tRNA FRAGMENTS AS POSSIBLE INDICATORS OF DIFFERENT STAGES OF CELL DIFFERENTIATION

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Motivation

Stem cells can self-renew, thus maintaining an undifferentiated state, or differentiate into various cell types. **Differentiation requires dynamic protein synthesis and higher translational rates**. Transfer RNAs (tRNAs) decode messenger RNA (mRNA) codons into amino acids, enabling protein synthesis. Beyond their role in genome decoding, tRNA sequence variants (isoacceptors and isodecoders), as well as tRNA base modifications, are involved in regulatory pathways linked to tRNA fragmentation events. **tRNA fragments (tRFs)** fall into different classes based on their length and fragmentation site: **5'-tRFs, 3'-tRFs**, **i-tRFs** and **tRNA halves** (Figure 1).

tRFs regulate several biological processes, including **protein synthesis** and **cell fate determination**[1], with their modifications adding an extra layer of control that dynamically directs their activity as regulatory molecules during the acquisition of a proper cell state**. tRFs can behave similarly to microRNAs (miRNAs)**, competing for incorporation into Argonaute (AGO) proteins and forming RNA-induced silencing complexes (RISC) to regulate gene expression by interacting with the 3'UTR of mRNAs. Understanding how **distinct tRFs are associated with different stages of cell differentiation** could uncover new regulatory mechanisms and identify potential biomarkers for distinguishing different cell states.

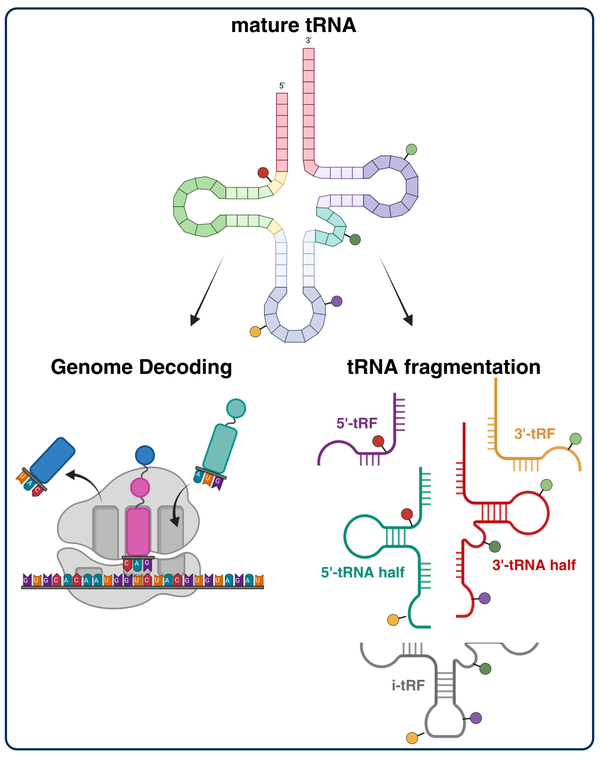


Figure 1. Chemical modifications regulate tRNA's canonical and non-canonical functions. tRNA modifications can promote or protect tRNAs from cleavage by specific endonucleases, which impacts the generation of tRNA fragments (tRFs). These tRFs are classified based on their length and cleavage site, including 5'-tRFs, 3'-tRFs, i-tRFs, and tRNA halves.

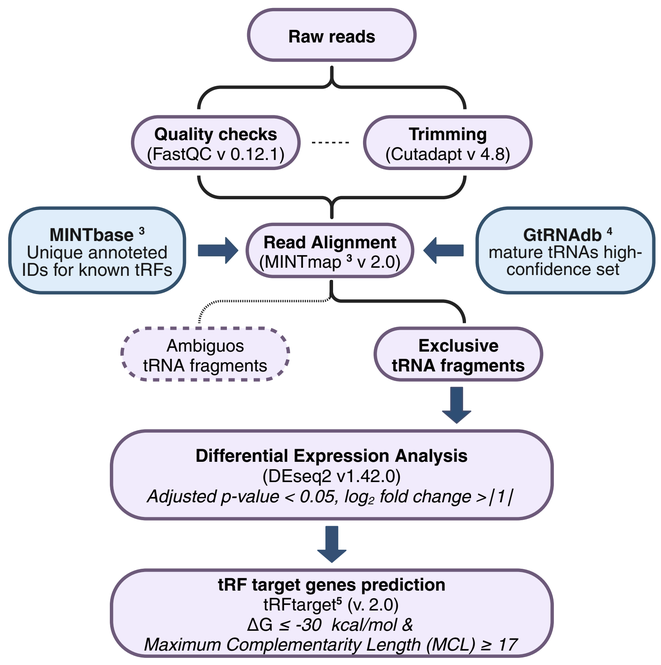
Aims of the study

- Investigate whether distinctive **tRNA fragment profiles are associated with specific cellular states and guide differentiation differently** in pluripotent stem cells.
- Explore and predict the **miRNA-like behavior of specific tRNA fragments in gene expression regulation** to assess their potential role in guiding or modulating cellular differentiation.

Data and Methods

- **Publicly available small-RNAseq dataset** [2]

- **Bioinformatics Workflow**



Results

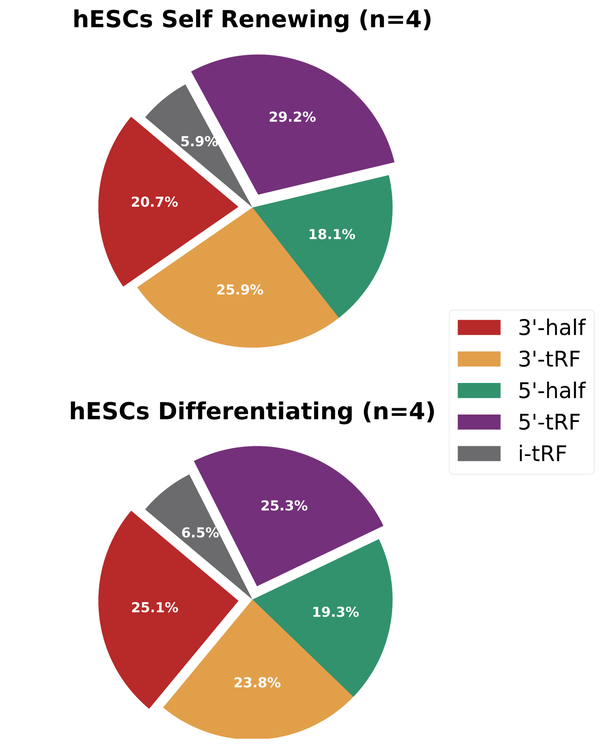


Figure 2. Distribution of tRNA fragments (tRFs) classes in hESCs Self Renewing and Differentiating. Proportion of each tRF class normalized to the total tRF counts for each cell state, with four biological replicates per group.

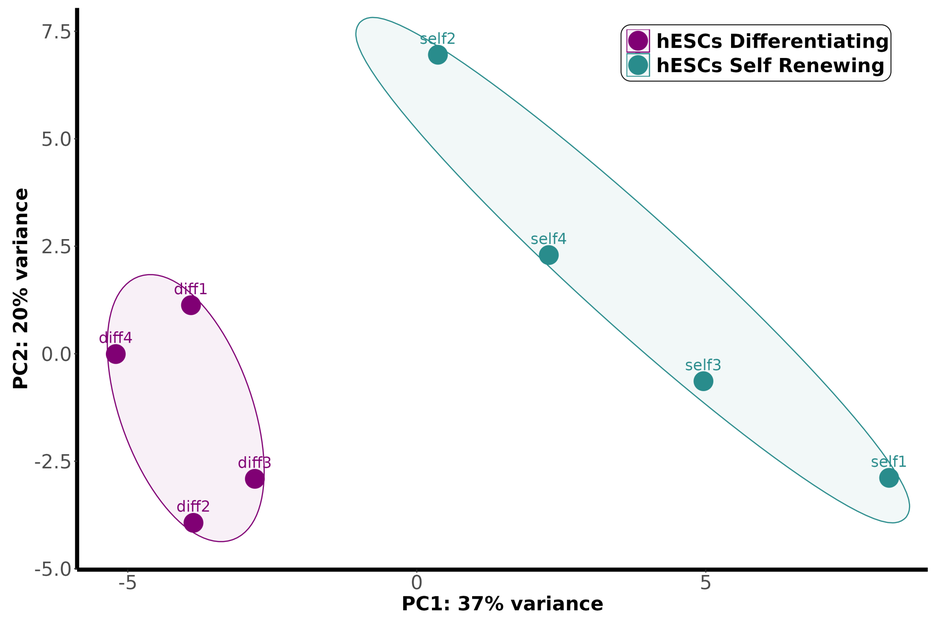


Figure 3. Principal Component Analysis (PCA) plot showing the variance distribution of the tRNA fragments in hESCs Self Renewing and Differentiating. The ellipses indicate the clustering of samples, demonstrating distinct separation between Self Renewing and Differentiating states.

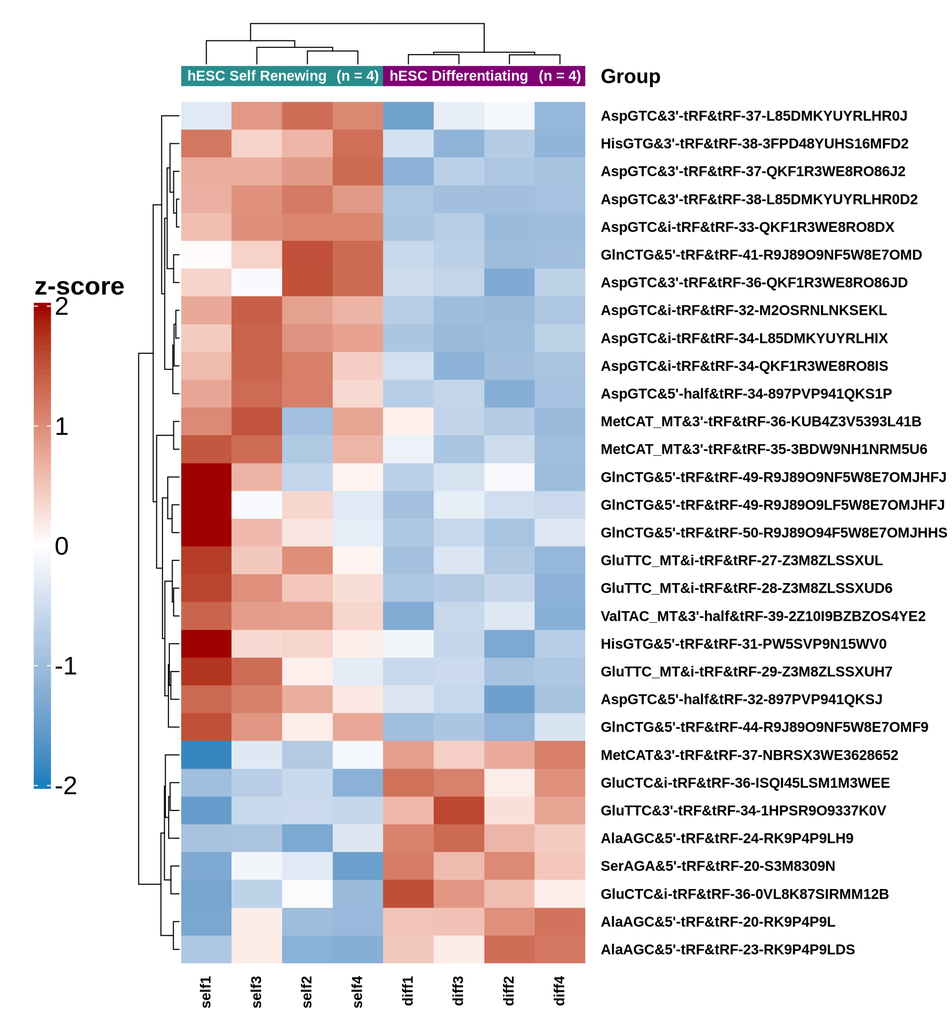


Figure 4. Hierarchical clustering heatmap analysis for differentially expressed tRNA fragments. Each row represents one tRNA fragment, and each column represents a sample, grouped by cell state. A total of 8 tRNA fragments are upregulated and 23 are downregulated in hESCs Differentiating. The analysis was filtered based on an adjusted p-value < 0.05 and a $\log_2$ fold change > |1|. The annotations of the tRNA fragments follow the license plate nomenclature provided by MINTbase[3].

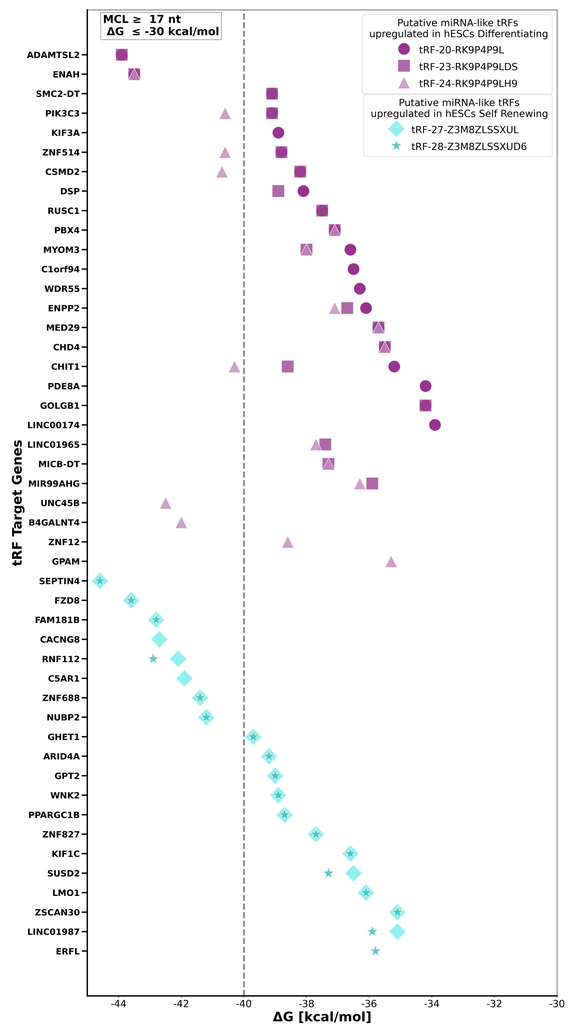


Figure 5. Predicted target genes for the putative miRNA-like differentially expressed tRNA fragments. The analysis was performed using tRFtargetv2.0 software, filtering for RNA-RNA interactions with a Gibbs Free Energy (ΔG) ≤ -30 kcal/mol and a Maximum Complementarity Length (MCL) ≥ 17 nucleotides. Interactions with ΔG ≤ -40 kcal/mol are likely to reflect strong RNA-RNA binding.

Conclusions & Future directions

This analysis enabled the **mapping and classification of tRNA fragments (tRFs)** within a small RNA-seq dataset. While tRF class distribution showed no differences across cell populations, **specific tRFs allowed for separation**, resulting in a **distinct cluster formation in differentiating cells**. The **prediction of tRFs' miRNA-like behavior revealed five differentially expressed tRNA fragments**, which are **predicted to target two separate sets of genes**, based on their upregulation in self renewing or differentiating cells. The putative target genes are implicated in key processes such as **cytoskeletal organization**, **cell cycle regulation**, **transcriptional control**, and **cellular signaling pathways**.

Future studies should **validate these predictions using high-throughput assays**, with a focus on assessing RNA stability in the predicted target genes. Additionally, these associations should be further confirmed by directly studying cell phenotypes. Overall, our data suggest that **distinct tRF profiles are specific to different cellular states**. Validating these observations could be pivotal in establishing a **potential role for tRNA fragments in regulating transitions between cell stages**.

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