Cell Reports

Supplemental Information

Organization of Mitochondrial Gene Expression in Two Distinct Ribosome-Containing Assemblies

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Extended experimental procedures

Separation of mitochondrial lysates on sucrose gradients

All steps were performed at 4 °C. 1 mg mitochondria were washed in SH buffer (10.000 x*g*, 10 min, 4°C) and lysed for 10 min in 285 µl lysis buffer (1% DDM, 10-300 mM KOAc, 5 mM MgOAc, 0.8 mM EDTA, 5 mM β- mercaptoethanol, 1 mM PMSF, 1 mM spermidine, 1x complete protease inhibitor (roche, Sweden), 10 mM Tris/HCl, pH 7.4, 5 % glycerol). After 10 min, the lysate was diluted 1:2 with dilution buffer (10-300 mM KOAc, 5 mM MgOAc, 0.8 mM EDTA, 5 mM β-mercaptoethanol, 1 mM PMSF, 1 mM spermidine, 10 mM Tris/HCl, pH 7.4, 5 % glycerol) and centrifuged for 5 min at 16.000 x*g*. Cleared lysate was then separated on a sucrose gradient (1 - 0.3 M sucrose, 0.1% DDM, 10-300 mM KOAc, 5 mM MgOAc, 0.8 mM EDTA, 5 mM β-mercaptoethanol, 1 mM PMSF, 1 mM spermidine, 10 mM Tris/HCl, pH 7.4) for 1 h at 370.000 x*g* in an SW60Ti rotor (Beckman Coulter, Fullerton, CA). Fractions were collected and proteins precipitated by addition of 12 % TCA.

Native purification of ribosomes and ribosome-associated complexes

Mitochondria from yeast strains expressing GFP-tagged Mrp1 or Mrpl4 were lysed for 10 min as above, cleared by centrifugation, and the lysate incubated for 60 min with GFP-Trap®_A beads (ChromoTek, Germany) at 4 °C. After incubation beads were allowed to sediment by gravity and washed four times with dilution buffer containing 0.1% DDM. Beads were resuspended in wash buffer with 50U TEV protease and incubated for 1 h at 4 °C and the eluted proteins analyzed by mass spectrometry and Western blotting.

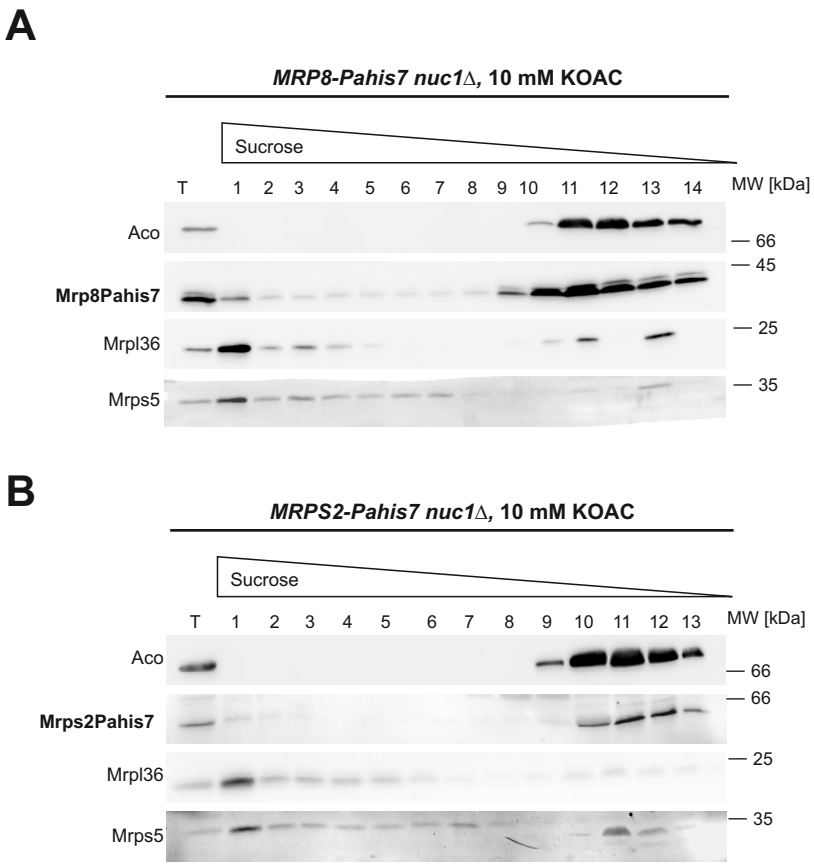
Immunofluorescence labeling and dual color STED super- resolution microscopy:

Yeast cells expressing GFP tagged proteins were grown in YPD medium to early exponential growth phase ($OD_{600\ nm}$ = 0.45 - 0.7). After fixation with 3.7% formaldehyde in growth

medium (RT, 20 min), cells were harvested by centrifugation (800 x*g*, 3 min) and washed in PBS/sorbitol (137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 10% (w/v) sorbitol, pH 7). Subsequently, the yeast cell wall was removed by zymolyase digestion (10 min, 30 °C). After washing in PBS/sorbitol, cells were attached to the surface of a poly-L-lysine coated cover slip. Following a blocking step (10 min, 2% (w/v) bovine serum albumine; 0.1% (v/v) Tween20/ SDS in PBS/ sorbitol), GFP tagged proteins and mtDNA were decorated using rabbit antibodies against GFP (abcam, Cambridge, UK) and a mouse antibody against dsDNA (Santa Cruz Biotechnology, Dallas, Tx, USA) (RT, 1h). The primary antibodies were decorated with secondary goat-anti-rabbit and sheep-anti-mouse antibodies (Jackson ImmunoResearch, West Grove, PA, USA) custom labelled with Alexa 594 and KK114. After several washing steps in blocking solution and sorbitol in PBS, the cells were mounted in mowiol containing DABCO.

STED images were recorded using a 2-color STED 595 QUAD scanning microscope (Abberior Instruments, Göttingen, Germany) in a frame sequential scanning scheme using 20 nm pixels, pixel dwell times of 40 µs, and a time gated detection (1.5 - 9.5 ns). Besides smoothing with a 20 nm Gaussian and contrast stretching no image processing was performed.

Figure S1, Mrp8 and Mrps2 are no structural companents of the ribosome. Related to figure 2.



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Name	Function	Ribosomal proteins part of the same regulon
Mrp8	Protein abundance increases in response to DNA replication stress	-
Mrps2	Protein with carboxyl methyl eserase activity	-
Ymr31	Subunit of alpha-ketoglutarate dehydrogenase	-

Figure S1: Mrp8, Mrps2 and Ymr31 are no structural components of the ribosome. Isolated mitochondria from the indicated strains were lysed for 10 min and the cleared lysates subjected to velocity centrifugation on a linear sucrose gradient. After separation of the lysate, fractions were collected starting from the bottom and analyzed by western blot. (A) Mrp8 does not co-migrate with the ribosome under low salt conditions. (B) Mrps2 does not co-migrate with the ribosome under low salt conditions. (C) Mrp8, Mrps2 and Ymr31 are not co-regulated with other ribosomal proteins. Co-regulation of Mrp8, Mrps2 and Ymr31 with ribosomal proteins was analyzed using SPELL (Hibbs et al. (2007)).

Figure S2. Interactions of mRNA metabolism factors with the ribosome. Related to figure 3.

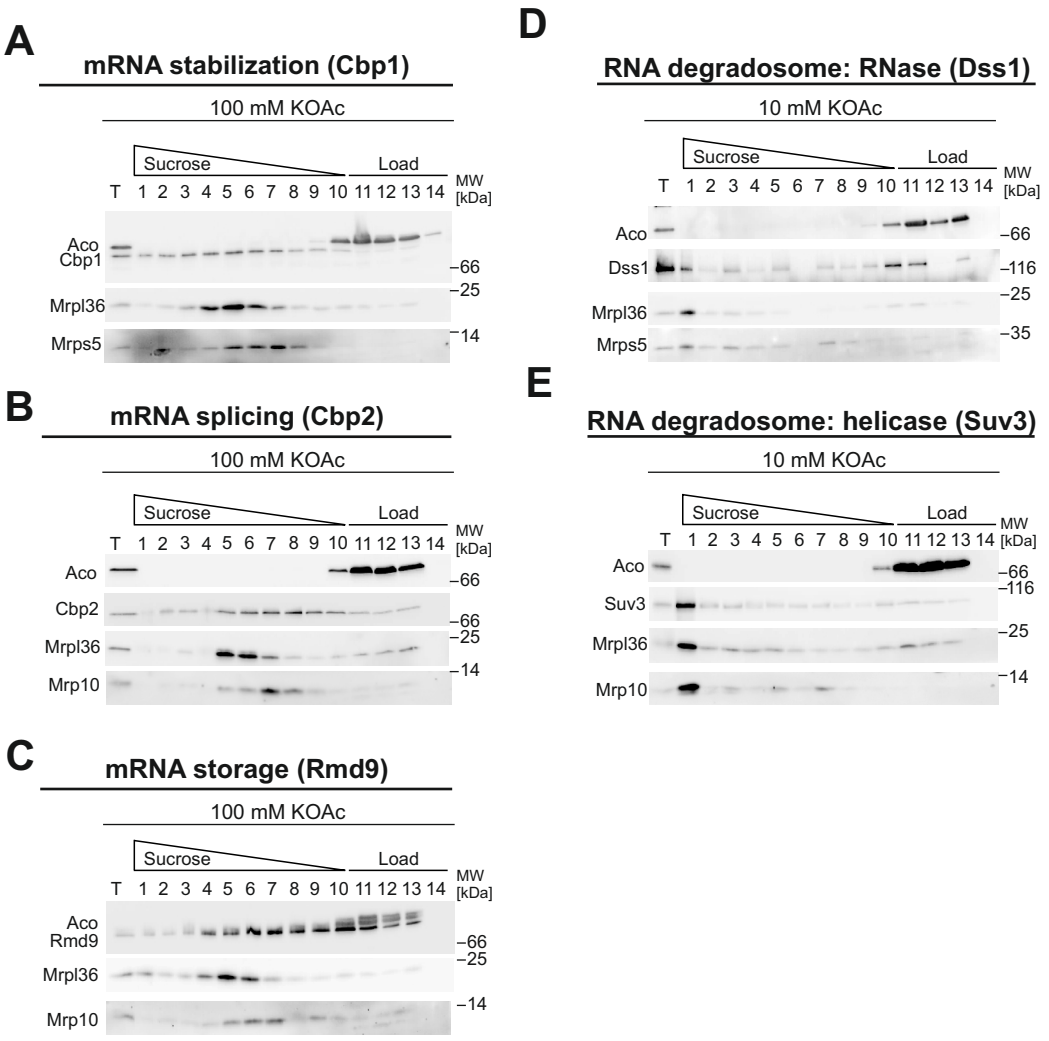

Figure S2: Interactions of selected factors supporting mRNA metabolism with mitochondrial ribosomes.
Isolated mitochondria from a strain expressing the indicated Prot.AHis7-tagged variant were lysed at the specified KOAc concentration for 10 min on ice and subjected to velocity centrifugation on a linear sucrose gradient. Fractions were analyzed by Western blotting.
T, total (representing 10% of the starting material); MW, molecular weight.

Figure S3, Mrpl4-GFP is part of both MIOREX complexes. Related to figure 5.

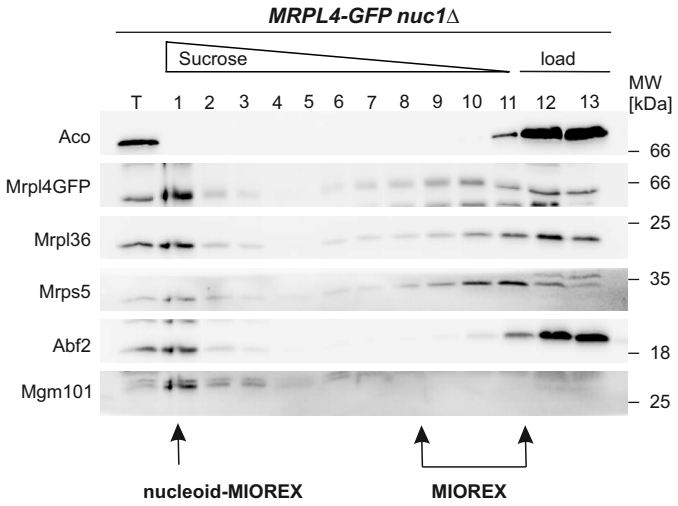

Figure S3: Mrpl4-GFP is part of the nucleoid-MIOREX and the peripheral MIOREX complexes.
Isolated mitochondria from a strain encoding a chromosomally GFP-tagged variant of Mrpl4 were lysed for 10 min on ice and subjected to velocity centrifugation on a linear sucrose gradient. Fractions were analyzed by Western blotting.
T, total (representing 10% of the starting material); MW, molecular weight.

Supplemental Table S1: Mass spectrometry of ribosomes purified under low salt conditions. Table S1 is found as a separate excel-sheet. Related to figure 2.

Supplemental Table S2: Mass spectrometry of ribosomes purified under high salt conditions. Table S2 is found as a separate excel-sheet. Related to figure 2.

Table S3, List of strains used in this study. Related to Experimental Procedures.

Name	Parental strain	Nuclear genotype	References
MOY663 W303Δnuc1 Cbs2Pahis7	W303	Mata; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 CBS2Pahis7::HIS3 *nuc1*::URA3	This study
W303A		Mat a; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100	Thomas, B. J. & Rothstein, R. (1989)
MOY646 W303Δnuc1	W303	Mat a; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 *nuc1*::URA3	This study
MOY 670 W303Δnuc1 Mrp1GFP	W303	Mat a; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 MRP1GFP::TRP *nuc1*::URA3	This study
MOY942 W303Δnuc1 Rmd9GFP	W303	MATa; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 RMD9GFP::HIS3 *nuc1*::URA3	This study
MOY653 W303Δnuc1 Mrpl4GFP	W303	Mat a; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 MRPL4GFP::TRP *nuc1*::URA3	This study
MOY865 W303Δnuc1 Suv3Pahis7	W303	Mat a; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 SUV3Pahis7::HIS3 *nuc1*::URA3	This study
MOY668 W303Δnuc1 Cbp1Pahis7	W303	Ma ta; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 CBP1Pahis7::HIS3 *nuc1*::URA3	This study
MOY860 W303Δnuc1 Cbp2Pahis7	W303	Mat a; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 CBP2Pahis7::HIS3 *nuc1*::URA3	This study
MOY859 W303Δnuc1 Rmd9Pahis7	W303	Mat a; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 RMD9Pahis7::HIS3 *nuc1*::URA3	This study
MOY905 W303Δnuc1 Dss1Pahis7	W303	MATa; ura3-1; trp1Δ 2; leu2-3,112; his3-11,15; ade2-1; can1-100 DSS1Pahis7::HIS3 *nuc1*::URA3	This study
Atp4GFP	BY4741	MATa; his3Δ; leu2Δ0; met15Δ0; ura3Δ0; ATP4GFP::HIS3	Huh, WK et al Nature. (2003)