Methods 113 (2017) 27–33

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Determining the fidelity of tRNA aminoacylation via microarrays

Michael H. Schwartz, Tao Pan *

Department of Biochemistry & Molecular Biology, University of Chicago, Chicago, IL 60637, USA
Committee on Microbiology, University of Chicago, Chicago, IL 60637, USA

ARTICLE INFO

Article history:
Received 11 July 2016
Received in revised form 30 August 2016
Accepted 12 September 2016
Available online 14 September 2016

Keywords:
tRNA
Aminoacylation
Mistranslation
Microarray

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.

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).

* Corresponding author at: Department of Biochemistry & Molecular Biology, University of Chicago, Chicago, IL 60637, USA.
E-mail address: taopan@uchicago.edu (T. Pan).

http://dx.doi.org/10.1016/j.ymeth.2016.09.004
1046-2023/

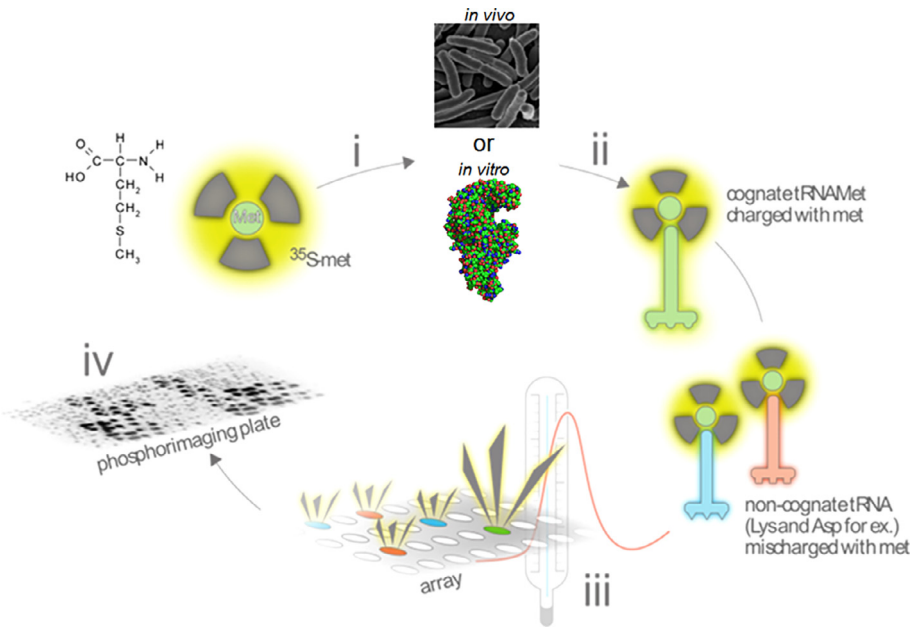

Fig. 1. *tRNA microarray methodology.* (i) A single radiolabeled amino acid (^{35}S-methionine is depicted) is used to charge purified total tRNA *in vitro* by an aminoacyl-tRNA synthetase. Alternatively, cells are pulse-labeled with the radiolabeled amino acid, which cells uptake and charge to tRNAs *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.

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 μL 200 mM KCl/50 mM KOAc, pH 7.
2. Transfer the dissolved oligonucleotide to a labeled 1.5 mL tube and add 1 mL of ethanol before refrigerated centrifugation (4 °C) at max speed for 30 min.
3. Aspirate the supernatant, speedvac dry briefly, and resuspend each oligonucleotide pellet in 100 μL of deionized water before spectrophotometrically measuring the concentrations either by nanodrop or dilution in a 96-well UV compatible plate and measuring UV_{260} 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)