NON-CODING RNA

Emerging roles of tRNA in adaptive translation, signalling dynamics and disease

Sebastian Kirchner[1,2] *and Zoya Ignatova*[1,3]

Abstract | tRNAs, nexus molecules between mRNAs and proteins, have a central role in translation. Recent discoveries have revealed unprecedented complexity of tRNA biosynthesis, modification patterns, regulation and function. In this Review, we present emerging concepts regarding how tRNA abundance is dynamically regulated and how tRNAs (and their nucleolytic fragments) are centrally involved in stress signalling and adaptive translation, operating across a wide range of timescales. Mutations in tRNAs or in genes affecting tRNA biogenesis are also linked to complex human diseases with surprising heterogeneity in tissue vulnerability, and we highlight cell-specific aspects that modulate the disease penetrance of tRNA-based pathologies.

tRNAs are ubiquitous nucleic acid entities that are the most abundant of all small non-coding RNA molecules, constituting 4–10% of all cellular RNA. They are a fundamental component of the translation machinery in that they deliver amino acids to the ribosome to translate the genetic information in an mRNA template-directed manner into a corresponding polypeptide chain[1]. The discovery of this canonical function of tRNA as an adaptor molecule in translation is considered to be one of the pioneering triumphs of molecular biology. However, various studies have revealed that far from being a simple adaptor molecule, tRNA has a surprising range of functions. For example, high-resolution structural data of the translating ribosome have brought new insights into the substantial conformational plasticity of tRNAs (reviewed in REF. 2), and global approaches using high-throughput sequencing technologies have provided important insights into tRNA diversity, both in terms of variations of the tRNA genes among species and in terms of the unexpected diversity of tRNA-derived fragments that are not merely tRNA degradation debris but have active roles in stress signalling[3,4]. Furthermore, several diseases are caused by dysfunctions of numerous aspects of tRNA biology, including mutations in tRNAs themselves and in the auxiliary proteins involved in tRNA biogenesis and modifications[5–9].

Several excellent reviews describing tRNA biogenesis, structure, modifications and its function in translation have been published[1,2,10–13]. Here, we focus on emerging concepts regarding the dynamics of tissue-specific tRNA expression and abundance, and present updated views concerning the mechanisms of tRNA involvement in stress regulation, with a particular emphasis on stress-induced tRNA fragmentation as a signalling paradigm. We also provide examples of various tRNA-linked diseases. Strikingly, the tissues and organs affected by the pathology differ in each disease despite each tissue harbouring the same disease-causing mutation, and so we discuss their molecular mechanisms in the context of cell specificity and tissue specificity.

tRNA biogenesis and structure

tRNA molecules are synthesized as precursors that are then processed by a sequence of maturation events. These maturation events include removal of the 5′ leader, trimming of the 3′ trailer, splicing of introns, addition of the 3′-terminal CCA residues by a CCA-adding enzyme (reviewed in REF. 13) (FIG. 1a) and covalent modification of multiple nucleoside residues (BOX 1). Only correctly processed tRNAs leave the nucleus through a nuclear receptor-mediated export process, which serves as a checkpoint for sorting tRNAs with incorrectly processed termini.

Mature eukaryotic tRNAs are prepared to function as an adaptor in translation by the covalent attachment of an amino acid to the adenosine at the invariant 3′ CCA tail. This reaction is catalysed by 20 different aminoacyl tRNA synthetases (aaRSs), each of which is specific for

[1]*Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Strasse 24–25, 14476 Potsdam, Germany.*
[2]*Present address: Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, 120 University Place, Glasgow G12 8TA, UK.*
[3]*Present address: Institute of Biochemistry and Molecular Biology, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany.*
Correspondence to Z.I.
e-mail: zoya.ignatova@uni-hamburg.de
doi:10.1038/nrg3861
Published online
23 December 2014

Anticodon
Nucleotides 34, 35 and 36 of each tRNA that recognize a specific codon of mRNA.

A-site
The site of entry of the aminoacyl tRNA in the ribosome.

Codon
Three consecutive nucleotides on mRNA that encode one amino acid.

the 20 different amino acids[14]. aaRSs discriminate cognate tRNAs primarily by their anticodon loop and the discriminator base (N73) located before the 3′ CCA tail (FIG. 1b), and specificity is 'fine-tuned' by interactions with structural elements that are unique to particular tRNAs[14]. Charged with its cognate amino acid and in a complex with the elongation factor (EF1A in eukaryotes and archaea, and EF-Tu in bacteria), each tRNA reaches the A-site of the ribosome and base-pairs its anticodon with the corresponding mRNA codon. Thus, to fit the same ribosomal site, the architecture of all tRNAs conform to a narrow set of structural parameters constrained by common identity rules[2] and structural features[15]; however, they differ enough to serve their unique roles in decoding a specific codon (FIG. 1b). In contrast to nuclear-encoded tRNAs, the mitochondrial-encoded tRNAs (mt-tRNAs) show broader structural heterogeneity[8] as a result of the rapid evolution and minimization of the mitochondrial genome[16].

Shaping the tRNAome in cells and tissues

tRNA repertoires in genomes. Of the 64 possible codons (triplets), 61 codons are sense codons that collectively encode 20 amino acids, whereas the remaining 3 codons are nonsense (stop) codons. In occasional contexts, stop codons are read through and used to incorporate

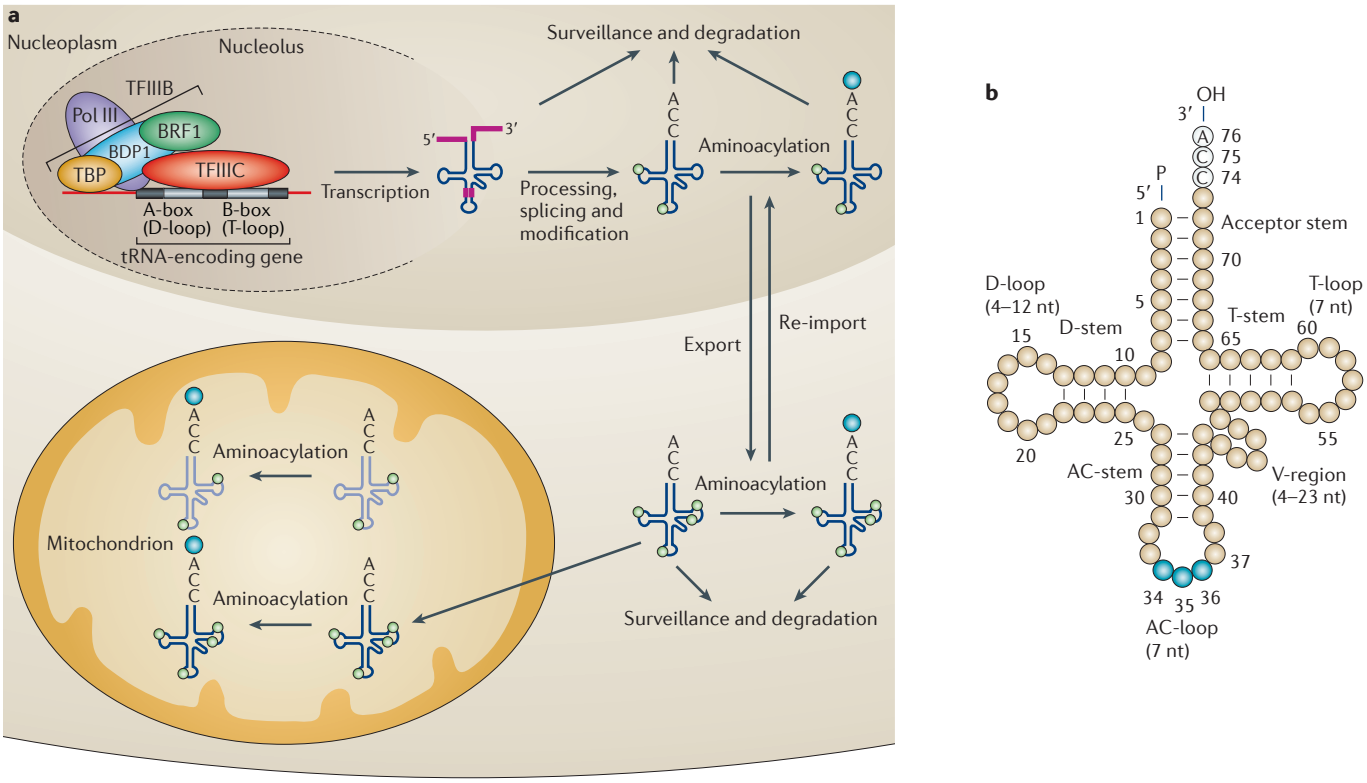

Figure 1 | **tRNA biogenesis and architecture. a** | tRNA biogenesis comprises multiple processes, including transcription, processing, splicing, post-transcriptional modification of nucleotides (green circles), CCA addition and aminoacylation (blue circle), nuclear-to-cytoplasmic shuttling and import into mitochondria (more details on each biogenesis process are reviewed in REF. 13). Transcription of tRNA genes is a concerted action of binding of the transcription factor TFIIIC to intragenic A-box and B-box (which encode parts of the D- and T-stems and loops, respectively), followed by recruitment of TFIIIB to the upstream regions, which directs the recruitment of RNA polymerase III (Pol III). The three subunits of TFIIIB — B double prime 1 (BDP1), B-related factor 1 (BRF1) and TATA-binding protein (TBP) — are shown. Aberrantly processed pre-tRNAs are eliminated through a nuclear surveillance pathway by degradation of their 3′ ends, whereas mature tRNAs lacking modifications are degraded from their 5′ends in the cytosol[13]. Aminoacylation can also occur in the nucleus, albeit to a much lower extent than in the cytoplasm, but it is unclear whether these aminoacyl-tRNAs directly participate in cytoplasmic translation. The reverse process, deaminoacylation, is also possible and occurs spontaneously to a very low extent. As only correctly processed tRNAs can be substrates of aminoacyl tRNA synthetases (aaRSs), nuclear aminoacylation is suggested to serve as a final proofreading step for the structural integrity of newly synthesized tRNAs[136].The mitochondrial genomes encode tRNA species (tRNA body shown in lighter blue) that are more bacteria-like, and their 3′ CCA tails are not encoded in the gene sequence. Mitochondrial import of nuclear-encoded tRNAs (tRNA body shown in darker blue) compensates for incomplete mitochondrial encoded tRNA (mt-tRNA) sets in some eukaryotes or is redundant in others with a full mt-tRNA set; however, only a maximum of a few nuclear-encoded tRNAs are imported into mitochondria[79]. For some organisms (for example, yeast and plants), the tRNA biogenesis pathway might differ from the depicted pathway (for more details refer to REF. 13). **b** | tRNA identity elements and structure (derived from the crystal structure; RSCB Protein Data Bank ID: 1EHZ) are shown. The predominantly nuclear-encoded eukaryotic tRNAs, as well as bacterial tRNAs, that function in translation have a length of 73–90 nucleotides (nt) and adopt a 'clover leaf'-shaped secondary structure. The acceptor stem is 7 bp long, the D-stem is 3–4 bp, and the anticodon (AC)-stem is 5 bp. Although in some tRNA species the D-loop can be 4–12 nt long and/or the variable (V) region, starting at residue 44, can be 4–23 nt long, the AC-loop (blue) is always numbered 34–36 and the CCA tail (white) is always numbered 74–76.

Translational frameshifting
A shift in the linear readthrough of mRNA in which the ribosome reads the second or the third nucleotide of a codon as the first nucleotide.

Box 1 | **Post-transcriptional tRNA modifications**

Among all RNA species, tRNAs undergo by far greatest number of and the most chemically diverse post-transcriptional modifications. Modifications are found at nearly 12% of all residues, with a median of 8 modifications per tRNA[117]. In eukaryotes, more than 100 chemically diverse modifications have been identified so far, many of which are conserved among organisms (see MODOMICS database[120]) and between tRNAs encoded in the nuclear or organelle genomes (see the table). Many modifications in mitochondrial-encoded-tRNAs are of bacterial origin[8,118].

Modifications in the stem–loops are crucial for tRNA structure and stability, whereas modifications in the anticodon loop affect the tRNA function in translation and increase the accuracy in translation by preventing translational frameshifting[11,33,119]. Modifications at position 34 (FIG. 1b) are typically associated with increasing the diversity of codon recognition through codon–anticodon wobbling[13,119]. Modification at this site is conserved in bacteria and eukaryotes, which has evolved in parallel to the species-specific genomic GC content to adapt tRNA gene populations for optimal translation[33]. Eukaryotes contain both U34- and C34-containing isoacceptors[18]. Furthermore, post-transcriptional modifications at base 37 adjacent to the anticodon loop (FIG. 1b) tune the stability of codon–anticodon interactions. Finally, tRNA modifications modulate the sensitivity of cells to various types of stress and might have implications in many human pathologies[119].

Positions	tRNA modification		Function
	Nuclear-encoded tRNA	Mitochondrial-encoded tRNA	
1, 29, 30, 35, 36 and 65	Ψ	Unknown	Unknown
4	Cm and Am	Unknown	Unknown
9	m^1G	m^1G and m^1A	tRNA folding
10	Unknown	m^2G	Unknown
12	ac^4C	Unknown	Unknown
16, 17 and 47	D	Unknown	Unknown
18	m^2G	Unknown	Unknown
20 and 20a-b	D	D	Unknown
26	m^2_2G	m^2_2G and m^2G	Unknown
27 and 31	Unknown	Ψ	Unknown
28, 39, 55 and 67	Ψ	Ψ	Unknown
32	Ψ, 2′*O*-methylribose and Cm	Ψ and m^3C	Unknown
34	I, Ψ, m^5C, Cm, Gm, 2′*O*-methylribose, Q, mcm^5U, ncm^5U, ncm^5Um and mcm^5s^2U	τm^5U, τm^5s^2U, f^5C, s^2U and Q	Wobble base-pairing (codon–anticodon interaction)
37	ms^2t^6A, yW, m^1I, I^6A and m^1G	m^1G, t^6A, i^6A, ms^2A and ms^2i^6A	Stabilization of codon–anticodon interaction and prevention of frameshifting
38	Ψ and m^5C	Unknown	Unknown
40 and 50	m^5C	Ψ	Unknown
41	m^5U	Unknown	Unknown
44	Um	Unknown	Unknown
46	m^7G	Unknown	Unknown
48	m^5C	Unknown	Unknown
49	m^5C	m^5C	Unknown
54	m^5U and rT	m^5U	tRNA stability
58	m^1A	m^1A	Unknown
64	Ar(p)	Unknown	Discrimination between initiator and elongator $tRNA^{Met}$

Conventional abbreviations are used for the modifications: τm^5s^2U, 5-taurinomethyl-2-thiouridine; τm^5U, 5-taurinomethyluridine; Ψ, pseudouridine; ac^4C, N^4-acetylcytidine; Am, 2′-*O*-methyladenosine; Ar(p), 2′-*O*-ribosyladenosine (phosphate); Cm, 2′-*O*-methylcytidine; D, dihydrouridine; f^5C, 5-formylcytidine; Gm, 2′-*O*-methylguanosine; I, inosine; I^6A, N^6-isopentenyladenosine; m^1A, 1-methyladenosine; m^1G, 1-methylguanosine; m^1I, 1-methylinosine; m^2G, N^2-methylguanosine; m^2_2G, N^2,N^2-dimethylguanosine; m^3C, 3-methylcytidine; m^5C, 5-methylcytidine; m^5U, 5-methyluridine; m^7G, 7-methylguanosine; mcm^5s^2U, 5-methoxycarbonylmethyl-2-thiouridine; mcm^5U, 5-methoxycarbonylmethyluridine; ms^2A, 2-methylthioadenosine; ms^2I^6A, 2-methylthio-N^6-isopentenyladenosine; ms^6t^2A, 2-methylthio-N^6-threonyl carbamoyladenosine; ncm^5U, 5-carbamoylmethyluridine; ncm^5Um, 5-carbamoylmethyl-2′-*O*-methylurid ine; Q, queuosine; rT, ribothymidine; s^2U, 2-thiouridine; t^6A, N^6-threonylcarbamoyladenosine; Um, 2′-*O*-methyluridine; yW, wybutosine. *Modifications were retrieved from the MODOMICS database[120], some of which are reviewed in REFS 8,117,119.

selenocysteine, pyrrolysine or phosphoserine[17]. Different tRNAs cumulatively decode all 61 sense codons; however, wobbling at the first position of some anticodons reduces the total number of tRNA isoacceptors required. The minimum set of isoacceptors is 30, but only a few bacterial and archaeal organisms encode ~30 tRNAs[18]. The actual number of nuclear-encoded tRNA genes varies considerably among organisms, even among closely related species, and generally increases with the complexity of the organism. Nuclear genomes of eukaryotic organisms contain between 171 (for *Schizosaccharomyces pombe*) and 12,794 (for zebrafish) tRNA genes, encoding 41–55 distinct isoacceptors in different eukaryotes[18]. In humans, there are 513 nuclear-encoded tRNA genes for 49 isoacceptors, in addition to 22 mt-tRNA genes[18]. Moreover, tRNA gene copy numbers show inter-individual variability in humans and can even differ among individuals who share the same ancestry[19,20]. The large number of tRNA genes arises because the isoacceptors are frequently encoded by an entire family of genes, and hence isoacceptors can be further subdivided into multiple tRNA isodecoders that bear the same anticodon but differ in sequence outside the anticodon[21]. The isodecoders are not simply a result of genetic expansions and neutral drift in larger genomes (see below); they also exist in bacteria and archaea. tRNA isodecoders may serve biologically non-redundant roles, and their expression may depend on the cell type and cell state[22].

Given the large numbers of tRNA genes in many organisms, it is metabolically wasteful to express all tRNA genes at the same time. What rules and concepts govern the expression of tRNAs, and what is the effect of differential tRNA expression levels on the cellular proteome?

Variation in tRNA transcription. Eukaryotic tRNA genes are dispersed throughout the linear genome, but characterization of their three-dimensional nuclear organization has revealed that they cluster in the nucleolus[23]. This shared nucleolar environment of tRNA genes raises the possibility of coordinated regulation of their transcription. The tRNA transcription cycle involves RNA polymerase III (Pol III) and is primarily guided by two multisubunit transcription factors: TFIIIC and TFIIIB. TFIIIC binds to the intragenic A-box and B-box that encode parts of the D- and T-stems and loops, respectively (FIG. 1). Conversely, TFIIIB — which is a complex consisting of B double prime 1 (BDP1), B-related factor 1 (BRF1) and TATA-binding protein (TBP) — is recruited upstream of the transcription start site (FIG. 1a). Overall, the sequence diversity among tRNA genes in the 5′ upstream region modulates the strength of Pol III binding[22] and is probably responsible for the variability in expression levels among tRNA isoacceptors in a given tissue, which can vary by up to tenfold[24]. Pol III interactions are likely to underlie the differential expression of particular tRNA genes among tissues of an organism. For example, various animal tissues differentially express multiple isoforms of BDP1 and BRF1 (REF. 25), which may modulate the strength of TFIIIB interactions with the 5′ regions of different isodecoder subgroups and thereby tune the tRNA tissue-specific expression. Consistent with this idea, subsets of mammalian isodecoders within one isoacceptor family share common motifs in their 5′ regions that might reflect coordinated regulation[25]. Moreover, the silk-producing glands of *Bombyx mori* specifically express a unique isodecoder of $tRNA^{Ala}$(AGC), the sequence of which contains a G40U substitution relative to the ubiquitously expressed $tRNA^{Ala}$(AGC) isoform[26]. This single-nucleotide substitution is outside the intragenic TFIIIC-interacting region or the TFIIIB-recognition sequences in the upstream region, suggesting that the mechanisms determining the tissue-specific tRNA expression are far more complex than merely TFIIIB- and TFIIIC-mediated Pol III binding.

tRNAome composition influences protein biogenesis. The unequal abundance of the tRNA isoacceptors within one cell shapes the non-uniform rate of translation (reviewed in REFS 27,28). Ribosomal speed is precisely regulated along the mRNA through the evolutionary selection of codons pairing to low-abundance tRNAs at specific positions. The transient pauses of ribosomes when these codons are translated facilitate co-translational protein folding (by allowing single domains of multidomain proteins to fold independently) and translocation (by facilitating interactions between the nascent protein chain and accessory factors)[28]. Consequently, synonymous substitutions can alter the kinetics of translation and ultimately influence protein structure and function[28–30].

In less complex organisms, including unicellular eukaryotes, tRNA abundance correlates with the codon usage of highly expressed genes[31], and tRNA gene copy numbers can be used as a proxy for cellular tRNA concentrations[32]. In higher eukaryotes, the correlation between the genomic codon usage and tRNA gene frequencies is relatively poor, but is significantly improved when species-specific covalent modifications at the U34 base in the anticodon are considered[33]. However tRNA gene frequencies cannot explain the tissue-specific pattern of tRNA expression[34]. The differential tissue-specific expression of human tRNAs[34] mirrors the codon bias of tissue-specific proteins[35], indicating that tRNA expression is tightly coordinated with the translational needs of the cell type[34]. This idea is supported by studies demonstrating that for paralogous genes the paralogue with a codon bias that is most compatible with the tRNA pools in a particular tissue[36] is preferentially expressed[37]. Moreover, the tRNA pools of proliferating versus differentiating human cells show reversed expression signature (that is, tRNAs expressed in proliferating cells are typically repressed in differentiating cells), which reflects the codon usage of the protein-coding genes that are selectively expressed in each cell type[35]. Interestingly, in each cell type the chromatin landscape (such as histone modification patterns) changes similarly at Pol II-transcribed genes encoding tissue-specific mRNAs and at the Pol III-transcribed tRNA genes that reflect the codon enrichment of these mRNAs; this suggests coordinated transcriptional regulation of an mRNA set and their corresponding tRNAs so that tRNA supply matches demand[35,38,39].

Wobbling
Non-Watson–Crick base pairing between the third base in the codon with the first nucleotide of the tRNA anticodon (nucleotide 34 in tRNA numbering).

tRNA isoacceptors
Different tRNA species carrying the same amino acids but with different anticodon sequences.

tRNA isodecoders
Distinct tRNA species bearing the same amino acids and anticodons but with sequence variations in the tRNA body.

Transcription factors
Proteins that bind to specific sequences in DNA and control the transcription of a gene.

Synonymous substitutions
Substitutions of nucleotides in the exons of protein-coding genes that do not change the encoded amino acid.

Codon bias
The difference in occurrence of codons encoding the same amino acid.

Paralogous genes
Genes that arose from a duplication event but have diverged from a parent copy by mutation and selection drift; they may evolve new functions.

Evolutionary malleability of tRNA copies. The greater number and divergence of tRNA genes in vertebrates relative to single-celled organisms may be a result of genomic expansion and neutral drift[40]. However, tRNA diversity is not merely a functionless evolutionary relic: selection of isodecoders with altered sequence of the tRNA bodies modulates the efficiency of aminoacylation and interactions with ribosomes[41,42]. The question that is then raised is what drives the asymmetry of copy numbers between tRNAs in each organism. A possible explanation is that some tRNAs within particular isoacceptor families are selected to remain at low gene copy numbers (and therefore probably low expression levels) to provide an adaptive and important benefit through shaping the non-uniform translation speed of mRNA to facilitate protein folding and biogenesis[28]. In support of this view, increasing the copy number of low-abundance tRNAs alters the folding and solubility of many cellular proteins and decreases cell fitness[43].

Some isoacceptors are present in only one copy[18,19], and a mutation in a singleton isoacceptor, specifically in the anticodon, may be detrimental for the cell. Indeed, a deletion of a single-copy tRNA induces proteotoxic stress, whereas deletion of a tRNA gene from families with multiple copies has a mild effect[44]. In *Saccharomyces cerevisiae*, each tRNA isoacceptor family consists of 1–16 copies, with 6 tRNAs having only 1 copy. Interestingly, in response to the experimental deletion of a single-copy $tRNA^{Arg}$(CCU) isoacceptor, this *S. cerevisiae* strain evolved over the following 200 generations to compensate for the defect through a single-nucleotide mutation in the anticodon of 1 of 11 isodecoders of $tRNA^{Arg}$(UCU) from UCU to CCU to substitute for the missing singleton $tRNA^{Arg}$(CCU)[45]. Thus, mutation-based codon shifting of an isoacceptor from the same isoacceptor family enables rapid compensation of tRNA loss[45]. However, naturally occurring tRNA isodecoders vary in their functionality, including in their levels of aminoacylation and stability[46], implying that only some isodecoders might be suited for such compensations. Mutations in the anticodon to re-adjust the ratios of tRNA pools within the same isoacceptor family are more frequent than duplications and deletions of tRNA genes; the latter may carry negative effects because of the duplication and/or deletion of adjacent unrelated genetic features[45]. This rapid adaptability of tRNA pools provides evolutionary plasticity to counteract mutations and to maintain translational balance.

Stress-induced dynamics of tRNA pools

Suboptimal growth conditions and various types of environmental stress require rapid and adequate reprogramming of gene expression at different regulatory levels and timescales[47]. Various stress factors alter tRNA pools, and such changes are traditionally viewed as modulating tRNA pools to mirror the codon usage of stress-related genes. However, emerging evidence indicates that tRNAs participate more centrally in stress responses by functioning directly as signalling molecules (FIG. 2).

tRNA cleavage: signalling versus translational inhibition? The earliest reports on tRNA deactivation through endonuclease-induced cleavage in the anticodon loop to produce 5′ and 3′ tRNA halves come from studies on *Escherichia coli*, in which bacteriophage infection is offset by cleavage of an *E. coli* tRNA[48]. tRNA fragments resulting from cleavage in the anticodon loop were generally considered to be debris of the tRNA surveillance and clearance pathways that degrade tRNAs with altered structural and functional integrity[13]. However, tRNA fragments are now known to occur in organisms spanning all domains of life, in which they perform crucial and conserved functions in regulated responses to different types of stress (for example, nutrient deprivation, oxidative and thermal stress, and upon innate immunoactivation)[49–53].

The cleavage events are executed by dedicated stress-inducible endonucleases, such as Rny1 (a member of the RNase T2 family) in yeast[54] and angiogenin (a member of the RNase A family) in human cells[55]. Notably, the level of mature, full-length tRNAs does not decline significantly[4,56], implying that stress-induced cleavage in the anticodon loop is unlikely to be a mechanism of translational repression merely through a depletion of the cellular tRNA pool. The primary targets of angiogenin are tRNAs with CA sequences in their anticodon loop[57]. 5′ tRNA halves with a terminal oligo-G motif displace eukaryotic translation initiation factor 4E (eIF4E) and eIF4G, and interfere with translation initiation[58,59] (FIG. 2Aa) in a manner similar to microRNA-mediated and small interfering RNA (siRNA)-mediated RNA interference. Moreover, 5′ tRNA halves, but not 3′ tRNA halves, act as stress signalling entities[60], and specifically reduce translation[59] and induce stress granule formation in mammalian cells[58]. A recent study has uncovered an additional signalling function of cleaved tRNAs during heat stress: tRNA-derived fragments facilitate the expression of stress-response genes by interfering with their siRNA-mediated silencing[61]. 5′ tRNA halves (and occasionally 3′ tRNA halves) are found in the serum of mice even under normal growth conditions, and their composition changes with ageing[62]. They circulate in the serum as a part of a large nucleoprotein complex, which highlights their potential role as signalling molecules and argues against them being simply debris from the cytoplasmic surveillance machinery.

In general, the specificity of angiogenin for single-stranded RNAs suggests that in addition to the anticodon loop, T-loops and D-loops are also potential cleavage targets for angiogenin, although post-transcriptional modifications at residues in the D-loops and T-loops may mask the nuclease recognition site. Shorter tRNA fragments of 13–30 nucleotides (designated as 5′tRFs or 3′tRFs), resulting from cleavage in either the T-loop or the D-loop, have been identified in organisms spanning all domains of life. It is unclear whether these shorter tRNA fragments also result from nuclease-induced cleavage, but they repress translation via association with the components of the RNA interference machinery and exert microRNA-like functions[3].

Proteotoxic stress
A collective term to describe the intracellular stress caused by toxic protein aggregation.

tRNA isoacceptor family
A family of all tRNA isoacceptors carrying the same amino acid.

Endonuclease
An enzyme that hydrolyses the phosphodiester bond between two nucleotides in a sequence.

RNA interference
A process by which short RNA sequences block gene expression by binding to specific mRNAs to cause their destruction.

Although CA motifs are present in the anticodon loops of only a small subset of tRNAs[57], all tRNA species carry the ubiquitous CCA sequence at their 3′ ends. Indeed, in response to severe oxidative stress, angiogenin deactivates all tRNAs by endonucleolytic cleavage within their 3′CCA termini[57]. This response occurs on a much faster timescale than the generation of the tRNA halves (FIG. 2B) and adds another layer of control to protein translation under stress through a global depletion of all translationally competent tRNAs (FIG. 2Ab). Translation is among the most energy-consuming cellular processes, and so a global translational shutdown might be an energy- and resource-saving programme and can be rapidly annulled upon stress relief. Indeed, the angiogenin-induced deactivation of the 3′CCA termini is a dynamically reversible process, and the 3′CCA termini are rapidly repairable by the ubiquitous CCA-adding enzyme at low metabolic cost[57]. This is in contrast to the metabolically expensive regeneration of tRNAs with cleaved anticodon loops because they can only be replaced through a new transcription cycle.

Alternative roles of tRNAs in stress signalling. The most potent stress-induced inhibition of translation is the repression of translation initiation by kinase-induced phosphorylation of Ser51 of eIF2α, which selectively represses the translation of mRNAs with scanning or cap-dependent translation[63]. Stress-specific signatures of the response are shaped by the activation of distinct kinases for each type of stress — for example, haem-regulated inhibitor kinase (HRI) by oxidative stress, general control nonderepressible 2 (GCN2) kinase by amino acid-deprivation stress and double-stranded RNA-activated protein-like ER kinase (PERK) by unfolded protein stress[64].

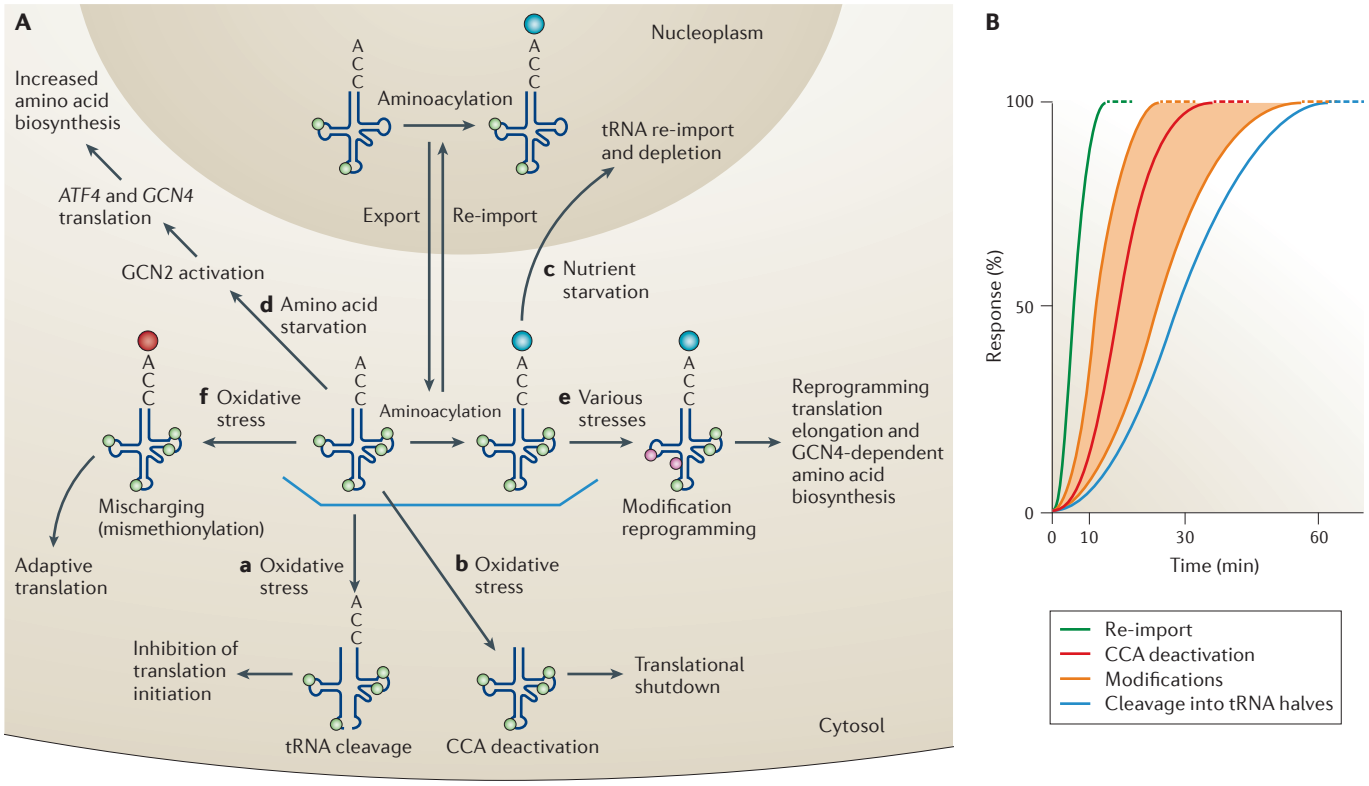

Figure 2 | **Stress-induced dynamics of tRNA pools.** **A** | The response to environmental stress activates different pathways. **a** | tRNA-derived fragments repress translation initiation through eukaryotic translation initiation factor 4E (eIF4E) and eIF4G displacement[58,59]. **b** |Oxidative stress-induced deactivation of the CCA tail shuts down global translation[57]. **c** | Nutrient starvation induced by glucose, nitrogen, amino acid or inorganic phosphate deprivation causes tRNA retrograde transport into the nucleus[65]. Mechanistically, nuclear tRNA import provides a means of global translational shutdown by depleting translation-competent tRNAs when nutrients are limited. **d** | Amino acid starvation activates the general control nonderepressible 2 (GCN2) kinase pathways. This leads to increased translation of activating transcription factor 4 (*ATF4*) and *GCN4* mRNAs to enhance amino acid biosynthesis[66,67]. **e** | Stress-induced modification reprogramming triggers a reprogramming of translation elongation[68,69]. **f** | Oxidative stress-induced mismethionylation triggers enhanced methionine incorporation through methionylation of non-Met-tRNA[75–77]. **B** | Kinetics of stress-induced alterations in tRNA pools is shown. The graph is a schematic but is based on the current understanding of the timescales of each process of tRNA regulation. tRNA re-import into the nucleus upon amino acid starvation is the fastest process and is accomplished within 10 minutes[65]. The oxidative stress-induced angiogenin-mediated cleavage of the 3′ CCA tails of tRNAs occurs within 30 minutes[57]. The cleavage of the anticodon loop of some tRNAs[57] is a much slower process and is accomplished in a timescale of 60 minutes. The timescale of reprogramming covalent modifications may vary (orange shaded area) depending on the organism, the type of modification and the presence of any adjacent modifications. (P. Dedon, Massachusetts Institute of Technology, Cambridge, USA, and T. Begley, University at Albany Institute, New York, USA, personal communication).

Under deprivation of either amino acids or other nutrients, a proportion of mature cytoplasmic tRNAs are rapidly re-imported into the nucleus as a mechanism for global translational repression[65] (FIG. 2Ac). In addition, during amino acid starvation, non-charged tRNAs activate GCN2 through binding to its carboxy-terminal domain, which is homologous to histidyl tRNA synthase. GCN2-induced eIF2α phosphorylation activates the translation of mRNAs encoding activating transcription factor 4 (ATF4) in mammals and GCN4 in yeast (FIG. 2Ad), which are required for the selective transcription of genes in amino acid biosynthesis pathways to increase amino acid biosynthesis[66]. Translation of *ATF4* and *GCN4* are regulated through upstream open reading frames (uORFs) in these mRNAs. The uORFs are only translated in unstressed conditions, which interfere with the translation of the principal *ATF4* or *GCN4* ORFs downstream. During amino acid deprivation, eIF2α phosphorylation results in the inhibition of $tRNA_i^{Met}$–eIF2α–GTP ternary complex formation, which enables 40S ribosomes to scan past uORFs and instead to initiate translation at the start codons of the *GCN4* or *ATF4* principal ORFs[67].

Reprogramming of tRNA modifications. Varying sources of stress dynamically shift the population of covalent tRNA modifications (BOX 1) with a specific signature for each type of stress[68] (FIG. 2Ae). The sequences of stress-response genes often display a significant bias towards certain amino acids or synonymous codons; thus, stress-related alterations in tRNA modification are commonly viewed as an adaptive mechanism to specifically enhance the expression of those genes. For example, in *S. cerevisiae*, exposure to oxidative stress increases Trm4-mediated 5-methylcytosine (m^5C) modification at the wobble C34 base in $tRNA^{Leu}$(CAA), allowing selective translation of stress-related genes with over-represented Leu-TTG codons[69].

However, recent findings argue against this classical view that tRNA modifications primarily fine-tune the expression of stress-related proteins and suggests instead that tRNA modifications have a direct role in stress signalling[70]. The wobble U34 base undergoes the largest number of modifications in cells: addition of carbamoylmethyl (ncm^5), methoxycarbonylmethyl (mcm^5) or concomitant mcm^5 and 2-thio (mcm^5s^2) groups enhances the *in vivo* ability of tRNAs to decode G- and A-ending codons of the six VAA and VAG codons (where V represents A, C or G nucleotides)[71]. Loss of mcm^5, mcm^5s^2 or s^2 modifications at U34 in tRNAs containing these modifications does not compromise translation fidelity but instead weakens the anticodon–codon interactions and reduces the rate of translation of Watson–Crick VAA and wobble VAG codons[72]. Using ribosomal profiling, which is a genome-wide approach for precisely determining the position of ribosomes on transcripts[73], Gilbert and co-workers[70] detected differences of the ribosomal distribution over VAA and VAG codons in yeast cells lacking the mcm^5s^2 modification at U34 compared with the wild-type cells; however, this change of ribosomal distribution was insufficient to affect the protein output. Instead, tRNAs lacking the mcm^5s^2 modification were less efficiently aminoacylated[14], and these uncharged species accumulated in the nucleus[70], activating *GCN4* translation[67] (FIG. 2Ae) and consequently amino acid biosynthesis[70].

tRNA mischarging. aaRSs are generally highly accurate, with an error rate of 1×10^{-4} to 1×10^{-5} per reaction[14]; however, there are notable exceptions for which lower fidelity charging results in tRNAs bearing non-cognate amino acids. *Candida albicans* is a notable example of a species that uses mistranslation. *C. albicans* regulates the adhesion properties of its cell surface proteins and consequently its virulence by modulating the Ser:Leu ratio incorporated though a single CUG codon that is translated by both Ser-$tRNA^{Ser}$(CAG) and Leu-$tRNA^{Ser}$(CAG) that are aminoacylated by either SerRS or LeuRS[74].

Another example of tRNA mischarging in various species is MetRS, which frequently mismethionylates non-Met-tRNAs[75]. In mammalian cells, the basal mismethionylation of non-Met-tRNAs has been estimated to be ~1% and increases up to ~10% upon viral infections or exposure to oxidative stress[76]. Similarly, oxidative stress enhances the mismethionylation of tRNAs in *E. coli*[77] and yeast[75], defining it as a conserved response (FIG. 2Af). Although enhanced Met incorporation through mismethionylated-tRNAs may increase the aggregation propensity of the synthesized peptides and proteins, the beneficial role of enhanced Met misincorporation for cells is to protect them against oxidative damage[75], which may offset the damage from protein aggregation. Consistent with the idea of the reactive oxygen species (ROS)-scavenging capacity of Met is the extensive utilization of Met as a building block in the mitochondrial respiratory chain complexes, which offsets the effects of highly oxidative conditions in the inner mitochondrial membrane[78].

tRNAs in disease

Genetic disorders in which tRNA alterations are thought to play a direct part can be classified into two groups of pathogenic mutations: within the tRNAs or in the tRNA processing and modifying enzymes. Additionally, many diseases that do not have a direct mutational link to tRNAs and their associated enzymes nevertheless display alterations in tRNA pools, albeit usually as a secondary effect of the altered disease biology. Intriguingly, the disease phenotypes of many of tRNA-based pathologies are potentiated in a tissue-specific manner, which we specifically emphasize in this section.

Mutations in tRNA genes. Strikingly, for all the identified cases in which human disease are directly linked to mutations in tRNAs, these mutations occur in mt-tRNAs. A possible explanation is that a mutated mt-tRNA is unlikely to be compensated for by other tRNAs: each mitochondrial genome bears a single copy of only 22 mt-tRNAs (there are generally no isodecoders to compensate), and importing a nuclear-encoded tRNA is rare, as such import in humans exists for only $tRNA^{Gln}$(UUG) (reviewed in REF. 79) (FIG. 1a). The

number of mitochondria per cell varies from hundreds to thousands depending on the energetic needs of each cell. If all mitochondrial genomes in one cell carry a mutation, a condition known as homoplasmy, the effect of this mutation is enhanced. However, a pathogenic mutation in mt-tRNA may also affect a proportion of the mt-tRNA copies, a condition known as heteroplasmy. Manifestation of a clinical phenotype depends on the threshold of mutation-affected mitochondria; this threshold varies among tissues and typically needs to exceed 85–90% to cause organ dysfunction[8]. Whole-genome analyses of patients with these genetic disorders led to the identification of a wide range of mt-tRNA aberrancies with as yet unresolved molecular mechanisms; these mutations are catalogued in variety of databases, such as MITOMAP and Online Mendelian Inheritance in Man (OMIM)[5,7,8] (TABLE 1).

A mutation that alters the tRNA anticodon would have severely deleterious effects. However, only few pathogenic mutations occur at one of the anticodon nucleotides; such mutations might be incompatible with early development stages and are likely to be lethal in embryogenesis[80]. In a patient suffering from mitochondrial myopathy and abnormal mitochondrial proliferation in the muscle (ragged-red fibres), the G36 nucleotide within the mt-tRNA^{Pro}(UGG) anticodon was substituted to UGA encoding Ser[81]. The authors do not report whether this mutation leads to misincorporation of Pro at Ser UCA codons[81], but it is likely because the discriminator base (N73) and the structural identity of the tRNA for the aaRS remain unaltered[14]; therefore, the ProRS might still charge tRNA^{Pro}(UGA) with Pro.

Anticodon structure and tRNA decoding efficiency can be changed through alterations of modifications in the anticodon. The most common mutations (BOX 1) in two mitochondrial encephalopathies — mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), and myoclonus epilepsy associated with ragged-red fibres (MERRF) — are linked to alterations in the modification pattern of U34 in the anticodons of mt-tRNA^{Leu}(UAA) and mt-tRNA^{Lys}(UUU), respectively. In MELAS, mt-tRNA^{Leu}(UAA) with mutations in the mt-tRNA body, A3243G (A14 in the D-loop in tRNA numbering)[76] (FIG. 1b) or T3271C (U40 in the anticodon stem in tRNA numbering)[82,83] (FIG. 1b), lack taurinomethyl (τm^5) modification at U34, which destabilizes U:G wobble interaction and decreases the fidelity of decoding Leu UUG but not Leu UUA[84]. In MERRF, mutation A8344G (A54 in the T-loop in tRNA numbering) (FIG. 1b) of the mt-tRNA^{Lys}(UUU) prevents 5-taurinomethyl-2-thiouridine ($\tau m^5 s^2$) modification at the U34 position, rendering the tRNA unable to translate both Lys codons, AAA and AAG[85] (FIG. 3a). Although both diseases share similar molecular traits — a mutation in a loop that is distant from the anticodon loop perturbs the U34-thiolation of a single tRNA — their pathologies differ substantially. The effect of MERRF mutations is probably more detrimental for the translation of mitochondrial proteins than MELAS; that is, MERRF mutations cause perturbation of the two Lys AAA and AAG codons, whereas MELAS mutations alter the reading of Leu UUG only[8]. This notion is supported by the loss of cytochrome *c* oxidase (COX) histochemical activity in MERRF, which is preserved in MELAS[86,87]. Unique to MELAS is the respiratory defect arising from deficiency of respiratory chain complex I — probably due to the enrichment of Leu UUG codons in the genes encoding this complex — and the presence of eponymous stroke-like episodes that can be linked to COX positivity because the absolute amount of COX in the positive blood vessels[87] (the second tissue together with muscles of MELAS and MERRF manifestation) is substantially higher than normal[7].

There are numerous additional examples of mutations in tRNA genes causing disease. A homoplasmic mutation A4263G in pre-tRNA^{Ile}(GAU) alters its 5′ processing by RNase P and reduces the level of the mature form to ~46%, as well as total mitochondrial translation to 32%, of normal levels[88]. Why is the tRNA^{Ile}(GAU) mutation connected to hypertension? A possible explanation is that lower tRNA^{Ile}(GAU) levels reduce the amount of respiratory complexes I, III and IV, which are significantly enriched in AUC and/or AUU codons that pair to tRNA^{Ile}(GAU), thereby lowering substrate-dependent oxygen consumption to 70–80% of normal levels[88]. Consequently, the overall respiratory capacity is reduced, which in turn increases the level of ROS and leads to hypertension[88]. Hypertension in several animal models is alleviated by inhibition of mitochondrial generation of ROS[89], which supports a direct link between hypertension and oxidative stress. Additionally, a homoplasmic mutation A4435G (A37 in the anticodon loop in tRNA numbering (FIG. 1b)) considerably decreases the level of the mitochondrial tRNA^{Met}(CAU) and consequently reduces translation in mitochondria, which is considered to be an inherent risk factor for hypertension[90].

Beyond human disease, a C50T mutation in the T-stem loop (FIG. 1b) of the brain-specific isodecoder tRNA^{Arg}(UCU) (encoded by the nuclear gene *n-Tr20*) was recently identified as a background mutation in the C57BL/6J mouse strain; this mutation affects the processing of and significantly decreases the aminoacylation of this tRNA[91]. Consequently, this dramatic reduction of translationally competent tRNA^{Arg}(UCU) causes anomalous stalling of ribosomes over Arg AGA codons, but this defect is typically compensated for by the surveillance machinery (including release and ribosome recycling factors). However, C57BL/6J mice that also lack GTP-binding protein 2 (GTPBP2), which assists in the rescue of stalled ribosomes, develop progressive neurodegeneration[91]. Intriguingly, *n-Tr20* is specifically expressed in the central nervous system (CNS) and constitutes ~60% of the total amount of all tRNA^{Arg}(UCU) isodecoders in the brain[91]; thus, disease manifestation is confined to the CNS despite the non-tissue-specific expression of *Gtpbp2*. Orthologues of *Gtpbp2* and *n-Tr20* have been identified in humans, although it is not yet known whether mutations would resemble the effect in mice. However, the reported results[91] underscore that a mutation in a nuclear-encoded tRNA may itself be phenotypically silent but epistatically exacerbate the deleterious effect of another mutation.

Homoplasmy
The presence of a single mitochondrial-encoded tDNA genotype in a cell.

Heteroplasmy
The presence of a mixture of more than one mitochondrial-encoded tDNA genotype in a cell.

Table 1 | **tRNA-related diseases***

Disease type	Disease	Affected gene	Pathological effect	Refs
Mutations in tRNA genes				
Mitochondrial	Combined oxidative phosphorylation defect (COXPD)	*MT-TW*	Reduced $tRNA^{Trp}$(UCA) levels	137
		MT-TR	Reduced $tRNA^{Arg}$(UCG) levels	137
	Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS)	*MT-TL*	Impaired 3′ processing and reduced $tRNA^{Leu}$(UAA) levels	138
		MT-TL1	Lack of τm^5U34 modification and impaired translation of Leu codon UUG	84
		MT-TL1	Reduced activity of $tRNA^{Leu}$(UAA)	139, 140
		MT-TH	Mutation in the D-stem leads to tRNA misfolding	141
	Myoclonic epilepsy with ragged-red fibres (MERRF)	*MT-TL1*	Lack of τm^5s^2U34 modification and impaired translation of Lys codons AAA and AAG	142
	Cardiomyopathy	*MT-TI*	Mutation in the D-stem leads to reduced tRNA levels	142
	Chronic ophthalmoplegia	*MT-TI*	T-stem mutations leads to misfolding and improper 3′ end processing	143,144
	Ragged-red fibres (RRFs)	*MT-TP*	Impaired mitochondrial function	81
	Cataract, spastic paraparesis and ataxia	*MT-TE*	Mutation in the T-stem disrupts conserved base pairing	81
	Neonatal death	*MT-TV*	Reduced tRNA levels	145
	Ataxia	*MT-TV*	Predicted to alter tRNA structure and function	146
		MT-TS2	Predicted to alter tRNA structure and function	147
	Myopathy	*MT-TD*	Unknown	148
		MT-TM	Impaired tRNA folding and reduced charging level	149
	Leigh syndrome	*MT-TW*	Unknown	150
	Hypertension	*MT-T1*	tRNA misprocessing and reduced $tRNA^{Ile}$(GAU) levels	88
		MT-TM	Reduced $tRNA^{Met}$(CAU) levels	90
Mutations in tRNA processing, charging and modification enzymes				
Metabolic	Type 2 diabetes mellitus	*CDKAL1*	Mistranslation of Lys codons AAA and AAG	105,106
		LARS2	Reduced charged $tRNA^{Leu}$ levels	151
Cancer	Breast cancer	*TRMT12*	Altered tRNA modification	102
Mitochondrial	Myopathy, lactic acidosis and sideroblastic anaemia (MLASA)	*YARS2*	Reduced aminoacylation	152
	Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL)	*DARS2*	Reduced aminoacylation	153
	Recessive ataxia	*MARS2*	Reduced aminoacylation and reduced protein synthesis	153
	Myopathy and infantile Charcot–Marie–Tooth syndrome	*AARS2*	Reduced aminoacylation	9
Neurological	Intellectual disability	*ADAT3*	Impaired A-to-I editing at tRNA position 34	100
	Dubowitz syndrome	*NSUN2*	Impaired modification of $tRNA^{Asp}$(GTC)	101
	Charcot–Marie–Tooth syndrome	*GARS*	Impaired aminoacylation	98
		AARS	Reduced aminoacylation and mischarging	9,95
		KARS	Impaired aminoacylation	9
	Dominant intermediate Charcot–Marie–Tooth syndrome	*YARS*	Gain of function of mutant YARS	9
	Pontocerebellar hypoplasia	*CLP1*	tRNA misprocessing and reduced tRNA levels	92,93
Others	Perrault syndrome	*HARS*	Reduced aminoacylation	154

Table 1 (cont.) | **tRNA-related diseases***

Disease type	Disease	Affected gene	Pathological effect	Refs
Alterations in the tRNA pool accompanying diverse disease states				
Metabolic	Type 2 diabetes mellitus	Not directly related to specific tRNA-associated mutation	Increased aminoacylation of tRNAs	114
Cancer	Breast cancer	Not directly related to specific tRNA-associated mutation	Upregulation of tRNAs carrying polar and charged amino acids	110
	Multiple myeloma	Not directly related to specific tRNA-associated mutation	Increased tRNA levels	111
	Various carcinomas	Not directly related to specific tRNA-associated mutation	Increased translation of oncogenic genes	155
Neurological	Huntington disease	Not directly related to specific tRNA-associated mutation	Reduced charged $tRNA^{Gln}$(CUG) levels and reduced generation of *trans*-frame encoded species	112
Infection	Influenza A	NA	Alterations in translationally active tRNA pool	156
	Vaccinia	NA	Alterations in translationally active tRNA pool	156
	West Nile virus	NA	Increased aaRS expression	157
	Japanese encephalitis virus	NA	Increased aaRS expression	157
	HIV	NA	Alterations in the tRNA pool	158

$\tau m^5 s^2 U34$, 5-taurinomethyl-2-thiouridine34; $\tau m^5 U34$, 5-taurinomethyluridine 34; aaRS, aminoacyl tRNA synthetase; *AARS*, alanyl tRNA synthetase; *AARS2*, mitochondrial alanyl-tRNA synthetase 2; *ADAT3*, adenosine deaminase tRNA-specific 3; *CDKAL1*, CDK5 regulatory subunit associated protein 1-like 1; *CLP1*, cleavage and polyadenylation factor I subunit 1; *DARS2*, mitochondrial aspartyl-tRNA synthetase 2; *GARS*, glycyl-tRNA synthetase; *HARS*, histidyl-tRNA synthetase; *KARS*, lysyl-tRNA synthetase; *LARS2*, mitochondrial leucyl-tRNA synthetase 2; *MARS2*, mitochondrial methionyl-tRNA synthetase 2; *MT-Tx*, mitochondrially encoded tRNA x (where x= single letter amino acid code); NA, not applicable; *NSUN2*, NOP2/Sun domain family, member 2 encoding cytosine 5 RNA methyltransferase; *TRMT12*, tRNA methyltransferase 12 homologue; *YARS*, tyrosyl-tRNA synthetase; *YARS2*, mitochondrial tyrosyl-tRNA synthetase 2. *This is a selection of mutations; the complete list is in MITOMAP and OMIM. Additional examples of mitochondrial tRNA-related diseases are included in REF. 159, human diseases related to tRNA modifications are reviewed in REF. 119, and human cancer related to mutations in aaRSs are discussed in REF. 9.

Mutations in tRNA processing, charging and modification enzymes. CLP1, which is a newly identified mammalian RNA kinase, is involved in the degradation circuit of some pre-tRNA species in the nucleus. An R140H mutation in human *CLP1* impairs interactions of the tRNA with the tRNA splicing endonuclease complex (TSEN) and decreases pre-tRNA processing in fibroblasts and neurons[92,93]. Affected individuals exhibit brain malformations, microcephaly, developmental delays and intellectual disabilities with symptoms similar to pontocerebellar hypoplasia[93]. CLP1 kinase-dead mice also show a progressive loss of lower motor neurons and fatal deterioration of motor functions due to enhanced accumulation of introns derived from aberrant processing of the $tRNA^{Tyr}$ precursor[94]. The pre-tRNAs, which escape the cellular clearance system, do not interfere with the translation machinery, but instead sensitize cells[93] (through a currently unknown molecular mechanism) to oxidative stress-induced activation of the p53 tumour suppressor pathway[94]. Intriguingly, neurodegeneration shows specific involvement of hindbrain and forebrain in humans[93] and motor neurons in mice[94]. Cerebellar Purkinje cells and spinal motor neurons are the largest neuronal cells and are likely to be the most metabolically challenged among all neuronal cells, which may enhance their susceptibility across the mutation spectrum[93].

Mutations within aaRS genes reduce their aminoacylation activity and are associated with a range of pathological conditions[9] (FIG. 3b; TABLE 1). Charcot–Marie–Tooth (CMT) syndrome is a hereditary motor neuropathy linked to mutations in several aaRS genes (TABLE 1). One form of CMT in which some patients also show significant neuronal degeneration[95] is caused by mutations in *AARS* (which encodes AlaRS). The resulting defect in AlaRS leads to low $Ala\text{-}tRNAs^{Ala}$ levels[95], although an additional $Ser\text{-}tRNAs^{Ala}$ mischarging might also contribute to human CMT pathology[9]. This is similar to the mouse sticky mutation ($Aars^{sti/sti}$)[96], which is a missense mutation in *Aars* that causes misacylation of $tRNAs^{Ala}$ with Ser. The resulting excessive misincorporation of non-cognate amino acids in the cellular proteome causes protein misfolding[96] and leads to neuronal degeneration with overt ataxia[97]. The neuronal degeneration of human CMT syndrome and in mice harbouring the sticky mutation is only observed in specific types of cells: neuromuscular synapses in CMT[98] or Purkinje cells in mice with the sticky mutation[97]. One possible explanation for the vulnerability of specific cells to disease is that postmitotic cells, particularly neurons, seem to be extremely sensitive to the deleterious effects of misfolded proteins[99], possibly because misfolded proteins cannot be diluted by cell division or because postmitotic cells might be inherently less efficient at degrading them.

Mutations in multiple proteins involved in tRNA modifications have been identified in patients and associated with disease[100–102] (TABLE 1), although causal molecular evidence for their link to disease pathology is missing for most of them. In one well-characterized example, microcephaly in humans and mice is linked to mutations in the cytosine-5 RNA methyltransferase *NSUN2*, which leads to site-specific loss of m^5C modification in tRNAs[103]. Lack of these modifications at positions 48–50 (BOX 1) increases the angiogenin-mediated endonucleolytic cleavage in the anticodon loop of tRNAs and reduces protein translation[103]. Intriguingly, although mouse skin cells maintain normal skin homeostasis and develop only high sensitivity to stress, neuronal tissues undergo increased apoptosis and cell shrinkage[103]. Dysregulation of translation alters local protein synthesis that is crucial for synapse development[104], which may explain the higher susceptibility of neuronal cells to damage.

In another example of a defective tRNA modification enzyme underlying disease, mutations in the CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*) gene are frequently associated with an increased risk for developing type 2 diabetes mellitus in humans and mice. CDKAL1 catalyses the 2-methylthio-N^6-threonylcarbamoyladenosine (ms^2t^6A) modification of A37 in $tRNA^{Lys}(UUU)$[105,106] (BOX 1); this modification is crucial for codon–anticodon interaction and for preventing translational misreading. *Cdakl1*-deficient mouse β-cells display a significantly reduced incorporation of Lys residues (which is indicative of misreading of Lys codons AAA or AAG[105,107]) and altered glucose-induced proinsulin biosynthesis and folding (FIG. 3a).

Alterations in the tRNA pool accompany diverse disease states. Even in the absence of specific alterations in genes closely linked to tRNA biology, various disease states

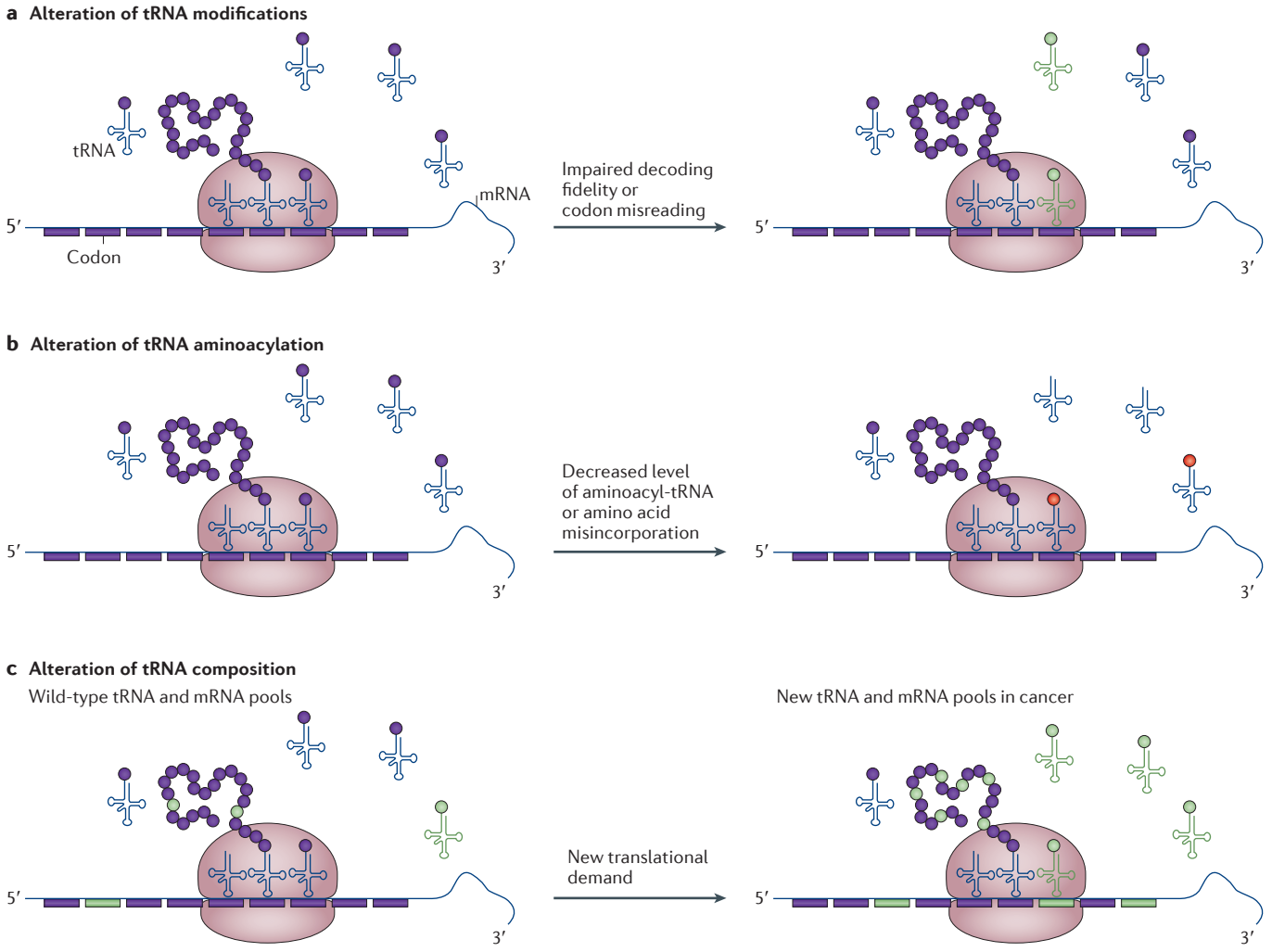

Figure 3 | **tRNA alterations and disease.** **a** | Mutation-based alterations in tRNA or auxiliary enzymes participating in tRNA modifications impair the translation of proteins enriched in codons read by the mismodified tRNA or lead to misreading of a codon and incorporation of an incorrect amino acid. **b** | Mutations in the aminoacyl tRNA synthetase (aaRS) or stress-induced alterations in aaRS fidelity cause the aaRS to mischarge or lose the ability to charge tRNA, leading to misincorporation of an amino acid or termination of translation, respectively. **c** | In tumour cells, the composition and concentration of the tRNAome are modified to meet the new translational demands. Cognate tRNAs (cloverleaf structure), amino acids (circles) and codons (rectangles) are shown in matching colours, whereas mismatched colours indicate miscoding.

show disruptions to levels and distributions of tRNAs in the tRNA pool. To maintain tumorigenic properties, such as elevated proliferation rates, increased expression of anti-apoptotic factors and increased energy demands, cancer cells extensively adjust translational apparatus and the cellular tRNA pool to meet this altered translational demand[108]. Tumour cells overexpress components of the TFIII complex[109], which is required for enhanced transcription of tRNA genes. In breast tumour samples and breast cancer-derived cell lines, levels of nuclear-encoded tRNAs increase by up to threefold, and levels of mt-tRNAs increase by up to fivefold[110], which mirrors the increased proliferation and elevated levels of protein synthesis of tumour cells (FIG. 3c). The tRNA levels of malignant plasma cells in multiple myeloma are significantly elevated; however, this accommodates the high translation levels of its tumour-specific monoclonal antibodies[111]. It is noteworthy that tRNA upregulation is not the primary cause for cancer and seems more likely to arise from secondary effects of the altered tumour biology and/or to be a biomarker of cancer.

In some cases, the aminoacylation step can render highly abundant tRNAs to become limited or can modulate the distributions of charged tRNAs in the tRNA pool. Translation of the mutation-expanded poly(CAG) tract of huntingtin in Huntington disease leads to a depletion of charged $tRNA^{Gln}$(CUG) and shifts the ribosome to an aberrant *trans* frame[112]. Intriguingly, the concentration of $tRNA^{Gln}$(CUG) differs in mouse brain tissues, with the lowest levels in hippocampal and striatal homogenates[112], suggesting that frameshifting might be potentiated in these neuronal tissues. The low $tRNA^{Gln}$(CUG) levels in striatum, in general, imply a lower capacity of this neuronal tissue to translate homopolymeric CAG codons than other neuronal tissues and may provide a link to the observation that the striatum is an early target of Huntington disease pathology[113]. As an additional example, chronic demand for insulin in type 2 diabetes mellitus induces endoplasmic reticulum stress which, similar to amino acid deprivation stress, leads to an ATF4-mediated boost in the translation of proteins regulating amino acid uptake and biosynthesis, and of aaRS proteins[66,114]. Increased plasma levels of Ile, Leu, Val, Tyr and Phe in normoglycaemic human subjects may drive the increased net uptake of branched amino acids (particularly Leu) through ATF4-mediated biosynthesis of transporter proteins, which enhances translational load and additionally increases endoplasmic reticulum stress, promoting β-cell dysfunction and the development of diabetes[114].

Conclusions

tRNA genes are more diverse than currently understood, and their functions extend far beyond that of a canonical translation adaptor molecule (BOX 2). With advances in technology and experimental approaches, we are beginning to appreciate the variety of programmes that operate to coordinate tRNA expression to tune translation and protein biogenesis in a cell- and tissue-specific manner, and the precision with which stress response is coordinated at the level of translation. tRNA dynamics provides the necessary plasticity to mount different regulatory layers to cope with stress and promote multiple biological responses.

The availability of genome sequencing data from patients with diverse conditions has already resulted in the identification of several mutations in tRNA genes and in genes involved in tRNA biogenesis (TABLE 1). The task now is to ascertain whether these mutations are the core of the molecular pathology of the diseases. An important factor to note is that multiple mutations may accumulate — for example, in the case of type 2 diabetes mellitus (TABLE 1) — which could epistatically modulate the severity of each disease[91]. Recent advances in targeted gene disruption in mammalian cells[115] offer a useful tool to assess the contribution of single genes, including tRNA genes, to disease pathology.

Some organs seem to be affected more than others despite the uniform genomic information (that is, mutations) among the cells. Variations in tRNA expression and abundance among different tissues may modulate the effect of tRNA aberrancies in a tissue-specific manner, underscoring the need for accurate quantification of tRNA expression and modification pattern for each specific cell type. This is a key area of study that will enable us to understand more clearly the genotype–phenotype relationship. Despite recent developments in RNA sequencing, high-throughput

Box 2 | **Additional roles of tRNA**

In addition to their essential role in protein translation, tRNAs participate in a plethora of non-translational activities in eukaryotes, bacteria and archaea. Here, we provide a brief overview of some examples. Although discovered early on (in the 1970s), some of these non-translational functions of tRNAs were considered as fortuitous and random owing to the high abundance of tRNA species in each cell. tRNA genes are highly transcribed and represent one of the most abundant transcripts in the cell; for example, a yeast cell contains ~3 million tRNA molecules[121] and, in *Escherichia coli* growing with a doubling time of 40 minutes, ~200,000 tRNA molecules are present in one cell[122].

In bacteria, tRNAs bind to Hfq RNA-binding protein and participate in the regulation of the small interfering RNA (siRNA)-driven mRNA silencing during stress[123]. For example, $tRNA^{Gly}$ is a structural element of the cell wall[124], Phe-$tRNA^{Phe}$ and Leu-$tRNA^{Leu}$ are substrates of aminoacyl-L/F-transferases that attach Leu or Phe to the amino termini of proteins and mark them for degradation[125], and $tRNA^{Lys}$ and $tRNA^{Ala}$ are used to aminoacylate membrane lipids to control cellular permeability to cationic antibiotics[126].

In eukaryotes, examples of non-translational functions of tRNAs include the following: $tRNA^{Glu}$ takes part in the first step of haem and chlorophyll biosynthesis[127]; $tRNA^{Arg}$-mediated amino-terminal arginylation is a tag for degradation[128]; mitochondrial encoded-tRNAs interfere with a cytochrome *c*-mediated apoptotic pathway and promote cell survival[129]; tRNA genes act as insulators in the human genome where they help to separate actively transcribed chromatin domains from silenced ones[130]; and specific cellular tRNAs or tRNA fragments are primers for reverse transcription of viruses[131,132]. Furthermore, tRNAs participate in energy metabolism and amino acid biosynthesis, functions that are conserved across all domains of life[133]. Additionally, a predictive, machine-learning approach identified multiple tRNA interaction partners and revealed a much broader set of putative cellular activities of tRNAs[134]. For example, the interaction with farnesyl-transferase and histone H3 lysine 9 methyltransferase may coordinate the global translation activity with protein modification[134]. Within the 1000 Genomes Project, some tRNA-like species that fold differently from the standard tRNA architecture were identified[20]. Some isodecoders bind to embedded Alu mRNA elements in 3′ untranslated regions and cause a global structural rearrangement of this region to alter the stability of the cognate mRNA[135].

tRNAome
The collective definition of the entire set of tRNAs expressed in a cell, a tissue or an organism at a given time.

sequencing of the tRNAome offers high power in identifying tRNA species[116] but is an inadequate approach for quantification owing to the presence of post-transcriptional modifications, which interfere with the quantitative PCR-based cDNA synthesis. Deep sequencing of only the 3′ tRNA fragments, the least modified parts of tRNAs, may offer unbiased quantification of the tRNAs. tRNA-based microarray technology is an alternative, albeit limited, methodology[34] to revealing relative concentrations of tRNAs. With quantitative knowledge of the tRNA biology in different tissues, we will gain a more accurate understanding of the tissue-specific aspects that modulate disease severity and progression.

1. Rodnina, M. V. & Wintermeyer, W. The ribosome as a molecular machine: the mechanism of tRNA–mRNA movement in translocation. *Biochem. Soc. Trans.* **39**, 658–662 (2011).
2. Giege, R. Toward a more complete view of tRNA biology. *Nature Struct. Mol. Biol.* **15**, 1007–1014 (2008).
3. Gebetsberger, J. & Polacek, N. Slicing tRNAs to boost functional ncRNA diversity. *RNA Biol.* **10**, 1798–1806 (2013).
4. Thompson, D. M. & Parker, R. Stressing out over tRNA cleavage. *Cell* **138**, 215–219 (2009).
5. Abbott, J. A., Francklyn, C. S. & Robey-Bond, S. M. Transfer RNA and human disease. *Front. Genet.* **5**, 158 (2014).
6. Blanco, S. & Frye, M. Role of RNA methyltransferases in tissue renewal and pathology. *Curr. Opin. Cell Biol.* **31C**, 1–7 (2014).
7. Schon, E. A., DiMauro, S. & Hirano, M. Human mitochondrial DNA: roles of inherited and somatic mutations. *Nature Rev. Genet.* **13**, 878–890 (2012). **This review highlights crucial aspects of somatic mutations in some human pathologies.**
8. Suzuki, T., Nagao, A. & Suzuki, T. Human mitochondrial tRNAs: biogenesis, function, structural aspects, and diseases. *Annu. Rev. Genet.* **45**, 299–329 (2011).
9. Yao, P. & Fox, P. L. Aminoacyl-tRNA synthetases in medicine and disease. *EMBO Mol. Med.* **5**, 332–343 (2013).
10. Durdevic, Z. & Schaefer, M. tRNA modifications: necessary for correct tRNA-derived fragments during the recovery from stress? *Bioessays* **35**, 323–327 (2013).
11. El Yacoubi, B., Bailly, M. & de Crecy-Lagard, V. Biosynthesis and function of posttranscriptional modifications of transfer RNAs. *Annu. Rev. Genet.* **46**, 69–95 (2012).
12. Gustilo, E. M., Vendeix, F. A. & Agris, P. F. tRNA's modifications bring order to gene expression. *Curr. Opin. Microbiol.* **11**, 134–140 (2008).
13. Phizicky, E. M. & Hopper, A. K. tRNA biology charges to the front. *Genes Dev.* **24**, 1832–1860 (2010).
14. Ibba, M. & Soll, D. Aminoacyl-tRNA synthesis. *Annu. Rev. Biochem.* **69**, 617–650 (2000).
15. Ramakrishnan, V. Ribosome structure and the mechanism of translation. *Cell* **108**, 557–572 (2002).
16. Lang, B. F., Gray, M. W. & Burger, G. Mitochondrial genome evolution and the origin of eukaryotes. *Annu. Rev. Genet.* **33**, 351–397 (1999).
17. Ambrogelly, A., Palioura, S. & Soll, D. Natural expansion of the genetic code. *Nature Chem. Biol.* **3**, 29–35 (2007).
18. Chan, P. P. & Lowe, T. M. GtRNAdb: a database of transfer RNA genes detected in genomic sequence. *Nucleic Acids Res.* **37**, D93–D97 (2009).
19. Iben, J. R. & Maraia, R. J. tRNA gene copy number variation in humans. *Gene* **536**, 376–384 (2014).
20. Parisien, M., Wang, X. & Pan, T. Diversity of human tRNA genes from the 1000-genomes project. *RNA Biol.* **10**, 1853–1867 (2013).
21. Goodenbour, J. M. & Pan, T. Diversity of tRNA genes in eukaryotes. *Nucleic Acids Res.* **34**, 6137–6146 (2006).
22. Kutter, C. *et al.* Pol III binding in six mammals shows conservation among amino acid isotypes despite divergence among tRNA genes. *Nature Genet.* **43**, 948–955 (2011).
23. Thompson, M., Haeusler, R. A., Good, P. D. & Engelke, D. R. Nucleolar clustering of dispersed tRNA genes. *Science* **302**, 1399–1401 (2003).
24. Dong, H., Nilsson, L. & Kurland, C. G. Co-variation of tRNA abundance and codon usage in *Escherichia coli* at different growth rates. *J. Mol. Biol.* **260**, 649–663 (1996).
25. Zhang, G., Lukoszek, R., Mueller-Roeber, B. & Ignatova, Z. Different sequence signatures in the upstream regions of plant and animal tRNA genes shape distinct modes of regulation. *Nucleic Acids Res.* **39**, 3331–3339 (2011).
26. Ouyang, C., Martinez, M. J., Young, L. S. & Sprague, K. U. TATA-binding protein–TATA interaction is a key determinant of differential transcription of silkworm constitutive and silk gland-specific tRNA(Ala) genes. *Mol. Cell. Biol.* **20**, 1329–1343 (2000).
27. Fredrick, K. & Ibba, M. How the sequence of a gene can tune its translation. *Cell* **141**, 227–229 (2010).
28. Zhang, G. & Ignatova, Z. Folding at the birth of the nascent chain: coordinating translation with co-translational folding. *Curr. Opin. Struct. Biol.* **21**, 25–31 (2011).
29. Sauna, Z. E. & Kimchi-Sarfaty, C. Understanding the contribution of synonymous mutations to human disease. *Nature Rev. Genet.* **12**, 683–691 (2011).
30. Zhou, M. *et al.* Non-optimal codon usage affects expression, structure and function of clock protein FRQ. *Nature* **495**, 111–115 (2013).
31. Novoa, E. M. & Ribas de Pouplana, L. Speeding with control: codon usage, tRNAs, and ribosomes. *Trends Genet.* **28**, 574–581 (2012).
32. Plotkin, J. B. & Kudla, G. Synonymous but not the same: the causes and consequences of codon bias. *Nature Rev. Genet.* **12**, 32–42 (2011).
33. Novoa, E. M., Pavon-Eternod, M., Pan, T. & Ribas de Pouplana, L. A role for tRNA modifications in genome structure and codon usage. *Cell* **149**, 202–213 (2012).
34. Dittmar, K. A., Goodenbour, J. M. & Pan, T. Tissue-specific differences in human transfer RNA expression. *PLoS Genet.* **2**, e221 (2006). **This paper presents quantitative assessment of tRNA abundance and reports broad variations in the tissue-specific expression of tRNA species.**
35. Gingold, H. *et al.* A dual program for translation regulation in cellular proliferation and differentiation. *Cell* **158**, 1281–1292 (2014).
36. Plotkin, J. B., Robins, H. & Levine, A. J. Tissue-specific codon usage and the expression of human genes. *Proc. Natl Acad. Sci. USA* **101**, 12588–12591 (2004).
37. Lampson, B. L. *et al.* Rare codons regulate KRAS oncogenesis. *Curr. Biol.* **23**, 70–75 (2013).
38. Barski, A. *et al.* Pol II and its associated epigenetic marks are present at Pol III-transcribed noncoding RNA genes. *Nature Struct. Mol. Biol.* **17**, 629–634 (2010).
39. Oler, A. J. *et al.* Human RNA polymerase III transcriptomes and relationships to Pol II promoter chromatin and enhancer-binding factors. *Nature Struct. Mol. Biol.* **17**, 620–628 (2010).
40. McFarlane, R. J. & Whitehall, S. K. tRNA genes in eukaryotic genome organization and reorganization. *Cell Cycle* **8**, 3102–3106 (2009).
41. Ledoux, S., Olejniczak, M. & Uhlenbeck, O. C. A sequence element that tunes *Escherichia coli* $tRNA^{Ala}_{GGC}$ to ensure accurate decoding. *Nature Struct. Mol. Biol.* **16**, 359–364 (2009).
42. Wohlgemuth, I., Pohl, C., Mittelstaet, J., Konevega, A. L. & Rodnina, M. V. Evolutionary optimization of speed and accuracy of decoding on the ribosome. *Phil. Trans. R. Soc. B* **366**, 2979–2986 (2011).
43. Fedyunin, I. *et al.* tRNA concentration fine tunes protein solubility. *FEBS Lett.* **586**, 3336–3340 (2012).
44. Bloom-Ackermann, Z. *et al.* A comprehensive tRNA deletion library unravels the genetic architecture of the tRNA pool. *PLoS Genet.* **10**, e1004084 (2014).
45. Yona, A. H. *et al.* tRNA genes rapidly change in evolution to meet novel translational demands. *Elife* **2**, e01339 (2013).
46. Geslain, R. & Pan, T. Functional analysis of human tRNA isodecoders. *J. Mol. Biol.* **396**, 821–831 (2010).
47. de Nadal, E., Ammerer, G. & Posas, F. Controlling gene expression in response to stress. *Nature Rev. Genet.* **12**, 833–845 (2011).
48. Levitz, R. *et al.* The optional *E. coli prr* locus encodes a latent form of phage T4-induced anticodon nuclease. *EMBO J.* **9**, 1383–1389 (1990).
49. Haiser, H. J., Karginov, F. V., Hannon, G. J. & Elliot, M. A. Developmentally regulated cleavage of tRNAs in the bacterium Streptomyces coelicolor. *Nucleic Acids Res.* **36**, 732–741 (2008).
50. Hsieh, L. C., Lin, S. I., Kuo, H. F. & Chiou, T. J. Abundance of tRNA-derived small RNAs in phosphate-starved *Arabidopsis* roots. *Plant Signal Behav.* **5**, 537–539 (2010).
51. Jochl, C. *et al.* Small ncRNA transcriptome analysis from *Aspergillus fumigatus* suggests a novel mechanism for regulation of protein synthesis. *Nucleic Acids Res.* **36**, 2677–2689 (2008).
52. Thompson, D. M., Lu, C., Green, P. J. & Parker, R. tRNA cleavage is a conserved response to oxidative stress in eukaryotes. *RNA* **14**, 2095–2103 (2008).
53. Wang, Q. *et al.* Identification and functional characterization of tRNA-derived RNA fragments (tRFs) in respiratory syncytial virus infection. *Mol. Ther.* **21**, 368–379 (2013).
54. Thompson, D. M. & Parker, R. The RNase Rny1p cleaves tRNAs and promotes cell death during oxidative stress in *Saccharomyces cerevisiae*. *J. Cell Biol.* **185**, 43–50 (2009).
55. Yamasaki, S., Ivanov, P., Hu, G. F. & Anderson, P. Angiogenin cleaves tRNA and promotes stress-induced translational repression. *J. Cell Biol.* **185**, 35–42 (2009).
56. Saikia, M. *et al.* Genome-wide identification and quantitative analysis of cleaved tRNA fragments induced by cellular stress. *J. Biol. Chem.* **287**, 42708–42725 (2012).
57. Czech, A., Wende, S., Morl, M., Pan, T. & Ignatova, Z. Reversible and rapid transfer-RNA deactivation as a mechanism of translational repression in stress. *PLoS Genet.* **9**, e1003767 (2013).
58. Emara, M. M. *et al.* Angiogenin-induced tRNA-derived stress-induced RNAs promote stress-induced stress granule assembly. *J. Biol. Chem.* **285**, 10959–10968 (2010).
59. Ivanov, P., Emara, M. M., Villen, J., Gygi, S. P. & Anderson, P. Angiogenin-induced tRNA fragments inhibit translation initiation. *Mol. Cell* **43**, 613–623 (2011). **This paper reports the first observation that tRNA fragments specifically displace eIF4G and eIF4F initiation factors and inhibit protein initiation.**
60. Zhang, Y. *et al.* Identification and characterization of an ancient class of small RNAs enriched in serum associating with active infection. *J. Mol. Cell. Biol.* **6**, 172–174 (2014).
61. Durdevic, Z., Mobin, M. B., Hanna, K., Lyko, F. & Schaefer, M. The RNA methyltransferase Dnmt2 is required for efficient Dicer-2-dependent siRNA pathway activity in *Drosophila*. *Cell Rep.* **4**, 931–937 (2013).
62. Dhahbi, J. M. *et al.* 5′ tRNA halves are present as abundant complexes in serum, concentrated in blood cells, and modulated by aging and calorie restriction. *BMC Genomics* **14**, 298 (2013).
63. Holcik, M. & Sonenberg, N. Translational control in stress and apoptosis. *Nature Rev. Mol. Cell Biol.* **6**, 318–327 (2005).
64. Donnelly, N., Gorman, A. M., Gupta, S. & Samali, A. The eIF2α kinases: their structures and functions. *Cell. Mol. Life Sci.* **70**, 3493–3511 (2013).
65. Whitney, M. L., Hurto, R. L., Shaheen, H. H. & Hopper, A. K. Rapid and reversible nuclear accumulation of cytoplasmic tRNA in response to nutrient availability. *Mol. Biol. Cell* **18**, 2678–2686 (2007).
66. Murguia, J. R. & Serrano, R. New functions of protein kinase Gcn2 in yeast and mammals. *IUBMB Life* **64**, 971–974 (2012).
67. Qiu, H. *et al.* Defects in tRNA processing and nuclear export induce GCN4 translation independently of phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2. *Mol. Cell. Biol.* **20**, 2505–2516 (2000).

68. Chan, C. T. *et al.* A quantitative systems approach reveals dynamic control of tRNA modifications during cellular stress. *PLoS Genet.* **6**, e1001247 (2010). **This study describes a highly sensitive mass spectrometry-based approach to simultaneously detect tRNA modification in yeast.**
69. Chan, C. T. *et al.* Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. *Nature Commun.* **3**, 937 (2012).
70. Zinshteyn, B. & Gilbert, W. V. Loss of a conserved tRNA anticodon modification perturbs cellular signaling. *PLoS Genet.* **9**, e1003675 (2013). **Using translatome-wide analysis, this study shows that stress-induced mismodification of wobble position U34 reduces global gene expression by activating GCN4-mediated stress response and not by altered codon–anticodon pairing.**
71. Johansson, M. J., Esberg, A., Huang, B., Bjork, G. R. & Bystrom, A. S. Eukaryotic wobble uridine modifications promote a functionally redundant decoding system. *Mol. Cell. Biol.* **28**, 3301–3312 (2008).
72. Chen, C., Tuck, S. & Bystrom, A. S. Defects in tRNA modification associated with neurological and developmental dysfunctions in *Caenorhabditis elegans* elongator mutants. *PLoS Genet.* **5**, e1000561 (2009).
73. Ingolia, N. T. Ribosome profiling: new views of translation, from single codons to genome scale. *Nature Rev. Genet.* **15**, 205–213 (2014).
74. Miranda, I. *et al. Candida albicans* CUG mistranslation is a mechanism to create cell surface variation. *MBio* **4**, e00285-13 (2013).
75. Wiltrout, E., Goodenbour, J. M., Frechin, M. & Pan, T. Misacylation of tRNA with methionine in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **40**, 10494–10506 (2012).
76. Netzer, N. *et al.* Innate immune and chemically triggered oxidative stress modifies translational fidelity. *Nature* **462**, 522–526 (2009). **This study describes a new mechanism that protects cells against oxidative stress by misincorporation of methionine through non-Met-tRNAs.**
77. Jones, T. E., Alexander, R. W. & Pan, T. Misacylation of specific nonmethionyl tRNAs by a bacterial methionyl-tRNA synthetase. *Proc. Natl Acad. Sci. USA* **108**, 6933–6938 (2011).
78. Bender, A., Hajieva, P. & Moosmann, B. Adaptive antioxidant methionine accumulation in respiratory chain complexes explains the use of a deviant genetic code in mitochondria. *Proc. Natl Acad. Sci. USA* **105**, 16496–16501 (2008).
79. Schneider, A. Mitochondrial tRNA import and its consequences for mitochondrial translation. *Annu. Rev. Biochem.* **80**, 1033–1053 (2011).
80. Brandon, M. C. *et al.* MITOMAP: a human mitochondrial genome database — 2004 update. *Nucleic Acids Res.* **33**, D611–D613 (2005).
81. Moraes, C. T. *et al.* A mitochondrial tRNA anticodon swap associated with a muscle disease. *Nature Genet.* **4**, 284–288 (1993).
82. Flierl, A., Reichmann, H. & Seibel, P. Pathophysiology of the MELAS 3243 transition mutation. *J. Biol. Chem.* **272**, 27189–27196 (1997).
83. Suzuki, T., Wada, T., Saigo, K. & Watanabe, K. Taurine as a constituent of mitochondrial tRNAs: new insights into the functions of taurine and human mitochondrial diseases. *EMBO J.* **21**, 6581–6589 (2002).
84. Kirino, Y. *et al.* Codon-specific translational defect caused by a wobble modification deficiency in mutant tRNA from a human mitochondrial disease. *Proc. Natl Acad. Sci. USA* **101**, 15070–15075 (2004).
85. Yasukawa, T., Suzuki, T., Ishii, N., Ohta, S. & Watanabe, K. Wobble modification defect in tRNA disturbs codon-anticodon interaction in a mitochondrial disease. *EMBO J.* **20**, 4794–4802 (2001).
86. Hasegawa, H., Matsuoka, T., Goto, Y. & Nonaka, I. Cytochrome-C-oxidase activity is deficient in blood-vessels of patients with myoclonus epilepsy with ragged-red fibers. *Acta Neuropathol.* **85**, 280–284 (1993).
87. Naini, A. *et al.* Hypocitrullinemia in patients with MELAS: an insight into the "MELAS paradox". *J. Neurol. Sci.* **229–230**, 187–193 (2005).
88. Wang, S. *et al.* Maternally inherited essential hypertension is associated with the novel 4263A > G mutation in the mitochondrial tRNAIle gene in a large Han Chinese family. *Circ. Res.* **108**, 862–870 (2011).
89. Liang, M. Hypertension as a mitochondrial and metabolic disease. *Kidney Int.* **80**, 15–16 (2011).
90. Liu, Y. *et al.* Mitochondrial transfer RNAMet 4435A > G mutation is associated with maternally inherited hypertension in a Chinese pedigree. *Hypertension* **53**, 1083–1090 (2009).
91. Ishimura, R. *et al.* RNA function. Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. *Science* **345**, 455–459 (2014). **This study reports that a mutation in a nuclear-encoded tRNA gene, which is specifically expressed in the CNS, may itself be phenotypically silent but epistatically exacerbates the deleterious effect of the mutation in a partner of the ribosome recycling protein Pelota.**
92. Karaca, E. *et al.* Human *CLP1* mutations alter tRNA biogenesis, affecting both peripheral and central nervous system function. *Cell* **157**, 636–650 (2014).
93. Schaffer, A. E. *et al. CLP1* founder mutation links tRNA splicing and maturation to cerebellar development and neurodegeneration. *Cell* **157**, 651–663 (2014). **References 92 and 93, which were simultaneously published, reveal a new link between a mutation in CLP1 kinase, impairment in pre-tRNA processing and human pathology.**
94. Hanada, T. *et al.* CLP1 links tRNA metabolism to progressive motor-neuron loss. *Nature* **495**, 474–480 (2013).
95. Latour, P. *et al.* A major determinant for binding and aminoacylation of $tRNA^{Ala}$ in cytoplasmic Alanyl-tRNA synthetase is mutated in dominant axonal Charcot–Marie–Tooth disease. *Am. J. Hum. Genet.* **86**, 77–82 (2010).
96. Guo, M. *et al.* Paradox of mistranslation of serine for alanine caused by AlaRS recognition dilemma. *Nature* **462**, 808–812 (2009).
97. Lee, J. W. *et al.* Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. *Nature* **443**, 50–55 (2006).
98. Sleigh, J. N., Grice, S. J., Burgess, R. W., Talbot, K. & Cader, M. Z. Neuromuscular junction maturation defects precede impaired lower motor neuron connectivity in Charcot–Marie–Tooth type 2D mice. *Hum. Mol. Genet.* **23**, 2639–2650 (2014).
99. Finsterer, J. Central nervous system manifestations of mitochondrial disorders. *Acta Neurol. Scand.* **114**, 217–238 (2006).
100. Alazami, A. M. *et al.* Mutation in *ADAT3*, encoding adenosine deaminase acting on transfer RNA, causes intellectual disability and strabismus. *J. Med. Genet.* **50**, 425–430 (2013).
101. Martinez, F. J. *et al.* Whole exome sequencing identifies a splicing mutation in *NSUN2* as a cause of a Dubowitz-like syndrome. *J. Med. Genet.* **49**, 380–385 (2012).
102. Rodriguez, V. *et al.* Chromosome 8 BAC array comparative genomic hybridization and expression analysis identify amplification and overexpression of *TRMT12* in breast cancer. *Genes Chromosomes Cancer* **46**, 694–707 (2007).
103. Blanco, S. *et al.* Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. *EMBO J.* **33**, 2020–2039 (2014). **This paper shows that a mutation in a tRNA-modifying enzyme leads to mismodification of tRNAs and enhances their susceptibility to angiogenin-mediated cleavage with an enhanced effect in neuronal tissues.**
104. Darnell, J. C. Defects in translational regulation contributing to human cognitive and behavioral disease. *Curr. Opin. Genet. Dev.* **21**, 465–473 (2011).
105. Wei, F. Y. *et al.* Deficit of $tRNA^{Lys}$ modification by Cdkal1 causes the development of type 2 diabetes in mice. *J. Clin. Invest.* **121**, 3598–3608 (2011).
106. Zhou, B. *et al.* Identification of a splicing variant that regulates type 2 diabetes risk factor CDKAL1 level by a coding-independent mechanism in human. *Hum. Mol. Genet.* **23**, 4639–4650 (2014).
107. Wei, F. Y. & Tomizawa, K. Functional loss of Cdkal1, a novel tRNA modification enzyme, causes the development of type 2 diabetes. *Endocr. J.* **58**, 819–825 (2011).
108. Stumpf, C. R. & Ruggero, D. The cancerous translation apparatus. *Curr. Opin. Genet. Dev.* **21**, 474–483 (2011).
109. White, R. J. RNA polymerase III transcription and cancer. *Oncogene* **23**, 3208–3216 (2004).
110. Pavon-Eternod, M. *et al.* tRNA over-expression in breast cancer and functional consequences. *Nucleic Acids Res.* **37**, 7268–7280 (2009).
111. Zhou, Y., Goodenbour, J. M., Godley, L. A., Wickrema, A. & Pan, T. High levels of tRNA abundance and alteration of tRNA charging by bortezomib in multiple myeloma. *Biochem. Biophys. Res. Commun.* **385**, 160–164 (2009).
112. Girstmair, H. *et al.* Depletion of cognate charged transfer RNA causes translational frameshifting within the expanded CAG stretch in huntingtin. *Cell Rep.* **3**, 148–159 (2013).
113. Landwehrmeyer, G. B. *et al.* Huntington's disease gene: regional and cellular expression in brain of normal and affected individuals. *Ann. Neurol.* **37**, 218–230 (1995).
114. Krokowski, D. *et al.* A self-defeating anabolic program leads to β-cell apoptosis in endoplasmic reticulum stress-induced diabetes via regulation of amino acid flux. *J. Biol. Chem.* **288**, 17202–17213 (2013).
115. Kim, H. & Kim, J. S. A guide to genome engineering with programmable nucleases. *Nature Rev. Genet.* **15**, 321–334 (2014).
116. Puri, P. *et al.* Systematic identification of tRNAome and its dynamics in *Lactococcus lactis*. *Mol. Microbiol.* **93**, 944–956 (2014).
117. Phizicky, E. M. & Alfonzo, J. D. Do all modifications benefit all tRNAs? *FEBS Lett.* **584**, 265–271 (2010).
118. Suzuki, T. A complete landscape of post-transcriptional modifications in mammalian mitochondrial tRNAs. *Nucleic Acids Res.* **42**, 7346–7357 (2014).
119. Torres, A. G., Batlle, E. & Ribas de Pouplana, L. Role of tRNA modifications in human diseases. *Trends Mol. Med.* http://dx.doi.org/10.1016/j.molmed.2014.01.008 (2014).
120. Czerwoniec, A. *et al.* MODOMICS: a database of RNA modification pathways. 2008 update. *Nucleic Acids Res.* **37**, D118–121 (2009).
121. Waldron, C. & Lacroute, F. Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acids in yeast. *J. Bacteriol.* **122**, 855–865 (1975).
122. Persson, B. C., Gustafsson, C., Berg, D. E. & Bjork, G. R. The gene for a transfer-RNA modifying enzyme, M5u54-methyltransferase, is essential for viability in *Escherichia-coli*. *Proc. Natl Acad. Sci. USA* **89**, 3995–3998 (1992).
123. Lee, T. & Feig, A. L. The RNA binding protein Hfq interacts specifically with tRNAs. *RNA* **14**, 514–523 (2008).
124. Navarre, W. W. & Schneewind, O. Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiol. Mol. Biol. Rev.* **63**, 174–229 (1999).
125. Graciet, E. *et al.* Aminoacyl-transferases and the N-end rule pathway of prokaryotic/eukaryotic specificity in a human pathogen. *Proc. Natl Acad. Sci. USA* **103**, 3078–3083 (2006).
126. Roy, H. & Ibba, M. RNA-dependent lipid remodeling by bacterial multiple peptide resistance factors. *Proc. Natl Acad. Sci. USA* **105**, 4667–4672 (2008).
127. Jahn, D., Verkamp, E. & Soll, D. Glutamyl-transfer RNA: a precursor of heme and chlorophyll biosynthesis. *Trends Biochem. Sci.* **17**, 215–218 (1992).
128. Karakozova, M. *et al.* Arginylation of β-actin regulates actin cytoskeleton and cell motility. *Science* **313**, 192–196 (2006).
129. Hou, Y. M. & Yang, X. Regulation of cell death by transfer RNA. *Antioxid. Redox Signal* **19**, 583–594 (2013).
130. Raab, J. R. *et al.* Human tRNA genes function as chromatin insulators. *EMBO J.* **31**, 330–350 (2012).
131. Miller, S. B., Yildiz, F. Z., Lo, J. A., Wang, B. & D'Souza, V. M. A structure-based mechanism for tRNA and retroviral RNA remodelling during primer annealing. *Nature* **515**, 591–595 (2014).
132. Ruggero, K. *et al.* Small noncoding RNAs in cells transformed by human T-cell leukemia virus type 1: a role for a tRNA fragment as a primer for reverse transcriptase. *J. Virol.* **88**, 3612–3622 (2014).
133. Sheppard, K. *et al.* From one amino acid to another: tRNA-dependent amino acid biosynthesis. *Nucleic Acids Res.* **36**, 1813–1825 (2008).
134. Parisien, M. *et al.* Discovering RNA–protein interactome by using chemical context profiling of the RNA–protein interface. *Cell Rep.* **3**, 1703–1713 (2013).
135. Rudinger-Thirion, J., Lescure, A., Paulus, C. & Frugier, M. Misfolded human tRNA isodecoder binds and neutralizes a 3′ UTR-embedded Alu element. *Proc. Natl Acad. Sci. USA* **108**, E794–802 (2011).
136. Lund, E. & Dahlberg, J. E. Proofreading and aminoacylation of tRNAs before export from the nucleus. *Science* **282**, 2082–2085 (1998).

137. Smits, P. *et al.* Functional consequences of mitochondrial tRNA Trp and tRNA Arg mutations causing combined OXPHOS defects. *Eur. J. Hum. Genet.* **18**, 324–329 (2010).
138. Levinger, L., Morl, M. & Florentz, C. Mitochondrial tRNA 3′ end metabolism and human disease. *Nucleic Acids Res.* **32**, 5430–5441 (2004).
139. Wittenhagen, L. M. & Kelley, S. O. Dimerization of a pathogenic human mitochondrial tRNA. *Nature Struct. Biol.* **9**, 586–590 (2002).
140. Chomyn, A., Enriquez, J. A., Micol, V., Fernandez-Silva, P. & Attardi, G. The mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episode syndrome-associated human mitochondrial $tRNA^{Leu}_{UUR}$ mutation causes aminoacylation deficiency and concomitant reduced association of mRNA with ribosomes. *J. Biol. Chem.* **275**, 19198–19209 (2000).
141. Calvaruso, M. A. *et al.* New mitochondrial tRNA HIS mutation in a family with lactic acidosis and stroke-like episodes (MELAS). *Mitochondrion* **11**, 778–782 (2011).
142. Shoffner, J. M. *et al.* Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA $tRNA^{Lys}$ mutation. *Cell* **61**, 931–937 (1990).
143. Schaller, A. *et al.* Impairment of mitochondrial tRNAIle processing by a novel mutation associated with chronic progressive external ophthalmoplegia. *Mitochondrion* **11**, 488–496 (2011).
144. Souilem, S. *et al.* A novel mitochondrial $tRNA^{Ile}$ point mutation associated with chronic progressive external ophthalmoplegia and hyperCKemia. *J. Neurol. Sci.* **300**, 187–190 (2011).
145. McFarland, R. *et al.* Multiple neonatal deaths due to a homoplasmic mitochondrial DNA mutation. *Nature Genet.* **30**, 145–146 (2002).
146. Sacconi, S. *et al.* Complex neurologic syndrome associated with the G1606A mutation of mitochondrial DNA. *Arch. Neurol.* **59**, 1013–1015 (2002).
147. Lynn, S. *et al.* Mitochondrial diabetes: investigation and identification of a novel mutation. *Diabetes* **47**, 1800–1802 (1998).
148. Seneca, S. *et al.* A mitochondrial tRNA aspartate mutation causing isolated mitochondrial myopathy. *Am. J. Med. Genet. A* **137A**, 170–175 (2005).
149. Jones, C. N., Jones, C. I., Graham, W. D., Agris, P. F. & Spremulli, L. L. A disease-causing point mutation in human mitochondrial tRNAMet rsults in tRNA misfolding leading to defects in translational initiation and elongation. *J. Biol. Chem.* **283**, 34445–34456 (2008).
150. Tulinius, M. *et al.* Leigh syndrome with cytochrome-c oxidase deficiency and a single T insertion nt 5537 in the mitochondrial *tRNATrp* gene. *Neuropediatrics* **34**, 87–91 (2003).
151. t Hart, L. M. *et al.* Evidence that the mitochondrial leucyl tRNA synthetase (*LARS2*) gene represents a novel type 2 diabetes susceptibility gene. *Diabetes* **54**, 1892–1895 (2005).
152. Bonnefond, L. *et al.* Crystal structure of human mitochondrial tyrosyl-tRNA synthetase reveals common and idiosyncratic features. *Structure* **15**, 1505–1516 (2007).
153. Scheper, G. C. *et al.* Mitochondrial aspartyl-tRNA synthetase deficiency causes leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. *Nature Genet.* **39**, 534–539 (2007).
154. Pierce, S. B. *et al.* Mutations in mitochondrial histidyl tRNA synthetase *HARS2* cause ovarian dysgenesis and sensorineural hearing loss of Perrault syndrome. *Proc. Natl Acad. Sci. USA* **108**, 6543–6548 (2011).
155. Begley, U. *et al.* A human tRNA methyltransferase 9-like protein prevents tumour growth by regulating LIN9 and HIF1-α. *EMBO Mol. Med.* **5**, 366–383 (2013).
156. Pavon-Eternod, M. *et al.* Vaccinia and influenza A viruses select rather than adjust tRNAs to optimize translation. *Nucleic Acids Res.* **41**, 1914–1921 (2013).
157. Clarke, P., Leser, J. S., Bowen, R. A. & Tyler, K. L. Virus-induced transcriptional changes in the brain include the differential expression of genes associated with interferon, apoptosis, interleukin 17 receptor A, and glutamate signaling as well as flavivirus-specific upregulation of tRNA synthetases. *MBio* **5**, e00902–e00914 (2014).
158. Pavon-Eternod, M., Wei, M., Pan, T. & Kleiman, L. Profiling non-lysyl tRNAs in HIV-1. *RNA* **16**, 267–273 (2010).
159. Scheper, G. C., van der Knaap, M. S. & Proud, C. G. Translation matters: protein synthesis defects in inherited disease. *Nature Rev. Genet.* **8**, 711–723 (2007).

Acknowledgements
The authors acknowledge careful reading of the manuscript and numerous suggestions by R. Smock and laboratory members. Work in the authors' laboratory on tRNA and translation is supported by grants from Deutsche Forschungsgemeinschaft, German Federal Ministry of Education and Research, and Marie–Curie Training Network of the European Union.

Competing interests statement
The authors declare no competing interests.

FURTHER INFORMATION
Compilation of mitochondrial tRNA genes: http://mamit-trna.u-strasbg.fr
Genomic tRNA gene database: http://lowelab.ucsc.edu/GtRNAdb
MITOMAP: http://www.mitomap.org/MITOMAP
MODOMICS: http://mods.rna.albany.edu/home
OMIM: http://www.omim.org
RSCB Protein Data Bank: http://www.rcsb.org/pdb/home/home.do
tRNA database: http://trnadb.bioinf.uni-leipzig.de/DataOutput

ALL LINKS ARE ACTIVE IN THE ONLINE PDF