

Determining the fate of engineered tRNAs in human cells by direct RNA sequencing

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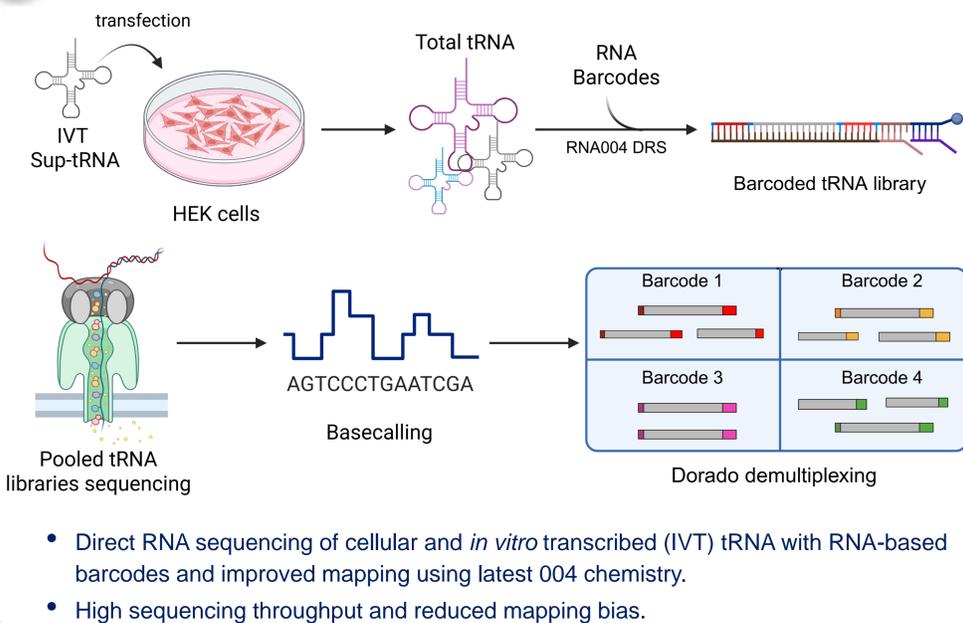
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Summary

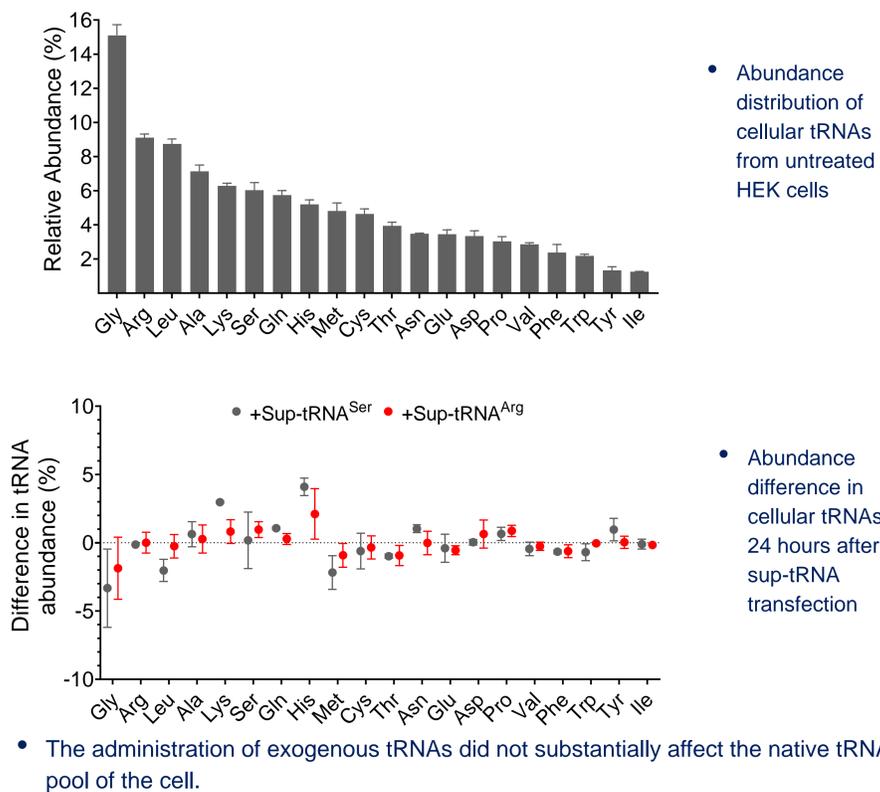
Engineered tRNAs, such as suppressor tRNAs (sup-tRNA), have a great therapeutic potential to target a variety of genetic diseases. However, the effects of these tRNAs on the cell are still unknown. We used Nanopore direct RNA sequencing with RNA-based demultiplexing and an optimized tRNA mapping strategy to assess the fate of sup-tRNAs and their effect on the cellular tRNAome. We observed that the cellular tRNA pool is not affected by exogenous sup-tRNAs. Using a basecalling error based approach we detected several modifications on the engineered sup-tRNAs. We also characterized the *in cellulo* stability and the kinetics of the modification acquisition by sup-tRNAs. These results provide a framework in application of these new sup-tRNA therapeutics.

1 Direct tRNA sequencing workflow

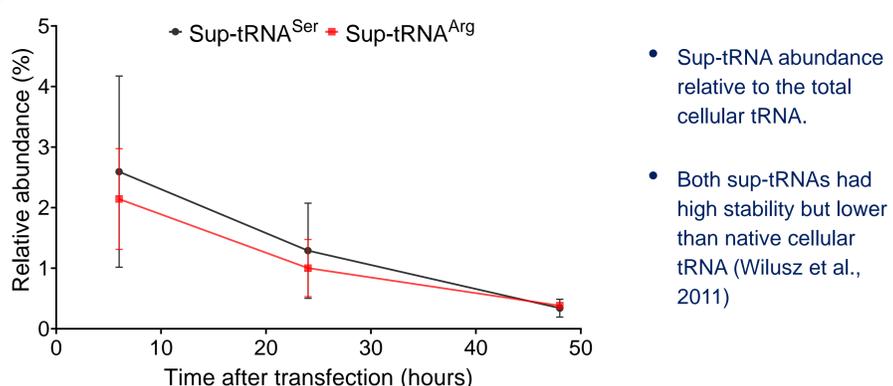


2 Cellular tRNAome is not affected by exogenous sup-tRNAs

Two different sup-tRNAs, based on serine and arginine, were transfected into HEK cells and the total tRNA pool was sequenced.

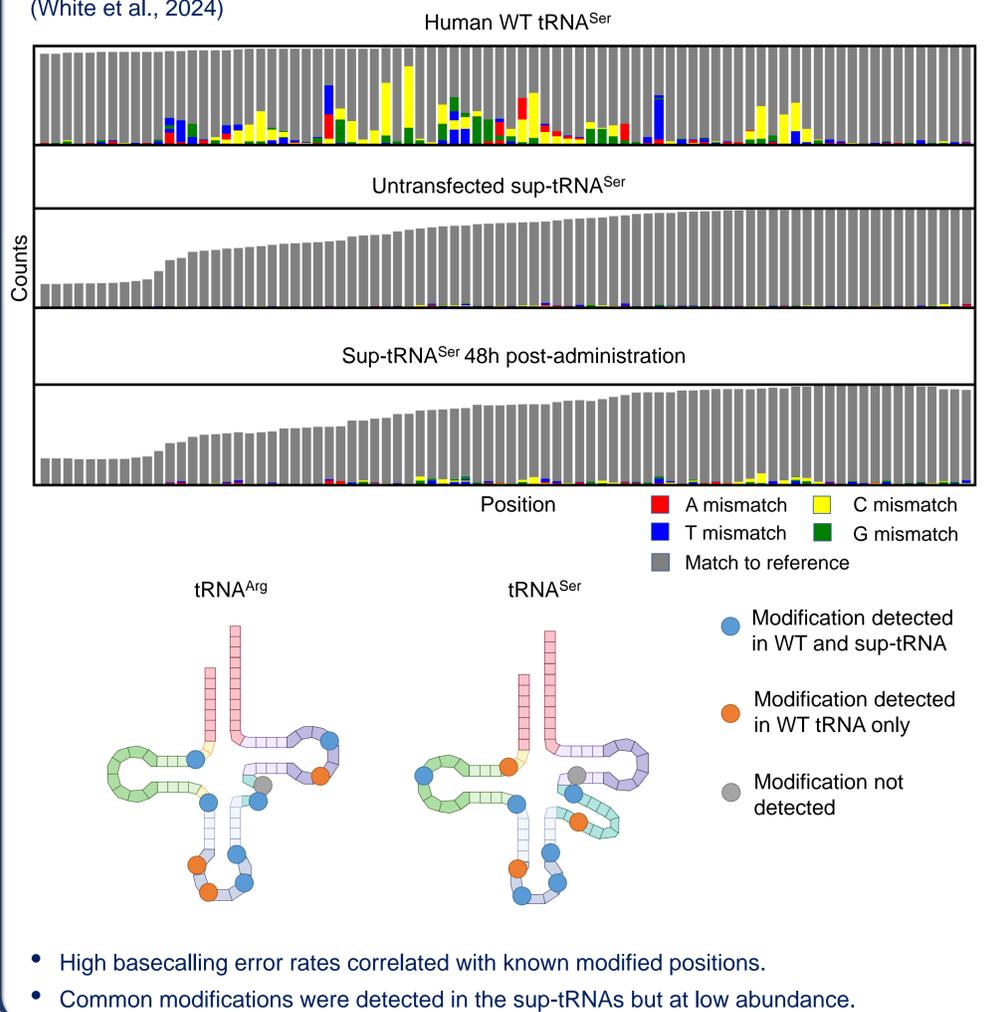


3 Sup-tRNAs are highly stable in cells

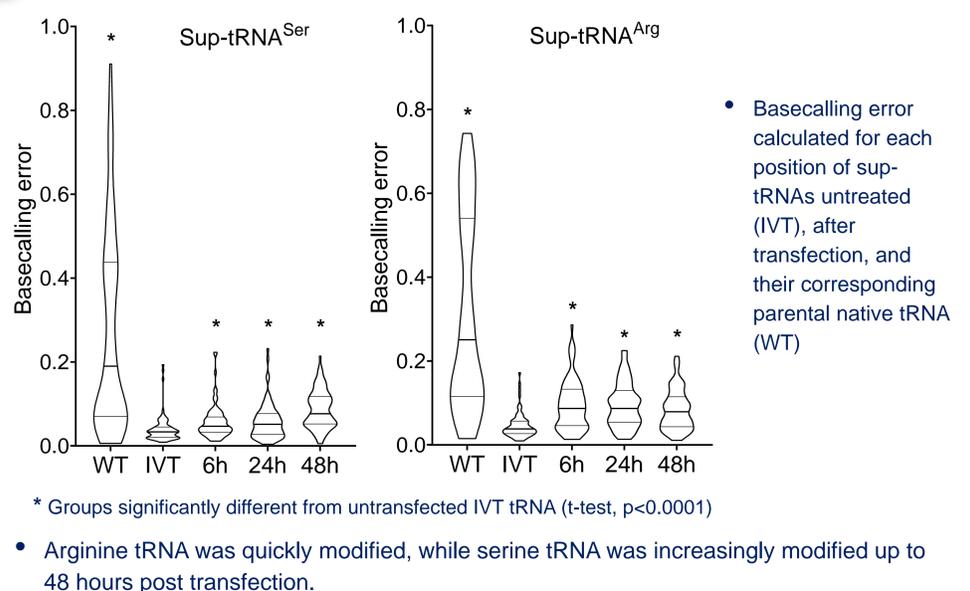


4 Sup-tRNAs are only slightly modified inside cells

Basecalling error was calculated for each position to identify modified nucleotides (White et al., 2024)



5 Sup-tRNAs are differentially modified over time



6 Conclusions

- Sup-tRNAs don't affect the native tRNAome and are stable in cells, which correlates with previous functional studies (Albers et al., 2023).
- Sup-tRNAs are modified only at a low stoichiometry by the cell machinery, suggesting that pre-administration modification of sup-tRNAs could improve their activity.
- Nanopore direct RNA sequencing is a promising tool for tRNA study and design.