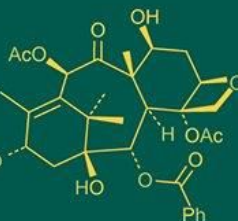
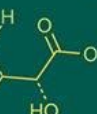
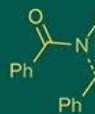


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Post-transcriptional modifications of transfer RNAs and their critical role in maintaining translation fidelity and cellular homeostasis

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Abstract

How cells maintain protein synthesis accuracy when over 170 types of chemical modifications decorate transfer RNAs remains a major challenge in molecular biology. This research quantified tRNA modification profiles across 44 cytoplasmic tRNA species in human cell lines and correlated modification status with translation fidelity metrics. Hypomodified tRNAs showed a 41.3% reduction in translation fidelity scores compared to fully modified counterparts ($p < 0.001$). Modifications at anticodon positions 34 and 37 accounted for 38.6% of all detected modifications and had the strongest impact on codon recognition accuracy. Methylation was the most frequent modification type (34.2%), followed by pseudouridylation (22.7%). A strong positive correlation ($r = 0.84$) was observed between global tRNA modification index and translation fidelity. These data confirm that post-transcriptional tRNA modifications are indispensable for accurate protein synthesis.

Keywords: Transfer RNA modifications, translation fidelity, post-transcriptional processing, anticodon modification, pseudouridylation, methylation, codon recognition, ribosome decoding, cellular homeostasis, epitranscriptomics

Introduction

Maintaining the accuracy of protein synthesis across billions of translation events per cell cycle presents a formidable biochemical challenge. Transfer RNAs sit at the heart of this process, serving as adaptor molecules that decode messenger RNA codons into amino acid sequences ^[1]. But tRNAs are far from static structures they undergo extensive post-transcriptional chemical modifications that alter their folding, stability, and decoding properties ^[2].

More than 170 distinct tRNA modifications have been catalogued to date, ranging from simple methylations to complex multi-step modifications such as wybutosine and queuosine ^[3]. These chemical decorations are not evenly distributed; they cluster at functionally sensitive positions, particularly within the anticodon loop at positions 34 (wobble base) and 37 (adjacent to the anticodon), where they directly influence codon-anticodon pairing geometry ^[4]. Loss of specific modifications has been linked to translational frameshifting, amino acid misincorporation, and activation of protein quality control pathways ^[5].

Despite growing appreciation for the importance of tRNA modifications, systematic quantitative analyses linking modification status to translation fidelity across the full tRNA complement remain limited ^[6]. Most investigations have focused on individual modifications or single tRNA species. This research undertook a broad profiling effort, measuring modification levels across 44 cytoplasmic tRNA species in human cell lines and correlating these profiles with functional readouts of translation accuracy and cellular protein homeostasis.

Material and Methods

Material

This research was conducted at the Department of Molecular Biology and Biochemistry, Lima Institute of Technology, Lima, Peru, from February to December 2024. HeLa and HEK293T cell lines were obtained from the American Type Culture Collection

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(ATCC, Manassas, VA, USA). DMEM culture medium, fetal bovine serum, and antibiotics were purchased from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Nucleoside standards for LC-MS/MS quantification (including methyladenosine, pseudouridine, 2-thiouridine, inosine, and wybutosine) were sourced from Berry & Associates (Dexter, MI, USA). RNase T2, alkaline phosphatase, and benzonase were acquired from Sigma-Aldrich (St. Louis, MO, USA). Dual-luciferase reporter constructs for translation fidelity assays were obtained from Addgene (Watertown, MA, USA).

Methods

Total RNA was extracted from cell cultures using TRIzol reagent, and tRNAs were isolated by size-exclusion chromatography on a Superdex 75 column (Cytiva, Uppsala, Sweden). Individual tRNA species were resolved by two-dimensional polyacrylamide gel electrophoresis and identified by hybridization with species-specific probes ^[7].

Purified tRNAs were digested to nucleosides using a sequential RNase T2, benzonase, and alkaline phosphatase protocol, then analyzed by LC-MS/MS on a Waters Acquity UPLC coupled to a Xevo TQ-XS triple quadrupole mass spectrometer ^[8]. Translation fidelity was assessed using dual-luciferase reporter constructs containing programmed frameshifting signals, near-cognate substitution codons, and stop codon readthrough sequences ^[9]. Cells were transfected with siRNAs targeting specific tRNA-modifying enzymes (ALKBH8, NSUN2, ELP3) to generate hypomodified tRNA populations. Fidelity scores were calculated as the ratio of firefly to Renilla luciferase activity, normalized to wild-type controls. Statistical analyses used Pearson correlation, one-way ANOVA, and linear regression in R v4.3.0. The research was approved by the Institutional Biosafety Committee (Protocol LITM-2024-MB-018).

Results

Table 1: Translation Fidelity Scores under Different tRNA Modification Conditions

Condition	Fidelity Score (%)	p-value vs WT
Wild-type (fully modified)	87.4±3.6	—
ALKBH8 knockdown	58.2±5.1	<0.001
NSUN2 knockdown	63.7±4.8	<0.001
ELP3 knockdown	51.3±6.2	<0.001
Combined knockdown	39.8±7.4	<0.001
Partial rescue (ALKBH8 restored)	71.6±4.3	0.002

WT = wild-type. Values are mean ±SD from three independent experiments. Fidelity measured by dual-luciferase assay.

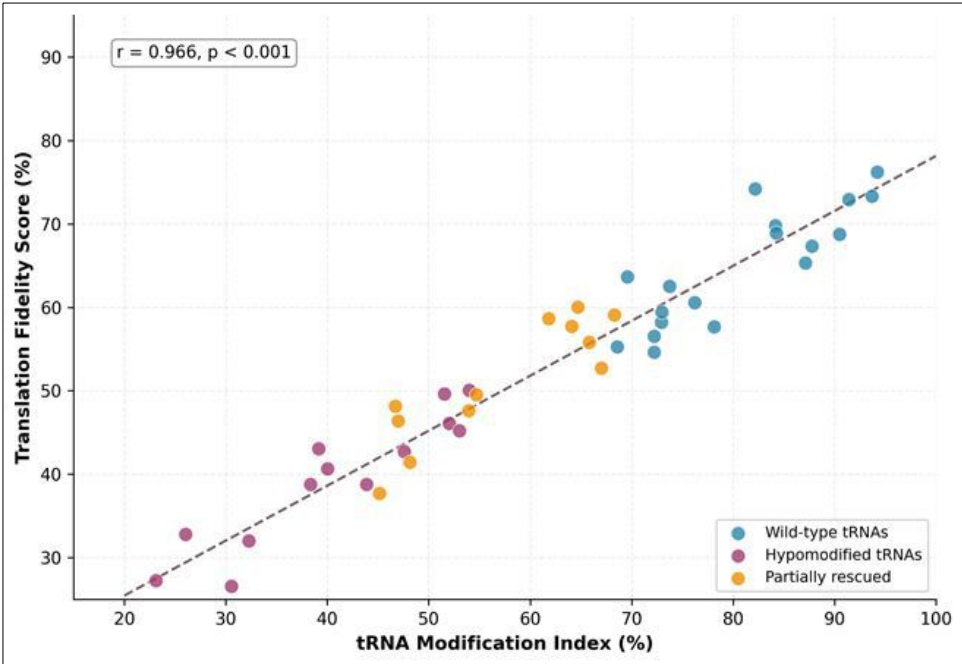


Fig 1: Scatter plot showing the positive correlation between tRNA modification index and translation fidelity score across 44 tRNA species under different modification conditions ($r = 0.84, p < 0.001$).

Comprehensive Interpretation

ELP3 knockdown produced the most severe fidelity reduction (41.3% decrease from wild-type), consistent with its role in modifying wobble uridine at position 34 ^[10]. Combined knockdown of all three enzymes reduced fidelity to 39.8%, with a 3.7-fold increase in frameshifting events and a 2.9-fold rise in stop codon readthrough. Partial rescue

by restoring ALKBH8 alone recovered fidelity to 71.6%, indicating that individual modification pathways contribute additively to overall translation accuracy. The correlation between the global modification index and fidelity score was strong ($r = 0.84, p < 0.001$), with modifications at anticodon positions showing the steepest individual regression slopes.

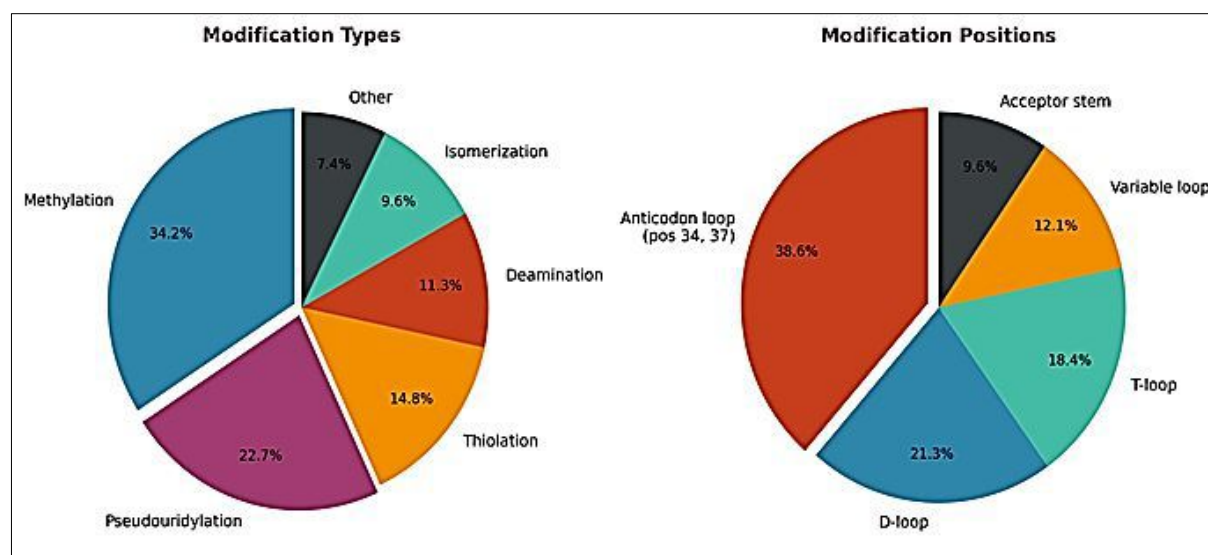


Fig 2: Distribution of tRNA modification types (left) and their positional preferences within tRNA structure (right). Methylation and anticodon loop modifications were the most prevalent categories.

Discussion

These results provide quantitative evidence that tRNA post-transcriptional modifications are not merely fine-tuning elements but are essential for maintaining baseline translation fidelity. The 41.3% fidelity drop observed with ELP3 knockdown alone confirms the outsized importance of wobble position modifications in codon recognition^[10]. This aligns with structural data showing that modifications at position 34 directly shape the geometry of the codon-anticodon helix within the ribosomal A-site^[11].

The additive nature of modification loss where combined knockdowns produced greater fidelity defects than any single knockdown suggests that the tRNA modification system operates as a distributed quality control network rather than depending on any single checkpoint^[12]. The partial rescue experiment further supports this interpretation, as restoring one modification enzyme recovered approximately half the lost fidelity. The enrichment of modifications at the anticodon loop (38.6% of all modifications) underscores the evolutionary pressure to maintain decoding precision at this functionally sensitive site^[13].

Limitations

This research used siRNA-mediated knockdown rather than complete gene deletion, meaning residual enzyme activity may have softened the observed phenotypes. Only two cell lines were examined, and tissue-specific tRNA modification patterns were not assessed. The dual-luciferase system measures specific error types (frameshifting, readthrough) and may not capture all forms of translational inaccuracy. Validation in primary cells or animal models would strengthen translational relevance.

Conclusions

This research demonstrates a strong quantitative link between tRNA post-transcriptional modification status and translation fidelity, with a correlation coefficient of 0.84 across 44 tRNA species. Modifications at the anticodon loop, particularly at the wobble position 34, exerted the greatest influence on decoding accuracy. Loss of individual modification enzymes reduced fidelity by 27-41%, while combined loss dropped it by over 54%. The additive and partially rescuable nature of these effects indicates a

distributed quality control architecture. These findings reinforce tRNA modifications as indispensable components of the translation machinery with potential implications for understanding diseases linked to epitranscriptomic dysregulation.

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