MitomiRs as the common regulators of gene silencing

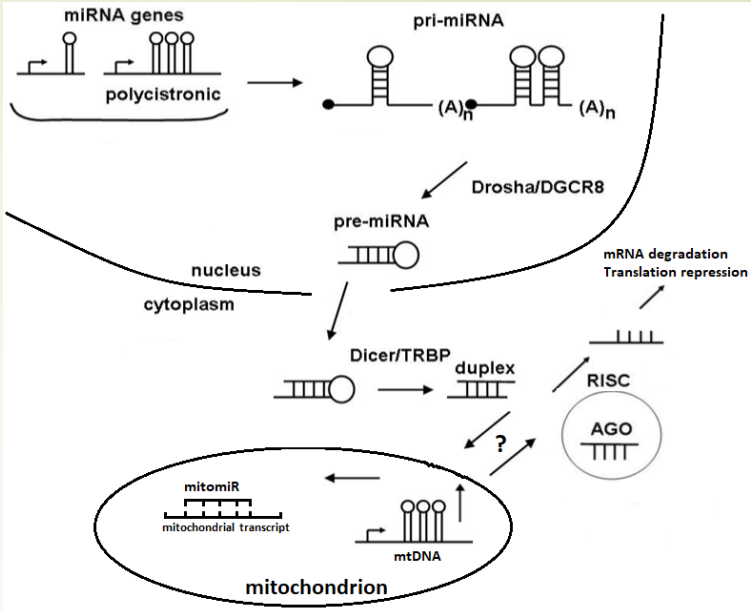
Vorozheykin Pavel[1], Vishnevsky Oleg[2], Titov Igor[1,2]

[1] *Novosibirsk State University, Novosibirsk, Russia*

[2] *Institute of Cytology and Genetics (IC&G), Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia*

Motivation and aim:

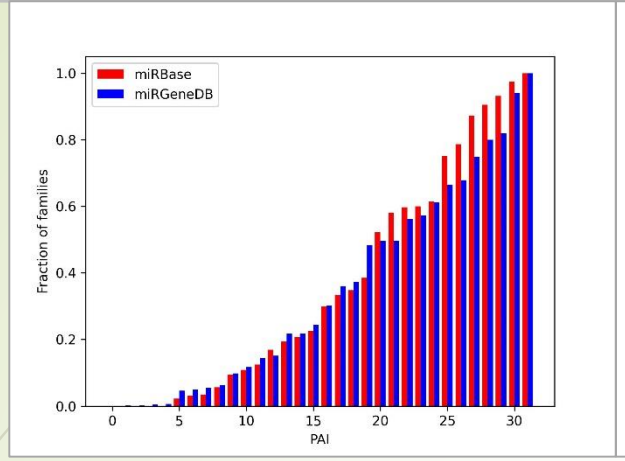
Short RNA sequences are presented in mitochondria. These observations may reveal both a nucleus miRNA translocation to mitochondria and an existence of a miRNA maturation process within mitochondria. The existence of such mitochondria associated miRNAs (the so-called mitomiRs) raises questions about their function and origin. *It is still unknown, is there a specific functions of different miRNA classes according to their cell location? Are mitomiRs originally evolved or have appeared only recently? Is there a sequence or structure difference between the mitomiRs and non-mitomiRs that provides a mitomiR translocation to the mitochondria?*

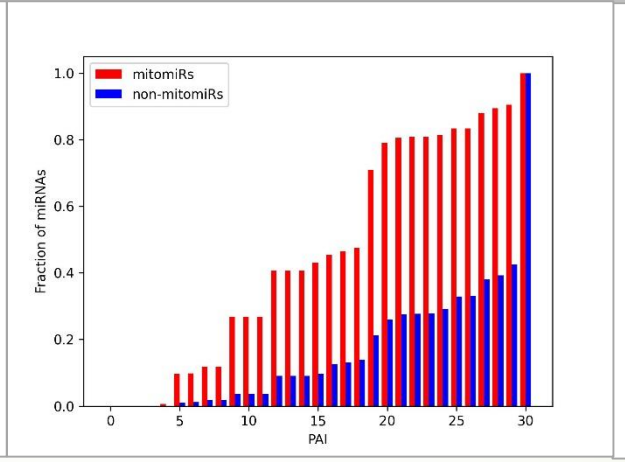


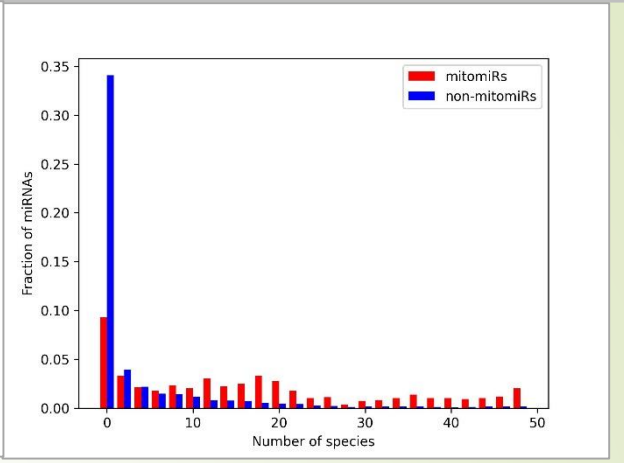
Methods and Algorithms:

- The miRNA sequences were downloaded from the miRBase database (rel.22.1) and the miRGeneDB database (rel.2.1).
- According to experimental studies we construct the set of human, mouse and rat ***mitomiRs***: the miRNA sequences that presented within mitochondria from various organisms, cell types and tissue (652 sequences). The other miRNAs of three selected species were considered as ***non-mitomiRs*** (4766 sequences).
- The homology search was carried out by comparing Hamming distance of the aligned miRNAs and selecting those miRNA pairs that differ less than 10% of the alignment length.
- To investigate the sequence and structure differences of miRNA classes we searched for statistically overrepresented oligonucleotide motifs by ARGO-program [1].
- To compare functions we explore the mitomiR and non-mitomiR targets from the experimentally validated miRNA-associated gene database miRTarBase (miRTarBase.cuhk.edu.cn, release 8.0) and investigated the relationship of the miRNA targets with the gene ontology (GO) terms from the GeneOntology.org database (on the date 2022-03-2)

1. Vishnevsky O., Kolchanov N. ARGO: a web system for the detection of degenerate motifs and large-scale recognition of eukaryotic promoters. *Nucleic Acids Research*. 2005;1(33):W417-22

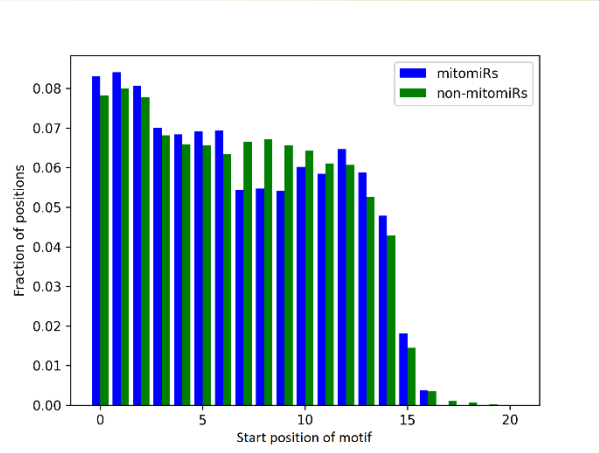






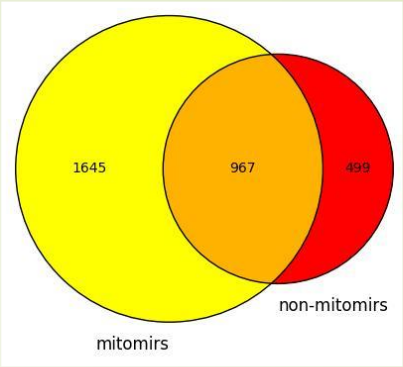
We divided all miRBase miRNAs into two sets according to their homology with the mitomiR/non-mitomiR sequences. Also we prepared two sets of the miRBase/miRGeneDB annotated pre-miRNA families. To estimate the evolution age of each mitomiRs/non-mitomiRs/miRBase/miRGeneDB classes we calculated phylostratigraphic age index (PAI, proposed in [2]) for their sequences (grouped by homology or by database annotation) and show their distribution by the PAI-values (figures left and center). Additionally for the sets of mitomiR/non-mitomiR homologs we considered the fractions of homologous miRNAs according to the number of species in which they appear (figure right). The lower PAI-value corresponds to the earlier appearance of the miRNA sequences in the course of the organismal evolution. Minimal value of the PAI (4, Metazoa) is observed only for the mitomiR dataset. We concluded that the mitomiRs are used to be more widely distributed among species than the non-mitomiRs and than an average of all miRNAs in two databases. This supports the hypothesis that mitomiRs may be recruited into mitochondria during their domestication.

Comparing the sequences of mitomiRs and non-mitomiRs we discovered several statistically overrepresented, independent oligonucleotide motifs in mitomiRs: *HASHWSBD*, *HRVRNTSH*, *RHASHWSB*, *MNTVCANK*, *HSVYDGDN* and others. For these motifs we explored location and their involvement in the pre-miRNA secondary structure. We found that the motifs tend to start in 1-3 positions of miRNA (figure left) rather then in other positions. The observations have significantly decreased in 7-9 positions of mitomiRs. Probably, the reason is that the motifs preferable cover the seed-region which represent the most conservative region of the miRNA. These motifs may be the signals for a specific miRNA processing (for example, a mitomiR translocation to the mitochondria) or may directly affect the mitochondria gene regulation. We found no patterns of secondary structure of the pre-miRNAs where the selected motifs were housed.



2. Mustafin Z.S., Zamyatin V.I., Konstantinov D.K. et all. Phylostratigraphic Analysis Shows the Earliest Origination of the Abiotic Stress Associated Genes in *A. thaliana*. *Genes.* **2019**;*10*:963.

Comparing the targets of mitomiRs and non-mitomiRs we concluded that mitomiRs are associated with a greater number of target genes than the non-mitomiRs even though the number of mitomiRs is much less than the number of non-mitomiRs (652 vs 4766). At the same time both classes have a vast set of common targets (figure right).



To explore the functions of the considered miRNAs, we performed the GO enrichment analyses for the targets lists of human mitomiRs and non-mitomiRs in three general categories "biological process", "molecular function", and "cellular component".
About 889 GO terms are related only to the mitomiRs' targets which indicates the unique features of mitomiRs. For the common GO terms of mitomiRs and non-mitomiRs targets we calculated the Fisher's exact test (p-value ≤ 0.05) and received 290 GO terms which are highly associated with one list of genes comparing to the other. All discovered GO terms are specifically enriched in list of non-mitomiR target genes.

Therefore, the mitomiR sequences can be identified as the common regulators while the non-mitomiRs appear to be specific ones.

The description of the significant miRNA features can shed light on the miRNA's evolutionary origin and on the specificities of the pri-/pre-/miRNA processing and context-structural characteristics of their sequences

Acknowledgements:

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Thank you for your attention