Mapping the yeast mitochondrial transcriptomes by direct RNA sequencing

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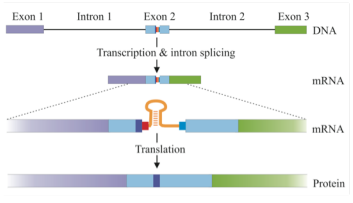
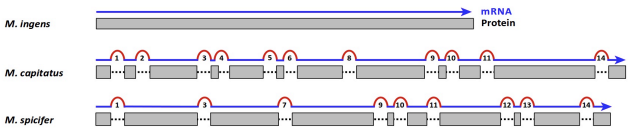
Mitochondrial genomes exhibit surprising diversity in terms of molecular architecture as well as genetic organization. In this study, we investigated two non-conventional yeast species *Magnusiomyces capitatus* and *Magnusiomyces spicifer* belonging to a deeply branching lineage of the subphylum Saccharomycotina. Their mitochondrial genomes comprise essentially the same set of highly conserved genes. Our previous studies [1, 2] revealed that the protein-coding genes contain numerous insertion elements (25-54 bp long) dubbed byps that introduce frameshifts and/or internal stop codons interrupting the reading frames. In contrast to introns, the byps are not excised from the primary transcripts during the processing but are ignored by the translational machinery via programmed translational bypassing mechanism. To characterize the mitochondrial transcriptomes of both species and assess, if all byp elements are retained in mature mRNAs, we isolated total RNAs from purified mitochondria and analyzed them by direct RNA sequencing on a MinION device. In total, we obtained 421 Mbp (*M. capitatus*) and 746 Mbp (*M. spicifer*) of basecalled sequence in 1 and 1.4 million reads, respectively. Using these datasets, we have assembled the reference mitochondrial transcriptomes for both species, defined individual transcription units, identified promoter motifs, intron-exon boundaries and 3' end processing sites. We also demonstrated that all byp elements remain in the mRNA molecules.



Programmed translational bypassing in the *cob* gene [1]. Left panel: The *cob* gene in *M. capitatus* mtDNA contains two group I introns and a single 41-nt long byp element (shown as multicolored box with a hairpin structure) inserted in the exon 2. After the transcription, both introns are removed by RNA splicing, but the byp element is retained in the matured mRNA. During the protein synthesis, mitochondrial ribosomes ignore the byp sequence via programmed translational bypassing mechanism. **Right panel:** Detailed structures of the *cob*-byp1 elements from *M. capitatus* and *M. spicifer*.

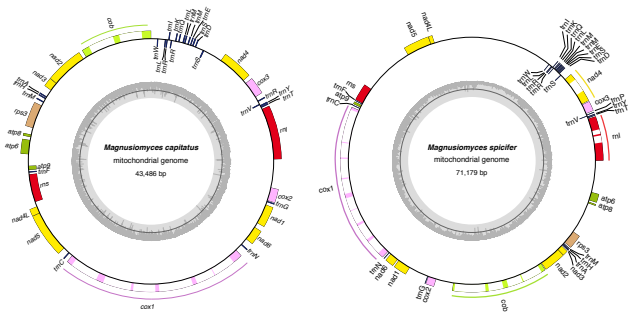


Distribution of byp elements in the *nad2* gene [2]. While *M. ingens nad2* lacks byp elements, *M. capitatus* and *M. spicifer* homologs contain 11 and 9 byps, respectively. The byps are shown as red arcs in mRNA molecules. Identical numbers indicate the presence of a byp in the same position in the sequence.

Experimental design:

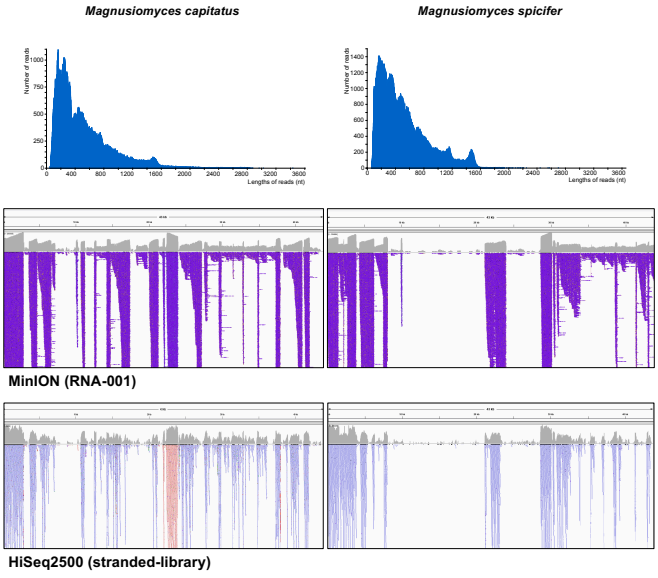
- Yeast mitochondria were isolated from O/N cultures grown in complex medium with 2% galactose (YPGal) and purified by sucrose-gradient centrifugation
- RNA was extracted from purified mitochondria by a Direct-zol RNA miniprep kit (Zymo Research)
- Sample QC: 360 ng/µl (Nanodrop), $A_{260/280}$ = 2.3, $A_{260/230}$ = 2.4; 89 ng/µl (Qubit)
- RNA molecules were polyadenylated at 3' ends using poly(A) polymerase (NEB)
- Sequencing library was prepared from 600 ng of polyA-tailed RNA using an RNA-001 kit and loaded into a FLO-MIN106 (R9.4) in a MinION device (Mk-1B)
- Basecalling was performed by Albacore (version 2.0.2). The reads were aligned to the reference genome using last [3]

Mitochondrial genomes:

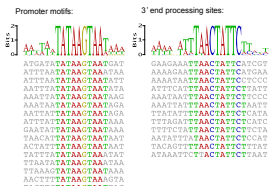


Mitochondrial transcriptome mapping:

	Raw data [Mbp]	Reads	Mean length	Median length
Magnusiomyces capitatus	**421.3**	**1019394**	**413.3**	**259**
Magnusiomyces spicifer	**746.1**	**1395771**	**534.6**	**383**



Promoter motifs and 3' end processing sites in *M. capitatus* mtDNA:



References:

1. Lang B.F., *et al.* (2014) Massive programmed translational jumping in mitochondria. *Proc. Natl. Acad. Sci. USA* 111(16): 5926-5931.
2. Nosek J., *et al.* (2015) Programmed translational bypassing elements in mitochondria: structure, mobility and evolutionary origin. *Trends Genet.* 31(4): 187-194.
3. Kiełbasa S.M., *et al.* (2011) Adaptive seeds tame genomic sequence comparison. *Genome Res.* 21(3): 487-493.

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