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

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Engineered tRNAs designed to suppress nonsense mutations (sup-tRNAs) have a great therapeutic potential for a variety of genetic diseases. However, the effects of these tRNAs on the cell translation machinery, especially in the native tRNA pool, are still unknown. Furthermore, it remains unclear whether the sup-tRNA undergoes the same modification processes as the natural tRNAs, and how those affect sup-tRNA activity and stability. We use Nanopore direct RNA sequencing of the tRNAome to study the effect of sup-tRNAs on them and the fate of these sup-tRNAs in human cells. We combine several samples by using unique barcode sequences in RNA adapters. This allows for multiplexing and specific mapping and detection of tRNAs. We observed that the cellular tRNA pool is unaffected by administration of exogenous sup-tRNAs. Additionally, we were able to detect several modifications of the sup-tRNAs using a basecalling-error based approach. We also characterized the cellular tRNA stability and the kinetics of modifications of distinct sup-tRNAs carrying different amino acids. These results provide a framework in understanding the effect of these new sup-tRNA therapeutics. Furthermore, our new workflows for tRNA analysis expands the possibilities of RNA sequencing with Nanopore.

Authors: Mr KOËSTER, Daniel (Universität Hamburg); ALARCON RODRIGUEZ PAIVA, Rodrigo (Universität Hamburg); Prof. IGNATOVA, Zoya (Universität Hamburg)

Presenter: Mr KOËSTER, Daniel (Universität Hamburg)

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