Perfectly accurate translation of mRNA into protein is not a prerequisite for life. Resulting from errors in protein synthesis, mistranslation occurs in all cells, including human cells. The human genome encodes >600 tRNA genes, providing both the raw material for genetic variation and a buffer to ensure that resulting translation errors occur at tolerable levels. On the basis of data from the 1000 Genomes Project, we highlight the unanticipated prevalence of mistranslating tRNA variants in the human population and review studies on synthetic and natural tRNA mutations that cause mistranslation or de-regulate protein synthesis. Although mitochondrial tRNA variants are well known to drive human diseases, including developmental disorders, few studies have revealed a role for human cytoplasmic tRNA mutants in disease. In the context of the unexpectedly large number of tRNA variants in the human population, the emerging literature suggests that human diseases may be affected by natural tRNA variants that cause mistranslation or de-regulate tRNA expression and nucleotide modification. This review highlights examples relevant to genetic disorders, cancer, and neurodegeneration in which cytoplasmic tRNA variants directly cause or exacerbate disease and disease-linked phenotypes in cells, animal models, and humans. In the near future, tRNAs may be recognized as useful genetic markers to predict the onset or severity of human disease.

Protein synthesis is an evolutionarily conserved process that is required by all life. In the interpretation of the genetic code, transfer RNAs (tRNAs) play a central role as they physically link tRNAs to ribosomes separately prepared with each of the possible 64 trinucleotide codons. These efforts established the standard set of 20.

Given the importance of cytosolic tRNAs to facilitate accurate synthesis of the proteome, surprisingly few examples have linked a cytosolic tRNA mutation to human disease thus far. Yet, recent examples directly connecting cytosolic tRNA mutations to disease in humans (19) and separately to neurodegeneration (20, 21) and cancer (22) in mice suggest that cytosolic tRNA variants play a greater role in disease than previously imagined. It is possible that significant changes to tRNA function are not usually tolerated in the genome or that defective tRNA alleles may be genetically buffered by multiple copies of each iso-decoder. Nevertheless, two empirical observations suggest tRNAs have a larger role in disease than previously recognized: (i) the unexpectedly large number of tRNA variants in the human population (Tables 1–3 and Table S1), and (ii) the fact that even a single nucleotide change in a single tRNA gene can cause mistranslation or stall translation leading to molecular...
lar and cellular defects (13, 21, 23). The majority of research connecting human tRNA functions to disease is focused on mutations in aminoacyl-tRNA synthetases (AARS) (24), on proteins that modify nucleotides in cytosolic tRNAs (25, 26), or on the smaller pool of mitochondrial tRNAs (27–29). Two major reasons for this are a relative lack of available sequence data for cytosolic tRNAs, and a long-held assumption that excessive tRNA copy number should “buffer” potential phenotypes resulting from a single mutant.

In this review, we outline the complexity of cytosolic tRNA function and regulation in eukaryotic cells. We then summarize recent studies demonstrating examples of single nucleotide

### Table 1

| tRNA gene | Variant | MAF (%)<sup>a</sup> | Variant count<sup>a</sup> | MAF (%)<sup>b</sup> | Variant count<sup>b</sup> | tRNA score<sup>c</sup> | Codon identity | tRNA identity | Expression | ARM<sup>e</sup> | CHIP<sup>e</sup> |
|-----------|---------|----------------------|------------------------|----------------------|------------------------|------------------------|----------------|----------------|------------|-------------|-------------|
| Ala-AGC-2–2 | G35A | 0.02 | 1 | 6.47 | 8130 | 84.7 | Val | Ala | + | + |
| Ala-AGC-6–1 | G35C | 6.55 | 328 | 42 | 56.7 | Thr | Ala | + | – |
| Ala-AGC-15–1 | _36T | 0.04 | 2 | 0.03 | 2 | 53.1 | Val | Ala | + | – |
| Ala-AGC-16–1 | G35A | 2.2 | 11 | 2.1 | 269 | 79.7 | Val | Ala | + | + |
| Ala-GGC-1–1 | G35T | 0.0008 | 1 | 80.5 | Val | Ala | + | + |
| Ala-TGC-1–1 | G35A | 0.0008 | 1 | 77.3 | Thr | Leu | + | + |
| Leu-CAA-3–1 | A35C | 0.0008 | 1 | 89.6 | Phe | Ser | + | + |
| Ser-AGA-2–2 | G35A | 0.02 | 0.003 | 22 | 90.4 | Leu | Ser | + | + |
| Ser-AGA-2–3 | G35A | 1.82 | 34 | 4 | 89.6 | Cys | Ser | + | + |
| Ser-AGA-2–4 | G35C | 0.03 | 1 | 89.6 | Phe | Ser | + | + |
| Ser-AGA-2–5 | _35T | 0.0008 | 1 | 89.7 | Gly | Ala | + | + |
| Ser-AGA-2–6 | A3G | 0.01 | 12 | 90.1 | Gly | Ala | + | + |
| Ser-TGA-2–1 | G35A | 0.04 | 2 | 0.02 | 22 | 90.4 | Leu | Ser | + | + |
| Ser-TGA-3–1 | _36T | 0.0008 | 1 | 89.7 | Gly | Ala | + | + |

<sup>a</sup> Data are from the 1000 Genomes Project (127, 128).

<sup>b</sup> Data are from the TOPMED sequencing project (128, 129).

<sup>c</sup> tRNA score was calculated using tRNA-Scan SE (128).

<sup>d</sup> ARM indicates ARM-seq data suggesting expression (128, 130).

<sup>e</sup> CHIP indicates CHIP-seq hits for at least 3 of 4 core transcription proteins (RPC155, POLR3G, BRF1, and BDP1) (46, 129, 131–133).

### Table 2

| tRNA gene | Variant | MAF (%)<sup>a</sup> | Variant count<sup>a</sup> | MAF (%)<sup>b</sup> | Variant count<sup>b</sup> | tRNA score<sup>c</sup> | Expression | ARM<sup>e</sup> | CHIP<sup>e</sup> |
|-----------|---------|----------------------|------------------------|----------------------|------------------------|------------------------|------------|-------------|-------------|
| Arg-ACG-1–3 | C70U | 0.02 | 2 | 68 | + | + |
| Cys-GCA-2–3 | C70U | 0.02 | 29 | 82 | + | + |
| Cys-GCA-1–1 | C70U | 0.02 | 86 | 100 | + | + |
| Cys-GCA-17–1 | C70U | 0.02 | 71 | 72 | + | + |
| Cys-GCA-12–1 | C70U | 0.02 | 16 | 72 | + | + |
| Gly-CCC-1–1 | G35A | 0.05 | 64 | 100 | + | + |
| Gly-CCC-2–4 | A3G | 0.05 | 63 | 81 | + | + |
| Gly-GCC-2–5 | A3G | 0.05 | 6 | 81 | + | + |
| Gly-GCC-2–1 | A3G | 0.02 | 20 | 81 | + | + |
| Gly-GCC-2–3 | A3G | 0.02 | 81 | 81 | + | + |
| Gly-GCC-1–1 | A3G | 1.2 | 1633 | 81 | + | + |
| Gly-GCC-2–1 | A3G | 0.005 | 6 | 81 | + | + |
| Gly-CCC-2–6 | C70U | 0.02 | 129 | 81 | + | + |
| Ser-AGA-2–6 | A3G | 0.01 | 90 | 81 | + | + |

<sup>a</sup> Data are from the 1000 Genomes Project (127, 128).

<sup>b</sup> Data are from the TOPMED sequencing project (128, 129).

<sup>c</sup> tRNA score was calculated using tRNA-Scan SE (128).

<sup>d</sup> ARM indicates ARM-seq data suggesting expression (128, 130).

<sup>e</sup> CHIP indicates CHIP-seq hits for at least 3 of 4 core transcription proteins (RPC155, POLR3G, BRF1, and BDP1) (46, 129, 131–133).

### Table 3

| tRNA gene | SNP ID | MAF (%)<sup>a</sup> | Variant count<sup>a</sup> | MAF (%)<sup>b</sup> | Variant count<sup>b</sup> | tRNA score<sup>c</sup> | Expression | ARM<sup>e</sup> | CHIP<sup>e</sup> |
|-----------|--------|----------------------|------------------------|----------------------|------------------------|------------------------|------------|-------------|-------------|
| Arg-ACG-1–1 | rs6939540 | 2.42 | 121 | 2.2 | 2765 | 67.6 | + | + |
| Arg-ACG-1–2 | rs186104107 | 0.02 | 1 | 0.01 | 14 | 67.6 | + | + |
| Arg-ACG-1–3 | rs11370043 | 16.6 | 831 | 20.2 | 24374 | 53.7 | + | + |
| Arg-TCT-5–1 | rs14333427 | 0.8 | 41 | 0.6 | 711 | 61.4 | + | + |

<sup>a</sup> Data are from the 1000 Genomes Project.

<sup>b</sup> Data are from the TOPMED sequencing project (128, 129).

<sup>c</sup> tRNA score was calculated using tRNA-Scan SE (128).

<sup>d</sup> ARM indicates ARM-seq data suggesting expression (128, 130).

<sup>e</sup> CHIP indicates CHIP-seq hits for at least 3 of 4 core transcription proteins (RPC155, POLR3G, BRF1, and BDP1) (46, 129, 131–133).
tRNA variants that elicit significant levels of amino acid misincorporation, which can be surprisingly well-tolerated in eukaryotic cells. Using data from the 1000 Genomes Project, we analyzed the location and frequency of naturally occurring human tRNA variants. These data reveal an abundance of mis-translating tRNAs in the human population. Finally, we summarize recent evidence linking tRNA mutations and de-regulated tRNA expression and nucleotide modification to disease in humans and model systems. Some of the studies point to the idea that tRNA mutations, which are otherwise tolerated or benign, contribute to disease in the context of other coincidental cellular defects.

tRNA function and regulation

The role of tRNA in decoding the genetic code

tRNAs are best known for their role in translation of RNA messages into proteins. tRNAs are relatively small RNA molecules, typically consisting of 76–90 nucleotides, and fold into a conserved three-dimensional structure in the shape of an upside-down L. (Fig. 1A). The anticodon resides at the long end of the L-shape and binds to cognate codons in the messenger RNA (mRNA) on the ribosome. On the opposite end of the tRNA, the amino acid is ligated to the 3’-terminal adenosine residue in the acceptor stem. Accurate tRNA aminoacylation and high-fidelity decoding of codons on the ribosome are key determinants to accurate protein production.

Codon recognition is determined by the tRNA anticodon, which base pair with tri-nucleotide codons in mRNAs during protein synthesis (Fig. 1B). The essential interaction between codon and anticodon is established not only by Watson-Crick base pairing, but also by nucleotide modifications in tRNAs (30, 31), competition between cognate and near-cognate decoding (32, 33), and wobble decoding. Generally, the first two positions of a codon form Watson-Crick pairs with the tRNA, whereas the third position is more flexible (30). In back-to-back publications, Crick hypothesized (34) what Söll et al. (35) determined experimentally that the third position of a codon can involve G:U or U:G wobble pairing with the 1st position of the anticodon at tRNA nucleotide 34. Indeed, the initial discoveries also included examples of extended wobble decoding in yeast arginine and alanine tRNAs that read codons ending in U, C, or A (35). Extended wobble decoding is facilitated by post-transcriptional tRNA modification, where adenosine residues at position 34 are modified to inosine, which pairs with U, C, or A in the third codon position (30). Additional nucleotide modifications in the anticodon loop (particularly at positions 34 and 37) also impact translation fidelity and reading-frame maintenance (36, 37). For example, in yeast, a 5-methoxycarbonylm-
ethyl-2-thiouridine modification at anticodon base U34 represses +1 frame-shifting. Lack of the modification or hypo-modification at this site in a variety of tRNAs leads to 1.5–3.0-fold increases in ribosomal frame-shifting (38). Similarly, absence of the modified base N6-threonylcarbamoyladenosine at the anticodon adjacent position 37 also increases frame-shifting in yeast by 2-fold (39).

The standard genetic code is composed of 61 sense codons that encode 20 amino acids and 3 stop codons (UGA, UAG, and UAA) that usually signal termination of protein synthesis. Because certain tRNAs decode up to three or four different codons, the theoretical minimum number of tRNAs for an organism to encode 20 amino acids is 32 (34). Soll et al. (35) observed that the “minimum number of sRNA [tRNA] molecules required for recognition of all of the meaningful codons is relatively small, and this conclusion in turn raises the question of redundancy in the sRNA pool of a cell.” The question as to why organisms encode apparently redundant tRNA genes, which was raised the year after the code was solved, is still unanswered today. As a result of the genome sequencing revolution, we now know that nature contains examples of organisms with tRNA gene complements that are well below and vastly greater than this apparent minimal requirement. There are examples of organelles (e.g. human mitochondria with 22 tRNA genes) and even parasitic microbes (e.g. Mycoplasma mobile with 28 tRNA genes (40)) with fewer than 32 tRNA genes. Their survival depends on importing the missing tRNAs from a different cellular compartment (41) or presumably a host cell.

E. coli encodes 88 tRNA genes, whereas yeast has a small tRNAome for a eukaryote at 275 tRNA genes. Eukaryotes typically have hundreds of tRNA genes that display a general trend to increase in number and sequence diversity with the complexity of the organism (42). Unicellular protozoans encode near the theoretical minimum of tRNA genes, such as the malaria parasite Plasmodium falciparum, which has only 35 tRNA genes (43). P. falciparum was recently found to import additional tRNAs from its host (44). Some species of fish have astonishingly high tRNA gene numbers (Table S2), such as the elephant shark (Callorhinchus milii), which encodes 13,724 tRNAs (43). As exemplified in a phylogenetic comparison of yeast and human alanine tRNAs (Fig. 2), the sequence variations among tRNA iso-acceptors appear to increase with complexity as well. Yeast has 16 tRNA\textsuperscript{Ala} iso-acceptors, including 11 identical genes with the AGC anticodon and 5 identical genes with the TGC anticodon. In contrast, humans encode 45 tRNA\textsuperscript{Ala} iso-acceptors with markedly greater sequence diversity than their yeast counterparts, including examples with CGC anticodons not seen in yeast (Fig. 2).

**tRNA regulation in human cells**

The number of expressed tRNA genes in human cells is not well defined. Of the 610 tRNA genes in humans, the genomic tRNA database predicts 417 genes in their high-confidence set, indicating the tRNA is likely to function in protein synthesis (43). Comprehensive profiling of RNAs in human serum suggests 411 expressed tRNA genes (45). According to CHIP-seq analysis of RNA polymerase III and transcription factor occupancy, ~350 tRNA genes are actively transcribed in a single human cell line (IMR90hTert) (46). Gogakos et al. (47) reported the expression of 288–349 tRNAs in HEK 293 cells based on two different RNA-Seq methods. Together, the data suggest 300–400 tRNA genes are expressed in any individual human cell.

The degree to which each human tRNA contributes to protein synthesis has not been determined, but evidence that cells regulate tRNA expression to control protein production is emerging. First, expression of individual tRNA genes varies between tissues (48, 49). Furthermore, the steady-state level of different tRNAs correlates with the expression of matched-codon biased mRNA transcripts (48). The observation suggests that cells can fine-tune tRNA expression profiles to match codon usage in expressed mRNAs. Indeed, the fact that efficient protein expression requires tRNA levels and decoding capacity

![Figure 2. Phylogenetic relationships of human and yeast tRNA\textsuperscript{Ala}](image-url)
matches the distribution of codons in mRNA is well known. Multiple E. coli strains and bioinformatic tools for codon adaptation were developed based on this principle to enhance the production of eukaryotic and other recombinant proteins in bacteria (50).

Once transcribed, tRNAs are processed via the removal and addition of nucleotides to produce a mature tRNA. Introns, 5′-leader, and 3′-trailer sequences in the original transcript are removed (51). Next, the CCA-adding enzyme elongates the pre-tRNA with the conserved CCA 3′-end. CCA-adding enzymes can append a second CCA to certain tRNAs with mismatches or excessive G:U pairs in their acceptor stems (52). The double CCA addition primes tRNAs for exonucleolytic digestion via the rapid tRNA decay pathway (53). The human CCA-adding enzyme (TRNT1) is implicated in disease. Complete loss-of-function in TRNT1 is embryonic lethal, and partial loss-of-function mutations cause congenital sideroblastic anemia with immunodeficiency, fevers, and developmental delay (54).

tRNAs are further processed with a variety of post-transcriptional nucleotide modifications, including 2′ O-methylation of the ribose, N-acetylation and N-methylation of the nucleotide bases, as well as more complex modifications that form bases such as wybutosine (55).

In eukaryotes, tRNAHis is post-transcriptionally edited to add an extra guanine (G−1) to the 5′-end of the tRNA, a unique feature recognized and required by the cognate histidyl-tRNA synthetase (56). Depleting the tRNAHis guanylyltransferase that catalyzes G−1 addition leads to accumulation of un-aminocylated and un-guanylated yet hyper-methylated tRNAHis in the nucleus (57). Subsequent studies point to tRNAHis m5C hypermethylation as a response to growth arrest in Saccharomyces cerevisiae, although the significance of the increase in m5C methylation is yet unclear (58). Monomethylation of the 5′-monophosphate of tRNAHis by bicoid-interacting 3 domain containing RNA methyltransferase (BCDIN3D) is thought to protect tRNAHis from degradation. BCDIN3D is overexpressed in breast cancer cells, and monomethylated tRNAHis is more abundant in breast cancer cells, yet the overall level of tRNAHis is not impacted (59). It is thought that monomethylation contributes to the formation of tRNAHis-derived fragments in breast cancer cells, which in turn regulate tumorigenic genes involved in breast cancers (60).

In fact, tRNAs are the most frequently modified noncoding RNA known, containing an average of 13 modifications per molecule (61). The combined number of expressed tRNA genes and their multiple modification states imply the existence of a large combinatorial number of tRNA microspecies in the human cell (62). Because tRNA modifications are important for translation fidelity and reading frame maintenance (34, 36, 37, 63), these microspecies have the potential to impact cellular function and disease. Modifications are also essential for regulating tRNA turnover (64) and for proper structure, folding, and stability of the tRNA (55). Indeed, many tRNA-modifying enzymes are already linked to disease (26). As described below, tRNA modification can also dynamically up- or down-regulate sets of tRNAs (65–67).

The mature and active tRNA is a substrate for amino acid ligation catalyzed by the AARS enzymes in an ATP-dependent reaction (68). Each AARS enzyme has specificity both for an amino acid and a distinct set of cognate tRNA iso-acceptors. Amino acids are ligated to the 3′-end of tRNAs, requiring the presence of a CCA 3′-tail. To ensure tRNA recognition fidelity, AARSs make essential contacts with nucleotides in their cognate tRNAs, called identity elements (69). Aminoacyl-tRNAs are then substrates for protein synthesis. The likelihood that a given aminoacyl-tRNA acts in translation depends upon many factors, including the stability of the tRNA, the number of aminoacylated-tRNAs competing for the same codon, and the expression of mRNAs containing codons read by the tRNA. tRNAs unfit for translation are degraded by the rapid tRNA decay pathway (70). Cytoplasmic tRNA levels are also regulated by export processes to other cellular compartments, including into mitochondria (71), or retrograde transport into the nucleus (72). As reviewed elsewhere (62), tRNAs perform additional functions outside of translation, either as whole tRNAs (73) or tRNA-derived fragments (74).

Phenotypes of mistranslating cells

Mistranslation occurs in all cells (75) as a result of multiple different mechanisms. Considering the small size, multitude of protein partners, and essential cellular role of tRNAs, single nucleotide changes can have a profound impact on their function and on the efficiency and fidelity of protein synthesis (12, 13, 23, 69). Proteins encoded by mRNAs containing rare codons or strongly biased codon compositions are most susceptible to the effects of tRNA variants. Loss-of-function mutations in tRNAs can cause ribosome stalling to de-regulate protein synthesis, whereas gain-of-function mutations in tRNAs can lead to mis-aminocacylation and mistranslation (12, 21).

Mistranslating tRNAs can arise from surprisingly minor changes to the nucleotide sequence. Although many tRNAs harbor major identity determinants in their anticodon, coupling aminocacylation fidelity to codon assignment, alanyl-, leucyl-, and seryl-tRNA synthetases do not recognize the anticodon nucleotides on their cognate tRNAs. Anticodon mutations in these tRNAs often elicit amino acid mis-incorporation (69). The accumulation of highly active tRNAHis anticodon mutants is toxic to yeast cells, causing proteome-wide mistranslation (23). In yeast, the degree of anticodon mutant toxicity varies, depending on competition with WT tRNAs, chemical properties of the amino acids, and tRNA modifications (76).

Santos et al. (22) analyzed tRNAHis variants containing Ala or Leu anticodons in murine NIH 3T3 cells grown in culture and subsequently xenografted to live mice. As determined by mass spectrometry in tumor samples recovered from the mice, the rate of mistranslation increased by ~2-fold in the cells expressing tRNAHis containing an alanine anticodon, but only marginally in cells expressing tRNAHis with a leucine anticodon. Mistranslation was not toxic to the NIH 3T3 cells when grown in culture as determined by cellular viability, necrosis, and proliferation assays, indicating that increased cytosolic tRNA-dependent mistranslation was initially well-tolerated. Interestingly, expressing the mistranslating tRNAs promoted the formation of foci in vitro, suggesting a link to tumorigenesis (22). Briefly, foci formation occurs when cancer-like cells form dense clusters resembling early-stage tumors on a Petri dish.
Mistranslating tRNAs promoted the activation of the oncogenic factors protein kinase B (Akt) and p38 when cells were treated with tumor necrosis factor-α, to a greater extent than cells expressing the WT tRNAs. Furthermore, cells mistranslating alanine codons with serine promoted angiogenesis in a chick chorioallantoic membrane assay and were highly tumorigenic when introduced in mice. Compared with the parent cells in culture, expression of the mistranslating tRNAs increased ~8-fold in cells recovered from mouse tumors. Although mistranslating tRNA variants had undetectable cytotoxicity in cells in culture, the mutant tRNAs exacerbated or accelerated cellular pathways to cancer in a mammalian model of disease (22).

Identity element mutations are another route to mistranslating tRNAs. The phenotypic consequences of a single tRNA variant of this type are the subject of a number of recent studies. AlaRS recognizes two critical identity determinants at the 3rd codon base, and these include both common and rare variants across mammalian genomes (79). Some human tRNA<sup>CVs</sup> and tRNA<sup>Thr</sup> species with G<sub>4</sub>:U<sub>69</sub> are natural alanine acceptors, and cysteine to alanine mistranslation was detected in HEK 293 cells (79). An Animalia-specific tRNA deacylase was recently discovered that co-occurs with tRNA<sup>Thr</sup> G<sub>4</sub>:U<sub>69</sub> variants in animal genomes and de-acylates mis-charged Ala-tRNA<sup>Thr</sup> (80). This enzyme may protect human cells from alanine mistranslation at threonine codons.

In our work on tRNA-dependent mistranslation, we expressed a mutant of human tRNA<sup>Pro</sup> containing a G<sub>3</sub>:U<sub>70</sub> base pair in human cells. The human tRNA<sup>Pro</sup> mutant was an efficient alanine acceptor in vitro that no longer accepted proline. Our previous work in yeast demonstrated that a homologous tRNA<sup>Pro</sup> mutant mistranslated multiple proline codons with alanine by creating these identity elements are common in mammalian genomes (79). Some human tRNA<sup>CVs</sup> and tRNA<sup>Thr</sup> species with G<sub>4</sub>:U<sub>69</sub> are natural alanine acceptors, and cysteine to alanine mistranslation was detected in HEK 293 cells (79). An Animalia-specific tRNA deacylase was recently discovered that co-occurs with tRNA<sup>Thr</sup> G<sub>4</sub>:U<sub>69</sub> variants in animal genomes and de-acylates mis-charged Alanine-tRNA<sup>Thr</sup> (80). This enzyme may protect human cells from alanine mistranslation at threonine codons.

To visualize the nature and extent of human tRNA variation, we analyzed data from the 1000 Genomes Project and plotted the number of unique variants at each position in an alignment of all human tRNA genes (Fig. 3, A and B). The number of unique variants were mapped on the tRNA secondary structure (Fig. 3A). We also plotted the frequency of occurrence of each of these tRNA mutations in the human population (Fig. 3C). Some tRNAs, such as those for leucine, serine, and selenocysteine, have significantly larger variable loops; variation from these regions was not included.

No position in the tRNA genes is immune to variation, yet some positions are far more variable than others (Fig. 3, A and B). The allele frequency of these variants indicates that common (>5% allele frequency) and rare variants (<5% allele frequency) are distributed across nearly all sites in the tRNA (Fig. 3C). Some sites, however, lack common variants. Although restricted to rare variants, variation is observed at position 73; this “discriminator” base is a key identity element for many AARSs. The anticodon shows variation at all three bases, albeit reduced compared with other regions of the tRNA. The data from 1000 Genomes Project suggest that across all ~600 tRNA loci there are 25–30 unique nucleotide variants at each anticodon base, and these include both common and rare variants in the population (Fig. 3C).

Positions within the acceptor stem contain large numbers of unique variants. Many AARSs recognize acceptor stem nucleotides to ensure aminoacylation fidelity (69); thus, acceptor stem variants have the potential to elicit mistranslation or lead to a defective tRNA. Another compelling observation is that several important sites of tRNA modification display significant variation (e.g. position 37 in the anticodon loop) (Fig. 3). Consistent with this observation, mutations in tRNA-modifying enzymes that act at these positions are implicated in disease (26).

As mentioned above, most human AARSs recognize identity determinants in the tRNA anticodon except for AlaRS, LeuRS, and SerRS (69); thus, nonsynonymous anticodon variants in Ala, Leu, and Ser tRNAs are likely to mistranslate. Correspond-
Figure 3. tRNA variants observed in the 1000 Genomes Project. A, variants that occur within tRNA genes (defined by GtRNAdb (128)) were downloaded from the 1000 Genomes Project phase 3 dataset (142). Insertions and deletions were removed, as were variants with no allele frequency available. Each variant was mapped to its corresponding tRNA position, according to standardized numbering (139), using an in-house Perl script. High-confidence tRNAs were defined as tRNAs with a tRNAscan-SE score of >50 (128). For the high confidence tRNA set, unique mutations are mapped to each position in the tRNA. B, same data in A are plotted for the high-confidence set (cyan dashed line) and for all human tRNA sequences (red line). C, allele frequencies (log scale) of all variants that occur at each tRNA position are represented in box and whisker plots. Boxes outline quartiles of the allele frequency distribution; filled circles depict the median allele frequency; whiskers show 1.5× quartile range; and open circles depict raw data, i.e. the allele frequencies for each unique tRNA variant at the indicated position.
ingly, the tRNA variants that have already been shown to elicit mistranslation in human cells have mutations in the tRNA anticodon or create the identity determinants for AlaRS (12, 78, 79). Anticodon mutations in other tRNAs typically reduce or ablate aminoacylation (69). However, the degree of aminoacylation loss is not known for all anticodon positions in all tRNAs, and in some cases, efficient aminoacylation can be retained even when some identity determinants are mutated (87).

Certainly, mistranslating tRNAs can arise from a variety of mechanisms. Mutations at identity elements, which ensure cognate aminoacylation, or mutations at anti-determinants, which prevent non-cognate aminoacylation, have the potential to convert any tRNA into a mistranslator. Although such mutants likely occur in the human population, there is a paucity of biochemical data regarding human tRNA identity elements and anti-determinants, thus challenging confident identification of such variants as mistranslating tRNAs from sequence alone. Anticodon mutations in Ser, Ala, and Leu, however, will undoubtedly lead to amino acid mis-incorporation. For this reason, our discussion of specific mistranslating tRNA examples from human genomes focused on these “obvious” mistranslating tRNAs.

To assess the prevalence of likely tRNA mistranslators in the human population, we searched the Genomic tRNA database (GtRNAdb) for high-confidence Ala, Leu, and Ser tRNAs with anticodon mutations (Table 1) or mutations in the 3rd or 4th acceptor stem bp that create the G3:U70 (Table 1) or G4:U69 (Table S1) AlaRS identity element. In total, among the human Ala, Leu, and Ser iso-acceptor groups reported in GtRNAdb there are 27 unique anticodon variants. Of these, there are 14 unique nonsynonymous (Table 1) and 13 synonymous anticodon variants. Most nonsynonymous anticodon variants are rare, but three variants occur in >1% of the population. One alanine tRNA variant containing a glycine anticodon occurs in over 6% of sequenced individuals. The common occurrence of these mutations in cytosolic tRNAs is striking; analogous variants in mitochondrial tRNAs are embryonic lethal (27). Although we found a similar number of unique synonymous anticodon variants, none were found in >1% of sequenced individuals. While still encoding the “correct” amino acid, such mutants may more or less efficiently read synonymous codons for a particular amino acid, altering translation rates.

Further complicating this scenario, certain apparently synonymous anticodon variants may become mistranslators through nucleotide modification. For example, tRNAs normally containing an A34 are modified to inosine (I34) by the action of adenine deaminases acting on tRNAs (88). As noted above, I34 enables expanded wobble decoding to codons ending in U, C, or A; thus, A34 containing tRNAs are normally restricted to those amino acids with synonymous codons ending in U, C, and A. However, human genomes include examples of tRNAs bearing A34 that, if modified to I34, would lead to mistranslation (e.g. tRNA-Ser-GCT-GCT-5–1 single nucleotide polymorphism (SNP) rs550301646; tRNAAsn-ATT-1–1 and tRNA-Tyr-ATA-1–1 are in the human reference genome). In the case of tRNAAsn, A34I would incorporate Asn at Lys AAA codons. This type of phenomenon was recently examined with anticodon variants of Methanocaldococcus jannaschii tRNA^{Tyr} expressed in E. coli (89, 90). In this case, a tRNA^{Tyr} mutant with an AUG anticodon decoded histidine CAU and CAC codons with tyrosine at approximately equal efficiency (2–3%); the mutant tRNA^{Tyr} AUG anticodon was indeed partially modified to IUG (89). Perhaps as a natural defense against mistranslation and the resulting abundant Gln-tRNA^{Gln1_UUG} mis-incorporation of tyrosine at glutamine CAA codons was not detected in E. coli (89).

Adding to the complexity of human tRNA variation, tRNA genes are particularly susceptible to transcription-associated mutagenesis (TAM) (91), and thus, their sequence can change more rapidly than other genes. Thornlow et al. (91) demonstrated that tRNA genes experience 7–10-fold higher rates of TAM compared with the genome-wide average. TAM occurs when DNA strands are separated during transcription, and the nontemplate strand becomes temporarily isolated and more accessible to mutagens (92). Although tRNA variation is generally selected against on a population scale, this implies that the sequence of tRNA genes within individual cells could change throughout life and that perturbations that increase tRNA expression could further increase mutation rates.

**tRNA variation and disease**

Like the involvement of mitochondrial tRNA variants in disease (28), recent studies have identified specific cytosolic tRNA mutants as drivers or modifiers of disease in humans and mice. In addition, tRNA mis-modification and imbalanced tRNA expression also contribute to disease. Here, we highlight examples of defective tRNA function in genetic disorders, cancers, and neurodegeneration.

**tRNA mutants linked to disease**

Kobayashi et al. (140) identified the first human tRNA associated with disease in 1990. The mutation, a variant of a mitochondrial tRNA^{Aeu} gene, leads to the degradation of the tRNA and causes a rare disorder characterized by stroke and dementia: mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes. Shortly after, a mutation in mitochondrial tRNA^{Lys} was found to cause another rare neurological disorder, myoclonic epilepsy and ragged-red fiber disease (93). Several other mitochondrial tRNA variants are implicated in major human diseases, including heart disease (94), hypertension (95), metabolic disease (96), and deafness (97).

Two clear examples have emerged where a cytosolic tRNA variant either contributes to or directly causes disease. One case (described under “tRNA variants in neurodegeneration”) involves a mutation in a single tRNA^{Aeg} gene that causes widespread neurodegeneration in mice when associated with a second mutation in a protein-coding gene that sensitizes cells to ribosome stalling (21). The other case (detailed under “tRNA modification defects in disease”) is a single nucleotide mutation in the only functional human tRNA^{Sec} gene (98, 99) that causes abdominal pain, fatigue, muscle weakness, and low plasma selenium levels in a homozygous patient (19). In this case, the tRNA^{Sec} variant appears to be the primary driver of disease (19).
Imbalanced tRNA expression and disease

tRNA copy number variation—the copy number of tRNA genes varies between individuals. Iben and Mariaa (100) assessed copy number variation among nuclearily encoded tRNAs from whole-genome sequencing data obtained in the 1000 Genomes Project. Their study focused on two sets of two parents and a child, from which >15-fold read coverage was obtained. Although the high similarity of tRNA iso-acceptors complicates this type of analysis, significant copy number variation in at least 11 tRNA gene loci among the six individuals was reported. Furthermore, they validated a homozygous deletion encoding tRNA_{GUU}^{ Thr} on chromosome 7 in one individual. Interestingly, modification defects in this tRNA associate with type 2 diabetes in mice. The deletion is common in the human population and has no known indication of an associated phenotype (101).

Tissue-specific tRNA expression—Dittmar et al. (48) demonstrated the tissue dependence of tRNA expression using a tRNA microarray that probed 42 nuclearily encoded and 21 mitochondrial encoded tRNAs from eight different tissues. They revealed that human tissues express different sets of cytosolic tRNAs. Comprehensive analysis based on RNA polymerase III occupancy of tRNA genes in mice support this finding (102).

The relevance of tissue-specific tRNA expression to disease was demonstrated by the link between tRNA abundance and cystic fibrosis (103). In this work, Kirchner et al. (103) characterized a synonymous SNP in the cystic fibrosis transmembrane conductance regulator (CFTR), which substitutes an ACT Thr codon with ACC. This synonymous mutation results in a cell type-dependent alteration of CFTR protein levels that are not explained by a change in mRNA stability or splicing. The authors discovered that tRNA_{CGU}^{ Thr} is a low abundance tRNA in the cystic fibrosis model and human bronchial epithelial cell lines. The polymorphism not only reduces CFTR expression in human bronchial epithelial cells, but also impairs the folding, localization, and membrane conductance of CFTR. The findings point to a translation rate-dependent mechanism, where ribosome stalling on the ACC codon, which is read by a low abundance tRNA, causes the protein product to mis-fold and malfunction.

Phenotypic defects from synonymous codon mutations are observed in numerous other disease-relevant proteins—coding genes (104). Examples include multidrug resistance 1 (MDR1) (105), estrogen receptor α (106, 107), and surfactant protein-D (108). Thus, expression of specific tRNA iso-decoders is an important consideration when synonymous mutations result in a phenotype, particularly if the protein synthesis burden is shifted to a low abundance or possibly defective tRNA. Conceivably, tRNA synonymous anticodon variants (noted above) could have a similar effect on translation rates and cellular phenotypes.

De-regulated tRNA expression—tRNA expression can change dynamically in disease. Pavon-Eternod et al. (109) demonstrated that tRNA expression increases from 3- to 10-fold in breast cancer tumors. Oncogenic factors such as RAS and C-MYC promote RNA polymerase (pol) III transcription, whereas tumor suppressors such as retinoblastoma protein (RB) and P53 inhibit pol III transcription, providing a link between common cancer mechanisms and pol III-dependent tRNA expression (110). Although cause or effect has not been established in these cases, tRNA expression changes in cancer may occur through global tRNA up-regulation to facilitate increased protein synthesis requirements in tumor cells (111).

Dysregulation of specific tRNA iso-acceptors is also implicated in cancer. Overexpression of the initiator tRNA_{Met} promotes translation reprogramming and cell proliferation in the human breast epithelial cell lines 184A1 and MCF10A (109). This was corroborated in a comprehensive study that quantified tRNA expression profiles using tRNA microarrays and histone modification mapping across 470 patient-derived tissue samples representing various states of proliferation (112). Gingold et al. (112) demonstrated that tRNA_{Met} expression is highest in the most proliferating samples and lowest in the differentiating cells.

In contrast, reduced tRNA_{Sec} expression was observed in many proliferating and especially cancerous cell samples (112). tRNA_{Sec} is required for the production of selenocysteine-containing proteins. Depending on the context, selenoprotein synthesis can either prevent or promote cancer (113); thus, up- or down-regulation of tRNA_{Sec} may have relevance to disease. tRNA expression changes can also promote cancer through roles for tRNAs beyond protein synthesis. A recent review highlighted examples of tRNAs or tRNA-derived fragments from at least 16 iso-acceptor groups that are specifically de-regulated in cancer (110).

The tRNA expression profile in a particular cell will lead to more or less efficient translation of certain mRNAs depending on codon usage (114, 115). Differential expression of tRNAs also promotes cancer through favoring particular “translation programs.” The study of Gingold et al. (112) profiled codon usage in transcripts associated with cell cycle versus differentiation. The authors observed a dichotomy where codons with A or U in the 3rd codon position are generally more common in proliferation-associated mRNAs, and G- or C-ending codons are more common in differentiation-associated mRNA transcripts (112). The emerging view is that cells dynamically switch between “programs” of protein synthesis, in part by coordinating the transcription of tRNAs with anticodons matching the codon bias in expressed mRNAs.

Differential expression of specific tRNA iso-decoders—the expression of specific tRNA iso-decoders promotes metastasis in breast cancer model cell lines (112). The authors measured the relative abundance of different tRNA iso-decoders in cell lines selected for high rates of metastasis (MDA-LM2 and CN-LM1) and parental cell lines (MDA-231 and CN34). Two tRNAs (tRNA_{CGG}^{ Thr} and tRNA_{GUU}^{ Glu} ) were highly up-regulated in both metastatic lines. These tRNAs were then overexpressed in MDA-231 cells to assess changes in the proteome resulting from their increased expression. The abundance of proteins encoded by transcripts enriched in the matching codons (GGC and GAR) increased. As measured by ribosome profiling, two such mRNAs (encoding EXOSC2 and GRIPAP1) showed higher rates of active translation in the cells overexpressing tRNA_{GUU}^{ Glu}. RNAi-mediated knockdown of these mRNAs reduced in vitro invasion capacity of the cells, suggesting that
EXOSC2 and GRIPAP1 are required for tRNA\textsubscript{Glu}-promoted metastasis. Hence, the coordinated expression of tRNA iso-decoders facilitates translational reprogramming in cancer cells and is implicated in the promotion of proliferation as well as metastasis.

**tRNA modification defects in disease**

As mentioned previously, post-transcriptional modifications are important for tRNA function and stability. Hypo-modification can lead to rapid tRNA decay (116), and many tRNAs require anticodon modifications to ensure faithful codon recognition (34, 63). Over 50 different nucleotide modifications occur in euukaryotic tRNAs (117), and in humans, tRNAs contain an average of 13 modifications per molecule (61). Accordingly, tRNA modification defects are implicated in numerous diseases, including neurological, cardiac, respiratory, and metabolic diseases, as well as cancer and mitochondria-linked disorders (26). Most diseases that result from defects in tRNA modification are due to mutations in protein-coding genes or in mitochondrial tRNA genes, rather than cytosolic tRNA genes.

A recent example, however, provides compelling evidence of a cytosolic tRNA mutant and de-regulated nucleotide modification in human disease. A C65G mutation in tRNA\textsubscript{Sec} was identified in a patient exhibiting abdominal pain, fatigue, muscle weakness, and low plasma selenium levels (28). Although humans encode two tRNA\textsubscript{Sec} genes, apparently only one is functional. A mutation in this gene has the potential to impact all 25 human selenoproteins, which are essential for normal development (118). Selenoproteins may be categorized into two groups: housekeeping and stress-related. Synthesis of housekeeping selenoproteins depends on a 5-methoxycarbonylmethyluridine (mcm\textsuperscript{5}U) modification at position 34 of tRNA\textsubscript{Sec}, whereas further modification to 5-methoxycarbonylmethyl-2′-O-methyluridine (mcm\textsuperscript{6}Um) promotes synthesis of stress-related selenoproteins (119). The tRNA\textsubscript{Sec} C65G variant only impaired expression of stress-related selenoproteins. This is attributed to the fact that the variant has markedly reduced levels of both the mcm\textsuperscript{5}U and mcm\textsuperscript{6}Um modification at position 34 and the N\textsuperscript{ε}-isopentenyl adenosine modification at position 37 (19). The finding underscores the complexity of nucleotide modification in tRNA function by showing that a mutation at one site in tRNA can impact modification at other locations in the tRNA body. In this case, a single nucleotide variant in the T-arm altered modifications in the anticodon stem loop (Fig. 1). Although the mechanism is not yet defined, presumably the C65G mutant inhibits or reduces the methyltransferase activity of the multifunction ALKBH8 gene product that catalyzes conversion of mcm\textsuperscript{5}U to mcm\textsuperscript{6}Um at position 34 (120).

Modifications can also drive or favor specific translation programs (65, 66). For example, melanomas harboring the V600E mutation in the proto-oncogene B-RAF depend on translational reprogramming controlled by up-regulation of U34 tRNA-modifying enzymes (67). Similar to the modulation of tRNA expression in metastatic breast cancer (121), the mechanism relies on coordinated regulation of both tRNAs and associated codon-biased transcripts. These modification tunable transcripts are sensitive to particular tRNA modification states (65). U34 tRNA modification promotes decoding of the “-AA” ending codons AAA, GAA, and CAA (122). Remarkably, up-regulation of U34-modifying enzymes promotes survival of melanomas dependent on hypoxia inducible factor 1α (HIF-1α) metabolism. Elevated levels of HIF-1α correlate with tumor metastasis and poor patient prognosis as well as tumor resistance to therapy (123). Indeed, the HIF-1α mRNA is enriched in AAA, GAA, and CAA codons (67). When U34-modifying enzymes ELP3, CTU1, or CTU2 were knocked down, HIF-1α protein levels decreased even though HIF-1α mRNA levels were unchanged. Thus, cancer cells are able to regulate tRNA modification enzymes to ultimately tune protein synthesis rates and protein levels in favor of oncogenesis.

**tRNA variants in neurodegeneration**

A common attribute of disorders linked to defective protein homeostasis is the accumulation of mistranslated or misfolded proteins in cells (20, 124). In many cell types, this problem can be counteracted through apoptosis or cell division (124). However, post-mitotic cells such as those found in the heart and brain are incapable of diluting misfolded proteins through division and lack the regenerative capacity to replace apoptotic cells readily (124). Furthermore, protein quality control decreases in post-mitotic tissues with age (125). Post-mitotic tissues may be particularly vulnerable to the consequences of tRNA variants and increasingly so with age.

Girstmair et al. (141) proposed a role for cytosolic tRNAs in Huntington’s disease (HD). HD is caused by an expanded Gln repeat in the huntingtin protein, encoded by a stretch of 40–100 repeated CAG codons (126). In some cases, shorter CAG repeats appear to also cause HD, suggesting there are additional disease modifiers. Continuous translation of the repeat depletes charged tRNA\textsubscript{Gln} which results in more frequent frameshifting in the translation of the huntingtin gene, possibly exacerbating the disease phenotype (141). Although tRNA\textsubscript{Gln} variants are not yet known to exacerbate HD, these findings illustrate the importance of tRNA function and abundance in pathologies of the brain.

Indeed, naturally occurring tRNA variants have the potential to deplete the abundance of a brain-specific tRNA that is essential for health. Ishimura et al. (21) uncovered a synthetic toxic effect involving a single cytosolic tRNA variant that causes widespread neurodegeneration in mice (21). Mutations in Gtpbp2 (encoding a protein that rescues stalled ribosomes) and Tr20 (encoding tRNA\textsubscript{Arg}) were found to co-occur in mice identified in a phenotypic screen for neurodegeneration. Mice carrying both mutations exhibit rapid neurodegeneration and die at 8–9 weeks. At 3 weeks, the mutant mice are indistinguishable from WT. The C50T mutation (n-Tr20) prevents tRNA\textsubscript{Arg} maturation and, in combination with the loss of GTPBP2, leads to ribosome stalling. Despite many "redundant" tRNA\textsubscript{Arg} iso-decoders in the cell, the lack of function of this single tRNA causes ribosome stalling. The authors measured a 3-fold increase in AGA pauses in the n-Tr20 mutant compared with a mouse containing the WT tRNA. Fascinatingly, tRNA\textsubscript{Arg} C50T variants also occur in the human population, including in a TCT iso-acceptor (Table 3).

This work exemplifies the ways in which tRNA variants can exacerbate pathways to disease. Two observations from
this work may have broader applicability to understanding the roles for tRNA variants in disease. First, the phenotype was tissue-specific, because expression of the n-Tr20–encoded tRNA is only observed in the central nervous system. Second, a coincident mutation in another gene sensitized cells to the loss-of-function mutation in a single tRNA gene. Together, these mutations caused disease in the animal model.

Conclusion

Humans display a remarkable array of both common and rare tRNA mutants, some with the obvious potential to mistranslate the genetic code (Tables 1 and 2 and Table S1) or create defective tRNAs (e.g. Table 3). Indeed, such tRNA variants can elicit significant levels of mistranslation in human cells and influence protein synthesis and protein homeostasis (Fig. 4). Above, we highlighted recent examples showing how tRNA variants and defective tRNA genes contribute to disease. In addition to causing disease, tRNA variants act synergistically with other disease-causing alleles by placing additional stress on protein quality control mechanisms or biasing translation programs that drive disease. Furthermore, tissue-specific tRNA expression and de-regulated tRNA expression or modification contribute to disease and phenotypic defects at the cellular level. Together, these observations suggest that cytosolic tRNA mutations may have greater importance in disease than previously recognized. We hope that the evidence provided in this review will stimulate new interest in considering cytoplasmic tRNA variants as an important factor in human genetic variation and disease.

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References

1. Crick, F. H. C. (1955) On degenerate templates and the adaptor hypothesis. A Note for the RNA Tie Club, 1–17
2. Hoagland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I., and Zamec-
1. Böck, A., and Stadtman, T. C. (1988) Selenocysteine, a highly specific component of certain enzymes, is incorporated by a UGA-directed translational mechanism. Biofactors 1, 245–250 CrossRef Medline

2. Berry, M. J., Martin, G. W., 3rd, Tujejabeva, R., Grundner-Culemann, E., Mansell, J. B., Morozova, N., and Harney, J. W. (2002) Selenocysteine insertion sequence element characterization and selenoprotein expression. Methods Enzymol. 347, 17–24 CrossRef Medline

3. Bröcker, M. J., Ho, J. M., Church, G. M., Soll, D., and O'Donoghue, P. (2014) Recoding the genetic code with selenocysteine. Angew. Chem. Int. Ed. Engl. 53, 319–323 CrossRef Medline

4. Schoenmakers, E., Carlson, B., Agostini, M., Moran, C., Rajanayagam, O., Bohukova, E., Tobe, R., Peat, R., Gevers, E., Muntoni, F., Guicheny, P., Schoenmakers, N., Farooqi, S., Lyons, G., Hatfield, D., and Chatterjee, K. (2016) Mutation in human selenocysteine transfer RNA selectively disrupts selenoprotein synthesis. J. Clin. Invest. 126, 992–996 CrossRef Medline

5. Kapur, M., and Ackerman, S. L. (2018) mRNA translation gone awry: translation fidelity and neurological disease. Trends Genet. 34, 218–231 CrossRef Medline

6. Soll, D., Ohtsuka, E., Jones, D. S., Lohrmann, R., Hayatsu, H., Nishimura, N., Akimoto, S., and Soll, D. (2013) UGA is an additional codon that specifies selenocysteine. Angew. Chem. Int. Ed. 52, 158–194 CrossRef Medline

7. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

8. Böck, A., Bankier, A. T., and Volkov, M. D. (1988) The importance of higher order structure in the recognition of transfer RNA. J. Mol. Biol. 203, 299–314 CrossRef Medline

9. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

10. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

11. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

12. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

13. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

14. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

15. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

16. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

17. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

18. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

19. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

20. Klug, A., and Engh, R. A. (1996) Phaseless refinement of electron density maps. J. Mol. Biol. 263, 341–355 CrossRef Medline

21. Ishimura, R., Nagy, G., Dotu, I., Zhou, H., Yang, X. L., Schimmel, P., Senju, S., Nishimura, Y., Chuang, J. H., and Ackerman, S. L. (2014) Ribosome stalling induced by mutation of a CNS-specific tRNA causes neuromuscular disease. Science 345, 455–459 CrossRef Medline

22. Santos, M., Pereira, P. M., Varanda, A. S., Carvalho, J., Azevedo, M., Mateus, D. D., Mendes, N., Oliveira, P., Trindade, F., Pinto, M. T., Bordeira-Carrio, R., Carneiro, F., Vitorino, R., Oliveira, C., and Santos, M. A. S. (2018) Codon misreading tRNAs promote tumor growth in mice. RNA Biol. 15, 773–786 CrossRef Medline

23. Berg, M. D., Hoffman, K. S., Genereaux, J., Mian, S., Trussler, R. S., Hampshire, D. B., O'Donoghue, P., and Brandl, C. J. (2017) Evolving mistranslating tRNAs through a phenotypically ambivalent intermediate in Saccharomyces cerevisiae. Genetics 206, 1865–1879 CrossRef Medline

24. Mayer-Schuman, R., and Antonellis, A. J. (2017) Emerging mechanisms of aminoacyl-tRNA synthetase mutations in recessive and dominant human disease. Hum. Mol. Genet. 26, R114–R127 CrossRef Medline

25. Bednářová, A., Hanna, M., Durham, I., VanCleave, T., England, A., Chaudhuri, A., and Krishnan, N. (2017) Lost in translation: defects in transfer RNA modifications and neurological disorders. Front. Mol. Neurosci. 10, 135 CrossRef Medline

26. Torres, A. G., Batlle, E., and Ribas de Pouplana, L. (2014) Role of tRNA modifications in human diseases. Trends Mol. Med. 20, 306–314 CrossRef Medline

27. Kirchner, S., and Ignatova, Z. (2015) Emerging roles of tRNA in adaptive translation, signalling dynamics and disease. Nat. Rev. Genet. 16, 98–112 CrossRef Medline

28. Abbott, J. A., Franklyn, C. S., and Robey-Bond, S. M. (2014) Transfer RNA and human disease. Front. Genet. 5, 158 CrossRef Medline

29. Yasukawa, T., Suzuki, T., Iishi, N., Ohta, S., and Watanabe, K. (2001) Wobble modification defect in tRNA disturbs codon-anticodon interaction in a mitochondrial disease. EMBO J. 20, 4794–4802 CrossRef Medline

30. Agris, P. F., Vendeix, F. A., and Graham, W. D. (2007) tRNA’s wobble decoding of the genome: 40 years of modification. J. Mol. Biol. 366, 1–13 CrossRef Medline

31. Joshi, K., Bhatt, M. J., and Farabaugh, P. J. (2018) Codon-specific effects of tRNA codon loop modifications on translational misreading errors in the yeast Saccharomyces cerevisiae. Nucleic Acids Res. 46, 10331–10339 CrossRef Medline

32. Pavlov, M. Y., and Ehrenberg, M. (2018) Substrate-induced formation of ribosomal decoding center for accurate and rapid genetic code translation. Annu. Rev. Biophys. 47, 525–548 CrossRef Medline

33. O’Donoghue, P., Prat, L., Heinemann, I. U., Ling, J., Odoi, K., Liu, W. R., and Soll, D. (2012) Near-cognate suppression of amber, opal and quadruplet codons competes with aminoacyl-tRNA synthetase for genetic code expansion. FEBS Lett. 586, 3931–3937 CrossRef Medline

34. Crick, F. H. (1966) Codon–anticodon pairing: the wobble hypothesis. J. Mol. Biol. 19, 548–555 CrossRef Medline

35. Soll, D., Jones, D. S., Ohtsuka, E., Faulkner, R. D., Lohrmann, R., Hayatsu, H., and Khairi, H. G. (1966) Specificity of tRNA for recognition of codons as studied by the ribosomal binding technique. J. Mol. Biol. 19, 556–573 CrossRef Medline

36. Agris, P. F., Narendran, A., Sarachan, K., Väre, V. Y. P., and Erusalim, E. (2017) The importance of being modified: the role of tRNA modifications in translational fidelity. Enzyme Res. 41, 1–50 CrossRef Medline

37. Gustilo, E. M., Vendeix, F. A., and Agris, P. F. (2008) tRNA’s modifications bring order to gene expression. Curr. Opin. Microbiol. 11, 134–140 CrossRef Medline

38. Tükenmez, H., Xu, H., Esberg, A., and Byström, A. S. (2015) The role of wobble uridine modifications in translational frameshifting in eukaryotes. Nucleic Acids Res. 43, 9489–9499 CrossRef Medline

39. Lin, C. A., Ellis, S. R., and True, H. L. (2010) The Suax5 protein is essential for normal translational regulation in yeast. Mol. Cell. Biol. 30, 354–363 CrossRef Medline

40. Jaffe, J. D., Stange-Thomann, N., Smith, C., DeCaprio, D., Fisher, S., Butler, J., Calvo, S., Elkins, T., FitzGerald, M. G., Halez, N., Kodira, C. D., Major, J., Wang, S., Wilkinson, J., Nicol, R., Nusbaum, C., et al. (2004)
The complete genome and proteome of <i>Mycoplasma mobile</i>. Genome Res. 14, 1447–1461 CrossRef Medline

41. Chatterjee, K., Nostramo, R. T., Wan, Y., and Hopper, A. K. (2018) RNA dynamics between the nucleus, cytoplasm and mitochondrial surface: location, location, location. Biochim. Biophys. Acta 1861, 373–386 CrossRef

42. Goodenbour, J. M., and Pan, T. (2006) Diversity of tRNA genes in eukaryotes. Nucleic Acids Res. 34, 6137–6146 CrossRef Medline

43. Chan, P. P., and Lowe, T. M. (2005) Depletion of 5-methylcytidine levels increase in response to several growth arrest conditions in <i>Saccharomyces cerevisiae</i>. Mol. Cell. Biol. 25, 8191–8201 CrossRef Medline

44. Chabuksvriri, K., and Bahrani, A. (2018) tRNA modification in distinct cellular contexts. Crit. Rev. Biochem. Mol. Biol. 52, 205–219 CrossRef Medline

45. Elmore, S. (2018) Conditional accumulation of toxic tRNAs to cause amino acid misincorporation. <i>Nucleic Acids Res.</i> 46, 7831–7843 CrossRef Medline

46. Alvarez, A., Barisone, G. A., and Diaz, E. (2014) Focus formation: a cell-based assay to determine the oncogenic potential of a gene. J. Vis. Exp. 94, e51742 CrossRef

47. Hou, Y.-M., Franklyn, C., and Schimmel, P. (1989) Molecular dissection of a transfer RNA and the basis for its identity. Trends Biochem. Sci. 14, 233–237 CrossRef Medline
80. Kuncha, S. K., Mazeed, M., Singh, R., Kattula, B., Routh, S. B., and San-kanarayan, R. (2018) A chiral selectivity relaxed paradigm of DTD for proofreading tRNA mischarging in Animalia. Nat. Commun. 9, 511

CrossRef Medline

81. Pezo, V., Metzgar, D., Hendrickson, T. L., Waas, W. F., Hazebruck, S., Döring, V., Marlèire, P., Schimmel, P., and De Crécy-Lagard, V. (2004) Artificially ambiguous genetic code confers growth yield advantage. Proc. Natl. Acad. Sci. U.S.A. 101, 8593–8597 CrossRef Medline

82. Netzer, N., Goodenour, J. M., David, A., Dittmar, K. A., Jones, R. B., Schneider, J. R., Boone, D., Eves, E. M., Rosner, M. R., Gibbs, J. S., Embry, A., Dolan, B., Das, S., Hickman, H. D., Berglund, P., et al. (2009) Innate immune and chemically triggered oxidative stress modifies translational fidelity. Nature 462, 522–526 CrossRef Medline

83. Moghal, A., Mohler, I., and Ibba, M. (2014) Mistranslation of the genetic code. FEBS Lett. 588, 4305–4310 CrossRef Medline

84. Wu, J., Fan, Y., and Ling, J. (2014) Mechanism of oxidant-induced mistranslation by threonyl-tRNA synthetase. Nucleic Acids Res. 42, 6523–6531 CrossRef Medline

85. Lee, J. Y., Kim, D. G., Kim, B. G., Yang, W. S., Hong, J., Kang, T., Oh, Y. S., Kim, K. R., Han, B. W., Hwang, B. J., Kang, B. S., Kang, M. S., Kim, M. H., Kwon, N. H., and Kim, S. (2014) Promiscuous methionyl-tRNA synthetase mediates adaptive mistranslation to protect cells against oxidative stress. J. Cell Sci. 127, 4234–4245 CrossRef Medline

86. Gomes, A. C., Kordala, A. J., Strack, R., Wang, X., Geslain, R., Delaney, K., Clark, W. C., Keenan, R., and Pan, T. (2016) A dual fluorescence reporter for the investigation of methionine mistranslation in live cells. RNA 22, 467–476 CrossRef Medline

87. Fechter, P., Rüdinger-Thirion, J., Tukalo, M., and Gigé, R. (2001) Major tyrosine identity determinants in Methanococcus jannaschii and Saccharomyces cerevisiae tRNA(Tyr) are conserved but expressed differently. Eur. J. Biochem. 268, 761–767 CrossRef Medline

88. Torres, A. G., Piñeiro, D., Rodríguez-Escribá, M., Camacho, N., Reina, O., Saint-Léger, A., Filonova, L., Batlle, E., and Ribas de Pouplana, L. (2015) Inosine modifications in human tRNAs are incorporated at the precursor tRNA level. Nucleic Acids Res. 43, 5145–5157 CrossRef Medline

89. Biddle, W., Schmitt, M. A., and Fisk, J. D. (2016) Modification of orthogonal tRNAs: unexpected consequences for sense codon reassignment. Nucleic Acids Res. 44, 10042–10050 CrossRef Medline

90. Biddle, W., Schmitt, M. A., and Fisk, J. D. (2015) Evaluating sense codon reassignment with a simple fluorescence screen. Biochemistry 54, 7355–7364 CrossRef Medline

91. Thornlow, B. P., Hough, J., Roger, J. M., Gong, S., Lovell, T. M., and Corbett-Detig, R. B. (2018) Transfer RNA genes experience exceptionally elevated mutation rates. Proc. Natl. Acad. Sci. U.S.A. 115, 8996–9001 CrossRef Medline

92. Jinks-Robertson, S., and Bhagwat, A. S. (2014) Transcription-associated mistranslation of the deafness-associated mitochondrial 12S rRNA 1555A>G mutation. J. Biol. Chem. 293, 3321–3334 CrossRef Medline

93. O’Neill, V. A., Eden, F. C., Pratt, K., and Hatfield, D. L. (1985) A humanopal suppressor tRNA gene and pseudogene. J. Biol. Chem. 260, 2501–2508 Medline

94. Santesteban, D., Mariotti, M., and Guigó, R. (2017) Computational identification of the selenocysteine tRNA (tRNAsec) in genomes. PLoS Comput. Biol. 13, e1005383 CrossRef Medline

95. Iben, J. R., and Maraia, R. J. (2014) tRNA gene copy number variation in humans. Nature 536, 376–384 CrossRef Medline

96. Wei, F.-Y., Suzuki, T., Watanabe, S., Kimura, S., Kaitsuka, T., Fujimura, A., Matsu, H., Atta, M., Michiue, H., Fontecave, M., Yamagata, K., Suzuki, T., and Tomizawa, K. (2011) Deficit of tRNAlys modification by Dck1l causes the development of type 2 diabetes in mice. J. Clin. Invest. 121, 3598–3608 CrossRef Medline

97. Kutter, C., Brown, G. D., Gonçalves, A., Wilson, M. D., Watt, S., Brazma, A., White, R. J., and Odom, D. T. (2011) Pol III binding in six mammals shows conservation among amino acid isotypes despite divergence among tRNA genes. Nat. Genet. 43, 948–955 CrossRef Medline

98. Kirchner, S., Cai, Z., Rauscher, R., Kastelic, N., Anding, M., Czech, A., Kleizen, B., Ostedgaard, L. S., Braakman, I., Sheppard, D. N., and Ignatova, Z. (2017) Alteration of protein function by a silent polymorphism linked to tRNA abundance. PLoS Biol. 15, e2000779 CrossRef Medline

99. Rauscher, R., and Ignatova, Z. (2018) Timing during translation matters: synonymous mutations in human pathologies influence protein folding and function. Biochim. Biochim. Acta. 467, 937–944 CrossRef Medline

100. Kimchi-Sarfaty, C., Oh, J. M., Kim, I. W., Sauna, Z. E., Calcagno, A. M., Ambudkar, S. V., and Gottesman, M. M. (2007) A “Silent” polymorphism in the MR1 gene changes substrate specificity. Science 315, 525–528 CrossRef Medline

101. Fernández-Calero, T., Cabrera-Cabrera, F., Ehrlich, R., and Marín, M. (2016) Silent polymorphisms: can the tRNA population explain changes in protein properties? Life 6, 9 CrossRef

102. Horjales, S., Cota, G., Señorale-Pose, M., Roivra, C., Román, E., Artagaveitia, N., Ehrlich, R., and Marín, M. (2007) Translational machinery and protein folding: evidence of conformational variations of the estrogen receptor α. Arch. Biochem. Biophys. 467, 139–143 CrossRef Medline

103. Foreman, M. G., Kong, X., DeMeo, D. L., Pillai, S. G., Hersh, C. P., Bakke, P., Guliev, A., Lomas, D. A., Litonjua, A. A., Shapiro, S. D., Tal-Singer, R., and Silverman, E. K. (2011) Polymorphisms in surfactant protein-D are associated with chronic obstructive pulmonary disease. Am. J. Respir. Cell Mol. Biol. 44, 316–322 CrossRef Medline

104. Pavon-Eterno, M., Gomes, S., Rosner, M. R., and Pan, T. (2013) Overexpression of initiator methionine tRNA leads to global reprogramming of tRNA expression and increased proliferation in human epithelial cells. RNA 19, 461–466 CrossRef Medline

105. Huang, S. Q., Sun, B., Xiong, Z. P., Shu, Y., Zhou, H. H., Wang, Z., Xiong, J., and Li, Q. (2018) The dysregulation of tRNAs and tRNA derivatives in cancer. J. Exp. Clin. Cancer Res. 37, 101 CrossRef Medline

106. Mahlab, S., Tuller, T., and Linial, M. (2012) Conservation of the relative tRNA composition in healthy and cancerous tissues. RNA 18, 640–652 CrossRef Medline

107. Gingold, H., Tehler, D., Christoffersen, N. R., Nielsen, M. M., Asmar, F., Kooistra, S. M., Christophersen, N. S., Christensen, L. L., Borre, M., Sørensen, K. D., Andersen, L. D., Andersen, C. L., Hullemann, E., Wurdinger, T., Raffiker, E., et al. (2014) A dual program for translation regulation in cellular proliferation and differentiation. Cell 158, 1281–1292 CrossRef Medline

108. Hatfield, D. L., Yoo, M.-H., Carlson, B. A., and Gladyshev, V. N. (2009) Selenoproteins that function in cancer prevention and promotion. Biochim. Biophys. Acta 1790, 1541–1545 CrossRef Medline

109. Quax, T. E., Claassen, N. J., Söll, D., and van der Oost, J. (2015) Codon bias as a means to fine-tune gene expression. Mol. Cell 59, 149–161 CrossRef Medline

110. Frumkin, I., Lajoie, M. J., Gregg, C. J., Hornung, G., Church, G. M., and Pilpel, Y. (2018) Codon usage of highly expressed genes affects proteome-wide translation efficiency. Proc. Natl. Acad. Sci. U.S.A. 115, E4940–E4949 CrossRef Medline
116. Alexandrov, A., Chernyakov, I., Gu, W., Hiley, S. L., Hughes, T. R., Grayhack, E. J., and Phizicky, E. M. (2006) Rapid tRNA decay can result from lack of nonessential modifications. Mol. Cell 21, 87–96 CrossRef Medline

117. Boccaletto, P., Machnicka, M. A., Purta, E., Piatkowski, P., Baginski, B., Wirecki, T. K., de Crécy-Lagard, V., Ross, R., Limbach, P. A., Kotter, A., Helm, M., and Bujnicki, J. M. (2018) MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic Acids Res. 46, D303–D307 CrossRef Medline

118. Schweizer, U., and Fradejas-Villar, N. (2016) Why 21? The significance of human tRNA genes from the 1000-genomes UCSC genome browser database: 2018 update. JBC REVIEWS: Pathways to disease from natural tRNA variations

119. Carlson, B., Yoo, M.-H., Tsuji, P., Gladyshev, V. N., and Hatfield, D. L. (2015) Mouse models targeting selenocysteine tRNA expression for elucidating the role of selenoproteins in health and development. Molecules 14, 3509–3527 CrossRef Medline

120. Songe-Møller, L., van den Born, E., Leihne, V., Vågbo, C. B., Kristoffer, T. K., de Crécy-Lagard, V., Ross, R., Limbach, P. A., Kotter, A., Helm, M., and Bujnicki, J. M. (2018) MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic Acids Res. 46, D303–D307 CrossRef Medline

121. Goodarzi, H., Nguyen, H. C. B., Zhang, S., Dill, B. D., Molina, H., and Schweizer, U., and Fradejas-Villar, N. (2016) Why 21? The significance of human tRNA genes from the 1000-genomes UCSC genome browser database: 2018 update. JBC REVIEWS: Pathways to disease from natural tRNA variations

122. Nedialkova, D. D., and Leidel, S. A. (2015) Optimization of codon translation rates via tRNA modifications maintains proteome integrity. Cell 165, 1416–1427 CrossRef Medline

123. Josefson, R., Andersson, R., and Nyström, T. (2017) How and why do human tRNA genes lack of nonessential modifications. Nature 549, 5294–5308 CrossRef Medline

124. Hrabeta-Robinson, E., Marcus, E., Cozen, A. E., Phizicky, E. M., and Bujnicki, J. M. (2018) MODOMICS: a database of RNA modification pathways. 2017 update. Nucleic Acids Res. 46, D303–D307 CrossRef Medline