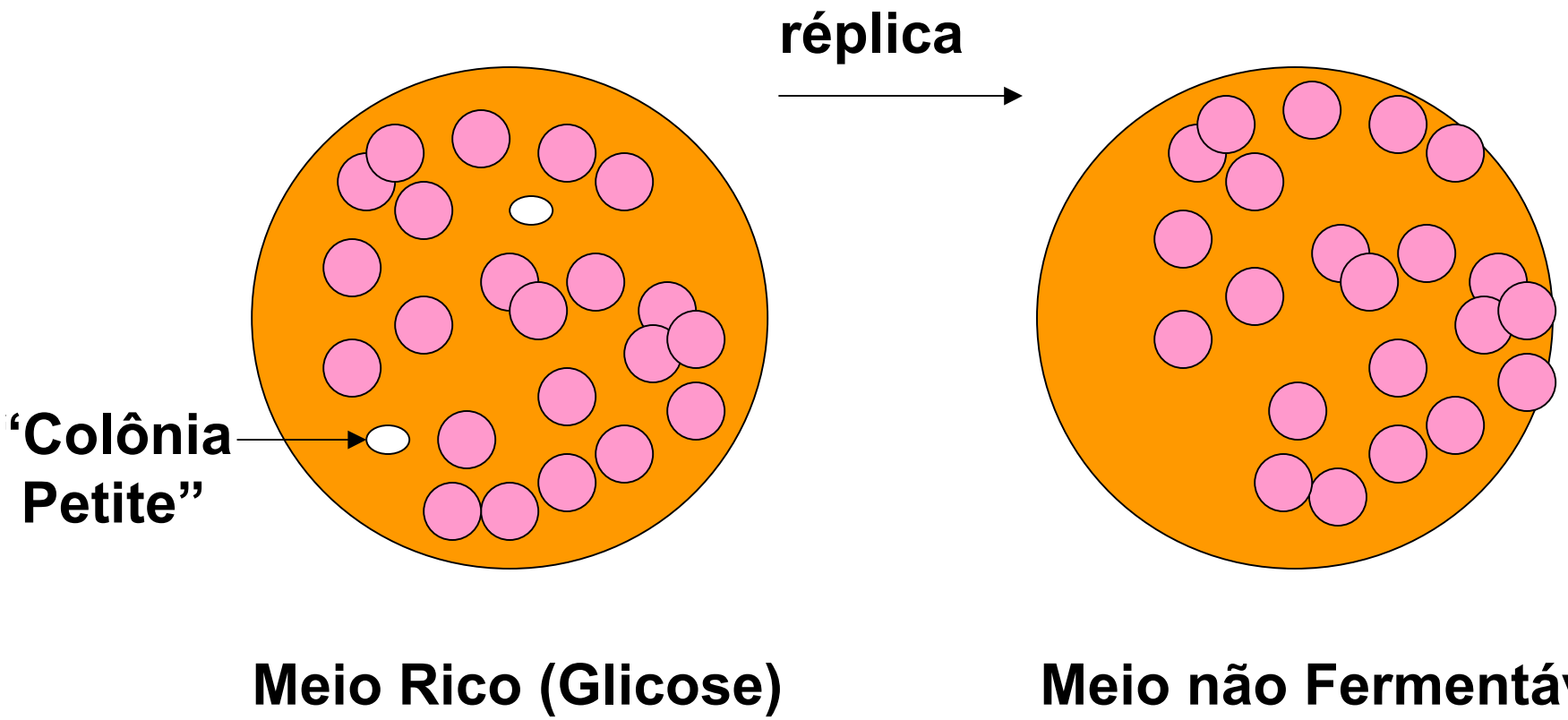
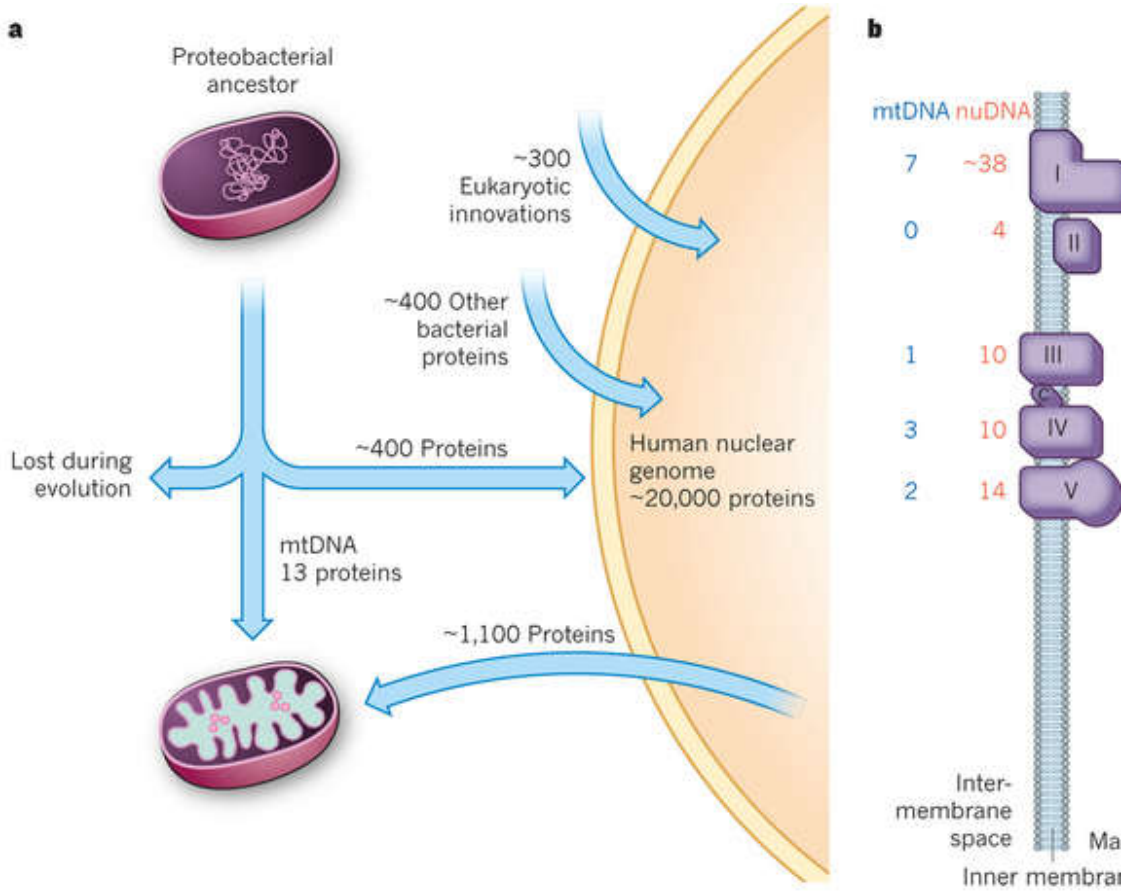
Characterization of new factors required for mitochondrial translation in yeast.

Mario H. Barros
ICB-USP
mariohb@usp.br

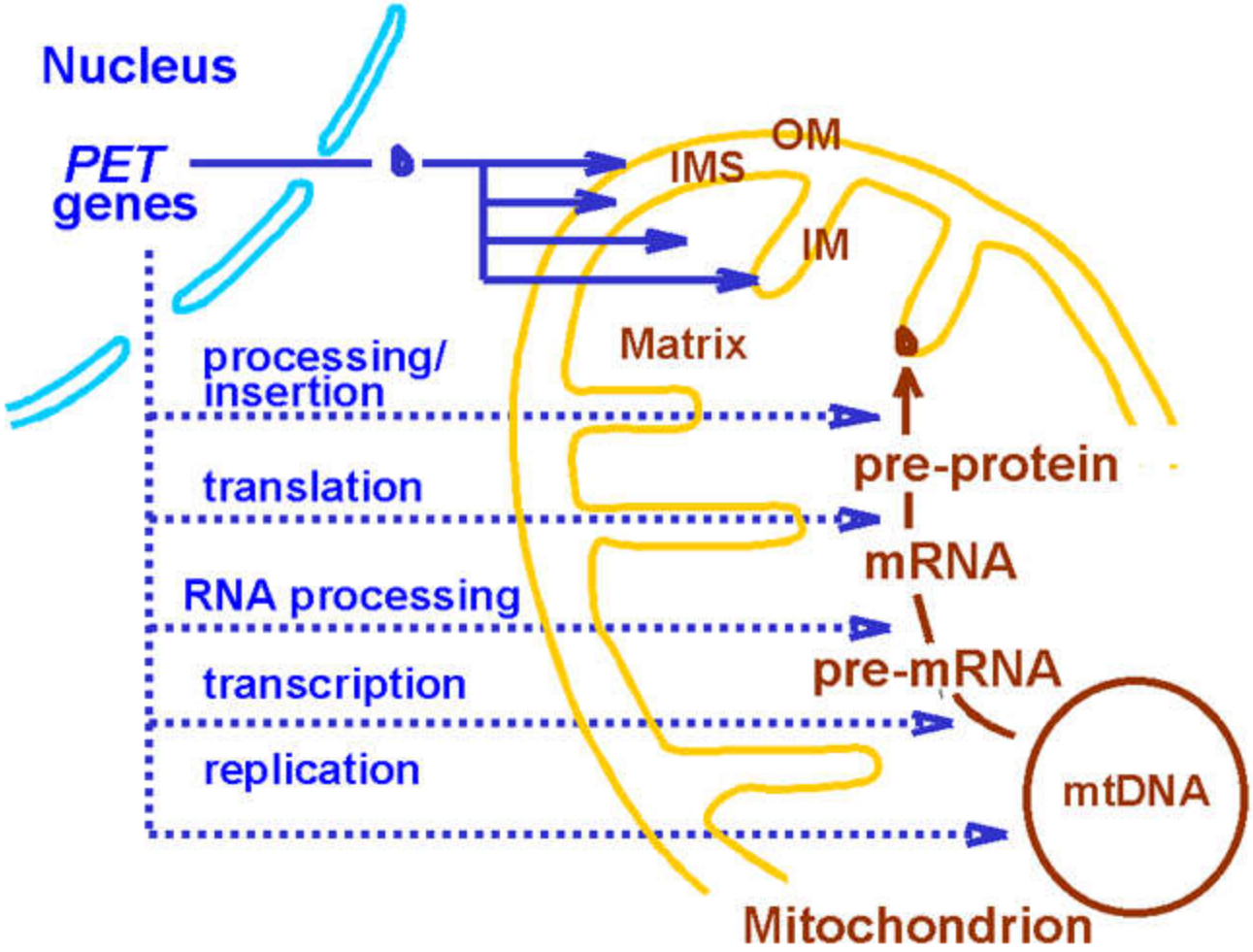
Ephrussi, 1949 – Mutantes "petites", *Saccharomyces cerevisiae.* Os mutantes "petites" apresentam crescimento lento e, somente crescem em meio de cultura contendo açúcares fermentáveis, como glicose. A análise genética desses mutantes revelou que o fenótipo "petite" não era determinado por tipo de herança nuclear



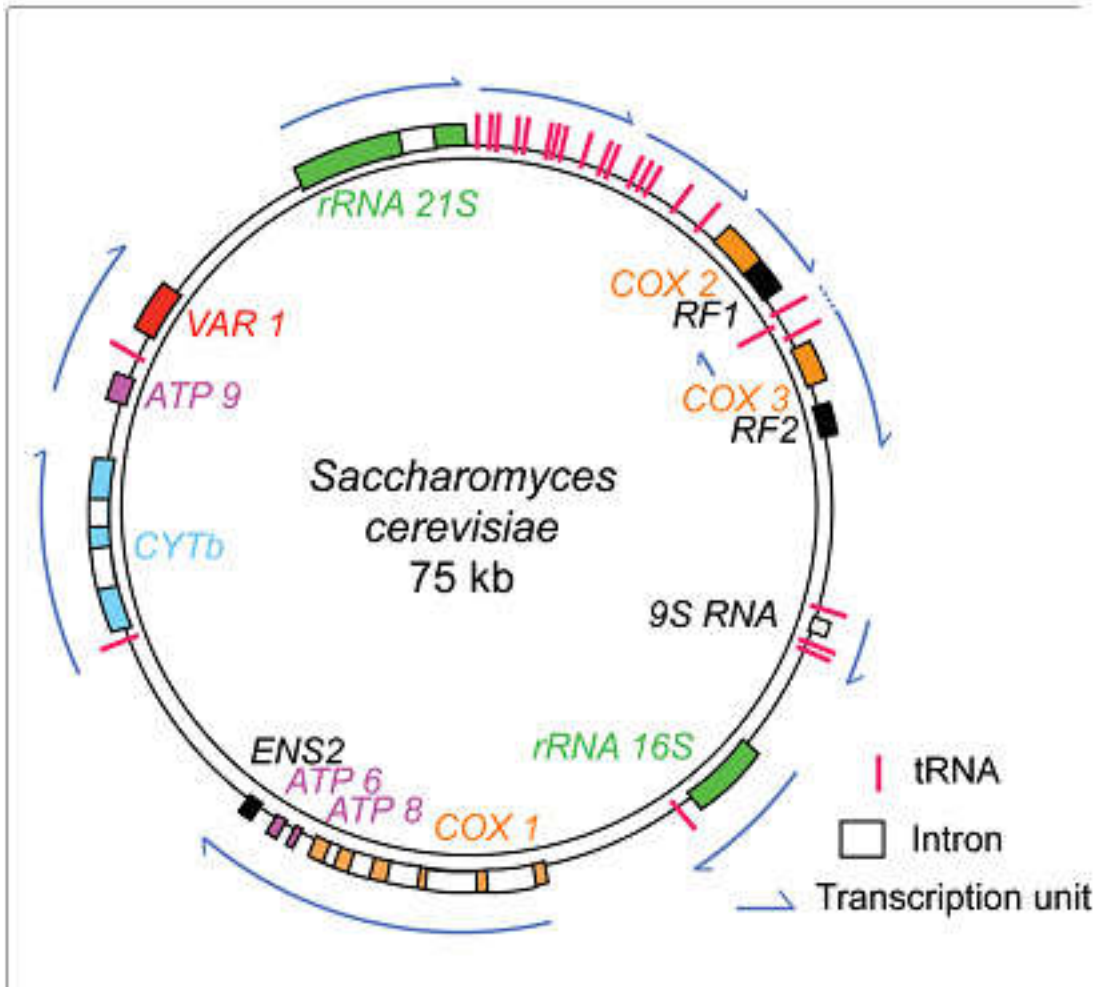
Evolution



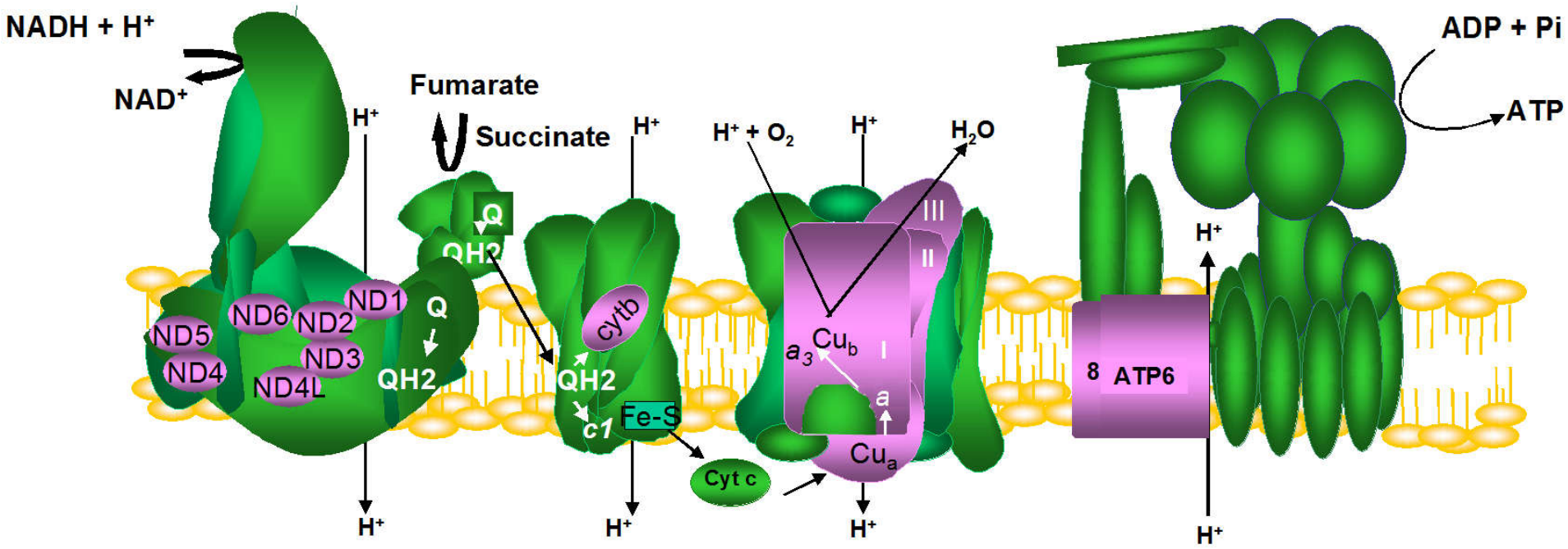
Mitochondrial disorders as windows into an ancient organelle
Scott B. Vafai & Vamsi K. Mootha
Nature 491, 374–383 (15 November 2012)


Nucleus
PET genes
OM
IMS
IM
Matrix
processing/ insertion
translation
RNA processing
transcription
replication
pre-protein
mRNA
pre-mRNA
mtDNA
Mitochondrion

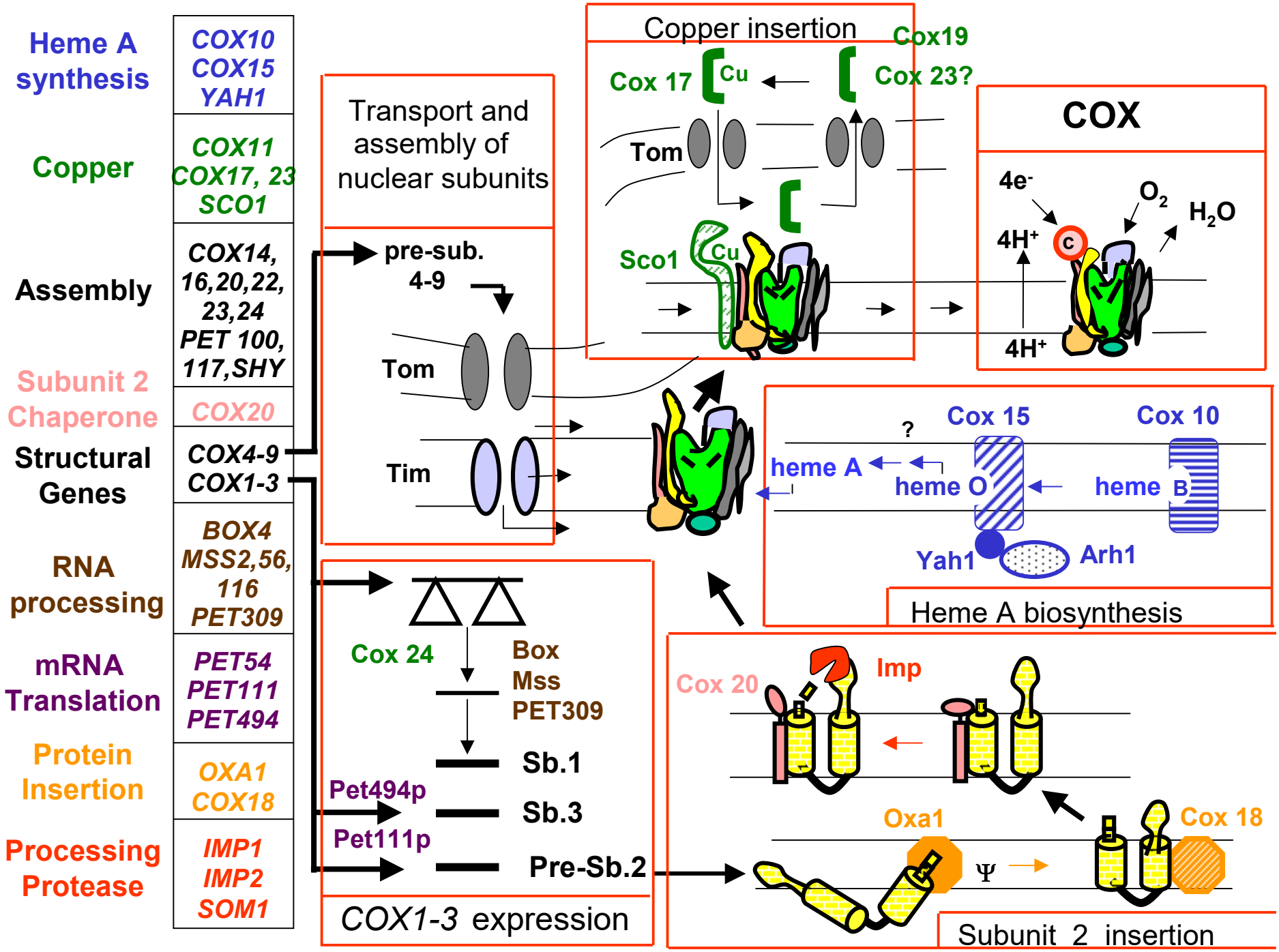
Yeast mt DNA → 11 RNA transcripts

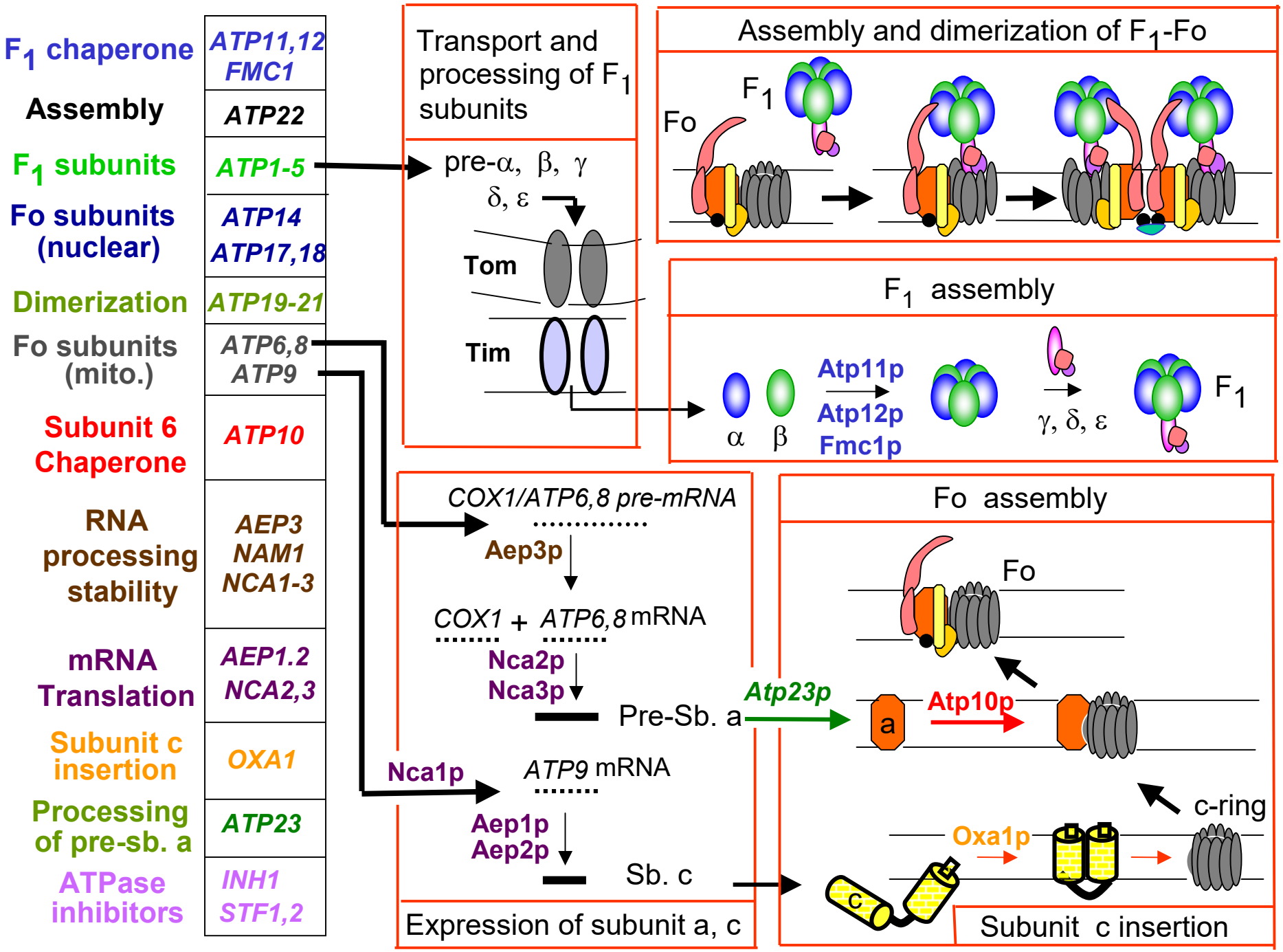


RNA Biology 10:9, 1477–1494; September 201

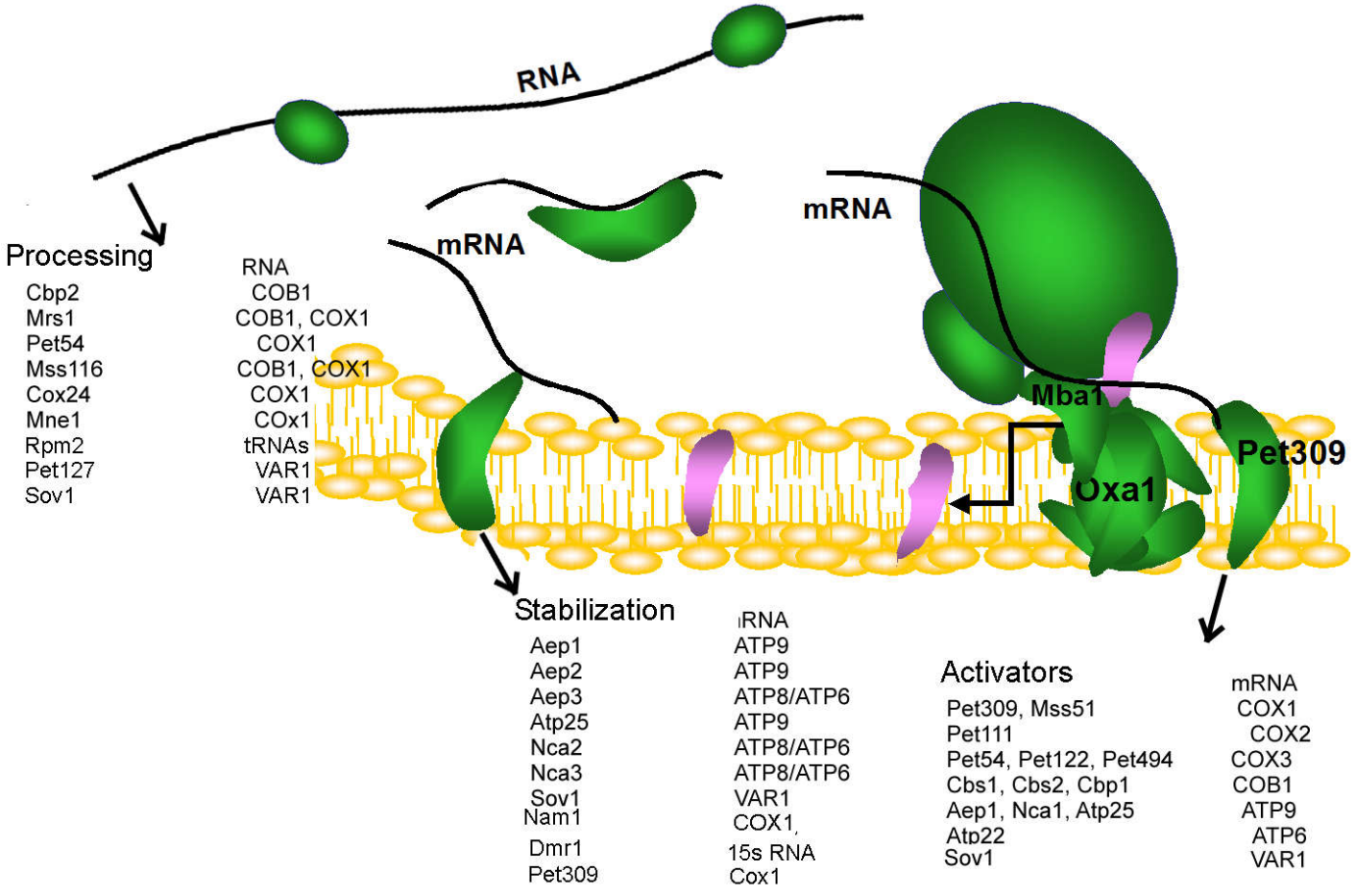


complex	I	II	III	IV	V
mt DNA subunits	7		1	3	2
nDNA subunits	38	4	10	10	14


Heme A synthesis
COX10
COX15
YAH1
Copper
COX11
COX17, 23
SCO1
Assembly
COX14, 16,20,22, 23,24
PET 100, 117,SHY
Subunit 2 Chaperone
COX20
Structural Genes
COX4-9
COX1-3
RNA processing
BOX4
MSS2,56, 116
PET309
mRNA Translation
PET54
PET111
PET494
Protein Insertion
OXA1
COX18
Processing Protease
IMP1
IMP2
SOM1
Transport and assembly of nuclear subunits
pre-sub. 4-9
Tom
Tim
Copper insertion
Cox19
Cox 17
Cu
Cox 23?
Tom
Sco1
Cu
COX
4e-
O2
H2O
4H+
c
4H+
Cox 15
Cox 10
?
heme A
heme O
heme B
Yah1
Arh1
Heme A biosynthesis
Cox 24
Box
Mss
PET309
Sb.1
Pet494p
Sb.3
Pet111p
Pre-Sb.2
COX1-3 expression
Cox 20
Imp
Oxa1
Ψ
Cox 18
Subunit 2 insertion

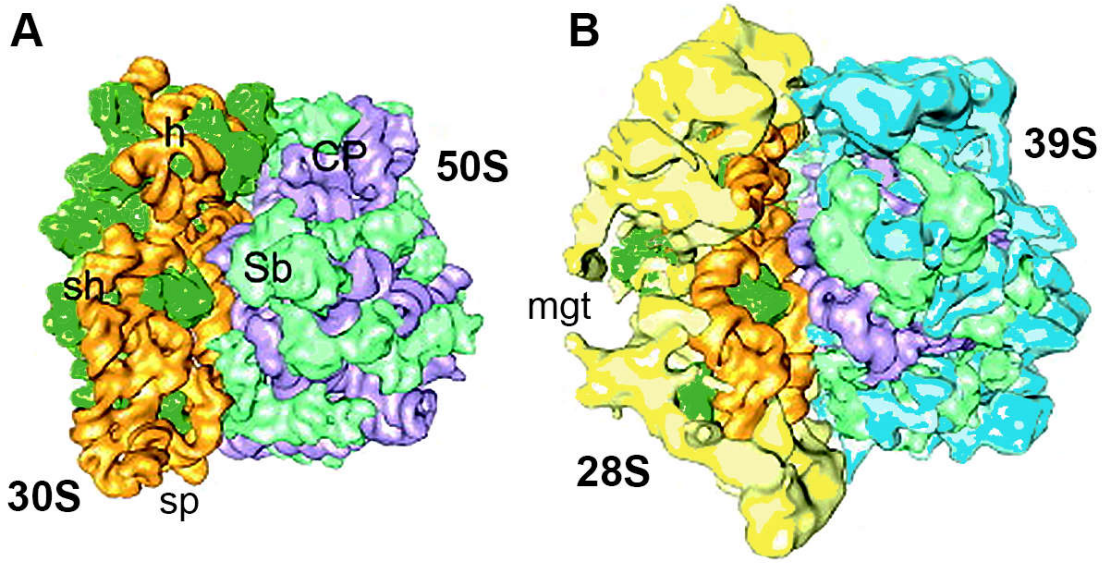

F1 chaperone
ATP11,12
FMC1
Assembly
ATP22
F1 subunits
ATP1-5
Fo subunits (nuclear)
ATP14
ATP17,18
Dimerization
ATP19-21
Fo subunits (mito.)
ATP6,8
ATP9
Subunit 6 Chaperone
ATP10
RNA processing stability
AEP3
NAM1
NCA1-3
mRNA Translation
AEP1.2
NCA2,3
Subunit c insertion
OXA1
Processing of pre-sb. a
ATP23
ATPase inhibitors
INH1
STF1,2
Transport and processing of F1 subunits
pre-α, β, γ
δ, ε
Tom
Tim
Assembly and dimerization of F1-Fo
F1
Fo
F1 assembly
α
β
Atp11p
Atp12p
Fmc1p
γ, δ, ε
F1
COX1/ATP6,8 pre-mRNA
Aep3p
COX1 + ATP6,8 mRNA
Nca2p
Nca3p
Pre-Sb. a
Atp23p
Nca1p
ATP9 mRNA
Aep1p
Aep2p
Sb. c
Expression of subunit a, c
Fo assembly
Fo
a
Atp10p
c-ring
Oxa1p
c
Subunit c insertion

Mt RNA transcripts need to be processed, stabilized and translated



Bacterial *vs* mitochondrial ribosomes

~7Å Resolution



Bacterial (*E. coli*) 70S ribosome

Mammalian (*B. taurus*) 55S mitoribosome

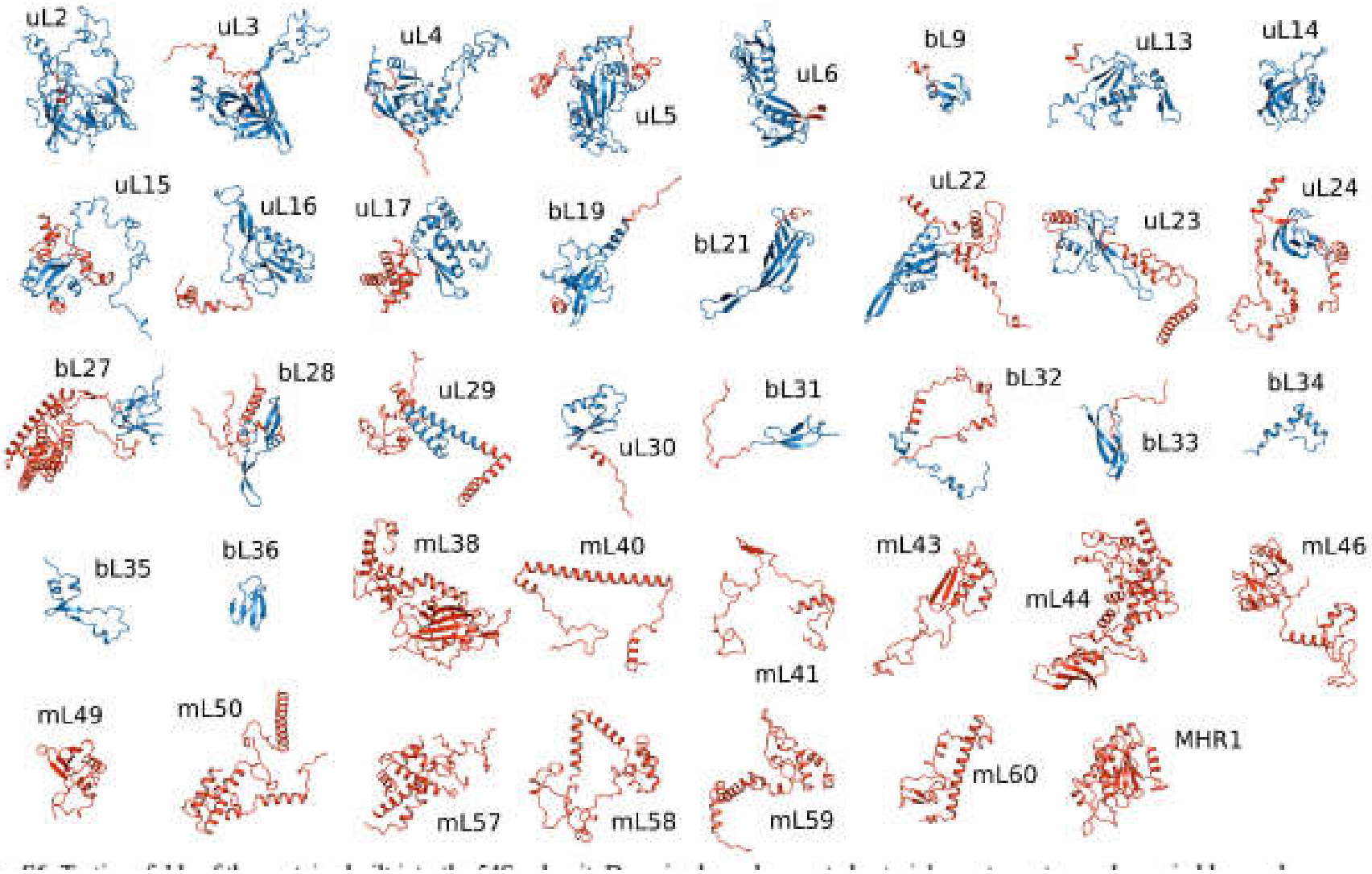
- The rRNAs: SSU (**orange**) and LSU (**purple**)
- Ribosomal proteins: SSU (**green**) and LSU (aquamarine)
- Mito-specific r-proteins: SSU (**yellow**) and LSU (blue)

			Yeast mitorib
Molecular mass	2.3MDa	2.7MDa	
Sedimentation Coefficient	70S	55S	74S
RNA : Protein ratio	**~2:1**	**~1:2**	**~1:1**

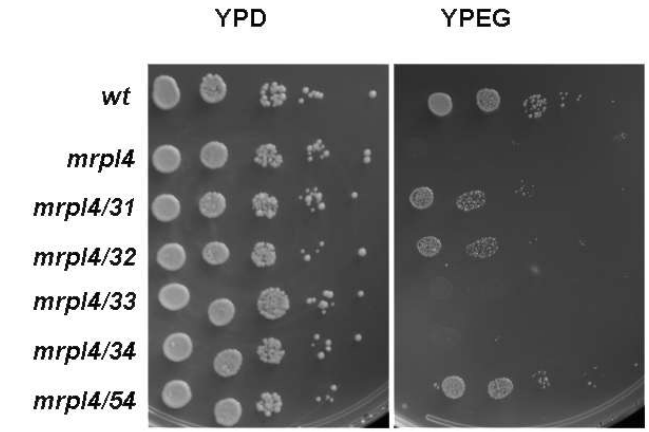
Rajendra Agrawal' s group

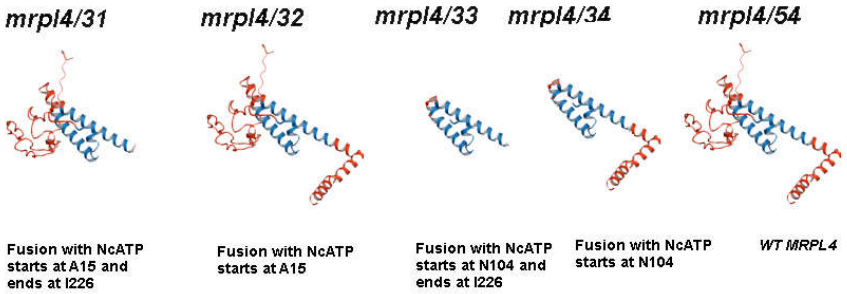
Current opinion in structure biology, Vol 22, 797–803, 2012

Proteins of the large subunit of the mitoribosome

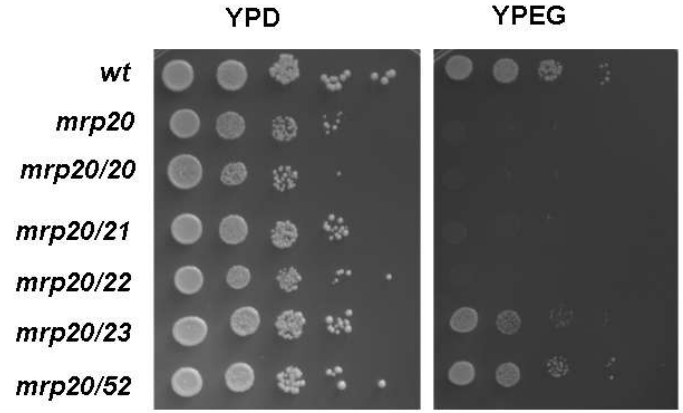


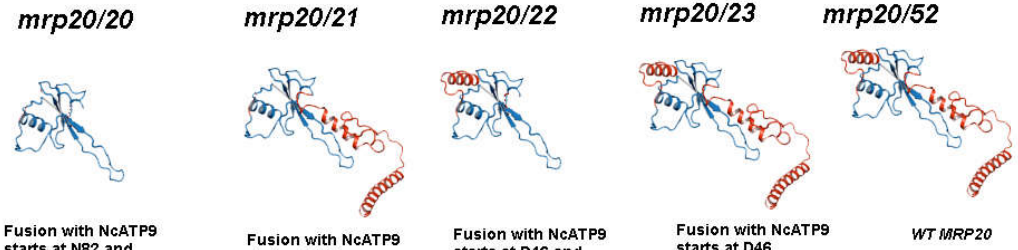
% ρ^+


YPD
YPEG
wt
mrpl4
mrpl4/31
mrpl4/32
mrpl4/33
mrpl4/34
mrpl4/54
100
55.5
43
55.5
99


mrpl4/31
mrpl4/32
mrpl4/33
mrpl4/34
mrpl4/54
Fusion with NcATP starts at A15 and ends at I226
Fusion with NcATP starts at A15
Fusion with NcATP starts at N104 and ends at I226
Fusion with NcATP starts at N104
WT MRPL4

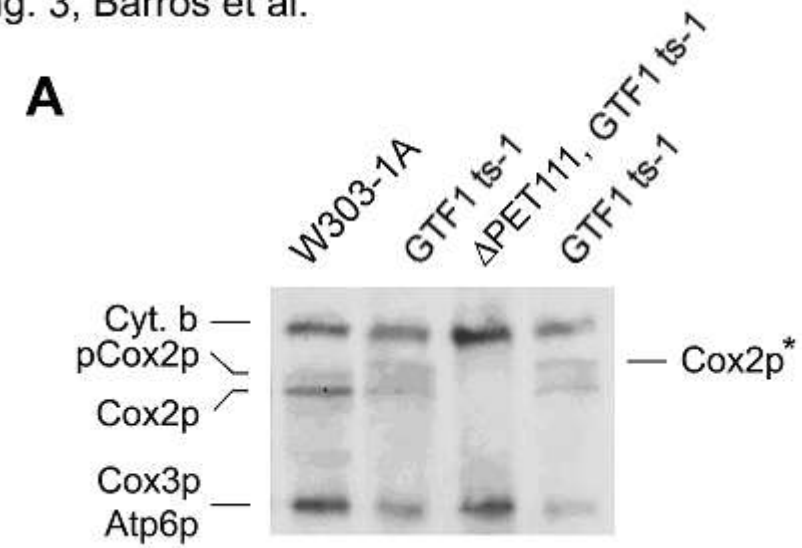
% ρ^+


YPD
YPEG
wt
mrp20
mrp20/20
mrp20/21
mrp20/22
mrp20/23
mrp20/52
84
98
78
96
100

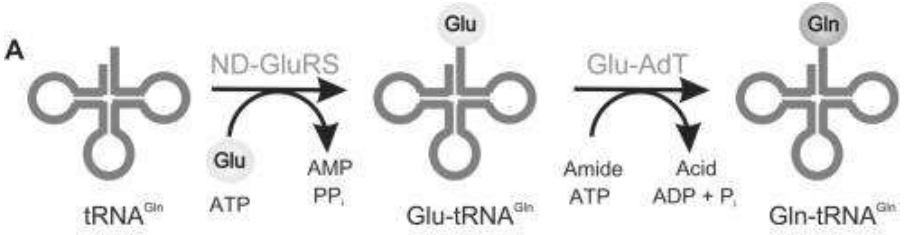

mrp20/20
mrp20/21
mrp20/22
mrp20/23
mrp20/52
Fusion with NcATP9 starts at N82 and ends at R175
Fusion with NcATP9 starts at N82
Fusion with NcATP9 starts at D46 and ends at R175
Fusion with NcATP9 starts at D46
WT MRP20

	Bacteria	Mitochondria	cytosol
Sensitivity to aminoglycosides	sensitive	sensitive	Resistant
Translation Initiation factors	IF1, IF2, IF3	mIF2, mIF3	eIFs...
Initial $tRNA^{Met}$	Formylated	Formylated	Normal
Aminoacyl tRNA sintetases	unspecific for Q and N	unspecific for Q	Specific for all

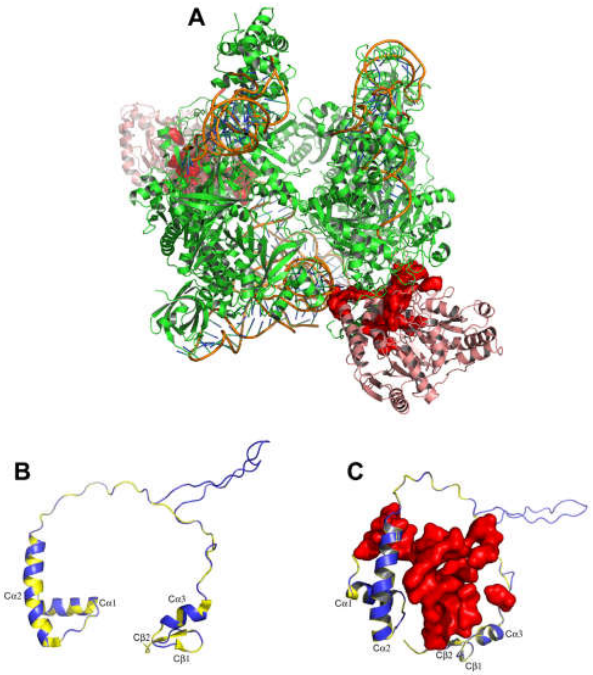
Fig. 3, Barros et al.



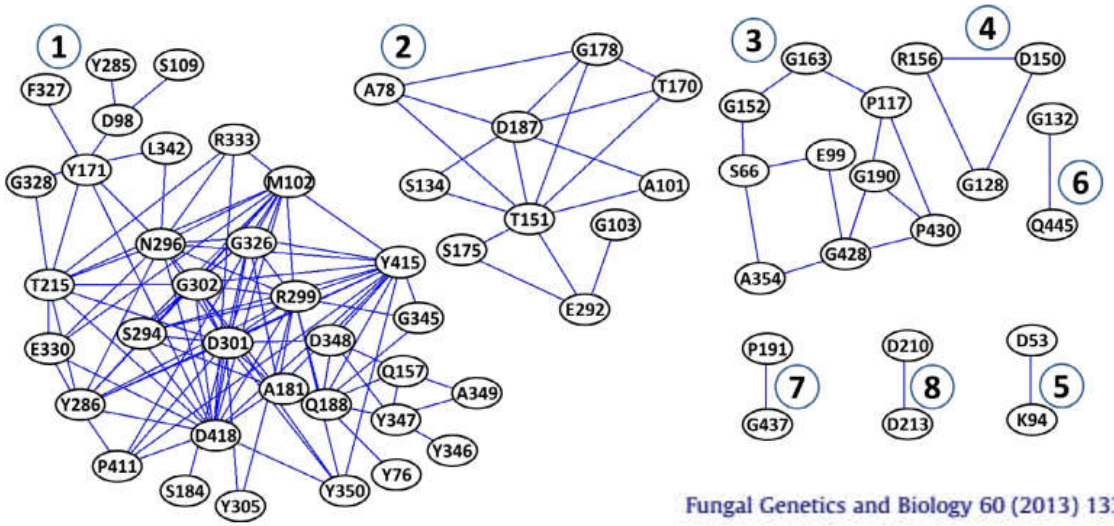
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 3



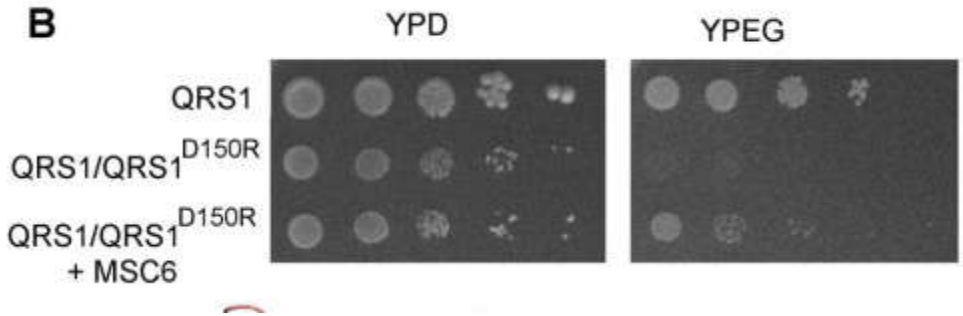
Nucleic Acids Res. 2008 Apr; 36(6):1813-1825



Fungal Genetics and Biology 60 (2013) 133–139

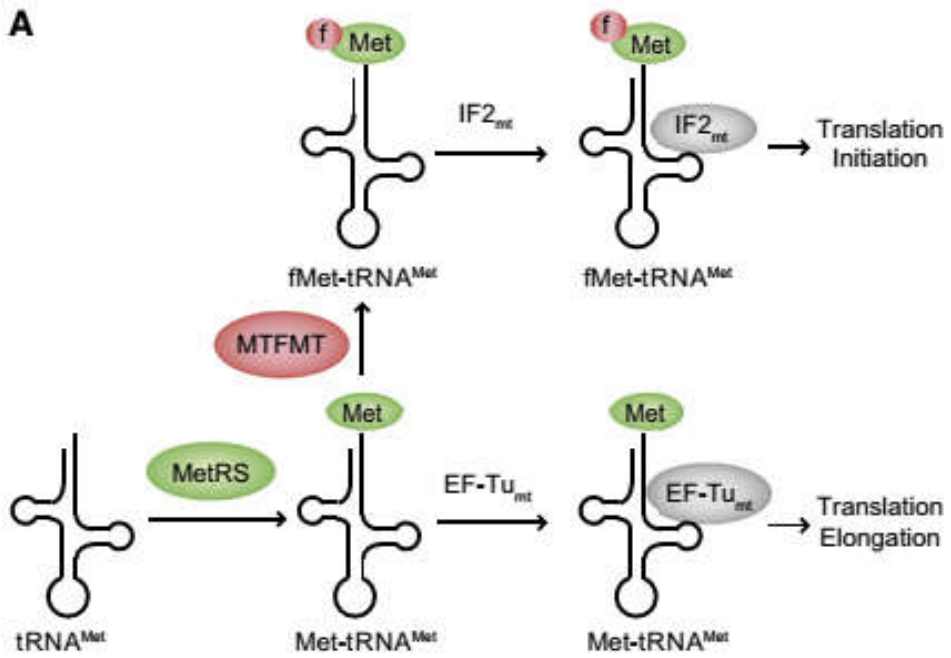


Fungal Genetics and Biology 60 (2013) 133–139



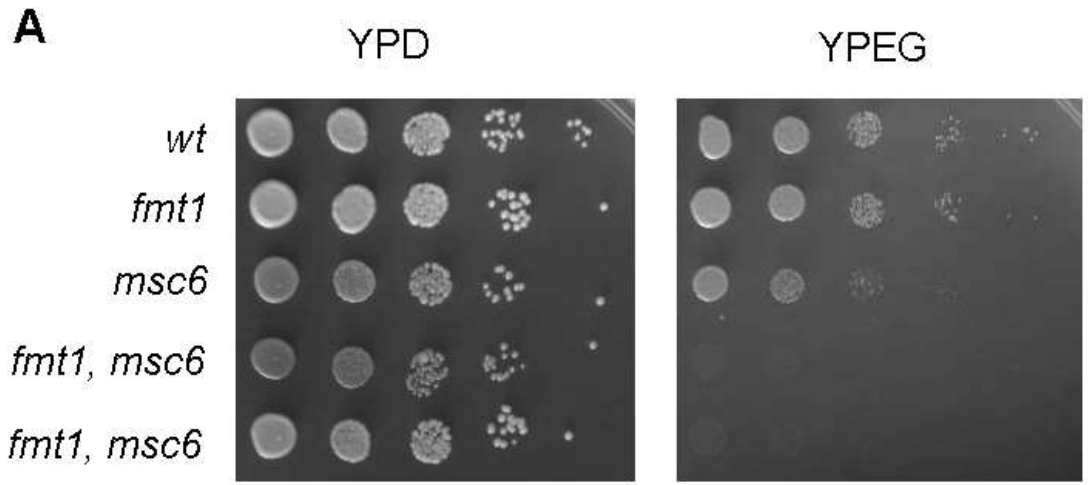
Curr Genet (2016) 62:607–617

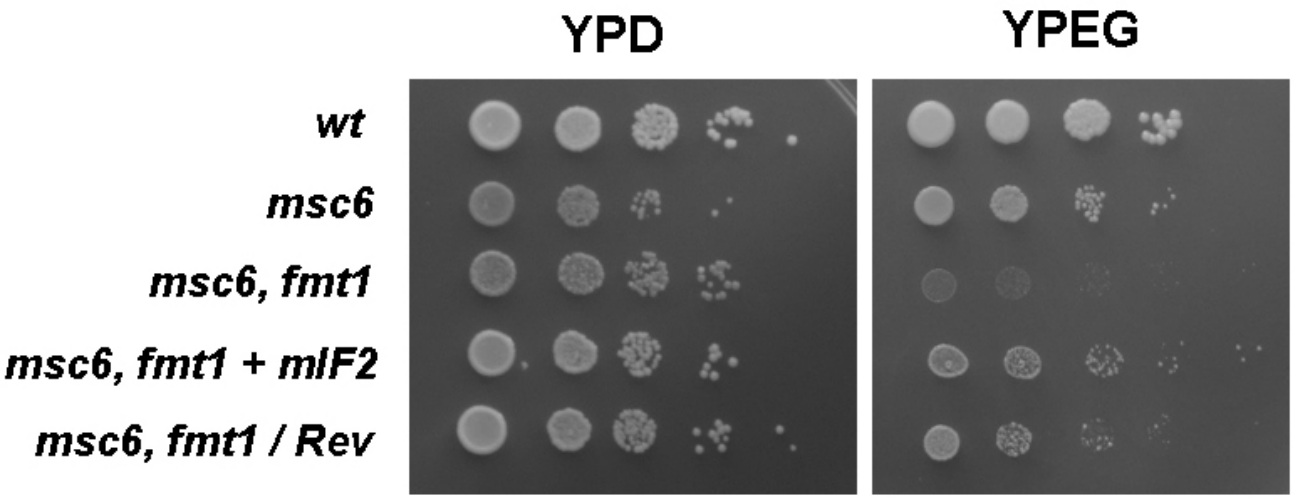
Translation initiation depends on formylation of Met-tRNA^{met}



Cell Metabolism *14*, 428–434, September 7, 2011

msc6 and *fmt1* double mutants are respiratory deficient





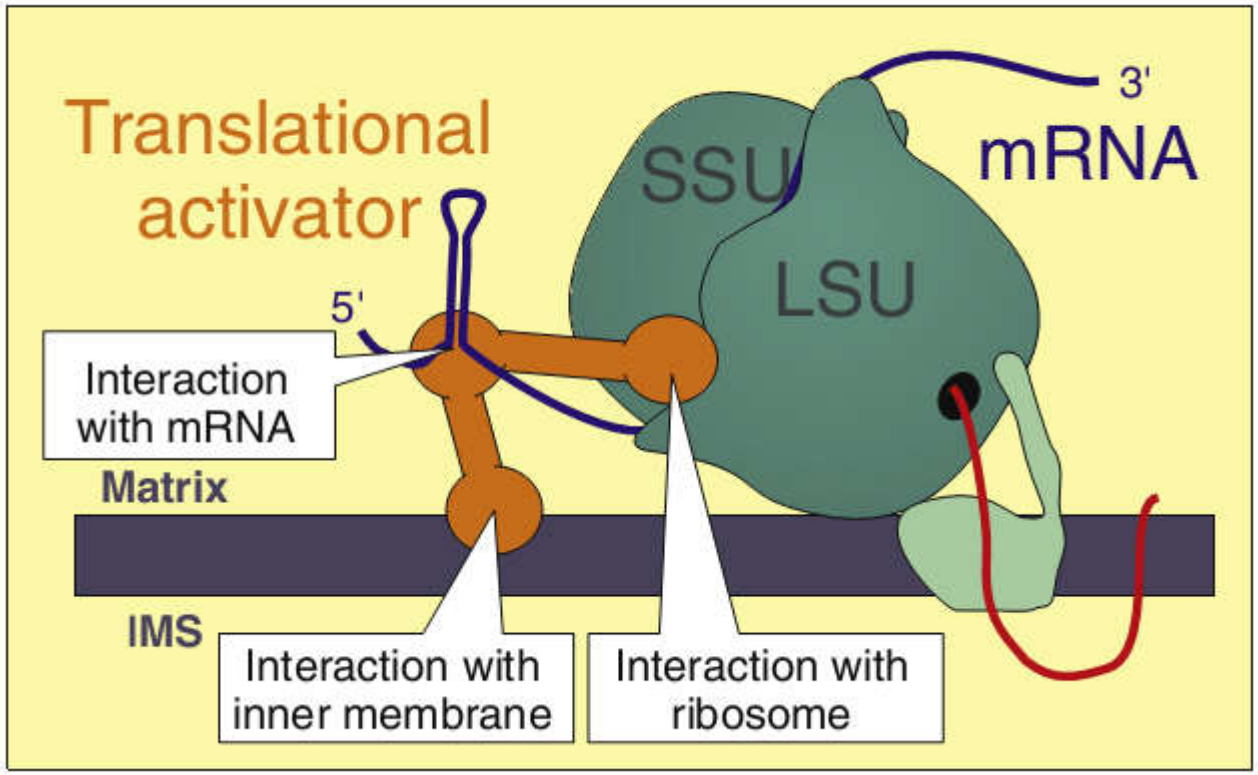
Summary on *MSC6*

→ 1) Overexpression suppresses defective translation in an AdT mutant

→ 2) The null mutant presents respiratory deficiency if combined with *fmt1* mutant

→3) The respiratory deficiency of the double mutant msc6, fmt1 is suppressed by mIF-2 excess.

Yeast Mt Genes	TranslationalActivators
ATP6	ATP22
ATP8	?
ATP9	AEP1
COX1	PET309, MSS51
COX2	PET111
COX3	PET54/122/494
COB1	CBP1, CBS1/2/3/6
VAR1	SOV1 (?)


Translational activator
3'
mRNA
SSU
LSU
5'
Interaction with mRNA
Matrix
IMS
Interaction with inner membrane
Interaction with ribosome

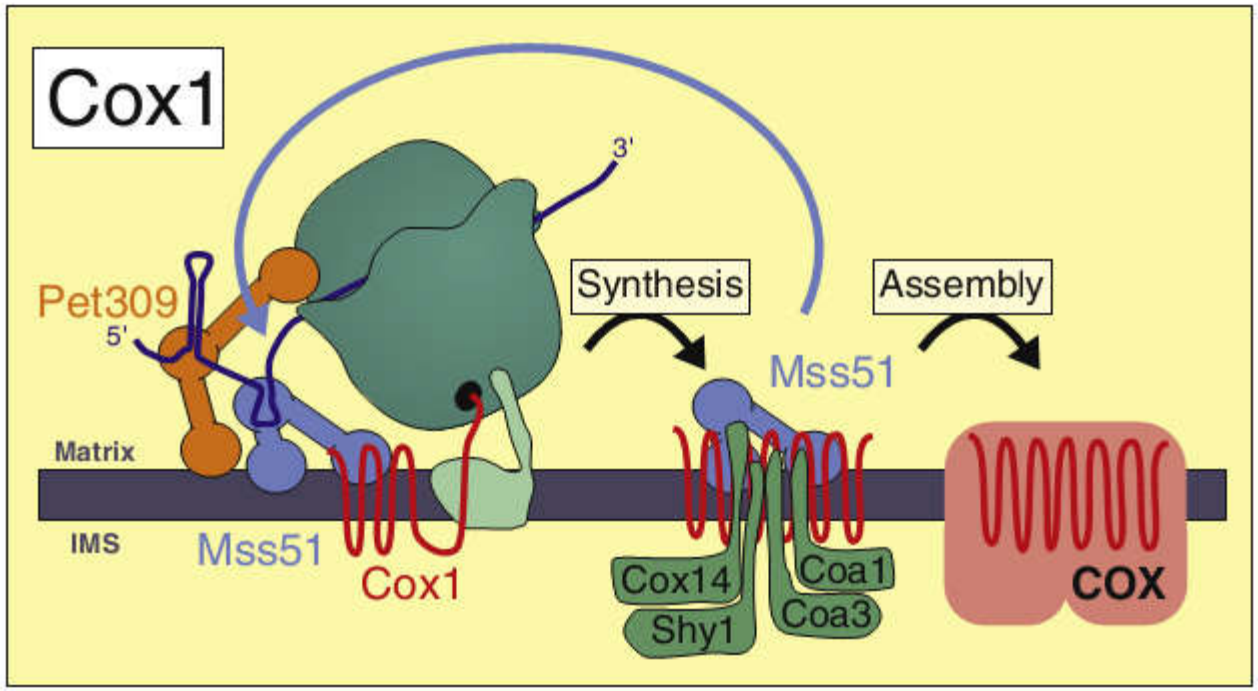
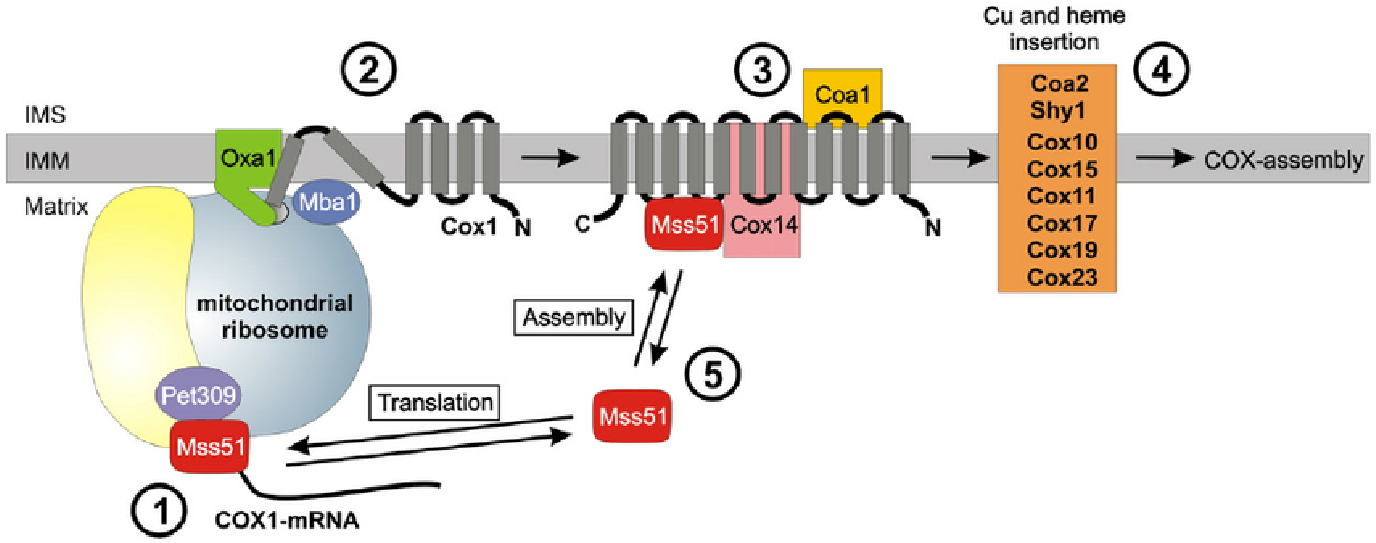


Fig. 3. Feedback control of Cox1 synthesis. The *COX1* mRNA is the target of two translational activators, Pet309 and Mss51. The membrane-associated protein Mss51 probably binds to the *COX1* mRNA at its 5′-UTR region. Moreover, it binds the newly synthesized Cox1 protein and stabilizes it together with a number of other assembly factors. As long as unassembled Cox1 and these assembly factors are bound to Mss51, Mss51 cannot stimulate translation of *COX1*. Only upon assembly of Cox1 with other subunits of the cytochrome *c* oxidase complex is Mss51 released to activate *COX1* translation.



Nature Reviews Molecular Cell Biology **12**, 14-20

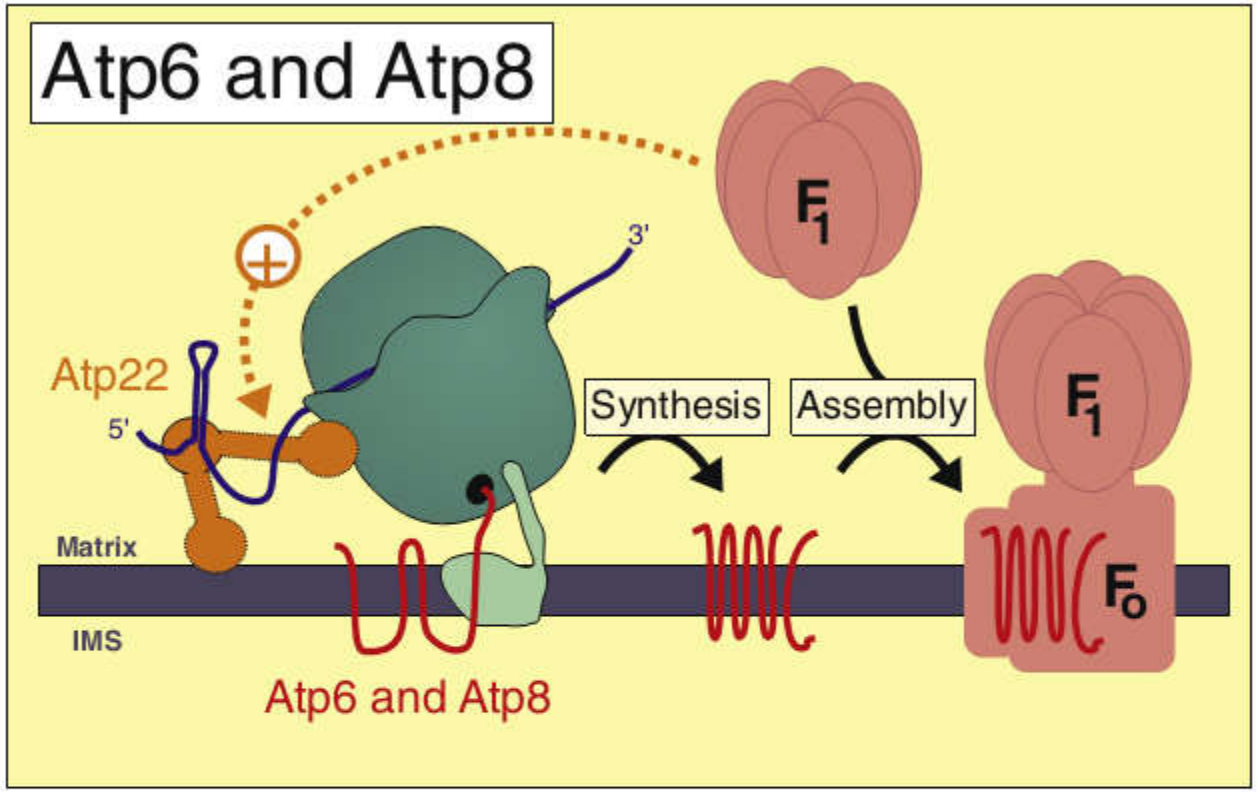
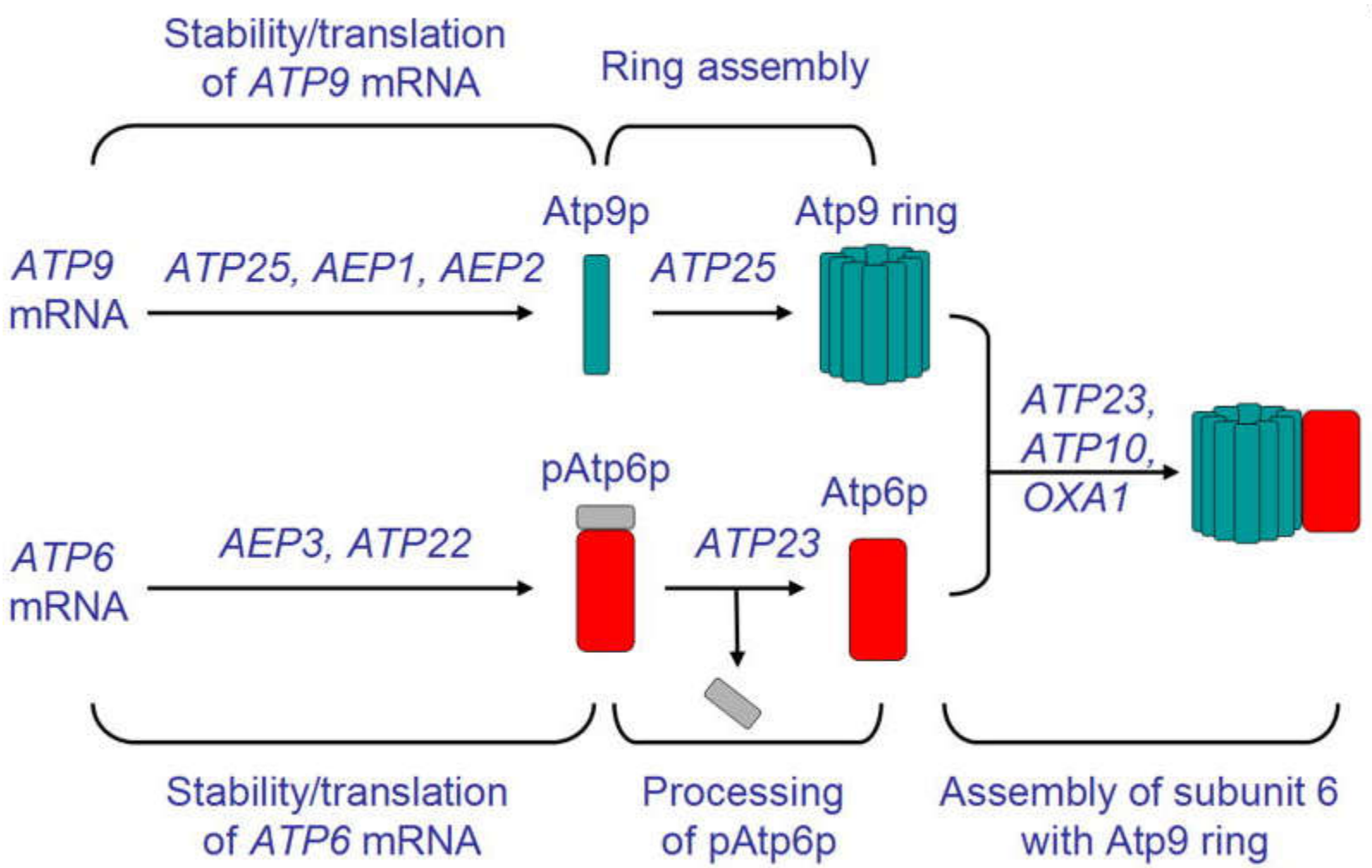
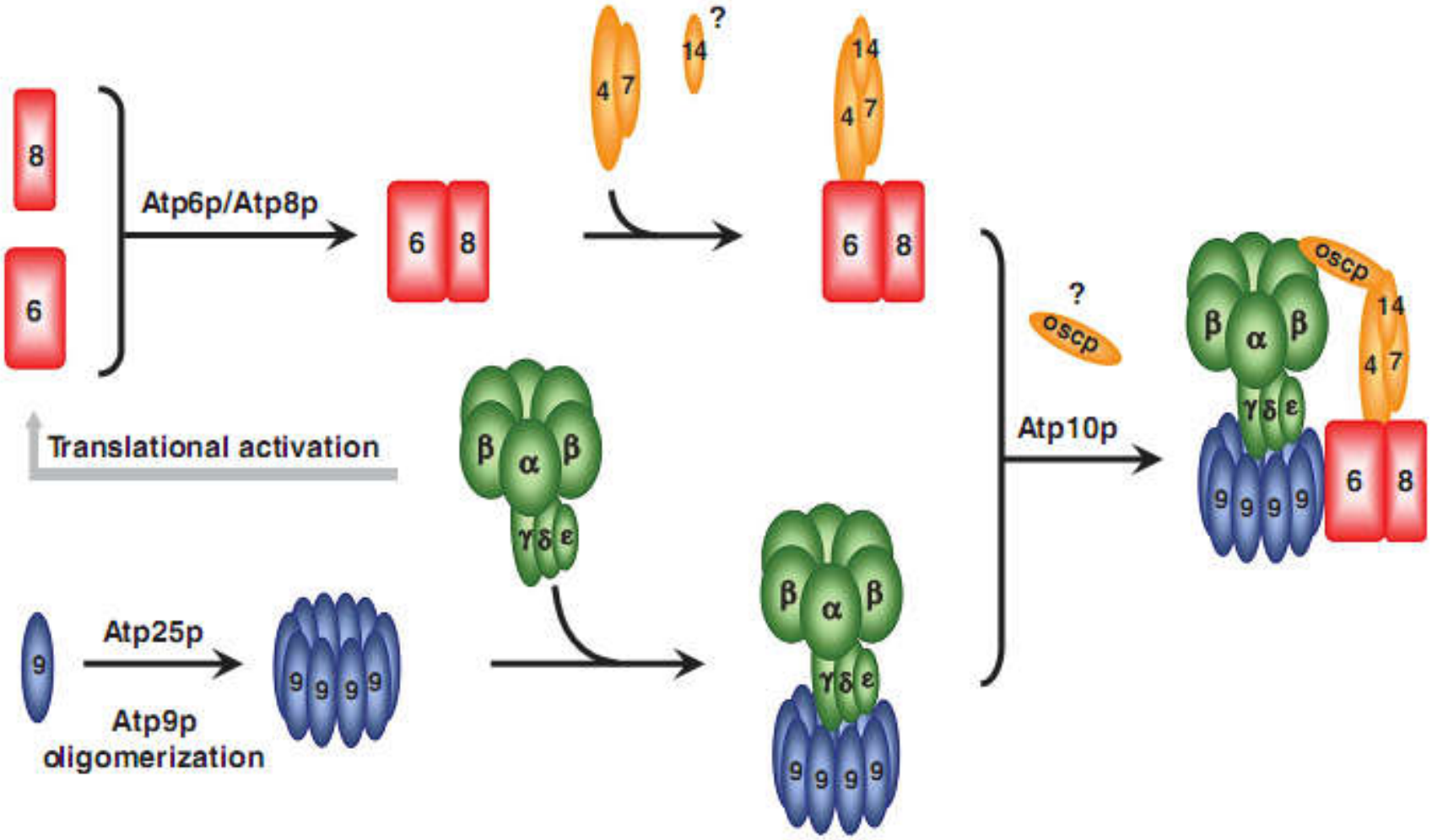
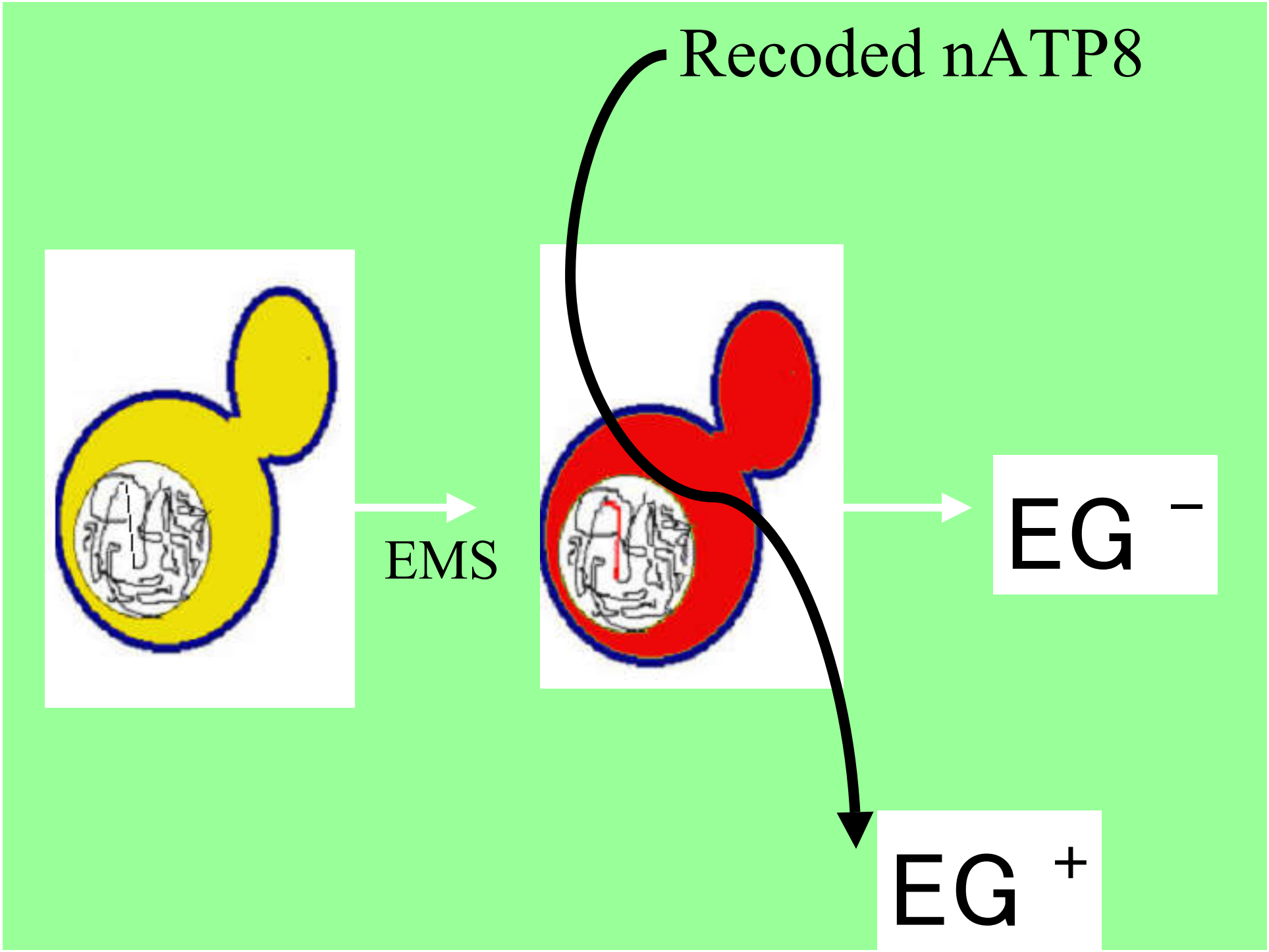


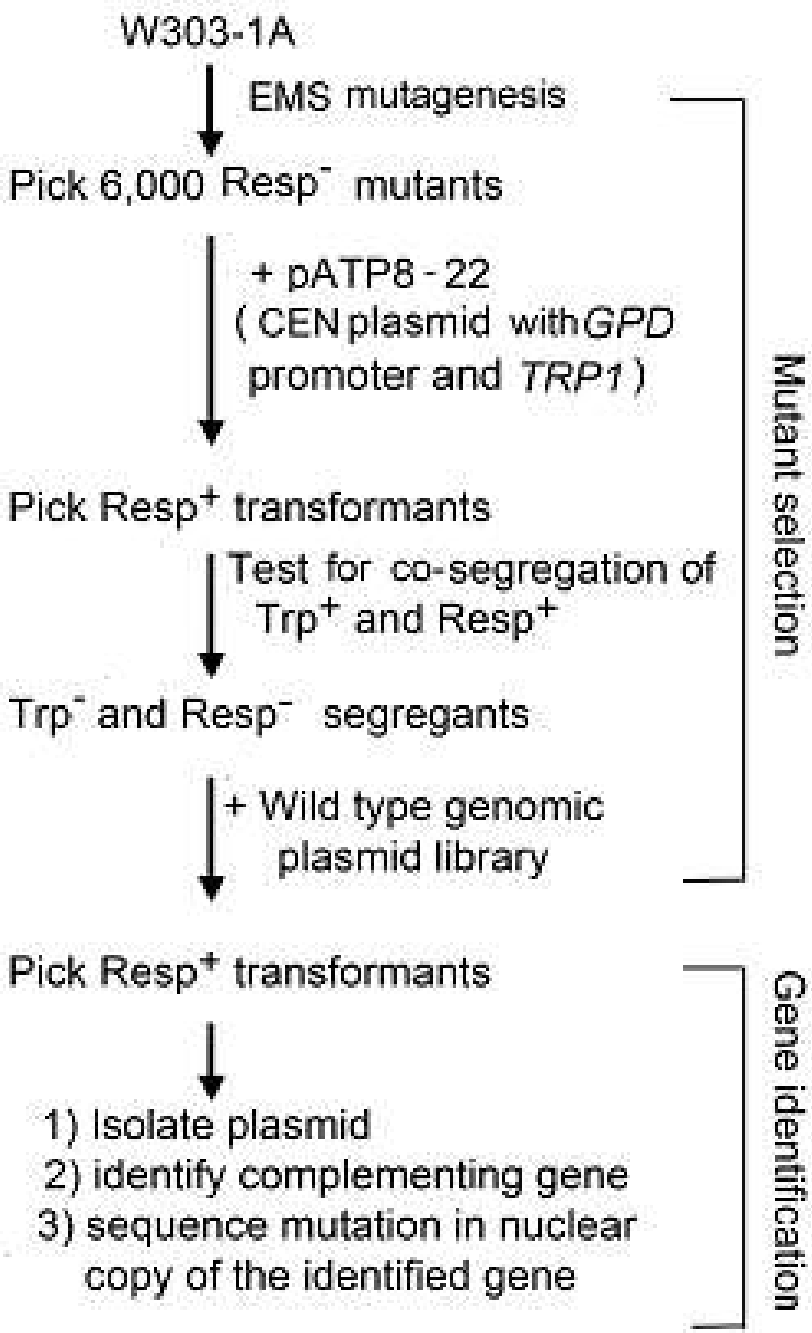
Fig. 5. Feedback control of Atp6 and Atp8 synthesis. Atp6 and Atp8 represent two subunits of the F_o part of the mitochondrial F_oF_1-ATPase. Their synthesis is strongly stimulated in the presence of F_1 pre-complexes which exclusively consist of nuclear encoded subunits. Mutants that fail to produce or assemble F_1 subunits produce only low amounts of Atp6 and Atp8. Overexpression of the translational activator Atp22 relieves this block suggesting that Atp22 is – directly or indirectly – activated by the presence of F_1 precomplexes. This regulatory feedback loop adapts the levels of mitochondrially encoded Atp6 and Atp8 to the levels of assembled nuclear encoded subunits of the ATPase.



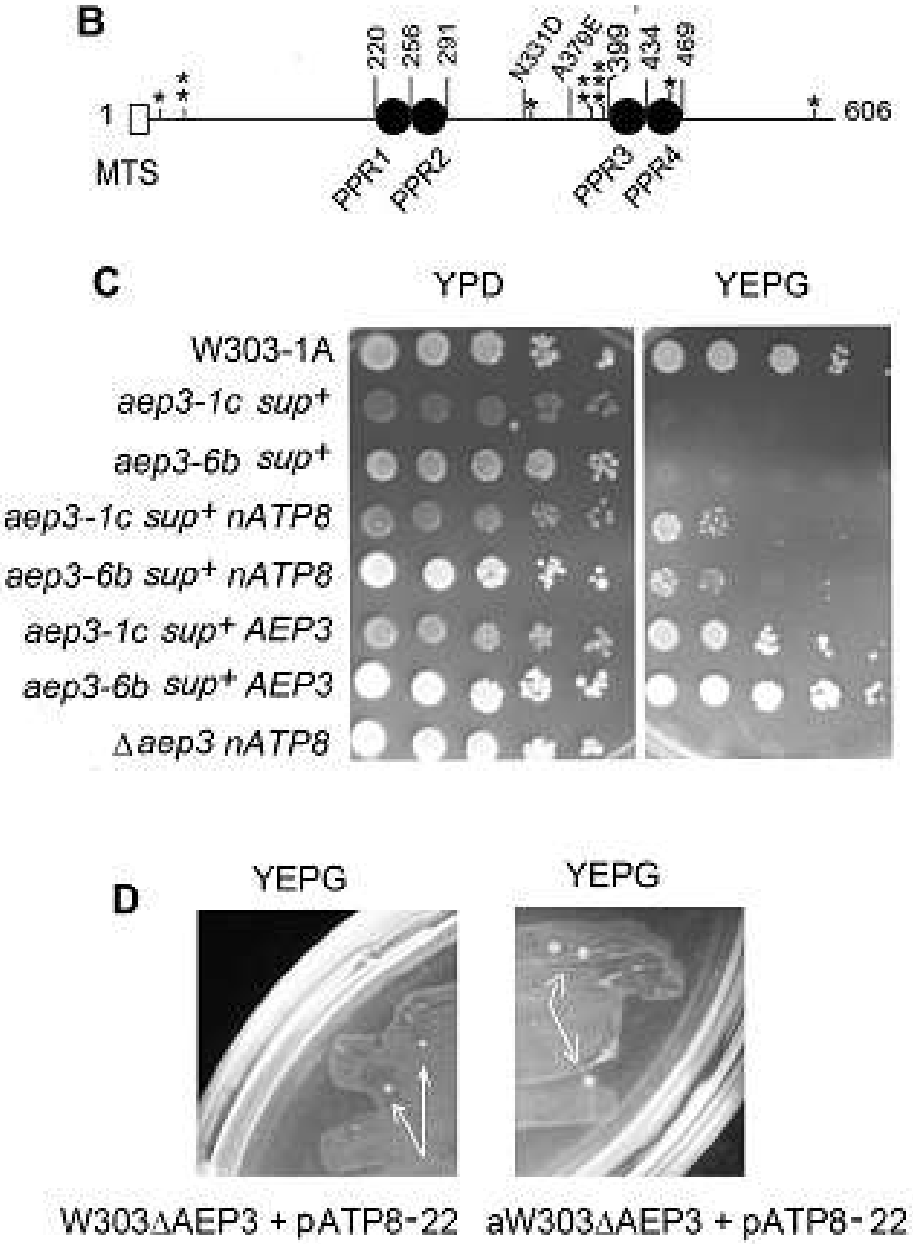
Biochim Biophys Acta. 2009 Jan;1793(1):108-16.


8
6
Atp6p/Atp8p
6 8
4 7
14
?
14
4 7
6 8
?
oscp
Atp10p
oscp
14
4 7
β α β
γ δ ε
9 9 9 9
6 8
Translational activation
β α β
γ δ ε
9
Atp25p
9 9 9 9
Atp9p
oligomerization
β α β
γ δ ε
9 9 9 9


Recoded nATP8
EMS
EG –
EG +


W303-1A
EMS mutagenesis
Pick 6,000 Resp- mutants
+ pATP8 - 22
(CEN plasmid with GPD
promoter and TRP1)
Pick Resp+ transformants
Test for co-segregation of
Trp+ and Resp+
Trp- and Resp- segregants
+ Wild type genomic
plasmid library
Pick Resp+ transformants
1) Isolate plasmid
2) identify complementing gene
3) sequence mutation in nuclear
copy of the identified gene
Mutant selection
Gene identification

Growth of *aep3* mutants in the presence of *nATP8*



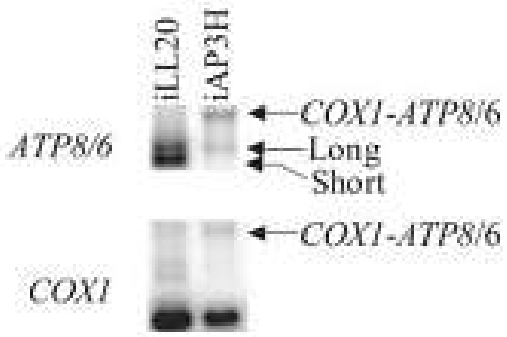
THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 279, No. 16, Issue of April 16, pp. 15728–15733, 2004
Printed in U.S.A.

Aep3p Stabilizes the Mitochondrial Bicistronic mRNA Encoding Subunits 6 and 8 of the H^+-translocating ATP Synthase of *Saccharomyces cerevisiae**

Received for publication, December 24, 2003, and in revised form, January 21, 2004
Published, JBC Papers in Press, January 23, 2004, DOI 10.1074/jbc.M314162200

Timothy P. Ellis‡, Kevin G. Helfenbein§, Alexander Tzagoloff§, and Carol L. Dieckmann‡¶

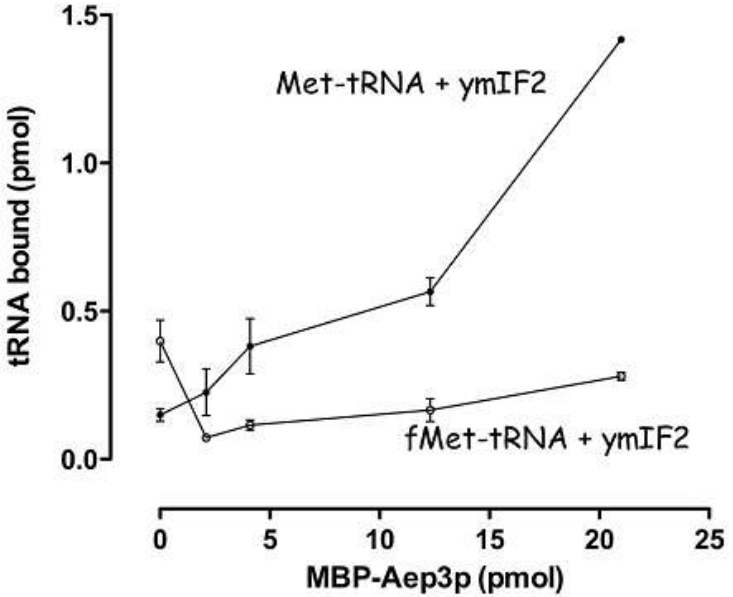


THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 284, NO. 49, pp. 34116–34125, [illegible]

Yeast AEP3p Is an Accessory Factor in Initiation of Mitochondrial Translation*

Received for publication, August 13, 2009, and in revised form, October 19, 2009 Published, JBC Papers in Press, October 20, 2009, DOI 10.1074/jbc.M109.05[illegible]

Changkeun Lee[1], Anne S. Tibbetts, Gisela Kramer, and Dean R. Appling[2]



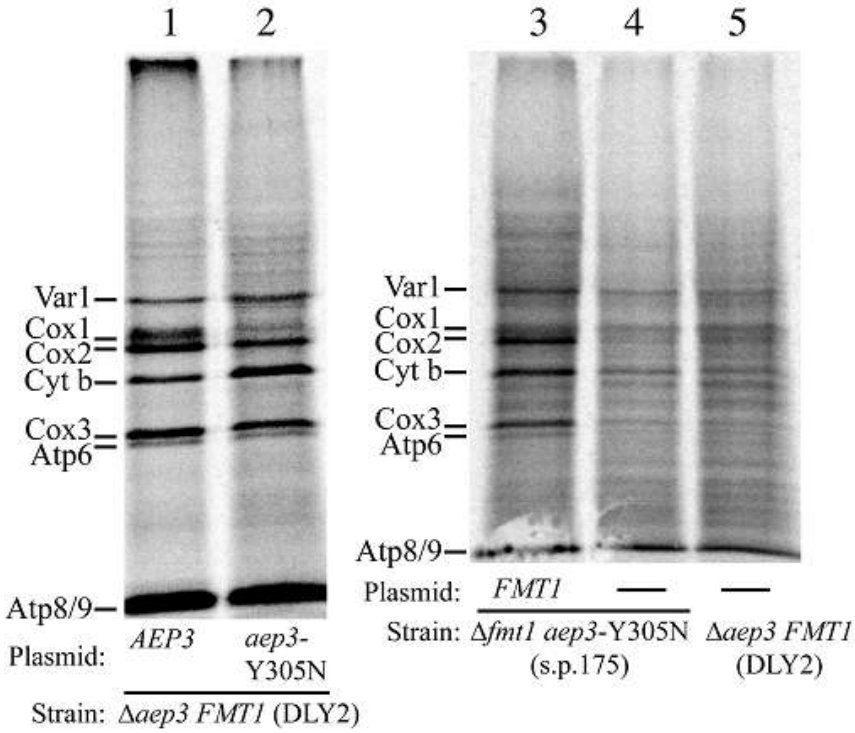
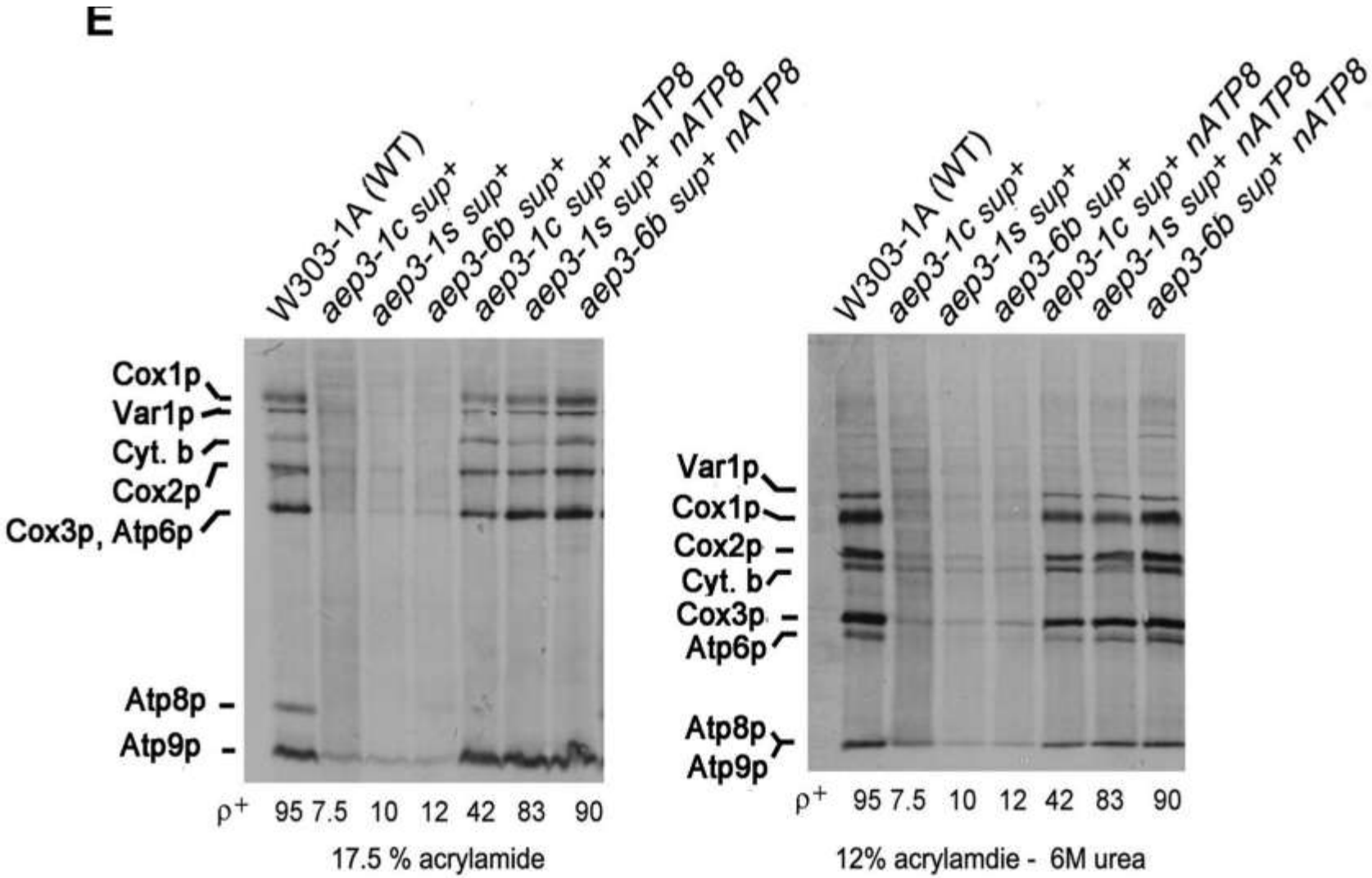
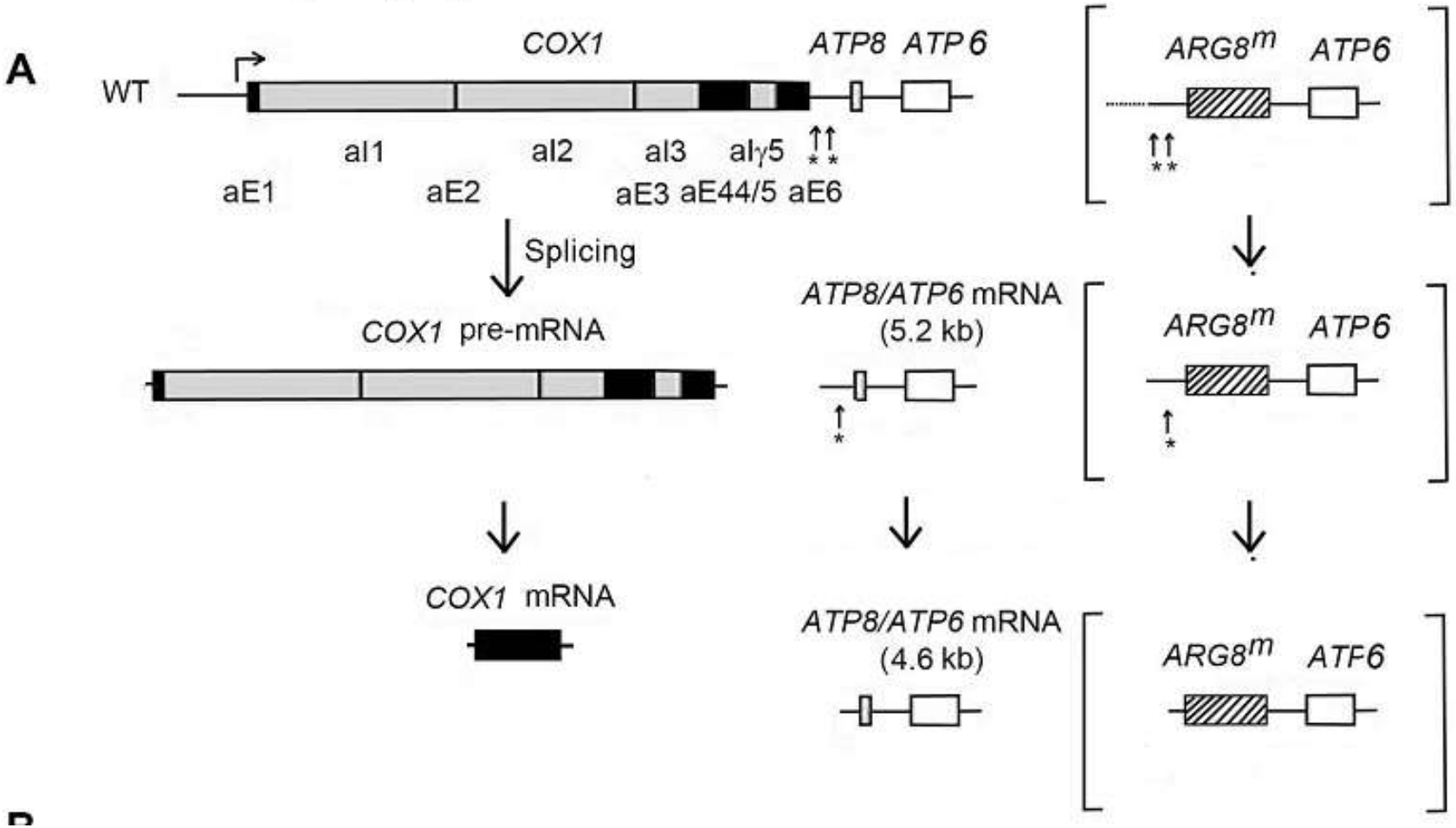


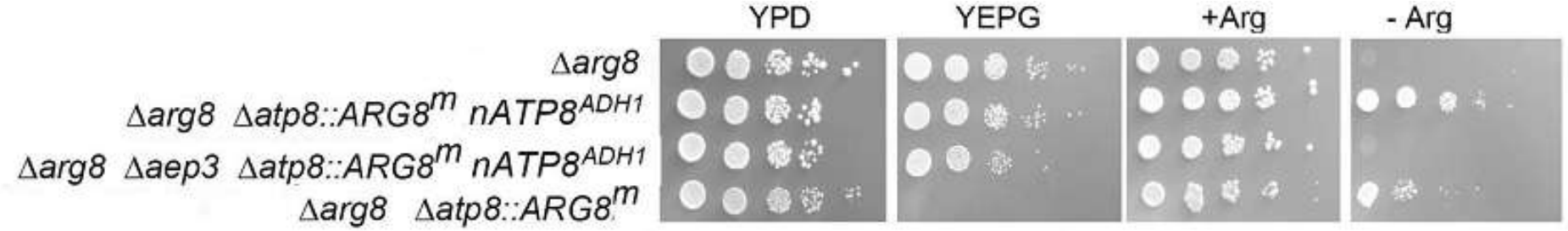
FIGURE 6. **Aep3p stimulates the binding of Met-tRNA$_f^{Met}$, but not fMet-tRNA$_f^{Met}$, to ymIF2.** Each filter binding assay contained 7 pmol of ymIF2 and increasing amounts of MBP-Aep3p fusion protein with either 5 pmol of [^{35}S]fMet-tRNA$_f^{Met}$ (○) or 4 pmol of [^{35}S]Met-tRNA$_f^{Met}$ (●). Nonspecific binding to the filters in the absence of protein was subtracted. Results shown are the

Restoration of mitochondrial translation in aep3 mutants by nATP8

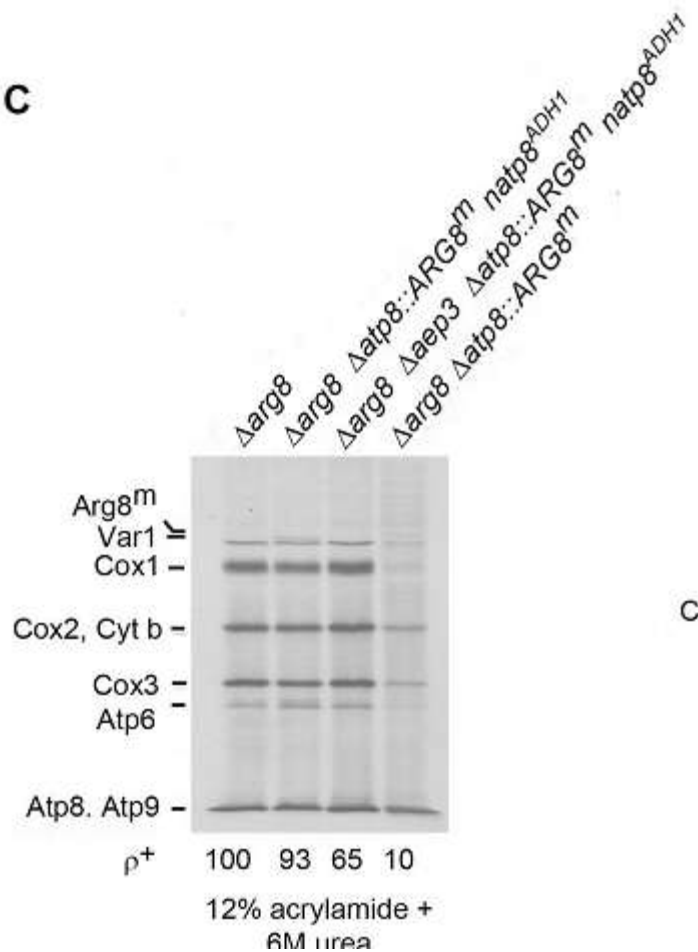


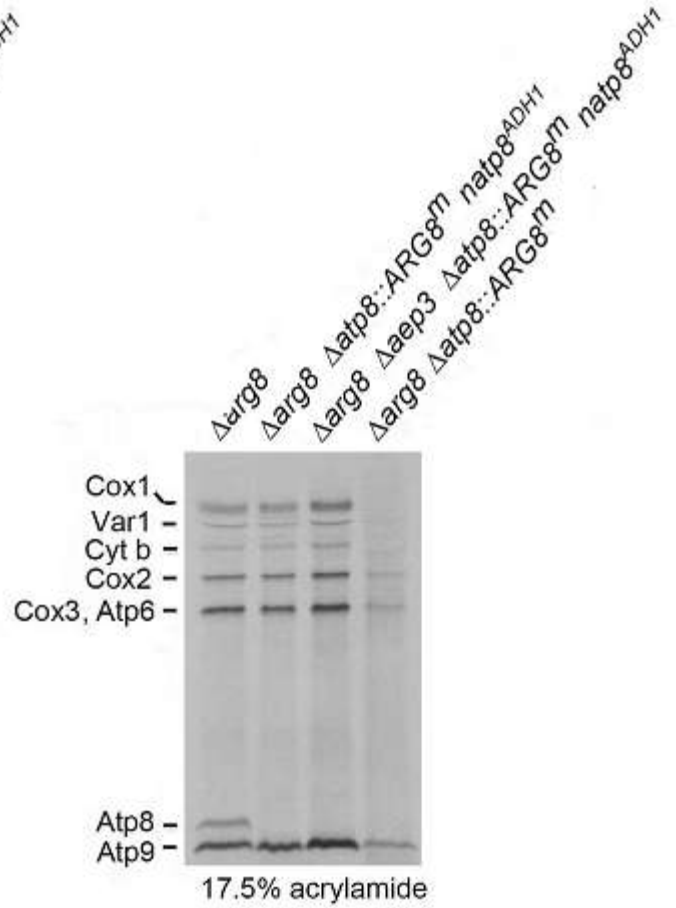
Barros and Tzagoloff, Fig. 2



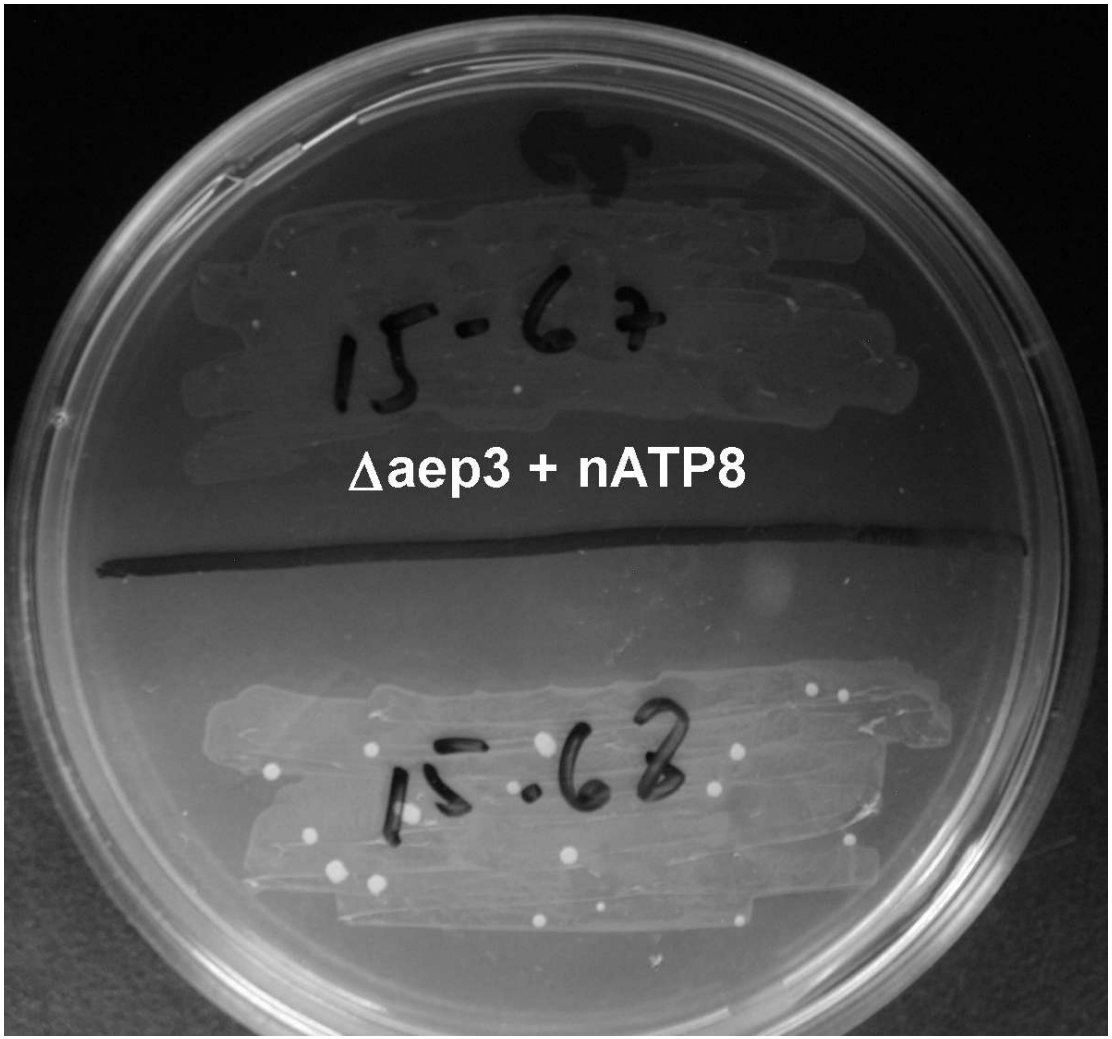

YPD
YEPG
+Arg
- Arg
Δarg8
Δarg8 Δatp8::ARG8m nATP8ADH1
Δarg8 Δaep3 Δatp8::ARG8m nATP8ADH1
Δarg8 Δatp8::ARG8m

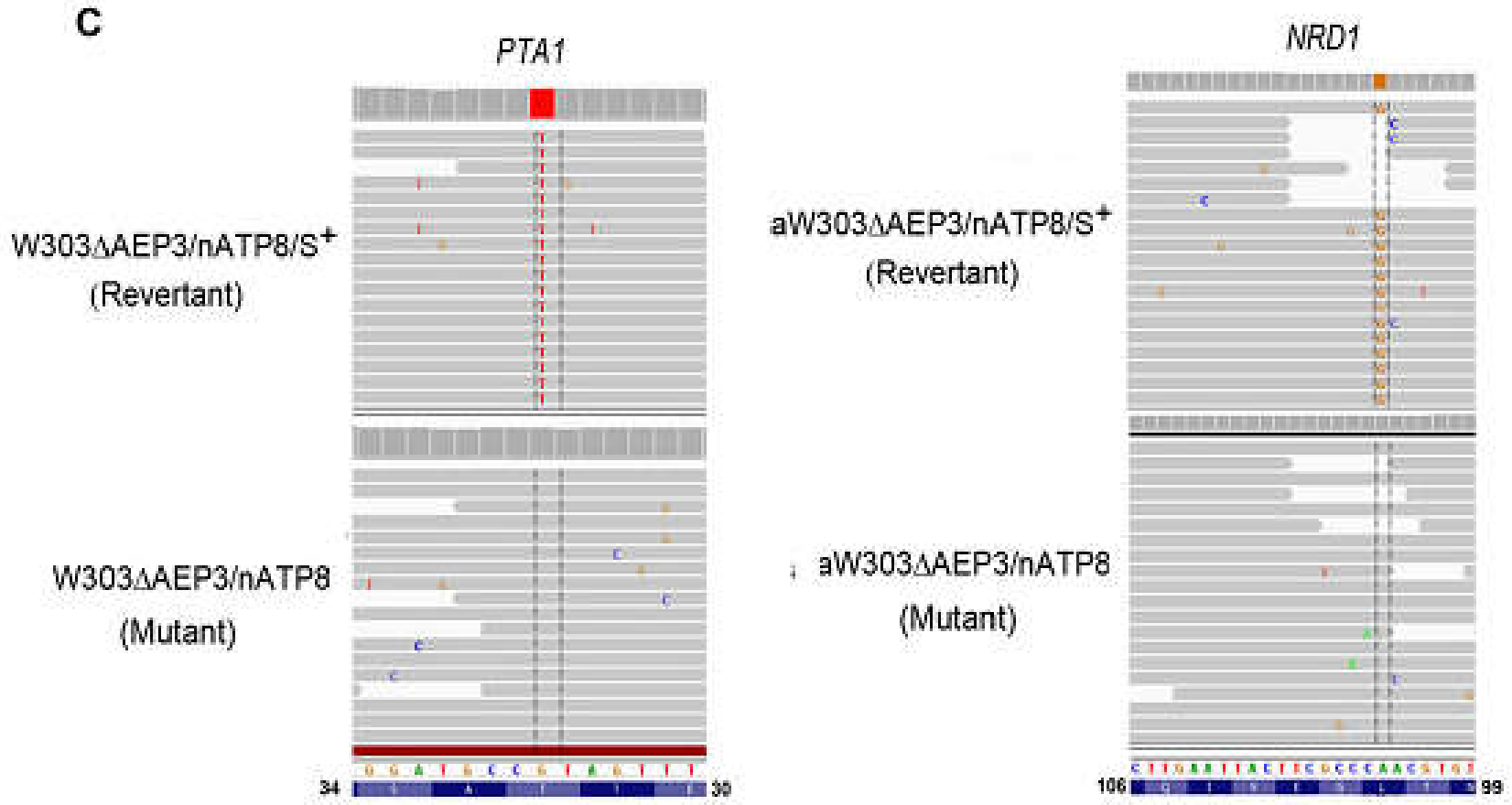
C

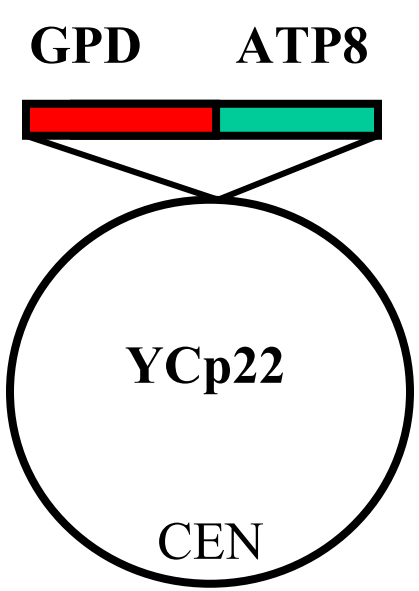

Δarg8
Δarg8 Δatp8::ARG8m natp8ADH1
Δarg8 Δaep3 Δatp8::ARG8m natp8ADH1
Δarg8 Δatp8::ARG8m
Arg8m
Var1
Cox1
Cox2, Cyt b
Cox3
Atp6
Atp8. Atp9
ρ+ 100 93 65 10
12% acrylamide + 6M urea

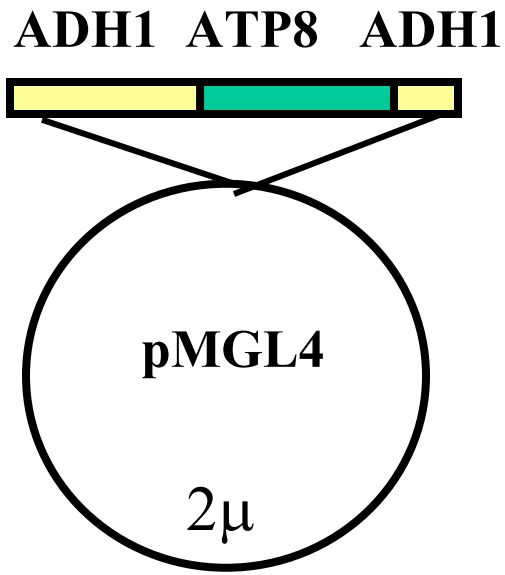

Δarg8
Δarg8 Δatp8::ARG8m natp8ADH1
Δarg8 Δaep3 Δatp8::ARG8m natp8ADH1
Δarg8 Δatp8::ARG8m
Cox1
Var1
Cyt b
Cox2
Cox3, Atp6
Atp8
Atp9
17.5% acrylamide

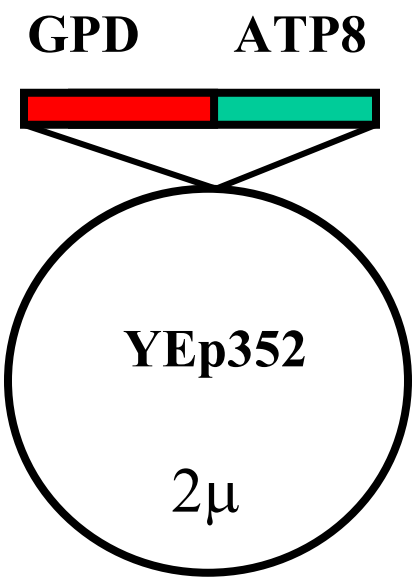
Rescue of respiratory capacity of *aep3* mutants by *nATP8-22* depends on a secondary mutation

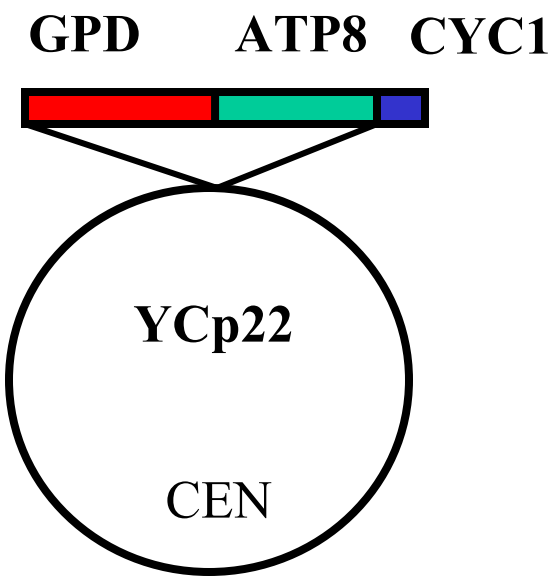


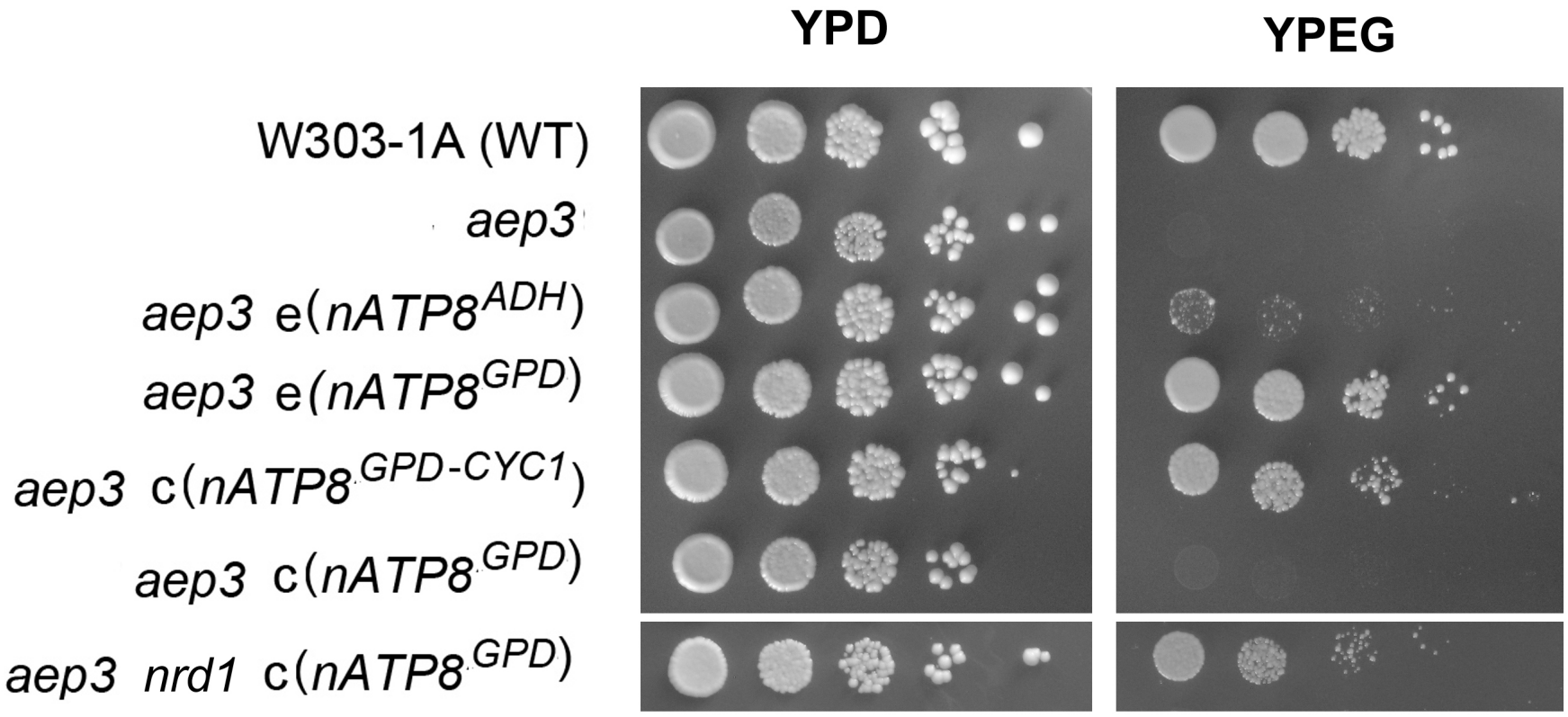

C
PTA1
NRD1
W303ΔAEP3/nATP8/S+
(Revertant)
W303ΔAEP3/nATP8
(Mutant)
aW303ΔAEP3/nATP8/S+
(Revertant)
aW303ΔAEP3/nATP8
(Mutant)
34
30
106
99


GPD
ATP8
YCp22
CEN


ADH1
ATP8
ADH1
pMGL4
2μ


GPD
ATP8
YEp352
2μ


GPD
ATP8
CYC1
YCp22
CEN


YPD
YPEG
W303-1A (WT)
aep3
aep3 e(nATP8ADH)
aep3 e(nATP8GPD)
aep3 c(nATP8GPD-CYC1)
aep3 c(nATP8GPD)
aep3 nrd1 c(nATP8GPD)

Conclusions of Aep3p trifunctional:

→ 1) Stabilizes ATP6/8 transcripts

→ 2) Interacts with IF-2 – synthetic respiratory with Δfmt1

→ 3) Aep3p is specifically required for *ATP8* translation

Thank you