tRNA Dynamics between the Nucleus and Cytoplasm

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The retrograde nuclear import is an important and conserved step in tRNA quality control in eukaryotes and is taken advantage of by retroviruses such as HIV to infect cells. Retrograde import occurs as the tRNA is brought back into the nucleus after being spliced. Several proteins including Mtr10 and Ssa2 were predicted to be retrograde tRNA nuclear importers according to previous fluorescence assays. Since certain types of tRNAs are methylated only when they return to the nucleus, if a protein is an actual retrograde importer, a decrease in nuclear import due to its absence would cause a decrease in methylated tRNAs. Here a real-time quantitative PCR (qPCR) and endpoint PCR assays were developed to measure the amount of tRNAs being moved in and out of the nucleus. Because methylation inhibits reverse transcription, if less tRNA is being imported, more PCR product would be detected. Endpoint PCR showed that tRNA Pro, LeuCAA, LeuUAG, and Phe followed the expected patterns of an increase in detected PCR products when the importer Mtr10 was deleted. The endpoint PCR results also showed that tRNA Trp, which receives no modification, lacks any major changes in relative concentration following PCR, thus confirming the mechanism. Results were inconclusive, however, for Ssa2. The same pattern in relative concentrations was also seen in qPCR results. In confirming previous fluorescence data, these assays show potential in accelerating retrograde import research. They may also be used for genome-wide screens in the future, improving our understanding of the pathways utilized by retroviruses.

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