12L.3 RNA processing, stability and turnover in yeast mitochondria – from genetics to evolution and systems biology

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As a legacy of their endosymbiotic eubacterial origin, mitochondria possess a residual genome, encoding only a few proteins and dependent on a variety of factors encoded by the nuclear genome for its maintenance and expression. As a facultative anaerobe with well understood genetics and molecular biology, yeast is the model system of choice for studying nucleo-mitochondrial genetic interactions. Expression of the yeast mitochondrial genome is regulated mostly at the post-transcriptional level, and involves many general and gene-specific factors regulating splicing, RNA processing and stability and translation. Among those, a prominent group are the pentatricopeptide repeat (PPR) proteins, which form the largest known RNA-binding protein family, and are found in all eukaryotes, being particularly abundant in higher plants. PPR proteins localize mostly in mitochondria and chloroplasts, and many were shown to modulate organellar genome expression on the posttranscriptional level. While the genomes of land plants encode hundreds of PPR proteins, only a few were identified in Fungi and Metazoa. Using a novel bioinformatic tool we assigned 12 new proteins to the PPR family in the genome of *S. cerevisiae*, and provided an exhaustive catalog of this family in other yeast genomes. A very interesting aspect of the yeast mitochondrial system is the relationship between genome maintenance and gene expression. Deletions of genes involved in many different aspects of mitochondrial gene expression, notably translation, result in an irreversible loss of functional mtDNA. The mitochondrial genetic system viewed from the systems biology perspective is therefore very fragile and lacks robustness compared to the remaining systems of the cell. This lack of robustness could be a legacy of the reductive evolution of the mitochondrial genome, but explanations involving selective advantages of increased evolvability have also been postulated.

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12L.4 Evolution and disease converge in the mitochondrion

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Mitochondrial DNA (mtDNA) mutations are long known to cause diseases but also underlie tremendous population divergence in humans. It was assumed that the two types of mutations differ in one major trait: functionality. However, evidence from disease association studies, cell culture and animal models support the functionality of common mtDNA genetic variants, leading to the hypothesis that disease-causing mutations and mtDNA genetic variants share considerable common features. Here we provide evidence showing that the two types of mutations obey the rules of evolution, including random genetic drift and natural selection. This similarity does not only converge at the principle level; rather, disease- causing mutations could recapitulate the ancestral DNA sequence state. Thus, the very same mutations could either mark ancient evolutionary changes or cause disease.

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Posters

12P.1 Fluorescence *in situ* hybridization of mitochondrial DNA and RNA

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Details of mitochondrial genetics are still poorly understood, including transcription and replication of mitochondrial DNA (mtDNA). In order to visualize mtDNA in co-localization with loci of its transcription, we used two different approaches for labelling mt nucleic acids. First, a molecular beacon with Cy3 fluorophore and BHQ2 quencher was designed and verified for specific mtRNA staining of either ND5 mRNA or the complementary mRNA permanently attached to the D-loop. The second approach employed a specific hybridization ND5 probe conjugated with Promofluor 647 *via* dUTP which replaced dTTP in about 100 locations. Experiments were done using HeLa cells transfected with mitochondria-targeted GFP to confirm mitochondrial location and HEK cells. Our results show that the ND5 probes have visualized mtDNA in these cells, while the D-loop beacon targeting RNA in RITOLs and triple structures clearly demonstrated the existence of transcription „clouds", which again co-localized with mitochondrial matrix space as indicated by confocal microscopy. This system provides a powerful tool for visualization of mtDNA and their triplicate structures such as RITOLs.

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12P.2 Polyadenylation of mt mRNA: Identification of novel deadenylase of human mitochondria

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The polyadenylation of mRNA is an essential step in gene expression. Unlike RNA encoded by the genomes of prokaryotes, human mitochondrial transcripts possess stable 3' end poly(A) tails, similar to mRNA in the eukaryotic cytosol. These poly(A) tails are necessary to create functional stop codons in some mt mRNAs; however, very little is known about their role in mitochondrial translation and RNA turnover. Also the factors responsible for poly (A) tails metabolism are yet to be discovered. Here we describe identification of a novel deadenylase in mitochondria, named hmtDAD (human mitochondrial deadenylase). This protein belongs to the endonuclease/exonuclease/phosphatase (EEP) family and shares 20-30% identity with well-characterised cytosolic deadenylases. In this study we show that hmtDAD localises to mitochondria and has a role in the turnover in poly(A) tails. Overexpression of hmtDAD subsequently yields mRNA species with shortened poly(A) tails. This is in agreement with *in vitro* studies showing a poly(A)-specific ribonuclease activity of recombinant hmtDAD. Removal of poly(A) tails by hmtDAD has a varied impact on mt mRNA stability and protein synthesis. In light of these new findings, the role of polyadenylation in mt mRNA metabolism and translation will be discussed.

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