doi 10.18699/CRISPR-2025-157

Mitochondrial genome manipulation using a programmable pAgo nuclease

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Key words: mitochondria; D-loop; prokaryotic argonaute proteins; mtDNA copy number

Efficient editing of the mitochondrial genome holds great promise for treating rare inherited diseases. However, current enzymatic tools are limited by the impermeability of mitochondrial membranes [1]. In this study, we introduce an alternative strategy based on prokaryotic Argonaute (pAgo) proteins, which offer key advantages over CRISPR-Cas nucleases, including smaller size, PAM-independent targeting, and the ability to use either RNA or ssDNA guides [2].

We report, for the first time, that a programmable pAgo fused to a mitochondrial targeting sequence (MTS) can be efficiently imported into human mitochondria, as demonstrated by immunocytochemical analysis. To assess whether pAgo remains functionally active after mitochondrial import, we performed a proof-of-concept experiment targeting the mitochondrial control region – specifically, the D-loop and R-loop structures – which play a central role in regulating mtDNA replication. Upon transfection with short synthetic guide RNAs designed to hybridise to these single-stranded regions, we observed a reproducible ~3-fold reduction in mtDNA copy number in HEK293T cells, as quantified by qPCR. Importantly, catalytically inactive pAgo mutants and pAgo variants lacking the mitochondrial targeting sequence did not affect mtDNA levels, indicating that both mitochondrial localisation and nuclease activity are required for the observed effect.

While further optimisation is required, these findings demonstrate the potential of programmable Argonaute systems for targeted manipulation of mitochondrial DNA and lay the groundwork for future development of compact, PAM-independent genome editing tools suited for mitochondrial applications.

Funding: The study is supported by the Russian Science Foundation, No. 25-24-00078.

References

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