Supplemental Materials

Molecular Biology of the Cell

Box et al.

SUPPLEMENTARY MATERIAL

Box, Kaur & Stuart, MBoC

SUPPLEMENTARY MATERIAL AND METHODS

Mass spectrometry analysis: Mass spectrometry analysis was performed by the Mass Spectrometery Facility for Proteomics at the Medical College of Wisconsin (MCW) by the late Dr. Kate Noon. Protein bands excised from SDS-PAGE gels and samples were subjected to in-gel digestion with trypsin, then purified for mass spectrometry analysis on the AB Sciex Ekspert 425 HPLC-LTQ system using standard Facility protocols. The LC-MS/MS data were processed with the MCW Workflow using Sequest software for searching against a current UniProt yeast database.

***In vivo* chase analysis**: Yeast strains, MrpL35^{WT}, *mrpL35*^{Y275A} and *mrpL35*^{Y275D} were grown and passaged at the permissive temperature (30 ^{o}C) in minimal media lacking histidine and containing lactate (0.5%) and galactose (2%). Cells were transferred to fresh media containing chloramphenicol (400 ug/ml) to prevent further mitochondrial translation and were further incubated at the non-permissive temperature of 37 ^{o}C. At indicated time points indicated, equivalent amounts of cells were reisolated and total cellular proteins were extracted. Samples were subsequently analyzed by SDS-PAGE, Western blotting and immunedecoration.

Table S1:

Protein Assigned	Gene Name	Mr (kDa) [a]	Calculated pI[a]	Sequence Coverage (%)	Peptides Matched (#)	Max X_{corr} [b]
MrpL7	YDR237W	33.0	9.48	55.3	11	8.1
MrpL17	YNL252C	32.2	8.86	62	25	5.9
Mam33	YIL070C	30.1	4.5	39	14	5.95
MrpL28	YDR462W	17.3	10.34	41	9	4.2
Rim1	YCR028C-A	15.3	7.83	31.9	4	4.5

Table S1: **MrpL35$_{His}$ interacting proteins identified following SDS-PAGE, gel excision and mass spectrometry analysis**. Silver stained bands were excised from SDS-PAGE gel and subjected to trypsin digestion and mass spectrometry analysis, as described in Supplementary Materials and Methods. Note, the co-association of MrpL7, MrpL17 with MrpL35 (and with Mrp7 and MrpL36) was verified using reciprocal affinity purification experiments (Figure 6B). The Mam33 and Rim1 association was verified through reciprocal pull downs and antibody decoration, respectively, and their association was found not to be specific for the MrpL35$_{His}$ subcomplex, as they were also recovered in the MrpL25$_{His}$/Mrp20 subcomplex. [a] Calculated Mr (or pI) of precursor form; [b] Max X_{corr}: Highest X_{corr} value of all the scans from all the matched peptides (X_{corr}: Estimation of the degree of correlation between the matched MS/MS spectrum and the predicted MS/MS spectrum)

Figure S1

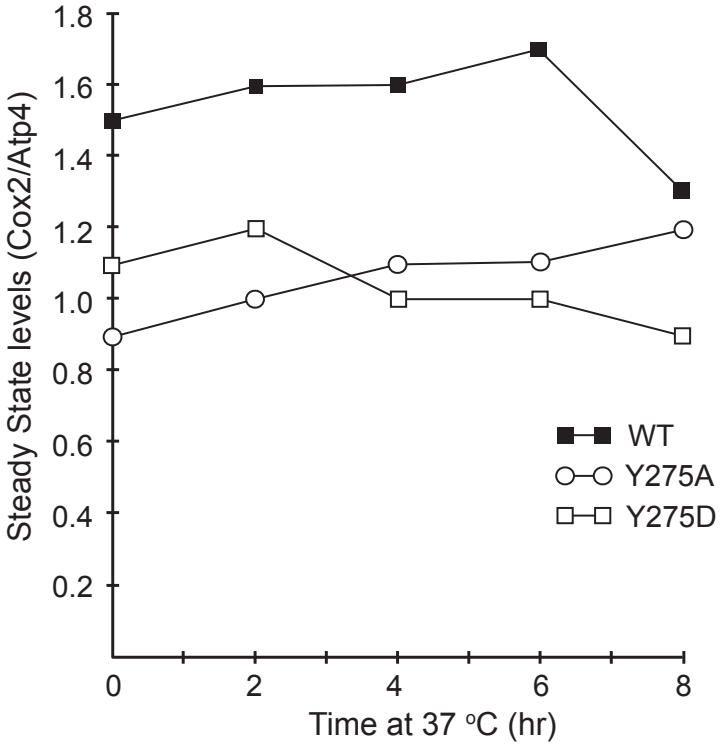

Figure S1: The COX complex assembled at permissive temperature in the *mrpL35* mutants does not display enhanced proteolytic instability when shifted to 37 °C. MrpL35^{WT}, *mrpL35*^{Y275A} and *mrpL35*^{Y275D} mutant strains were initially maintained at 30 °C (on minimal media supplemented with galactose). Cultures were inoculated into fresh media containing chloramphenicol and incubated at 37 °C for up to 8 hours. At time points indicated equivalent amounts of cells were reisolated, and total cellular proteins were extracted. Samples were subsequently analyzed by SDS-PAGE, Western blotting and immunedecoration with antibodies specific to Cox2 of the COX complex and Atp4 of the F_1F_o-ATP synthase. The amounts of Cox2 and Atp4 at each time point were quantified, as described in Materials and Methods and expressed as a ratio of each other (Cox2/Atp4).