonso et al. 1995; Andersen et al. 2006). It is unclear why yeast ribosomes require eEF3 when similar ATPases are neither required for translation nor are obviously present in the genomes of higher eukaryotes.

eIF5A

In addition to eEF1A/EF-Tu and eEF2/EF-G, a third universally conserved factor, eIF5A/EF-P, also functions in translation elongation. eIF5A and EF-P were originally identified based on their abilities to stimulate the yield of methionyl-puromycin in a model assay of first peptide bond formation (Glick and Ganoza 1975; Kemper et al. 1976), and, so, eIF5A was considered an initiation factor with a critical role in first peptide bond formation. However, it is noteworthy that puromycin is a poor substrate because of unfavorable positioning in the PTC (Youngman et al. 2004; Wohlgemuth et al. 2008). Thus, eIF5A stimulation of the puromycin reaction might reflect an ability of eIF5A to enhance the

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reactivity of a poor substrate like puromycin rather than a role for eIF5A in first peptide bond synthesis. The results of dipeptide synthesis assays employing canonical aminoacyl-tRNA substrates argue strongly against a critical role for eIF5A in first peptide bond formation. The dipeptides Met-Phe and Met-Pro, or related polypeptides initiating with these residues, were efficiently synthesized in fully reconstituted yeast in in vitro translation assays lacking eIF5A, and addition of the factor resulted in only a modest stimulation in peptide yield (Gutierrez et al. 2013; Schuller et al. 2017; Shin et al. 2017). The absence of a strong eIF5A dependence for synthesis of these peptides indicates that first peptide bond synthesis does not impose a heightened requirement for the factor. An in vivo study supporting a role for eIF5A in first peptide bond synthesis reported that depletion of eIF5A in yeast resulted in reduced levels of large polysomes and accumulation of smaller polysomes and monosomes, suggestive of an initiation defect (Henderson and Hershey 2011). At odds with this finding, a separate study in yeast using a different degron to deplete eIF5A reported the maintenance of polysomes upon depletion of eIF5A, even in the absence of the elongation inhibitor cycloheximide (Saini et al. 2009). Thus, depletion of eIF5A mimicked cycloheximide treatment and was suggestive of impaired translation elongation. Moreover, this latter study also reported that rapid inactivation of temperature-sensitive eIF5A mutants in yeast resulted in the accumulation of polysomes in the absence of cycloheximide (Saini et al. 2009). These findings indicate a rate-limiting role for eIF5A in translation elongation rather than translation initiation or first peptide bond formation. In further support for a role for eIF5A in translation elongation or termination, inactivation of eIF5A resulted in increased ribosomal transit times (Gregio et al. 2009; Saini et al. 2009). Consistent with the findings in these latter in vivo studies, addition of eIF5A stimulated the rate of peptide synthesis in in vitro elongation assays and of release in termination assays in the presence of eRF1 and eRF3 (Saini et al. 2009). Taken together, the findings from the in vitro peptide synthesis assays using authentic aminoacyl-tRNA substrates and from the eIF5A inactivation studies in yeast argue that the factor plays a critical role in translation elongation, but not in translation initiation or first peptide bond formation.

Further studies into the function of EF-P revealed that the factor stimulated the synthesis of proteins containing runs of consecutive proline residues (Doerfel et al. 2013; Ude et al. 2013). Complementary studies in yeast cells revealed that inactivation of eIF5A impaired translation of reporter genes containing runs of polyproline residues and that eIF5A and its hypusine modification are required for the synthesis of polyproline peptides in vitro (Gutierrez et al. 2013). Consistent with these results, the synthesis of native yeast proteins containing polyproline motifs was impaired in eIF5A mutants (Gutierrez et al. 2013; Li et al. 2014a).

Whereas the critical role of eIF5A to promote translation of polyproline motifs is consistent with previous reports suggesting that eIF5A stimulates the translation of only a subset of mRNAs in the cell (Kang and Hershey 1994), polysome profile analyses in cells depleted of eIF5A revealed a pervasive elongation defect affecting a substantial fraction of cellular mRNAs (Saini et al. 2009). Moreover, recent ribosomal profiling analyses revealed that eIF5A functions globally to promote translation elongation and that its function is not restricted to polyproline motifs (Pelechano and Alepuz 2017; Schuller et al. 2017). Importantly, the stimulation of non-polyproline peptide synthesis by eIF5A was also observed in vitro (Schuller et al. 2017). In addition to detecting ribosomal pausing during translation elongation, the ribosomal profiling studies of cells depleted for eIF5A revealed a pronounced accumulation of ribosomes at stop codons (Schuller et al. 2017). Using in vitro peptide release assays, eIF5A was shown to promote the eRF1 and eRF3-dependent translation termination (Schuller et al. 2017). Taken together, these new findings reveal a genome-wide role for eIF5A in translation elongation and termination. Interestingly, in contrast to eIF5A, ribosomal profiling of bacteria lacking EF-P did not detect any impact on translation termination (Elgamal et al. 2014; Woolstenhulme et al.

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2015); the differences between eukaryotic and bacterial translation termination that underlie the stimulatory effect of eIF5A are unclear.

Recent structural and biochemical studies have provided insights into how eIF5A and EF-P may stimulate translation. The amino- and carboxy-terminal domains of eIF5A resemble domains I and II of EF-P; however, the bacterial factor possesses a carboxy-terminal domain III that is not present in eIF5A (Dever et al. 2014; Lassak et al. 2016). Two isoforms of eIF5A are differentially expressed in both yeast and mammalian cells (Dever et al. 2014; Mathews and Hershey 2015). Although no biochemical studies have reported functional differences between eIF5A isoforms, the eIF5A2 isoform has been linked to cancer (Mathews and Hershey 2015). It is noteworthy that the amino acid hypusine is formed posttranslationally on eIF5A by transfer of an *N*-butylamine group from spermidine to the ε-amino group of a conserved Lys residue forming deoxyhypusine (Park et al. 2010). Hydroxylation of deoxyhypusine completes the modification. The modified Lys residue resides at the tip of a loop in domain I of eIF5A (Kim et al. 1998), and the corresponding Lys or Arg residue in EF-P is modified by the addition of hydroxylated β-lysine, 5-aminopentanol, or the sugar rhamnose in different species (Dever et al. 2014; Lassak et al. 2016). The posttranslational modification of eIF5A and EF-P is required for stimulation of methionyl-puromycin synthesis (Park et al. 1991, 2012), polyproline synthesis (Gutierrez et al. 2013; Ude et al. 2013; Doerfel et al. 2015), and translation termination (Schuller et al. 2017). As in the X-ray structure of EF-P bound to the bacterial ribosome (Blaha et al. 2009), cryo-EM (Schmidt et al. 2016), and X-ray (Melnikov et al. 2016b) structures of eIF5A bound to the yeast ribosome revealed the factor binding in the E site. In these structures, the factor abuts the peptidyl-tRNA in the P site and the hypusine residue interacts with the acceptor arm of the peptidyl-tRNA. Exploiting the heightened requirement for eIF5A/EF-P for polyproline synthesis, biochemical studies using misacylated tRNAs revealed that the imino acid proline rather than $tRNA^{Pro}$ imposes the requirement for eIF5A (Shin et al. 2017), although in bacteria the D-arm of the $tRNA^{Pro}$ contributes to EF-P stimulation of translation (Katoh et al. 2016). Further analysis of bacterial translation revealed that EF-P provides an entropic benefit to peptide synthesis (Doerfel et al. 2015), and studies in yeast revealed that a more flexible proline analog lessened the requirement for eIF5A in peptide synthesis (Shin et al. 2017). These findings support a model in which the hypusine side chain acts sterically to position the acceptor arm of the P-site tRNA for favorable interaction with the A-site substrate in the ribosome PTC. Although this repositioning is likely to assist synthesis of all peptide bonds, some substrates like polyproline may show a greater requirement because of their inherently poor positioning in the PTC.

Assuming that eIF5A and deacyl-tRNA cannot simultaneously occupy the E site, as would be predicted based on the structures of the relevant ribosomal complexes, eIF5A binding to the E site is likely restricted until after dissociation of the deacyl-tRNA. While single-molecule studies in a human eukaryotic translation elongation system indicate that dissociation of the deacyl-tRNA from the E site is slow (Ferguson et al. 2015), it is noted that these studies were performed in the absence of eIF5A and using bacterial tRNAs. Given the high abundance of eIF5A in both yeast and mammalian cells (Duncan and Hershey 1986; Firczuk et al. 2013), more than twofold greater than the concentration of total ribosomes, and its strong affinity for the ribosome (unpublished data), eIF5A is predicted to rapidly fill the E site following deacyl-tRNA release and contribute to each peptide bond and termination reaction. Finally, biochemical studies in both mammalian and yeast systems have revealed an interplay between eIF5A and polyamines. Inclusion of eIF5A lowered the optimum Mg^{2+} concentration for globin mRNA translation in mammalian assays lacking spermidine (Schreier et al. 1977). Moreover, whereas most of the eIF5A activities in yeast assays can be attributed to the hypusine residue, suggesting that the body of eIF5A might function simply as a hypusine delivery agent, it is notable that unhypusinated eIF5A can substitute for polyamines in the stimulation of general translation in vitro (Shin et al. 2017). Whereas

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the function of polyamines in translation elongation is not clear, these results suggest that polyamines, like the body of eIF5A, may interact with the peptidyl-tRNA to facilitate its proper and stable positioning required for peptide bond formation.

TRANSLATIONAL RECODING

Recoding Definition

It is generally assumed that (1) all codons encode identical information in all organisms (with few exceptions [Ling et al. 2015]), and (2) the reading frame is invariant. Beginning in the mid-1970s, mRNA elements were discovered that direct ribosomes to reassign the meanings of codons, induce ribosomes to slip into alternative reading frames (programmed ribosomal frameshifting [PRF]), and even bypass long stretches of mRNA sequence (ribosome shunting). All of these were eventually subsumed under the general heading of "translational recoding," defined as instances in which "…the rules for decoding are temporarily altered through the action of specific signals built into the mRNA sequences" (Gesteland and Atkins 1996).

A Unifying Mechanistic Concept: Recoding Is Driven by Kinetic Traps

At the biophysical level, translational recoding events are driven by *cis*-acting elements on mRNAs that alter the processivity kinetics of elongating ribosomes. These "kinetic traps" alter rates of kinetic partitioning between the normal "forward reaction" (i.e., canonical decoding) and "side reactions" (i.e., recoding events). Typically, these kinetic traps direct ribosomes to pause at a specific location on an mRNA. Most studies suggest that directed pausing is critical; the sequence over which a ribosome is stopped is thought to lower the energy barrier to a particular alternative coding solution. Some *cis*-acting elements can be very simple "flat" sequences (i.e., defined by primary sequence alone and not by the ability to form higher-order structures). More often, recoding elements involve complex mRNA topological features that induce ribosomal pausing. These can be in *cis* (i.e., entirely composed of mRNA), in *trans* (i.e., composed of proteins and/or other RNAs that interact with specific mRNA sequences), or a combination of the two. The combination of the sequence at which the ribosome is paused plus the kinetic substep that is affected determines the functional output of the ribosome (i.e., the nature and extent of the recoding event).

Molecular Mechanisms of Recoding

Recoding Directed by "Flat" cis-*Acting Sequence Elements*

This term refers to recoding elements in which *cis*-acting mRNA structural elements do contribute to defining the recoding signal. Typically, in these cases, low abundance of a translation factor that would normally be required for the next step of the normal coding program induces ribosome pausing at a specific recoding sequence. For example, +1 PRF by the yeast *Ty1* retrotransposable element is effected by the simple heptameric sequence CUU AGG C (where the incoming 0 frame is indicated by spaces). Here, the kinetic trap is supplied by the rare 0 frame A-site AGG codon, which is decoded by the very low abundance Arg-tRNA^{CCU} tRNA. Ribosomal pausing at this codon allows the P-site tRNA to slip from the 0 frame CUU to the +1 frame UUA (Belcourt and Farabaugh 1990). Evidence of this mechanism was first based on the observation that high-copy episomal expression of this tRNA caused a 50-fold decrease in +1 PRF, while its deletion from the chromosome caused +1 PRF efficiency to approach 100% (Kawakami et al. 1993). Biochemical studies further demonstrated that mutant yeast ribosomes with altered affinities for tRNAs in the P site displayed changes in *Ty1*-mediated +1 PRF (Meskauskas and Dinman 2001; Rhodin and Dinman 2010; Musalgaonkar et al. 2014) and that these effects could be antagonized by sparsomycin, an antibiotic that increases the affinity of ribosomes for peptidyl-tRNA (Meskauskas and Dinman 2001). The +1 frameshifts of *Ty2* and *Ty4*, other members of the *copia* family of retrotransposable elements, as well as the yeast

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ABP140 and *EST3* mRNAs are also thought to use this mechanism of tRNA slippage (Morris and Lundblad 1997; Asakura et al. 1998; Farabaugh et al. 2006). Interestingly, the *Ty3* GCG AGU U slippery site appears to function quite differently because the 0 frame P-site tRNA is not able to base pair with the +1 frame codon (Vimaladithan and Farabaugh 1994). Rather, *Ty3*-directed +1 PRF requires skipping the first A of the 0 frame P-site codon and to instead recognize the +1 frame GUU codon. This is dependent on an unknown feature of the Ala-$tRNA^{UGC}$, which is shared by four additional tRNAs.

Stop codons can also function as kinetic traps to drive recoding. These kinetic traps are driven by release factor abundance. This is particularly well documented in protozoa and mitochondria where loss of genes encoding a stop codon–specific (typically UGA) release factor leads to this codon being decoded by a suppressor tRNA (Alkalaeva and Mikhailova 2017; Lobanov et al. 2017).

Recoding Directed by cis*-Acting Topological Features*

Most known translational recoding signals include *cis*-acting mRNA structural elements, typically mRNA stem-loops and pseudoknots. In PRF, both classes of elements are thought to direct ribosomes to pause at special slippery sequences, the nature of which allows re-pairing of tRNAs that are already within the ribosome to shift into a different reading frame (reviewed in Dinman 2012b). Shifting thus requires unpairing of the tRNAs from the initial reading frame, an event that inevitably requires energetic input. Contextualization of PRF within the elongation cycle reveals two translation factors capable of providing the needed energy by virtue of their GTP hydrolysis activities: EF-Tu/eEF1A and EF-G/eEF2 (Harger et al. 2002). Computational kinetic modeling of −1 PRF revealed three steps during the elongation cycle at which this may occur: (1) during translocation of the ribosome into the slippery site; (2) during accommodation of tRNA into a ribosome paused at the slippery site; and (3) during translocation out of the slippery site (Liao et al. 2008). All three of these mechanisms are supported by data generated using molecular genetics, structural biology, and biochemical analyses in multiple different systems (Jacks et al. 1988a,b; Weiss et al. 1989; Yelverton et al. 1994; Plant et al. 2003; Baranov et al. 2004; Namy et al. 2006; Leger et al. 2007; Caliskan et al. 2014). Thus, we suggest that rather than a monolithic molecular mechanism, −1 PRF should be viewed as a functional outcome that can result from at least three different kinetic pathways.

mRNA pseudoknot stimulation of recoding has been proposed to occur via a torsional restraint model, in which supercoiling of stem 2 forces ribosomes to pause over the slippery site (Plant and Dinman 2005). This model can account for how elongating ribosomes are directed to the slippery site but it does not address the actual mechanism of slippage. A mechanistic model of −1 PRF (Plant et al. 2003) is based on observations that the mRNA pseudoknot region is “pulled into” the ribosome by one base during the process of aa-tRNA accommodation (Noller et al. 2002). This movement pulls the entire mRNA in the 5′ direction (i.e., into the ribosome by this distance). This model is supported by ribosome toe printing studies showing that the lengths of reverse transcriptase primer extension products are reduced by one base after aa-tRNA accommodation (Fredrick and Noller 2002) (i.e., the mRNA is pulled into the ribosome by the distance of one base during this event). The 9 Å model of −1 PRF (Plant et al. 2003) posits that the placement of the downstream stimulatory structure in the ribosome’s mRNA entry tunnel impedes this one base movement of the mRNA into the ribosome, stretching the segment of mRNA located between the slippery site and the downstream stimulatory structure. The resulting local region of tension in the mRNA can be resolved either by unwinding the stimulatory structure or by −1 slippage. This mechanism also applies to −1 PRF events that occur during translocation (Caliskan et al. 2014; Chen et al. 2014; Kim et al. 2014; Kim and Tinoco 2017). Regardless of whether it occurs during translocation or aa-tRNA accommodation, the active stretching of

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the spacer region between the slippery site and downstream stimulatory element followed by tRNA unpairing and slippage of the mRNA into an alternative reading frame can be subsumed under the heading of the "tension model." Structural and kinetic analyses using purified *E. coli* ribosomes and elongation factors also revealed that the downstream pseudoknot in the mRNA can impair the closing movement of the large subunit head, delaying dissociation of the translocase EF-G and the release of deacylated tRNA. Release of the tension by ribosomal slippage accelerates completion of translocation, providing a lower energy path for the ribosome to continue translation (Caliskan et al. 2014).

Do downstream stimulatory structures play active or passive roles in directing recoding? Numerous studies suggest that dynamic mRNA structural remodeling helps to physically "push" ribosomes to slip (Ritchie et al. 2012, 2014, 2017; Tinoco et al. 2013; Gupta and Bansal 2014; Moomau et al. 2016; Tsai et al. 2016; Zhong et al. 2016; Kendra et al. 2017). Coordination of base triples in both major and minor grooves provide mechanical resistance to pseudoknot unwinding, and stretches of adenosines confined along the minor groove of a helix prevent it from unwinding. Together, these molecular features contribute to ribosome pausing at the slippery site to help stimulate −1 PRF (Chen et al. 2017). Thus, although it was initially thought that downstream stimulatory structures were mere passive "roadblocks," the most recent research suggests that they play active roles in recoding.

Given the existence of *cis*-acting recoding stimulatory elements, it is logical to assume that elements with the opposing activity may also exist. Indeed, *cis*-acting mRNA structural elements that attenuate −1 PRF activity have been described in coronaviruses (Su et al. 2005; Cho et al. 2013). These consist of stem-loop structures located immediately 5′ of the slippery site sequences. These hairpins are first unwound by elongating ribosomes as they approach the frameshift signal. As they enter the slippery site, however, the ribosome moves past the sequence, enabling the stem-loop to re-form. It is reasoned that this structure can then resist the backward slippage of the ribosome caused by the −1 PRF signal. This regulation of translational recoding via formation of a stem-loop after ribosome clearance is reminiscent of Rho-independent transcription termination in bacteria (reviewed in Henkin and Yanofsky 2002).

Recoding Directed by trans-*Acting Factors*

Translational recoding is also subject to regulation through the action of *trans*-acting factors. These can be divided into three general classes: small molecules, nucleic acids, and proteins.

Small molecules. Programmed +1 ribosomal frameshifting on mRNAs encoding ornithine decarboxylase antizyme (OAZ) is stimulated by polyamines (Ivanov et al. 2000). OAZ +1 PRF is autoregulated by the availability of small molecules in the form of polyamines (i.e., the products of the synthetic pathway controlled by OAZ). OAZ downregulates polyamine synthesis by stimulating ubiquitin-independent degradation of ornithine decarboxylase (ODC), the enzyme that catalyzes the first step in polyamine biosynthesis. When polyamine levels are low, +1 PRF on the OAZ mRNA is low, thus downregulating OAZ synthesis and resulting in increased levels of polyamines. These levels of polyamines, in turn, feed back to increase +1 PRF and OAZ synthesis, negatively feeding back on polyamine synthesis.

***Trans*-acting proteins.** There is a growing list of *trans*-acting proteins that stimulate translational recoding in all domains of life. Synthesis of bacterial release factor 2 (RF2), one of the two versions of the protein involved in termination codon recognition in bacteria, requires a +1 PRF event (Larsen et al. 1995). RF2 is required for decoding of the UGA stop codon and additionally contributes to the recognition of some fraction of the UAA stop codons. Importantly, the *prfB* genes encoding RF2 in approximately 87% of bacterial species harbor an in-frame UGA codon located approximately 26 codons downstream from the AUG start codon, with the remainder of the protein coding sequence in the +1 frame (Craigen et al. 1985). This allows for an autoregulatory feedback system: when RF2 lev-

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els are high, termination is efficient, whereas when RF2 levels are low, termination on the UGA stop codon is inefficient. Thus, low RF2 levels enhance ribosomal pausing at the termination codon, and this kinetic pause enhances formation of a frameshift-inducing SD-like/anti-SD interaction between the ribosome and the mRNA.

Whereas RF2 technically functions in the prfB system, it only does so through its absence. In contrast, selenocysteine recoding actively requires *trans*-acting proteins. SECIS-binding proteins (SBPs in archaea, SBP2 in eukaryotes) interact with a special domain of a specialized elongation factor eEFsec to enhance recruitment of Sec-tRNA$^{(Ser)Sec}$ to the SECIS element and thus to an elongating ribosome. An additional protein, SECp43, methylates the 2′-hydroxylribosyl moiety in the wobble position of the selenocysteyl-tRNA$^{(Ser)Sec}$ to enhance selenoprotein expression (Ding and Grabowski 1999). In porcine reproductive and respiratory syndrome virus, an unusual −1/−2 PRF mechanism is stimulated in the absence of any apparent downstream RNA structural element, by the binding of a *trans*-acting protein complex composed of the virus-encoded nsp1β replicase subunit and the cellular poly(C) binding protein (Fang et al. 2012) to the mRNA sequence CCCANCUCC located 11 nucleotides 3′ of the GGGUUUUU shift site (Li et al. 2014b). Binding of this complex to the target sequence induces ribosome pausing over this −1/−2 slippery site (Napthine et al. 2016). Encephalomyocarditis virus protein 2A similarly functions to direct −1 PRF so as to decrease expression of its nonstructural gene products and up-regulate structural protein production, during the late phase of its replication cycle (Napthine et al. 2017). The possibility that this mechanism may be employed by many picornaviruses (e.g., hepatitis C virus, poliovirus, and rhinoviruses) suggests a novel target for antiviral therapeutic interventions. In each case, a *trans*-acting protein binding to the mRNA impacts the output of gene expression through modulation of a frameshifting event.

***Trans*-acting nucleic acids.** Hybridization of small synthetic nucleic acids to mRNAs 3′ of canonical slippery sites has been demonstrated to *trans*-activate efficient frameshifting in vitro (Aupeix-Scheidler et al. 2000; Howard et al. 2004; Olsthoorn et al. 2004; Henderson et al. 2006; Yu et al. 2010). The spacing between slippery sites and the downstream region of hybridization is important, supporting the idea of mRNA tension as causative (Lin et al. 2012). In live cells, the interaction of a microRNA (miRNA) with a −1 PRF-stimulating mRNA pseudoknot in the human CCR5 mRNA was shown to stimulate −1 PRF (Belew et al. 2014). It is hypothesized that this interaction renders the downstream element even more difficult to resolve, enhancing the probability of kinetic partitioning to the −1 frame at the slippery site. The proposed base-pairing interaction between the −1 PRF signal and miRNA in this case provides the potential for sequence-specific regulation of −1 PRF and, hence, a means to control expression of the CCR5 gene product. Preliminary studies reveal that miRNAs impact frameshifting at other human slippery sites, suggesting that this may be a widely used strategy to regulate gene expression in higher eukaryotes (Belew et al. 2014).

Functional Outcomes of Recoding

Two-for-one. All viruses with positive-sense plus-stranded RNA [(+) ssRNA] genomes, and many with double-stranded RNA genomes, face a common problem: their (+) strands have to serve as both mRNA and as a template for genome replication. Thus, maximization of protein coding information must be achieved in ways that do not alter the genetic information that will be passed to the next generation. Translational recoding is one solution to this problem. The simplest such solution can be thought of as "two-for-one," where PRF or termination suppression mechanisms are used to produce carboxy-terminally extended fusion proteins in addition to the peptides synthesized by canonical translation. Numerous such examples are well documented in many virus families (reviewed in Dinman 2012a; Firth and Brierley 2012). Many studies have shown that viruses have evolved to optimize recoding rates so as to optimize ratios of viral proteins, and that altering

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recoding efficiency has deleterious effects on viral propagation (reviewed in Dinman 2012b). As such, recoding is a potential target for antiviral therapeutics (Dinman et al. 1998). Efforts targeting the HIV-1 −1 PRF signal in particular have identified promising candidates for therapeutic development (Lonnroth et al. 1988; Dinman et al. 1997; Hung et al. 1998; Aupeix-Scheidler et al. 2000; McNaughton et al. 2007; Dulude et al. 2008; Marcheschi et al. 2009, 2011; Kobayashi et al. 2010; Palde et al. 2010; Ofori et al. 2014; Cardno et al. 2015; Hilimire et al. 2016; Hu et al. 2016). As a note of caution, however, in light of the finding that ~10% of chromosomally encoded genes harbor potential −1 PRF signals (Belew et al. 2008), and that global dysregulation of −1 PRF has deleterious effects on cell growth and replication (reviewed in Dinman 2012b), drug development efforts must be tailored to specific recoding elements as opposed to a broad targeting of all recoding.

mRNA destabilizing elements. Analysis of −1 PRF signals located in chromosomally encoded mRNAs revealed the counterintuitive finding that >99% of all predicted frameshifts would direct elongating ribosomes to premature termination codons (Jacobs et al. 2007; Belew et al. 2008). This prompted the hypothesis that these elements might serve to limit gene expression through the nonsense-mediated mRNA decay (NMD) pathway. Further, the ability of −1 PRF stimulatory elements to cause ribosomes to pause for relatively long periods of time (Heller et al. 1976; Caliskan et al. 2014) suggested that these elements may also render mRNAs substrates for degradation through the No-Go mRNA decay (NGD) pathway. Both of these mechanisms were validated using endogenous −1 PRF signals in yeast (Belew et al. 2011). Moreover, −1 PRF-directed NMD has been shown to control gene expression in human cells on many genes (Belew et al. 2014). Evidence is emerging that this strategy is used to control telomere maintenance in yeast (Advani et al. 2013), the cell cycle (Belew and Dinman 2015), and many more cellular pathways (Advani and Dinman 2016; Meydan et al. 2017). Further, global dysregulation of −1 PRF may be linked to a wide variety of human diseases (Jack et al. 2011; Hekman et al. 2012; Sulima et al. 2014; Belew and Dinman 2015; De Keersmaecker et al. 2015; Paolini et al. 2017).

CONCLUDING REMARKS

Fulfilling the fundamental role of decoding the genetic code, high-fidelity translation elongation is critical for proper cellular function. Recent studies have provided new insights into the general mechanism of translation elongation, its regulation, and the means to exploit the process for alternative decoding events. As is typical in biology, complex regulation is achieved through the modest manipulation of the core events of the process, not through the acquisition of wholly novel elements that redirect the system. As such, continued progress in obtaining high-resolution structural images of translation elongation intermediates, combined with rigorous biochemical and kinetic dissection of the partial reactions in elongation, and further exploitation of ribosomal profiling strategies to interrogate the translation elongation process, offer the exciting opportunity for even greater insights in the near future.

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