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Pathways to disease from natural variations in human cytoplasmic tRNAs

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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 amino acids to codons. Crick's adaptor hypothesis predicted the existence of tRNAs in 1955: "*each amino acid would combine chemically, at a special enzyme, with a small molecule which, having a specific hydrogen-bonding surface, would combine specifically with the nucleic acid template.*" (1). Just 3 years later, the first tRNAs were discovered as soluble RNAs involved in protein synthesis (2). Working with yeast, Holley *et al.* (3) isolated tRNAs and determined the first tRNA sequence. The first codons were mapped to amino acids using repeating polyribonucleotides as templates for protein synthesis (4, 5). The laboratories of Nirenberg *et al.* (6) and Khorana *et al.* (7) then raced to solve the complete codon catalogue using a technique that monitored the binding of radiolabeled aminoacyl-tRNAs to ribosomes separately prepared with each of the possible 64 trinucleotide codons. These efforts established the standard genetic code table for which Holley, Khorana, and Nirenberg were awarded the Nobel prize in Physiology or Medicine in 1968.

Fascinatingly, by the time the prize was awarded, exceptions to the genetic code had already been identified. In 1966, Yanofsky and co-workers (8) demonstrated tRNA mutants enabled missense suppression or amino acid mis-incorporation using a defective tryptophan synthase A gene in *Escherichia coli*. Early in 1968, Atkins (9) identified the first exception to triplet decoding with the discovery of frame-shifting in *Salmonella typhimurium*. Indeed, although the genetic code is *nearly* universal in the living world, several exceptions to the standard code occur in diverse organisms (10). Genome sequences and genetic and biochemical data reveal that in organisms from microbes to humans, codons can be ambiguously decoded (8, 11–13), reassigned (14, 15), or site-specifically recoded (16–18) to incorporate unexpected amino acids or amino acids beyond 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 molecu-

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This article contains Tables S1 and S2 and supporting Refs. 1–8.

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Table 1
Human tRNA anticodon variants
MAF is minor allele frequency. Inserted nucleotides are indicated by, *e.g.* _35T.

tRNA gene	Variant	MAF (%)[a]	Variant count[a]	MAF (%)[b]	Variant count[b]	tRNA score[c]	Codon identity	tRNA identity	Expression	
									ARM[d]	CHIP[e]
Ala-AGC-2–2	G35A	0.02	1			84.7	Val	Ala	+	+
Ala-AGC-6–1	G35C	6.55	328	6.47	8130	74.1	Gly	Ala	+	+
Ala-AGC-15–1	C36T	0.04	2	0.03	42	56.7	Thr	Ala	+	−
Ala-AGC-16–1	G35A	2.2	11	2.1	269	53.1	Val	Ala	+	−
Ala-CGC-1–1	G35T			0.0008	1	79.7	Glu	Ala	+	+
Ala-TGC-1–1	G35A			–		80.5	Val	Ala	+	+
Leu-CAA-3–1	A35C			0.0008	1	77.3	Trp	Leu	+	+
Ser-AGA-2–2	G35A	0.02		0.003	4	89.6	Phe	Ser	+	+
Ser-AGA-2–3	G35A	1.82	91	1.87	2347	89.6	Phe	Ser	+	+
Ser-AGA-2–4	G35C			0.003	4	89.6	Cys	Ser	+	+
Ser-AGA-2–5	_35T			0.0008	1	89.6			+	+
Ser-CGA-2–1	_36T			0.03	34	94			+	−
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			+	+

[a] Data are from the 1000 Genomes Project (127, 128).
[b] Data are from the TOPMED sequencing project (128, 129).
[c] tRNA score was calculated using tRNA-Scan SE (128).
[d] ARM indicates ARM-seq data suggesting expression (128, 130).
[e] 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
Human tRNA variants that introduce a G3:U70 base pair
MAF is minor allele frequency.

tRNA gene	Variant	MAF (%)[a]	Variant count[a]	MAF (%)[b]	Variant count[b]	tRNA score[c]	Expression	
							ARM[d]	CHIP[e]
Arg-ACG-1–3	C70U			0.002	2	68	+	+
Cys-GCA-2–3	C70U			0.02	29	82	+	+
Cys-GCA-1–1	C70U	0.02	1	0.07	86	84	+	−
Cys-GCA-17–1	C70U			0.002	2	71	+	+
Cys-GCA-12–1	C70U	0.02	1	0.01	16	72	+	+
Gly-CCC-2–1	G70U			0.05	64	75	+	+
Gly-GCC-2–4	A3G	0.08	4	0.05	63	81	+	−
Gly-GCC-2–5	A3G	0.02	1	0.005	6	81	+	−
Gly-GCC-2–1	A3G	0.02	1	0.02	20	81	+	+
Gly-GCC-2–3	A3G					81	+	+
Gly-GCC-1–5	A3G	1.2	61	1.3	1633	81	−	−
Gly-GCC-5–1	A3G	0.02	1	0.1	129	55	−	−
Gly-TCC-2–6	C70U	0.03	14	0.09	111	74	+	+
Ser-AGA-2–6	A3G			0.01	12	90	+	+

[a] Data are from the 1000 Genomes Project (127, 128).
[b] Data are from the TOPMED sequencing project (128, 129).
[c] tRNA score was calculated using tRNA-Scan SE (128).
[d] ARM indicates ARM-seq data suggesting expression (128, 130).
[e] 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
Human $tRNA^{Arg}$ C50T variants

tRNA gene	SNP ID	MAF (%)[a]	Variant count[a]	MAF (%)[b]	Variant count[b]	tRNA score[c]	Expression	
							ARM[d]	CHIP[e]
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-TCG-6–1	rs113170043	16.6	831	20.2	24,374	53.7	+	−
Arg-TCT-5–1	rs143334272	0.8	41	0.6	711	61.4	+	−

[a] Data are from the 1000 genomes project.
[b] Data are from the TOPMED sequencing project (128, 129).
[c] tRNA score was calculated using tRNA-Scan SE (128).
[d] ARM indicates ARM-seq data suggesting expression (128, 130).
[e] CHIP indicates CHIP-seq hits for at least 3 of 4 core transcription proteins (RPC155, POLR3G, BRF1, and BDP1) (46, 129, 131–133).

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 (AARSs)[4] (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

[4] The abbreviations used are: AARS, aminoacyl-tRNA synthetase; SNP, single nucleotide polymorphism; TAM, transcription-associated mutagenesis; CFTR, cystic fibrosis transmembrane conductance regulator; mcm^5U, 5-methoxycarbonylmethyluridine; mcm^5Um, 5-methoxycarbonylmethyl-2′-*O*-methyluridine; HIF, hypoxia-inducible factor; GtRNAdb, Genomic tRNA database; pol III, polymerase III; HD, Huntington's disease.

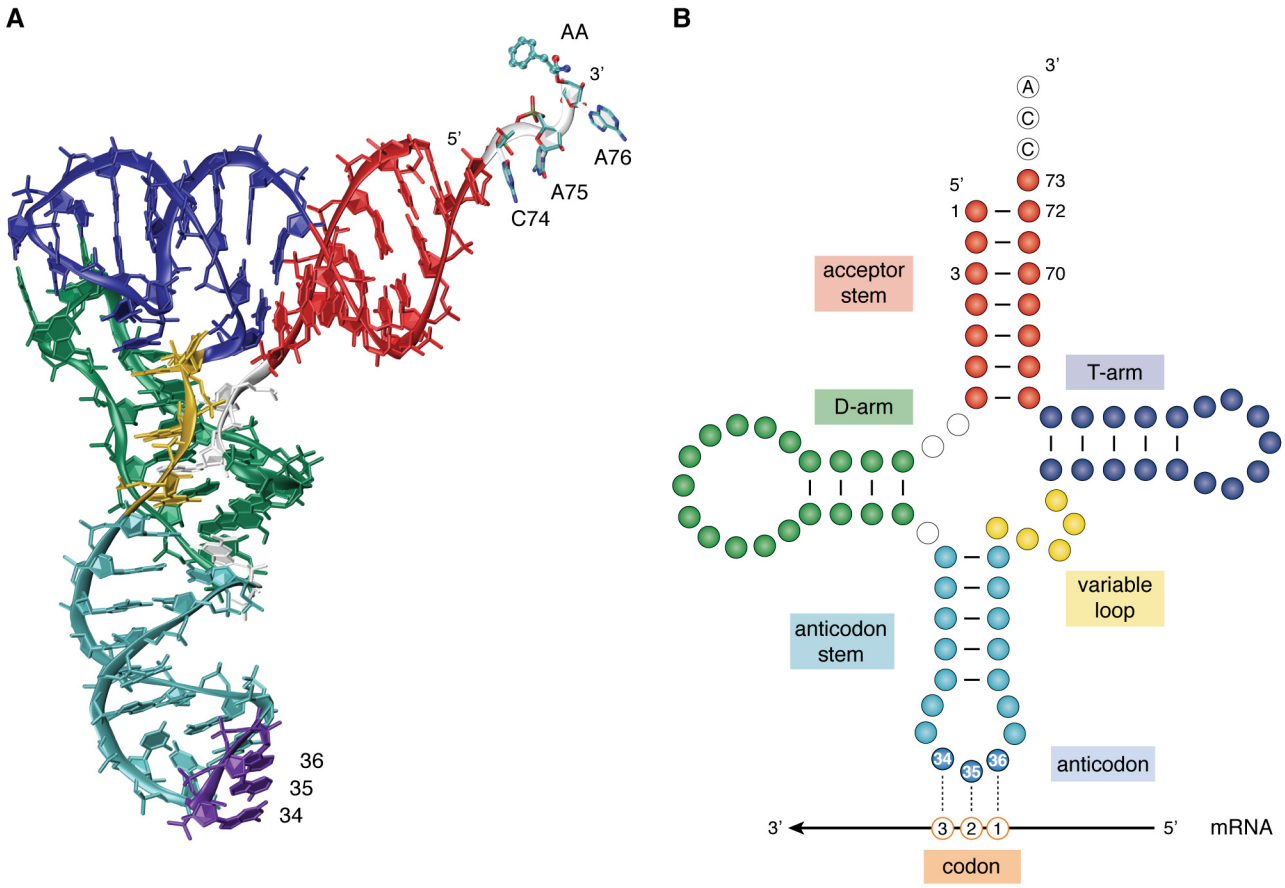

Figure 1. tRNA structure. *A,* tRNAs fold into an L-shaped three-dimensional structure with extensive intramolecular base-pairing. This $tRNA^{Phe}$ structure (Protein Data Bank code 1OB5) (134) is aminoacylated with phenylalanine (*AA*). *B,* in a two-dimensional representation, tRNA resembles a cloverleaf. In both diagrams, the tRNA is colored by structural elements: acceptor stem (*red*), dihydrouridine (D)-arm (*green*), anticodon stem (*cyan*), anticodon (bases 34–36 in *purple*), variable loop (*yellow*), TΨC (T) arm (*navy*), and the conserved CCA-3′ end (*white*). A schematic mRNA is shown *below* the tRNA diagram to indicate the tRNA nucleotides that base pair with each codon position.

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 mistranslating 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. 1*A*). 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. 1*B*). 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 hypomodification 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 N^6-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). Söll *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 know now 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 astoundingly 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 $tRNA^{Ala}$ iso-acceptors, including 11 identical genes with the AGC anticodon and 5 identical genes with the TGC anticodon. In contrast, humans encode 45 $tRNA^{Ala}$ iso-acceptors with markedly greater sequence diversity than their yeast counterparts, including examples with CGC anticodons not seen in yeast (Fig. 2).

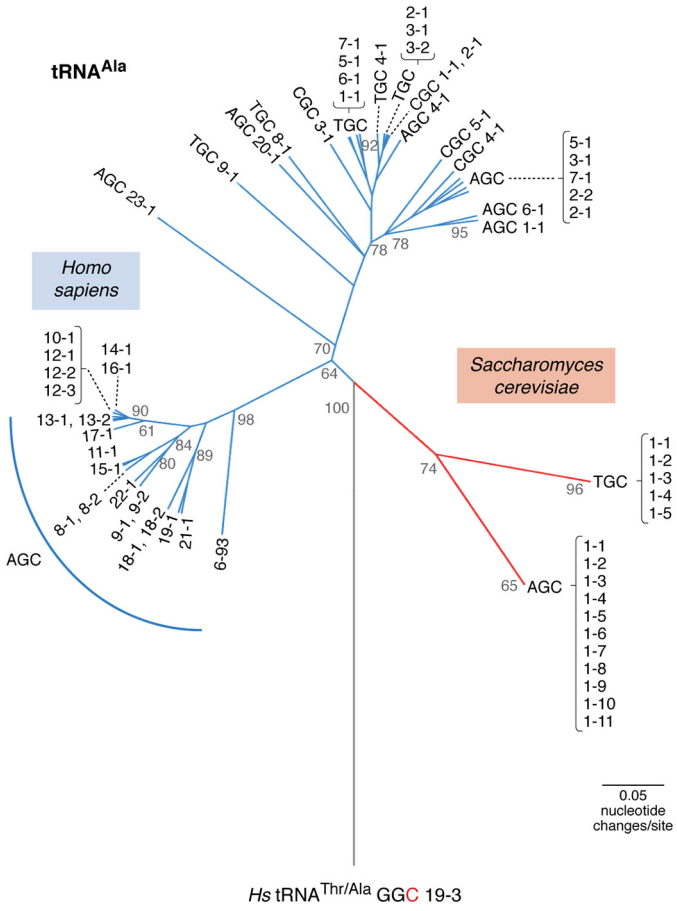

Figure 2. Phylogenetic relationships of human and yeast $tRNA^{Ala}$. The tree is based on an alignment of all known human and yeast $tRNA^{Ala}$ iso-acceptors. The vastly expanded number and greater diversity of human $tRNA^{Ala}$ genes compared with their yeast counterparts are evident. The tRNAs are labeled according to gene names in the genomic tRNA database (43), which include anticodon sequence followed by a numbering system where the first number indicates similar sequences, and the second number indicates a gene copy identifier. The human reference genome contains a misannotated $tRNA^{Ala}$, which was used to root the tree. This gene, tRNAAla-GGC-19-3, is a $tRNA^{Thr}$ with a mutation (T36C) endowing the tRNA with an alanine anticodon. This is the only example of the alanine GGC anticodon in humans. *Scale bar* indicates the number of nucleotide changes per site in the tRNA sequences. The tree was calculated similarly as before (135). Briefly, a starting tree computed in MultiSeq 2.0 (136) was optimized to identify the maximum likelihood tree using PhyML 3.1 (137). Statistical branch support (out of 100) was calculated based on an approximate likelihood ratio test method (138) as implemented in PhyML.

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

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, $tRNA^{His}$ 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 $tRNA^{His}$ guanylyltransferase that catalyzes G^{-1} addition leads to accumulation of un-aminoacylated and un-guanylated yet hyper-methylated $tRNA^{His}$ in the nucleus (57). Subsequent studies point to $tRNA^{His}$ m^5C hypermethylation as a response to growth arrest in *Saccharomyces cerevisiae*, although the significance of the increase in m^5C methylation is yet unclear (58). Monomethylation of the 5′-monophosphate of $tRNA^{His}$ by bicoid-interacting 3 domain containing RNA methyltransferase (BCDIN3D) is thought to protect $tRNA^{His}$ from degradation. BCDIN3D is overexpressed in breast cancer cells, and monomethylated $tRNA^{His}$ is more abundant in breast cancer cells, yet the overall level of $tRNA^{His}$ is not impacted (59). It is thought that monomethylation contributes to the formation of $tRNA^{His}$-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-aminoacylation 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 aminoacylation 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 $tRNA^{Ser}$ 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 $tRNA^{Ser}$ 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 $tRNA^{Ser}$ containing an alanine anticodon, but only marginally in cells expressing $tRNA^{Ser}$ 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

(77). 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 base pair in the acceptor stem (G3:U70) (78), and also aminoacylates tRNAs bearing the GU pair at the 4th acceptor stem base pair (76). tRNA variants that convert nonalanine tRNAs to alanine accepting tRNAs by creating these identity elements are common in mammalian genomes (79). Some human $tRNA^{Cys}$ and $tRNA^{Thr}$ species with G4:U69 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 $tRNA^{Thr}$ G4:U69 variants in animal genomes and de-acylates mis-charged Ala-$tRNA^{Thr}$ (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 $tRNA^{Pro}$ containing a G3:U70 base pair in human cells. The human $tRNA^{Pro}$ mutant was an efficient alanine acceptor *in vitro* that no longer accepted proline. Our previous work in yeast demonstrated that a homologous $tRNA^{Pro}$ mutant mistranslated multiple proline codons with alanine (13). We developed a GFP reporter (D129P) that fluoresces in response to mistranslation at the Pro-129 codon. In HEK 293 cells (12), we did not observe significant mistranslation in rich media compared with cells expressing WT $tRNA^{Pro}$. When the cells were starved of serum and glucose over a period of days, mistranslation accumulated to 2–5% according to the GFP reporter. Strikingly, and similar to the report of Santos *et al.* (22), we did not observe a loss in cellular viability or induction of the heat-shock response compared with cells expressing the WT tRNA, indicating that this level of mistranslation was well tolerated. In yeast, a mistranslating tRNA did show synthetic slow growth when coupled with deletion mutants lacking the proteome regulatory transcription factor Rpn4 (13). The above examples illustrate how phenotypes driven by a mistranslating tRNA variant can remain hidden; yet, in conditions of stress or in models of disease, an otherwise neutral tRNA variant can interact synthetically with a stressor or a second mutation to cause phenotypic defects.

Errors in protein synthesis are normally thought to be deleterious, yet mistranslation is an adaptive response in diverse organisms from bacteria (81) to yeast (13) to human cells (82). In some of these cases mistranslation provides a selective advantage. As reviewed elsewhere (83), a recurrent finding is that stress conditions reduce aminoacylation fidelity (84). For example, upon oxidative stress, methionyl-tRNA synthetase increases its mis-aminoacylation of noncognate tRNAs up to 10-fold in bacteria and mammalian cells (82, 85, 86); the additional methionine in proteins is thought to protect the proteome from oxidative damage.

tRNA variation in humans

Human tRNA variants that likely mistranslate can be readily identified in publicly available sequence data (Tables 1 and 2, S1) (43). Some of these natural variants (79) or similar variants designed in the laboratory (12, 22) do in fact cause mistranslation in human cells. In addition, tRNA variants, which simply impair tRNA folding or function, also impact the proteome. The absence of even a single tRNA gene product can alter the tRNA pool and limit the rate of protein synthesis by causing ribosome stalling (21). In both capacities, mutations in tRNA-encoding genes represent an important class of potential disease modifiers that could increase the severity of other disease-causing alleles.

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. 3*A*). We also plotted the frequency of occurrence of each of these tRNA mutations in the human population (Fig. 3*C*). 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 human 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. 3*C*). 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. 3*C*).

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-

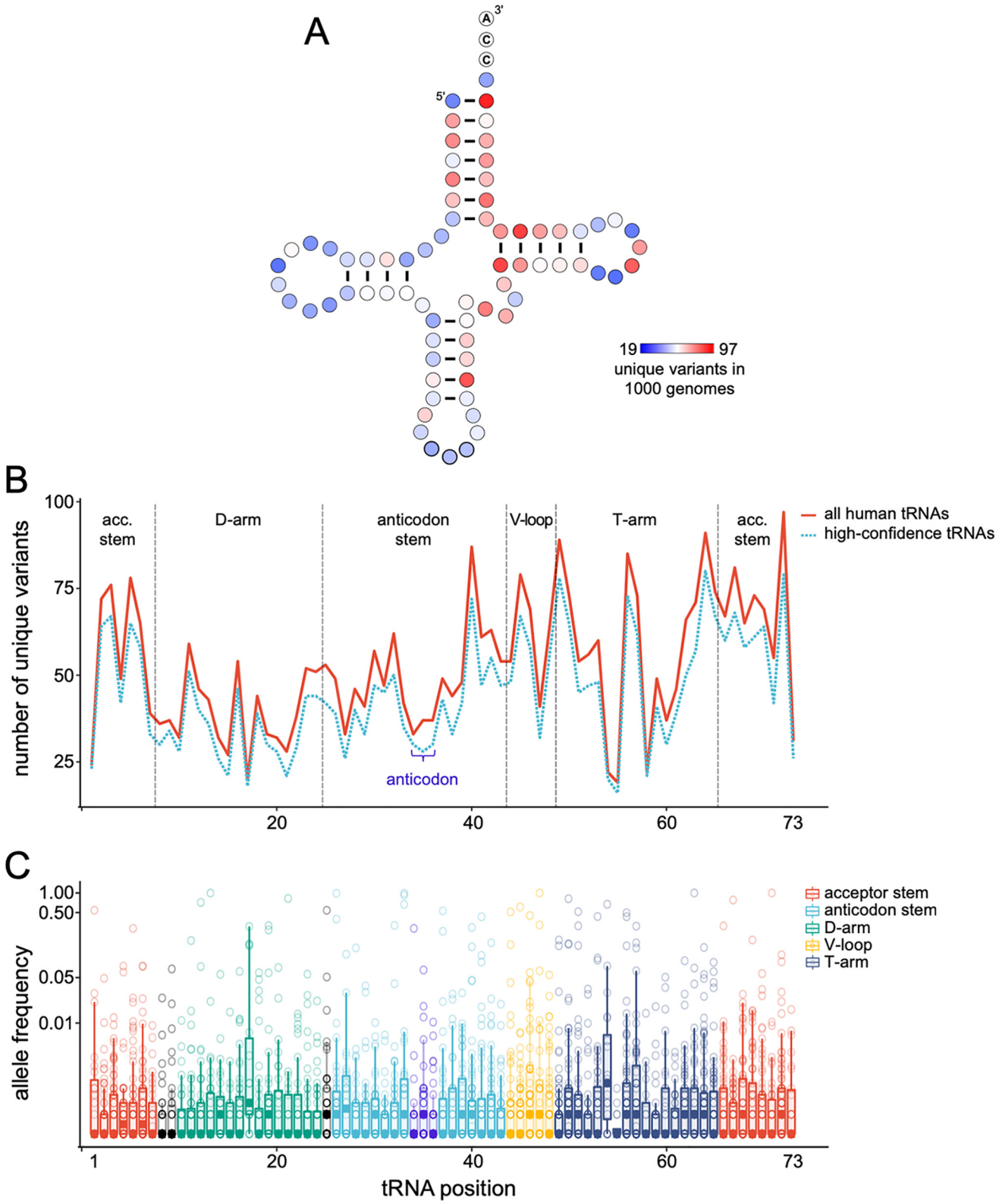

Figure 3. tRNA variants observed in the 1000 Genomes Project. *A,* variants that occur within tRNA genes (defined by GtRNAdb (128)) were downloaded from the 1000 Genomes Project phase 3 dataset (142). Insertions and deletions were removed, as were variants with no allele frequency available. Each variant 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 ($\log_2$ 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-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 $tRNA^{Asn}$, 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 $tRNA^{Leu}$ 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 $tRNA^{Arg}$ 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 Maraia (100) assessed copy number variation among nuclearly 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 $tRNA^{Lys}_{UUU}$ 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 phenotypic defect (101).

Tissue-specific tRNA expression—Dittmar *et al.* (48) demonstrated the tissue dependence of tRNA expression using a tRNA microarray that probed 42 nuclearly encoded and 21 mitochondrially 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 ACG. 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 $tRNA^{Thr}_{CGU}$ 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 ACG 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 protein–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 $tRNA^{Met}_{i}$ 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 ($tRNA^{Arg}_{CCG}$ and $tRNA^{Glu}_{UUC}$) 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 $tRNA^{Glu}_{UUC}$. RNAi-mediated knockdown of these mRNAs reduced *in vitro* invasion capacity of the cells, suggesting that

EXOSC2 and GRIPAP1 are required for $tRNA^{Glu}_{UUC}$-promoted metastasis. Hence, the coordinated expression of tRNA isodecoders 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 eukaryotic 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 $tRNA^{Sec}_{UCA}$ was identified in a patient exhibiting abdominal pain, fatigue, muscle weakness, and low plasma selenium levels (28). Although humans encode two $tRNA^{Sec}$ 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^5U) modification at position 34 of $tRNA^{Sec}$, whereas further modification to 5-methoxycarbonylmethyl-2′-*O*-methyluridine (mcm^5Um) promotes synthesis of stress-related selenoproteins (119). The $tRNA^{Sec}$ 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^5Um modification at position 34 and the N^6-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^5U to mcm^5Um 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-oncoprotein 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 $tRNA^{Gln}_{CUG}$, which results in more frequent frameshifting in the translation of the huntingtin gene, possibly exacerbating the disease phenotype (141). Although $tRNA^{Gln}_{CUG}$ 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 $tRNA^{Arg}_{UCU}$) 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 $tRNA^{Arg}_{UCU}$ maturation and, in combination with the loss of GTPBP2, leads to ribosome stalling. Despite many "redundant" $tRNA^{Arg}$ 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, $tRNA^{Arg}$ 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

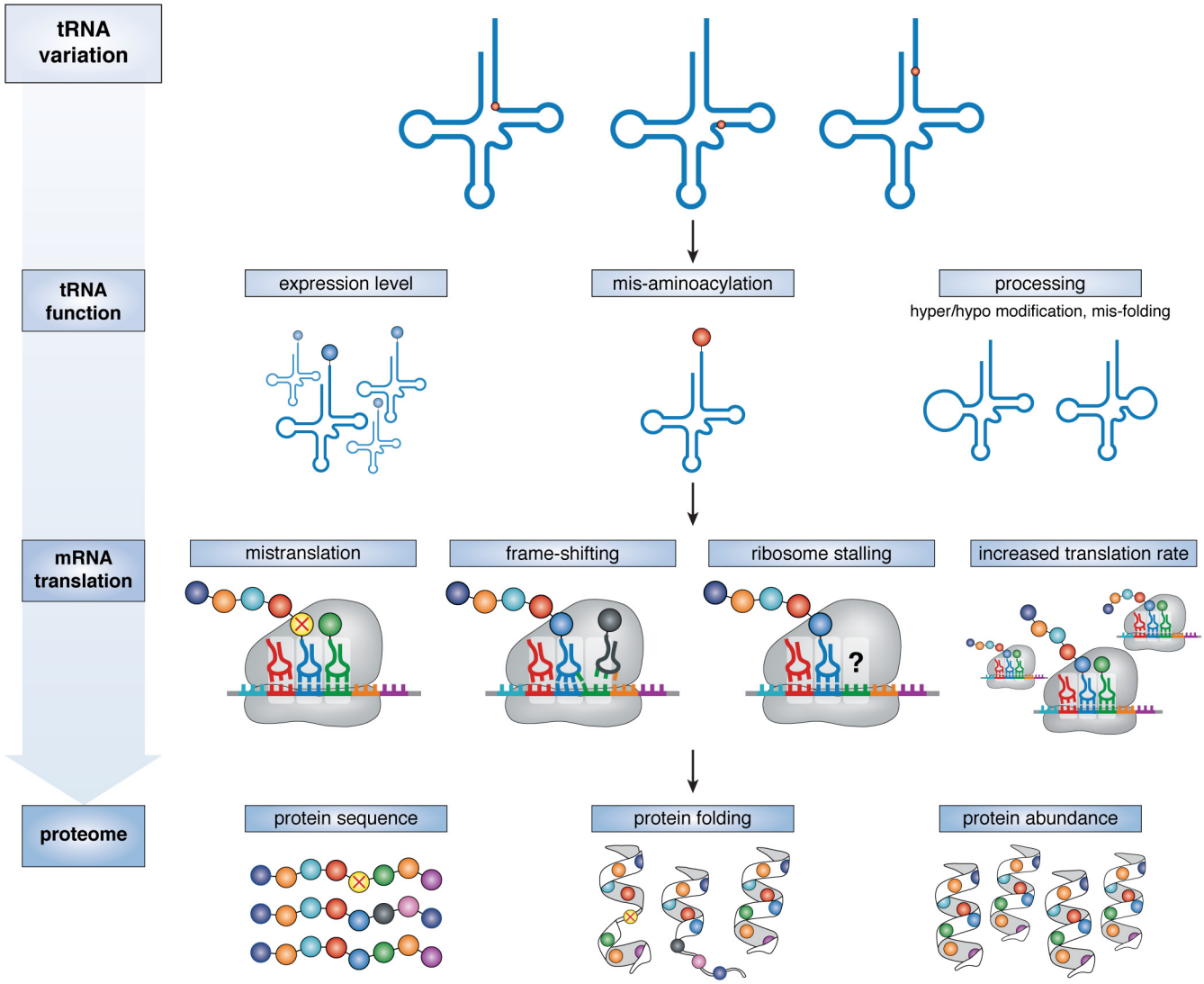

Figure 4. Phenotypic consequences of tRNA variation. tRNA variation can impact expression, aminoacylation, or processing and maturation of tRNAs, including nucleotide modification and tRNA folding. Alterations in the functional tRNA pool can impact mRNA translation by causing mistranslation, frame-shifting, ribosome stalling, or increased expression of codon-biased transcripts. These alterations can in turn alter the amino acid sequence, folding, and abundance of proteins across the entire proteome.

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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