The emerging role of mitochondrially encoded small interfering RNAs to regulate nuclear gene expression.

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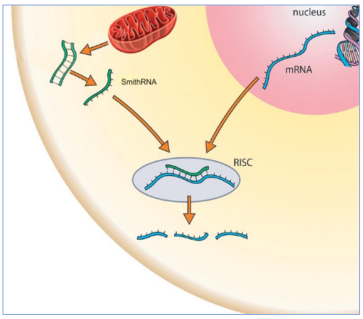
What mitocondria can do. An unexpected function.

The possibility that **mitochondrial DNA (mtDNA) can act on nuclear gene expression** has been suggested only recently: mtDNAs have been found to produce small noncoding RNAs (sncRNAs), long non-coding RNAs (lncRNAs) and peptides, all of them suggested or demonstrated to interact via different pathways with the nucleus. Our research aims at characterizing the retrograde mitochondrial-to-nucleus signaling by **mitochondrially encoded small RNAs** (called small mitochondrial highly transcribed RNAs, **smithRNAs**), in a comparative way.

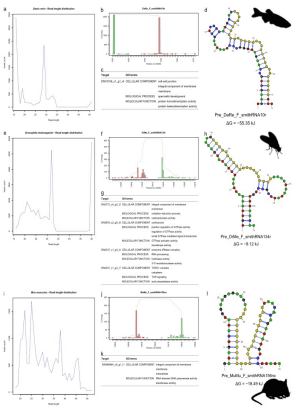


SmithRNAs regulate nuclear gene expression through Mitochondrial Retrograde Response. SmithRNAs were the first mitochondrial sncRNAs to be explicitly investigated for this role and to give positive results through *in silico* association with nuclear mRNA targets. We have already proved functionality of two smithRNAs in *Ruditapes Philippinarum* (Mollusca, Bivalvia), in which they are likely involved in gonad formation and sex determination related to Doubly Uniparental Inheritance.

We also found putative smithRNAs in gonad samples of three model species: *Drosophila melanogaster* (Ecdysozoa, Protostomia), *Danio rerio* and *Mus musculus* (Vertebrata, Deuterostomia), and many other species are under investigation at the moment, evidencing that **smithRNAs are not an 'exotic' feature of few animals, but rather a fairly distributed feature of metazoans' mitochondrial genomes.**

Overall, smithRNAs are emerging as a fast-evolving generalized new form of retrograde signaling and, potentially, a common feature among metazoans, adding a brand-new functionality level of mitochondria in the eukaryotic cell.

1 Phylogenetics distribution of smithRNAs among Metazoa.



We applied our pipeline to other metazoan species in order to conservatively recover good smithRNA candidates from other systems. *Drosophila melanogaster*, belongs to Ecdysozoa, while *R. philippinarum* belongs to Lophotrochozoa. *Mus musculus*, contrastingly with the two aforementioned protostome species, belongs to Deuterostomia. Other Metazoans species are now under investigation.

Putative smithRNAs genes are found in almost all Metazoans analyzed so far.

2 SmithRNAs role in mito-nuclear incompatibilities and speciation.

SmithRNAs must coevolve with their nuclear targets. This requires a finely tuned coevolution, which might be a trigger of speciation (through Dobzasky-Mueller postzygotic incompatibilities). We are analyzing the circum-Mediterranean genus *Bacillus* stick insects, which presents many parthenogenetic/hybridogenetic hybrids. These hybrids are always asymmetric, meaning that only *B. rossius* is the maternal species, while no stable hybrid is known to have mitochondrial genomes of the other two species, *B. atticus* and *B. grandii*. We are investigating smithRNAs of these stick insects to see if the observed hybrids features may be due to **incompatibilities based on this peculiar mitonuclear coevolution mechanism.**

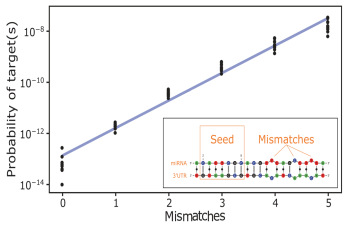
The ongoing researches

While we have developed a new and more efficient bioinformatic pipeline to detect and analyze smithRNAs in metazoans, we present new data on:

1. smithRNAs' phylogenetic distribution among Metazoa;
2. their role in mito-nuclear incompatibilities and speciation;
3. their evolvability in the context of the polycistronic maturation of mtDNA;
4. their possible ways to escape mitochondria to deliver their function in the cytoplasm;
5. their maturation processes, to understand whether they can be ascribed to the known classes of small interfering RNAs (i.e. miRNAs, siRNAs or piRNAs), or to a new unknown one.

3 Smith RNAs evolvability in the context of the polycistronic maturation of mtDNA.

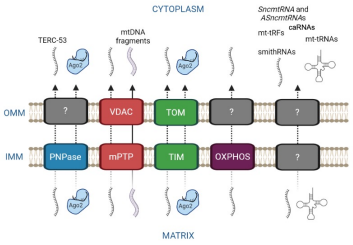
We estimate the probability with which a newly arisen smithRNA finds a suitable target in the nuclear transcriptome. Simulations with transcriptomes of 12 bivalve species suggest that **this probability is high and not species specific**. We propose that novel smithRNAs may easily evolve as exaptation of the preexisting mitochondrial RNAs. In turn, the ability of evolving novel smithRNAs may have played a pivotal role in mito-nuclear interactions during animal evolution.



Frequency of miRNA-like simulated molecules that found at least one suitable target on 3' UTRs of a given species.

4 SmithRNAs ways to escape mitochondria to deliver their function in the cytoplasm.

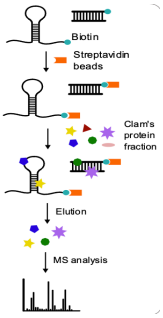
RNA-mediated Mitochondrial Retrograde Response requires these molecules to exit the mitochondrion, a process that is still mostly unknown. We suggest that the proteins/ complexes TIM, PNPase, mPTP, and the subunits of OXPHOS complexes may be responsible for RNA export.



Mechanisms of RNA export from the mitochondrion. Dotted arrows, suggested mechanisms for RNA export from the mitochondrial matrix to the cytoplasm.

5 SmithRNAs maturation processes.

The biogenesis of smithRNAs is not well defined yet. We performed pull-down analysis with biotinylated smithRNAs (122nca and 145t), one nuclear miRNA (let7), and their preliminary structures, to identify which proteins interact with these RNAs in *R. philippinarum*. Preliminary results show that the smithRNA pathway shares some proteins with the microRNA pathway. Moreover, according to *in silico* analyses, smithRNAs can interact with proteins like AGO2, DGCR8 and DROSHA.



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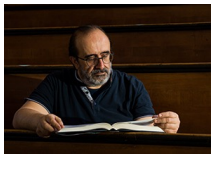

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