Modulating Mistranslation Potential of tRNA\textsuperscript{Ser} in \textit{Saccharomyces cerevisiae}

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ABSTRACT Transfer RNAs (tRNAs) read the genetic code, translating nucleic acid sequence into protein. For tRNA\textsuperscript{Ser} the anticodon does not specify its aminoacylation. For this reason, mutations in the tRNA\textsuperscript{Ser} anticodon can result in amino acid substitutions, a process called mistranslation. Previously, we found that tRNA\textsuperscript{Ser} with a proline anticodon was lethal to cells. However, by incorporating secondary mutations into the tRNA, mistranslation was dampened to a nonlethal level. The goal of this work was to identify second-site substitutions in tRNA\textsuperscript{Ser} that modulate mistranslation to different levels. Targeted changes to putative identity elements led to total loss of tRNA function or significantly impaired cell growth. However, through genetic selection, we identified 22 substitutions that allow nontoxic mistranslation. These secondary mutations are primarily in single-stranded regions or substitute G:U base pairs for Watson–Crick pairs. Many of the variants are more toxic at low temperature and upon impairing the rapid tRNA decay pathway. We suggest that the majority of the secondary mutations affect the stability of the tRNA in cells. The temperature sensitivity of the tRNAs allows conditional mistranslation. Proteomic analysis demonstrated that tRNA\textsuperscript{Ser} variants mistranslate to different extents with diminished growth correlating with increased mistranslation. When combined with a secondary mutation, other anticodon substitutions allow serine mistranslation at additional nonserine codons. These mistranslating tRNAs have applications in synthetic biology, by creating “statistical proteins,” which may display a wider range of activities or substrate specificities than the homogenous form.

KEYWORDS serine tRNA; tRNA modifications; mistranslation; synthetic biology; statistical proteins

MISTRANSLATION occurs when an amino acid that differs from that specified by the “standard” genetic code is incorporated into nascent proteins during translation. Although considered less frequently than protein modification, mistranslation plays a significant role in generating protein diversity. Mistranslation naturally occurs at a frequency of \(~1\) in every \(10^4\)–\(10^5\) codons (Kramer and Farabaugh 2007; Drummond and Wilke 2009), with some conditions increasing this frequency (Santos et al. 1999; Bacher et al. 2007; Javid et al. 2014). Woese (1965) predicted that mistranslation was substantially greater during the evolution of the translational machinery, creating diversity that would allow proteins to probe phase-space. Mistranslation is also used in several systems as an adaptive response (Ling and Söll 2010; Moghal et al. 2014; Wu et al. 2014; Wang and Pan 2016). For example, in response to oxidative stress, \textit{Escherichia coli}, yeast, and mammalian cells mistranslate methionine into proteins to sequester reactive oxygen species and protect cells from oxidative damage (Netzer et al. 2009; Wiltrout et al. 2012; Lee et al. 2014; Gomes et al. 2016; Schwartz and Pan 2017). In the archaean \textit{Aeropyrum pernix}, transfer RNA (tRNA)\textsuperscript{Leu} is misaminoacylated by methionyl-tRNA synthetase at low temperatures, which enhances enzyme activity (Schwartz and Pan 2016). \textit{Mycoplasma} species use editing-defective synthetases to generate diversity and escape the
host defense systems (Li et al. 2011). In other cases, mistranslation results in nearly complete codon reassignment. Yeasts of the Candida genus naturally evolved a tRNA\textsuperscript{Ser} variant that ambiguously decodes the leucine CUG codon mainly as serine (Massey et al. 2003; Paredes et al. 2012).

The first specificity step of translation is aminocaylation of a tRNA by its corresponding aminocayl-tRNA synthetase [aaRS; reviewed in Pang et al. (2014)]. Each aaRS recognizes its cognate tRNAs from a pool of tRNAs with similar structure using structural elements and nucleotides within the tRNA called identity elements (Rich and RajBhandary 1976; de Duve 1988; Giegé et al. 1998). For many tRNA-aaRS interactions, the specificity is determined in large part by the anticodon. In yeast the exceptions to this are tRNA\textsuperscript{Ser} and tRNA\textsuperscript{Ala} (Giegé et al. 1998). The major identity elements for tRNA\textsuperscript{Ser} and tRNA\textsuperscript{Ala} are the variable arm, positioned 3’ to the anticodon stem, and a G3:U70 base pair, respectively. Because of the latter, inserting a G3:U70 base pair into other tRNAs results in misaminoacylation with alanine (McClain et al. 1998). For many tRNA-aaRS interactions, the specificity is determined in large part by the anticodon. In yeast the exceptions to this are tRNA\textsuperscript{Ser} and tRNA\textsuperscript{Ala} (Giegé et al. 1998). The major identity elements for tRNA\textsuperscript{Ser} and tRNA\textsuperscript{Ala} are the variable arm, positioned 3’ to the anticodon stem, and a G3:U70 base pair, respectively. Because of the latter, inserting a G3:U70 base pair into other tRNAs results in misaminoacylation with alanine (McClain et al. 1998). For many tRNA-aaRS interactions, the specificity is determined in large part by the anticodon. In yeast the exceptions to this are tRNA\textsuperscript{Ser} and tRNA\textsuperscript{Ala} (Giegé et al. 1998).

Mistranslation has applications in synthetic biology. tRNAs that misincorporate amino acids expand the diversity of expressed proteins, resulting in what Woese described as “statistical proteins” [Woese 1965; reviewed in Schimmel (2011)]. Statistical proteins have the potential to display a wider range of activities or substrate specificities than the homogeneous form. For example, generating antibodies that are heterogeneous mixtures, with each molecule containing one or two amino acid variants, could expand antigen recognition and be valuable for rapidly evolving antigens. Although tolerated and sometimes beneficial to cells, too much mistranslation can be lethal (Berg et al. 2017). Therefore, for mistranslation to have biological applications the activity of the mistranslating tRNA must be tuned so that it is below a toxicity threshold. Zimmerman et al. (2018) exploited the rapid tRNA decay (RTD) pathway to control tRNA levels in yeast. In this approach, mistranslating tRNAs are mutated to become substrates of the RTD pathway by destabilizing the acceptor stem (Whipple et al. 2011). The RTD pathway is controlled using an inducible \textit{MET22} gene, where repression of \textit{MET22} inhibits the RTD pathway and induces mistranslation by the mutant tRNA. Although effective, this approach also influences the levels of endogenous tRNAs.

Regulating other steps along the tRNA pathway allows the levels and/or activity of a tRNA to be controlled. These include steps during biosynthesis (e.g., transcription, processing, 3’ CCA addition, and splicing), nuclear export, aminocaylation, and interaction with the translational machinery (Nissen et al. 1996; Dreher et al. 1999; Phizicky and Hopper 2010; Hopper 2013). tRNAs are also extensively modified in both the nucleus and cytoplasm (Jackman and Alfonzo 2013). The modified bases are identity elements for aaRS enzymes (Giegé et al. 1998), regulate codon-anticodon pairing (Gustilo et al. 2008; Wei et al. 2011), maintain the reading frame during decoding (Urbonavičius et al. 2001), and regulate the tRNA structure (Lorenz et al. 2017). The final aspect of tRNA regulation is their degradation through either the RTD pathway mentioned above, which degrades hypomodified and unstable tRNAs (Chernyakov et al. 2008; Whipple et al. 2011), or the nuclear exosome, which monitors tRNA modifications and 3’ end maturation (Kadaba et al. 2004; Schneider et al. 2007; Schmid and Jensen 2008).

Because of their toxicity (Berg et al. 2017), the applications of mistranslating tRNAs to research and biotechnology requires that their activity be regulated. The goal of this work was to identify a range of base changes in tRNA\textsuperscript{Ser} that would dampen tRNA function and fine-tune the extent of mistranslation for use in different applications and with a number of anticodon substitutions. Using a genetic suppression system that requires a proline codon be mistranslated as serine, we selected mistranslating tRNA\textsuperscript{Ser}_{UGG} variants with a range of activities after random mutagenesis. Many of these had increased toxicity at low temperature and upon inhibiting the RTD pathway, suggesting that they destabilize the tRNA, and enabling temperature-sensitive induction of mistranslation. Through targeted changes to predicted identity elements, we also identified substitutions in the acceptor stem and discriminator base that diminish lethality and allow mistranslation. Proteomic analysis demonstrated that tRNA\textsuperscript{Ser} variants mistranslate to different extents with diminished growth correlating with increased mistranslation. Thus, by altering nucleotides in tRNA\textsuperscript{Ser} it is possible to decrease tRNA\textsuperscript{Ser} function and mistranslate with various efficiencies. In addition, we demonstrate that in combination with the correct secondary mutation, the anticodon of the tRNA\textsuperscript{Ser} can be mutated to mistranslate arginine, glutamine, phenylalanine, and ochre stop codons, expanding the mistranslation potential of this tRNA.

Materials and Methods

Yeast strains and growth

All yeast strains are derivatives of the wild-type haploid strains BY4741 and BY4742 (Supplemental Material, Table S1; Winzeler and Davis 1997). The \textit{tti2} disruption strains covered by either \textit{TTI2} (CY6963), \textit{tti2-L187P} (CY7020), or \textit{tti2-Q276TAA} (CY6874) on a centromeric plasmid have been described (Hoffman et al. 2016). The \textit{tti2} disruption strain with \textit{tti2-L187R} and tRNA\textsuperscript{Ser}_{UCU}-G26A was made by transforming \textit{tti2-L187R} on an \textit{LEU2} centromeric plasmid into CY6963 along with \textit{sup17(UUC)-G26A on an HIS3 centromeric plasmid. The wild-type \textit{TTI2} on a \textit{URA3} plasmid was lost by counter selection on 5-fluoroorotic acid to generate CY8150. The \textit{met22A} strain (CY8588) and its isogenic \textit{MET22} control
(CY8589) were derived from a spore colony of the magic marker strain in the BY4743 diploid background (Tong et al. 2001).

Yeast strains were grown in yeast peptone media containing 2% glucose or synthetic media supplemented with nitrogenous bases and amino acids at 30°C unless otherwise indicated. For spot assays on plates, strains were grown to saturation in selective medium, OD600 was normalized, and cultures were spotted in 10-fold serial dilutions. To quantitate growth on solid media, cells were plated after dilution to obtain single colonies. Colony size was measured using ImageJ (v1.52h; Schneider et al. 2012). Growth curves were generated by diluting saturated cultures to OD600 ~0.1 in minimal media and incubating at 30°C. OD600 was measured every 15 min using a BioTek Epoch 2 microplate spectrophotometer for 24 hr. Doubling time was calculated using the R package “growthcurver” (Sproufsky and Wagner 2016).

**Plasmid constructs**

**SUP17** (pCB3076) and **sup17(UGG)** (pCB3082) expressed in YCplac33 have been previously described (Berg et al. 2017). Derivatives of YCplac33-sup17(UGG) with mutations at G9A (pCB4020), A20bG (pCB4021), G26A (pCB4023), and C40T (pCB4022) were obtained previously (Berg et al. 2017), whereas A4G (pCB4097), C7ST (pCB4080), C12T (pCB4102), G15A (pCB4106), G18A (pCB4114), T20C (pCB4088), A29G (pCB4087), T33G (pCB4084), A38C (pCB4081), T39G (pCB4093), T44G (pCB4074), G45A (pCB4075), Ge23A (pCB4090), C21T (pCB4072), C48T (pCB4079), T51C (pCB4101), A59G (pCB4098), and T60G (pCB4089) were obtained through genetic selection.

sup17(UGG)-VAΔ was engineered using two-step mutagenic PCR with primers UG5953/VL5002 and UG5954/VL5003, and template pCB3082. The product was used as template to introduce the T69A mutation using inside primers WF1165/WF1164 and template pCB3082. The final product was used as template to introduce the T69A mutation using inside primers WF1165/WF1166. The product was cloned into YCplac33 as an HindIII/EcoRI fragment to give pCB4333.

**sup17(UCU)** and **sup17(UCU)-G9A** were made by two-step mutagenic PCR with outside primers UG5953/U5954 and inside primers VJ2409/VJ2410 from template pCB3082 or pCB4020, respectively. The products were cloned as EcoRI fragments into pRS303 (Sikorski and Hieter 1989) after first subcloning into pGEM-Teasy (Promega), giving pCB4215 or pCB4216, respectively. sup17(UCU)-G9A was also cloned into YCplac33 to give pCB4120. sup17(UCU)-G26A was similarly made using template pCB3082, and inside primers VL4943/VL4945 giving pCB4257. The following derivatives were made by two-step PCR using outside primers UG5953 and UG5954. Final products were cloned into YCplac33 as an EcoRI fragment after first subcloning into pGEM-Teasy (Promega). Template, inside primers, and construct are in parentheses: sup17(UUA) (pCB3082, VK4595/VK4596, pCB4235), sup17(UUA)-G9A (pCB4020, VK4595/VK4596, pCB4236), sup17(UUG)-G26A (pCB4023, VF7765/VF7766, pCB4311), and sup17(GAA)-G26A (pCB3082, WC8504/WC8505, pCB4358).

TTI2, ttt2-L187P, and ttt2-Q276TAA constructs have been described (Hoffman et al. 2016). The ttt2-L187R construct was made by two-step PCR using outside primers 5693-1/5693-2, inside primers 6856-1/6856-2, and template pCB2134. The product was cloned into the wild-type DED1pr-TTI2 vector as a NotI-Sacl fragment to give pCB2865.

The centromeric plasmid containing HSE-eGFP was kindly provided by Onn Brandman (Stanford University) (Brandman et al. 2012). The URA3 marker on the plasmid was switched to HIS3 using pUH7 (Cross 1997).

**Selection of variants of sup17(UGG) that suppress ttt2-L187P**

Selection of mutant sup17(UGG) alleles that support viability and suppress ttt2-L187P was performed as previously described (Berg et al. 2017). Briefly, YCplac33-sup17(UGG) were UV-irradiated and transformed into CY7020. Ura+ transformants were screened for growth on YPD containing 5% ethanol. The YCplac33 plasmids were then isolated, sequenced, and transformed back into CY7020 to analyze growth.

**Fluorescence heat shock reporter**

Yeast strains containing the heat shock response element (HSE)-eGFP reporter and a YCplac33-sup17(UGG) allele were grown to stationary phase in medium lacking histidine and uracil, diluted 1:20 in the same medium and grown for 6 hr
at 30°C. Cell densities were normalized to OD_{600} before measuring fluorescence. Fluorescence was measured with a BioTek Synergy H1 microplate reader at an emission wavelength of 528 nm using Gen5 2.08 software.

**Mistranslation quantification using mass spectrometry**

Starter cultures of yeast strains containing YCplac33-sup17(UGG) variants were grown to stationary phase in medium lacking uracil, diluted 1:20 in 10 ml of the same medium and grown for 19 hr at 30°C. Cell pellets from the resulting 10 ml of yeast culture were resuspended in a denaturing lysis buffer (8 M urea, 50 mM Tris, pH 8.2, 75 mM NaCl). Cells were lysed by bead-beating with 0.5 mm glass beads at 4°C. Lysates were cleared by centrifugation at 21,000 × g for 10 min at 4°C and protein concentration was determined by BCA assay (Pierce, Thermo Fisher Scientific). Proteins were reduced with 5 mM dithiothreitol for 30 min at 55°C, alkylated with 15 mM iodoacetamide for 30 min at room temperature, and the alkylation was quenched with additional 5 mM dithiothreitol for 15 min at room temperature. For each sample, 100 μg of protein was diluted 1:2 with 50 mM Tris, pH 8.9, and digested overnight at room temperature with 1 μg LysC (Wako Chemicals). Digests were acidified to pH 2 with trifluoroacetic acid and desalted over Empore C18 stage tips (Rappsilber et al. 2007).

Peptide samples were resuspended in 4% acetonitrile, 3% formic acid, and subjected to liquid chromatography coupled to tandem mass spectrometry. Samples were loaded onto a fused silica capillary column packed with 1.9 μm Reprosil-Pur C18 AQ reversed-phase resin and separated using a gradient of 8–30% acetonitrile in 0.125% formic acid delivered at 250 nl/min over 95 min, with a total 120-min acquisition time. Peptides were analyzed online on the linear ion trap Orbitrap (LTQ Velos Orbitrap; Thermo Fisher Scientific) hybrid mass spectrometer using a data-dependent acquisition method. For each cycle, one full mass spectrometry scan was acquired from 350 to 1500 m/z at 60,000 resolution on the Orbitrap, with fill target of 3E6 ions and maximum injection time of 500 msec, followed by up to 20 tandem mass spectrometry on the 20 most-intense precursor ions fragmented by collision-induced dissociation and acquired in the ion trap with a 3E4 fill target and 100 msec of maximum injection time.

Raw files were converted to the open mzXML format and searched against the *Saccharomyces* Genome Database yeast protein sequence database (downloaded in 2014) using Comet (release 2015.01; Eng et al. 2013). The false discovery rate (FDR) was estimated using a target-decoy strategy (Elias and Gygi 2007). Data were filtered to 1% FDR at the peptide-spectrum match level using Percolator (2017; Käll et al. 2007). To identify peptides with serine substitutions at proline sites, the search was conducted with a variable modification corresponding to the mass shift of a proline to serine substitution at proline positions. A maximum of two proline to serine substitutions per peptide were allowed, as the low rate of substitution we observed was considered unlikely to produce detectable peptides with multiple substitutions.

Additional search parameters were cleavage C-terminal to lysine with a maximum of two missed cleavages, constant modification of carbamidomethylation on cysteines, variable modifications of methionine oxidation and N-terminal protein acetylation, tolerance of 50 ppm for precursor masses, and 0.36 kDa with 0.11 offset for fragment ions.

To estimate the frequency of substitution, we calculated the fraction of unique peptides containing the proline sites for which the serine-substituted version of the peptide was also detected. To minimize the FDR among mistranslated peptides, we applied additional, more stringent filtering. Serine-substituted peptides with additional modifications (methionine oxidation or N-terminal acetylation) or those in which the corresponding wild-type peptide was not present in the data set were filtered out. For codon specific substitution analysis, an additional filtering step was applied. Only peptides with a single proline instance were considered so as to rule out potential site localization issues.

**Data availability**

Strains and plasmids are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables. File S1 contains all supplemental figures and tables. Supplemental material available at FigShare: https://doi.org/10.25386/genetics.9742577.

**Results**

tRNAs that mistranslate at different codons and with a range of efficiencies will enable broad applications of mistranslation. We previously demonstrated that SUP17, the gene encoding tRNA^Ser, is lethal when modified with a UGG anticodon and transformed into a wild-type yeast strain (Berg et al. 2017). Second-site mutations (e.g., G9A and G26A) that cripple the tRNA allow the mistranslation to be tolerated. By showing that the tRNAs suppress the stress sensitivity of a tti2-L187P mutation, we demonstrated that they mistranslate proline codons, a result confirmed by mass spectrometry. In addition, these mistranslating tRNA^SerUGG variants induce a cellular heat-shock response.

To determine the range of mistranslation induced by tRNA^Ser{UGG}-G9A and -G26A, we analyzed the cellular proteome by mass spectrometry. The frequency of mistranslation detected at CCA codons in a strain containing a wild-type tRNA^Ser was 0.04% (Figure 1A, number of peptides identified can be found in Table S3). The extent of mistranslation for tRNA^Ser{UGG}-G9A and -G26A was 0.4 and 5.2%, respectively, at the CCA codon. We note that the difference between the G9A variant and the wild-type tRNA was statistically significant (Welch’s t-test; *P < 0.05*), although the values do approach the estimated FDR. The majority of mistranslated codons were the cognate CCA codon, but we also observed mistranslation by tRNA^SerUGG at the wobble codon CCG and the CCU codon. Decoding of CCU may be due to modification of tRNA^SerUGG, since tRNA^ProUGG in *Salmonella enterica*
decodes this codon after modification of U34 to 5-oxyacetic acid (Näsvall et al. 2004). The difference in extent of mistranslation by tRNA\textsuperscript{Ser}\textsubscript{UGG-G9A} and tRNA\textsuperscript{Ser}\textsubscript{UGG-G26A} was consistent with the lesser effect of the G9A variant on cell growth (Figure 1B). We also observed previously that while both mistranslating variants induce a heat-shock response, G26A induces a greater heat-shock response relative to G9A (Berg et al. 2017). Although suppression of tti2-L187P is a sensitive method to detect mistranslation, it is not a quantitative measure of mistranslation (Figure 1C), likely because growth of the tti2-L187P strain under conditions of stress is a balance of both suppression by the tRNA and the toxicity caused by mistranslation.

Our goal was to obtain a set of tRNA variants that mistranslate at a broader range of frequencies. Regulating the aminoacylation of a tRNA provides a possible method to modulate functionality (Giegé et al. 1998). Based on studies in E. coli and Thermus thermophilus, the identity elements for tRNA\textsuperscript{Ser} fall within the discriminator base, first 4 bp of the acceptor stem, 1 bp in the D-arm, and unique extended variable arm (Normanly et al. 1986, 1992, Himeno et al. 1990, 1997; Sampson and Saks 1993; Asahara et al. 1994; Biou et al. 1994; Saks and Sampson 1996). We found mutations in the

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**Figure 1** tRNA\textsuperscript{Ser} with a proline UGG anticodon and various secondary mutations allow nonlethal levels of mistranslation. (A) Mass spectrometry analysis of the cellular proteome was performed on wild-type strain (BY4742) containing either wild-type tRNA\textsuperscript{Ser}, tRNA\textsuperscript{Ser}\textsubscript{UGG-G9A} or tRNA\textsuperscript{Ser}\textsubscript{UGG-G26A}. Mistranslation of serine at proline codons was quantified at all four proline codons. (B) Growth rates for each strain in A were determined from growth curves of the strains diluted to an OD\textsubscript{600} of ~0.1 in media lacking uracil and grown for 24 hr. Doubling time was calculated with the R package “growthcurve” (Sprouffske and Wagner 2016), normalized to the strain containing the wild-type tRNA and plotted against the percent mistranslation at all proline codons determined through whole proteome mass spectrometry. (C) Yeast strains containing tti2-L187P (CY7020) and either wild-type tRNA\textsuperscript{Ser}, tRNA\textsuperscript{Ser}\textsubscript{UGG-G9A} or tRNA\textsuperscript{Ser}\textsubscript{UGG-G26A} were grown to saturation in media lacking uracil and spotted in 10-fold serial dilutions on media lacking uracil or YPD containing 5% ethanol.
variable arm that either decreased the length of the arm, inverted the base pairs, or removed the arm entirely resulted in completely loss of tRNA function and no mis-translation as measured by suppression of \textit{tti2-L187P} and heat-shock induction (Figure S1). The same was true when we inverted the C11:G24 base pair within the D-arm (Figure S2).

We then investigated if bases within the acceptor stem could be mutated to modulate tRNA\textsubscript{Ser} function and allow mistranslation. Normanly \textit{et al.} (1992) converted a leucine accepting tRNA into a serine accepting tRNA in \textit{E. coli} by mutating positions 2, 3, 70, 71, and 72 within the tRNA\textsubscript{Leu} acceptor stem to C72, G2:C71 and A3:U70, all conserved within tRNA\textsubscript{Ser}, suggesting that these bases are identity elements for serylation. In addition, Saks and Sampson (1996) have shown that the charging of minihelices by SerRS requires the first 5 bp in the acceptor stem. Therefore, we constructed four alleles each inverting 1 bp in the acceptor stem (Figure 2A). Transformants were not obtained for base inversions at positions 2:71 and 4:69 (Figure S3), suggesting that these variants are highly functional and mistranslate at lethal levels. The variants with base inversions at positions 1:72 and 3:70 could be transformed. Both were partially toxic as measured by reduced growth (Figure 2B), suppressed \textit{tti2-L187P} (Figure 2C) and induced a heat-shock response (Figure 2D). Together these results suggest that the C1:G72 and G3:C70 base-pair inversions result in a partial loss of tRNA function. Interestingly, the G3:C70 variant was more toxic than the G26A variant but the G26A variant induced a greater heat-shock response.

**Figure 2**  Mutation of G1:C70 and C3:G70 reduces tRNA\textsubscript{Ser} function allowing a nonlethal level of mistranslation. (A) Structures of four tRNA\textsubscript{Ser}\textsubscript{UGG} alleles created by flipping base pairs at the first four positions in the acceptor stem. (B) Wild-type strain (BY4742) expressing wild-type tRNA\textsubscript{Ser}\textsubscript{UGA}, tRNA\textsubscript{Ser}\textsubscript{UGG}\textsubscript{G26A} or the viable tRNA\textsubscript{Ser}\textsubscript{UGG}-C1:G72 or -G3:C70 were grown to saturation in media lacking uracil and spotted in 10-fold serial dilutions on the same media. (C) Strains containing \textit{tti2-L187P} (CY7020) and one of the tRNAs described in B were grown to saturation in media lacking uracil and spotted in 10-fold serial dilutions on media lacking uracil or complete media containing 5% ethanol. (D) Wild-type strain BY4742 containing one of the tRNAs described in B and a fluorescence heat shock reporter were grown to saturation in media lacking uracil and histidine. Cells were diluted 1:20 in the same media and grown for 6 hr. Cell densities were normalized and fluorescence measured. Each point represents one biological replicate.
Next, we mutated the discriminator base (position 73), which plays an important role in aminoacylation of many tRNAs by making contacts with the aaRS (Hou 1997; Fukunaga and Yokoyama 2005). All tRNA\textsuperscript{Ser} isoacceptors have G as the discriminator base. We engineered tRNA\textsuperscript{Ser\_UGG} encoding alleles with the discriminator base converted to A or C (Figure 3A) and attempted to transform these alleles on centromeric plasmids into BY4742 (Figure S4). No transformants were obtained for the 73A variant, suggesting that it is highly functional and mistranslates at a lethal level. Transformants expressing the 73C variant were obtained. Consistent with mistranslation by this variant, the transformants grew at a reduced rate relative to the control wild-type tRNA\textsuperscript{Ser} (Figure 3B) and induced a 2.7-fold heat-shock response relative to the wild type (Figure 3C). We again note that similar to the G3:C70 variant, the extent of the heat-shock response did not correlate well with the effect of the G73C variant on growth. We were unable to transform the tRNA\textsuperscript{Ser\_UGG}-G73C containing plasmid into the tti2-L187P strain, likely reflecting the combined toxicity of mistranslation with the tti2 mutation.

In E. coli and Saccharomyces cerevisiae, the discriminator base for tRNA\textsuperscript{Ser} and tRNA\textsuperscript{Leu} is G and A, respectively. Normanly et al. (1992) found that changing the discriminator base of a leucine tRNA from A to the G allowed E. coli tRNA\textsuperscript{Leu} to be charged with serine. To test this for S. cerevisiae tRNA\textsuperscript{Ser}, we constructed tRNA\textsuperscript{Ser\_UGG} alleles with G73C and G73A mutations in the context of the wild-type UGA serine anticodon. If the tRNA\textsuperscript{Ser\_UGG} variants were mischarged, they should be partially toxic in a wild-type strain and induce a heat-shock response. We did not observe toxicity in growth assays nor did these tRNAs induce a heat-shock response (Figure S5), suggesting that the discriminator base variants are not mischarged. These discriminator base variants are only toxic in the context of a mistranslating tRNA\textsuperscript{Ser} with a noncognate anticodon.

\textit{tRNA\textsuperscript{Ser\_UGG} variants randomly selected to mistranslate at different levels}

The targeted mutations in tRNA\textsuperscript{Ser\_UGG} that mistranslated resulted in poor viability. To identify additional bases that, when mutated, lower tRNA\textsuperscript{Ser\_UGG} mistranslation to a variety of levels in a less biased manner, we mutagenized the \textit{sup17}(UGG)-containing plasmid with UV light and randomly selected mutants that suppress \textit{tti2}-L187P. Plasmids that allowed growth of the \textit{tti2}-L187P strain on media containing 5% ethanol were isolated, transformed back into the \textit{tti2}-L187P strain to verify activity, and sequenced (suppression of \textit{tti2}-L187P by the randomly selected variants is shown in Figure 3D).
Figure S6). We mapped the mutations onto the tRNA^Ser^UGG secondary structure (Figure 4A). We isolated 22 different mutations that dampen tRNA^Ser^UGG function to allow non-lethal levels of mistranslation, including mutations at G9, A20b, G26, and C40 that we had identified previously (Berg et al. 2017). Twelve mutations occur in single-stranded regions; 10 occur in stem structures. Of those occurring in stem structures, six change a Watson–Crick base pair to a G:U pair, including one in the variable arm. The three mutations that abolish base-pairing occur either at the beginning or end of a stem resulting in a shortened stem structure. tRNA^Ser^UGA contains 14 modified bases (Machnicka et al. 2013; colored red in Figure 4A). Seven of the mutations that dampen tRNA^Ser^UGG function occur at one of these modification sites.

To estimate the amount of mistranslation by the randomly selected derivatives, we examined their effect on growth in a wild-type strain (Figure 4B and Table 1) and their induction of a heat-shock response (Figure 4C and Table 1). The least toxic tRNA^Ser^UGG variants, containing a CSU, G9A, A20bG, or U44G mutation, had a doubling time similar to the control strain that contained wild-type tRNA^Ser^.

To better estimate mistranslation frequencies, we analyzed protein lysates by mass spectrometry for tRNA^Ser^UGG variants U44G, U33G, and U39G, which have ~1.5-, ~2-, and ~2-fold increases in doubling time, respectively. We predicted that these variants would mistranslate at different frequencies correlating to their effect on growth. In agreement with this, the U44G variant mistranslated at 0.3%, the U33G...
variant mistranslated at 3.0% and the U39G variant mistranslated at 4.0% (Figure 5).

To evaluate whether the secondary mutations in the randomly selected variants reduced the stability of the tRNAs, we compared their toxicity at 18°C and 30°C (Figure 6A with representative temperature curves shown in Figure 6B). Seventeen of the 22 mutations became more toxic at 18°C, suggesting that these mutations affect the stability or folding of the tRNA. Neither the G9A nor G26A variants were more toxic at lower temperatures (Figure 6B), suggesting that temperature induced toxicity is not due to changes in translation decoding at different temperatures. Furthermore, this result suggests that the extent of mistranslation can be regulated by growth temperature. To further evaluate if secondary mutations affect the stability of the tRNA, we determined the toxicity of the mistranslating tRNAs in a met22Δ strain where the RTD pathway is inhibited (Dichtl et al. 1997; Chernyakov et al. 2008). If a mutation reduces tRNA function by increasing the turnover rate of the tRNA, the tRNA should be more toxic in the met22Δ strain. Eighteen of the 22 randomly selected tRNASerUGG variants were more toxic in the met22Δ strain than the wild-type strain (Figure 7 and Figure S7). Of the four variants not affected by the RTD pathway, one changed an A:U base pair in the acceptor stem to a G:U and three had mutations in loop regions. We note that of the 22 variants, all but the A38G variant showed either temperature-dependent toxicity or increased toxicity when the RTD pathway was inhibited, or both.

### Table 1 Mutations to tRNA^{Ser}_{UGG} allowing nontoxic levels of mistranslation

| Mutationa | Doubling time (min) | Heat-shock induction (fold change) |
|------------|---------------------|-----------------------------------|
| tRNA^{Ser}_{UGA} | 65.5 | 1.0 | |
| A20Gb | 67.4 | 1.6 | |
| G9A | 68.3 | 1.2 | |
| U44G | 71.8 | 2.4 | |
| CSU | 75.5 | 1.9 | |
| Ce21Ub | 77.7 | 1.4 | |
| A4Gb | 77.9 | 1.7 | |
| G45A | 79.4 | 2.3 | |
| A38G | 80.4 | 2.2 | |
| A29G | 81.6 | 3.3 | |
| U19C | 85.3 | 3.4 | |
| A59G | 85.4 | 2.7 | |
| G26A | 88.9 | 3.2 | |
| U33G | 98.0 | 2.8 | |
| U51C | 101 | 3.8 | |
| G17A | 102 | 2.5 | |
| C12Ub | 105 | 2.8 | |
| G233Ub | 105 | 4.2 | |
| G15A | 107 | 2.9 | |
| C48U | 109 | 6.5 | |
| U60C | 113 | 1.7 | |
| C40U | 116 | 3.8 | |
| U39G | 129 | 2.4 | |

a With the exception of the wild-type tRNA^{Ser}_{UGA}, all tRNAs have the UGG anticodon.
b Refers to the extended variable arm, as numbered by Sprinzl et al. (1998).

As shown by Zimmerman et al. (2018) in yeast and by Geslain et al. (2010) in mammalian cells, the ability to change the anticodon of tRNA^{Ser} without affecting serylation makes it ideal to engineer mistranslation at many codons. Interestingly, Zimmerman et al. (2018) found that different amino acid substitutions are not equally toxic. To determine if secondary mutations dampen toxicity and allow mistranslation at other codons, we constructed tRNA^{Ser} expressing plasmids with anticodons to decode arginine, ochre, glutamine, and phenylalanine codons. tRNA^{Ser}_{UCU} to decode arginine was examined first. No transformants were obtained when we attempted to introduce a centromeric plasmid expressing tRNA^{Ser}_{UCU} into a wild-type yeast strain (Figure S8), indicating its toxicity. To decrease mistranslation frequency and allow viable levels of serine for arginine mistranslation, we engineered a plasmid expressing tRNA^{Ser}_{UCU} with a G9A secondary mutation, which transformed into cells (Figure S8). To verify mistranslation, we transformed the plasmid expressing tRNA^{Ser}_{UCU}-G9A into a strain deleted for TTI2, but with wild-type TTI2 on a URA3 plasmid and tti2-L187R on a LEU2 centromeric plasmid (CY8607). As shown by plasmid shuffling on 5-fluoroorotic acid medium (Figure 8A), tRNA^{Ser}_{UCU}-G9A suppresses tti2-L187R, in contrast to the control wild-type tRNA^{Ser}_{UGA}. Since Tti2 with serine at position 187 supports viability (Berg et al. 2017), this result indicates that tRNA^{Ser}_{UCU}-G9A mistranslates serine for arginine.

Previously, we identified an allele of tti2 where the glutamine codon at position 276 was mutated to an ochre stop codon (Hoffman et al. 2016). tti2-Q276TAA grows slowly on...
complete medium and is stress sensitive. We predict that tRNA<sup>Ser</sup> could be engineered to suppress ochre stop codons. As shown in Figure 8B, a wild-type tRNA<sup>Ser</sup> variant with an ochre anticodon (UUA) is not toxic and suppresses the <i>tti2-Q276TAA</i> slow growth and stress-sensitive phenotype. In this case, the tRNA<sup>Ser</sup>UUA lacking secondary mutations was not lethal, likely because of competition between the tRNA and release factor. The variant with the ochre anticodon and a G9A secondary mutation was less active, suppressing the slow growth of <i>tti2-Q276TAA</i> on complete media and only weakly restored growth on medium containing 5% ethanol.

We generated serine tRNAs with glutamine (CUG) or phenylalanine (GAA) anticodons in the context of a G26A secondary mutation, to prevent potential lethality. Since we do not have a reporter construct to detect mistranslation of glutamine or phenylalanine codons, we used heat-shock response and growth rates as proxies for mistranslation. Heat-shock response was measured in the wild-type BY4742 strain expressing wild-type tRNA<sup>Ser</sup>UGA or G26A variants that decode either glutamine, phenylalanine, proline, or arginine codons. All of the strains expressing a variant tRNA induced a heat-shock response, as compared to the strain containing the wild-type tRNA<sup>Ser</sup>UGA (Figure 8C). The effect of the tRNAs on growth is shown in Figure 8D. The serine tRNA with a glutamine anticodon did not affect growth, whereas the tRNA decoding arginine, proline, or phenylalanine significantly increased doubling time by 10, 20, and 50%, respectively (Welch’s t-test; <i>P</i> < 0.05), indicating that incorporating serine for different amino acids has different consequences. Our results also demonstrate that serine misincorporation at different codons can be achieved by pairing with an appropriate secondary mutation.

**Discussion**

tRNAs that mistranslate the genetic code have utility in a number of molecular and synthetic biology applications for both research and biotechnology. Because of its effects on the proteome, mistranslation can be toxic or even lethal, depending on the level of mistranslation and the amino acid substitution. For mistranslation to be useful in biological applications, it must be modulated to avert toxicity. Here, we identify secondary mutations within the tRNA to allow for a range of mistranslation levels for serine to proline substitution (all variants investigated are listed in Table S5). These same derivatives are applicable for substitutions at other codons.

Uses of mistranslation include expanding the genetic code to incorporate noncanonical amino acids, generating statistical proteins that have expanded activity or substrate specificity or inducing proteome wide translational errors to study the effects of amino acid substitutions on cellular functions. The range of efficiencies allows selection of the optimal variant for the application. For example, selecting the optimal tRNA<sup>Ser</sup> variant could allow incorporation of noncanonical amino acids or even selenocysteine in yeast, without resulting in a loss of fitness, which can lead to poor protein yields. Tuning of the mistranslation will also facilitate optimal mistranslation levels when using multiple tRNAs that substitute at different codons. The temperature-sensitive nature of the tRNAs variants we identified...
allows regulated mistranslation and applications such as the control of protein expression when used to suppress stop codons. For example, a stop codon could be incorporated early in the protein coding sequence of interest. In the presence of a corresponding temperature-sensitive tRNA^Ser derivative, at low temperatures the stop codon would be read through and the protein expressed. The conditional mistranslation also allows mistranslation at levels above the lethal threshold.

**Generating tRNAs that mistranslate at different levels**

We found that an unbiased genetic selection was the most efficient approach to identify tRNA^Ser bases with nonlethal levels of mistranslation. Bases altered in the targeted strategy were often essential and many of the variants did not mistranslate. By selecting for randomly induced mutations of tRNA^Ser<sub>UGG</sub> that suppressed trt2-L187P and were therefore mistranslating at a nonlethal level, we identified 22 single nucleotide mutations that induce mistranslation, including mutations on G9, A20b, G26, and C40 that we had identified previously (Berg et al. 2017). Most of these changes occur in single-stranded regions (12 variants) or create a G:U pair (six variants), and thus they likely maintain the overall tRNA structure. The mutations that abolish base pairing occur at the beginning or end of the stem resulting in its shortening. Despite this, we conclude that most of the secondary mutations have a role in tRNA stability and/or turnover, since 21 of the variants were more susceptible to the RTD pathway (18 variants) and/or showed enhanced toxicity at low temperature (19 variants). Included in the latter were base changes that altered base pairing (A4G, CSU, C12U, A29G, U39G, C40U, G45A, Ge23A, and Ce21U). Many of these create a G:U base pair, thus supporting the idea that although thermodynamically similar to a Watson–Crick pair, the G:U pair has different structural and chemical properties that alter tRNA structure (Varani and McClain 2000).

We considered that the enhanced toxicity of the mistranslating variants at lower temperatures may be due to a temperature-dependent effect of mistranslation on cells, rather than being an effect of tRNA stability. However, neither the G9A nor G26A variant (shown in the representative temperature curves in Figure 7B), with mistranslation frequencies of <1% and ~5%, were more toxic at lower temperatures. Their lack of increased toxicity suggests that the temperature-induced toxicity seen for other variants is a result of tRNA stability, rather than a temperature-dependent effect of mistranslation on the proteome.

The concentration of a tRNA in the cell depends upon both its synthesis and degradation. We note that six variants had base changes in the A box or B box region, required for transcription by RNA polymerase III (Hamada et al. 2001). The EufindtRNA algorithm, a tool that predicts likelihood of tRNA expression based upon a consensus sequence (Pavesi et al. 1994), predicts that all the variants will be expressed and only the G17A secondary mutation substantially decreases the Eufind score (Table S4). As these variants are all temperature sensitive, we suggest the base changes have a more pronounced effect on stability than synthesis.

Nucleotide changes that affect tRNA stability yet enable partial activity in vivo are difficult to predict. Therefore, using a genetic selection as we have done here or a screen for alleles that induce a partial heat-shock response appears to be the most efficient way to identify mistranslating tRNA variants.

**Features of the sequence-function relationships of S. cerevisiae tRNA^Ser**

With the exception of mitochondrial tRNA^Ser in metazoans (Helm et al. 2000), the long variable arm is conserved in all tRNA^Ser molecules and is a key element in recognition by SerRS (Normanly et al. 1986, 1992; Sampson and Saks 1993; Asahara et al. 1994; Biou et al. 1994; Himeno et al. 1997; Bilokapic et al. 2006). Our results indicate the importance of both the length and sequence orientation of the variable arm for function of tRNA^Ser in S. cerevisiae. As little as a single base-pair deletion impaired function below a detectable level in the sensitive trt2-L187P suppression assay. Similarly, reversing the orientation of the G:C base pairs in the variable arm eliminated function. Our results also confirm the essential nature of the D-arm C11:G24 base pair of tRNA^Ser<sub>UGG</sub>, previously identified for its role in serylation in E. coli (Normanly et al. 1986, 1992; Asahara et al. 1994).
SerRS is a class II synthetase that contains a seven-sheet, antiparallel β-fold (Artymiuk et al. 1994). In this fold, motif 2 interacts with the acceptor stem (Burke et al. 2000) and models of the interaction position motif 2 close to base pairs 3:70 of the acceptor stem (Eichert et al. 2011; Berg et al. 2018). Our results indicate that base pairs 1:72 and 3:70 play partial roles in tRNA\textsubscript{Ser} function in \textit{S. cerevisiae}, whereas base pairs 2:71 and 4:69 have lesser roles. This is consistent with a previous study, where we found that cross-species differences in the function of human and yeast tRNA\textsubscript{Ser} are the result of differences at the 3:70 position (Berg et al. 2018).

Modifications play many roles in tRNA function, from facilitating accurate decoding to maintaining the correct tRNA structure (Lorenz et al. 2017). Fourteen bases in tRNA\textsubscript{Ser} are modified (Machnicka et al. 2013). In our random selection for reduced function, we identified mutations at seven of these modified bases. Six were more toxic at lower temperatures and were turned over by the RTD pathway consistent with the modifications enhancing folding and protecting the tRNA from degradation at higher temperatures. Also consistent with this idea, thermophiles have more abundant and diverse tRNA modifications, including thiolations and methylations, which rigidify the tRNA structure (Watanabe et al. 1976; Kawai et al. 1992; Kowalak et al. 1994; McCloskey et al. 2001).

**Cellular effects of mistranslation**

The effect of the tRNA\textsubscript{Ser}\textsubscript{UGG} variants on doubling time varied from almost no change in growth to a twofold increase in doubling time. The change in growth correlated well with the frequencies of mistranslation as estimated by mass spectrometry for the five mistranslating tRNAs that were tested. Based on this correlation, we predict mistranslation rates as high as 8% could be achieved with a 90% decrease in fitness. This is consistent with other measures for the maximum tolerable amount of mistranslation. For example, Ruan et al. (2008) found \textit{E. coli} can tolerate up to 10% mismade protein and Mohler et al. (2017) found yeast can tolerate 8% tyrosine incorporation at phenylalanine codons. We also note that based on the correlation we observed, growth rate provides a better proxy for mistranslation than level of heat-shock response.

We compared the growth characteristics of strains mistranslating at proline, arginine, glutamine, and phenylalanine codons. Of these, misincorporation at phenylalanine codons resulted in the most pronounced decrease in growth rate. A similar result was obtained by Zimmerman et al. (2018), who found that tRNA\textsubscript{Ser} with phenylalanine anticodons were depleted from pools of cells containing tRNA\textsubscript{Ser} with randomized anticodons. Zimmerman et al. (2018) discuss possible

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**Figure 8** tRNA\textsubscript{Ser} variants mistranslate at nonserine codons. (A) \textit{tti2-L187R} and either a wild-type tRNA\textsubscript{Ser}\textsubscript{UGA}, or tRNA\textsubscript{Ser}\textsubscript{UCU}-G9A that mistranslates serine at arginine codons were transformed into the \textit{tti2} disruption strain CY6963 with \textit{TTI2} on a \textit{URA3} plasmid. Strains were grown in media lacking histidine and leucine and plated in 10-fold serial dilutions on 5-fluoroorotic acid containing medium to select against colonies containing wild-type \textit{TTI2}. (B) Wild-type tRNA\textsubscript{Ser}\textsubscript{UGA}, an ochre suppressor serine tRNA (tRNA\textsubscript{Ser}\textsubscript{UUA}) or tRNA\textsubscript{Ser}\textsubscript{UUA}-G9A were transformed into a \textit{tti2} disruption strain containing \textit{tti2-Q276(TAA)}. Strains were spotted in 10-fold serial dilutions on media lacking uracil or on YPD containing 5% ethanol. (C) Mistranslating tRNAs induce a heat-shock response. Wild-type strain (BY4742) containing a fluorescence heat shock reporter was transformed with wild-type tRNA\textsubscript{Ser}\textsubscript{UGA}, tRNA\textsubscript{Ser}\textsubscript{UGG}-G26A, tRNA\textsubscript{Ser}\textsubscript{CUG}-G26A, tRNA\textsubscript{Ser}\textsubscript{UCU}-G26A, or tRNA\textsubscript{Ser}\textsubscript{GAA}-G26A. Strains were grown to saturation in selective minimal medium, diluted 1:20 in the same media, and grown for 6 hr. Cell densities were normalized and fluorescence measured. Each point represents one biological replicate. (D) Strains containing either wild-type tRNA\textsubscript{Ser} or mistranslating serine tRNAs from C were grown to saturation in media lacking uracil, diluted to an OD\textsubscript{600} of ~0.1 in the same media and grown for 24 hr. OD\textsubscript{600} was measured every 15 min. Doubling time was calculated with the R package "growthcurver" (Sprouffske and Wagner 2016). Each point represents one biological replicate.
reasons for differences in toxicity noting that level of misincorporation does not correlate well with toxicity when comparing different amino acid substitutions. Reasons include whether the amino acid substitution is conservative or non-conservative, features of codon usage, and the ratio of the mistranslating tRNA to the native isodecoder. Zimmerman et al. (2018) investigated the effect of competition between a mistranslating tRNA and native cognate tRNA on toxicity, finding that overexpressing a wild-type cognate tRNA reduced the toxicity of mistranslation. The mistranslating serine derivatives generated here that mistranslate at phenylalanine, proline, arginine, or glutamine codons all compete with approximately the same number of wild-type cognate tRNAs (10 for GAA phenylalanine, 10 for UGG proline, 11 for UCU arginine, and 10 for GUC glutamine). We believe that the specific chemistry of the substitution explains the varying toxicities of mistranslation at different codons, although we recognize that the analysis is complicated by difficulties quantitating all of the parameters in cells; for example, the extent and efficiency of wobble.

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Literature Cited

Artyymiuk, P. J., D. W. Rice, A. R. Poirrette, and P. Willet, 1994 A tale of two synthetases. Nat. Struct. Biol. 1: 758–760. https://doi.org/10.1038/nsb1194-758

Asahara, H., H. Himeno, K. Tamura, N. Nameki, T. Hasegawa et al., 1994 Escherichia coli Seryl-tRNA synthetase recognizes tRNAser by its characteristics tertiary structure. J. Mol. Biol. 236: 738–748. https://doi.org/10.1006/jmbi.1994.1186

Bacher, J. M., W. F. Waas, D. Metzgar, V. De Crécy-Lagard, and P. Schimmel, 2007 Genetic code ambiguity confers a selective advantage on Acinetobacter baylyi. J. Bacteriol. 189: 6494–6496. https://doi.org/10.1128/JB.00622-07

Berg, M. D., K. S. Hoffman, J. Genereaux, S. Mian, R. S. Trussler et al., 2017 Evolving mistranslating tRNAs through a phenotypically ambivalent intermediate in Saccharomyces cerevisiae. Genetics 206: 1865–1879. https://doi.org/10.1534/genetics.117.202322

Berg, M. D., J. Genereaux, Y. Zhu, S. Mian, G. B. Gloor et al., 2018 Acceptor stem differences contribute to species-specific use of yeast and human tRNAser. Genes (Basel) 9: 612. https://doi.org/10.3390genes9120612

Bilokapic, S., T. Maier, D. Ahel, I. Gruic-Sovulj, D. Soll et al., 2006 Structure of the unusual seryl-tRNA synthetase reveals a distinct zinc-dependent mode of substrate recognition. EMBO J. 25: 2498–2509. https://doi.org/10.1038/sj.emboj.7601129

Biou, V., A. Yaremchuk, M. Tukalo, and S. Cusack, 1994 The 2.9 A crystal structure of T. thermophilus seryl-tRNA synthetase complexed with tRNA(Ser). Science 263: 1404–1410. https://doi.org/10.1126/science.8128220

Brandman, O., J. Stewart-Ornstein, D. Wong, A. Larson, C. C. Williams et al., 2012 A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. Cell 151: 1042–1054. https://doi.org/10.1016/j.cell.2012.10.044

Burke, B., F. Yang, F. Chen, C. Stehlin, B. Chan et al., 2000 Evolutionary coadaptation of the Motif 2–acceptor stem interaction in the class II prolyl-tRNA synthetase system. Biochemistry 39: 15540–15547. https://doi.org/10.1021/bi001835p

Chapeville, P., F. Lipmann, G. Ehrenstein, B. Weisblum, W. J. Ray et al., 1962 On the role of soluble ribonucleic acid in coding for amino acids. Proc. Natl. Acad. Sci. USA 48: 1086–1092. https://doi.org/10.1073/pnas.48.6.1086

Chernyakov, I., J. M. Whipple, L. Kotelawala, E. J. Grayhack, and E. M. Phizicky, 2003 Degradation of several hypomodified mature tRNA species in Saccharomyces cerevisiae is mediated by Met22 and the 5′-3′ exonucleases Rat1 and Xrn1. Genes Dev. 22: 1369–1380. https://doi.org/10.1101/gad.1654308

Cross, F. R., 1997 “Marker swap” plasmids: convenient tools for budding yeast molecular genetics. Yeast 13: 647–653. https://doi.org/10.1002/ps.5580130608

Dichtl, B., A. Stevens, and D. Torliver, 1997 Lithium toxicity in yeast is due to the inhibition of RNA processing enzymes. EMBO J. 16: 7184–7195. https://doi.org/10.1038/embj.16.23.7184

Dreher, T. W., O. C. Uhlenbeck, and K. S. Browning, 1999 Quantitative assessment of EF-1alpha.GTP binding to aminoacyl-tRNAs, aminoacyl-viral RNA, and tRNA shows close correspondence to the RNA binding properties of EF-Tu. J. Biol. Chem. 274: 666–672. https://doi.org/10.1074/jbc.274.2.666

Drummond, D. A., and C. O. Wilke, 2009 The evolutionary consequences of erroneous protein synthesis. Nat. Rev. Genet. 10: 715–724. https://doi.org/10.1038/nrg2662

Eichert, A., D. Oberthuer, C. Betzel, R. Geßler, V. A. Erdmann et al., 2011 The Seryl-tRNA synthetase/tRNA Ser acceptor stem interface is mediated via a specific network of water molecules. Biochem. Biophys. Res. Commun. 412: 532–536. https://doi.org/10.1016/j.bbrc.2011.07.030

Elias, J. E., and S. P. Gygi, 2007 Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. Nat. Methods 4: 207–214. https://doi.org/10.1038/nmeth1019

Eng, J. K., T. A. Jahan, and M. R. Hoopmann, 2013 Comet: an open-source MS/MS sequence database search tool. Proteomics 13: 22–24. https://doi.org/10.1002/pmc.201200439

Franklyn, C., and P. Schimmel, 1989 Aminoacylation of RNA minihelices with alanine. Nature 337: 478–481. https://doi.org/10.1038/337478a0

Fukunaga, R., and S. Yokoyama, 2005 Aminoacylation of RNA minihelices with alanine. Nature 337: 478–481. https://doi.org/10.1038/337478a0

Geslain, R., L. Cubells, T. Bori-Sanz, R. Alvarez-Medina, D. Rossell et al., 2010 Chimeric tRNAs as tools to induce proteome
damage and identify components of stress responses. Nucleic Acids Res. 38: e30. https://doi.org/10.1093/nar/gkp1083

Giege, R., M. Sisler, and C. Florentz, 1998 Universal rules and idiosyncratic features in tRNA identity. Nucleic Acids Res. 26: 5017–5035. https://doi.org/10.1093/nar/26.22.5017

Gomes, A. C., A. J. Kordala, R. Strack, X. Wang, R. Geslain et al., 2016 A dual fluorescent reporter for the investigation of methionine mistranslation in live cells. RNA 22: 467–476. https://doi.org/10.1016/j.rna.2015.04.013

Gustilo, E. M., F. A. Vendeix, and F. A. Agris, 2008 tRNAs modifications bring order to gene expression. Curr. Opin. Microbiol. 11: 134–140. https://doi.org/10.1016/j.mib.2008.02.003

Hamada, M., Y. Huang, and T. M. Lowe, 2001 Widespread use of TATA elements in the core promoters for RNA polymerases III, II, and I in fission yeast. Mol. Cell. Biol. 21: 6870–6881. https://doi.org/10.1128/MCB.21.20.6870-6881.2001

Helm, M. H. Brulé, D. Friese, R. Giege, D. Pütz et al., 2000 Search for characteristic structural features of mammalian mitochondrial tRNAs. RNA 6: 1356–1379. https://doi.org/10.1010/s1355583300000137

Himeno, H., T. Hasegawa, T. Ueda, K. Watanabe, and M. Shimizu, 1990 Conversion of aminoacyl-specificity from tRNAryto tRNAser in vitro. Nucleic Acids Res. 18: 6815–6819. https://doi.org/10.1093/nar/18.23.6815

Himeno, H., S. Yoshida, A. Soma, and K. Nishikawa, 1997 Only one nucleotide insertion to the long variable arm confers an efficient serine acceptor activity upon Saccharomyces cerevisiae tRNA(Leu(UUR)) in vitro. J. Mol. Biol. 268: 704–711. https://doi.org/10.1016/j.jmb.1997.09.091

Hoffman, K. S., M. L. Duennwald, J. Karagiannis, J. Genereaux, A. S. McCartney et al., 2016 Saccharomyces cerevisiae Tt2 regulates PIK3 proteins and stress response. G3 (Bethesda) 6: 1649–1659. https://doi.org/10.1534/g3.115.029520

Hoffman, K. S., M. D. Berg, B. H. Shilton, C. J. Brandl, and P. O’Donoghue, 2017 Genetic selection for mistranslation rescues a defective co-chaperone in yeast. Nucleic Acids Res. 45: 3407–3421. https://doi.org/10.1093/nar/gkw1021

Hopper, A. K., 2013 Transfer RNA post-transcriptional processing, turnover, and subcellular dynamics in the yeast Saccharomyces cerevisiae. Genetics 194: 43–67. https://doi.org/10.1534/g3.112.147470

Hou, Y. M., 1997 Discriminating among the discriminator bases of tRNAs. Chem. Biol. 4: 93–96. https://doi.org/10.1016/S1074-5521(97)90252-0

Jackman, J. E., and J. D. Alfonzo, 2013 Transfer RNA modifications: nature’s combinatorial chemistry playground. Wiley Interdiscip. Rev. RNA 4: 35–48. https://doi.org/10.1002/wrna.1144

Javia, B., F. Sorrentino, M. Toosky, W. Zheng, J. T. Pinkham et al., 2014 Mycobacterial mistranslation is necessary and sufficient for rifampicin phenotypic resistance. Proc. Natl. Acad. Sci. USA 111: 1132–1137. https://doi.org/10.1073/pnas.1317580111

Kadaba, S., A. Krueger, T. Trice, A. M. Krecic, A. G. Hinnebusch et al., 2004 Nuclear surveillance and degradation of hypomodified initiator tRNAMet in S. cerevisiae. Genes Dev. 18: 1227–1240. https://doi.org/10.1101/gad.1183804

Käll, L., J. D. Canterbury, J. Weston, W. S. Noble, and M. J. MacCoss, 2007 Semi-supervised learning for peptide identification from shotgun proteomics datasets. Nat. Methods 4: 923–925. https://doi.org/10.1038/nmeth1113

Kawai, G., Y. Yamamoto, T. Kamimura, T. Masegi, M. Sekine et al., 1992 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 31: 1040–1046. https://doi.org/10.1021/bi00119a012

Kowalak, J. A., J. J. Dalluge, J. A. McCloskey, and K. O. Stetter, 1994 The role of posttranscriptional modification in stabilization of transfer RNA from hyperthermophiles. Biochemistry 33: 7869–7876. https://doi.org/10.1021/bi00191a014

Kramer, E. B., and P. J. Farabaugh, 2007 The frequency of translational misreading errors in E. coli is largely determined by tRNA competition. RNA 13: 87–96. https://doi.org/10.1261/rna.294907
Normany, J., R. C. Ogden, S. J. Horvath, and J. Abelson, 1986 Changing the identity of a transfer RNA. Nature 321: 213–219. https://doi.org/10.1038/321213a0

Normany, J., T. Olick, and J. Abelson, 1992 Eight base changes are sufficient to convert a leucine-inserting tRNA into a serine-inserting tRNA. Proc. Natl. Acad. Sci. USA 89: 5680–5684. https://doi.org/10.1073/pnas.89.12.5680

Pang, Y. L. J., K. Poruri, and S. A. Martinis, 2014 tRNA synthetase: tRNA aminoacylation and beyond. Wiley Interdiscip. Rev. RNA 5: 461–480. https://doi.org/10.1002/wrna.1224

Paredes, J. A. L. Carreto, J. Simões, A. B. Bezerra, A. C. Gomes et al., 2012 Low level genome mistranslations deregulate the transcriptome and translatome and generate proteotoxic stress in yeast. BMC Biol. 10: 55. https://doi.org/10.1186/1741-7007-10-55

Pavesi, A., F. Conterio, A. Bolchi, G. Dieci, and S. Ottonello, 1994 Identification of new eukaryotic tRNA genes in genomic DNA databases by a multistep weight matrix analysis of transcriptional control regions. Nucleic Acids Res. 22: 1247–1256. https://doi.org/10.1093/nar/22.7.1247

Phizicky, E. M., and A. K. Hopper, 2010 tRNA biology charges to the front. Genes Dev. 24: 1832–1860. https://doi.org/10.1101/gad.1956510

Rappsilber, J., M. Mann, and Y. Ishihama, 2007 Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. Nat. Protoc. 2: 1896–1906. https://doi.org/10.1038/nprot.2007.261

Rich, A., and U. L. RajBhandary, 1976 Transfer RNA: molecular structure, sequence, and properties. Annu. Rev. Biochem. 45: 805–860. https://doi.org/10.1146/annurev.bi.45.070176.004105

Ruan, B., S. Palioura, J. Sabina, L. Marvin-Guy, S. Kochhar et al., 2016 Quality control despite mistranslation caused by an ambiguous genetic code. Proc. Natl. Acad. Sci. USA 105: 16502–16507. https://doi.org/10.1073/pnas.1519719105

Saks, M. E., and J. R. Sampson, 1996 Variant minihelix RNAs reveal sequence-specific recognition of the helical tRNA(Ser) acceptor stem by E. coli seryl-tRNA synthetase. EMBO J. 15: 2843–2849. https://doi.org/10.1002/j.1460-2075.1996.tb00454.x

Sampson, J. R., and M. E. Saks, 1993 Contributions of discrete tRNAser domains to aminoacylation by E. coli seryl-tRNA synthetase: a kinetic analysis using model RNA substrates. Nucleic Acids Res. 21: 4467–4475. https://doi.org/10.1093/nar/21.19.4467

Santos, M. A. S., C. Cheesman, V. Costa, P. Moradas-Ferreira, and M. F. Tuite, 1999 Selective advantages created by codon ambiguity allowed for the evolution of an alternative genetic code in Candida spp. Mol. Microbiol. 31: 937–947. https://doi.org/10.1046/j.1365-2958.1999.01233.x

Schimmel, P., 2011 Mistranslation and its control by tRNA synthetases. Philos. Trans. R. Soc. B Biol. Sci. 366: 2965–2971. https://doi.org/10.1098/rstb.2011.0158

Schmid, M., and T. H. Jensen, 2008 The exosome: a multipurpose RNA-decay machine. Trends Biochem. Sci. 33: 501–510. https://doi.org/10.1016/j.tibs.2008.07.003

Schneider, C., J. T. Anderson, and D. Tollerre, 2007 The exosome subunit Rrp44 plays a direct role in RNA substrate recognition. Mol. Cell 27: 324–331. https://doi.org/10.1016/j.molcel.2007.06.006

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri, 2012 NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9: 671–675. https://doi.org/10.1038/nmeth.2089

Schwartz, M. H., and T. Pan, 2016 Temperature dependent mistranslation in a hyperthermophile adapts proteins to lower temperatures. Nucleic Acids Res. 44: 294–303. https://doi.org/10.1093/nar/gkv1379

Schwartz, M. H., and T. Pan, 2017 tRNA misacylation with methionine in the mouse gut microbiome in situ. Microb. Ecol. 74: 10–14. https://doi.org/10.1007/s00248-016-0928-0

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122: 19–27.

Sprinzl, M., C. Horn, M. Brown, A. Loudovitch, and S. Steinberg, 1998 Compilation of tRNA sequences and sequences of tRNA genes. Nucleic Acids Res. 26: 148–153. https://doi.org/10.1093/nar/26.1.148

Sproufske, K., and A. Wagner, 2016 Growthcurver: an R package for obtaining interpretable metrics from microbial growth curves. BMC Bioinformatics 17: 172. https://doi.org/10.1186/1471-2199-11-105

Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader et al., 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294: 2364–2368. https://doi.org/10.1126/science.1065810

Urbonavičius, J., Q. Qian, J. M. B. Durand, T. G. Hagervall, and G. H. Björk, 2001 Improvement of reading frame maintenance is a common function for several tRNA modifications. EMBO J. 20: 4863–4873. https://doi.org/10.1093/emboj/dco177

Varani, G., and W. H. McClain, 2000 The G×U wobble base pair. A fundamental building block of RNA structure crucial to RNA function in diverse biological systems. EMBO Rep. 1: 18–23. https://doi.org/10.1093/embo-reports/kvd001

Wang, X., and T. Pan, 2016 Stress response and adaptation mediated by amino acid misincorporation during protein synthesis. Adv. Nutr. 7: 773S–779S. https://doi.org/10.3945/an.115.010991

Watanabe, K., M. Shinma, T. Oshima, and S. Nishimura, 1976 Heat-induced stability of tRNA from an extreme thermophile, Thermus thermophilus. Biochem. Biophys. Res. Commun. 72: 1137–1144. https://doi.org/10.1016/S0006-291X(76)80250-1

Wei, F., T. Suzuki, S. Watanabe, S. Kimura, T. Kaitusuka et al., 2011 Deficit of tRNA(Lys) modification by Cdkal1 causes the development of type 2 diabetes in mice. J. Clin. Invest. 121: 3598–3608. https://doi.org/10.1172/JCI85056

Whipple, J. M., E. A. Lane, I. Chernyakov, S. D’Silva, and E. M. Phizicky 2011 The yeast rapid tRNA decay pathway primarily monitors the structural integrity of the acceptor and T-stems of mature tRNA genes. Dev. Cell 25: 1173–1184. https://doi.org/10.1016/j.devcel.2010.08.012

Wittlout, E. J., M. Goodenbour, M. Fréchin, and T. Pan, 2012 Misacylation of tRNA with methionine in Saccharomyces cerevisiae. Nucleic Acids Res. 40: 10494–10506. https://doi.org/10.1093/nar/gks805

Winzeler, E. A., and R. W. Davis, 1997 Functional analysis of the yeast genome. Curr. Opin. Genet. Dev. 7: 771–776. https://doi.org/10.1016/S0959-437X(97)80039-1

Woese, C. R., 1965 On the evolution of the genetic code. Proc. Natl. Acad. Sci. USA 54: 1546–1552. https://doi.org/10.1073/pnas.54.6.1546

Wu, J., Y. Fan, and J. Ling, 2014 Mechanism of oxidant-induced mistranslation by threonyl-tRNA synthetase. Nucleic Acids Res. 42: 6523–6531. https://doi.org/10.1093/nar/gku271

Zimmerman, S. M., Y. Kon, A. C. Hauke, B. Y. Ruiz, S. Fields et al., 2018 Conditional accumulation of toxic tRNAs to cause amino acid misincorporation. Nucleic Acids Res. 46: 7831–7843. https://doi.org/10.1093/nar/gky623

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