Supplemental Materials

Molecular Biology of the Cell

Barros and Tzagoloff

SUPPLEMENTAL MATERIAL

Table S1. Properties of different *aep3* mutants

Genotype	Mutation	Phenotype % $\rho^{-/o}$	Mito. Translation	Ref
aep3	Δ36–661	~90	Atp8p and Atp6p more affected than other mit. gene products	Ellis et al., 2004
Δ*aep3 nATP8*^{GPD}	Δ36–661	~90	Atp6p and other mitochondrial gene products very low because of high percentage of $\rho^{-/0}$ mutants	This study
Δ*aep3 sup*$^{+}$*nATP8*^{GPD}	Δ36–661	~20	Normal except Atp8p is expressed from allotropic *nATP8*	This study
Δ*aep3 nATP8*$^{GPD\text{-}CYC1}$	Δ36–661	ND	Normal except Atp8p is expressed from allotropic *nATP8*	This study
aep3-6b	A379E	~90	Severely depressed	This study
aep3-6b sup^{+} *nATP8*^{GPD}	A379E	~10	Normal except Atp8p is expressed from allotropic *nATP8*	This study
aep3-1s	Δ416-606	~90	Severely depressed	This study
aep3-1s sup^{+} *nATP8*^{GPD}	Δ416-606	~10	Normal except Atp8p is expressed from allotropic *nATP8*	This study
aep3-1c	A379E, N331E Δ416-606	~90	Severely depressed	This study
aep3-1c sup^{+}*nATP8*^{GPD}	A379E, N331E Δ416-606	~60	Normal except Atp8p is expressed from allotopic *nATP8*	This study
aep3	Y305N	Not reported	Not affected in wild type background. General decrease in a *fmt1* mutant	Lee et al., 2009
Δfmt1	Deletion	ND	Normal	This study

Δ*aep3* Δ *fmt1*	Δ36–661 Deletion	ND	Severely depressed	This study
Δ*aep3* Δ *fmt1 nATP8*	Δ36–661 Deletion	ND	Severely depressed	This study
Δ*aep3* Δ *fmt1 sup*$^{+}$ *nATP8*[2]	Δ36–661 Deletion	ND	Normal except Atp8p is expressed from allotropic *nATP8*	This study

[1] The percentage of ρ^{-} and ρ^{0} mutants differs in different strains. The difference in the values reported for the *aep3* null and the point mutants could stem from the differences in the genetic background of iLL20 and W303, respectively.
[2]The suppressor in this strain has not been determined.

Table S2. Genotypes and Sources of *Saccharomyces cerevisiae* Strains

Strain	Genotype	Source
W303-1A	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1*	a
W303-1B	MATα *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1*	a
DFK/W303	MATα *kar1-1 ade2-101 leu2Δ ura3-52 lys2 Δarg8::URA3 with W303 mtDNA*	This study
MR10	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 arg8::HIS3 atp6::ARG8^{m}*	Rak et al., 2007
MR6	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 arg8::HIS3*	Rak et al., 2007
MR6ΔATP8	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 arg8::HIS3 atp8::ARG8^{m}*	Barros et al., 2011
MR6ΔATP8/ST4	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 arg8::HIS3 atp8::ARG8^{m}* + *pATP8/ST4* (*nATP8^{ADH1}*)	Barros et al., 2011
a/αW303	MATa/α *ade2-1/ade2-1 his3-1,15/his3-1,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1*	a
aW303 ΔAEP3	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1ura3-1 aep3::HIS3*	Ellis et al., 2004
aW303 ΔAEP3/S^{+}/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1ura3-1 aep3::HIS3 nrd1* + pATP8-22	This study
W303 ΔAEP3/S^{+}/22	MATα *ade2-1 his3-1,15 leu2-3,112 trp1-1ura3-1 aep3::HIS3 pta1* + pATP8-22	This study
a/αW303ΔAEP3/22	MATa/α *ade2-1/ade2-1 his3-1,15/his3-1,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 aep3::HIS3/aep3::HIS3* + pATP8-22 (*nATP8*)	This study
a/αW303ΔAEP3/ST4	MATa/α *ade2-1/ade2-1 his3-1,15/his3-1,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1 aep3::HIS3/aep3::HIS3* + pATP8/ST4 (*nATP8^{ADH1}*)	This study
W303ΔFMT1	MATα *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 fmt1::ura3*	This study
W303 ΔAEP3ΔFMT1	MATα *ade2-1 his3-1,15 leu2-3,112 trp1-1ura3-1 fmt1::ura3 aep3::HIS3*	This study
aW303 ΔAEP3ΔFMT1/ S^{+}/ 22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 fmt1::ura3 aep3::HIS3* + pATP8-22 *sup^{+}*	This study

aW303 ΔAEP3ΔFMT1/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 fmt1::ura3 aep3::HIS3* + pATP8-22	This study
W303 ΔAEP3ΔSMT1	MATα *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 smt1::ura3 aep3::HIS3*	This study
aW303ΔAEP3/ S^+/ SMT1/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3::HIS3 LEU2::SMT1 sup*$^+$ + pATP8-22	This study
W303 ΔAEP3ΔSMT1/22	MATα *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 smt1::ura3 aep3::HIS3* + pATP8-22	This study
aW303/1c	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 leu2::aep3-1c + nrd1*	This study[b]
aW303/1c/S^+/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3-1c nrd1* + pATP8-22	This study
aW303ΔAEP3/1c/ S^+/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3::HIS3 leu2::aep3-1c sup*$^+$ + pATP8-22	This study
aW303ΔAEP3/1c/ S^+ /SMT1/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3::HIS3 leu2::aep3-1c LEU2::SMT1sup*$^+$ + pATP8-22	This study
aW303ΔAEP3/1s/S^+/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3::HIS3 leu2::aep3-1s sup*$^+$ + pATP8-22	This study[b]
aW303/6b	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 trp1::aep3-6b nrd1*	This study[c]
aW303/6b/S^+/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3-6b sup*$^+$ + pATP8-22	This study
aW303ΔAEP3/6b/ S^+/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3::HIS3 trp1::aep3-6b sup*$^+$ + pATP8-22	This study
aW303ΔAEP3/6b/ S^+ /SMT/22	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3::HIS3 trp1::aep3-6b LEU2::SMT11 sup*$^+$ + ATP8-22	This study
aW303ΔAEP3/ST4	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3::HIS3* + pATP8/ST4 (*nATP8*^{ADH1})	This study
aW303ΔAEP3/ST5	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3::HIS3 pta1* + pATP8-ST5[d] (*nATP8*^{GPD})	This study
αW303ΔAEP3/22T	MATα *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 aep3::HIS3* + pATP8-22T (*nATP8*^{GPD}-^{CYC1})	This study

MR6ΔATP8 ΔAEP3/ST4	MATa *ade2-1 his3-1,15 leu2-3,112 trp1-1 ura3-1 arg8::HIS3 aep3::HIS3 atp8::ARG8*^{m} + pATP8/ST4 (*nATP8*^{ADH1})	This study

[a] Dr. R. Rothstein, Department of Human Genetics, Columbia University, New York, NY
[b] The *aep3-1c* and *aep3-1s* alleles were cloned in YIp351 (a *LEU2* integrative plasmid).
[c] The *aep-6b* allele was cloned in YIp349 (a *TRP1* integrative plasmid).
[d] pATP8/ST5 consists of *nATP8* downstrem of the *GPD* promoter in the high copy plasmid YEp352.

Barros and Tzagoloff, Fig. S1

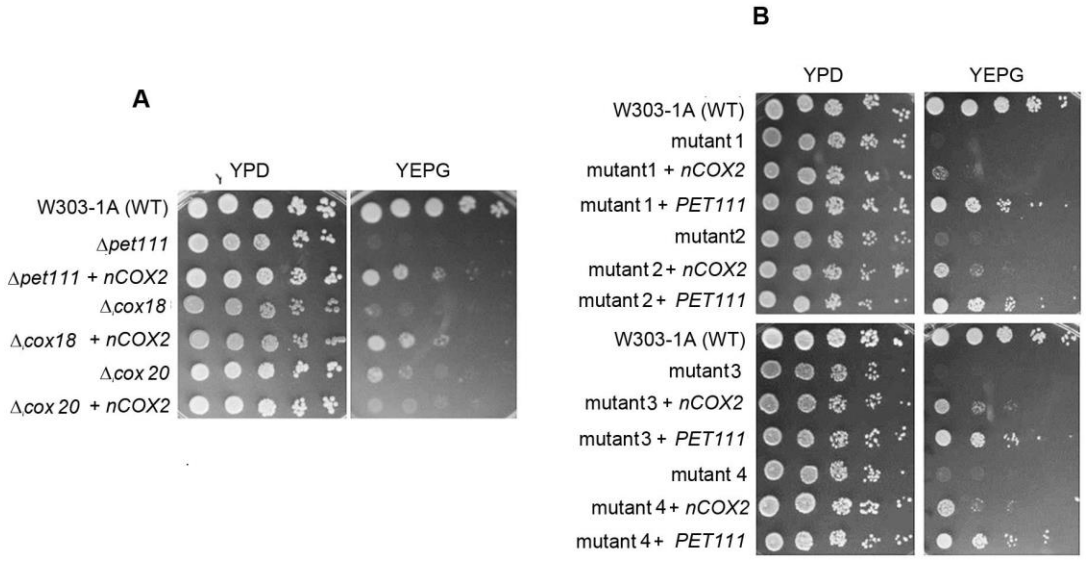

Figure S1 Proof of principle for selection of mutants involved in mitochondrial gene expression. Respiratory growth of mutants involved in mitochondrial *COX2* expression is partially complemented by *nCOX2*, a recoded nuclear version of the mitochondrial gene. n*COX2* contained the W56R in the first trans-membrane domain, which was shown to be needed for complementation of *cox2* mutations (Supekova et al, 2010). **A.** Mutants with null alleles in the *COX2* translational activator (Δ*pet111*) and chaperone of pre-Cox2p processing/translocation (Δcox18 and Δcox20) were transformed with allotopic *nCOX2*. Growth on YPD (rich glucose) and YEPG (rich ethanol/glycerol) was photographed after two day incubation at 30^{o}C. **B**. Mutants 1, 2, 3 and 4 obtained by EMS mutagenesis were transformed with *nCOX2* and as a control with *PET111*. Growth was measured as in **A**.

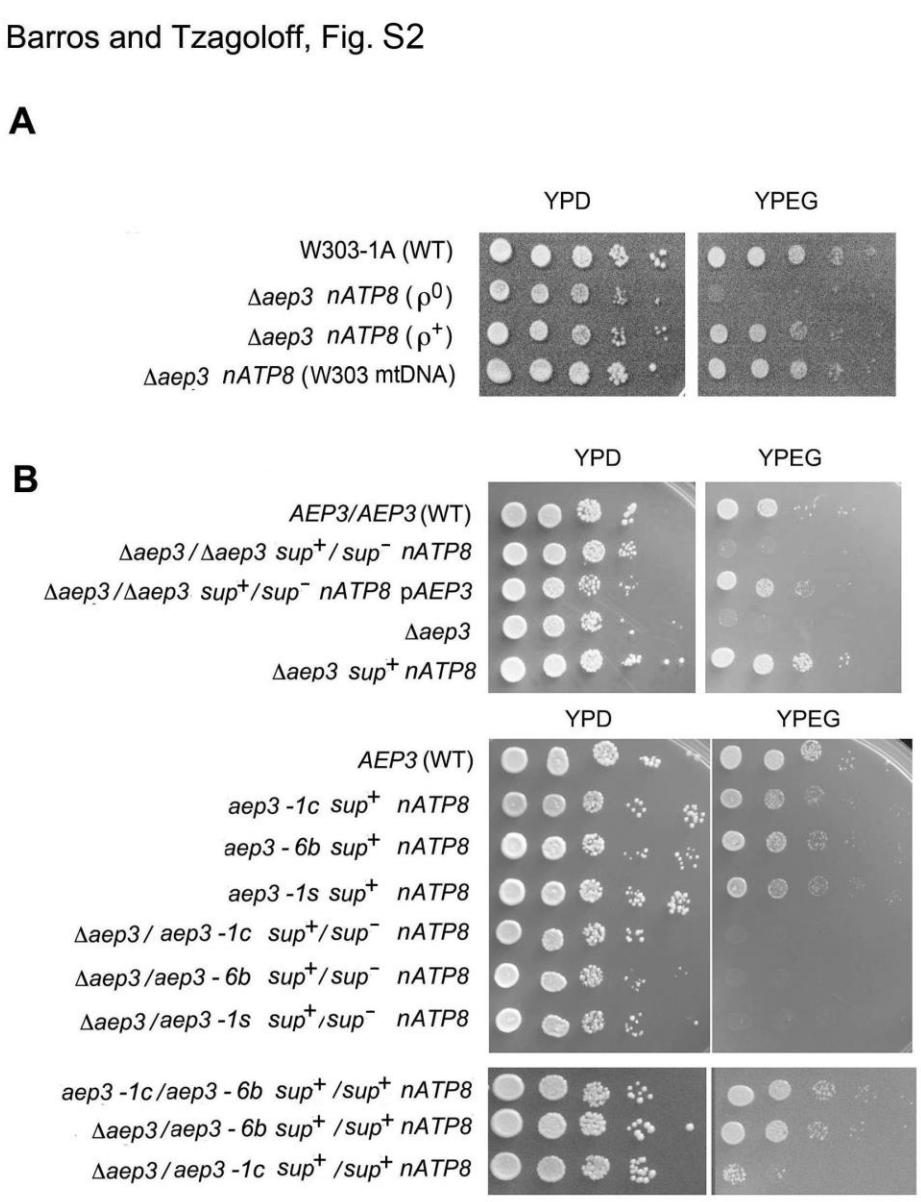

Figure S2. Genetic properties of *aep3* revertants. **A**. Test of the spontaneous suppressor for dominance and recessiveness. The wild type diploid and a diploid strain homozygous for the *aep3* null allele but heterozygous for the suppressor (Δ*aep3*/Δ*aep3 sup*$^+$/*sup*$^-$ *nATP8*) was obtained from a cross of the *aep3* null mutant W303ΔAEP3 (*Δaep3 sup*$^-$*)* to the respiratory competent strain aW303ΔAEP3/nATP8/sup (Δ*aep3 sup*$^+$ *nATP8*). The diploid strain obtained from this cross was also transformed with wild type *AEP3* (Δ*aep3*/Δ*aep3 sup*$^+$*/ sup*$^-$ *nATP8 pAEP3*). The respiratory deficient haploid strain aW303ΔAEP3 (Δ*aep3*) and the respiratory competent revertant aW303ΔAEP3/nATP8/sup (Δ*aep3 sup+ nATP8)* used in the cross are also shown. The mutants and revertant were serially diluted and spotted on YPD and YPEG. The two plates were incubated at 30^{o}C for 3 days. **B**. Upper panel: Same as **A** except the mutants

had the *aep3* 1c, 6b and 1s alleles. Lower panel: growth of diploid cells with the indicated genotypes on YPEG indicates they contain the same suppressor.

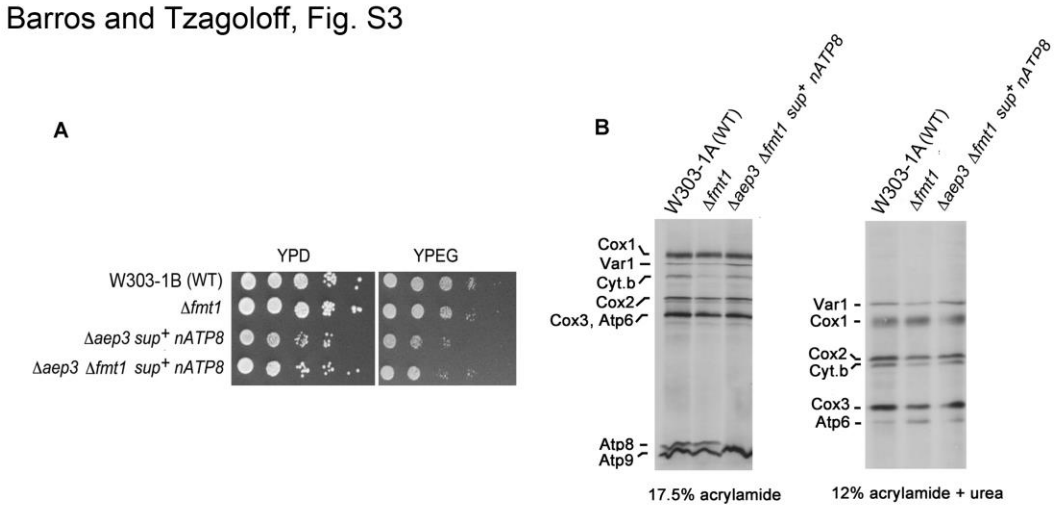

Figure S3. Growth and mitochondrial translation of *aep3-fmt1* double mutants. **A.** Serial dilutions of the wild type strain W303-1B, the *fmt1* null mutant (*Δfmt1*), the revertant of the *aep3* null mutant (Δ*aep3 sup*$^+$ *nATP8*) and the double mutant Δ*aep3* (Δ*fmt1 sup*$^+$ *nATP8*). Serial dilutions of each strain were spotted on YPD and YPEG and photographed 3 days after incubation at 30^{o}C **B)** The strains described in **A** were labeled with [^{35}S] in the presence of cycloheximide as described in the Materials and Methods section and total mitochondrial protein separated on 17.5% and 12% polyacrylamide gel containing 5M urea. The urea gel resolves Cox2p and Atp6p. Proteins were transferred to nitrocellulose and the blot exposed to X-ray film. The mitochondrial translation products are identified in the left-hand margin of each gel.

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