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Review

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.

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.

1. 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

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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 non-protein 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 non-cognate 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.

2. 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