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Determining the fidelity of tRNA aminoacylation via microarrays

Michael H. Schwartz^{1,2} and Tao Pan^{1,2,*}

¹Department of Biochemistry & Molecular Biology, University of Chicago, Chicago, IL 60637, USA

²Committee on Microbiology, University of Chicago, Chicago, IL 60637, USA

Abstract

The fidelity of tRNA aminoacylation is a critical determinant for the ultimate accuracy of protein synthesis. Although aminoacyl-tRNA synthetases are assumed to consistently maintain high tRNA charging fidelity, recent evidence has demonstrated that the fidelity of the aminoacylation reaction can be actively regulated and liable to change. Accordingly, the ability to conveniently assay the fidelity of tRNA charging is becoming increasingly relevant for studying mistranslation. Here we describe a combined radioactivity and microarray based method that can quantitatively elucidate which individual cognate or noncognate tRNA isoacceptors are charged with amino acid. In this technique, *in vitro* tRNA charging reactions or *in vivo* pulse-labeling is performed using a radiolabeled amino acid and tRNA microarrays are used to distinguish tRNA isoacceptors in total tRNA. During the tRNA array hybridization, each tRNA will hybridize to its unique probe and subsequent phosphorimaging of the array can determine which tRNAs were aminoacylated with the radiolabeled amino acid. The method can be used to assess the fidelity of tRNA charging *in vivo* or *in vitro* and can be applied to any organism with annotated tRNA genes.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) are the enzymes responsible for continually ligating amino acids to tRNAs for protein synthesis. Each amino acid has a designated tRNA synthetase that is responsible for exclusively charging a single type of amino acid to its cognate tRNA isoacceptors [1]. To maintain the fidelity of the genetic code, each amino acid and each tRNA should only be a substrate for a single aaRS. Although cells were initially thought to maintain high translational fidelity at all times, it is becoming increasingly evident that mistranslation is a regulated process that can be beneficial under certain conditions [2, 3]. While mistranslation can be engendered in any of the processes that mediate the conversion of DNA to protein, the most prominent mistranslational processes have so far been shown to occur during mRNA translation via ribosomal miscoding or tRNA mischarging [4–6]. However, the difficulty of detecting significant mistranslational processes has resulted in a significant lack of information regarding the occurrence and functional consequences of mistranslation.

*correspondence to: taopan@uchicago.edu.

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Mistranslation engendered at the level of tRNA charging is convenient to study since this reaction can be reduced to an aaRS, tRNAs, and amino acid. Since aaRSs use both a tRNA and an amino acid as substrates in the aminoacylation reaction, tRNA mischarging can occur if an aaRS accepts either a noncognate amino acid or a noncognate tRNA [7]. The regulated misacylation of noncognate tRNAs among methionyl-tRNA synthetases has been shown to be of particular physiological importance [8-13]. For instance, the methionyl-aaRS from mammalian cells is inherently accurate, but can accept noncognate tRNAs in response to a post-translational modification and this adaptable substrate specificity is important for the oxidative stress response [11]. Additionally, the methionyl-tRNA synthetase from the hyperthermophilic archaeon *Aeropyrum pernix* can conditionally accept tRNA^{Leu} at lower physiological temperatures and this process can enhance the function of proteins at lower temperatures [8]. Furthermore, oxidation of the threonyl-tRNA synthetase from *E. coli* has been shown to reduce its amino acid substrate specificity during oxidative stress allowing it to charge serine to tRNA^{Thr} and mistranslation has been shown to activate the oxidative stress response in *E. coli* [14, 15]. Other analogous mistranslational processes most likely exist at the tRNA charging level in nature, but their discovery will require convenient and sensitive detection tools. This review describes the methodology and application of custom tRNA microarrays, which have proven to be effective at determining the fidelity of the aminoacylation reaction both *in vitro* and *in vivo* (Fig. 1).

2. Method

2.1 Manually printing tRNA microarrays

The tRNA microarray method relies on custom microarrays that can be made for any organism with a sequenced genome and annotated tRNA genes. Arrays are created using DNA oligonucleotide probes that are spotted and crosslinked on amine coated array slides. Each probe consists of a DNA oligonucleotide that is the reverse complement of each tRNA sequence in an organism. Probes cover the entire length of tRNA minus the 3'CCA and are sufficient to distinguish tRNAs that differ by about 8 or more residues [16].

tRNA sequence information can be obtained from the Genomic tRNA Database (GtRNAdb) [17] or from the Pathosystems Resource Integration Center (PATRIC) [18]. Microbial organisms contain fewer tRNA genes and fewer tRNA isodecoder genes (tRNAs with the same anticodon and different body sequences) than eukaryotes. Therefore, in most microbial instances, one probe can be made for each unique tRNA isoacceptor sequence, but keep in mind that many organisms can have multiple genes for the same tRNA isoacceptor. Conversely, eukaryotic organisms contain multiple tRNA isoacceptor genes that differ by less than 8 nucleotides, or multiple tRNA isodecoder genes that differ by more than 8 nucleotides. Consequently, one probe may cover sequences from different isoacceptors, or multiple probes are needed for different tRNA isodecoders [19, 20].

The similarity between tRNA genes and thus their propensity to hybridize to other probes can be determined by aligning tRNA genes with Clustal alignment software (e.g. <http://www.ebi.ac.uk/Tools/msa/clustalo/>). Generally, a tRNA probe contains the direct reverse complement of a tRNA, however, probes can be designed to selectively exclude or include the hybridization of similar tRNAs by making selective nucleotide changes to the tRNA

reverse complement. It is important to remember that eukaryotic and archaeal tRNA genes contain introns, and these sequences must be trimmed from the annotated tRNA genes prior to designing the probes [21]. Lastly, the 3' CCA is excluded from the probe sequence since it is a common element that will not aid sequence specific tRNA binding. However, eukaryotes, archaea, and certain gram-positive bacteria do not have the CCA sequence contained within the tRNA gene itself [22].

After tRNA sequences have been obtained and their introns and possible CCA sequences removed, tRNA sequence reverse complements are obtained by using a reverse complement generator (e.g. http://www.bioinformatics.org/sms/rev_comp.html). It is advisable to include control tRNA probes from a different organism on the array, which should not hybridize to any tRNAs in the eventual sample of interest. The reverse complement sequences are then ordered as DNA oligonucleotides. For large orders, oligonucleotides can be purchased in a 96-well plate, which significantly reduces the cost.

- 1.) Ethanol precipitate the oligonucleotides to help remove small molecule impurities from the synthesis by first resuspending each oligonucleotide in 500 μ L 200mM KCl/50mM KOAc, pH 7.
- 2.) Transfer the dissolved oligonucleotide to a labeled 1.5mL tube and add 1mL of ethanol before refrigerated centrifugation (4°C) at max speed for 30 minutes.
- 3.) Aspirate the supernatant, speedvac dry briefly, and resuspend each oligonucleotide pellet in 100 μ L of deionized water before spectrophotometrically measuring the concentrations either by nanodrop or dilution in a 96-well UV compatible plate and measuring UV₂₆₀ in a plate reader.

The array printing apparatus consists of three main parts. First, the Floating Pin Replicator (V&P Scientific, VP478A) has 24 individual pins that dip into a 384-well plate containing the tRNA probes and transfers a small volume of diluted oligonucleotide solution (~10 nL) to the glass array. Second, the Glass Slide Indexing System (V&P Scientific, VP470) serves as a guide for the probe applicator to print on the array and allows for reproducible probe spotting. Third, the Microplate Indexing System (V&P Scientific, VP472A) plus cooling block guides the Floating Pin Replicator into the 384-well printer plate and cools the plate to reduce evaporation. Other components include the Glass Slide Replicator Wash & Blot Station (V&P Scientific, VP475) and Replicator Pin Drier (V&P Scientific, VP 474), which washes and dries the Floating Pin Replicator, respectively. Array printing is best done in a clean area with higher humidity; a plexiglass hood (V&P Scientific, VP473) provides a dust-free, protected environment to print arrays. Also, a small travel humidifier and hygrometer in the plexiglass hood will allow the humidity to be measured and controlled for reproducible array printing (50% humidity provides high-quality array spots). See Fig. 2 for array printing station set-up.

The Glass Slide Indexing System, which guides the Floating Pin Replicator to different positions on the array, allows for a user devisable probe pattern to be created. A standard

array layout is available on the V&P scientific website (<http://www.vp-scientific.com/Technotes/46.pdf>) or from Fig. 3. The 384-well plate can be divided into 12 sections for probe application (A8-D8, E1-H8, I8-L8, M1-P8, etc.), which are selectable with the Microplate Indexing System and each section prints 24 different probes on the array. The number of sections required depends on the number of probes required for an array. We have made arrays with as many as 186 probes (with 4 replicate spots, can reach a maximum of 192 probes) and as few as 32 (with 6 replicate spots). The array can accommodate 768 individual spots.

- 4.) Each concentrated oligonucleotide is then diluted to 50 μ M in 3x SSC, pH 7, 0.01% SDS and 40 μ L is placed in each well of a Genetix X7020 384-well plate in the desired layout. We generally randomize spot placement, so similar probes are not in close proximity.
- 5.) Turn on the small travel humidifier prior to printing arrays and allow the humidity to reach about 50% and fill the four wells of the Replicator Wash Station from right to left with 5% bleach (20mL), deionized water (22.5mL), deionized water (25mL), and isopropanol (27.5mL).
- 6.) Place completed 384-well printer plate containing the tRNA probes on the cooling block and insert into the Microplate Indexing system.
- 7.) Position the amine coated array slides (Molecular Devices, K2615) into the Glass Slide Indexing System and etch the type of array and its number in the unprinted, frosted section of the array with a Diamond Tip Pencil (Fisher 22268912). We print arrays on the face of the array which does not contain the frosted glass surface.
- 8.) Set the first set of coordinates on the Glass Slide indexing system. Next, position the Floating Pin Replicator on the first position of the Microplate Indexing System and press down to submerge the pins in the probes and immediately position the Floating Pin Replicator on the Glass Slide Indexing System and press down so the pins contact the glass slide.
- 9.) Set the next set of coordinates on the Glass Slide Indexing System and then return the Floating Pin Replicator to the Microplate Indexing System and reapply probes to the pins before each new probe application to the array. Repeat steps 8 and 9 until all coordinate positions for the given printer plate section have been applied to the arrays.
- 10.) Once the first 24 probes have been applied in adequate replication, wash the Floating Pin Replicator with the Replicator Wash & Blot Station and Replicator Pin Drier by positioning

the Floating Pin Replicator on the wash station and pressing down to submerge the pins in each of the wash solutions moving from left to right. Dry the pins on the Floating Pin Replicator with the Replicator Pin Drier before printing the next set of probes.

- 11.) Move the position on the Microplate Indexing System to the next set of 24 probes and continue printing as in step 8. Always wash the Floating Pin Replicator as in step 10 before printing a new set of probes to prevent cross contamination.
- 12.) When the array printing process is completed, allow the arrays to dry in a clean area outside the plexiglass hood. Drying time can vary significantly based on the temperature and humidity in the lab. The 384-well printer plate can be sealed with an aluminum foil lid (e.g. Beckman Coulter 538619) and stored at -20°C for future use.
- 13.) Once the arrays have dried, crosslink at 254nm at $1\text{J}/\text{cm}^2$ in a UV crosslinker.
- 14.) After crosslinking, block the arrays in BlockIt Blocking Buffer (Arrayit, BKTHT) in a slide rack within the Microarray Wash Station (Arrayit, HTW) on a magnetic stirrer with light stirring overnight at room temperature.
- 15.) After the arrays have been blocked, remove the blocking solution (can be reused 2x) and fill the Microarray Wash Station with deionized water and wash for 2 minutes with light stirring. Repeat the wash step.
- 16.) Remove the water from the Microarray wash station, and remove the excess water from the arrays before brief centrifugation at low speed to completely dry the arrays.
- 17.) Arrays are now ready for sample analysis and are stable long-term (over 1 year) if stored in a desiccation cabinet or container.

2.2 Charging tRNAs with radiolabeled amino acids

The tRNA array microarray can separate tRNA isoacceptors in a complex total tRNA sample, yet resolution of the arrays requires a method of general or selective tRNA detection. Fluorescence has been utilized as the detection method to determine tRNA charging and abundance [23-25], but we will focus on radiolabeled detection here. The tRNA microarrays described above are designed with large spots (~500 microns in diameter) for radioactivity detection via autoradiography. Total ^{32}P labeling of a tRNA followed by array hybridization can determine the relative abundances and identity of tRNAs in a sample [26, 27]. This total labeling method can be useful for determining the identity of a subset of tRNA isoacceptors that perform a specific function, such as selectively binding a particular protein.

Analysis of tRNA charging fidelity relies on the same principle of total ^{32}P radiolabeled detection. However, since the assay utilizes a radiolabeled amino acid (i.e. ^{35}S , ^{14}C , ^3H) rather than total labeling, only tRNAs that have been charged with the radiolabeled amino acid will be selectively detected via autoradiography. The fidelity of tRNA charging can be analyzed *in vivo* by pulse-labeling cells with a radiolabeled amino acid or *in vitro* by using a radiolabeled amino acid to charge purified tRNA with a tRNA synthetase. The general protocol for *in vivo* pulse-labeling involves an optional short amino acid starvation period, concentration of cells, pulse-labeling with radiolabeled amino acid, and subsequent charged tRNA isolation. Protocols for *in vivo* pulse labeling and total charged tRNA isolation of different organisms can be found in the literature and are easily adapted to new organisms [8, 10, 13, 28]. Since *in vivo* pulse labeling methods vary based on the organism and this review aims to describe on the array method itself, we will focus on the standard assessment of the fidelity of recombinant synthetases *in vitro* [8, 10, 12]. The reaction parameters for optimal *in vitro* aminoacylation have been established for many aaRSs in different species. These optimized reaction conditions should be used when available.

- 1.) Purify tRNA and the tRNA synthetase of interest:
 - tRNA: Purified total tRNA can be purchased from Roche for *Escherichia coli* (10109541001) and for *Saccharomyces cerevisiae* (10109517001). For other organisms, total tRNA can be purified through cell lysis, total RNA isolation, and gel purification and elution [8].
 - Aminoacyl-tRNA synthetase: Purified tRNA synthetase can be obtained by tagged, recombinant overexpression and purification of the enzyme [8, 10]. Alternatively, large eukaryotic tRNA synthetase complexes can be isolated by sucrose gradient fractionation via ultracentrifugation of cell lysates [29].
- 2.) *In vitro* tRNA charging reactions can be run in 50mM HEPES KOH pH 7.5, 100mM potassium glutamate, 10mM magnesium acetate, and 2mM ATP. It is convenient to make a 4x buffer stock of the tRNA charging buffer and store at -20°C . We have found this buffer to provide significantly improved tRNA charging for a variety of synthetases from different organisms. (Note: due to the high concentration of potassium glutamate, this buffer is insufficient for assaying the glutamate tRNA synthetase. Instead use a conventional NH_4Cl based buffer, which has been previously described [10].)
- 3.) We generally run 16 μL reactions derived from a master mix containing the following components added to the tRNA charging buffer described above:
 - DTT: Freshly prepared DTT should be added to the reactions at a final concentration of 1 mM.

•Purified total tRNA: 12.5-50 μ M should be charged per 16 μ L reaction, which is sufficient for an array.

•Radiolabeled amino acid: Higher concentrations are preferable; we generally make the radiolabeled amino acid 25% of the total charging reaction (i.e. 4 μ L for a 16 μ L reaction). The amino acid concentration will vary according to the radiolabeled amino acid being used.

•tRNA synthetase: Purified enzyme should be added to the reaction components to a final concentration of 100-500nM.

- 4.) Reactions should be run at the conventional temperature used to cultivate the organism from which the synthetase originates and should be run for 5-10 minutes.
- 5.) Each tRNA charging reaction should be stopped by adding cold 45 μ L 0.3M NaOAc/AcOH, 10mM EDTA pH 4.8 and an equal volume of acetate saturated phenol/chloroform before being mixed by pipetting or brief vortexing. It is important to maintain the charged tRNA in acidic conditions to maintain the aminoacylated tRNA.
- 6.) Centrifuge the samples in a refrigerated tabletop centrifuge at maximum speed for 5 minutes at 4°C.
- 7.) Remove the top aqueous layer and transfer to a new tube. Add 1 μ L of 10mg/mL Poly-A RNA and 2 μ L of 10mg/mL salmon sperm DNA (these are nonspecific nucleic acids, which aid the array hybridization process and also aid the ethanol precipitation of tRNA).
- 8.) Add 3 volumes of ice-cold ethanol and place at -20°C for 20 minutes before centrifugation at maximum speed for 30 minutes at 4°C.
- 9.) Resuspend the pellet in 135 μ L of 2x SSC pH 4.8. The tRNA sample is now ready to be analyzed via array hybridization.

2.3 Analyzing charged tRNAs via microarrays

tRNAs are hybridized to the manually printed arrays using an array hybridization machine. An array hybridization machine evenly heats the array hybridization mixture to initially denature the tRNAs and maintains an elevated temperature so tRNAs will hybridize to the arrays rather than refold. Additionally, certain array hybridization machines will agitate the sample during hybridization and wash away the unbound sample after hybridization is complete. New hybridization machines of different array capacities with washing abilities can be obtained from Digilab (Hyb4 or Hyb12). Alternatively, similar, used machines can be obtained refurbished equipment retailers under the older brand names GeneTAC, Gene

Machines or Genomic Solutions. The following protocol describes hybridization using a Hyb4, but could be adapted for use with other machines.

- 1.) Wash the station with warm water before and after each use and wash/dry the array cassettes well before use.
- 2.) Clean and refresh the rubber array cassette gaskets by boiling them in a beaker with deionized water for several minutes before inserting them into the cleaned array cassette.
- 3.) Clean the printed arrays before use by boiling them for about 1 minute in deionized water in a beaker. Allow the arrays to dry before inserting them into the array cassette and placing them in the machine.
- 4.) Use the following program to hybridize tRNAs to the array:
 - a.) Introduce the 135 μ L tRNA sample onto the array at 60°C.
 - b.) Denature tRNAs at 90°C for 2 minutes with agitation (if possible).
 - c.) Hybridize denatured tRNAs to array at 60°C for 50 minutes for charged tRNAs (longer hybridization time can reduce the signal due to the loss of the charged amino acid) or up to 16 hours for measuring tRNA abundance using ³²P-labeled tRNAs with agitation (if possible)
 - d.) Wash arrays in 2x SSC, 0.1% SSC pH4.8 for 20 seconds at 50°C (can be done with the hybridization machine or performed manually in a 50mL conical tube).
 - e.) Wash arrays in 0.1% SSC pH4.8 for 20 seconds at 50°C (can be done with the hybridization machine or performed manually in a 50mL conical tube).
- 5.) Allow slides to dry. This can be accelerated by brief centrifugation at low speed.
- 6.) For slides using ³⁵S or ¹⁴C radiolabeled amino acids, wrap slides in Spex Sample Prep 0.12 mil Mylar clear film (3516) and affix the film to the array using a strip of double-sided tape on the back of the array. Due to the low energy β particle

emission of ^3H , the film wrapping (which normally prevents direct contact with the phosphorimaging screen) is omitted since it will reduce the already low signal for ^3H arrays.

- 7.) Expose the arrays to a phosphorimaging screen in a cassette. Expose arrays utilizing ^3H amino acid to a tridium phosphorimaging screen (e.g. Fuji BAS TR2040). Since ^3H arrays will make direct contact with the phosphorimaging screen and ^3H half-life is 12.3 years, make sure to note which positions on the screen have been used, since these areas may not be reusable in the future.
- 8.) Image phosphorimaging screens with a phosphorimager after the desired amount of exposure, which will depend on the isotope being utilized. Overnight exposure should be sufficient to obtain adequate signal for arrays utilizing ^{35}S radiolabeled amino acids, expose arrays using ^{14}C radiolabeled amino acids for a couple days, and expose arrays using ^3H amino acids for at least three weeks to obtain potential signal for noncognate tRNAs, which will be a fraction of the cognate tRNA signal. However, exposing arrays utilizing any isotope for longer periods of time will increase signal and may allow for even faint noncognate signals to be observed.
- 9.) Once the arrays have been exposed, quantify the signals corresponding to different tRNA isoacceptors that have been charged with amino acid with image software (e.g. Quantity One, ImageLab). Free software such as ImageJ64 can also be used to quantify arrays.

3. Results

We made an array to analyze the tRNA charging fidelity in *E. coli*. This array design allows for 64 different tRNA probes each repeated with 6 individual spots in a consecutive L-shaped pattern. This array type is sufficient for most organisms and will only require probes to be aliquoted in the first two sections of the 384-well plate in order to print. The array probe distribution in the 384-well plate, printing coordinates for the Glass Slide Indexing System, and eventual layout of this array type is shown in Fig. 3. The *E. coli* tRNA probe locations on the array are shown in Table 1. The remaining array probes are complementary to selected yeast tRNAs that do not crosshybridize with *E. coli* tRNAs. The array probe sequences have been deposited to Geo database (accession number GSE2065) [24].

Here we provide an example of how to analyze and deduce factors that might modify the fidelity of the aminoacylation reaction. Previous results in the lab from an *in vitro* translation system had implicated calcium in reducing the fidelity of tRNA aminoacylation. To determine how calcium might affect which tRNA isoacceptors are charged with methionine *in vitro*, we ran several tRNA charging reactions with *E. coli* methionyl-tRNA synthetase,

total *E. coli* tRNA, and ^{35}S -methionine at varying concentrations of CaCl_2 . The tRNA charging and array analysis protocols outlined in sections 2.2 and 2.3 were followed.

The *E. coli* methionyl-tRNA synthetase has already been shown to accept nonmethionyl-tRNAs *in vitro* in the absence of calcium [12], yet addition of CaCl_2 to *in vitro* charging reaction considerably decreases the fidelity of aminoacylation (Fig. 4). In the array diagram, the locations of initiator (fmet) and elongator tRNA^{Met} are shown and all other signals represent ^{35}S -methionine that has been charged to nonmethionyl-tRNAs. The degree to which noncognate tRNAs are charged increases with calcium and additional noncognate tRNA isoacceptor species are charged with increasing concentrations of calcium. It is known that Mg^{2+} stabilizes tRNA/aaRS complexes [30-33] and the similar divalent ion Ca^{2+} could function to stabilize noncognate tRNA complexes with MetRS and therefore aid noncognate tRNA aminoacylation. This same analysis could be performed during *in vivo* tRNA charging to determine if addition of calcium to the growth medium similarly reduces the fidelity of tRNA charging and is therefore relevant to translational accuracy within the cell. Either way, the array approach is a convenient tool for deducing ions or other factors that may alter translational fidelity through tRNA mischarging.

4. Discussion

Once the initial barrier of obtaining array printing and hybridization equipment is surmounted, the array based method is an easy and cost-effective method to assess tRNA charging fidelities. The average cost of the microarray, oligonucleotide probes, and chemicals needed is less than \$10 per array. We have found that tRNA misacylation can vary tremendously based on specific cellular or *in vitro* conditions/perturbations. The fidelity of tRNA synthetases has been shown to be regulated by post-translational modifications [11], oxidation [14, 34], and temperature [8], although additional methods of regulating tRNA synthetase substrate specificity surely exist. In addition to the systems dedicated to regulating mistranslation for potential benefit, there are other extraneous factors—such as calcium—which may alter tRNA misacylation; the array method should be sufficient to elucidate these processes at the tRNA charging level.

Determining which amino acids are charged to which tRNA isoacceptors can help elucidate the amino acid substitutions and corresponding extents of mistranslation we should expect to see in proteins. This information greatly reduces the difficulty of searching for such mistranslational events in proteins through methods such as mass spectrometry and fluorescence detection [28]. Furthermore, since mistranslation can significantly alter the function and activity optima of a protein [8, 9, 35], elucidating specific mistranslational processes can help determine how specific amino acid substitutions may affect protein function as well as determine how these mistranslation might affect cellular physiology.

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References

- [1]. Ibba M, Soll D. Aminoacyl-tRNA synthesis. *Annual review of biochemistry*. 2000; 69:617–50.
- [2]. Ribas de Pouplana L, Santos MA, Zhu JH, Farabaugh PJ, Javid B. Protein mistranslation: friend or foe? *Trends in biochemical sciences*. 2014; 39(8):355–62. [PubMed: 25023410]
- [3]. Pan T. Adaptive translation as a mechanism of stress response and adaptation. *Annual review of genetics*. 2013; 47:121–37.
- [4]. Reynolds NM, Lazazzera BA, Ibba M. Cellular mechanisms that control mistranslation. *Nature reviews. Microbiology*. 2010; 8(12):849–56. [PubMed: 21079633]
- [5]. Ling J, O'Donoghue P, Soll D. Genetic code flexibility in microorganisms: novel mechanisms and impact on physiology. *Nature reviews. Microbiology*. 2015; 13(11):707–21. [PubMed: 26411296]
- [6]. Zaher HS, Green R. Fidelity at the molecular level: lessons from protein synthesis. *Cell*. 2009; 136(4):746–62. [PubMed: 19239893]
- [7]. Ling J, Reynolds N, Ibba M. Aminoacyl-tRNA synthesis and translational quality control. *Annual review of microbiology*. 2009; 63:61–78.
- [8]. Schwartz MH, Pan T. Temperature dependent mistranslation in a hyperthermophile adapts proteins to lower temperatures. *Nucleic acids research*. 2016; 44(1):294–303. [PubMed: 26657639]
- [9]. Wang X, Pan T. Methionine Mistranslation Bypasses the Restraint of the Genetic Code to Generate Mutant Proteins with Distinct Activities. *PLoS genetics*. 2015; 11(12):e1005745. [PubMed: 26709516]
- [10]. Wiltout E, Goodenbour JM, Frechin M, Pan T. Misacylation of tRNA with methionine in *Saccharomyces cerevisiae*. *Nucleic acids research*. 2012; 40(20):10494–506. [PubMed: 22941646]
- [11]. Lee JY, Kim DG, Kim BG, Yang WS, Hong J, Kang T, Oh YS, Kim KR, Han BW, Hwang BJ, Kang BS, Kang MS, Kim MH, Kwon NH, Kim S. Promiscuous methionyl-tRNA synthetase mediates adaptive mistranslation to protect cells against oxidative stress. *Journal of cell science*. 2014; 127(Pt 19):4234–45. [PubMed: 25097229]
- [12]. Jones TE, Alexander RW, Pan T. Misacylation of specific nonmethionyl tRNAs by a bacterial methionyl-tRNA synthetase. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(17):6933–8. [PubMed: 21482813]
- [13]. Netzer N, Goodenbour JM, David A, Dittmar KA, Jones RB, Schneider JR, Boone D, Eves EM, Rosner MR, Gibbs JS, Embry A, Dolan B, Das S, Hickman HD, Berglund P, Bennink JR, Yewdell JW, Pan T. Innate immune and chemically triggered oxidative stress modifies translational fidelity. *Nature*. 2009; 462(7272):522–6. [PubMed: 19940929]
- [14]. Ling J, Soll D. Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(9):4028–33. [PubMed: 20160114]
- [15]. Fan Y, Wu J, Ung MH, De Lay N, Cheng C, Ling J. Protein mistranslation protects bacteria against oxidative stress. *Nucleic acids research*. 2015; 43(3):1740–8. [PubMed: 25578967]
- [16]. Dittmar KA, Mobley EM, Radek AJ, Pan T. Exploring the regulation of tRNA distribution on the genomic scale. *Journal of molecular biology*. 2004; 337(1):31–47. [PubMed: 15001350]
- [17]. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic acids research*. 1997; 25(5):955–64. [PubMed: 9023104]
- [18]. Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJ, Yoo HS, Zhang C, Zhang Y, Sobral BW. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic acids research*. 2014; 42:D581–91. (Database issue). [PubMed: 24225323]
- [19]. Goodenbour JM, Pan T. Diversity of tRNA genes in eukaryotes. *Nucleic acids research*. 2006; 34(21):6137–46. [PubMed: 17088292]
- [20]. Geslain R, Pan T. Functional analysis of human tRNA isodecoders. *Journal of molecular biology*. 2010; 396(3):821–31. [PubMed: 20026070]

- [21]. Yoshihisa T. Handling tRNA introns, archaeal way and eukaryotic way. *Frontiers in genetics*. 2014; 5:213. [PubMed: 25071838]
- [22]. Hou YM. CCA addition to tRNA: implications for tRNA quality control. *IUBMB life*. 2010; 62(4):251–60. [PubMed: 20101632]
- [23]. Dittmar KA, Goodenbour JM, Pan T. Tissue-specific differences in human transfer RNA expression. *PLoS genetics*. 2006; 2(12):e221. [PubMed: 17194224]
- [24]. Dittmar KA, Sorensen MA, Elf J, Ehrenberg M, Pan T. Selective charging of tRNA isoacceptors induced by amino-acid starvation. *EMBO reports*. 2005; 6(2):151–7. [PubMed: 15678157]
- [25]. Zaborske JM, Narasimhan J, Jiang L, Wek SA, Dittmar KA, Freimoser F, Pan T, Wek RC. Genome-wide analysis of tRNA charging and activation of the eIF2 kinase Gcn2p. *The Journal of biological chemistry*. 2009; 284(37):25254–67. [PubMed: 19546227]
- [26]. Parisien M, Wang X, Perdriest G 2nd, Lamphear C, Fierke CA, Maheshwari KC, Wilde MJ, Sosnick TR, Pan T. Discovering RNA-protein interactome by using chemical context profiling of the RNA-protein interface. *Cell reports*. 2013; 3(5):1703–13. [PubMed: 23665222]
- [27]. Starck SR, Jiang V, Pavon-Eternod M, Prasad S, McCarthy B, Pan T, Shastri N. Leucine-tRNA initiates at CUG start codons for protein synthesis and presentation by MHC class I. *Science*. 2012; 336(6089):1719–23. [PubMed: 22745432]
- [28]. Gomes AC, Kordala AJ, Strack R, Wang X, Geslain R, Delaney K, Clark WC, Keenan R, Pan T. A dual fluorescent reporter for the investigation of methionine mistranslation in live cells. *RNA*. 2016; 22(3):467–76. [PubMed: 26729921]
- [29]. Wolfe CL, Warrington JA, Davis S, Green S, Norcum MT. Isolation and characterization of human nuclear and cytosolic multisynthetase complexes and the intracellular distribution of p43/EMAPII. *Protein science : a publication of the Protein Society*. 2003; 12(10):2282–90. [PubMed: 14500886]
- [30]. Hammamieh R, Yang DC. Magnesium ion-mediated binding to tRNA by an amino-terminal peptide of a class II tRNA synthetase. *The Journal of biological chemistry*. 2001; 276(1):428–33. [PubMed: 11035022]
- [31]. Krauss G, Pingoud A, Boehme D, Riesner D, Peters F, Maas G. Equivalent and non-equivalent binding sites for tRNA on aminoacyl-tRNA synthetases. *European journal of biochemistry / FEBS*. 1975; 55(3):517–29.
- [32]. Rigler R, Pachmann U, Hirsch R, Zachau HG. On the interaction of seryl-tRNA synthetase with tRNA Ser. A contribution to the problem of synthetase-tRNA recognition. *European journal of biochemistry / FEBS*. 1976; 65(1):307–15.
- [33]. Pachmann U, Cronvall E, Rigler R, Hirsch R, Wintermeyer W, Zachau HG. On the specificity of interactions between transfer ribonucleic acids and aminoacyl-tRNA synthetases. *European journal of biochemistry / FEBS*. 1973; 39(1):265–73.
- [34]. Wu J, Fan Y, Ling J. Mechanism of oxidant-induced mistranslation by threonyl-tRNA synthetase. *Nucleic acids research*. 2014; 42(10):6523–31. [PubMed: 24744241]
- [35]. Javid B, Sorrentino F, Toosky M, Zheng W, Pinkham JT, Jain N, Pan M, Deighan P, Rubin EJ. Mycobacterial mistranslation is necessary and sufficient for rifampicin phenotypic resistance. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111(3):1132–7. [PubMed: 24395793]

Fig. 1. tRNA microarray methodology

(i) A single radiolabeled amino acid (^{35}S -methionine is depicted) is used to charge purified total tRNA *in vitro* by an aminoacyl-tRNA synthetase. Alternatively, cells are pulse-labeled with the radiolabeled amino acid, which cells uptake and charge to tRNAs *in vivo*. (ii) The radiolabeled amino acid can be charged to cognate or noncognate tRNAs depending on the conditions. (iii) Total tRNA that has been charged with a radiolabeled amino acid is hybridized to a tRNA array, which contains probes for all tRNAs within an organism. (iv) The array is exposed to a phosphorimaging plate and signals will be obtained for cognate or noncognate tRNAs that have been charged with the radiolabeled amino acid.

Fig. 2. tRNA microarray printing station

The station shown is underneath a small plexiglass hood to prevent dust accumulation and also to control humidity during the printing process. During array printing, the Floating Pin Replicator dips its pins into the 384-well printer plate containing the tRNA probes. The Glass Slide Indexing System then guides the pins of the Floating Pin Replicator to apply the probes to precise locations on the array. The Glass Slide Replicator Wash & Blot station is used to wash the pins of the Floating Pin Replicator when new section of the 384-well printer plate is being utilized in order to prevent probe cross-contamination. The Replicator Pin Drier accelerates the pin drying after they are washed.

Fig. 3. Example printer plate and corresponding array layout

A. Diagram of a 384-well printer plate showing a 64 probe arrangement in the first two sections (labeled A and B) of the plate. The individual array printing coordinates (X = horizontal, Y = vertical coordinates) used for each section of the 384-well plate (A&B) on the Glass Slide Indexing System to replicate the probes are shown. **B.** The corresponding array layout from the probe distribution and array printing scheme shown in panel **A**.

Fig. 4. Calcium decreases the fidelity of the *E. coli* methionyl-tRNA synthetase in vitro
E. coli tRNA microarrays showing the increase in nonmethionyl-tRNA signal corresponding to the increased misacylation of noncognate tRNAs with methionine upon increasing Ca^{2+} concentration. The array layout on the right shows the locations of the initiator (fMet) and elongator tRNA^{Met} probes. At zero Ca^{2+} , the two dominant mischarged tRNAs are tRNA^{Arg}(CCU) and tRNA^{Thr}(CGU) as described previously [12]. Complete identity and location of tRNA array probes can be found in Table 1.

Table 1

All tRNAs isoacceptors with unique sequences in the *E. coli* genome with their corresponding nomenclature in the literature, anticodon, gene copy number, and position on the array designed in Fig. 3 and subsequently utilized in Fig. 4.

Amino Acid	tRNA Name	Anticodon	Gene copy number	Array probe position
Ala	Ala-1	UGC	3	2
	Ala-2	GGC	2	1
Arg	Arg-2	ICG	4	4
	Arg-3	CCG	1	5
	Arg-4	UCU	1	3
	Arg-5	CCU	1	6
	Asn	GUU	4	8
Asp	Asp	GUC	3	7
Cys	Cys	GCA	1	9
Gln	Gln-1	UUG	2	12
	Gln-2	CUG	2	12
Glu	Glu	UUC	4	11
Gly	Gly-1	CCC	1	14
	Gly-2	UCC	1	15
	Gly-3	GCC	4	13
His	His	GUG	1	10
Ile	Ile-1	GAU	3	16
	Ile-2	CAU	2	17
Leu	Leu-1	CAG	4	19
	Leu-2	GAG	1	20
	Leu-3	UAG	1	22
	Leu-4	CAA	1	18
	Leu-5	UAA	1	21
Lys	Lys	UUU	6	23
Met	fMet	CAU	4	24
	Met	CAU	2	25
Phe	Phe	GAA	2	29
Pro	Pro-1	CGG	1	27
	Pro-2	GGG	1	28
	Pro-3	UGG	1	26
Sec	Sec	UCA	1	34
Ser	Ser-1	UGA	1	33
	Ser-2	CGA	1	30
	Ser-3	GCU	1	31
	Ser-5	GGA	2	32
Thr	Thr-1/3	GGU	2	35
	Thr-2	CGU	2	36

Amino Acid	tRNA Name	Anticodon	Gene copy number	Array probe position
Trp	Thr-4	UGU	1	37
	Trp	CCA	1	39
	Tyr	GUA	3	38
	Val-1	UAC	5	41
	Val-2A	GAC	1	40
	Val-2B	GAC	1	40