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Repertoires of tRNAs: The Couplers of Genomics and Proteomics

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Keywords

transfer RNA, tRNA, anticodon, genomic organization, translation fidelity, protein abundance, codon usage

Abstract

The pool of transfer RNA (tRNA) molecules in cells allows the ribosome to decode genetic information. This repertoire of molecular decoders is positioned in the crossroad of the genome, the transcriptome, and the proteome. Omics and systems biology now allow scientists to explore the entire repertoire of tRNAs of many organisms, revealing basic exciting biology. The tRNA gene set of hundreds of species is now characterized, in addition to the tRNA genes of organelles and viruses. Genes encoding tRNAs for certain anticodon types appear in dozens of copies in a genome, while others are universally absent from any genome. Transcriptome measurement of tRNAs is challenging, but in recent years new technologies have allowed researchers to determine the dynamic expression patterns of tRNAs. These advances reveal that availability of ready-to-translate tRNA molecules is highly controlled by several transcriptional and posttranscriptional regulatory processes. This regulation shapes the proteome according to the cellular state. The tRNA pool profoundly impacts many aspects of cellular and organismal life, including protein expression level, translation accuracy, adequacy of folding, and even mRNA stability. As a result, the shape of the tRNA pool affects organismal health and may participate in causing conditions such as cancer and neurological conditions.

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INTRODUCTION

Transfer RNA (tRNA) molecules are the decoders of the genetic code. The synthesis of a new protein by the ribosome is done by base pairing of the trinucleotide codon, as encoded by the mRNA sequence, with the trinucleotide anticodon of a tRNA molecule that carries a specific amino acid. The repertoire of tRNA molecules that operates in any living cell allows the ribosome to decode genetic information while translating the transcriptome to the proteome. Following the deciphering of tRNA biochemistry, function, and structure, more than half a century ago the omics and systems biology fields began to explore the tRNA repertoire of many organisms. New types of analyses reveal basic new exciting biology. Genomics now allows one to detect and characterize all the genes that encode tRNAs in any fully sequenced species. Algorithms that detect tRNA genes and classify them according to anticodon type specificity are now available. Analyses of the tRNA repertoires of species reveal interesting similarities and differences across the tree of life. Across all genomes analyzed, one observes between approximately 30 and 50 tRNA types, each defined by a unique anticodon nucleotide triplet. Interestingly, genes encoding certain types of tRNAs can appear in many dozens, and even more, copies per genome, but out of the 61 possible anticodons in the genetic code, between a sixth and a half are absent in any genome.

The expression of tRNAs is more difficult to resolve than that of other RNA types because tRNA molecules are highly structured and possess many chemical modifications. Yet in recent years, the development of specialized tools has enabled researchers to sequence tRNA molecules and to determine their expression patterns and multiple levels of regulation ranging from production to modification, amino acid loading, and degradation. The availability of ready-to-translate (RtT) tRNA molecules is determined via complicated and regulated processes in which several posttranscriptional regulatory phases take place. Owing to technological developments, we can estimate the pool of available tRNAs in many organisms and physiological states, including tissues

and organs; in various stages of life such as development and aging; and in pathologies. It is evident that the tRNA pool can exert profound impacts on the proteome and, as a consequence, affects aspects of cellular and organismal life. The tRNA pool not only participates in determining the expression level of each protein in the proteome but also affects aspects of gene regulation. Data regarding the various stages of tRNA processing reveal that the state of the tRNA pool can determine the speed and efficiency with which proteins are made and that the accuracy of the translation process and the adequacy of protein folding also depend on a cell's tRNA repertoire. Furthermore, tRNA availability is associated with the stability of mRNAs. The tRNA pool also participates in health-related conditions, for example, cancer and neurological conditions. With further advances in new technologies to decipher the tRNA pool, we envisage a growing recognition of the role of these genetic entities in coupling organismal genotypes and phenotypes.

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DIVERSE CELLULAR FACTORS THAT SHAPE THE READY-TO-TRANSLATE tRNA POOL

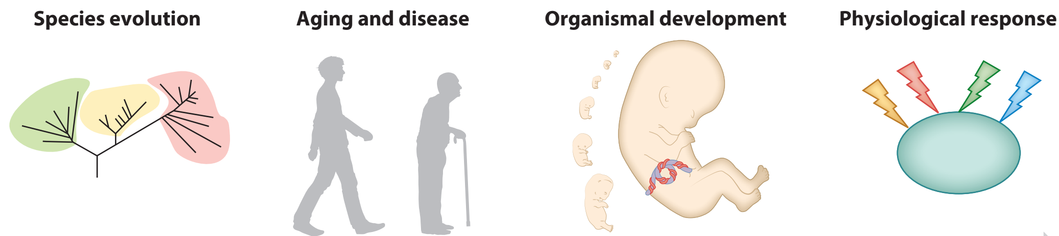
Many factors and processes affect the composition of RtT tRNAs in living cells. Processes that operate on many timescales, from millions of years to minutes, control dynamism in the tRNA repertoire. On the longest timescale—the evolutionary one, which operates over millions of years—the tRNA pool shows dynamic evolution. In expanding the repertoire, tRNA genes are born, mostly through duplications of genes or genomic segments, and potentially also due to other processes, e.g., reverse transcription (see below). tRNA genes can also die, mostly upon gene deletion or as they accumulate mutations and cease to function. Although some evolutionarily remote organisms may show similar proportions of the various tRNAs' gene copy numbers, some species evolved very peculiar repertoires. Over evolutionary timescales, tRNAs and the codon usage of genes in the open reading frames adapt to one another, potentially ensuring adequacy of translation. tRNA genes can change in expression over years during organisms' life spans, for example, during aging or in cancer. Over shorter timescales of months to hours, tRNA expression can change in developmental processes, e.g., when cells differentiate. Lastly, tRNA expression, amino acid loading, and modifications change the tRNA pool in a manner of minutes and even seconds, for example, in response to environmental cues (**Figure 1**).

In this section we review the major factors that determine the tRNA pool composition (**Figure 2**, upper panel).

tRNA Gene Copy Numbers in Genomes

A classical and the simplest way to characterize the tRNA pool of any organism whose genome has been fully sequenced is by the gene copy number of each tRNA anticodon type (i.e., isoacceptor). Such characterization shows several interesting features of the tRNA pool, as detailed below.

First, a minimal tRNA pool for the standard genetic code that utilizes the classical wobble rules, which enables additional non-Watson-Crick base pairing between the 5′ nucleotide of the anticodon and the 3′ nucleotide of the codon, should consist of 32 tRNA anticodon species. This minimalist set was originally predicted from a simple count: With 20 amino acids, two amino acids are coded by one codon and require only one tRNA for decoding, and nine amino acids are coded by two codons (two-codon boxes) and also require only one tRNA. Six amino acids that are coded by three or four codons (three/four-codon boxes) require two tRNAs each, and three amino acids that are coded by six codons (six-codon boxes) add another three tRNAs each. The maximal tRNA set could have contained tRNA for each of the 61 sense codons, but in reality in very few known genomes does this number exceed 50. Thus, some codons do not have a fully matched



	Millions of years	Dozens of years	Weeks	Minutes and hours
Dynamics in tRNA pool	tRNA repertoire changes by gene duplication and loss and anticodon switching	tRNA transcription changes, changes in epigenetic marks in chromatin in tRNA vicinity		Changes in transcription; amino acid loading; and tRNA cleavage, modification, and processing
Biological response	Codon usage adaptation	Maintenance of proteome integrity	Tissue-specific protein regulation, cell status control	Rapid response of the proteome to environmental changes (metabolic shifts, biotic and abiotic stress, amino acid starvation)
Technologies	Gene copy number prediction by genome scanning algorithms	tRNA type evaluation by deep sequencing, ChIP sequencing for RNA polymerase III occupancy and histone modification		Deep sequencing and mass spectrometry methods for detecting tRNA charging and modification

Figure 1

The dynamics of tRNA changes spans diverse timescales. Changes in the tRNA pool can be viewed on a continuum of timescales. Evolutionarily, the tRNA pool changes over millions of years as tRNA genes are gained and lost. On this timescale, the tRNA pool and the protein-coding sequences of a genome can adapt to one another such that highly expressed genes' codon usage is better adapted to the abundant tRNAs. During the life of a multicellular organism, the tRNA pool can change at the transcription level, the chromatin epigenetic level, and perhaps also at the gene copy number level. Processes involved in such changes include aging, cancer development, and the progression of chronic diseases. Likewise, at the timescale of developmental processes, e.g., upon stem cell differentiation, the tRNA pool changes in expression and at the level of chromatin epigenetics. At the shortest timescales, as short as minutes, RtT tRNA abundance can change at all gene expression levels, including transcription, modifications, splicing, and amino acid loading. The various levels of tRNA regulation can be evaluated by the corresponding technologies and methods (see **Supplemental Text**).

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Supplemental Material >

tRNA and need to rely on wobble interactions with nonperfectly matching tRNAs to be properly decoded (Grosjean et al. 2010). There is a set of 16 anticodon types that are generally avoided in the eukaryotes, there is a partially overlapping set of 16 anticodon types that are avoided in Archaea, and 19 types are either completely avoided or are partly absent in Bacteria. The absence of these so-called disallowed tRNAs indicates that they could be deleterious. Specifically, disallowed tRNAs that correspond to codons in the two-codon boxes of the genetic table can potentially introduce the wrong amino acid during translation (**Figure 3**).

Second, the existing tRNA types can be coded by a range of gene copy numbers, resulting in a high number of tRNA coding genes in a typical genome. For example, in the human genome there are 417 functional tRNA genes (Chan & Lowe 2016). An interesting question pertains to the function of tRNA gene copy multiplicity. One obvious possibility is that all copies of tRNA genes of a certain anticodon type contribute to the total expression level of that tRNA. Thus, multiplicity of copies of each tRNA type can enable higher expression of highly demanded tRNAs. In addition, alternative copies of same tRNA may be subject to differential regulation, e.g., across physiological conditions or tissues. Furthermore, having several copies of tRNA genes for the

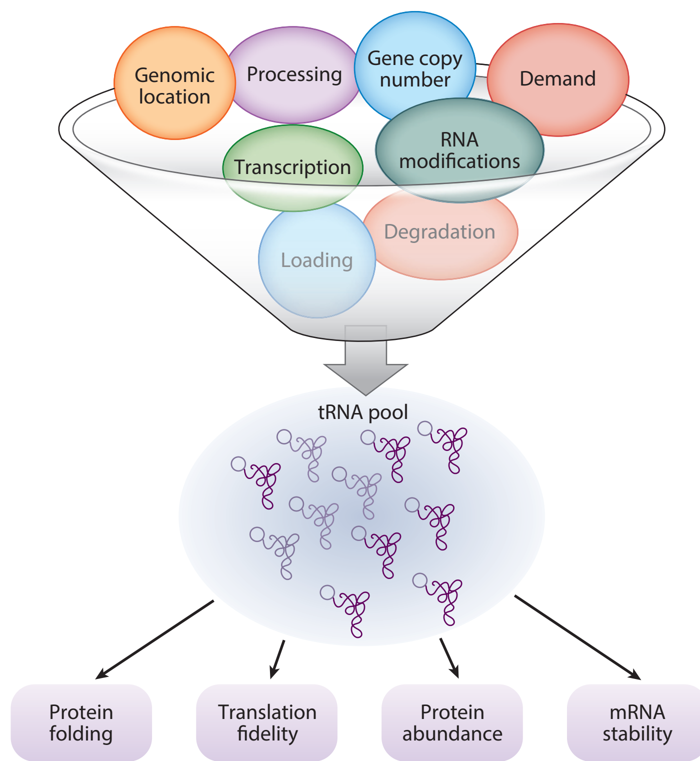

Figure 2

Mechanisms that affect ready-to-translate (RtT) tRNA availability and cellular processes affected by tRNA pool shape. Within a cell, many factors acting at different levels determine the availability and dynamics of mature RtT tRNAs. At the genomic level, the composition of the tRNA pool is defined by the gene copy number that codes each type of tRNA. Additional important genomic features that influence tRNA expression are regulatory motifs within tRNA genes and the adjacent surrounding as well as the location of the gene on the chromosome. Chromatin epigenetic modifications in the vicinity of tRNA genes, and various transcription regulation factors, can further shape the tRNA transcription landscape. RtT tRNAs are formed by posttranscriptional processes, including splicing, base modifications, and amino acid loading, that together add to the levels of regulation. Furthermore, RtT tRNAs are consumed according to their demand and are subjected to degradation. The shape of the repertoire of RtT tRNAs has diverse effects on cellular physiology, on the transcriptomes, and on the proteomes. The tRNA pool regulates translation; determines protein production; affects ribosome movement and allocation along transcripts; and influences protein abundance, protein folding, and translation fidelity. In addition, the stability of the mRNA transcript can be affected by tRNA availability.

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same anticodon could provide organisms with the ability to further evolve their tRNA repertoire (**Figure 4**).

Third, in addition to containing functional tRNA genes, most genomes also contain tRNA genes that appear to be nonfunctional. Such pseudo-tRNA genes can be identified and annotated as low-confidence tRNA since their sequence resembles that of canonical tRNAs, although with many imperfections (see below).

Fourth, in general, the total number of functional tRNA genes in a genome correlates with genome size (Bermudez-Santana et al. 2010). For example, in *Escherichia coli* there are 86 tRNA genes, while in *Saccharomyces cerevisiae* there are 275 genes (Chan & Lowe 2009); the ratio between

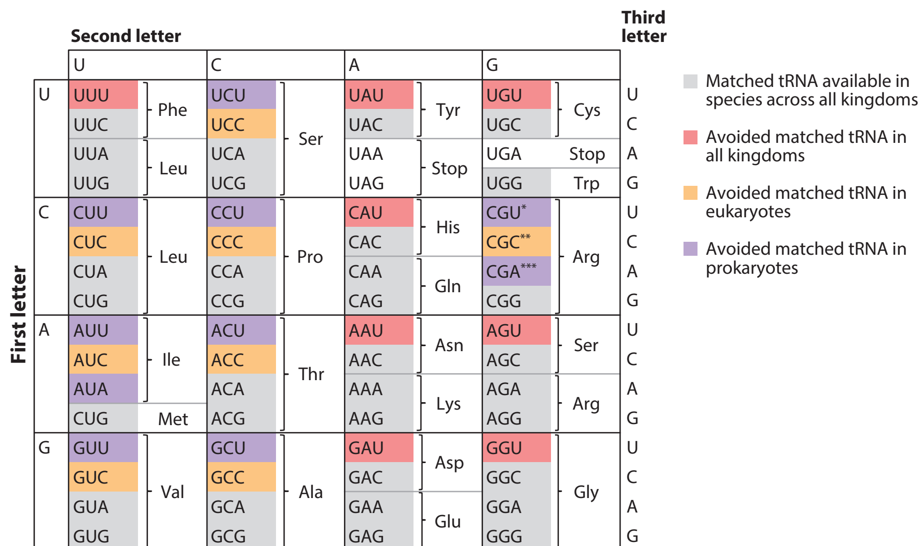

Figure 3

Allowed and disallowed tRNAs in evolution. Genomes feature disallowed tRNAs. Certain codons do not have corresponding anticodon tRNA genes in many genomes. In particular, in most genomes at least 16 codons do not have a fully matched tRNA and are decoded by wobble interactions. The colors marked on codons indicate whether their cognate tRNAs are present in genomes of all kingdoms of life, avoided in all genomes of all kingdoms of life, or avoided uniquely in eukaryotes or prokaryotes. A tRNA is avoided in a clade if it is absent in at least 90% of the analyzed species from that clade, as annotated in tRNAdb (Chan & Lowe 2016). Two recurring patterns emerge. First, in all two-codon boxes of the genetic code table (that share the first two nucleotides and code for two amino acids), tRNA that carries an anticodon that matches the upper codon (*XY*U) is avoided in the majority of species across all domains. Second, in most four-codon boxes of the genetic code table, the *XY*U tRNA is avoided in the prokaryotes, and the *XY*C's tRNAs are avoided in the eukaryotes. There are two exceptions to this rule: In the GGN glycine four-codon box, the GGU's tRNA is avoided across all kingdoms. In the CG*X* arginine box, one asterisk denotes a tRNA that is avoided only in Archaea, two asterisks denote a tRNA that is avoided in both Eukarya and Bacteria, and three asterisks denote a tRNA that is avoided only in Bacteria.

these numbers scales with the difference in genome size (0.38). The correlation becomes even stronger for pseudo-tRNA genes (Bermudez-Santana et al. 2010). In higher eukaryotes, the tRNA gene copy number has a greater range, spanning two orders of magnitude between different organisms with a lower correlation with the genome size (Chan & Lowe 2009, Goodenbour & Pan 2006).

Fifth, the differences in tRNA gene copy number between organisms cannot be completely explained by the phylogenetic relationships between species. For example, in yeast, the phylogenetic tree generated on the basis of tRNA gene copy number does not recapitulate the phylogenetic tree generated from the 18S rRNA sequence, suggesting a rapid change in tRNA composition (Iben & Maraia 2012). These rapid changes can be mediated through lateral gene transfer (McDonald et al. 2015); through anticodon switching, a phenomenon in which the anticodon of a tRNA mutates into that of another (Rogers & Griffiths-Jones 2014, Yona et al. 2013); or through gene and chromosome duplication. Even within the scale of the human genome, tRNA gene copy number varies in the population (Iben & Maraia 2014).

Finally, in addition to the tRNAs needed to code the classical 20 amino acids, the tRNA pool features several unique tRNA types, including suppressor tRNAs (which recognize stop codons), a unique tRNA for selenocysteine, and the initiator methionine tRNA.

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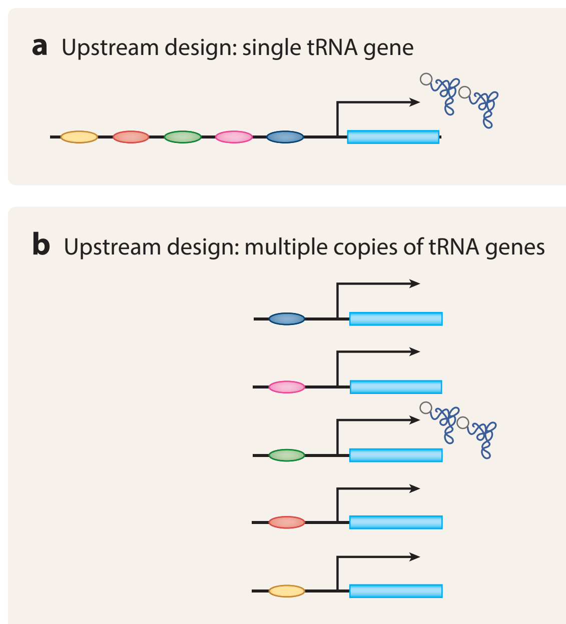

Figure 4

Two putative designs of a tRNA promoter, a potential evolutionary explanation for a multiple-gene-copy tRNA pool. Illustrated here are two alternative regulation modes of tRNA expression by different upstream region designs. (*a*) A design of a single-copy tRNA gene with an elaborate upstream region with various motifs that support alternative expression in diverse tissues, organs, and developmental stages. (*b*) A design of multiple identical gene copies, each with a simple and shorter upstream region that contains only one of the alternative regulatory features. Such a design supports simple and binary expression regulation that has the advantage of being less complex and may be more suitable for transcription by RNA polymerase III. This design could also provide backup for mutations in particular copies and could support the evolution of new tRNA sequences and function.

As described above, examining tRNA gene copy number in various organisms reveals a complex and intriguing picture, but what is the physiological impact of different tRNA repertoires? tRNA gene copy numbers correlate with codon usage of genes. Anticodons that correspond to codons that are highly represented in transcriptomes are often represented by a high copy number of their tRNAs. Indeed, a popular measure to capture the translation efficiency of genes—the tRNA Adaptation Index (tAI), which uses the tRNA gene copy number as well as the strength of codon-anticodon interactions—builds on this notion (dos Reis et al. 2004). It remains an open question whether all copies of the same isoacceptor family are functionally identical. Even seemingly identical copies can contribute differently in expression and may differ in, for example, essentiality (Bloom-Ackermann et al. 2014), tissue of expression (Dittmar et al. 2006, Ishimura et al. 2014, Sagi et al. 2016), and time of expression during development (Sagi et al. 2016) (**Figure 4**).

Viral genomes, particularly phages, often contain genes encoding just a selection of tRNAs; the remaining tRNAs are supplied solely by the host cell. The selection of tRNA genes that are encoded in viral genomes appears to be highly nonrandom. For example, in several phages the viral encoded tRNAs correspond to the codons that are enriched in the virus's most highly expressed genes (Bailly-Bechet et al. 2007, Limor-Waisberg et al. 2011). Interestingly, viruses of higher organisms such as humans appear not to encode any tRNA genes, and hence such viruses must rely on the supply of tRNAs from the host.

Both mitochondrial and chloroplast genomes encode a self-sufficient set of tRNA genes. Owing to wobble interaction rules for codons and anticodons, these organelles can translate all their proteins with their encoded tRNA pools. Those organelles' tRNA pools have some unique features. For example, in animals there are 22 tRNA types in the mitochondria. This minimalist number is presumably realized through more relaxed wobble rules [uracil (U) in the first anticodon position can pair with all four nucleotides], a modified genetic code, and a versatile initiator-elongator methionine tRNA. The mitochondrial tRNAs are shorter and lack the classical cloverleaf structure (Anderson et al. 1981). In mitochondria, tRNAs are charged by mitochondria-specific aminoacyl tRNA synthetases (aaRSs) that are encoded in the nuclear genome. Interestingly, many of the mitochondrial aaRS genes are associated with human diseases (reviewed by Diodato et al. 2014). Chloroplasts encode more tRNAs, typically 30. Seven out of 30 tRNA types have two copies that are duplicated due to an inverted repeat that occurred in the chloroplast genome (Maréchal-Drouard et al. 1991).

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Genomic Organization of tRNAs

Depending on the organism, tRNA genes can be scattered across the genome (for example, in *S. cerevisiae*) or can be organized in clusters. In extreme cases, clusters can contain more than 100 tRNA genes. Neighboring tRNA genes are typically of different anticodon specificities (Horton et al. 2004). Interestingly, the extent of similarity in the identity and organization of tRNA genes within clusters varies in different sets of related organisms (Bermudez-Santana et al. 2010). In addition to linearly clustering on the genome, tRNAs also cluster in 3D at the nuclear space, as shown in yeasts. This organization may be related to their reversible movement into the nucleolus, thus regulating their expression (Duan et al. 2010, Thompson et al. 2003). tRNA genes can appear as stand-alone transcription units within genomes, but especially in higher eukaryotes tRNA genes may also be contained within other genes, mostly within introns (Sagi et al. 2016). The tRNA can be encoded on the coding strand of the hosting gene or in the opposite orientation. This gene-within-a-gene arrangement raises the possibility, especially in the case in which the two genes are encoded on the same strand, that the tRNA is transcribed through an independent transcription event or with the host gene and is presumably cleaved out of the intron.

Transcription and Epigenetic Regulation of tRNAs

In eukaryotes, tRNAs are transcribed by a special RNA polymerase, RNA Pol III. In all organisms, the promoter of tRNA genes is internal and is composed of two motifs, or boxes, the A-box and the B-box, that are present inside the tRNA gene (Willis 1993). In recent years, it has become evident that tRNA expression levels can vary in response to environmental cues and stresses (Pang et al. 2014), across tissues (Dittmar et al. 2006, Ishimura et al. 2014), during development and aging (Sagi et al. 2016), and in pathologies such as cancer (Gingold et al. 2014, Goodarzi et al. 2016, Pavon-Eternod et al. 2009). Comparing RNA Pol III occupancy in different mouse tissues revealed changes in occupancy at the level of individual tRNA genes (Kutter et al. 2011), and comparing HeLa cells to T cells revealed a remarkable change in RNA Pol III occupancy on different genes (Barski et al. 2010). Yet, at least in the case of mouse tissues, these differences are reduced when tRNA genes are grouped into isoacceptor families (Kutter et al. 2011). Interestingly, there are no known transcription factors whose sole role is in regulating tRNAs, and little is known about transcription factors that regulate both protein-coding genes and tRNAs. Genome-wide data on localization of transcription factors on genomes (Dunham et al. 2012) may shed more light on the potential involvement of transcription factors in regulating tRNA expression. Regulatory motif

analysis suggests that the upstream regions of tRNA genes contain information that affects their transcription, perhaps through transcription factor binding. For example, regulatory motifs were discovered upstream of yeast tRNA genes (Giuliodori et al. 2003, Zhang et al. 2011), and their presence differs in isoacceptor family members that vary in their essentiality (Bloom-Ackermann et al. 2014). The tRNAs that are contained within introns, e.g., in the case of *Caenorhabditis elegans*, tend to show a lower association with RNA Pol III relative to stand-alone tRNA genes (irrespective of the strand in which they are encoded), suggesting potential interference between the host gene's transcription and the tRNA's transcription (Sagi et al. 2016).

In addition to the core transcription mechanism, the chromatin status of tRNA genes can contribute to the regulation of transcription. Similar to epigenetic regulation of protein-coding genes, the same histone marks that are associated with active transcription of RNA Pol II genes are also associated with active transcription of tRNAs, and the same was shown for inhibition-associated marks (Barski et al. 2010, Oler et al. 2010). The epigenetic signature of histone marks upstream of tRNA genes can be modified in development and in cancer; in particular, certain tRNAs that are needed more in certain conditions show enhanced signals of chromatin marks associated with activation (Gingold et al. 2014).

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Processing and Splicing of tRNAs

tRNAs are transcribed in the nucleolus as longer initial transcripts (pre-tRNAs), which then undergo a series of processing events, including trimming, splicing, modification, and addition of a CCA tail. The trimming of the 5′ leader sequence and the 3′ trailer occurs via endo- and exonucleolytic cleavages (Phizicky & Hopper 2010). Some tRNAs contain introns; e.g., in *S. cerevisiae* 22% of the tRNA genes contain an intron, and notably, in mouse and human this percentage is just 5%. Another interesting difference is the cellular location in which splicing occurs: While in vertebrates, splicing occurs in the nucleus, in yeast, tRNA splicing takes place on the outer membrane of the mitochondria (Chatterjee et al. 2018). In addition to the different tRNA processing steps that are mediated by nucleases, the fate of the pre-tRNA is dependent on the binding of the La protein, which is the first protein that recognizes the pre-tRNA molecule immediately upon transcription (Stefano 1984). This binding event facilitates the proper folding of the pre-tRNA molecule and affects the tRNA's stability (Copela et al. 2006). Interestingly, the La protein may mediate the expansion of the eukaryotic tRNA gene pool by allowing for the buffering of mutations due to its chaperone activity (Maraia & Arimbasseri 2017). All the above processes have a crucial effect on the shape of the mature tRNA pool, and they can be regulated in different physiological states. Nonetheless, not much is known about such dynamic regulation, and further research is needed to determine how environmental changes can influence this level.

tRNA Modifications and Editing

Another key feature that determines the RtT levels of tRNAs is the rich world of chemical modifications. tRNA molecules are highly modified; on average, 12 of the 72 nucleotide positions can feature a modification (Björk 1995). More than 100 types of modifications are known to occur in tRNAs, effectively increasing the size of the nucleotide alphabet used by tRNAs (Machnicka et al. 2013). The various nucleotide modifications can affect tRNA folding and stability, amino acid loading, and codon-anticodon base pairing of tRNAs. Wobble U, for example, is modified across all organisms and governs the wobble interaction with guanine (G) in the third codon position (Patil et al. 2012, Schaffrath & Leidel 2017). The existence of the modification affects the tRNA structure itself (Copela et al. 2006, Väre et al. 2017). Another chemical change that tRNA bases

can undergo is editing. A common editing event is deamination, which, when it occurs on an adenine (A), converts the base into an inosine (I). A-to-I editing takes place in anticodons of tRNAs and can affect their potential for wobble codon interaction. For example, A-to-I editing on the wobble position of the tRNA-Arg^{ACG}, the only tRNA editing known in the bacterial world, facilitates wobble interaction of arginine codons (Wolf et al. 2002). In eukaryotes, A-to-I editing of tRNAs is more prevalent and may occur in other tRNA types and in other positions along the molecules (Su & Randau 2011). Modifications on the wobble position can both broaden and restrict codon-anticodon interactions and may therefore be involved in evolutionary shaping of the tRNA pool, such as in the case of Mollicutes. In these parasitic bacteria, which are under strong selection to minimize their genome, eliminating the editing on tRNA-Arg^{ACG} increased the wobble potential of this tRNA type, allowing it to decode all four CGN arginine codons. This in turn resulted in elimination of the tRNA-Arg^{CCG} from the genome of this bacterial clade (Yokobori et al. 2013). Similar to all factors that affect the RtT tRNAs, modification and editing patterns are dynamic in that they can change across physiological conditions (Damon et al. 2015, Endres et al. 2015, Gu et al. 2014), in cancer (for review, see Close et al. 2018, Rapino et al. 2017), and during development (Zaborske et al. 2014).

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Aminoacylation of tRNAs

A key step in executing the genetic code is the proper loading of tRNAs with their cognate amino acids. Similar to all other steps in tRNA biology, loading levels respond to stimuli from the environment (Subramaniam et al. 2013, Zhou et al. 2009b). There are 20 aaRSs, one for each amino acid type. Thus, the various isoacceptor tRNAs compete with each other on the same aaRS. Notably, the loading efficiency of different isoacceptors by a particular aaRS is not identical, leading to some tRNAs being more highly loaded than others (Dittmar et al. 2005, Evans et al. 2017). Selective charging differs depending on physiological conditions. In particular, a model predicts that, upon amino acid starvation, loading patterns change, featuring a redistribution of loading among the various tRNAs for the same amino acid. This redistribution was predicted to result in high loading for tRNAs that recognize rare codons (Elf et al. 2003). Current methods that measure the amino acid charging status of tRNAs support the theory of redistribution of charging upon amino acid starvation, which in turn affects the translation efficiency of genes containing the corresponding codons (Dittmar et al. 2005, Subramaniam et al. 2013). In addition to the global loading status of tRNAs, translation efficiency was also suggested (but see Gingold & Pilpel 2011, Hussmann & Press 2014) to depend on the local availability of charged tRNAs. Immediately upon exit from the ribosome, tRNAs may be recycled by recharging with amino acid. In support of these theories, aaRSs have been shown to interact with the ribosome (Godinic-Mikulcic et al. 2014, Kaminska et al. 2009). The recycling theory generates the specific prediction that in cases in which the same amino acid is encoded twice in close proximity within a gene, it is encoded by the same codon. The synthetic gene approach provided some support for this prediction (Cannarrozzi et al. 2010).

We now discuss two processes that reduce the concentration of RtT tRNAs in the cell. The first is the participation of tRNAs in translation, in which they give away their loaded amino acid, and the second is tRNA degradation and cleavage.

Demand and Consumption of tRNAs

RtT tRNAs are constantly consumed by ribosomes. The extent to which different tRNAs are utilized is determined by their demand, i.e., the presence of corresponding codons among the

actively translated mRNAs in the cell. Since the expression level of genes is highly variable across tissues and physiological and environmental conditions, the demand for tRNAs also changes dynamically. Furthermore, different sets of genes, expressed in various conditions, exhibit different codon usage. For example, the codon preference of proliferation-related genes differs from that of genes expressed during vertebrate cell differentiation (Gingold et al. 2014), further increasing the dynamics of RtT tRNA demand. Likewise, the demand for the various tRNAs changes dynamically in yeast, when it experiences various environmental changes (Gingold et al. 2012).

While the demand affects the RtT tRNA, the efficiency of translation and even cellular well-being appear to depend on the balance between tRNA availability and usage of the actively translated codon, i.e., the supply-to-demand ratio. In unicellular organisms, these two factors are highly correlated. In general, codon demand, as determined by the choice of codons in the genome, correlates with tRNA gene copy numbers and with tRNA level measurements (Dong et al. 1996, Percudani et al. 1997). Furthermore, in higher eukaryotes such correlations were even found in different tissues and cellular states (Dittmar et al. 2006, Gingold et al. 2014). A significant observation is that the highly expressed genes in every genome feature strong codon usage bias that favors the usage of codons that match the most abundant tRNAs (Kanaya et al. 1999, Sharp & Li 1987). Several processes can explain this correlation. First, the higher translation speed of optimal codons suggests that some proteins enjoy higher expression levels because they use efficiently translated codons (Gardin et al. 2014, Sørensen et al. 1989). In addition, highly expressed genes are also thought to exhibit strong, evolutionarily driven codon optimization relative to less expressed genes because the fitness cost of not optimizing codons is greater. Finally, the presence of nonoptimal codons in highly expressed genes may be selected against since it may hamper protein synthesis of the rest of the proteome. A recent study that used extensive genome engineering in bacteria showed that converting codons of highly expressed genes from optimal to nonoptimal did not appreciably affect the expression of these genes but did affect the expression of other genes in the genome, predominantly genes that often used these nonoptimal codons that were now highly demanded (Frumkin et al. 2018). Regardless of the exact mechanism, such coordination between tRNA supply and demand ensures the efficient production of genes and is thus important for cell functionality. Indeed, evolution has optimized the translation efficiency of different gene sets in various organisms to match the differences in lifestyle and phenotypic divergence between organisms (Botzman & Margalit 2011, Jiang et al. 2008, Man & Pilpel 2007, Zaborske et al. 2014).

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Degradation of tRNAs

tRNA molecules are highly stable due to their rigid tertiary structure and abundant nucleotide modifications. Nonetheless, those molecules can be degraded by separate pathways that target either normal or malfunctioning tRNA molecules (reviewed in Hopper et al. 2010). The turnover process of normally structured and modified tRNAs provides cells with the means to regulate the tRNA pool and protein translation in response to stress (Thompson & Parker 2009). The key example of dynamic cleavage of tRNAs is angiogenin-mediated cleavage. Angiogenin is an RNase A protein that induces cleavage of all tRNA molecules by targeting the anticodon loop (Fu et al. 2009, Saxena et al. 1992). Several studies have shown that angiogenin-mediated cleavage is a dynamic process that can be enhanced in response to amino acid starvation and oxidative stress in eukaryotes ranging from yeast to human (Fu et al. 2009, Lee & Collins 2005, Thompson & Parker 2009, Yamasaki et al. 2009). The cleavage can be regulated by a specific modification that can protect or target the tRNA for cleavage (Blanco et al. 2014, Schaefer et al. 2010). The

cleavage products, i.e., tRNA halves, have the potential to have additional cellular roles such as serving as inhibitory RNAs or sequestering tRNA processing enzymes and the translation machinery (Ivanov et al. 2011, Telonis et al. 2016, Yamasaki et al. 2009). Surprisingly, additional stress-induced inhibition of tRNAs by angiogenin is achieved by a different type of cleavage, of the 3′ CCA of all tRNA molecules. This general deactivation-under-stress pathway can be rapidly reversed upon release from the stressing condition by a readdition of the CCA tail by the tRNA nucleotidyltransferase (Czech et al. 2013).

In addition to degradation of proper tRNAs, pathways exist for the degradation of damaged tRNAs, providing cells with a quality control mechanism that can ensure suitable protein translation. Rapid tRNA decay is a key tRNA degradation pathway for damaged tRNA (Alexandrov et al. 2006, Chernyakov et al. 2008, Kadaba et al. 2004, Whipple et al. 2011). The selective degradation of malfunctioning tRNAs is achieved by discriminative binding of key factors such as EF1A and aaRSs. This is a degradation-by-default mechanism in which the tRNA binding proteins protect proper tRNAs from degradation by this pathway, exposing the improper ones (Turowski et al. 2012).

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tRNA-Derived Molecules

It is now becoming evident that, in addition to the RtT and premature tRNA molecules discussed above, most organisms contain additional types of molecules and genes similar but not identical to functional tRNAs.

tRNA fragments. Sequencing of small RNAs often reveals fragments of a few dozens of nucleotides that map to tRNA gene sequences. There are at least three potential sources of such fragments. First, genomes often contain short loci (typically shorter than 20 nucleotides) with sequences that match parts of full-length tRNA genes. For example, the human genome contains more than 10,000 such segments, approximately 2,000 of which map to the 3′ end of mature tRNAs. Thus, some fragments of tRNA molecules may represent transcription products emanating from such loci (Pliatsika et al. 2018). Interestingly, the fragment that matches the 3′ end of the tRNA end with the CCA nucleotide triplet is added posttranscriptionally in mature tRNA but, for tRNA fragments, is hard-coded in the genomic sequence (Telonis et al. 2016). How might such loci appear in genomes? Aside from the nonnegligible possibility of mere chance appearance of such short fragments, such loci may have been added to the genome as a consequence of transcription followed by reverse transcription of tRNA genes. The appearance of a CCA at these fragments' 3′ ends certainly supports this scenario. In further support of this hypothesis, some tRNAs served as a primer for the reverse transcription of retroelements [for example, in the case of Ty1 in yeast (Chapman et al. 1992)] and retroviruses [such as HIV (Kleiman 2002)], demonstrating the potential for this sequence to be reintegrated into the genome.

The second potential source of tRNA fragments is cleavage products of mature tRNAs. The result of a cleavage event could be two parts of the molecule, of which the one that is detected by sequencing is the more stable. One common type of tRNA fragment that likely results from such cleavage is roughly half the length of a mature tRNA molecule. The tRNA halves represent cleavage that occurs around the anticodon loop. Such cleavage events are mediated by the enzyme angiogenin (see section titled Degradation of tRNAs). Nonetheless, additional types of cleavage events of both mature and pre-tRNAs can result in fragments of varying sizes (Kumar et al. 2014, 2015).

The third potential source of tRNA fragments appearing in RNA sequencing involves a technical explanation. The fragments may represent artifacts resulting from drop-off of the reverse

transcription enzyme during the preparation of RNA for sequencing. Yet, as such drop-offs are prone to happen at modified tRNA bases, the fragmentation could be informative in mapping certain modifications (see **Supplemental Text**). While some of the fragments detected in sequencing may be due to technical artifacts, recent research has clarified that many of the tRNA fragments detected by deep sequencing result from real tRNA fragments within cells. The existence of tRNA fragments suggests new exciting possibilities for biological function. tRNA fragments can be considered tRNA-derived microRNA and were shown to be involved in cellular processes such as gene regulation by the RNAi machinery (Burroughs et al. 2011, Loss-Morais et al. 2013, Maute et al. 2013) and to be associated with cancer progression (Goodarzi et al. 2015, Kumar et al. 2015, Maute et al. 2013, Pliatsika et al. 2018). Another exciting role for tRNA fragments relates to retrotransposon inhibition (Martinez 2017, Schorn et al. 2017). Lastly, tRNA halves are involved in spermatogenesis and may contribute to epigenetic inheritance (Chen et al. 2016, Sharma et al. 2016).

Supplemental Material >

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Pseudo-tRNAs. When tRNA detection algorithms [such as tRNAscan (Lowe & Eddy 1997)] are used to identify all tRNA genes in a particular genome, these algorithms often detect, in addition to high-scoring tRNA genes, genomic segments with borderline scores. For example, the tRNAscan algorithm (Lowe & Eddy 1997), when run on the human genome, will detect a couple hundred such segments whose tRNA score is certainly above that of the genome's background but is not quite as high as that of bona fide tRNA genes. Such segments resemble tRNAs but fall short of the canonical signature. Indeed, such tRNA look-alikes are sometimes transcribed as detected by tRNA deep sequencing, but their expression levels are often significantly lower than those of high-scoring tRNA genes (N. Hefetz, I. Frumkin, O. Dahan, R. Rak & Y. Pilpel, in preparation).

A recent analysis of extensive mutagenesis of a tRNA gene in yeast has indicated that, beyond a couple of mutations, the mutated tRNA molecule is typically nonfunctional (Li et al. 2016). Thus, it is expected that the borderline scoring look-alikes are not functional tRNAs and may well represent pseudo-tRNAs. These pseudogenes may have originated from evolutionary decay of previously functional tRNAs, but they may represent tRNAs to be, i.e., segments that in the evolutionary future may turn into (or return to being) functional tRNAs when accumulating the suitable mutations. An intriguing possibility, analogous to one raised about other types of pseudogenes (Poliseno et al. 2010), is that these pseudo-tRNAs, even in their current status, may exert physiological effects on the cell. Transcribed tRNA pseudogenes could affect other tRNAs, e.g., by competing with them on shared resources such as aaRSs. It will thus be interesting to include such pseudo-tRNAs in genome-wide analyses of the tRNA pool.

DIVERSE CELLULAR EFFECTS OF THE tRNA POOL

Above we review the diverse means by which the shape of the tRNA pool in cells is determined. We survey evolutionary processes that shape genomes' tRNA pools and many regulatory physiological processes that govern tRNA transcription, tRNA modification and processing, amino acid loading, tRNA consumption, and tRNA degradation. These processes ultimately result in the absolute and relative amounts of RtT tRNAs of each type that can be dynamically regulated in response to external and internal cues. The tRNA pool is now ready to translate mRNAs into proteins, and in doing so it has fundamental effects on the proteome and cellular physiology.

Protein Abundance

One key factor that is dependent on RtT tRNAs is the translation elongation rate, which affects the abundance of proteins in the cell. It is generally assumed that frequently used codons, which usually

represent highly abundant matching tRNAs, are translated at a faster rate (Gardin et al. 2014, Gorochowski et al. 2015, Varenne et al. 1984). This idea that codons that correspond to highly abundant tRNAs are translated rapidly was recently discussed in view of ribosome profiling data (reviewed in Hanson & Coller 2017). In particular, ribosome profiling data reveal that ribosomes more rapidly translate codons that correspond to abundant tRNAs (Weinberg et al. 2016).

Across many genomes analyzed, a good correlation is typically seen between the tAI of a gene and its protein level (Botzman & Margalit 2011, Jiang et al. 2008, Krisko et al. 2014, Lithwick & Margalit 2003, Man & Pilpel 2007). While this correlation could reflect a direct effect of the tRNA availability on expression level, it could also arise from an evolutionary effect. Highly expressed genes (i.e., genes that owe their high expression to intense transcription) may have been selected for evolutionarily to optimize their codons' adaptation to the highly abundant tRNAs because lack of such adaptation in these genes would have been detrimental for the cell. In support of the evolutionary cause for this correlation is the positive correlation observed between mRNA levels and tAI (see section titled mRNA Degradation: Slowness-Mediated Decay).

In the more artificial and technological context, heterologous proteins that are transformed into a foreign genome are typically reencoded using codons that correspond to the abundant tRNAs of the host cell. Numerous studies on codon optimization of such proteins revealed that such codon optimization is essential for achieving high protein expression (Burgess-Brown et al. 2008, Gustafsson et al. 2004). Recent research has made clear that the effect of translation elongation efficiency as determined by the interplay between codon choice and RtT tRNA availability is more complicated than simply choosing optimal codons. It was demonstrated that codon choice can provide cells with a rich set of options to control protein levels. An example of the effect of codon choice on protein expression is the observation that, in subsequent occurrences of the same amino acids, genes tend to deliberately use codons that are translated by the same tRNA type. Such codon arrangement was hypothesized to be an efficient means to recycle tRNAs and was shown to increase the translation of reporter proteins (Cannarrozzi et al. 2010).

The choice between synonymous codons and the interplay between codons and RtT tRNA availability can be a means to dynamically regulate protein abundance in response to various environmental and physiological cues. For example, under starvation for amino acids, differential loading of tRNAs coding for the same amino acid results in altered expression of proteins enriched for the corresponding codon (Subramaniam et al. 2013). The challenge of optimizing codons to ensure proper protein abundance is more pronounced in multicellular organisms since the same genes may have to reach different protein levels in different tissues and developmental stages. Codon usage of genes is adapted to the RtT tRNA pool of the corresponding tissues in which they are expressed (Dittmar et al. 2006). Changes in cellular status, for example, when proliferating cells differentiate, reflect a coordinated change in both the tRNA pool and the codon bias of the expressed genes (Gingold et al. 2014). This balance between tRNA supply and demand may be a mechanism to ensure proper protein expression in the corresponding tissue or cellular status. Furthermore, differential codon bias for proteins involved in proliferation or differentiation, representing a dual translation program, may be a safety mechanism to control cellular status. The ability to translate a protein with codon bias that does not match the tRNA pool is reduced, and hence genes that do not match the current translation program of the cell will not be translated properly when not needed. Indeed, disturbing this balance may have physiological outcomes. Upregulating specific tRNAs promotes metastatic progression by enhancing expression of genes enriched with the matching codons (Goodarzi et al. 2016). Why are certain tRNAs induced in, and why do they promote, metastasis? Are the genes needed for metastasis enriched with particular codons that correspond to such tRNAs? And if so, what is the nonmetastatic, i.e., normal, function of these genes? It would be interesting to see whether a metastatic tumor's

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upregulation of a selection of tRNAs mimics a normal process, e.g., a cell-migratory program that might be naturally at work in organ development.

Another interesting arena that illustrates the interplay between tRNA supply and demand ratio and protein abundance is the case of virus protein expression. The dependency of virus protein production on the tRNA pool of the host cell is crucial for the proper life cycle of viruses. In many cases, the codon usage of viral genes is adapted to the specific organism/tissue that the viruses infected (Haas et al. 1996, Wong et al. 2010, Zhou et al. 1999). Furthermore, a possible antiviral mechanism of the infected cell is to discriminate against tRNAs that are needed for the virus. For example, an interferon-regulated gene in T cells, *schlafen 11*, binds tRNAs that are required for the production of HIV proteins (Li et al. 2012).

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Translation Fidelity

In addition to affecting protein abundance, the RtT tRNA pool can influence proteome integrity through its effect on translation fidelity, i.e., the accurate incorporation of the encoded amino acids. The rate of misincorporation of amino acids is estimated to be in the order of 10^{-4}–10^{-3} per codon (Bouadloun et al. 1983, Kurland & Ehrenberg 1987, Parker 1989). This rate represents both mispairing (errors caused by misincorporation of the wrong tRNA by the ribosome) and mischarging (noncognate charging by the aaRS, i.e., attaching the wrong amino acid on a tRNA). The ability to synthesize proteins with high accuracy is of great importance. Reduced viability (Nangle et al. 2002) and human diseases such as neurodegenerative pathologies (reviewed recently in Kapur et al. 2017, Mohler & Ibba 2017) are related to compromised synthesis. Remarkably, a recent work found that the rate of translation fidelity in long-lived rodents was higher than that observed in short-lived ones (Azpurua et al. 2013, Ke et al. 2017), implying that translation fidelity plays a role in aging.

Increasing translation error rate can also be beneficial. In all domains of life, methionine aaRS can misload other tRNAs and can lead to misincorporation of methionine in nonmethionine codons (Jones et al. 2011, Netzer et al. 2009, Wang & Pan 2015, Wiltrout et al. 2012). Such an increase in methionine content in the proteome can mitigate the damaging effect of oxidative stress when ROS content is high (Luo & Levine 2009). Indeed, the level of methionine misincorporation is elevated in response to cellular oxidative stress (Netzer et al. 2009). Another example of regulated incorporation of an alternative amino acid is the usage of codon ambiguity in certain yeasts such as *Candida albicans*. These organisms reassigned the CUG codon from leucine (Leu) to serine (Ser) by using a unique tRNA-Ser^{CAG} that contains a Leu CAG anticodon that is aminoacylated by the seryl-tRNA synthetase (Santos & Tuite 1995). Under standard growth conditions, 3% of Leu and 97% Ser are incorporated at CUG sites on a proteome-wide scale (Gomes et al. 2007). However, alteration of the relative levels of CUG-decoding tRNAs can shift the ratio between incorporated Ser and Leu in the proteome, thus generating phenotypic diversity and resulting in increased fitness under various growth conditions (Bezerra et al. 2013, Simões et al. 2016). Translation mistakes can also be used to suppress deleterious mutations. For example, a mutation in an essential yeast gene that results in a codon change can be suppressed by a tRNA mutation that alters the loading of an amino acid on that tRNA, thereby restoring the function of the mutated protein (Hoffman et al. 2016). Finally, elevated mistranslation can be a general way to increase diversity in the proteome when such increased diversity is needed, and it can be used to purge genetically encoded mutations (Bratulic et al. 2017, Ribas de Pouplana et al. 2014).

A delicate balance between ensuring high fidelity and allowing for translation plasticity might be achieved by modulation of the relative levels of various tRNAs. Assuring high accuracy is very costly, and there is a trade-off between protein quality and quantity (Conn & Qian 2013, Johansson

et al. 2012). A theoretical model, supported by experimental data, suggests an interesting trade-off between translation speed and accuracy that could be governed by tRNA supply: On one hand, high tRNA levels can accelerate translation and support higher protein levels, but on the other hand, fidelity results at least in part from proofreading by the ribosome, which requires slow translation at critical positions. Highly expressed genes thus confront the conflict of high speed for high expression and low speed for accuracy, since translation mistakes in highly expressed genes might be very detrimental. For highly expressed genes, the model predicts reduced elongation speed in favor of fidelity (Johansson et al. 2012).

From a tRNA-centric point of view, translation fidelity can be altered by several mechanisms: aaRSs need to recognize with high accuracy both the correct tRNA and the correct amino acid. The precise selection of tRNA by these enzymes is based on key positions within the tRNA, both in the anticodon loop and on other regions of the tRNA (Francklyn & Schimmel 1990, Galili et al. 2016, Giegé et al. 1998, McClain et al. 1994). Recognition of the cognate amino acids is based on a double screening mechanism: Amino acids with larger side chains are excluded in the first phase, and amino acids that are too small are disassembled in the second phase (Bacher et al. 2005, Nangle et al. 2002). Given the proofreading function, the error rates of aaRSs in vitro are 10^{-5} to 10^{-4} (Hopfield et al. 1976, Yamane & Hopfield 1977, Zaher & Green 2009). In addition to mistakes in tRNA loading by the synthetase, misincorporation of an amino acid can occur by mispairing by the ribosome, which can incorporate a near-cognate tRNA instead of the correct cognate tRNA. The difference in free energy between cognate and near-cognate interaction is low, and the predicted error ratio at chemical equilibrium is approximately 1:100 misincorporation events. The much lower translation error rate in vivo results from additional, energy-consuming proofreading processes (reviewed in Gromadski & Rodnina 2004, Rodnina & Wintermeyer 2001).

Although the overall rate of translation error ranges between 10^{-4} and 10^{-3} per amino acid, different positions along a gene and different codon choices can modulate the error rate even within a gene (Dix & Thompson 1989, Mordret et al. 2018, Thomas et al. 1988). Furthermore, the error rate of a specific codon as well as the type of amino acid substitution can be dynamically altered in response to environmental conditions such as temperature, the presence of antibiotics, and amino acid starvation, as well as mutations in the ribosome (Kramer & Farabaugh 2006, Meyerovich et al. 2010, Mordret et al. 2018). Therefore, in addition to the role of translation fidelity in regulating protein abundance, the choice of synonymous codons and hence tRNA type may influence translation fidelity at each position along a gene. In agreement with this notion, critical residues in proteins tend to be coded by preferable codons that presumably manifest higher translation fidelity (Akashi 1994, Drummond & Wilke 2008, Stoletzki & Eyre-Walker 2007), and conserved protein positions tend to have fewer translation mistakes (Mordret et al. 2018). In addition, the balance between the availability of RtT cognate tRNA and near-cognate tRNA may play a major role in regulating the misincorporation of amino acids (Kramer & Farabaugh 2006). Finally, as mentioned above, examining the tRNA pool in various organisms revealed that some anticodons are not represented at all by tRNAs in most genomes. This phenomenon of presumably disallowed tRNA can result from the tendency of such tRNAs to introduce the wrong amino acid to a near-cognate codon. Such disallowance of certain tRNAs is especially seen in the genetic code among the two-codon box codons, in places where two amino acid types share the first two nucleotides of their codons (**Figure 3**). In all these cases, the disallowed tRNA for one in the amino acid pair would have been wobble compatible with a codon of the other amino acid. Thus, in these cases evolution seems to have selected against such potential causes of translational mistakes.

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Protein Folding

In all organisms, folding of proteins happens cotranslationally. Cotranslational folding depends on the rate of translation, which in turn is dictated, as discussed above, by factors such as the availability of RtT tRNAs in the cell and by other determinants such as mRNA secondary structure (for review, see Komar 2009). Can the tRNA pool affect protein folding? Can expression of certain tRNAs affect and even modify the folded structure of the proteins they are translating?

Since data on tRNA availability are only beginning to emerge (see **Supplemental Text**), analysis of codon usage along genes was initially used to approximate the effects of translation speed on folding. Thus, an interesting body of research examined patterns of codon usage, specifically the allocation of optimal and nonoptimal codons, along proteins. In this context, codon optimality is often referred to as the representation in the genome of that codon that was hypothesized to correlate with availability of the corresponding tRNA. In the mid-1990s, researchers observed that rare codons tend to encode the amino acids that reside in the boundaries of protein domains (Thanaraj & Argos 1996). It was hypothesized that such a choice for rare codons can provide a pause in the translation process. Such pauses may promote the proper folding of an already translated domain before the translation of the next one (Purvis et al. 1987). While the statistical significance of the enrichment of rare codons in domain boundaries has been questioned (Chaney et al. 2017), experimental evidence suggests that the presence of rare codons in domain boundaries may facilitate proper protein folding (Hess et al. 2015). In contrast, translationally optimal codons, which would often correspond to abundant tRNAs, are associated with sites in proteins that tend to be buried within domains and to be structurally sensitive to mutations (Zhou et al. 2009a). Changing an abundant codon to a rare one causes protein misfolding. Synonymous mutation in *MDR1* that changed a single codon from an abundant codon to a rare one resulted in a misfolded, inactive protein (Kimchi-Sarfaty et al. 2007). Additionally, disrupting the local rate at which strategic codons are synthesized, by increasing their optimality, disrupts the folding of a particular protein (Zhang et al. 2009). Thus, an emerging theme is that, to promote efficient folding of proteins, optimal codons are needed within domains, while nonoptimal codons are needed in their boundaries or within small structural motifs. Therefore, codon optimality may be under evolutionary selection in cases of both high- and low-optimality codons. Supporting this idea, analysis of closely related yeast species has shown that codon optimality is conserved, suggesting selective pressure for site-specific translational speed (Pechmann & Frydman 2013).

Supplemental Material >

Common to all the above research are that codons and their optimality were analyzed and the effects of their manipulation on translation and folding were measured. Can one directly manipulate the tRNA pool and obtain its effects on folding? An increase or decrease in tRNA expression could increase or decrease, respectively, the optimality of the corresponding codons throughout the genome, but without the undesired side effects of manipulation of the mRNA sequences. Indeed, Zhang et al. (2009) did not merely change the codons themselves in a gene of interest; they also increased the supply of a tRNA that corresponds to a nonoptimal codon and observed a reduction in protein folding in vitro. The importance of low tRNA abundance for global protein folding was further demonstrated in vivo in yeast: Overexpression of a rare tRNA, but not of an abundant one, caused general unfolding stress in the cell (Yona et al. 2013). This experiment, which effectively converted many occurrences of a low-optimality codon into a higher-optimality one without directly mutating any coding gene, showed that the tRNA pool caused protein folding across the proteome. The systematic deletion of most of the tRNA genes in yeast allowed Bloom-Ackermann et al. (2014) to examine effects of each tRNA on cell physiology upon diverse stresses. An expected result was that deletion of most tRNAs had rather mild effects on

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fitness in diverse conditions. More surprising was the observation that most of the deletion mutants showed enhanced growth (relative to wild type) when cells were treated with a reducing agent that caused proteotoxic stress. This result suggests that many tRNA deletion mutants experience a chronic protein unfolding problem prior to drug treatment. This chronic protein unfolding problem invokes the protein folding response, a state that preadapts the mutants to the ensuing proteotoxic drug (Bloom-Ackermann et al. 2014). Another factor that can alter decoding rate and globally affect protein stability is tRNA modifications. For example, inhibition of the wobble U34 modifications in eukaryotes led to ribosome stalling and increased proteotoxic stress (Nedialkova & Leidel 2015). The involvement of tRNAs in ensuring proper protein folding was recently demonstrated in relation to various pathologies. Among the many disease-causing mutations in *CFTR*, the cystic fibrosis–causing gene, one is a synonymous mutation that converts a codon that is recognized by an abundant tRNA into a codon that corresponds to a rare tRNA. Such change in codon identity reduces both translation speed and CFTR protein folding. Strikingly, CFTR misfolding could be reversed by overexpressing the matching tRNA (Kirchner et al. 2017). Another example is a naturally occurring mutation in a tRNA gene that is expressed mainly in the central nervous system in mice and results in ribosome stalling. When combined with loss of a ribosome-recycling protein, GTPBP2, the epistatic effect of both mutations increased ribosome stalling and led to neurodegeneration, presumably due to aberrant protein folding (Ishimura et al. 2014).

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mRNA Degradation: Slowness-Mediated Decay

In recent years, new surprising discoveries have linked translation rate to mRNA stability. On a genome-wide level, stable mRNAs are enriched with optimal codons (Presnyak et al. 2015). This connection between translation efficiency and mRNA stability was experimentally shown decades ago for a handful of reporter genes and endogenic genes (Caponigro et al. 1993, Hoekema et al. 1987), but genome-wide studies now reveal consistent trends and effects. In recent years, additional studies revealed a very similar trend at a genome-wide level in both prokaryotes and eukaryotes (Boël et al. 2016, Harigaya & Parker 2016). These studies also raise the question of the causal effect between codon optimality and mRNA stability. In principle, the correlation could be interpreted to indicate that slow translation causes mRNA degradation, but degradation may also affect ribosome speed, and there may be no direct effect between these two variables; e.g., evolution may have endowed fast translation genes with high mRNA stability. One way to address the question of causality is via perturbations. Indeed, in human cancer cells, mRNA stability responds to changes in the tRNA pool (Goodarzi et al. 2016). An interesting biological role for translation-dependent mRNA degradation was recently shown during maternal-to-zygotic transition in several metazoans. This developmental stage requires the massive degradation of maternally deposited mRNAs. Strikingly, codon composition shapes maternal mRNA clearance, and this selective degradation is dependent on translation (Bazzini et al. 2016, Mishima & Tomari 2016). By measuring mRNA stability of thousands of variants of a reporter gene, which differed in their codon usage, with and without active translation, Bazzini et al. (2016) identified codons that affect mRNA stability upon translation. Reassuringly, codons that stabilized the actively translated transcript corresponded to abundant tRNAs (Bazzini et al. 2016).

Slowness-mediated decay (SMD) is the term that we propose to describe this phenomenon in which mRNAs enriched with low-optimality codons are degraded more rapidly. This SMD term evokes the well-known term nonsense-mediated decay (NMD). Whereas in SMD a slowly elongating codon causes mRNA decay, in NMD a premature stop codon destines the transcript to degradation. A potential means to achieve SMD is the surveillance mechanism that senses

slowly translating ribosomes on mRNA (Shoemaker & Green 2012). The DEAD-box protein Dhh1p is recruited to such slowly translating ribosomes and targets their corresponding mRNAs for degradation (Radhakrishnan et al. 2016).

The connection between translation rate and RNA stability drastically increases the effect of optimal codon usage on gene regulation. Hence, the adaptation between codon choice on mRNAs and the tRNA pool has a dual regulatory role in gene expression, as it appears to determine both translation efficiency and mRNA stability. This coupling between codon optimality and mRNA stability also has important implications for two commonly used calculations of translation efficiency, the tAI and Codon Adaptation Index of genes, which typically show good correlation with protein levels. It is now becoming clearer that the capacity of these two measures to predict protein abundance partially reflects the effect of codon optimality on mRNA levels through an mRNA stabilization effect.

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CONCLUSIONS AND FUTURE PERSPECTIVES

In this review, we discuss the diverse cellular mechanisms that contribute to and are affected by the dynamics of the tRNA pool that can range across different timescales. The longest timescale is the evolutionary level, which is manifested by different tRNA gene compositions between different organisms, while the shortest timescale is cellular response to environmental cues, which is manifested by rapid changes in tRNA levels. We focus above on several transcriptional and posttranscriptional regulatory processes that govern the availability and composition of RtT tRNA molecules in cells. tRNA composition, in turn, affects protein expression level, translation accuracy, protein folding, and mRNA stability.

In recent years, new technologies have allowed researchers to determine the dynamic expression and modification patterns of tRNAs (see **Supplemental Text**). Using those new developments, we are now entering an era in which we can quantify with high precision the tRNA pool in many cell types and organisms and across different timescales and conditions. Further expanding our knowledge regarding the dynamics of the tRNA pool will have a profound impact on our understanding of how translation processes can be regulated in normal organismal life and in diseases.

Supplemental Material >

DISCLOSURE STATEMENT

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