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Mistranslation of the genetic code

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Abstract

During mRNA decoding at the ribosome, deviations from stringent codon identity, or “mistranslation,” are generally deleterious and infrequent. Observations of organisms that decode some codons ambiguously, and the discovery of a compensatory increase in mistranslation frequency to combat environmental stress have changed the way we view “errors” in decoding. Modern tools for the study of the frequency and phenotypic effects of mistranslation can provide quantitative and sensitive measurements of decoding errors that were previously inaccessible. Mistranslation with non-protein amino acids, in particular, is an enticing prospect for new drug therapies and the study of molecular evolution.

Keywords

Amino acid; mistranslation; protein synthesis; quality control; translation; tRNA

The flow of information from the genetic code to the protein code is an imperfect process. Errors in transcribing messenger RNA from the genomic template and in decoding mRNA at the ribosome typically occur at low levels, yielding protein populations with only minor sequence variability [1]. One of the primary effectors of this high degree of quality control is the aminoacyl-tRNA synthetase family of enzymes, which pair amino acids with their appropriate tRNAs to form aminoacyl-tRNAs for use as substrates in peptide synthesis by the ribosome [2]. Errors in aminoacyl-tRNA synthetase function can result in the production of mispaired aminoacyl-tRNAs and erroneous insertion of amino acids at codons in a manner not defined by the genetic code, a phenomenon called "mistranslation” [3, 4]. These deviations from the genetic code can be associated with a loss of protein structural and functional integrity as well as phenotypic defects and disease [5]. Recent work has demonstrated that mistranslation may benefit the cell in certain circumstances (see [6] and references therein) and that some organisms have selected for a higher degree of mistranslation than others [7–10]. Moreover, conditional cellular stress presents unique challenges to accurate aminoacyl-tRNA synthesis [11, 12], underscoring the emerging view of quality control as a dynamic process dependent on the cellular microenvironment and other evolutionary pressures.

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Introduction

Central to all life is the flow of information from a genetic code to an RNA and protein code [13]. Transcription of genetic information into an RNA code and translation of that RNA code into an amino acid sequence are processes that have long been thought to tolerate few errors. An inaccurately transcribed DNA base can result in an mRNA codon with different identity, and inaccurate decoding of mRNA codons at the ribosome can result in inappropriate amino acid insertion in a nascent peptide. Such errors in information flow can result in truncated and/or misfolded proteins, proteins with neutral or deleterious substitutions at critical residues [14], and an overall loss in protein function at the molecular and cellular levels [15].

Maintaining accurate ribosomal protein synthesis, in particular, is critical to all life. Atypical of most enzymatic processes in the cell, protein synthesis requires permissivity in the enzymatic binding site, allowing for dozens of substrate aminoacyl-tRNAs (aa-tRNAs) bearing the full complement of proteinogenic amino acids to be incorporated into proteins. The nature of genetically encoded amino acid sequences necessitates specificity at the ribosome for canonical aa-tRNAs, such that for each codon, only an aa-tRNA bearing the genetically encoded amino acid can bind and participate in protein synthesis. This specificity is achieved by ribosomal quality control mechanisms that rely on codon-anticodon interactions [16, 17] and discrimination against certain types of nonprotein amino acids (NPAs), which can be attenuated with ribosomal mutations [18, 19]. However, no such quality control mechanism exists in the ribosome to exclude aa-tRNAs formed from the linkage of a standard proteinogenic amino acid to a noncognate tRNA. In such a case, codon-anticodon interactions that pass the quality control steps at the ribosome will drive protein synthesis forward. Ribosomal quality control may instead act retrospectively, by increasing the frequency of errors in decoding a given mRNA, facilitating premature release of mis-synthesized peptides from the ribosome [20–22]. The phenomenon of amino acid insertion at a codon that codes for a different amino acid is termed “mistranslation,” and until recently has been thought to reflect a minor and infrequent imperfection in the protein synthesis machinery.

Mistranslation is typically limited to one erroneously inserted amino acid per 10^3–10^4 translated codons [1]. Many mutations and environmental conditions are known to elevate this error rate beyond tolerable limits [5, 23]. Recent studies have uncovered differences between organisms in the requirement for quality control in protein synthesis [7, 10, 24], suggesting that perfect decoding may not be inherently ideal. Mistranslation of the genetic code in response to cellular stress has been shown in some cases to serve as a clear benefit for the cell [23]. It is a misinterpretation of an ambiguous term to equate "mistranslation" with "mistakes" in all cases, as variability in decoding is sometimes evolutionarily conserved and favorable [25, 26]. In this review, we highlight challenges and recent advances in the way variability in decoding is measured, address environmental and evolutionary determinants of quality control in protein synthesis, and reevaluate the way we view “errors” in translational decoding to more accurately reflect the range of positive and negative effects that mistranslation has on the cell.

Mass Spectrometry as a Tool for Measuring Protein Mistranslation

One of the greatest challenges in studying mistranslation is quantitative measurement of amino acid substitutions, particularly low-frequency events. Traditionally, measurement of mistranslation has been carried out indirectly, by quantifying amino acid substitutions in exogenously expressed proteins, such as β-lactamase, green fluorescent protein, and others [27–29]. In these analyses, critical residues of the reporter protein of interest are mutated such that mistranslation of the codon of interest will restore the protein sequence and/or change the protein's functionality. Reporter protein activity is quantified under various conditions, and residue-specific mistranslation is inferred as a result.

There are several drawbacks to this kind of analysis. Replication of exogenous genetic material and expression of a protein reporter alter the metabolic profile of the host organism, potentially confounding studies of natural variation in decoding [30]. Moreover, biologically relevant low-frequency amino acid substitution events may be undetectable or underrepresented in these systems, and this type of analysis erroneously assumes that mistranslated peptides have comparable half-lives to the accurately translated form [31]. Perhaps most importantly, these techniques are used for detection of specific amino acid substitutions at a chosen codon, limiting the scope of study to a case-by-case analysis in a specific primary sequence context. Given the anticipated variables that determine mistranslation, another drawback of these types of analyses is the assumption that they reflect mistranslation of all relevant codons. As a result, it has long been difficult to properly and sensitively quantify typical amino acid substitution rates on a per-codon basis with multiple amino acid residues, and to address global rates and effects of mistranslation.

More recently, sophistication in analytical mass spectrometry has provided the means for direct, highly sensitive measurement of mistranslation at each codon with multiple amino acids. In particular, liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) with or without the use of multiple reaction monitoring (MRM) mode is the new technique of choice [32–35]. Proteolytically digested protein samples are separated by liquid chromatography and the eluent peptides are ionized via an electrospray source. In the case of a linear triple quadrupole setup, a target peptide of interest is mass-selected on the first quadrupole and is fragmented in a collision chamber. Resulting fragment ions are mass analyzed on a second quadrupole. MRM mode entails analysis of a selected few fragment ions on the second quadrupole, which contrasts full scan MS/MS, in which all resulting fragment ions are quantified. MRM yields greater sensitivity than full scan MS/MS, allowing for greater detection of low-frequency mistranslation. Alternatively, fragment ions may be mass analyzed with an orbitrap type mass analyzer, which also offers a high resolution and sensitivity. These techniques allow for measurement of normal levels of mistranslation at each codon in a global fashion, and have provided the tools to examine perturbations from the norm caused by changes in the cellular environment.

Industrial protein manufacturers have largely pioneered this analysis as a method to test the quality and homogeneity of their protein products. A common method of large-scale human antibody production involves exogenous expression in Chinese hamster ovary (CHO) cells [36]. Mammalian protein production is convenient, as post-translational modifications are

typically similar to the human protein products, and alternative synthetic chemistry methods are expensive and inefficient by comparison. The primary downside is that organismal protein synthesis is subject to typically low-level variability in decoding, resulting in a statistical population of protein products with heterogeneity in the primary sequence [33–35, 37].

Because variability in decoding can increase in certain environmental contexts, the exact growth media must be carefully controlled and quality control in protein products must be monitored frequently. Under conditions of tyrosine (Tyr) limitation, it was recently discovered that CHO cells suffer growth defects and accumulate phenylalanine (Phe) at Tyr codons in heterologously-produced monoclonal antibodies at frequencies as high as 0.7%, a value much greater than that quoted for typical mistranslation (~0.01%) [12, 34]. Upon further examination, it was discovered that tyrosyl-tRNA synthetase (CHO TyrRS), the enzyme responsible for producing Tyr-tRNA^{Tyr} in the CHO cytoplasm, exhibits inherently poor discrimination against Phe, which is similar in structure to cognate Tyr [12]. As a result, CHO TyrRS produces Phe-tRNA^{Tyr} which, when used as a substrate for protein synthesis at the ribosome, results in Phe misincorporated at Tyr codons. By increasing the bioavailability of tyrosine to CHO cells, this mistranslation is greatly decreased [38], indicating that amino acid starvation and poor discrimination by CHO TyrRS were responsible for mistranslation-dependent heterogeneity in the protein product.

Bacterial tyrosyl-tRNA synthetase (TyrRS) is highly specific for Tyr over Phe, even under Tyr limitation [39], so the poor discrimination exhibited by this higher eukaryote is surprising. This may be of particular interest to the study of diseases such as phenylketonuria, in which the ratio of Phe to Tyr is similarly affected [40], and results in human neurological defects similar to many diseases involving mutated aaRSs [5, 41]. Treatment of phenylketonurics includes dietary restriction of Phe and supplementation with Tyr such that normal intracellular Phe/Tyr ratios are maintained in the absence of adequate phenylalanine hydroxylase activity. In a broader sense, the absence of stringent quality control to limit mistranslation at Tyr codons in CHO cells is further evidence that the evolutionary determinants for accuracy in protein synthesis are apparently varied. Many specific and conditional mistranslation events are known [11, 12, 23, 26, 35, 37, 42, 43], and we are just beginning to scratch the surface of which types of amino acid substitutions occur in which organisms, under what conditions, by what mechanisms, and to what effect.

Evolutionary Surprises in Decoding Dynamics

Most organisms contain at least 20 aminoacyl-tRNA synthetases (aaRSs) [44], which pair tRNAs with each proteinogenic amino acid to produce aminoacyl-tRNAs (aa-tRNAs), the substrates for ribosomal protein synthesis (Figure 1). For the ribosome to perform its function accurately, aaRSs must bind only their appropriate, or "cognate," amino acids and tRNAs, lest misacylated aa-tRNAs be synthesized. aaRSs accomplish this discriminatory task primarily by exclusion of incorrect or "noncognate" amino acids and tRNAs. Noncognate amino acids and tRNAs that vary in size, shape, charge, and/or hydrophobicity will be excluded from respective binding pockets. Because tRNAs differ from one another by a larger surface area and more discriminatory functional groups than amino acids, tRNA

selection is typically the lesser challenge [45–47]. aa-tRNA synthesis is a two-step process [2]. In the first step, aaRSs catalyze the synthesis of activated amino acids, called aminoacyl-adenylates (aa-AMPs) using the chemical energy stored in ATP. These aa-AMPs are the substrates for the second catalytic step, whereby the aminoacyl moiety is transferred to the 3'-OH of a tRNA bound to the aaRS. In the event that a noncognate amino acid is not excluded in the binding pocket, it may be activated, forming an aa-AMP. Some aaRSs prevent subsequent aminoacyl-tRNA formation by hydrolyzing misactivated aa-AMP, a processed termed "pre-transfer editing" [48], which is tRNA-dependent in some cases [49]. If this does not occur, a mispaired aa-tRNA may be synthesized and released, providing a substrate for mistranslation at the ribosome. Approximately half of the aaRSs bear a catalytic domain independent of the canonical aa-tRNA synthetic site. This separate “editing” domain serves to hydrolyze misacylated tRNAs. Termed “post-transfer editing,” aa-tRNA hydrolysis may be performed by an aaRS in *cis*, prior to aa-tRNA release, or upon re-binding a misacylated aa-tRNA species in *trans* [50]. In addition, freestanding post-transfer editing domain homologues serve as an additional layer of quality control [51–54]. Post-transfer editing limits the population of mischarged tRNA species that may threaten accurate decoding at the ribosome. The types of aaRS editing have been reviewed extensively elsewhere [3].

The standard genetically encoded proteinogenic amino acids are not the only threats to quality control in aa-tRNA synthesis. Additional "non-protein" amino acids (NPAs) with similar physiochemical properties to proteinogenic amino acids must be discriminated against by relevant aaRSs. In the past year, post-transfer editing has been implicated to a greater degree as a mechanism by which the cell is protected from NPAs generated under conditions of stress. In *E. coli*, phenylalanyl-tRNA synthetase (PheRS) bears a post-transfer editing domain, the activity of which is dispensable under normal conditions. The near-cognate proteinogenic amino acid Tyr is not misacylated to $tRNA^{Phe}$ to a degree that threatens cellular viability, and PheRS editing-deficient mutants survive in the presence of Tyr. Under conditions that favor the formation of reactive oxygen species, however, PheRS editing is critical for cellular survival. It was discovered that the NPA *meta*-tyrosine (*m*-Tyr) accumulates under oxidative stress, is charged appreciably to $tRNA^{Phe}$ and is translated at Phe codons[11]. Taken together, this suggests that post-transfer editing by *E. coli* PheRS, while not necessary under normal conditions, is critical to protect the cell from cytotoxic mistranslation with a NPA under conditions of oxidative stress. In the yeast *Saccharomyces cerevisiae* cytosolic PheRS similarly bears post-transfer editing domain activity, whereas mitochondrial PheRS relies instead on high discrimination in the activation step to maintain quality control (Figure 2). Mutational attenuation of mitochondrial PheRS selective discrimination for Phe/Tyr from ~12,000:1 to ~700:1 limits growth on respiratory media and prevents mitochondrial biogenesis[55]. Ablation of cytoplasmic PheRS editing activity has no effect on Phe/Tyr selectivity, and mutants maintain viability but become sensitive to elevated Phe:Tyr ratios. The *S. cerevisiae* post-transfer editing mutant also exhibits a phenotypic loss of viability under conditions of oxidative stress (unpublished data). This suggests that post-transfer editing by *S. cerevisiae* cytosolic PheRS and *E. coli* PheRS may be conserved in part to protect the cell from mistranslation of Phe codons with the NPA *m*-Tyr that accumulates under conditions of oxidative stress.

Similarly, post-transfer editing by *E. coli* leucyl-tRNA synthetase (LeuRS) has been shown to typically be dispensable, and the near-cognate proteinogenic amino acid isoleucine (Ile) is not an efficient substrate for LeuRS [56]. Under conditions that favor the accumulation of certain near-cognate proteinogenic amino acids and NPAs, LeuRS post-transfer editing is critical [57, 58]. Norvaline is an efficient substrate for LeuRS and may represent a significant threat to quality control at leucine codons under conditions of oxygen limitation, which induce intracellular norvaline accumulation [56, 57]. Again, it seems that evolution may favor the conservation of post-transfer editing in part to protect the cell against cytotoxic mistranslation of the genetic code with NPAs, some of which are only biologically relevant threats to the cell under stress conditions. More examples of this phenomenon are likely to arise in the near future, and current models will be refined as broad analysis with new tools from mass spectrometry allow us to uncover additional mistranslated noncognates and we begin to better understand the role of NPAs.

Defining Mistranslation, Deviation from the Norm, and Effects on the Cell

In recent years, mistranslation has been viewed through a different lens than in previous decades. This is due in part to the observation of high degrees of mistranslation tolerated in certain species and the discovery of conditional increases in the frequency of mistranslation that mitigate environmental stress. Under conditions of oxidative stress, non-methionyl tRNAs can be methionylated by methionyl-tRNA synthetase from *E. coli* [25], yeast [26], and mammals [23]. Because methionine may spontaneously react with reactive oxygen species (ROS) that are formed under oxidative stress, methionine residues mistranslated at non-methionyl codons may serve as ROS "sinks," to be later safely reduced by methionine sulfoxide reductases [59]. Such “adaptive translation” is reviewed extensively in [6]. In one notable example the *Candida albicans* CUG codon is inherently decoded in an ambiguous manner, and the resulting proteomic and phenotypic diversity [9] may make this opportunistic pathogen a "moving target" for the host’s adaptive immune system [8].

Taken together, these examples illustrate the substantial difficulty in simply defining mistranslation, given the degree to which some organisms tolerate or benefit from codon ambiguity. “Mistranslation,” “errors” in protein synthesis, and “accuracy” in translation are terms that implicitly assign a negative value to deviations from stringent definitions of codon identity. Moreover, consideration of the basal level of mistranslation on a per-protein level is too limited in scope, as the full complement of proteins in the cell has, by the statistical nature of misincorporation, a wide range of primary sequences (Figure 3). This “statistical protein” model implies a frequency of misincorporation at every codon, such that any given amino acid has a certain probability of translation at a given codon. Quality control mechanisms inherent to the translation machinery limit these errors, but the system is imperfect and dynamic; perturbations in amino acid pools [11, 12], modification in the copy number or modification status of tRNAs [60–62], aaRSs [29], can all change the frequency of translated amino acids at a given codon. Expanding this picture to include every newly synthesized protein in new growth conditions and retaining the non-degraded protein from all previous growth conditions further complicates the picture of the mistranslation frequencies of protein sequences in the cell.

Because the primary sequence of a protein determines its fold and function, variation in the primary sequence can result in "neomorphic" proteins, which bear new and different functions in binding and catalysis than those of the parent protein. Variations in protein sequence due to genetic mutation are the basis of evolution, resulting in heritable allelic diversity. Mutation is typically deleterious or neutral at best, but occasionally refines or gives new beneficial function to a protein. These heritable mutant proteins may grant the organism a selective advantage and thus the new sequence becomes a feature of the species.

Mistranslation in a protein population instead results in non-heritable diversity at the protein level. A population of proteins with variability in their primary sequence may have varying degrees of mistranslation with many different amino acids at many different positions, potentially yielding neomorphic individual proteins. Proteins with neomorphic moonlighting functions implicated in disease have been discussed elsewhere [63, 64]. Under normal conditions, the effects of low-frequency mistranslation may be minimal. In conditions that increase the frequency of these replacement events, drastically varied peptide sequences may become a double-edged sword: whereas randomness in protein populations may decrease the binding and catalytic function of a protein population as a whole and can result in aggregation and growth defects [5], individual proteins with neomorphic properties may grant the cell access to new and beneficial binding partners and catalytic activities not derived from the genetically-encoded primary sequence. The cost-benefit calculus of protein quality control thus depends on environmental factors and the complement of tools for combating the negative effects of mistranslation at the disposal of the organism in question. Perhaps some NPAs conditionally charged to tRNAs will be discovered to have a role that benefits, rather than harms the cell. Such a conditional increase in the protein alphabet implies new modes of binding and, depending on the chemical properties of the NPA, possibly unknown modes of catalysis.

Outlooks

The fact of conditional mistranslation is no longer a surprise, but the tools for deeper study of its possible significance have been lacking until recently. Greater analytical power afforded by sensitive mass spectrometry and bioinformatic tools will soon make this the technique of choice for the study of mistranslation. Mass spectrometry can be used to directly detect and quantify low-level mistranslation as well as identify, in a codon-specific manner, the kinds of noncognate amino acids translated, and under what conditions. Particularly, knowledge of the role and prevalence of mistranslation with NPAs will be expanded; additional NPAs may be identified at multiple codons with multiple phenotypic effects. A global picture of mistranslation in wild-type organisms from multiple branches of the tree of life, under various conditions, will provide a picture of evolutionarily conserved quality control mechanisms and various degrees of permissive decoding. aaRSs in particular are deeply conserved cellular factors, due to their central role in translation, and it is expected that variation in aaRS specificities between organisms may provide targets for therapeutic and antibiotic treatment [65]. High throughput use of these techniques may be used to identify and treat protein disease on a per-patient basis. We may even see the design of synthetic NPAs that target specific aaRSs under certain conditions, a prospect that has many implications for cellular synthetic protein chemistry and drug discovery. Translation

of specific codons with NPAs has already been used to introduce novel chemistry into cells, which allows for new analytical techniques, protein-drug conjugation, and novel protein interactions *in vivo* [66–69].

Recently, it was discovered that the NPA β-aminomethylalanine (BMAA) is mistranslated at serine (Ser) codons in human tissue [43]. Media supplementation with serine drastically reduces the degree of this mistranslation, suggesting that noncognate BMAA competition with cognate Ser for seryl-tRNA synthetase may be the source of the error in protein synthesis. Ingestion of BMAA is associated with an increased risk for neurodegenerative disorders such as amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinsonism [70–72]. It is a tantalizing prospect to use dietary supplementation with cognate amino acids as a treatment for diseases such as these, as proteinogenic amino acids are cheap to produce, are widely available over the counter, and are normal human metabolites. It is conceivable that inducing specific changes in amino acid pools, chemically modifying the selectivity of aaRSs, or designed NPA treatment may be used in various combinations to combat many diseases with minimal deleterious effects on the patient.

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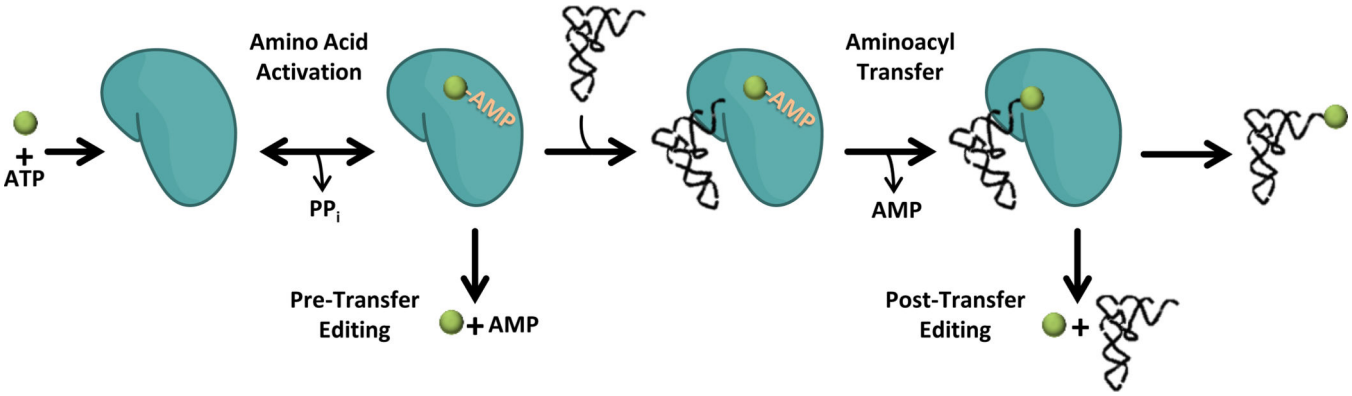

Figure 1. Aminoacyl-tRNA synthesis and quality control mechanisms

aa-tRNA synthesis is a two-step process, with checkpoints at each step to ensure proper product formation. An aminoacyl-adenylate formed by the activation of a noncognate amino acid may be hydrolyzed by pre-transfer editing mechanisms in the active site of the aaRS. Misactivated amino acids that escape pre-transfer editing may be acylated to tRNA, forming aa-tRNA. If an aaRS bears a post-transfer editing domain, mispaired aa-tRNA may be hydrolyzed, releasing the amino acid and tRNA.

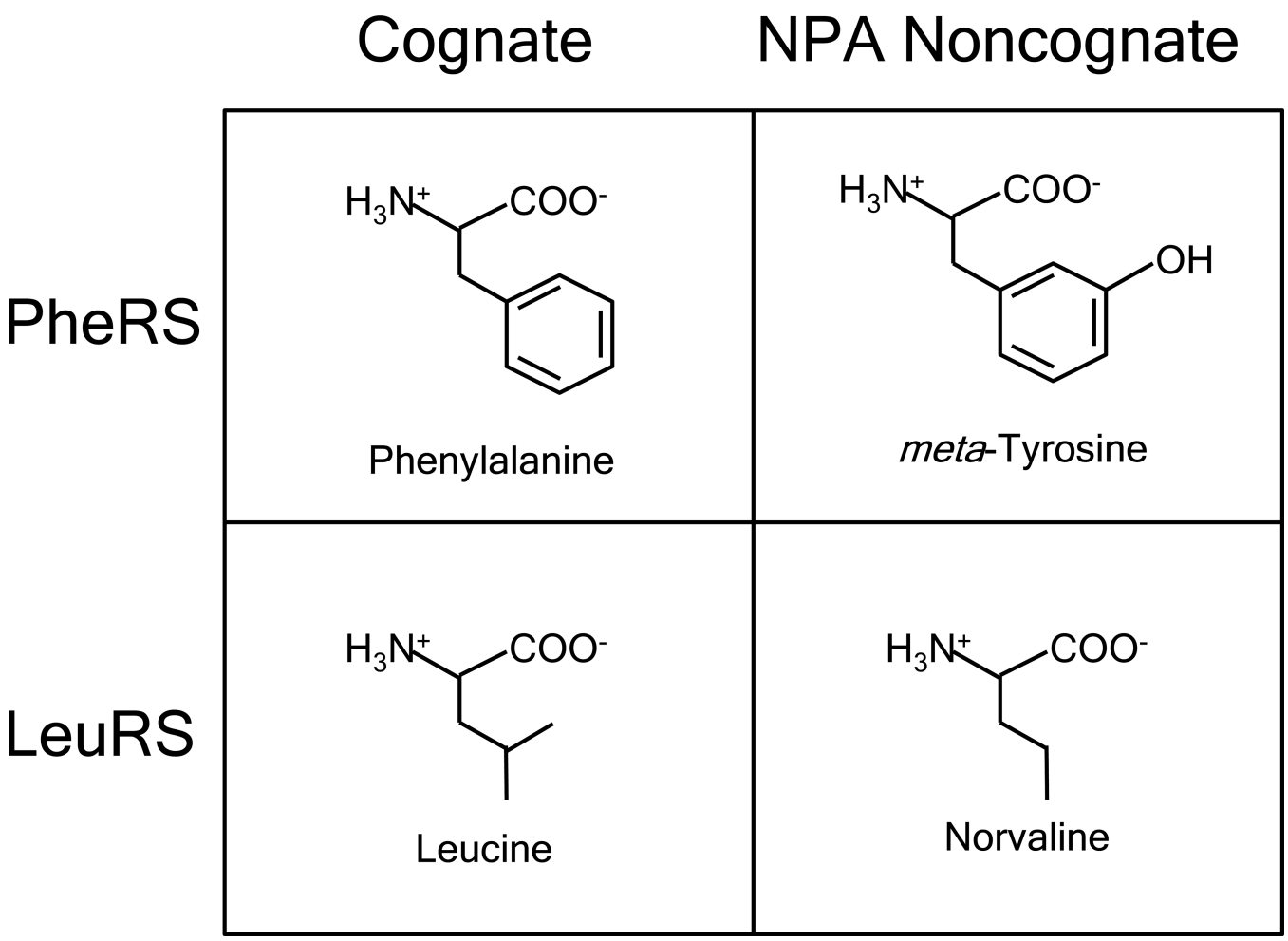

Figure 2. Non-protein amino acids as a threat to quality control in aminoacyl-tRNA synthesis

NPAs that pose a threat to translational fidelity bear similar physiochemical and structural features compared to cognate amino acids. *E. coli* PheRS post-transfer editing activity prevents release of $tRNA^{Phe}$ charged with the NPA *m*-Tyr, which differs from cognate Phe by a single oxygen atom [11]. *E. coli* LeuRS post-transfer editing activity prevents release of $tRNA^{Leu}$ charged with norvaline, which differs from cognate Leu by a methylene group [56, 58].

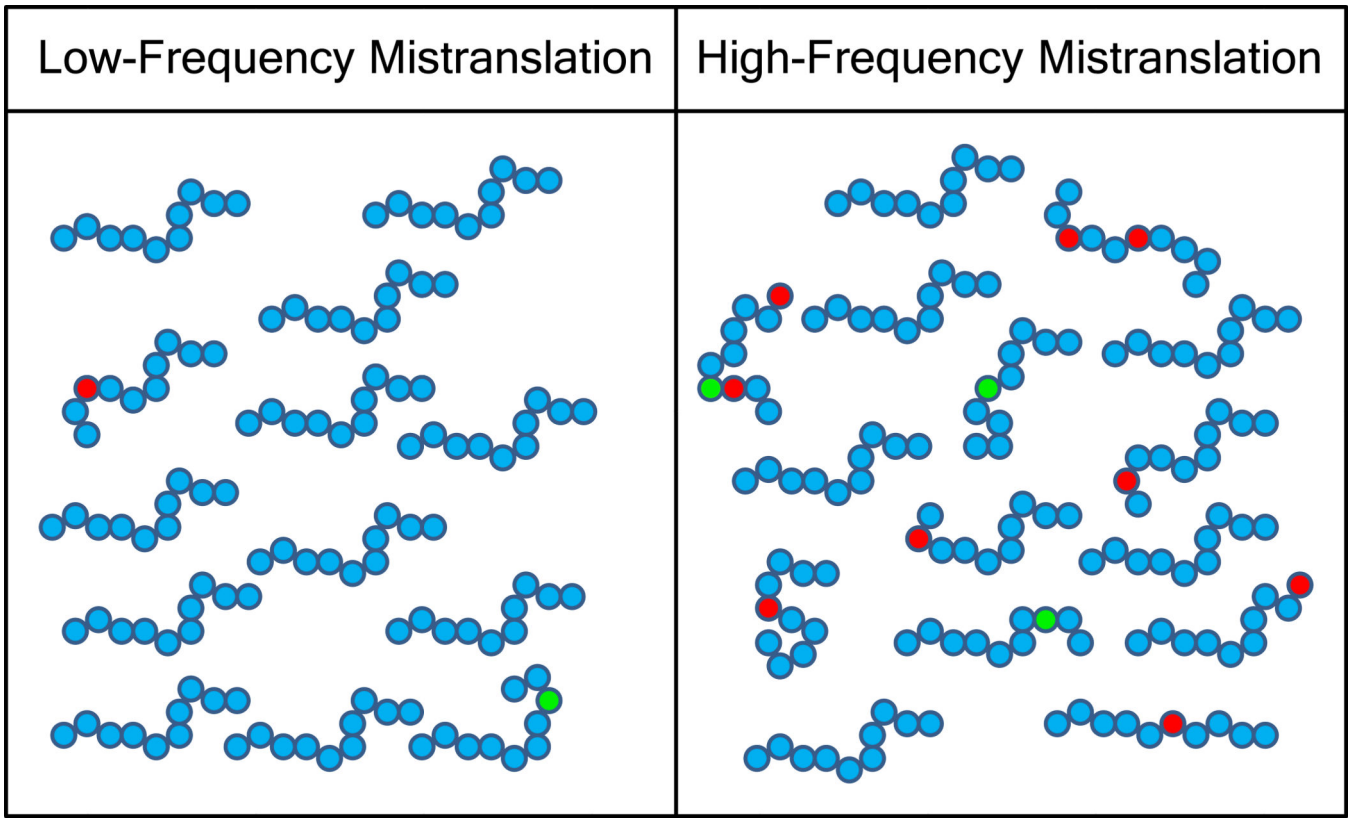

Figure 3. Mistranslation and the statistical proteome

Represented here are various copies of a single protein arising from translation in low- or high-frequency mistranslation systems. Amino acids inserted at appropriate codons are shown as blue. Inappropriately inserted amino acids are represented as green and red. Typical mistranslation is infrequent, resulting in protein populations with minor variability. In organisms that naturally mistranslate more frequently [7, 10], or in conditions that promote less stringent quality control [12, 23, 25, 26], protein populations become more diverse in their primary sequences. Proteins arising from “statistical proteomes” have various folding and binding properties, resulting in phenotypic diversity in the host organism. Expanded to include all proteins in a cell, the effects of mistranslation can be drastic at the molecular and cellular levels.