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tRNA misacylation with methionine in the mouse gut microbiome *in situ*

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Abstract

Global protein mistranslation with methionine has been shown to be a conserved biological process that affords distinct functional advantages in all three domains of life. In all instances, methionine mistranslation occurs through a regulated process where low-fidelity forms of methionyl-tRNA synthetase are conditionally induced to mischarge non-methionyl-tRNAs with methionine followed by the utilization of the misacylated tRNAs in translation. In mammals, methionine mistranslation contributes to oxidative stress response; in the hyperthermophilic archaeon *Aeropyrum pernix*, methionine mistranslation produces proteins that are better adapted to low temperature growth; in *E. coli*, methionine mistranslation increases resistance to antibiotics and chemical stressors. The phenotypic benefits conferred by tRNA mismethionylation suggest that it should be a widespread adaptational mechanism in diverse bacterial lineages, yet this response has only been described in *E. coli*. Furthermore, previous microbial investigations on this response have been confined to axenic laboratory cultures. It was unknown whether tRNA mismethionylation was relevant in a natural microbial habitat. Here we show that four abundant gut microbial genera belonging to the Firmicutes and Bacteroidetes phyla perform constitutive tRNA misacylation with methionine in the mouse cecum *in situ*. These results reveal the ubiquity of the tRNA mismethionylation process among bacteria and implicate the potential importance of this response for subsistence and adaptation in natural habitats.

Keywords

tRNA; misacylation; microbiome; mouse

Introduction

Inducible protein mistranslation with methionine is a cellular response that intentionally reduces translational fidelity on a global scale when activated. In all described cases, this response is mediated by the misacylation of noncognate tRNAs with methionine by the methionyl-tRNA synthetase [1–5]. Inducible tRNA misacylation and associated methionine

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Conflict of Interest

The authors declare that they have no conflict of interest.

mistranslation has been found in all three domains of life and a beneficial function for this process has been described in each case [2, 4–6]. tRNA misacylation has been extensively characterized in *E. coli* through axenic laboratory cultivation [5]. However, since *E. coli* naturally resides in the mammalian colon [7], laboratory findings are not an accurate representation of the conditions and frequencies with which this response would be naturally utilized. Intriguingly, *E. coli* performs constitutive tRNA misacylation under anaerobiosis in axenic culture, which is partly representative of the anaerobic conditions in its natural colonic habitat [8]. Despite being the most studied model organism, *E. coli* is a very minor component of the gut microbiome (<0.1%, [9]), which precludes its direct examination *in situ*. To determine whether methionine mistranslation could indeed be a relevant response for subsistence in the gut microbiome—as anaerobic tRNA misacylation in *E. coli* would suggest—we chose to examine potential tRNA misacylation with methionine in prevalent bacterial genera which are present in assayable quantities in the mouse cecum.

The ability to perform tRNA misacylation by the dominant gut microbiota would suggest that this response provides an advantage to organisms in the gut. Due to the complexity of the gut microbiome, few techniques are available to study the distinct physiologies of the resident microbes. Obtaining more comprehensive information about the microbial physiology that occurs within the gut microbiome requires new approaches [10, 11]. Here we adapt our tRNA microarray method that was extensively used for pure laboratory culture studies to examine tRNA mischarging with methionine in the mouse gut microbiome. These results report an activity in the microbiome that is not accessible through metagenomic sequencing.

Results and Discussion

To investigate whether the prevalent genera in the mouse gut microbiome are capable of mischarging methionine to nonmethionyl-tRNAs, we adapted our previously developed ³⁵S-methionine pulse-labeling and microarray method to the mouse gut microbiome consortium [1–3, 5, 12]. The fidelity of tRNA charging reactions for ³⁵S-methionine pulse-labeled samples can be determined via radioactive phosphorimaging of tRNA microarrays that can separate ³⁵S-methionine charged tRNA isoacceptors based on sequence specific array hybridization. To mimic the *in situ* anaerobic conditions of the mouse cecum microbiome, mouse intestine was tightly sealed with strings immediately upon surgery, and the cecal contents were transferred to test tubes in an anaerobic glove box prior to pulse labeling with ³⁵S-methionine. Total charged tRNA was then isolated for direct hybridization to custom made tRNA microarrays [12].

Our tRNA microarrays contain DNA probes that are complementary to the annotated full-length tRNAs from the organism of interest minus the universal 3' CCA of tRNA. This array design reduces the complications derived from the interference of tRNA modifications, but also reduces the resolution to ~8 nucleotide differences between tRNAs [13, 14]. Since tRNA genes cannot yet be fully annotated in microbiome samples, we focused on designing probes for four major bacterial genera known to be highly abundant in the mouse gut microbiome from meta-analysis [15]. These are *Parabacteroides* from the Bacteroidetes phylum and *Turicibacter*, *Lactobacillus*, and *Blautia* from the Firmicutes phylum (Figure

1A). The limit of 4 genera was necessary since our array can accommodate a maximum of 192 probes, each repeated 4 times for a total of 768 spots. We first identified tRNA sequences from one representative species from each genus that has complete genome sequences from the Genomic tRNA Database (www.gtrnadb.ucsc.edu) [16] and from the Pathosystems Resource Integration Center (www.patricbrc.org) [17], *distasonis* for *Parabacteroides*, *HGF1* for *Turicibacter*, *johnsonii* for *Lactobacillus*, and *hansenii* for *Blautia*. tRNA probes were then designed from these tRNA sequences, yielding a total of 186 probes for these 4 genera. We also added 4 mouse tRNA probes of tRNA^{iMet}, tRNA^{eMet}, tRNA^{Lys} CTT and tRNA^{Lys} TTT as well as tRNA^{iMet} and tRNA^{eMet} probes from *Saccharomyces cerevisiae* as controls. More detailed information on probe design is included in the Methods section under “microarray probe design” and in supplementary figure S1.

We analyzed the gut microbiome from the most commonly used laboratory strains of mice, BALB/c and Black 6, at both 10 weeks and 8 months to survey multiple representative mouse samples. Intriguingly, all microbiota analyzed collectively mischarged methionine to nonmethionyl-tRNAs *in situ* (Figure 1B). No signal was detected for any of the control probes, including the mouse tRNA^{iMet}, tRNA^{eMet} probes (Figure 1C), indicating that our RNA samples were not contaminated with those from the host cells. Like previous studies, we performed two controls to ensure that the signals derived from nonmethionyl-tRNA probes are from authentic, mischarged tRNAs. Firstly, crosshybridization of cognate tRNA^{Met}s is ruled out by the addition of a large excess of all unbound tRNA^{iMet} and tRNA^{eMet} probes during array hybridization. This eliminates all cognate tRNA^{Met} signals, but signals from non-cognate tRNA probes remain essentially unchanged (Figure 1D). To minimize the possibility that signals from non-Met tRNA probes are derived from crosshybridization of unknown bacterial tRNA^{Met} species, we expanded the tRNA^{iMet} and tRNA^{eMet} probes from 4 to 10 major genera on the array (Figure S1) [15]. tRNA^{iMet} and tRNA^{eMet} probes from all 10 genera were included in the crosshybridization control. Secondly, we ruled out signals derived from ³⁵S-methionine tRNA thio-modifications by performing chemical deacylation of the tRNA sample prior to array hybridization (Figure 1E).

Interestingly, each bacterial genus charged methionine to some common tRNAs as well as tRNAs of different amino acid identities and at variable degrees (Figure 2). Each genus had comparable types and degrees of tRNA misacylation in all mice investigated (Figure 2), which strongly suggests that tRNA misacylation is extensively present in the mouse gut microbiome. Despite the universal presence of tRNA misacylation in all mice investigated, there were discernable differences in the misacylation profiles between mice. Further investigation is required to determine if these differences in tRNA misacylation are in fact specific to certain mice strains or ages. In *E. coli*, tRNA mismethionylation is constitutively present when cultured under anaerobic conditions, but absent when cultured under aerobic conditions [5]. The pervasive mismethionylation by diverse genera in the anaerobic gut microbiome environment is consistent with tRNA misacylation observed during anaerobiosis in *E. coli*.

At this time, it remains unclear how tRNA mismethionylation is regulated in the microbiome. In the known cases, tRNA mismethionylation is regulated by the methionyl-tRNA synthetase (MetRS) whose fidelity is altered through serine phosphorylation, growth temperature, and lysine-succinylation [2, 4, 5]. These previous results indicate that multiple, diverse mechanisms may exist to tune MetRS fidelity in the microbiome. In *E. coli*, global tRNA misacylation is induced by anaerobiosis and antibiotic exposure and this response broadly increases stress resistance in the laboratory [5]. tRNA misacylation may also afford similar advantages to other bacteria in their natural habitats. Organisms must compete with one another for space and resources in the mammalian colon [18] and the constitutive utilization of tRNA misacylation suggests that this response may enhance fitness in complex environments such as the mammalian gut. The common presence of tRNA misacylation in these phylogenetically distant organisms from the Firmicutes and Bacteroidetes phyla suggests that tRNA misacylation and its potential role in mistranslation is a wide-spread bacterial response that may promote effective subsistence and adaptation in natural habitats.

Materials and methods

Microbiome tRNA charging

For microbiome pulse labeling, fresh mouse cecum was obtained from Taconic mice by first sealing both ends prior to harvest (to maintain anaerobic conditions) immediately after sacrifice. Sealed cecum was immediately transferred to an anaerobic glove box and cecal contents were transferred to a tube and pulse labeled for 4 minutes with 1mCi ^{35}S -methionine at 37°C before addition of 400μL of ice cold tRNA isolation buffer (0.3M NaOAc/HOAc, 10mM EDTA pH 4.8) and placement on ice. Cecum contents were pelleted briefly for 30 seconds at room temperature and washed once before resuspension in ice cold 400μL tRNA isolation buffer.

RNA extraction

0.5mL of 0.1mm glass beads was added to washed pulse-labeled microbiome samples. Mixture was vortexed for 1 minute and placed on ice for 1 minute four times for lysis. RNA was then extracted from lysed cells a total of five times by adding 400μL acetate saturated phenol chloroform pH 4.8 followed by 1 minute of vortexing and 5 minutes of centrifugation at 17,000rcf at 4°C before ethanol precipitation. RNA pellet was suspended in 10mM NaOAc/HOAc, 1mM EDTA pH 4.8.

Microarrays

tRNA microarray was created by using tRNA probes for all tRNAs from the representative gut microbiome species: *Parabacteroides distasonis*, *Turicibacter HFG1*, *Lactobacillus johnsonii*, and *Blautia hanenii* [15]. tRNA microarray analysis and controls were performed as previously described [1, 12]. For crosshybridization control, 250pmol of each tRNA^{iMet} (6 total) and tRNA^{eMet} (12 total) probes in solution were added to the RNA hybridization mix prior to array loading. 50μg RNA was loaded per array. Arrays were exposed to a phosphorimaging screen for 4 days and quantified with Image Lab 4.1 software (Bio Rad). tRNA charging calculations were performed using the median signal from the 4 individual array spots used for each isoacceptor. Percent misacylation was calculated by dividing the

median signal obtained from a tRNA isoacceptor by the combined median signal obtained from both the initiator (fMet) and elongator tRNA^{Met} (eMet) for each respective genus. Median isoacceptor signals corresponding to tRNAs of the same amino acid identity were added for percent misacylation.

Microarray probe design

Annotated tRNA sequences from representative species of bacterial genera analyzed here were obtained from Genomic tRNA Database (www.gtrnadb.ucsc.edu) [16] and from the Pathosystems Resource Integration Center (www.patricbrc.org) [17]. As previously described [13, 14], our array probes are complementary to the full-length tRNA minus the universal 3'CCA in tRNA. This design significantly reduces the interference of uncharacterized tRNA modifications in array hybridizations, but also reduces the resolution of ~8 nucleotides between tRNA sequences. An example of how this information is used for probe design is shown in Figure S1 for tRNA^{iMet} and tRNA^{eMet} from 10 bacterial genera included on the array. The sequences of these tRNA^{Met}s differ in such a way that 6 probes are sufficient to cover tRNA^{iMet} among all 10 genera, but 2 of the 6 probes represent more than one genus. Similarly, 12 probes are sufficient to cover tRNA^{eMet} among all 10 genera, but 2 of the 12 probes can represent more than one genus. This same principle is used for the probe design of all other tRNAs. All tRNA sequences and the 192 probe sequences used in this work are shown in Table S1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. tRNA misacylation in the mouse cecum

(A) Phylogenetic tree showing the relationship between genera analyzed in the mouse gut microbiome based on 16S sequences. (B) Microbiome tRNA microarray from 10 week BALB/c gut microbiome showing methionine misacylation to many tRNA species in all four genera; tRNA^{Met} and control probes are shown in the array grid. (C) Expanded array sections showing the absence of mouse tRNA^{Met} signals. (D) tRNA^{Met} cross-hybridization control using tRNA^{Met} probes from 10 bacterial genera shown in Figure S1. (E) tRNA thio-

modification control showing the elimination of all signals upon chemical deacylation of charged amino acids.

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