Published in final edited form as:

Wiley Interdiscip Rev RNA. 2015 July ; 6(4): 453–470. doi:10.1002/wrna.1287.

Controlling translation via modulation of tRNA levels

Jeremy E. Wilusz*

Department of Biochemistry and Biophysics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104

Abstract

Transfer RNAs (tRNAs) are critical adaptor molecules that carry amino acids to a messenger RNA template during protein synthesis. Although tRNAs have commonly been viewed as abundant ‘house-keeping’ RNAs, it is becoming increasingly clear that tRNA expression is tightly regulated. Depending on a cell’s proliferative status, the pool of active tRNAs is rapidly changed, enabling distinct translational programs to be expressed in differentiated versus proliferating cells. Here, I highlight several post-transcriptional regulatory mechanisms that allow the expression or functions of tRNAs to be altered. Modulating the modification status or structural stability of individual tRNAs can cause those specific tRNA transcripts to selectively accumulate or be degraded. Decay generally occurs via the rapid tRNA decay pathway or by the nuclear RNA surveillance machinery. In addition, the CCA-adding enzyme plays a critical role in determining the fate of a tRNA. The post-transcriptional addition of CCA to the 3′ ends of stable tRNAs generates the amino acid attachment site, whereas addition of CCACCA to unstable tRNAs prevents aminoacylation and marks the tRNA for degradation. In response to various stresses, tRNAs can accumulate in the nucleus or be further cleaved into small RNAs, some of which inhibit translation. By implementing these various post-transcriptional control mechanisms, cells are able to fine-tune tRNA levels to regulate subsets of mRNAs as well as overall translation rates.

Keywords

protein synthesis; post-transcriptional control; CCA-adding enzyme; RNA modification; rapid tRNA decay pathway; nuclear RNA surveillance; RNA stability; tRNA-derived RNA fragments; tRNA

During protein synthesis, genetic information encoded in messenger RNAs is used to direct the synthesis of a polypeptide composed of the corresponding amino acids. This decoding process occurs with great accuracy and speed on the ribosome, using transfer RNAs (tRNAs) as adaptor molecules that carry the amino acids to the mRNA template. Such adaptors were first proposed by Crick¹ and definitively identified by Hoagland and colleagues,² prompting a rush of seminal discoveries about how tRNAs function to read the genetic code (reviewed in ^{3, 4}). In brief, mature tRNAs are generally ~75 to 95 nucleotides in length and adopt a cloverleaf secondary structure⁵ that is further folded into a compact L-shaped tertiary structure (Figure 1).^{6, 7} The end of the long arm of the L contains the

*Corresponding Author: wilusz@mail.med.upenn.edu.

trinucleotide anticodon sequence, which recognizes the mRNA codon. The short arm ends in a single-stranded CCA sequence, onto which the appropriate aminoacyl-tRNA synthetase covalently attaches the amino acid (reviewed in ⁸). One by one, aminoacylated (or “charged”) tRNAs are then brought to the ribosome and their ability to properly base-pair to the mRNA codon is rapidly tested. Once the correct tRNA is in position, the ribosome catalyzes the linkage of the amino acid to the growing polypeptide chain, releasing a now uncharged tRNA so that it can be re-charged to function in subsequent decoding events (reviewed in ⁹). Beyond this canonical role in protein synthesis, certain tRNAs additionally function to deliver amino acids to other biochemical processes, such as antibiotic biosynthesis, bacterial cell wall biosynthesis, or as signaling molecules that regulate gene expression and cell survival (reviewed in ^{10, 11}).

Given the critical role for tRNAs in many processes, it is not surprising that cells commit significant energy and resources to tRNA production. In every yeast cell, there are ~3,000,000 tRNAs, representing ~10% of the total RNA.¹² These transcripts are generally very stable and tRNA half-lives have been estimated to be on the order of 2 days or longer.^{13, 14} This observation has led many to refer to tRNAs simply as abundant ‘house-keeping’ RNAs. However, it is becoming increasingly clear that tRNAs are highly regulated (reviewed in ¹⁵). For example, although multiple tRNAs may have the same anticodon (so-called isodecoders), there are often sequence differences elsewhere in the transcript, e.g. in the internal promoter regions.^{16, 17} These transcripts may, therefore, not be fully redundant with one another and, indeed, many tRNAs show tissue-specific expression patterns.^{17–19}

As the various tRNAs can be expressed at vastly different levels, codons corresponding to the same amino acid (e.g. UCU, UCC, UCA, UGA, AGU, and AGC all code for serine) are genetically synonymous but not functionally equivalent. Replacing a preferred codon (one decoded by high-abundance tRNAs) with a rare codon (one decoded by low-abundance tRNAs) can, for example, cause translation rates to be slowed by several fold.²⁰ This indicates that tRNAs can, in fact, be limiting for translation. Interestingly, changing a rare codon to a preferred codon is not always beneficial. Recent work has revealed that rare codons are purposely present in genes that regulate circadian cycles, as they act to slow translation in order for these growing polypeptides to properly fold.^{21, 22} Analogously, rare codons act to slow translation so that proteins destined for membranes or outside of the cell can be efficiently targeted by the signal recognition particle to the secretory pathway.²³ Differences in the expression of specific tRNAs thus help regulate cell behavior.

Here, I highlight a number of recent insights into how the levels of specific tRNAs can be modulated, with a particular focus on post-transcriptional regulatory mechanisms. Transcription of tRNAs can change in response to various stimuli (reviewed in ^{24, 25, 26}), especially when the cell’s proliferation status is altered. In addition, cells are able to post-transcriptionally modulate the modification status or structural characteristics of specific tRNAs to cause those tRNA transcripts to be selectively stabilized or degraded. In particular, the mechanism by which the CCA-adding enzyme gauges tRNA stability and determines tRNA fate will be detailed. In addition, I will discuss how cells respond to various stresses by altering the subcellular localization of tRNAs and/or cleaving tRNAs into small RNAs.

Through their combined activities, these post-transcriptional regulatory mechanisms aid in shaping the pool of active tRNAs present in cells, thereby driving translational programs.

Proliferating and differentiated cells have largely different tRNA repertoires

tRNA expression levels can vary drastically across tissues and development.²⁷ For example, ~50% of transcribed mouse tRNA genes (162 out of 311) were found to be selectively induced or repressed during mouse liver or brain development.¹⁹ Likewise, tRNA pools change significantly between normal and cancer cells, with the expression of some tRNA genes being reproducibly altered by more than 10-fold.^{28–30} These changes can be functionally relevant since slight (~1.4-fold) over-expression of the initiator methionine tRNA leads to increased cell metabolism and proliferation.^{31, 32} Selenocysteine tRNA, on the other hand, is often down-regulated in cancers, which is thought to prevent several selenocysteine-containing proteins from inhibiting cancer growth.³³ Interestingly, tRNAs appear to turn over faster in tumors than in normal tissues,³⁴ indicating that transcriptional and post-transcriptional mechanisms regulating tRNAs are altered in cancer cells.

As cells commit significant energy to producing tRNAs, it stands to reason that the amount of each distinct tRNA produced should be somewhat balanced with the number of times each codon is present in the mRNA transcriptome.^{35, 36} This would ensure that all translating ribosomes can be occupied by any given tRNA at any moment, while simultaneously preventing the synthesis of excess tRNAs. It is, however, important to note that tRNAs that decode rare codons can not be so scarce that diffusion to the ribosome severely limits translation. tRNA transcription by RNA polymerase III is directly regulated by numerous factors, including several oncogene and tumor suppressor pathways (reviewed in ^{25, 26, 37}). For example, the transcription factor Myc, which is over-expressed and amplified in many tumors, promotes tRNA transcription by directly binding to tRNA genes,³⁸ whereas the mTOR signaling complex inactivates the Pol III transcriptional repressor Maf1.²⁵ These factors can thus induce significant Pol III transcriptional changes and selectively induce or repress specific tRNAs, thereby helping the tRNA pool to adapt to the drastically different mRNA transcriptomes that are observed across tissues.³⁹ Consistent with this idea, the relative abundance of tRNAs within certain tissues has long been known to be correlated with the codon usage of the most highly expressed mRNAs.^{27, 40–42}

Recent work by Gingold and colleagues has extended these observations and elegantly revealed the extent to which tRNA repertoires are coordinated to dictate translational programs.³⁰ Using microarrays and histone modification maps, tRNA expression levels were measured in a wide variety of proliferating, differentiating/differentiated, and growth arrested human cell types. Surprisingly, upon comparing the expression data from each of the samples, a continuous spectrum of tRNA pools was observed that mirrored the proliferative status of the cell (Figure 2).³⁰ At one extreme of the spectrum were differentiated or arrested cells, while the other extreme included actively proliferating cells. These patterns were observed regardless of the tissue of origin and were driven at least partly by transcriptional changes. This is because the tRNA promoter sequences, especially the B box,⁴³ are distinct between tRNAs preferentially expressed in differentiated vs. proliferating cells.³⁰ Upon differentiating embryonic stem cells with retinoic acid, the tRNA

pool gradually transitioned from the proliferation tRNA signature to the differentiation/arrested tRNA signature. In contrast, inducing fibroblasts to proliferate after a period of serum starvation transitioned their tRNAs away from the differentiation/arrested signature towards the proliferation signature. These experimental manipulations thus confirmed that the levels of specific tRNAs can be rapidly changed.³⁰ Interestingly, other classes of RNAs are not similarly regulated by proliferation. For example, when cell types are clustered based on differences in mRNA expression, the tissue of origin dictates the clustering. Therefore, the mRNA and tRNA pools capture different information about the cell.³⁰

Why then are such different tRNA pools present in proliferating vs. differentiated cells? If tRNAs were simple 'house-keeping' RNAs, they should all be roughly equally required for the translation of every gene and thus any change in their expression should affect the translation of all genes in a similar direction. Under this model, it is especially unclear why the expression of certain tRNAs would be reduced, as such changes could slow translation elongation rates and be potentially detrimental to cellular fitness. Gingold and colleagues fascinatingly revealed that genes involved in proliferation and differentiation have distinct codon usage profiles.³⁰ Therefore, the observed tRNA expression changes are, in fact, highly relevant. For most amino acids, there is at least one codon that is preferentially used in proliferation-related genes (e.g. genes involved in the cell cycle, DNA replication, or nucleosome assembly) and at least one distinct codon that is preferentially used in differentiation-related genes (e.g. genes involved in cell adhesion, developmental patterning, or formation of the extracellular matrix) (Figure 2). As one would expect, those tRNAs induced upon cell proliferation correspond to codons enriched in the mRNAs that drive proliferation, allowing tRNA levels to be coordinated with the translational demand of the mRNA transcriptome.³⁰

Such differences in codon usage patterns are observed in humans, mice, and chickens, indicating that these translational programs that separate proliferating vs. differentiated cells are deeply rooted in vertebrate evolution.³⁰ Curiously, flies show a similar distinction in codon usage, but the actual codons enriched in proliferation vs. differentiation genes are different from those used in vertebrates. In contrast, *C. elegans* does not show this pattern, likely because cell differentiation is tightly tied to cell division during worm development.

In total, the coordination between tRNA levels and mRNA codon usage likely represents a key way that gene expression mechanisms are coupled.⁴⁴ By doing so, cells can optimize translation rates and ensure high expression of key genes that are driving the cell state. Furthermore, matching the levels of specific tRNAs to the mRNA transcriptome may help reinforce and maintain the cellular state as non-desired mRNAs, such as those generated via transcriptional noise,⁴⁵ likely will not match the tRNA pool and, therefore, be poorly translated.³⁰ Some of these changes in tRNA levels are driven by changes in their rate of transcription, although post-transcriptional mechanisms (as discussed below) likely also play equally critical roles in regulating tRNA levels and functions.

Nucleotide modifications are important for tRNA function and rapidly change upon stress

Once transcribed, precursor tRNAs are processed at their 5' and 3' ends, introns (if present) are removed, and the 3' terminal CCA sequence is added (reviewed in ⁴⁶) (Figure 3). In addition, numerous nucleotides are modified. Greater than 100 different modified nucleotides are known in nature, ranging in complexity from simple methylation events to structures generated via multi-step reactions. Modifications are found on 11.9% of the bases of sequenced tRNAs, with a median of 8 modifications per tRNA (Figure 4).^{47–49} In any given species, some modified bases are present in almost all tRNAs, such as dihydrouridine (D) and pseudouridine (ψ) in the D and TψC arms, respectively, whereas others are only present on a single tRNA. Although the exact functions for many of these modifications are not yet known, modified nucleotides in and around the anticodon triplet often affect translation or translation fidelity, whereas those in the tRNA body (away from the anticodon) are often critical for tRNA folding and stability.^{15, 50–54} For example, both pseudouridine and 2'-O methylation stabilize base stacking,^{55, 56} whereas dihydrouridine imparts flexibility.⁵⁷

Notably, a given modified nucleotide may be present in many tRNAs, but exert vastly different effects on the stability or function of each of those transcripts (reviewed in ⁴⁸). For example, the modified nucleotides ac⁴C₁₂ (N⁴ acetylation of C₁₂) and Um₄₄ (methylation of U₄₄) are present in all yeast serine tRNAs, yet deletion of key proteins responsible for their biogenesis (including the co-activator Tan1, which functions with the acetyltransferase Kre33,⁵⁸ and the methyltransferase Trm44) only affects tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} (Figure 4).⁵⁹ At high temperatures, hypomodified mature tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} transcripts are degraded by a rapid tRNA decay (RTD) pathway.⁶⁰ In contrast, there is no reduction in the levels of hypomodified tRNA^{Ser(IGA)} and tRNA^{Ser(GCU)} despite the fact that these tRNAs lack the same modifications in *trm44- tan1-* cells. Interestingly, the reason these transcripts are not degraded is that they have significantly more stable acceptor and TψC stems, indicating that the RTD pathway selects substrates by monitoring the overall stability of the tRNA transcript.⁶¹ Consistent with this model, the RTD pathway can be triggered by a lack of other sets of tRNA modifications⁵³ and fully modified tRNAs can be targeted for degradation if they contain mutations that destabilize any of the tRNA arms.⁶²

Whereas the RTD pathway surveys mature tRNAs, certain hypomodified pre-tRNAs are recognized and degraded by the nuclear RNA surveillance machinery. In particular, when the yeast initiator methionine precursor tRNA (pre-tRNA_iMet) lacks m¹A₅₈, it is polyadenylated by the Trf4 component of the TRAMP complex and degraded by the nuclear exosome.^{63, 64} However, other tRNAs that normally contain m¹A₅₈, including the elongator tRNA^{Met}, are not significantly affected when unmethylated. This suggests that the TRAMP complex specifically recognizes only pre-tRNA_iMet, possibly because it misfolds into an alternative tertiary structure. A somewhat analogous mechanism exists in *E. coli* where pre-tRNA^{Trp} is polyadenylated and degraded when it contains a mutation (G7 → A7) that disrupts the bottom of its acceptor stem.⁶⁵

The existence of these RNA decay mechanisms suggests a model in which alterations in specific tRNA modifications, e.g. by modulating the expression of the enzymes responsible for their biogenesis, can cause the expression levels or functions of specific tRNAs to change. Interestingly, tRNA modification enzymes are mis-regulated in various human diseases, including cancer and neurological disorders (reviewed in ⁶⁶). Specific modifications are also known to be regulated by growth rates,^{67, 68} DNA damage,⁶⁹ temperature,^{70–72} as well as other cellular stresses.⁷³ It stands to reason that these modification changes may alter the stability of specific tRNAs, causing these tRNA transcripts to be specifically degraded (or stabilized) under certain cellular conditions. In addition, modifications induced by stresses can change tRNA function. For example, when yeast are exposed to hydrogen peroxide, the level of m⁵C in the wobble position of the tRNA^{Leu(CAA)} anticodon increases.⁷³ This results in enhanced translational elongation of a number of genes that are enriched in UUG codons, including the ribosomal protein Rpl22a. Rpl22a protein is subsequently incorporated into ribosomes and likely promotes the translation of additional stress response genes.⁷⁴ When yeast are exposed to other stresses, including methylmethane sulfonate, arsenite, and hypochlorite, m⁵C levels are, on the other hand, largely unaffected.⁷³

Taken together, alterations in tRNA modifications are critical players in cellular stress responses and may allow tRNA pools to be shaped as appropriate. It is likely that analogous changes in the modification status of various tRNAs contribute to the differences in tRNA levels observed in differentiated vs. proliferating cells. With the ongoing development of high-throughput approaches for measuring modification levels, especially those using mass spectrometry,⁷⁵ we should soon have a broader understanding of the dynamics of tRNA modifications.

tRNA fate is controlled by CCA vs. CCACCA addition

Although all tRNAs require CCA at their 3' ends for amino acid attachment⁷⁶ (Figure 1) and correct positioning in the ribosome,⁷⁷ CCA is not encoded in nearly all eukaryotic tRNA genes or in many archaeal and bacterial genes.⁷⁸ Instead, the CCA-adding enzyme [ATP(CTP):tRNA nucleotidyltransferase], a member of the nucleotidyltransferase superfamily, post-transcriptionally adds CCA to the 3' ends of tRNAs (reviewed in ^{79, 80–82}). This enzyme operates without a nucleic acid template and uses CTP and ATP as substrates to extend the tRNA “primer.” In organisms whose tRNA genes do not encode the CCA sequence, the CCA-adding enzyme is, as expected, essential for viability.⁸³ Interestingly, mutations in the human CCA-adding enzyme that reduce its catalytic activity have recently been shown to cause certain forms of congenital sideroblastic anemia⁸⁴ and mitochondrial dysfunction.⁸⁵ Organisms such as *E. coli* that genomically encode the CCA sequence on their tRNAs still express the CCA-adding enzyme and its inactivation impairs cell growth.⁸⁶ This is because the enzyme reconstructs the CCA end of partially degraded tRNAs (e.g. due to hydrolytic damage). Furthermore, the CCA-adding enzyme plays a critical role in tRNA quality control (see below).

To ensure that CCA addition occurs in an error-free manner, the CCA-adding enzyme must tightly coordinate multiple steps. It must (i) specifically recognize tRNA and tRNA-like

substrates, (ii) use only CTP and ATP, while excluding UTP or GTP, (iii) switch the nucleotide specificity from C to A after the addition of two C nucleotides, and (iv) stop polymerization exactly after CCA. If any of these steps do not occur, a tRNA that is unable to be charged with an amino acid or function in translation is produced. Original models for CCA addition proposed the existence of multiple nucleotide binding sites, with the tRNA progressively moving to each of these active sites during polymerization. However, subsequent biochemical experiments^{87, 88} and crystal structures^{89, 90} revealed a single active site, with the tRNA 3' end progressively refolding as the body of the tRNA remains anchored to the enzyme. Curiously, CCA-adding enzymes are divided into two divergent classes with only the catalytic domains sharing significant homology.⁹¹ In class I enzymes, which are present in archaea, the enzyme and the tRNA collaboratively ensure that the proper nucleotide is selected for incorporation.^{90, 92} The size and shape of the nucleotide pocket successively changes during the CCA addition reaction, causing the nucleotide specificity to switch from C to A after two C's have been added and for polymerization to stop once CCA has been added (reviewed in ^{79, 93}). In contrast, for class II enzymes, which are present in eubacteria and eukaryotes, nucleotide selectivity is determined solely by the protein, as arginine and aspartate residues in the active site form Watson-Crick-like hydrogen bonds with incoming nucleotides.^{94, 95} Fidelity of the CCA addition cycle is further ensured because the enzyme inspects the integrity of the tRNA molecule, discriminates against nicked or damaged species,⁹⁶ and stops polymerization if an incorrect nucleotide has been incorporated.⁹⁷

As the tRNA body does not translocate, rotate, or slip during catalysis,^{87, 88} the CCA-adding enzyme was long assumed to efficiently stop polymerization once CCA had been added. Indeed, crystal structures confirmed that there is simply not enough space in the active site for the CCA end to reposition and attack another incoming nucleotide.⁹⁰ It was thus quite a surprise when we found CCACCA post-transcriptionally added to the MEN β tRNA-like transcript in human and mouse cells.⁹⁸ CCA-adding enzymes from all three kingdoms of life catalyzed CCACCA addition to this substrate *in vitro*, indicating that CCA or CCACCA can be specifically added to an RNA substrate depending on its characteristics.

By adding these three extra nucleotides to generate the CCACCA end, the CCA-adding enzyme completely changes the fate of the transcript. This is most clearly demonstrated by comparing the MEN β tRNA-like transcript with mascRNA (MALAT1-associated small cytoplasmic RNA), a highly similar (~70% identity) small RNA derived from the long noncoding RNA MALAT1 (Figure 5A). Whereas mascRNA is subjected to canonical CCA addition and accumulates as a stable transcript in the cytoplasm,⁹⁹ the MEN β small RNA receives CCACCA and is rapidly degraded in almost all human and mouse cells.¹⁰⁰ Unlike CCA, the CCACCA tail is long enough to be recognized by 3'-5' exonucleases, such as *E. coli* RNase R or the yeast exosome component Rrp44 (Dis3), which require single-stranded RNA tails to be threaded a significant distance to reach the enzyme's catalytic active sites.^{98, 101, 102} Additional exonucleases, including the 5'-3' exonucleases Xrn1 or Rat1, may also be recruited to transcripts ending in CCACCA *in vivo*, ensuring that degradation proceeds in a rapid and efficient manner.

By adding CCA or CCACCA, the CCA-adding enzyme is thus able to elegantly control the expression and fate of tRNAs and tRNA-like transcripts. Whereas CCA addition generates the amino acid attachment site, CCACCA addition blocks aminoacylation and triggers RNA decay. What then determines if CCA or CCACCA is added?

tRNA structural instability triggers CCACCA addition

The structures of mascRNA and the MEN β tRNA-like small RNA are highly similar, yet there are notable differences that cause the transcripts to be subjected to CCA vs. CCACCA addition. The ends of the acceptor stems of the mouse and human MEN β transcripts are unstable because of a C-A mismatch or multiple G-U wobbles, respectively (Figure 5A). Swapping the acceptor stem of mascRNA with that of MEN β was sufficient to convert the transcript from a CCA to a CCACCA target with near 100% efficiency *in vitro*⁹⁸ and *in vivo*.¹⁰³ Further detailed mutational analysis demonstrated that CCACCA is only added to transcripts that contain guanosines at the first and second positions (G₁G₂) and have a destabilized acceptor stem. Interestingly, the MEN β acceptor stem is poorly conserved across evolution and is stable in some species, such as Old World monkeys, resulting in MEN β homologs that receive CCA and accumulate in cells (Figure 5B).⁹⁸ Although the mouse MEN β tRNA-like small RNA is undetectable by Northern blots in most cell types, it is weakly detected in mouse liver where it appears to receive CCA.^{100, 104} This suggests that tissue-specific mechanisms, e.g. differential addition of nucleotide modifications, may act to counteract the inherent instability present in the MEN β acceptor stem. As the functions of mascRNA and the MEN β tRNA-like transcripts are unknown, it remains unclear why the various homologs are differentially regulated.

The CCA-adding enzyme is also able to add CCACCA to unstable canonical tRNAs. Between 30 and 45 percent of canonical tRNA genes in a given species begin with G₁G₂, indicating that many, but certainly not all tRNAs could be subjected to CCACCA addition.⁹⁸ However, under normal circumstances, tRNA acceptor stems are thought to be structurally stable and thus tRNAs are generally subjected to CCA addition. A handful of tRNAs have genomically encoded mismatches that are corrected by post-transcriptional editing events.^{105, 106} This process is likely proofread by the CCA-adding enzyme as the unedited transcripts are CCACCA targets.⁹⁸ Many other events can destabilize a tRNA, including transcriptional errors and nucleotide modifications that alter base-pairing strengths or the transcript's tertiary structure. Although changes anywhere in a tRNA can be detrimental (reviewed in ¹⁰⁷), it is particularly critical that the cell recognize tRNAs with possible errors in their acceptor stems. This is because aminoacylation determinants are often present in this region, e.g. a G3:U70 base pair is sufficient to cause aminoacylation with alanine,^{108, 109} and alterations in the acceptor stem could, therefore, cause mis-charging and downstream errors in translation.

Indeed, CCACCA addition and degradation of mutated tRNAs has been clearly demonstrated *in vitro* and *in vivo*.⁹⁸ In addition, some hypomodified yeast tRNAs undergoing the rapid tRNA decay (RTD) pathway are marked with CCACCA. When yeast are grown at high temperatures, some tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} transcripts lacking 2'-O-methylation of U₄₄ and N⁴ acetylation of C₁₂ (due to deletion of Trm44 and Tan1)

receive CCACCA and are degraded (Figure 4).^{59, 98} Strengthening the secondary structure of the hypomodified tRNAs, e.g. by increasing the stability of their acceptor or T ψ C stems, abolishes CCACCA addition and RNA degradation.^{61, 98} This indicates that the overall structural stability of a tRNA affects the choice between CCA vs. CCACCA addition.

Although not yet demonstrated, cells may purposely regulate the structural stability of tRNAs, e.g. by adding or removing nucleotide modifications, to modulate CCA vs. CCACCA choice and, therefore, tRNA levels. If true, CCACCA addition would thus not only function as a quality control mechanism to remove potentially deleterious tRNAs, but also as a key mechanism by which tRNA pools are modulated to regulate translation patterns.

The CCA-adding enzyme acts as a molecular vise to proofread tRNAs

How does the CCA-adding enzyme distinguish between structurally stable and unstable transcripts? By co-crystallizing the *Archaeoglobus fulgidus* CCA-adding enzyme with a tRNA minihelix (which is comprised of only the acceptor and T ψ C arms)¹¹⁰ in multiple states, we recently showed that the structural flexibility of the RNA controls the choice between CCA vs. CCACCA addition.¹¹¹ When initially binding to the enzyme, the tRNA does so in the same conformation regardless of the stability of its acceptor stem. The tRNA 3' end is bound to the enzyme's catalytic center, comprised of the head and neck domains, whereas the T ψ C loop interacts with the enzyme's tail domain (Figure 6).^{89, 90} Upon binding an incoming CTP nucleotide, the head domain exhibits a clockwise screw motion that causes the tRNA acceptor stem to become overwound and compressed.¹¹¹ Nucleotides are then sequentially added until the full CCA triplet is present on the 3' end of the transcript. At this point, CCA stacks against the head domain, causing it to rotate counter-clockwise and the acceptor stem to become decompressed. Further nucleotides are unable to be added to a tRNA in this conformation, and the decision to add CCA versus CCACCA is now made.

Although the terminal A of the CCA triplet is not positioned in a manner in which it can accept an additional nucleotide, there is sufficient space in the active site pocket for an incoming nucleotide to bind.¹¹¹ As above, nucleotide binding at this stage would induce the head domain to close and apply torque on the tRNA. If the bound tRNA is stable, multiple Watson-Crick base pairs would have to be broken for the head domain to fully close. This is energetically unfavorable, and thus a stable tRNA ending in CCA dissociates and is subsequently charged to function in protein synthesis. If, however, the tRNA begins with G₁G₂ and is unstable, the screw motion will trigger the 3' end of the RNA to refold and extrude a 3-nt bulge from the middle of the acceptor stem (Figure 6).^{98, 111} Notably, the tRNA 5' end and the T ψ C loop remain tightly bound to the enzyme during this refolding event, ensuring that the tRNA is not released. Only the conformation of the tRNA 3' end changes: the C's of the CCA sequence shift to form base pairs with G₁G₂, while the A of the CCA sequence becomes the new unpaired discriminator base. The A is now positioned in the enzyme's catalytic pocket, ready to undergo a second cycle of CCA addition using the same catalytic mechanism as the first. The second CCA triplet is added and, analogous to the CCA termination state, stacks against the head domain. Nucleotide binding could, in

principle, result in yet another round of on-enzyme RNA refolding, allowing CCACCACCA to be added. However, tails longer than CCACCA are generally not observed *in vitro* or in cells.⁹⁸ This is likely because the generation of a 6-nt bulge prevents the rest of the RNA from adopting a continuous helical conformation, which is required for recognition by the CCA-adding enzyme.¹¹¹ An unstable tRNA ending in CCACCA is instead released and subsequently degraded.

The CCA-adding enzyme thus exploits the structural characteristics of unstable transcripts to mark them for degradation. In fact, the instability (e.g. mutation or nucleotide modification) in the tRNA is never directly sensed by the enzyme's active site. The CCA-adding enzyme simply continues to add CCA triplets until the RNA substrate is unable to remain bound to the enzyme. In this way, the tRNA essentially proofreads itself.¹¹¹ As a result, CCA is added to structurally stable tRNAs, whereas CCACCA is added to a variety of structurally unstable tRNAs, thereby altering the fate of the tRNA.

tRNA structural instability triggers degradation through multiple mechanisms

It is becoming apparent that cells simultaneously employ multiple strategies to mark and degrade unstable tRNAs. For example, at least two distinct 3' end tails can be detected on hypomodified tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} transcripts undergoing RTD in yeast. Whereas some transcripts are post-transcriptionally marked with CCACCA by the CCA-adding enzyme (likely occurring in both the nucleus and the cytoplasm), a significant increase in tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} transcripts with short poly(A) tails added to their CCA ends (to generate CCA(A)_n tails) is also observed (Figure 7).⁹⁸ These polyadenylated CCA tails are likely generated in the nucleus by the Trf4 poly(A) polymerase of the TRAMP complex, indicating that multiple distinct sequences can be added to the 3' ends of tRNAs to trigger tRNA degradation. Although the exact sequences of the single-stranded tails are different, they all are predicted to block aminoacylation as well as allow recognition by 3'-5' exonucleases. Nevertheless, it is clear that 5'-3' decay by the exonucleases Xrn1 and Rat1 plays a major role in the RTD pathway,⁶¹ and significantly fewer hypomodified transcripts ending in CCACCA or polyadenylated CCA tails are observed when Xrn1 is deleted.⁹⁸ It has thus been proposed that both 5'-3' and 3'-5' exonucleases may cooperate to degrade transcripts undergoing RTD, although the exact mechanistic details are still unclear. It is, for example, possible that distinct pools (possibly due to differences in their subcellular localization) of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} may be marked differently for degradation, thereby explaining the variety of decay patterns observed.

Additional decay mechanisms besides the well-characterized RTD and nuclear RNA surveillance mechanisms are also likely used to degrade tRNAs. We previously showed that destabilized mascRNA transcripts beginning with G₁A₂ can not be marked with CCACCA, yet are efficiently degraded in HeLa cells. They instead have short U-rich tails post-transcriptionally added to their 3' ends.¹⁰³ Uridylation has been associated with the decay of numerous classes of small RNAs¹¹²⁻¹¹⁴ and mRNAs, including non-polyadenylated histone

mRNAs.^{103, 115–117} How and why transcripts are marked in distinct ways for RNA degradation is an area that clearly requires further investigation.

tRNAs can accumulate in the nucleus in response to stress

Besides simply degrading a tRNA to remove it from the tRNA pool, cells are able to regulate the function of a tRNA by changing its subcellular localization. Interestingly, unlike mRNAs which are generally processed co-transcriptionally at their site of transcription,¹¹⁸ primary tRNA transcripts are processed and matured at several distinct subcellular locations. In yeast, tRNA transcription occurs in the nucleolus, whereas splicing occurs at the surface of the mitochondria¹¹⁹ (in contrast, splicing occurs in the nucleus in vertebrates¹²⁰) and various modifications are added in the nucleolus, nucleoplasm, inner nuclear membrane, or cytoplasm (reviewed in^{15, 121}). There thus exists extensive machinery that shuttles tRNAs in and out of the nucleus and, in fact, tRNAs appear to move between cellular compartments in a constitutive manner.^{122, 123} When cells are stressed, tRNAs accumulate in the nucleus, likely because their ability to be re-exported is impaired.^{122, 124, 125} Although global translation is not significantly inhibited when tRNAs accumulate in the nucleus, specific mRNAs, such as those involved in certain amino acid biosynthetic pathways, become poorly translated when proper nuclear-cytoplasmic tRNA dynamics are disrupted.¹²⁶ Under certain circumstances, it is possible that only specific tRNAs may be retained in the nucleus, which would possibly allow translation to become biased towards genes whose codon bias matches that of the remaining cytoplasmic tRNA pool. Interestingly, it has been demonstrated that even in non-stressed cells, some hypomodified tRNAs are specifically imported to the nucleus.¹²⁷ Although the exact fate of these transcripts is unclear, it is possible that they are repaired or, alternatively, they may be targeted for degradation. This suggests a model in which changing tRNA modification levels can trigger changes in tRNA localization and/or tRNA levels, thereby influencing the pool of tRNAs that are able to function in the cytoplasm.

Activation of tRNA cleavage pathways in response to cellular state

tRNAs can also be cleaved into a variety of smaller molecules, collectively known as tRNA-derived RNA fragments (tRFs) (Figure 7). Some of these transcripts are constitutively expressed in cells, whereas many are produced in response to certain stimuli (reviewed in^{128–130}). When vertebrates are exposed to various stresses, including oxidative stress, heat shock, and UV irradiation, the RNase angiogenin is activated and endonucleolytically cleaves mature tRNAs.^{131–133} A recent report¹³⁴ suggests that angiogenin may first cleave within the CCA terminus that is present on all tRNAs, thereby rapidly blocking their ability to function in translation. Angiogenin subsequently cleaves a subset of tRNAs (<5%) in their anticodon loops, generating 5' and 3' tRNA halves. Cleavage within the CCA end can be repaired by the CCA-adding enzyme, thereby re-generating functional tRNAs when stress is relieved. Certain tRNA halves, on the other hand, inhibit translation initiation by displacing the cap-binding complex eIF4F from capped mRNAs and inducing the assembly of cytoplasmic stress granules.^{135, 136} As tRNA halves can additionally inhibit stress-induced apoptosis,¹³⁷ it appears that the generation of smaller RNA fragments from tRNAs

is a functionally important part of cellular stress responses. Nevertheless, the precise regulation and functions for most tRNA halves is still poorly understood.

Beyond cleavage within the anticodon loop, tRNAs can be cleaved at other positions to yield a diverse set of additional tRNA fragments, some of which are expressed at levels equivalent to abundant microRNAs.^{138, 139} Particularly common is cleavage of mature tRNAs in the D or T ψ C loops to yield 19-26 nt transcripts known as 5'- and 3'-tRFs, respectively.¹⁴⁰ Interestingly, the abundance of these smaller transcripts generally does not correlate with the abundance of their parent tRNAs, indicating that their biogenesis and half lives are tightly controlled. In higher eukaryotes, some of these tRNA fragments associate with Argonaute proteins^{141, 142} and have been shown to inhibit translation elongation.¹⁴³ Interestingly, a 5'-tRF derived from tRNA^{Val} in the archaeon *Haloferax volcanii* directly binds the ribosome to interfere with peptidyl transferase activity¹⁴⁴ and tRNA fragments from pumpkin phloem sap inhibit translation *in vitro*.¹⁴⁵ These results suggest that inhibition of translation may be an evolutionarily conserved function for tRFs. On the other hand, a tRF derived from the 3' trailer sequence of human pre-tRNA^{Ser(UGA)} is known to promote cell proliferation, although its molecular mechanism is unknown.¹⁴⁰ This suggests that some tRNA cleavage products may have functions completely unrelated to translation.

Perspectives

Considering that tRNA pools in cancer cells and proliferating cells are different from those observed in differentiated cells, it is quickly becoming clear that tRNA levels are highly regulated. Although changes in transcription allow new tRNAs to be produced, it is equally important that cells are able to adjust the levels of existing transcripts. Indeed, tRNAs are subjected to many post-transcriptional regulatory mechanisms, including pathways that trigger transcript degradation, alter tRNA localization, or cleave the transcript to generate small RNAs (Figure 7). We are beginning to understand some of the underlying events that can trigger each of these pathways, and future work will likely reveal an integrated view of how these mechanisms shape tRNA pools. Although often presented as separate processes, there is likely significant crosstalk between the various post-transcriptional control mechanisms. In fact, several pathways appear to be simultaneously active on the same set of transcripts, as is the case for the rapid tRNA decay pathway. tRNAs undergoing RTD can (i) have CCACCA or a poly(A) tail post-transcriptionally added to their 3' ends,⁹⁸ (ii) be degraded by 5'-3' exonucleases,⁶¹ (iii) be specifically trafficked away from the cytoplasm to the nucleus,¹²⁷ and/or (iv) be protected from decay by binding to elongation factors or their respective aminoacyl-tRNA synthetase.^{146, 147} However, some of these events are mutually exclusive (e.g. a transcript can have either CCACCA or a poly(A) tail added, not both) and thus an important future direction will be to understand how and why each regulatory pathway is employed.

Since tRNA modifications change in response to various stimuli, it appears that cells purposely alter the structural characteristics of individual tRNAs to either stabilize or degrade those tRNAs depending on the cellular state. Thereby, the overall tRNA pool changes as needed to influence translation patterns. With the advent of new technologies to study modification changes in cells, it will be particularly interesting to identify post-

transcriptional pathways that directly link cellular stimuli (e.g. oncogenic activation) to changes in tRNA levels. Beyond the mechanisms discussed here, it is also clear that tRNA functions can be altered in response to cellular cues. For example, mammalian cells contain a surprisingly large number of non-methionyl tRNAs that are mischarged with methionine, yet used for protein synthesis.^{148, 149} In summary, a remarkable variety of mechanisms by which tRNAs regulate major cellular functions are beginning to emerge. It is thus already clear that tRNAs can no longer be regarded simply as adaptors that match amino acids to mRNA codons in the process of protein synthesis. Instead, tRNAs function as critical regulators of proliferation and differentiation in health and disease as well as signaling molecules that help cells respond to their environment.

Acknowledgments

I thank Claus Kuhn and members of the Wilusz lab for suggestions and discussions. Research in the Wilusz lab is supported by NIH R00-GM104166.

References

1. Crick FH. On protein synthesis. *Symp Soc Exp Biol.* 1958; 12:138–163. [PubMed: 13580867]
2. Hoagland MB, Stephenson ML, Scott JF, Hecht LI, Zamecnik PC. A soluble ribonucleic acid intermediate in protein synthesis. *J Biol Chem.* 1958; 231:241–257. [PubMed: 13538965]
3. RajBhandary UL, Kohrer C. Early days of tRNA research: discovery, function, purification and sequence analysis. *J Biosci.* 2006; 31:439–451. [PubMed: 17206064]
4. Rich A, RajBhandary UL. Transfer RNA: molecular structure, sequence, and properties. *Annu Rev Biochem.* 1976; 45:805–860. [PubMed: 60910]
5. Holley RW, Apgar J, Everett GA, Madison JT, Marquisee M, Merrill SH, Penswick JR, Zamir A. Structure of a Ribonucleic Acid. *Science.* 1965; 147:1462–1465. [PubMed: 14263761]
6. Kim SH, Quigley GJ, Suddath FL, McPherson A, Sneden D, Kim JJ, Weinzierl J, Rich A. Three-dimensional structure of yeast phenylalanine transfer RNA: folding of the polynucleotide chain. *Science.* 1973; 179:285–288. [PubMed: 4566654]
7. Robertus JD, Ladner JE, Finch JT, Rhodes D, Brown RS, Clark BF, Klug A. Structure of yeast phenylalanine tRNA at 3 Å resolution. *Nature.* 1974; 250:546–551. [PubMed: 4602655]
8. Jakubowski H. Quality control in tRNA charging. *Wiley Interdiscip Rev RNA.* 2012; 3:295–310. [PubMed: 22095844]
9. Ramakrishnan V. Ribosome structure and the mechanism of translation. *Cell.* 2002; 108:557–572. [PubMed: 11909526]
10. Raina M, Ibba M. tRNAs as regulators of biological processes. *Front Genet.* 2014; 5:171. [PubMed: 24966867]
11. Dare K, Ibba M. Roles of tRNA in cell wall biosynthesis. *Wiley Interdiscip Rev RNA.* 2012; 3:247–264. [PubMed: 22262511]
12. Waldron C, Lacroute F. Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acids in yeast. *J Bacteriol.* 1975; 122:855–865. [PubMed: 1097403]
13. Nwagwu M, Nana M. Ribonucleic acid synthesis in embryonic chick muscle, rates of synthesis and half-lives of transfer and ribosomal RNA species. *J Embryol Exp Morphol.* 1980; 56:253–267. [PubMed: 7400745]
14. Kanerva PA, Maenpää PH. Codon-specific serine transfer ribonucleic acid degradation in avian liver during vitellogenin induction. *Acta Chem Scand B.* 1981; 35:379–385. [PubMed: 6914867]
15. Phizicky EM, Hopper AK. tRNA biology charges to the front. *Genes Dev.* 2010; 24:1832–1860. [PubMed: 20810645]
16. Chan PP, Lowe TM. GtRNAdb: a database of transfer RNA genes detected in genomic sequence. *Nucleic Acids Res.* 2009; 37:D93–97. [PubMed: 18984615]

17. Goodenbour JM, Pan T. Diversity of tRNA genes in eukaryotes. *Nucleic Acids Res.* 2006; 34:6137–6146. [PubMed: 17088292]
18. Ishimura R, Nagy G, Dotu I, Zhou H, Yang XL, Schimmel P, Senju S, Nishimura Y, Chuang JH, Ackerman SL. RNA function. Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. *Science.* 2014; 345:455–459. [PubMed: 25061210]
19. Schmitt BM, Rudolph KL, Karagianni P, Fonseca NA, White RJ, Talianidis I, Odom DT, Marioni JC, Kutter C. High-resolution mapping of transcriptional dynamics across tissue development reveals a stable mRNA-tRNA interface. *Genome Res.* 2014; 24:1797–1807. [PubMed: 25122613]
20. Sorensen MA, Kurland CG, Pedersen S. Codon usage determines translation rate in *Escherichia coli*. *J Mol Biol.* 1989; 207:365–377. [PubMed: 2474074]
21. Zhou M, Guo J, Cha J, Chae M, Chen S, Barral JM, Sachs MS, Liu Y. Non-optimal codon usage affects expression, structure and function of clock protein FRQ. *Nature.* 2013; 495:111–115. [PubMed: 23417067]
22. Xu Y, Ma P, Shah P, Rokas A, Liu Y, Johnson CH. Non-optimal codon usage is a mechanism to achieve circadian clock conditionality. *Nature.* 2013; 495:116–120. [PubMed: 23417065]
23. Pechmann S, Chartron JW, Frydman J. Local slowdown of translation by nonoptimal codons promotes nascent-chain recognition by SRP in vivo. *Nat Struct Mol Biol.* 2014; 21:1100–1105. [PubMed: 25420103]
24. Boguta M. Maf1, a general negative regulator of RNA polymerase III in yeast. *Biochim Biophys Acta.* 2013; 1829:376–384. [PubMed: 23201230]
25. Moir RD, Willis IM. Regulation of pol III transcription by nutrient and stress signaling pathways. *Biochim Biophys Acta.* 2013; 1829:361–375. [PubMed: 23165150]
26. Grewal SS. Why should cancer biologists care about tRNAs? tRNA synthesis, mRNA translation and the control of growth. *Biochim Biophys Acta.* 2014
27. Dittmar KA, Goodenbour JM, Pan T. Tissue-specific differences in human transfer RNA expression. *PLoS Genet.* 2006; 2:e221. [PubMed: 17194224]
28. Pavon-Eternod M, Gomes S, Geslain R, Dai Q, Rosner MR, Pan T. tRNA over-expression in breast cancer and functional consequences. *Nucleic Acids Res.* 2009; 37:7268–7280. [PubMed: 19783824]
29. Marshall L, White RJ. Non-coding RNA production by RNA polymerase III is implicated in cancer. *Nat Rev Cancer.* 2008; 8:911–914. [PubMed: 18987635]
30. Gingold H, Tehler D, Christoffersen NR, Nielsen MM, Asmar F, Kooistra SM, Christophersen NS, Christensen LL, Borre M, Sorensen KD, et al. A dual program for translation regulation in cellular proliferation and differentiation. *Cell.* 2014; 158:1281–1292. [PubMed: 25215487]
31. Pavon-Eternod M, Gomes S, Rosner MR, Pan T. Overexpression of initiator methionine tRNA leads to global reprogramming of tRNA expression and increased proliferation in human epithelial cells. *RNA.* 2013; 19:461–466. [PubMed: 23431330]
32. Rideout EJ, Marshall L, Grewal SS. *Drosophila* RNA polymerase III repressor Maf1 controls body size and developmental timing by modulating tRNAiMet synthesis and systemic insulin signaling. *Proc Natl Acad Sci U S A.* 2012; 109:1139–1144. [PubMed: 22228302]
33. Hatfield DL, Tsuji PA, Carlson BA, Gladyshev VN. Selenium and selenocysteine: roles in cancer, health, and development. *Trends Biochem Sci.* 2014; 39:112–120. [PubMed: 24485058]
34. Borek E, Baliga BS, Gehrke CW, Kuo CW, Belman S, Troll W, Waalkes TP. High turnover rate of transfer RNA in tumor tissue. *Cancer Res.* 1977; 37:3362–3366. [PubMed: 884680]
35. Gingold H, Pilpel Y. Determinants of translation efficiency and accuracy. *Mol Syst Biol.* 2011; 7:481. [PubMed: 21487400]
36. Brackley CA, Romano MC, Thiel M. The dynamics of supply and demand in mRNA translation. *PLoS Comput Biol.* 2011; 7:e1002203. [PubMed: 22022250]
37. White RJ. RNA polymerases I and III, non-coding RNAs and cancer. *Trends Genet.* 2008; 24:622–629. [PubMed: 18980784]
38. Gomez-Roman N, Grandori C, Eisenman RN, White RJ. Direct activation of RNA polymerase III transcription by c-Myc. *Nature.* 2003; 421:290–294. [PubMed: 12529648]

39. Wang ET, Sandberg R, Luo S, Khrebukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. *Nature*. 2008; 456:470–476. [PubMed: 18978772]
40. Comeron JM. Selective and mutational patterns associated with gene expression in humans: influences on synonymous composition and intron presence. *Genetics*. 2004; 167:1293–1304. [PubMed: 15280243]
41. Lavner Y, Kotlar D. Codon bias as a factor in regulating expression via translation rate in the human genome. *Gene*. 2005; 345:127–138. [PubMed: 15716084]
42. Kanaya S, Yamada Y, Kinouchi M, Kudo Y, Ikemura T. Codon usage and tRNA genes in eukaryotes: correlation of codon usage diversity with translation efficiency and with CG-dinucleotide usage as assessed by multivariate analysis. *J Mol Evol*. 2001; 53:290–298. [PubMed: 11675589]
43. Galli G, Hofstetter H, Birnstiel ML. Two conserved sequence blocks within eukaryotic tRNA genes are major promoter elements. *Nature*. 1981; 294:626–631. [PubMed: 7312050]
44. Maniatis T, Reed R. An extensive network of coupling among gene expression machines. *Nature*. 2002; 416:499–506. [PubMed: 11932736]
45. Struhl K. Transcriptional noise and the fidelity of initiation by RNA polymerase II. *Nat Struct Mol Biol*. 2007; 14:103–105. [PubMed: 17277804]
46. Betat H, Long Y, Jackman JE, Morl M. From End to End: tRNA Editing at 5'- and 3'-Terminal Positions. *Int J Mol Sci*. 2014; 15:23975–23998. [PubMed: 25535083]
47. Sprinzl M, Vassilenko KS. Compilation of tRNA sequences and sequences of tRNA genes. *Nucleic Acids Res*. 2005; 33:D139–140. [PubMed: 15608164]
48. Phizicky EM, Alfonzo JD. Do all modifications benefit all tRNAs? *FEBS Lett*. 2010; 584:265–271. [PubMed: 19931536]
49. Motorin Y, Helm M. tRNA stabilization by modified nucleotides. *Biochemistry*. 2010; 49:4934–4944. [PubMed: 20459084]
50. Urbonavicius J, Qian Q, Durand JM, Hagervall TG, Bjork GR. Improvement of reading frame maintenance is a common function for several tRNA modifications. *EMBO J*. 2001; 20:4863–4873. [PubMed: 11532950]
51. Agris PF, Vendeix FA, Graham WD. tRNA's wobble decoding of the genome: 40 years of modification. *J Mol Biol*. 2007; 366:1–13. [PubMed: 17187822]
52. Yarian C, Townsend H, Czeszkowski W, Sochacka E, Malkiewicz AJ, Guenther R, Miskiewicz A, Agris PF. Accurate translation of the genetic code depends on tRNA modified nucleosides. *J Biol Chem*. 2002; 277:16391–16395. [PubMed: 11861649]
53. Alexandrov A, Chernyakov I, Gu W, Hiley SL, Hughes TR, Grayhack EJ, Phizicky EM. Rapid tRNA decay can result from lack of nonessential modifications. *Mol Cell*. 2006; 21:87–96. [PubMed: 16387656]
54. Muramatsu T, Nishikawa K, Nemoto F, Kuchino Y, Nishimura S, Miyazawa T, Yokoyama S. Codon and amino-acid specificities of a transfer RNA are both converted by a single post-transcriptional modification. *Nature*. 1988; 336:179–181. [PubMed: 3054566]
55. Durant PC, Davis DR. Stabilization of the anticodon stem-loop of tRNA^{Lys,3} by an A+-C base-pair and by pseudouridine. *J Mol Biol*. 1999; 285:115–131. [PubMed: 9878393]
56. Kawai G, Yamamoto Y, Kamimura T, Masegi T, Sekine M, Hata T, Iimori T, Watanabe T, Miyazawa T, Yokoyama S. Conformational rigidity of specific pyrimidine residues in tRNA arises from posttranscriptional modifications that enhance steric interaction between the base and the 2'-hydroxyl group. *Biochemistry*. 1992; 31:1040–1046. [PubMed: 1310418]
57. Dalluge JJ, Hashizume T, Sopchik AE, McCloskey JA, Davis DR. Conformational flexibility in RNA: the role of dihydrouridine. *Nucleic Acids Res*. 1996; 24:1073–1079. [PubMed: 8604341]
58. Sharma S, Langhendries JL, Watzinger P, Kotter P, Entian KD, Lafontaine DL. Yeast Kre33 and human NAT10 are conserved 18S rRNA cytosine acetyltransferases that modify tRNAs assisted by the adaptor Tan1/THUMP1. *Nucleic Acids Res*. 2015; 43:2242–2258. [PubMed: 25653167]
59. Kotelawala L, Grayhack EJ, Phizicky EM. Identification of yeast tRNA Um(44) 2'-O-methyltransferase (Trm44) and demonstration of a Trm44 role in sustaining levels of specific tRNA(Ser) species. *RNA*. 2008; 14:158–169. [PubMed: 18025252]

60. Chernyakov I, Whipple JM, Kotelawala L, Grayhack EJ, Phizicky EM. Degradation of several hypomodified mature tRNA species in *Saccharomyces cerevisiae* is mediated by Met22 and the 5'-3' exonucleases Rat1 and Xrn1. *Genes Dev.* 2008; 22:1369–1380. [PubMed: 18443146]
61. Whipple JM, Lane EA, Chernyakov I, D'Silva S, Phizicky EM. The yeast rapid tRNA decay pathway primarily monitors the structural integrity of the acceptor and T-stems of mature tRNA. *Genes Dev.* 2011; 25:1173–1184. [PubMed: 21632824]
62. Guy MP, Young DL, Payea MJ, Zhang X, Kon Y, Dean KM, Grayhack EJ, Mathews DH, Fields S, Phizicky EM. Identification of the determinants of tRNA function and susceptibility to rapid tRNA decay by high-throughput in vivo analysis. *Genes Dev.* 2014; 28:1721–1732. [PubMed: 25085423]
63. Kadaba S, Krueger A, Trice T, Krecic AM, Hinnebusch AG, Anderson J. Nuclear surveillance and degradation of hypomodified initiator tRNAMet in *S. cerevisiae*. *Genes Dev.* 2004; 18:1227–1240. [PubMed: 15145828]
64. Kadaba S, Wang X, Anderson JT. Nuclear RNA surveillance in *Saccharomyces cerevisiae*: Trf4p-dependent polyadenylation of nascent hypomethylated tRNA and an aberrant form of 5S rRNA. *RNA.* 2006; 12:508–521. [PubMed: 16431988]
65. Li Z, Reimers S, Pandit S, Deutscher MP. RNA quality control: degradation of defective transfer RNA. *EMBO J.* 2002; 21:1132–1138. [PubMed: 11867541]
66. Torres AG, Batlle E, Ribas de Pouplana L. Role of tRNA modifications in human diseases. *Trends Mol Med.* 2014; 20:306–314. [PubMed: 24581449]
67. Emilsson V, Naslund AK, Kurland CG. Thiolation of transfer RNA in *Escherichia coli* varies with growth rate. *Nucleic Acids Res.* 1992; 20:4499–4505. [PubMed: 1383926]
68. Preston MA, D'Silva S, Kon Y, Phizicky EM. tRNAHis 5-methylcytidine levels increase in response to several growth arrest conditions in *Saccharomyces cerevisiae*. *RNA.* 2013; 19:243–256. [PubMed: 23249748]
69. Begley U, Dyavaiah M, Patil A, Rooney JP, DiRenzo D, Young CM, Conklin DS, Zitomer RS, Begley TJ. Trm9-catalyzed tRNA modifications link translation to the DNA damage response. *Mol Cell.* 2007; 28:860–870. [PubMed: 18082610]
70. Damon JR, Pincus D, Ploegh HL. tRNA Thiolation Links Translation to Stress Responses in *Saccharomyces cerevisiae*. *Mol Biol Cell.* 2014; 26:270–282. [PubMed: 25392298]
71. Han L, Kon Y, Phizicky EM. Functional importance of Psi38 and Psi39 in distinct tRNAs, amplified for tRNAGln(UUG) by unexpected temperature sensitivity of the s2U modification in yeast. *RNA.* 2015; 21:188–201. [PubMed: 25505024]
72. Alings F, Sarin LP, Fufezan C, Drexler HC, Leidel SA. An evolutionary approach uncovers a diverse response of tRNA 2-thiolation to elevated temperatures in yeast. *RNA.* 2015; 21:202–212. [PubMed: 25505025]
73. Chan CT, Dyavaiah M, DeMott MS, Taghizadeh K, Dedon PC, Begley TJ. A quantitative systems approach reveals dynamic control of tRNA modifications during cellular stress. *PLoS Genet.* 2010; 6:e1001247. [PubMed: 21187895]
74. Chan CT, Pang YL, Deng W, Babu IR, Dyavaiah M, Begley TJ, Dedon PC. Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. *Nat Commun.* 2012; 3:937. [PubMed: 22760636]
75. Su D, Chan CT, Gu C, Lim KS, Chionh YH, McBee ME, Russell BS, Babu IR, Begley TJ, Dedon PC. Quantitative analysis of ribonucleoside modifications in tRNA by HPLC-coupled mass spectrometry. *Nat Protoc.* 2014; 9:828–841. [PubMed: 24625781]
76. Sprinzl M, Cramer F. The -C-C-A end of tRNA and its role in protein biosynthesis. *Prog Nucleic Acid Res Mol Biol.* 1979; 22:1–69. [PubMed: 392600]
77. Nissen P, Hansen J, Ban N, Moore PB, Steitz TA. The structural basis of ribosome activity in peptide bond synthesis. *Science.* 2000; 289:920–930. [PubMed: 10937990]
78. Deutscher MP. tRNA nucleotidyltransferase. *The Enzymes.* 1982; 15:183–215.
79. Tomita K, Yamashita S. Molecular mechanisms of template-independent RNA polymerization by tRNA nucleotidyltransferases. *Front Genet.* 2014; 5:36. [PubMed: 24596576]
80. Betat H, Rammelt C, Morl M. tRNA nucleotidyltransferases: ancient catalysts with an unusual mechanism of polymerization. *Cell Mol Life Sci.* 2010; 67:1447–1463. [PubMed: 20155482]

81. Xiong Y, Steitz TA. A story with a good ending: tRNA 3'-end maturation by CCA-adding enzymes. *Curr Opin Struct Biol.* 2006; 16:12–17. [PubMed: 16364630]
82. Weiner AM. tRNA maturation: RNA polymerization without a nucleic acid template. *Curr Biol.* 2004; 14:R883–885. [PubMed: 15498478]
83. Aebi M, Kirchner G, Chen JY, Vijayraghavan U, Jacobson A, Martin NC, Abelson J. Isolation of a temperature-sensitive mutant with an altered tRNA nucleotidyltransferase and cloning of the gene encoding tRNA nucleotidyltransferase in the yeast *Saccharomyces cerevisiae*. *J Biol Chem.* 1990; 265:16216–16220. [PubMed: 2204621]
84. Chakraborty PK, Schmitz-Abe K, Kennedy EK, Mamady H, Naas T, Durie D, Campagna DR, Lau A, Sendamarai AK, Wiseman DH, et al. Mutations in TRNT1 cause congenital sideroblastic anemia with immunodeficiency, fevers, and developmental delay (SIFD). *Blood.* 2014; 124:2867–2871. [PubMed: 25193871]
85. Sasarman F, Thiffault I, Weraarpachai W, Salomon S, Maftai C, Gauthier J, Ellazam B, Webb N, Antonicka H, Janer A, et al. The 3' addition of CCA to mitochondrial tRNA^{Ser}(AGY) is specifically impaired in patients with mutations in the tRNA nucleotidyl transferase TRNT1. *Hum Mol Genet.* 2015
86. Zhu L, Deutscher MP. tRNA nucleotidyltransferase is not essential for *Escherichia coli* viability. *EMBO J.* 1987; 6:2473–2477. [PubMed: 3311729]
87. Yue D, Weiner AM, Maizels N. The CCA-adding enzyme has a single active site. *J Biol Chem.* 1998; 273:29693–29700. [PubMed: 9792681]
88. Shi PY, Maizels N, Weiner AM. CCA addition by tRNA nucleotidyltransferase: polymerization without translocation? *EMBO J.* 1998; 17:3197–3206. [PubMed: 9606201]
89. Tomita K, Fukai S, Ishitani R, Ueda T, Takeuchi N, Vassilyev DG, Nureki O. Structural basis for template-independent RNA polymerization. *Nature.* 2004; 430:700–704. [PubMed: 15295603]
90. Xiong Y, Steitz TA. Mechanism of transfer RNA maturation by CCA-adding enzyme without using an oligonucleotide template. *Nature.* 2004; 430:640–645. [PubMed: 15295590]
91. Yue D, Maizels N, Weiner AM. CCA-adding enzymes and poly(A) polymerases are all members of the same nucleotidyltransferase superfamily: characterization of the CCA-adding enzyme from the archaeal hyperthermophile *Sulfolobus shibatae*. *RNA.* 1996; 2:895–908. [PubMed: 8809016]
92. Tomita K, Ishitani R, Fukai S, Nureki O. Complete crystallographic analysis of the dynamics of CCA sequence addition. *Nature.* 2006; 443:956–960. [PubMed: 17051158]
93. Pan B, Xiong Y, Steitz TA. How the CCA-Adding Enzyme Selects Adenine over Cytosine at Position 76 of tRNA. *Science.* 2010; 330:937–940. [PubMed: 21071662]
94. Li F, Xiong Y, Wang J, Cho HD, Tomita K, Weiner AM, Steitz TA. Crystal structures of the *Bacillus stearothermophilus* CCA-adding enzyme and its complexes with ATP or CTP. *Cell.* 2002; 111:815–824. [PubMed: 12526808]
95. Cho HD, Verlinde CL, Weiner AM. Reengineering CCA-adding enzymes to function as (U,G)- or dCdCdA-adding enzymes or poly(C,A) and poly(U,G) polymerases. *Proc Natl Acad Sci U S A.* 2007; 104:54–59. [PubMed: 17179213]
96. Dupasquier M, Kim S, Halkidis K, Gamper H, Hou YM. tRNA integrity is a prerequisite for rapid CCA addition: implication for quality control. *J Mol Biol.* 2008; 379:579–588. [PubMed: 18466919]
97. Toh Y, Numata T, Watanabe K, Takeshita D, Nureki O, Tomita K. Molecular basis for maintenance of fidelity during the CCA-adding reaction by a CCA-adding enzyme. *EMBO J.* 2008; 27:1944–1952. [PubMed: 18583961]
98. Wilusz JE, Whipple JM, Phizicky EM, Sharp PA. tRNAs marked with CCACCA are targeted for degradation. *Science.* 2011; 334:817–821. [PubMed: 22076379]
99. Wilusz JE, Freier SM, Spector DL. 3' end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA. *Cell.* 2008; 135:919–932. [PubMed: 19041754]
100. Sunwoo H, Dinger ME, Wilusz JE, Amaral PP, Mattick JS, Spector DL. MEN epsilon/beta nuclear-retained non-coding RNAs are up-regulated upon muscle differentiation and are essential components of paraspeckles. *Genome Res.* 2009; 19:347–359. [PubMed: 19106332]

101. Lorentzen E, Basquin J, Tomecki R, Dziembowski A, Conti E. Structure of the active subunit of the yeast exosome core, Rrp44: diverse modes of substrate recruitment in the RNase II nuclease family. *Mol Cell*. 2008; 29:717–728. [PubMed: 18374646]
102. Vincent HA, Deutscher MP. Substrate recognition and catalysis by the exoribonuclease RNase R. *J Biol Chem*. 2006; 281:29769–29775. [PubMed: 16893880]
103. Wilusz JE, JnBaptiste CK, Lu LY, Kuhn CD, Joshua-Tor L, Sharp PA. A triple helix stabilizes the 3' ends of long noncoding RNAs that lack poly(A) tails. *Genes Dev*. 2012; 26:2392–2407. [PubMed: 23073843]
104. Zhang B, Arun G, Mao YS, Lazar Z, Hung G, Bhattacharjee G, Xiao X, Booth CJ, Wu J, Zhang C, et al. The lncRNA Malat1 Is Dispensable for Mouse Development but Its Transcription Plays a cis-Regulatory Role in the Adult. *Cell Rep*. 2012; 2:111–123. [PubMed: 22840402]
105. Leigh J, Lang BF. Mitochondrial 3' tRNA editing in the jakobid *Seculamonas ecuadoriensis*: a novel mechanism and implications for tRNA processing. *RNA*. 2004; 10:615–621. [PubMed: 15037771]
106. Lonergan KM, Gray MW. Editing of transfer RNAs in *Acanthamoeba castellanii* mitochondria. *Science*. 1993; 259:812–816. [PubMed: 8430334]
107. Yarham JW, Elson JL, Blakely EL, McFarland R, Taylor RW. Mitochondrial tRNA mutations and disease. *Wiley Interdiscip Rev RNA*. 2010; 1:304–324. [PubMed: 21935892]
108. Hou YM, Schimmel P. A simple structural feature is a major determinant of the identity of a transfer RNA. *Nature*. 1988; 333:140–145. [PubMed: 3285220]
109. McClain WH, Foss K. Changing the identity of a tRNA by introducing a G-U wobble pair near the 3' acceptor end. *Science*. 1988; 240:793–796. [PubMed: 2452483]
110. Shi PY, Weiner AM, Maizels N. A top-half tDNA minihelix is a good substrate for the eubacterial CCA-adding enzyme. *RNA*. 1998; 4:276–284. [PubMed: 9510330]
111. Kuhn CD, Wilusz JE, Zheng Y, Beal PA, Joshua-Tor L. On-Enzyme Refolding Permits Small RNA and tRNA Surveillance by the CCA-Adding Enzyme. *Cell*. 2015; 160:644–658. [PubMed: 25640237]
112. Choi YS, Patena W, Leavitt AD, McManus MT. Widespread RNA 3'-end oligouridylation in mammals. *RNA*. 2012; 18:394–401. [PubMed: 22291204]
113. Heo I, Joo C, Kim YK, Ha M, Yoon MJ, Cho J, Yeom KH, Han J, Kim VN. TUT4 in concert with Lin28 suppresses microRNA biogenesis through pre-microRNA uridylation. *Cell*. 2009; 138:696–708. [PubMed: 19703396]
114. Faehnle CR, Walleshauser J, Joshua-Tor L. Mechanism of Dis3l2 substrate recognition in the Lin28-let-7 pathway. *Nature*. 2014; 514:252–256. [PubMed: 25119025]
115. Slevin MK, Meaux S, Welch JD, Bigler R, Miliani de Marval PL, Su W, Rhoads RE, Prins JF, Marzluff WF. Deep sequencing shows multiple oligouridylations are required for 3' to 5' degradation of histone mRNAs on polyribosomes. *Mol Cell*. 2014; 53:1020–1030. [PubMed: 24656133]
116. Rissland OS, Norbury CJ. Decapping is preceded by 3' uridylation in a novel pathway of bulk mRNA turnover. *Nat Struct Mol Biol*. 2009; 16:616–623. [PubMed: 19430462]
117. Lim J, Ha M, Chang H, Kwon SC, Simanshu DK, Patel DJ, Kim VN. Uridylation by TUT4 and TUT7 Marks mRNA for Degradation. *Cell*. 2014; 159:1365–1376. [PubMed: 25480299]
118. Bentley DL. Coupling mRNA processing with transcription in time and space. *Nat Rev Genet*. 2014; 15:163–175. [PubMed: 24514444]
119. Yoshihisa T, Yunoki-Esaki K, Ohshima C, Tanaka N, Endo T. Possibility of cytoplasmic pre-tRNA splicing: the yeast tRNA splicing endonuclease mainly localizes on the mitochondria. *Mol Biol Cell*. 2003; 14:3266–3279. [PubMed: 12925762]
120. Paushkin SV, Patel M, Furia BS, Peltz SW, Trotta CR. Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3' end formation. *Cell*. 2004; 117:311–321. [PubMed: 15109492]
121. Hopper AK. Transfer RNA post-transcriptional processing, turnover, and subcellular dynamics in the yeast *Saccharomyces cerevisiae*. *Genetics*. 2013; 194:43–67. [PubMed: 23633143]

122. Shaheen HH, Hopper AK. Retrograde movement of tRNAs from the cytoplasm to the nucleus in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*. 2005; 102:11290–11295. [PubMed: 16040803]
123. Takano A, Endo T, Yoshihisa T. tRNA actively shuttles between the nucleus and cytosol in yeast. *Science*. 2005; 309:140–142. [PubMed: 15905365]
124. Whitney ML, Hurto RL, Shaheen HH, Hopper AK. Rapid and reversible nuclear accumulation of cytoplasmic tRNA in response to nutrient availability. *Mol Biol Cell*. 2007; 18:2678–2686. [PubMed: 17475781]
125. Hurto RL, Tong AH, Boone C, Hopper AK. Inorganic phosphate deprivation causes tRNA nuclear accumulation via retrograde transport in *Saccharomyces cerevisiae*. *Genetics*. 2007; 176:841–852. [PubMed: 17409072]
126. Chu HY, Hopper AK. Genome-wide investigation of the role of the tRNA nuclear-cytoplasmic trafficking pathway in regulation of the yeast *Saccharomyces cerevisiae* transcriptome and proteome. *Mol Cell Biol*. 2013; 33:4241–4254. [PubMed: 23979602]
127. Kramer EB, Hopper AK. Retrograde transfer RNA nuclear import provides a new level of tRNA quality control in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A*. 2013; 110:21042–21047. [PubMed: 24297920]
128. Thompson DM, Parker R. Stressing out over tRNA cleavage. *Cell*. 2009; 138:215–219. [PubMed: 19632169]
129. Anderson P, Ivanov P. tRNA fragments in human health and disease. *FEBS Lett*. 2014; 588:4297–4304. [PubMed: 25220675]
130. Gebetsberger J, Polacek N. Slicing tRNAs to boost functional ncRNA diversity. *RNA Biol*. 2013; 10:1798–1806. [PubMed: 24351723]
131. Yamasaki S, Ivanov P, Hu GF, Anderson P. Angiogenin cleaves tRNA and promotes stress-induced translational repression. *J Cell Biol*. 2009; 185:35–42. [PubMed: 19332886]
132. Fu H, Feng J, Liu Q, Sun F, Tie Y, Zhu J, Xing R, Sun Z, Zheng X. Stress induces tRNA cleavage by angiogenin in mammalian cells. *FEBS Lett*. 2009; 583:437–442. [PubMed: 19114040]
133. Thompson DM, Lu C, Green PJ, Parker R. tRNA cleavage is a conserved response to oxidative stress in eukaryotes. *RNA*. 2008; 14:2095–2103. [PubMed: 18719243]
134. 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*. 2013; 9:e1003767. [PubMed: 24009533]
135. Ivanov P, O'Day E, Emara MM, Wagner G, Lieberman J, Anderson P. G-quadruplex structures contribute to the neuroprotective effects of angiogenin-induced tRNA fragments. *Proc Natl Acad Sci U S A*. 2014; 111:18201–18206. [PubMed: 25404306]
136. Ivanov P, Emara MM, Villen J, Gygi SP, Anderson P. Angiogenin-induced tRNA fragments inhibit translation initiation. *Mol Cell*. 2011; 43:613–623. [PubMed: 21855800]
137. Saikia M, Jobava R, Parisien M, Putnam A, Krokowski D, Gao XH, Guan BJ, Yuan Y, Jankowsky E, Feng Z, et al. Angiogenin-cleaved tRNA halves interact with cytochrome c, protecting cells from apoptosis during osmotic stress. *Mol Cell Biol*. 2014; 34:2450–2463. [PubMed: 24752898]
138. Cole C, Sobala A, Lu C, Thatcher SR, Bowman A, Brown JW, Green PJ, Barton GJ, Hutvagner G. Filtering of deep sequencing data reveals the existence of abundant Dicer-dependent small RNAs derived from tRNAs. *RNA*. 2009; 15:2147–2160. [PubMed: 19850906]
139. Li Z, Ender C, Meister G, Moore PS, Chang Y, John B. Extensive terminal and asymmetric processing of small RNAs from rRNAs, snoRNAs, snRNAs, and tRNAs. *Nucleic Acids Res*. 2012; 40:6787–6799. [PubMed: 22492706]
140. Lee YS, Shibata Y, Malhotra A, Dutta A. A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). *Genes Dev*. 2009; 23:2639–2649. [PubMed: 19933153]
141. Zamudio JR, Kelly TJ, Sharp PA. Argonaute-bound small RNAs from promoter-proximal RNA polymerase II. *Cell*. 2014; 156:920–934. [PubMed: 24581493]
142. Maute RL, Schneider C, Sumazin P, Holmes A, Califano A, Basso K, Dalla-Favera R. tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-

regulated in B cell lymphoma. *Proc Natl Acad Sci U S A*. 2013; 110:1404–1409. [PubMed: 23297232]

143. Sobala A, Hutvagner G. Small RNAs derived from the 5' end of tRNA can inhibit protein translation in human cells. *RNA Biol*. 2013; 10:553–563. [PubMed: 23563448]
144. Gebetsberger J, Zywicki M, Kunzi A, Polacek N. tRNA-derived fragments target the ribosome and function as regulatory non-coding RNA in *Haloferax volcanii*. *Archaea*. 2012; 2012:260909. [PubMed: 23326205]
145. Zhang S, Sun L, Kragler F. The phloem-delivered RNA pool contains small noncoding RNAs and interferes with translation. *Plant Physiol*. 2009; 150:378–387. [PubMed: 19261735]
146. Dewe JM, Whipple JM, Chernyakov I, Jaramillo LN, Phizicky EM. The yeast rapid tRNA decay pathway competes with elongation factor 1A for substrate tRNAs and acts on tRNAs lacking one or more of several modifications. *RNA*. 2012; 18:1886–1896. [PubMed: 22895820]
147. Turowski TW, Karkusiewicz I, Kowal J, Boguta M. Maf1-mediated repression of RNA polymerase III transcription inhibits tRNA degradation via RTD pathway. *RNA*. 2012; 18:1823–1832. [PubMed: 22919049]
148. Netzer N, Goodenbour JM, David A, Dittmar KA, Jones RB, Schneider JR, Boone D, Eves EM, Rosner MR, Gibbs JS, et al. Innate immune and chemically triggered oxidative stress modifies translational fidelity. *Nature*. 2009; 462:522–526. [PubMed: 19940929]
149. Pan T. Adaptive translation as a mechanism of stress response and adaptation. *Annu Rev Genet*. 2013; 47:121–137. [PubMed: 23988117]
150. Maraia RJ, Lamichhane TN. 3' processing of eukaryotic precursor tRNAs. *Wiley Interdiscip Rev RNA*. 2011; 2:362–375. [PubMed: 21572561]

Figure 1. Conserved structure of tRNAs

Mature tRNAs fold into a cloverleaf secondary structure (A) that is further folded into an L-shaped tertiary structure (B). Aminoacyl-tRNA synthetases catalyze the covalent attachment of the cognate amino acid (denoted AA) to the tRNA 3' end, and the genetic code is read by base pairing between the tRNA anticodon and the mRNA codon. The acceptor stem is typically 7 base pairs long, the D stem 3–4 base pairs, the anticodon stem 5 base pairs, and the TΨC stem 5 base pairs. As the name suggests, different tRNAs have different numbers of nucleotides in the variable loop.

Figure 2. Opposing tRNA signatures in proliferating vs. differentiated cells

A continuous spectrum of tRNA pools is observed in human cells depending on the cell's proliferative status. As differentiated or arrested cells begin to proliferate, a gradual transition in tRNA expression is observed towards the proliferation tRNA signature.

Likewise, differentiation causes the tRNA pool to change in the opposite direction towards the differentiation tRNA signature. For many amino acids, there is at least one codon that is preferentially used in proliferation-related genes and at least one distinct codon in differentiation-related genes. For example, tRNA^{Arg(ACG)} and tRNA^{Arg(CCG)} are predominately expressed in differentiated cells, whereas tRNA^{Arg(TCG)} and tRNA^{Arg(TCT)} are more abundantly expressed in proliferating cells.³⁰

Figure 3. Maturation of tRNAs

Once transcribed, tRNAs are sequentially processed at their 5' and 3' ends to generate a mature tRNA that can be charged with an amino acid. Whereas the 5' leader sequence is almost universally cleaved off by the endonuclease RNase P, multiple mechanisms have been reported for tRNA 3' end processing.¹⁵⁰ In humans, the endonuclease RNase Z cleaves downstream of the unpaired discriminator base, releasing the 3' trailer sequence. Bacteria instead generally remove the 3' trailer by trimming using 3'-5' exonucleases (not shown). In humans and all other species that do not encode CCA on their tRNAs, CCA is then post-transcriptionally added by the CCA-adding enzyme to generate the amino acid attachment site.

Figure 4. tRNAs are highly modified post-transcriptionally

Yeast tRNA^{Ser(CGA)}, which is commonly modified at eleven positions, is shown as an example. When yeast are grown at high temperatures, tRNA^{Ser(CGA)} is degraded by the rapid tRNA decay pathway if ac⁴C₁₂ and Um₄₄ are absent due to deletion of Tan1, a co-activator of Kre33, and Trm44.⁵⁹ Structural instability in the acceptor and TΨC stems is highlighted.

Figure 5. CCA or CCACCA is specifically added to the 3' ends of tRNA-like transcripts
(A) Structures of mouse mascRNA as well as the mouse and human homologs of the MEN β tRNA-like small RNA are shown. Nucleotides added post-transcriptionally by the CCA-adding enzyme are in blue. A sequence alignment between mouse mascRNA and the mouse MEN β tRNA-like small RNA is shown in the middle. **(B)** In stark contrast to the mouse and human MEN β homologs, the top of the acceptor stem of the Rhesus monkey MEN β homolog is significantly more stable, resulting in CCA addition.

Figure 6. Proofreading by the CCA-adding enzyme determines tRNA fate

A primary tRNA transcript is first processed at its 5' and 3' ends prior to recognition by the CCA-adding enzyme. For simplicity, the D and anticodon arms are not shown in subsequent steps. Only the head and neck domains, which comprise the catalytic center, are shown for the CCA-adding enzyme. After the addition of CCA, nucleotide binding triggers closure of the head domain and either disassociation of structurally stable tRNAs or refolding of structurally unstable tRNAs.¹¹¹ Whereas transcripts ending in CCA can be aminoacylated and function in translation, transcripts ending in CCACCA are recognized for degradation. Additional details for each step are provided in the text.

Figure 7. Multiple mechanisms are employed to change the fate of a mature tRNA

Once generated, mature tRNAs can be prevented from functioning in translation via multiple mechanisms. tRNAs can be imported and selectively retained in the nucleus, cleaved to generate a variety of small RNAs, or marked for degradation via the post-transcriptional addition of 3' terminal tails.