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Translational fidelity and mistranslation in the cellular response to stress

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Abstract

Faithful translation of mRNA into the corresponding polypeptide is a complex multistep process, requiring accurate amino acid selection, transfer RNA (tRNA) charging and mRNA decoding on the ribosome. Key players in this process are aminoacyl-tRNA synthetases (aaRSs), which not only catalyse the attachment of cognate amino acids to their respective tRNAs, but also selectively hydrolyse incorrectly activated non-cognate amino acids and/or misaminoacylated tRNAs. This aaRS proofreading provides quality control checkpoints that exclude non-cognate amino acids during translation, and in so doing helps to prevent the formation of an aberrant proteome. However, despite the intrinsic need for high accuracy during translation, and the widespread evolutionary conservation of aaRS proofreading pathways, requirements for translation quality control vary depending on cellular physiology and changes in growth conditions, and translation errors are not always detrimental. Recent work has demonstrated that mistranslation can also be beneficial to cells, and some organisms have selected for a higher degree of mistranslation than others. The aims of this Review Article are to summarize the known mechanisms of protein translational fidelity and explore the diversity and impact of mistranslation events as a potentially beneficial response to environmental and cellular stress.

When the sequence of amino acids in a newly synthesized protein is different to the genetically encoded sequence, a gene is said to have been mistranslated. There are several steps where this alteration may occur. Mistakes during DNA replication are on the order of ~10^{-8} and are kept to this extremely low level by a robust suite of error prevention, correction and repair mechanisms[1–5], while transcription of DNA into mRNA has error rates ~10^{-5} that are also prevented by complex proofreading and degradation systems[6,7]. The next step in gene expression, protein synthesis, offers the greatest opportunity for errors, with mistranslation events routinely occurring at a frequency of ~1 per 10,000 mRNA codons translated[8] (Fig. 1).

An error rate of 10^{-4} during protein synthesis equates to around 15% of all proteins in the cell containing at least one misincorporated amino acid under optimal growth conditions[9,10].

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Competing interests

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While the elevated error rates observed during protein synthesis can in part be attributed to the complexity of translating mRNA into protein, as the ribosome must select the correct aminoacyl-transfer RNAs (aa-tRNAs) from a large pool of near-cognate substrates fast enough to sustain an elongation rate of 10–20 amino acids per second[11–13], there are mechanisms that exist at multiple checkpoints during protein synthesis to minimize the frequency of translational errors. However, when compared to earlier steps during gene expression, error rates remain relatively high. This raises the questions of why cells appear to be more tolerant of errors at the level of protein synthesis, and could such errors be directly or indirectly beneficial in some cases?

Mechanisms of translational fidelity and error

The translation of genetic information into functional proteins is a multistep process with regulatory mechanisms at each level to ensure accuracy (Fig. 2). First, amino acids are correctly paired with their cognate tRNAs by aminoacyl-tRNA synthetases (aaRSs)[14]. Aminoacylation occurs in a two-step reaction: cognate amino acids are activated within the aaRS catalytic domain to form an aminoacyl-adenylate (aa-AMP); then, the activated amino acid is transferred to the 3′ OH of the terminal adenosine on the tRNA acceptor stem of its cognate tRNA, forming an aa-tRNA[15]. Next, with the help of elongation factors (EFs), the ribosome selects the appropriate aa-tRNA substrate by matching the anticodon of the tRNA with the corresponding mRNA codon occupying the aminoacyl site (A site) of the ribosome. Peptide bond formation with the neighbouring aa-tRNA in the peptidyl site (P site) results in elongation of the polypeptide chain, and this process is repeated as mRNA is decoded using aa-tRNAs until synthesis of the protein is complete and the ribosome dissociates from the mRNA.

Amino acid selection

For the cell, the consequences of aaRSs failing to discriminate between amino acids is potentially disastrous and could lead to mistranslation of proteins and activation of cellular stress responses[16]. The challenge to correctly recognize the 20 proteinogenic amino acids centres around their limited diversity in chemical space, which makes it difficult to accurately select substrates from a pool of structurally similar constituents[17]. The main mechanism to preserve fidelity is aaRS active-site screening of the substrate pool using size and physicochemical properties[18]. However, the diversity of amino acid functional groups is insufficient for the completely accurate selection of some cognate amino acid substrates and can result in misactivation by aaRSs[18]. For example, Ala–tRNA synthetase (AlaRS) has difficulty discriminating between its cognate Ala and the near-cognates Ser (differing by a single hydroxyl group) and Gly (lacking the methyl group) based on the topology of the active site and size of the amino acid, respectively[19]. Consequently, AlaRS must counter the problem of non-cognate amino acid activation with a variety of proofreading mechanisms[20].

In addition to misactivation of genetically encoded proteinogenic amino acids (GPAs), cells also encounter non-proteinogenic amino acids (NPAs) environmentally or as metabolic by-products, and must discriminate against these substrates to prevent aberrant use in protein synthesis. NPAs include D-amino acids that arise during amino acid synthesis and amino

acids that are damaged by reactive oxygen species (ROS)[21,22]. In *Escherichia coli*, oxidation of Phe via the Fenton reaction produces a racemic mixture of *p*-Tyr, *o*-Tyr and *m*-Tyr, and *E. coli* PheRS is able to effectively prevent misactivation of *p*-Tyr and *o*-Tyr through a mechanism of steric exclusion involving a key Ala residue within the amino-acid-binding pocket. However, the *meta* position of the hydroxyl group of *m*-Tyr prevents steric hindrance, leading to misacylation of $tRNA^{Phe}$ with *m*-Tyr[23], which can substantially decrease cellular viability if *m*-Tyr is misincorporated into the proteome[24]. Proteomic incorporation of NPAs has been described in all domains of life (reviewed extensively in ref. 25), with recent focus on their role in human disease[26,27]. Work to date shows that the cytotoxicity of NPA misincorporation varies greatly depending on the chemical nature of both the NPA and the GPA being replaced.

Transfer-RNA selection

A diverse pool of tRNAs with different sequences are responsible for relaying specific amino acids to the ribosome. Beyond primary sequence differences, the structural and functional diversity of tRNAs is further expanded through post-transcriptional modifications[28] (see Box 1). This diversity poses a unique challenge to aaRSs, which must effectively discriminate against non-cognate tRNAs while still selecting for multiple isoacceptors (tRNAs that accept the same amino acid)[29]. tRNA sequence elements and modification states serve as identity elements for their cognate aaRS, allowing for accurate selection from the complex pool of tRNA substrates[30,31]. While highly effective in preventing misselection of tRNAs, selection based on modification leaves the cell sensitive to conditions that can alter the modification state of the tRNA pool; for example, the efficient aminoacylation of some $tRNA^{Leu}$ species in *E. coli* is dependent on i6A37 modification (attachment of an isopentenyl group to the adenine at position 37), which improves codon recognition. In cells that are defective in this modification pathway, RNA polymerase sigma S (rpoS) translation is perturbed due to an inability to efficiently decode transcripts (like rpoS) that contain a high abundance of rare Leu codons[32]. Environmental or nutritional stress has also been shown to alter the modification state of the tRNA pool[33]. During oxidative stress, ROS accumulate within the cell and oxidize tRNAs, leading to mistranslation (reviewed in ref. 34).

Beyond structural differences, the ability of aaRSs to accurately select tRNAs is also dependent on the overall composition of substrates in the tRNA pool[35]. In several cases, tRNA pool imbalance has been observed to directly contribute to mistranslation via increased competition for limited cognate tRNA substrates. Overexpression of GlnRS, for example, results in misacylation of $tRNA^{Tyr}$, which can be prevented by concomitantly increasing cognate $tRNA^{Gln}$ expression, demonstrating that not only the level of tRNA substrates within the tRNA pool, but also the ratio of aaRS to cognate tRNA substrate, help maintain aminoacylation fidelity[35].

Proofreading of aa-tRNA

aaRS proofreading ensures accurate aa-tRNA synthesis, which helps maintain the fidelity of translation and can occur at a pre-transfer step or post-transfer after catalytic linkage of an incorrect amino acid to a tRNA.

Pre-transfer editing activities vary widely between enzymes and remain poorly understood, but often involve either selective release of misactivated amino acids or enzymatic hydrolysis of the aa-AMP. Selective release of non-cognate aa-AMP is thought to depend on decreased binding affinity within the amino-acid-binding pocket, leading to premature release of the amino acid from the active site. Hydrolysis of misactivated aa-AMP has been observed in both tRNA-dependent and -independent reactions, which involve either conformational change to the aminoacylation active site or trans-location of the aa-AMP to an alternative editing site to facilitate specific recognition and hydrolysis of non-cognate aa-AMP[10,36–38].

In cases where pre-transfer editing does not eliminate misactivated amino acids, approximately half of the aaRSs possess additional proofreading activities that selectively deacylate non-cognate aa-tRNAs both in *cis* and *trans*. Resolution of misaminoacylated tRNAs in *cis* requires translocation of the aa-tRNA acceptor stem from the synthetic active site to a hydrolytic editing site. Once repositioned, the misaminoacylated tRNA is resolved through hydrolysis of the ester linkage and both amino acid and tRNA are released[39,40]. Much like the active site, aaRS post-transfer editing activity is mediated by the topology of the editing site, with size and steric hindrance precluding correctly paired aa-tRNAs from aberrant hydrolysis.

In addition to *cis*-editing, most cells possess *trans*-editing mechanisms, which allow resampling of aa-tRNA by aaRSs to monitor aa-tRNA pool fidelity and the use of stand-alone *trans*-editing factors, whose sole function is to resolve misaminoacylated tRNAs[29,41]. One of the best-defined examples of *trans*-editing is found in the AlaXp family of proteins, comprised of freestanding editing domains that specifically hydrolyse misaminoacylated Ser–tRNA^{Ala19}. In addition to proteinogenic amino acids, misaminoacylation of tRNAs with non-protein amino acids is also monitored by *cis*- and *trans*-editing factors. For example, D-aminoacyl-tRNA deacylases target and hydrolyse both D- and L-aminoacyl-tRNAs and in doing so prevent misincorporation of a wide range of amino acids during protein synthesis[42–44].

Errors in decoding at the ribosome

In the event that misaminoacylated tRNAs avoid *cis*- and *trans*-editing mechanisms, several mechanisms ensure correct aa-tRNA decoding at the ribosome. First, discrimination between correct and non-cognate aa-tRNAs occurs through interaction with translation factors. The prokaryotic and eukaryotic EFs, EF-Tu and eEF1a, respectively form a ternary complex with aa-tRNAs and guanosine triphosphate (GTP), which protects against premature deacylation and facilitates delivery to the ribosome[45]. The interaction between the aa-tRNA and EF is thermodynamically tuned to bind cognate amino acid:tRNA pairs, while misaminoacylation perturbs this interaction such that an increase in binding affinity may prevent release of the aa-tRNA, or a decrease may lead to premature release of the aa-tRNA[46–50] before ribosome delivery.

If misaminoacylated tRNA is successfully delivered to the ribosome, additional proofreading occurs within the A site of the ribosome based on aa-tRNA position and affinity[12,51–59]. During selection of the correct tRNAs to match the mRNA codons, aa-tRNA complexes

undergo structural fluctuations that allow them to scan the codon while remaining bound to the EF that is anchored to the large subunit of the ribosome. Selection of an incoming ternary complex depends primarily on codon:anticodon interactions[60]. Formation of the correct, fully complementary codon:anticodon complex locks the aa-tRNA:EF:GTP complex (the A/T state) and induces structural rearrangements in the decoding centre of the small ribosomal subunit. When correct aa-tRNA is present, GTP hydrolysis releases the EF from the aa-tRNA, thus allowing translocation of aa-tRNA to the P site and subsequent peptide-bond formation. Ribosomal interactions with additional tRNA-specific sequences and modifications facilitate accurate selection of aa-tRNAs based on kinetic discrimination during the initial selection stage and subsequent proofreading stage. For example, transversion of a $tRNA^{Ala}$ GGC-specific A–U pair at the top of the anticodon loop leads to misincorporation of Ala at near-cognate Val GUC codons[61].

Much like amino selection during tRNA charging, misreading of codons can also occur in response to stresses that disturb the balance of the cellular tRNA pool and the availability of aa-tRNAs[62]. As the ribosome primarily interacts with non-cognate tRNAs during translation, tRNA abundance is critical to the accuracy of protein synthesis, especially within the context of transcripts containing rare codons[55]. Codon usage varies and the impact of rare codons on translation fidelity is readily observed using recombinantly produced proteins. For example, production of yeast proteins containing the Arg AGA codon (rarely used in *E. coli*) results in misincorporation of Lys, but is abrogated by co-expression of $tRNA^{Arg}$[63]. Similarly, the stochastic nature of aa-tRNA sampling by the ribosome causes decoding errors during periods of amino acid limitation[64]. In an effort to maintain the high rates of translation necessary for cellular viability during periods of specific amino acid limitation, the cell may prevent ribosome stalling caused by deacylated tRNA entering the A site by instead selecting near-cognate aa-tRNAs that differ by a single base within the anticodon domain, resulting in missense translational error[65–67]. This phenomenon has been observed in response to Phe starvation where Leu is misincorporated at Phe codons due to preferential misreading of UUC codons, and during Asn starvation where Asn codons AAU and AAC are misread as Lys codons[68,69].

Mistranslation

The impact of failure in proofreading is mistranslation, which encompasses any action that results in a loss of fidelity while decoding genetic information during protein synthesis. Mistranslation may be specific for a single amino acid substitution at many near-cognate codon positions, or may be the result of random misincorporation of amino acids across the entire proteome[70] (Table 1). Depending on the source of mistranslation, the impact to the proteome can range from ‘local’ mistranslation (a change at a single codon) to ‘regional’ mistranslation (substitution of one amino acid for another, regardless of codon) to ‘global’ mistranslation (indiscriminate misincorporation of an amino acid). Regional mistranslation events, resulting in near-cognate amino acid substitutions, occur naturally in bacteria as a response to environmental stressors. Many antibiotics mechanistically target the bacterial ribosome, resulting in codon-specific local mistranslation events and aberrant protein synthesis[71,72]. For example, *in vitro* studies of streptomycin mechanisms of action revealed an increase in the frequency of misreading errors at the ribosome due to decreased fidelity of

pyrimidine recognition[73]. Observations in *E. coli* provided quantitative examples of ribosome-mediated decoding errors *in vivo* by monitoring the incorporation of Cys into flagellin (normally Cys-free), and found that pyrimidine misreading causes Arg CGU and CGC codons to be recognized as Cys UGU and UGC codons[74,75]. Global analyses of the *E. coli* proteome, made possible by recent advances in quantitative mass spectrometry, have yielded codon-specific measurements of mistranslation that range from 0.1% to 40% (Table 1).

Many mistranslation events (and corresponding proofreading mechanisms) are conserved across all domains of life. For example, in a similar fashion to *E. coli* LeuRS, yeast LeuRS misaminoacylates $tRNA^{Leu}$ with the near-cognate Ile, resulting in regional mistranslation events[76]. Far less is known about mistranslation in archaea, with a few studies showing *in vivo* regional mistranslation events mediated by aaRSs. For example, in the hyperthermophile *Aeropyrum pernix*, low-temperature stress causes ~2% substitution of Met at Leu codons due to misacylation of $tRNA^{Leu}$ by MetRS[77]. While the mechanisms of translation fidelity are probably conserved in archaea, as exemplified by the initial discovery of misaminoacylated tRNA *trans*-editing in archaea, the impact of protein mistranslation on archaeal physiology remains largely unexplored[78,79].

Beneficial mistranslation

While errors in protein synthesis are traditionally viewed as detrimental to cellular processes, emerging evidence suggests beneficial roles for mistranslation in certain biological contexts. In fact, many aaRSs possess broad polyspecificity for non-cognate amino acids, suggesting that absolute translational fidelity may not be completely necessary under many physiological conditions, and that under some, mistranslation is able to improve cellular viability in response to environmental, nutritional or immunological stress[80,81]. For example, the ability to accurately sense and mount an efficient response to stress is essential for the maintenance of cellular viability, and alterations in translational fidelity and protein structures can be utilized by cells to monitor and respond to adverse environmental conditions. Similarly, the ability to produce altered proteins may also enable cellular survival during these periods of stress.

Mistranslation enhancing cell viability

Fluctuations in temperature, osmolarity and chemical growth conditions may be perceived as stress events by the cell if they fall outside normal growth parameters[82,83]. Oxidative stress is of particular concern, with ROS arising through external factors, such as chemical stress, and internally as a byproduct of metabolic processes. As a counter to oxidative stress, cells across all domains of life have evolved a conserved adaptive response to limit proteome damage—MetRS phosphorylation increases during ROS stress, resulting in a decrease in specificity for tRNA substrates and global methionylation of non-methionyl tRNAs[70,84,85]. Increased Met misincorporation acts as a sink for ROS, thus decreasing detrimental oxidation of critical active site residues within the proteome[86]. In *E. coli*, MetRS post-translation modification instead limits tRNA mismethionylation, providing a mechanism by which mistranslation can be regulated, leading to increased stress resistance[87].

Amino acid limitation is a common source of nutritional stress, to which cells respond through transcriptional and translational reprogramming events[88,89]. During amino acid limitation, the response to excess near-cognate amino acid can be either detrimental or beneficial, depending on the amino acid involved. For example, strains of *E. coli* with proofreading-defective ValRS grow poorly in presence of non-cognates Thr and α-aminobutyrate[90]. Additionally, misincorporation of Ser at Ala codons in *E. coli* with AlaRS deficient in proofreading activity is conditionally lethal in the presence of excess Ser[20]. While amino acid misincorporation conferred a negative fitness cost to the cell in the above cases, the addition of Val or the NPA norvaline to Ile-depleted cultures of *E. coli* or *Acinetobacter baylyi* with proofreading-deficient IleRS conferred a growth advantage[91,92]. While little is known about the specific effects of amino acid substitutions, these observations underscore the importance of contextual amino acid identity and highlight the diversity of amino acid misincorporation responses to stress. This last point is well illustrated by recent studies of clinical isolates of *Mycobacteria tuberculosis*, where antibiotic resistance results from mutations in the glutamine amidotransferase GatCAB that lead to tRNA misacylation and subsequent mistranslation[93,94].

Beyond presumed metabolic impacts, mistranslation can also enhance survival by creating antigenic diversity in surface proteins for some pathogens[95]. Parallel exploration of mistranslation in two species of yeast, *Saccharomyces cerevisiae* and *Candida albicans*, has provided context to the role of mistranslation in pathogenicity. Local mistranslation in *C. albicans*, mediated by poor tRNA substrate recognition by aaRSs, results in 0.5% to 6% misincorporation of Ser at Leu (CUG) codons[96]. In addition, Ser misincorporation increases the antigenic diversity of *C. albicans*, facilitating evasion of host innate immune response[95,97]. More broadly, Ser misincorporation also provides a mechanism to promote adaptive phenotypic diversity, illustrative of a more general principle by which mistranslation can potentially be beneficial for cellular viability and survival[98,99]. Similar to *C. albicans*, several members of the Mycoplasmataceae aaRSs have evolved with degenerate (or absent) proofreading mechanisms that allow for misacylation of cognate tRNAs[100,101]. In both cases, the proteome diversity generated through these processes has been suggested to provide a mechanism to increase phenotypic diversity and evasion of host immune responses.

Aminoacyl-tRNA and translation-mediated stress response signaling

In addition to abnormal proteins having direct impacts on fitness, mistranslation can also alter stress response signalling. For example, deacylated tRNA accumulates within the cell during amino acid starvation, and as this deacylated tRNA enters the A site of the bacterial ribosome, the ribosome briefly pauses and transfers the tRNA to a ribosome-associated protein, RelA, to initiate the stringent response. On binding deacylated tRNA, RelA begins to synthesize (p)ppGpp, which serves as a global alarmone, triggering functional reprogramming of the cell in response to stress[102–104]. However, misaminoacylation of tRNA can mask amino acid starvation in sensing mechanisms such as the stringent response; in *E. coli*, defects in PheRS aa-tRNA proofreading lead to an increase in misaminoacylated Tyr–$tRNA^{Phe}$, reduced accumulation of deacylated tRNA, and subsequent misregulation of the stringent and other amino acid stress responses[24].

Eukaryotes use a similar mechanism to the stringent response to respond to amino acid starvation by monitoring accumulation of deacylated tRNA in the cytoplasm (Fig. 3). In the yeast general amino acid control (GAAC) pathway, deacylated tRNA interacts with the protein kinase general control non-depressible 2 (Gcn2p). This activates a cascade that results in a global decrease in translation while preferentially increasing the expression of *GCN4* (mechanism reviewed in ref. 105), a transcription factor that regulates expression of ~400 core stress response genes, including those for amino acid biosynthesis. Activation of this stress response programme is coordinated by a global decrease in translational capacity of the cell, and is critical to yeast's ability to respond to nutrient stress. Recently, work from our lab has demonstrated that reduction of aminoacylation fidelity through aaRS-mediated mistranslation events leads to dysregulation of the GAAC. In a yeast cytoplasmic PheRS aa-tRNA-proofreading-deficient strain, accumulation of misaminoacylated Tyr–$tRNA^{Phe}$ prevents accurate sensing of Phe starvation by the GAAC by limiting the pool of deacylated $tRNA^{Phe}$[106].

Adaptive versus non-adaptive mistranslation

Stress events can be segregated based on the characteristics and outcome of the stress event. Acute stress may be classified as any atypical cytotoxic stress (for example, antibiotic, chemical or ROS) that the cell must resolve to maintain viability. Resolution of acute stress events is achieved through transient cellular adaptation (for example, non-adaptive mistranslation), where modification of existing cellular mechanisms counteracts stress events. For example, when faced with an acute oxidative stress, oxidation of a critical Cys active site residue in *E. coli* ThrRS causes misaminoacylation of Ser–$tRNA^{Thr}$, which may provide a mechanism to sense oxidant levels in the environment[107].

In addition to acute responses, mistranslation can also serve to enable cellular reprogramming to coordinate metabolic responses to stress[108]. Cells regularly encounter nutrient limitation in the form of carbon limitation or amino acid starvation, which are typically cyclic in nature and mediated by autoregulatory biosynthesis mechanisms, ensuring that cellular resources are only being redirected during times of need[109,110]. Response to intermittent stress events, such as these, requires programmable adaptation while limiting the rate of overall cellular growth[111]. In this context, mistranslation through the use of near-cognate amino acids in place of cognate amino acids as substrates for protein synthesis allows the cell to maintain translation rates while it initiates a programmed adaptive response[112].

Cells may also encounter persistent stress that is unresponsive to transient or programmable adaptive responses. For example, a mutagenic event that reduces translation fidelity is one form of persistent stress resulting in a constitutive pressure that the cell must overcome to maintain viability. Several examples of aaRS mutations have been directly linked to observations of human disease and substantial fitness cost[113,114]. In other cases where mistranslation is very high, however, cells have been shown to develop robustness to increased error rates by increasing protein turnover to maintain proteome homeostasis[99]. In effect, if cells can maintain viability when translation fidelity is initially reduced, the accompanying increase in mistranslation (for example, adaptive mistranslation) can be

beneficial by promoting phenotypic heterogeneity and increasing the probability of successfully adapting to stress[115,116]. Given that specific mechanisms such as aaRS oxidation impair quality control on exposure to stress, adaptive mistranslation provides a potentially potent mechanism to promote survival (Fig. 4).

Outlook

As we consider the full spectrum of mistranslation, it is clear that some organisms can tolerate a substantial amount of misincorporation and that these events have the potential to confer selective fitness advantages at the organismal level. Recent work has started to provide data to support mechanistic models for the potential benefits of adaptive mistranslation, but to date these studies have been confined to individual mistranslation events. Recent technical advances now provide the opportunity to substantially broaden our understanding of the role of mistranslation by allowing measurement of the rates of both misaminoacylation and mistranslation *in vivo*[117,118]. Such accurate error rate measurements will provide a means to properly establish the relevance of mistranslation in physiological contexts by delineating how the level of mistranslation correlates with a cell's ability to adapt, survive and thrive under different conditions. Furthermore, conditions where mistranslation affords a distinct advantage will provide a context for defining mechanisms of adaptive mistranslation both at the population and single-cell levels. Studies at the single-cell level, while more challenging, are of particular importance as they have the potential to provide new mechanistic insights as to how mistranslation impacts phenotypic heterogeneity, which opens avenues to test the role of mistranslation in microbial evolution, antigen presentation, bacterial persistence and metabolic diversity.

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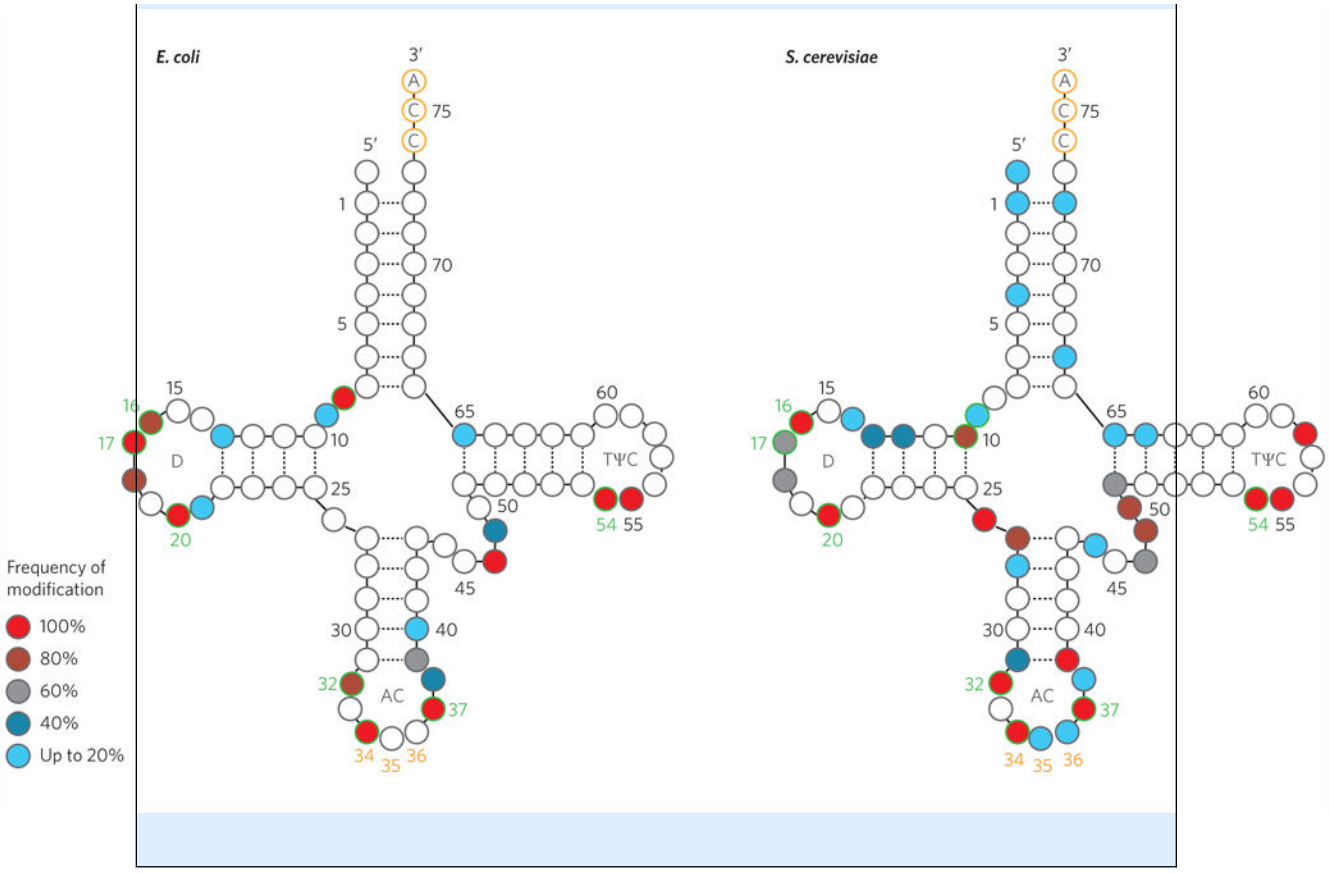
Box 1

Structure and modification of tRNA

Transfer RNAs are typically 70–90 nucleotides in length with a cloverleaf-like secondary structure characterized by anticodon (AC), dihydrouridine (D) and thymidine–pseudouridine–cytidine (TΨC) stem-loops and an acceptor stem (ACC, orange circles)[127]. Although transcribed with the same nucleotide bases as mRNA (A, U, C, G), ribonucleosides of tRNAs undergo extensive post-transcriptional chemical modification (PTrM) at an average of 9–11% of bases per mature tRNA, representing the most highly modified RNA species within the cell[128]. These modifications are very diverse, with more than 100 different structures identified to date in different tRNAs.

The occurrence and distribution of modifications across all tRNAs for a representative prokaryote (*E. coli*) and eukaryote (*S. cerevisiae*) are illustrated in the tRNA structures below. Modified positions marked with a green circle represent PTrMs known to be essential to the structure, activity and recognition of tRNA substrates. tRNAs from organisms across all domains of life share characteristic modification profiles in the AC loop at positions 32, 34 and 37 that ensure translational accuracy during decoding at the ribosome. PTrMs are also fundamental to the formation of the canonical tRNA tertiary structure: positions 16, 17 and 20 within the D loop and position 54 in the T loop are essential for tRNA structural stability and flexibility. While the role of PTrMs in the context of translation has been well characterized, investigation of tRNA-specific PTrM functions remains largely underexplored, leaving the roles of the majority of tRNA PTrMs unknown. Alteration to tRNA modifications within the AC loop often result in decoding error or decoding biases at the ribosome[33, 129]. In yeast, for example, stress-induced alteration in tRNA modification by Trm4p (tRNA:m5C methyltransferase) leads to an increase in the modification of m5C (5-methylcytosine) at the wobble position (34) of $tRNA^{Leu}$, resulting in preferential decoding of TTG codons and translation of transcripts with high occurrence[130]. Recent investigations have highlighted the implications of PTrM alteration by linking a number of human pathologies directly to defects in tRNA PTrMs (extensively reviewed in ref. 131).



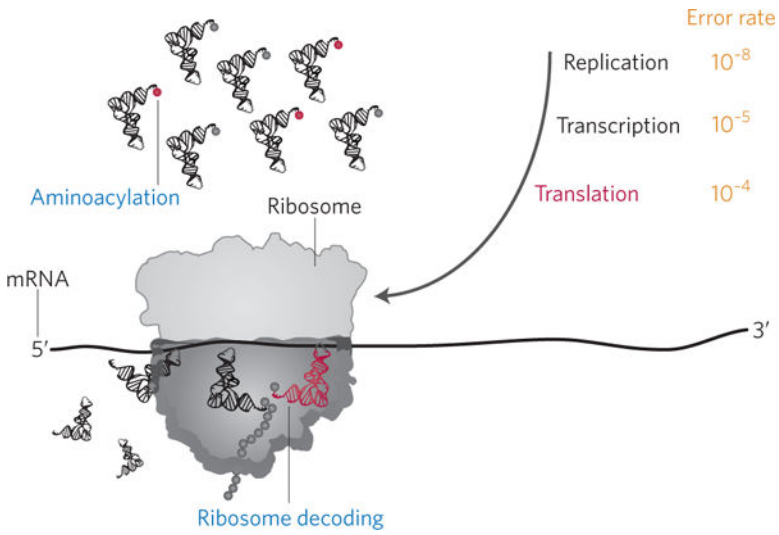


Figure 1. Opportunities for mistranslation

Protein mistranslation may result from errors accumulated at multiple steps within the cell's replicative cycle. During genome duplication, misreading by DNA polymerase leads to amino acid substitutions through direct alteration of the genetic code. Likewise, misreading of DNA templates by RNA polymerases may result in transcriptional errors that alter specific amino acid identity. More frequently, mistranslation occurs at the proteome level during translation, due to misacylation of tRNA (via defects in aaRS, tRNA modification or amino acid imbalance), or through decoding errors at the ribosome.

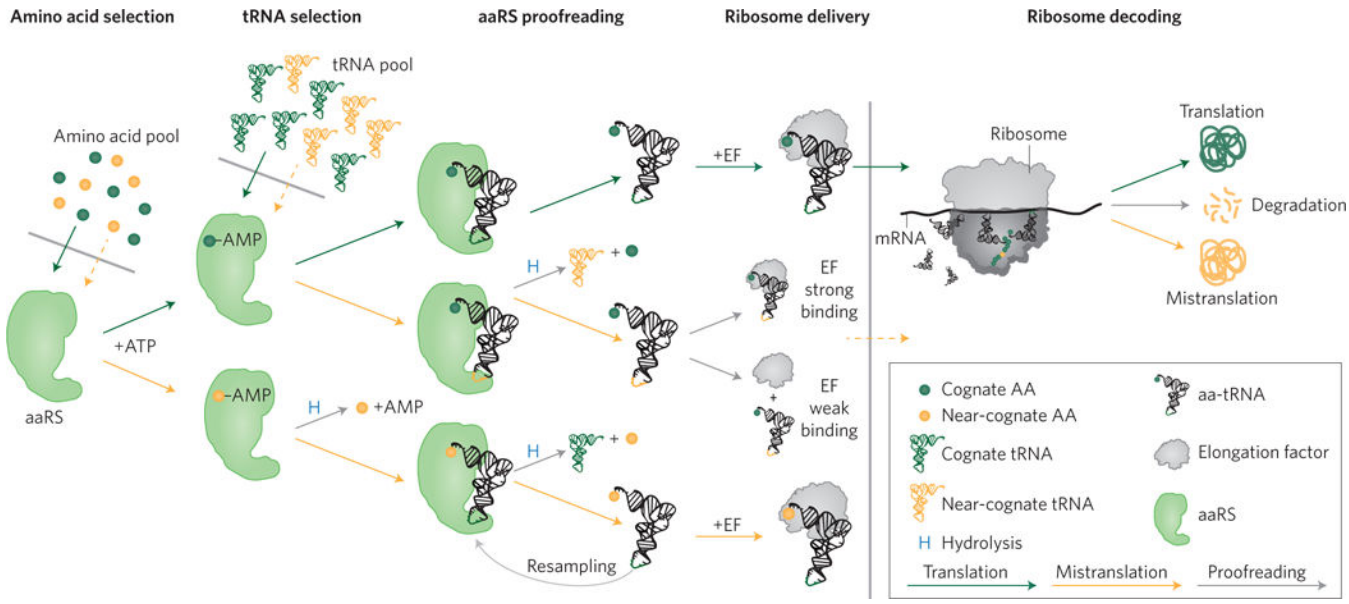


Figure 2. Aminoacylation and proofreading of tRNA

Usually, aaRSs efficiently select cognate amino acids and tRNAs from a pool of near-cognate substrates through structural and kinetic discrimination. However, occasionally, non-cognate amino acid substrates are activated, which may be resolved through hydrolysis of the aa-AMP (pre-transfer editing) at the active site. If misactivated amino acids are aminoacylated to tRNA (an issue common for near-cognate amino acids), an additional proofreading mechanism may hydrolyse the aminoacyl linkage (post-transfer editing) at the aaRS. Misaminoacylated tRNAs that initially escape proofreading mechanisms may be resampled and removed from the aa-tRNA pool by aaRSs and *trans*-editing factors. Aside from aaRS-mediated proofreading, misaminoacylated tRNAs are subjected to additional counter selection through thermodynamic interactions with EFs that deliver substrates to the ribosome and during codon decoding at the ribosome. The impact of misaminoacylated tRNAs that bypass these proofreading mechanisms (which results in amino acid misincorporation) may be further eliminated by degradation of the mistranslated protein. The surviving proteins that contain incorrectly incorporated amino acids are mistranslated, which may impact cellular survival and fitness.

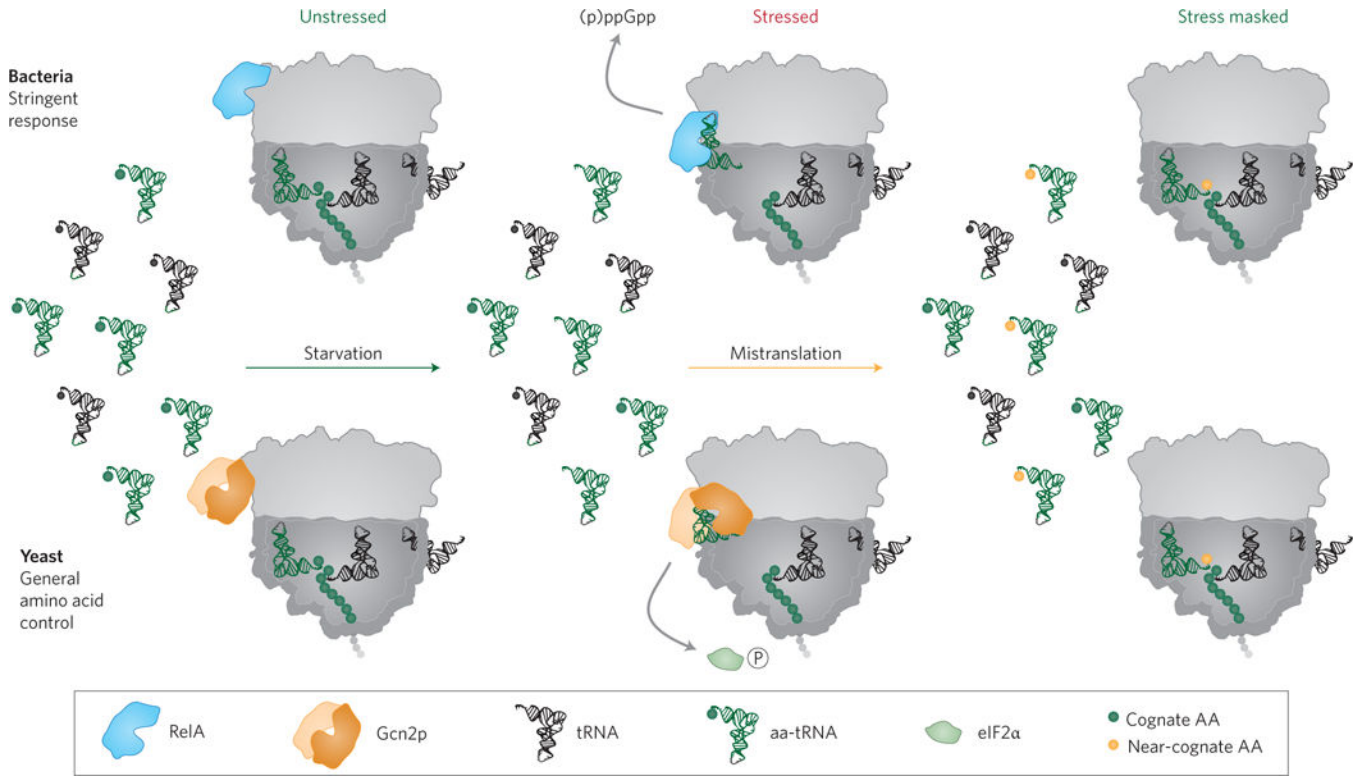


Figure 3. Misacylation of tRNA can mask amino acid starvation

Both prokaryotic and eukaryotic cells have evolved mechanisms to specifically monitor the levels of deacylated tRNA within the cell. In bacteria, during stress conditions such as amino acid starvation, deacylated tRNA within the A site of the ribosome is transferred to RelA, which synthesizes (p)ppGpp and leads to induction of the stringent response. In eukaryotes, the global level of deacylated tRNA is directly monitored by the protein kinase Gcn2p. When bound to deacylated tRNA, Gcn2p reduces the global translation rate, initiating the GAAC stress response to promote cell survival. In both cases, tRNA misaminoacylation may directly inhibit the accurate sensing of deacylated tRNA levels in the ribosome and delay stress response activation. eIF2α, eukaryotic initiation factor 2α; P, phosphorylation.

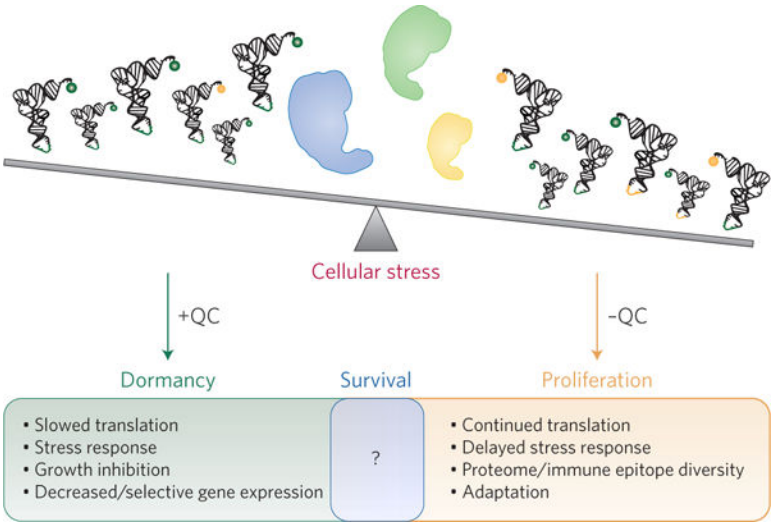


Figure 4. Strategies for adaptation and survival

Protein translation and the corresponding machinery are centrally positioned to assess the severity of stress and modulate the stress response, and quality control (QC) probably plays an important role in the maintenance of cellular homeostasis beyond the scope of proteome fidelity. In some instances, it may be appropriate for the cell to temporarily inactivate QC mechanisms to delay the stress response and maintain growth rates (proliferation) until the stress event has been resolved. However, as a cell encounters stress, the consequences of stress response activation must be weighed carefully against the deleterious effects of the stress itself, often leading to downregulation of cellular processes (dormancy). Observations of the cellular response to stress, however, are less polarized and are more accurately reflected as an intersection of proliferation and dormancy states governed by a practical balance of QC regulation, which ensures cellular viability (survival).

Table 1

Scope of mistranslation.

Mistranslation event	Error rate	Scope of mistranslation	Organism	Domain	Quantification method	Ref.
Ile → Val	10^{-4}	Regional	Rabbit	Eukaryota	Radiolabelled AA	119
Arg/Trp → Cys	10^{-3}	Global	*E. coli*	Bacteria	Radiolabelled AA	75
Leu → Met	~2%	Regional	*A. pernix*	Archaea	Mass spectrometry	77
Arg (CGU/C) Cys	10^{-5}–10^{-4}	Local	*E. coli*	Bacteria	Mass spectrometry	74
24 codons → Lys	10^{-4}	Global	*E. coli*	Bacteria	Biochemical	57
Tyr → His	10^{-6}	Regional	*S. cerevisiae*	Eukaryota	Biochemical	66
UGA readthrough	10^{-2}	Global	*E. coli*	Bacteria	Immunoblot	120
Asn → Asp	~10%	Regional	*E. coli*	Bacteria	Mass spectrometry	121
UAC → His	10^{-6}	Local	*S. cerevisiae*	Eukaryota	Biochemical	66
Global → His/Lys/Trp	10^{-3}–10^{-2}	Global	*E. coli*	Bacteria	Mass spectrometry	120
Stop-codon readthrough	~0.4%	Global	*B. subtilis*	Bacteria	Biochemical	122
Asn → Lys	10^{-4}–10^{-3}	Regional	*E. coli*	Bacteria	Isoelectric focusing	69
Leu/Val/Ser → Phe	10^{-5}–10^{-3}	Global	*In vitro*	–	Biochemical	123
Gly (GGC) → Ser	10^{-3}	Regional	*E. coli*	Bacteria	Biochemical	124
+1 Frameshift	10^{-5}	Global	*E. coli*	Bacteria	Biochemical	125
Phe → Tyr	~6%	Regional	*S. cerevisiae*	Eukaryota	Mass spectrometry	106
Global → Met	1–10%	Global	*S. cerevisiae*	Eukaryota	Radiolabelled AA	70
Asn → Asp	~0.8%	Regional	*M. smegmatis*	Bacteria	Biochemical	93
Gln → Glu	~0.8%	Regional	*M. smegmatis*	Bacteria	Biochemical	93
Ser (CUG) → Leu	3–5%	Local	*C. albicans*	Eukaryota	–	95
Phe → Leu	1–5%	Regional	*S. solfataricus*	Archaea	Radiolabelled AA	72
Thr → Ser	ND	Regional	*P. abyssi*	Archaea	Biochemical	42
Arg (AGA) → Lys	36–42%	Local	*E. coli*	Bacteria	Mass spectrometry	126

Protein mistranslation arises from defects in a number of cellular processes related to translation of the genetic code. Depending on the source of mistranslation, the impact to the proteome can range from local mistranslation (a change at a single codon) to regional mistranslation (substitution of one amino acid for another, regardless of codon) to global mistranslation (indiscriminate misincorporation of an amino acid). This table provides a snapshot of known mistranslation events across all domains of life. The methods used to monitor mistranslation within the cell include biochemical (*in vitro* or reporter-based kinetics), radiolabelled (following amino acids or proteins labelled with radioactive isotopes) and analytical chemistry methods (mass spectrometry and isoelectric focusing). Together, these techniques form a toolbox for the exploration of protein mistranslation. ND, not determined; radiolabelled AA, radiolabelled amino acids; *B. subtilis*, *Bacillus subtilis*; *M. smegmatis*, *Mycobacterium smegmatis*; *S. solfataricus*, *Sulfolobus solfataricus*; *P. abyssi*, *Pyrococcus abyssi*.