THE ASSEMBLY OF ATP SYNTHASE IS IMPAIRED IN HUMAN CELLS LACKING MITOCHONDRIAL IF3

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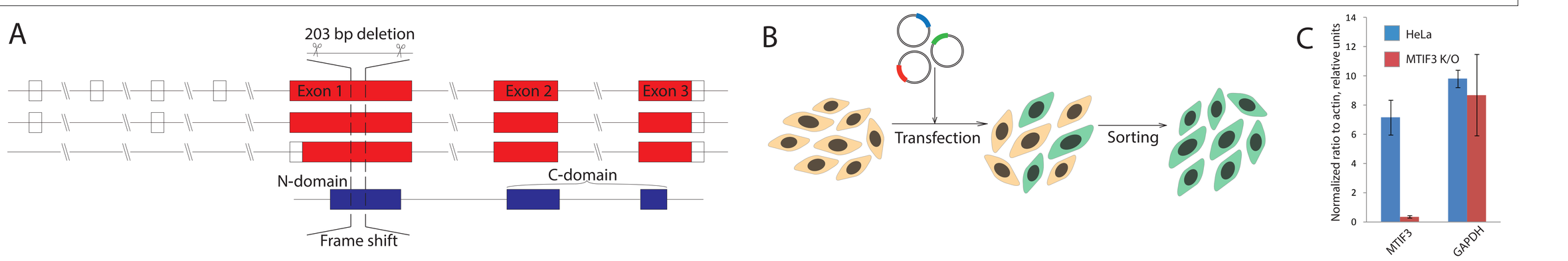
Project outline

Initiation of protein synthesis is ruled by three canonical factors, IF1, IF2 and IF3. They were studied in detail in bacteria, where they play essential role in the recruitment of the ribosome on the mRNA, correct selection and positioning of the start codon and initiator tRNA. This system persists in human mitochondria, however it functions in rather different way due to specialization and adaptation to the organellar micro-environment. We focused on human mitochondrial IF3 which was earlier studied in vitro, but no knock-out models up to date have been published. In this work we generated the human HeLa cell lines deficient in MTIF3 gene and analyzed their mitochondrial translation.

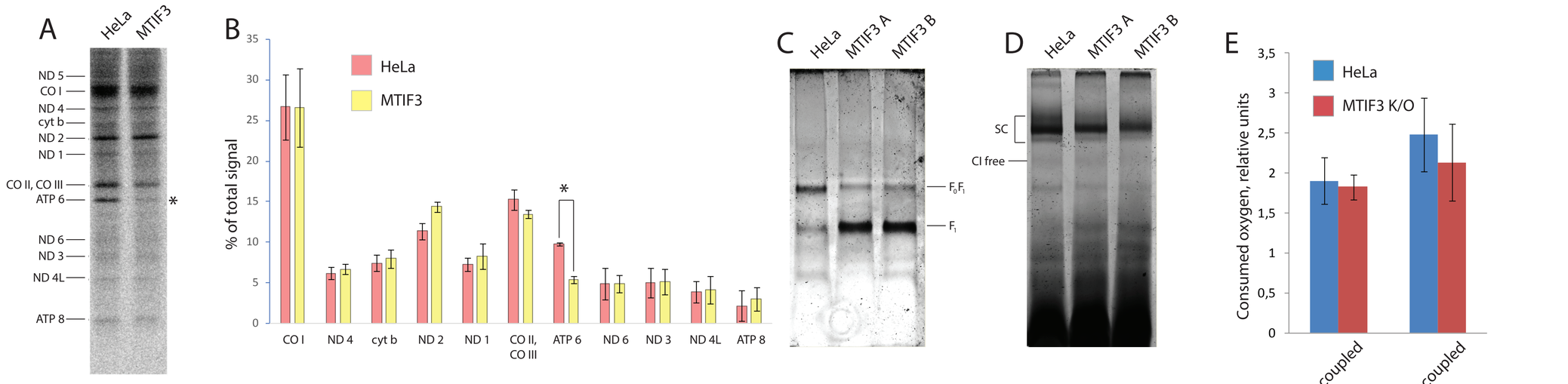
Results

Knock-out of MTIF3 gene is not lethal in human cultured cells

We applied the CRISPR/Cas9 genome editing technique to knock-out the MTIF3 gene. We designed two pairs of guide RNAs intended to cut the DNA locus of exon 1 and generate the deletion of 203 bp in the coding region, which produces the frame-shift soon after the start of translation. The sequences were inserted in gRNA expression vector pU6gRNA and transfected in HeLa cells and human fibroblasts with the vectors expressing Cas9 nuclease and EGFP. Transfected clones with bright EGFP fluorescence were selected and their DNA was analyzed by RT-qPCR and sequencing, which confirmed the presence of the intended deletion. The MTIF3 knock-out HeLa cells did not demonstrate any pronounced growth defects in normal conditions, therefore the deletion of MTIF3 is not lethal

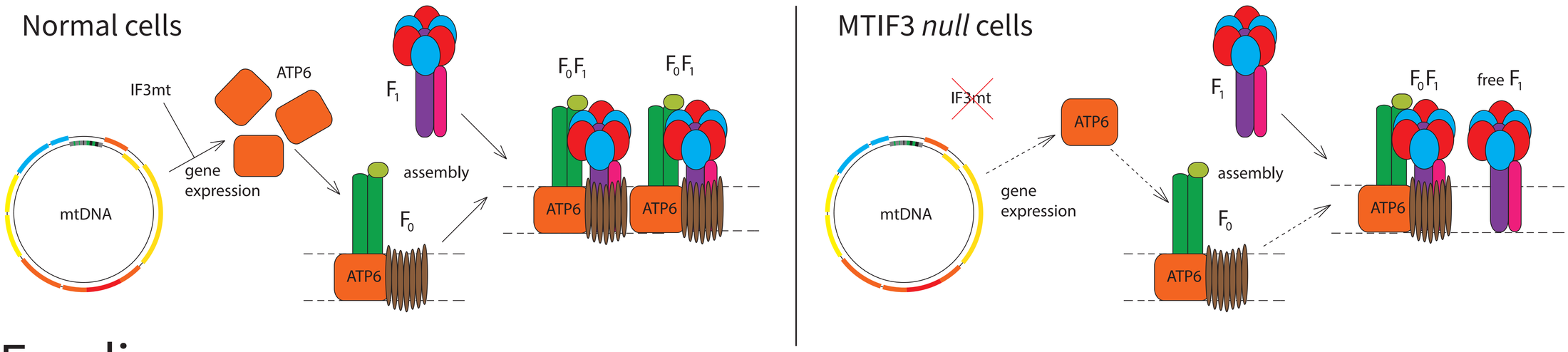


Knock-out of MTIF3 gene selectively decreases translation of ATP6 gene in mitochondria of human cultured cells and compromises the assembly of ATP synthase, but does not alter oxygen consumption and the supercomplexes



A. The profiles of mitochondrial translation. B. Calculations of the relative signals of for each translation product. C. In-gel activity of ATP synthase (complex V). D. In-gel activity of NADH-DH (complex I). E. Oxygen consumption

Current model



Funding

The work was funded by Russian Science Foundation (RSF, Grant 18-74-00016). The work of Dashinimaev E.B. was supported by a grant to creating the "Center for Precise Gene Editing and Genetic Technologies for Biomedicine" of the Ministry of Science and Higher Education of the Russian Federation. The work was supported in part by the MSU Program of Development (FACSAria SORP cell sorter).