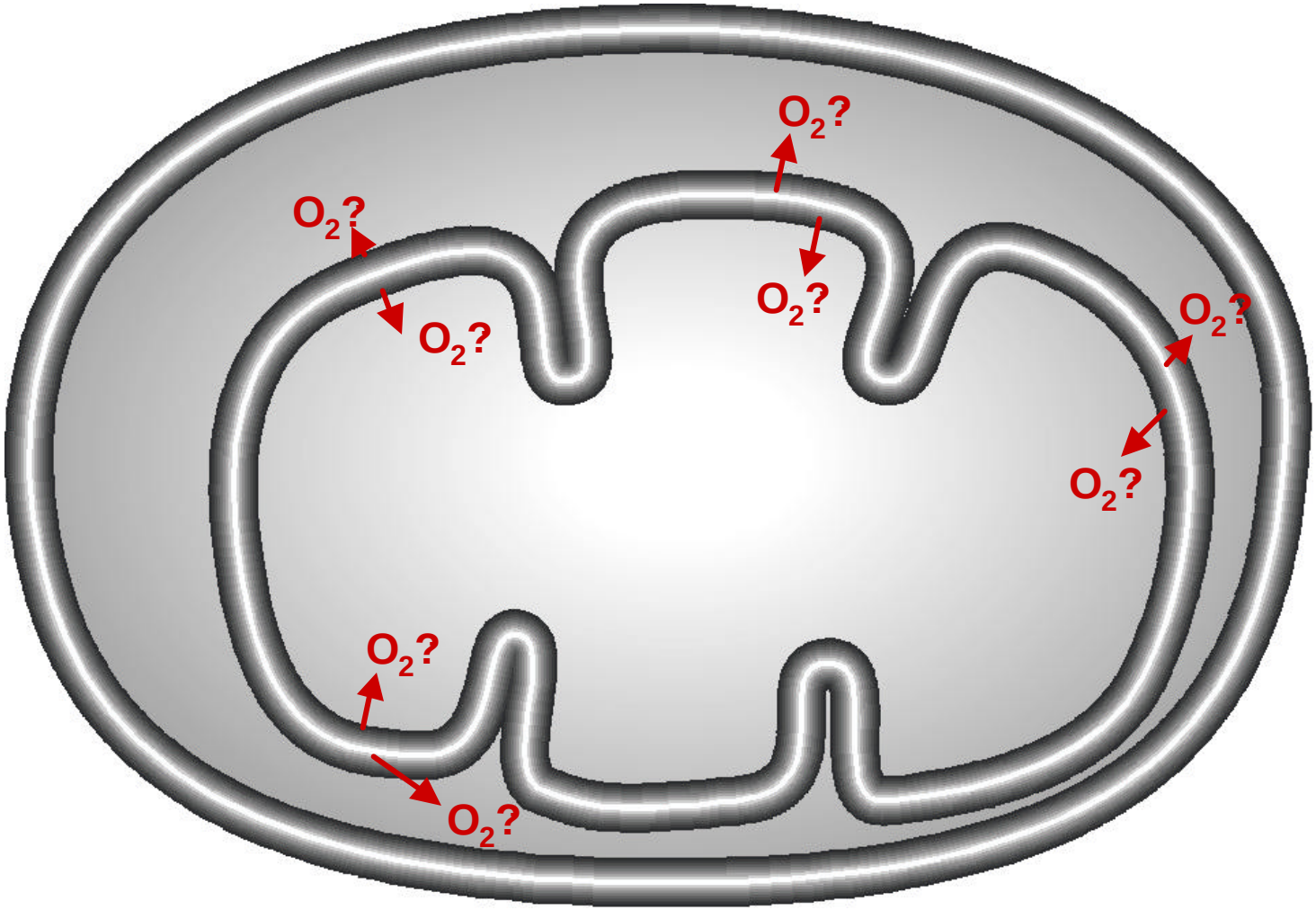
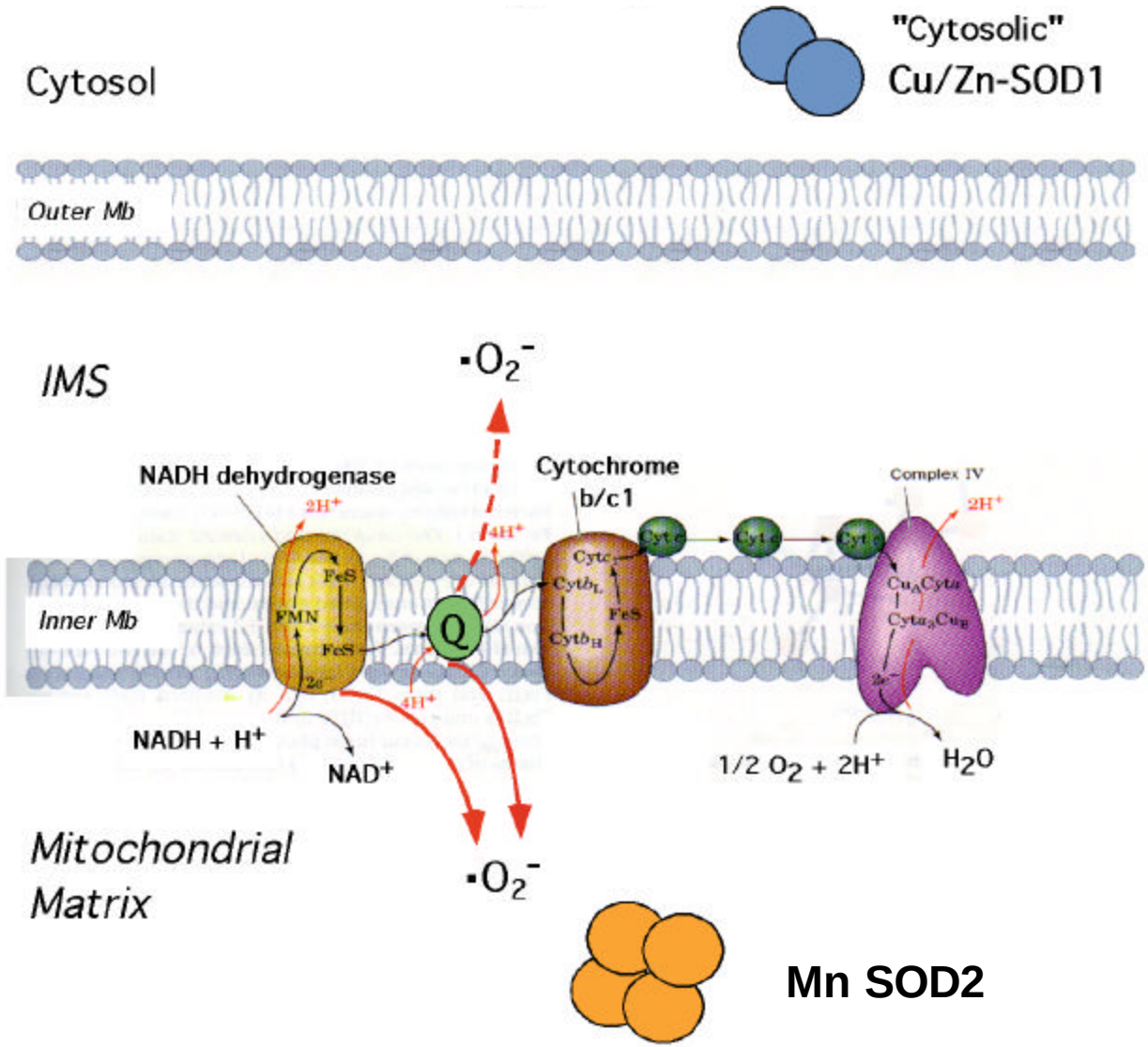
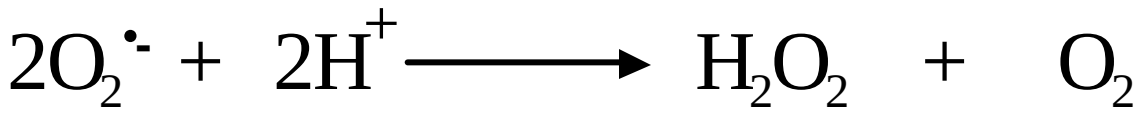
SODs and their metallochaperones in mitochondrial antioxidant defense

The mitochondria as a factory for ROS




Cytosol
"Cytosolic"
Cu/Zn-SOD1
Outer Mb
IMS
$\cdot O_2^-$
NADH dehydrogenase
Cytochrome
b/c1
Complex IV
2H+
4H+
FeS
FMN
Q
Inner Mb
NADH + H+
NAD+
1/2 O2 + 2H+
H2O
Mitochondrial
Matrix
$\cdot O_2^-$
Mn SOD2

The metal containing superoxide dismutases



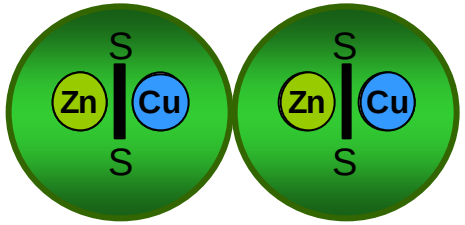
SOD1 = "cytosolic", copper-requiring
SOD2 = mitochondria, manganese-requiring

Cu/Zn SOD1

Highly conserved among eukaryotes

Maturation of SOD1:

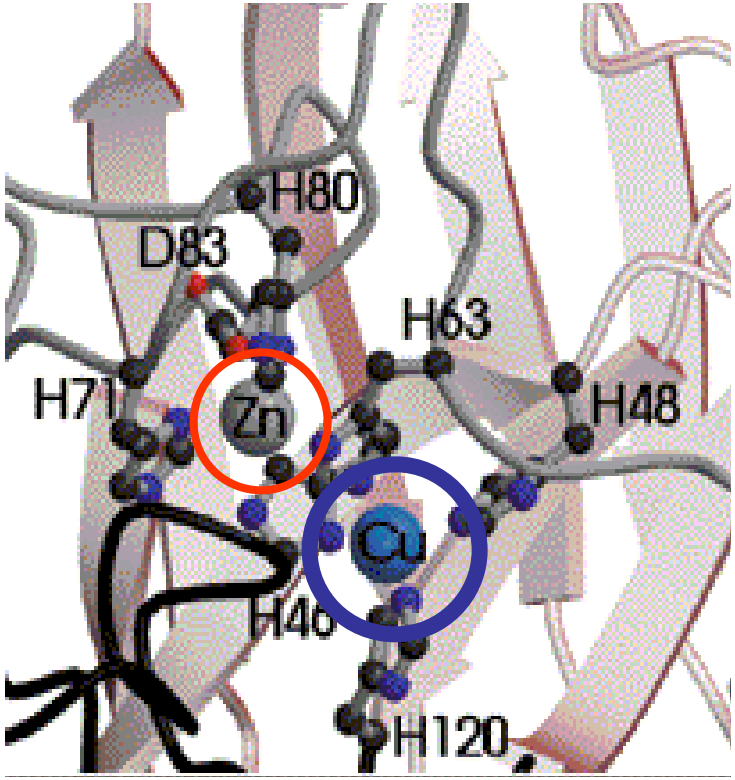
1. Zinc insertion
2. Copper insertion
3. Disulfide bond formation



SOD1 Homodimer

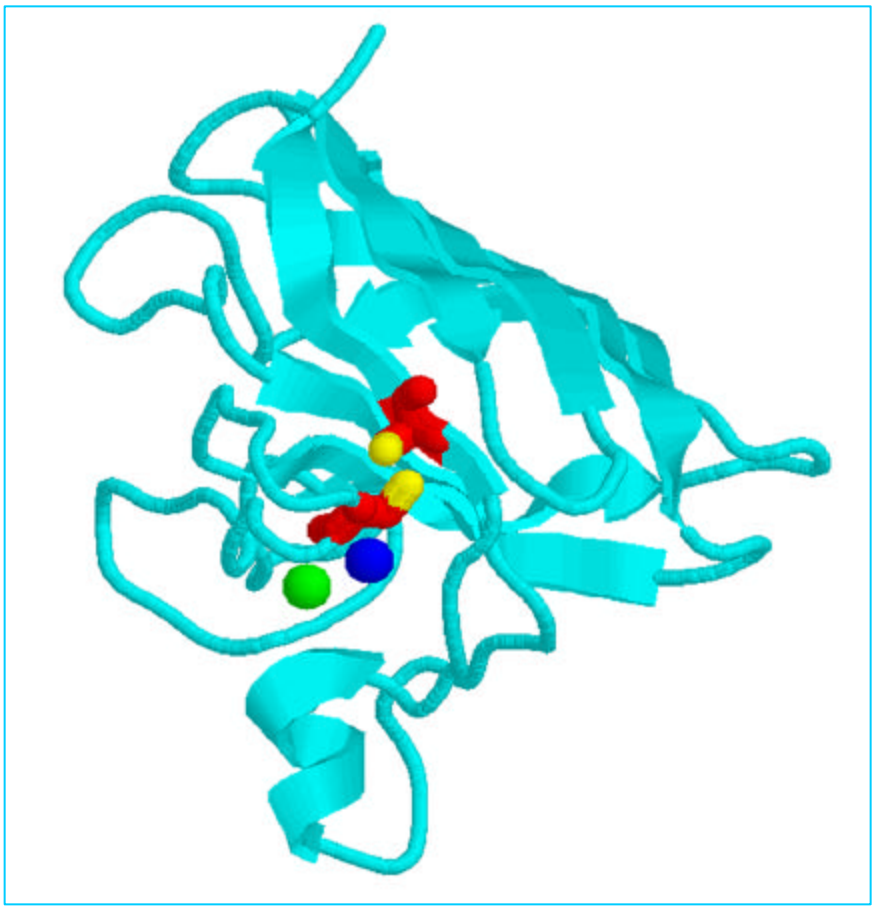
1) $O_2^{?-} + Cu^{++} \longrightarrow Cu^{+} + O_2$

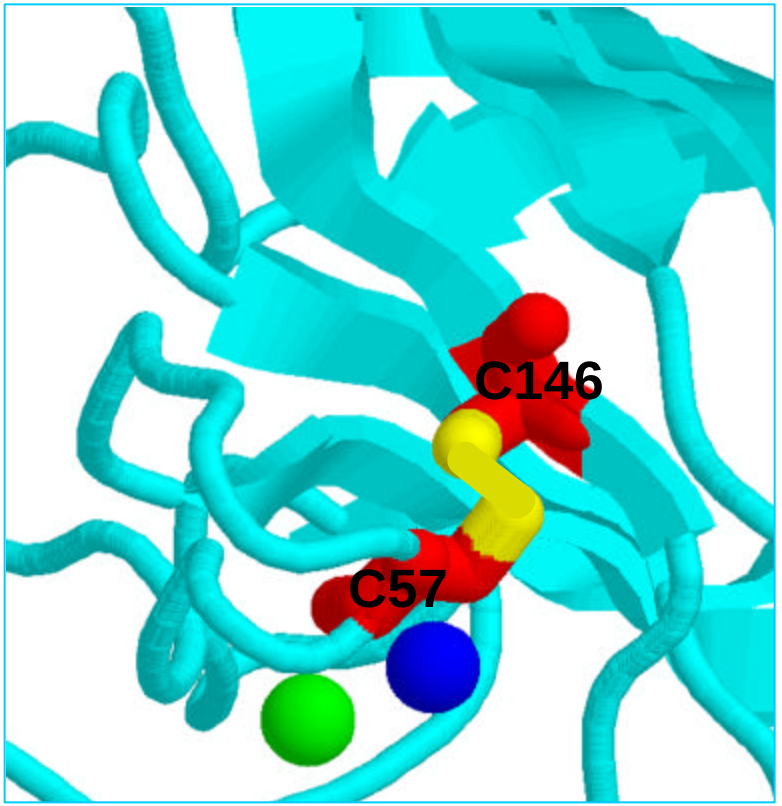
2) $O_2^{?-} + Cu^{+} + 2H^{+} \longrightarrow Cu^{++} + H_2O_2$



From Tainer et al., JMB 1982

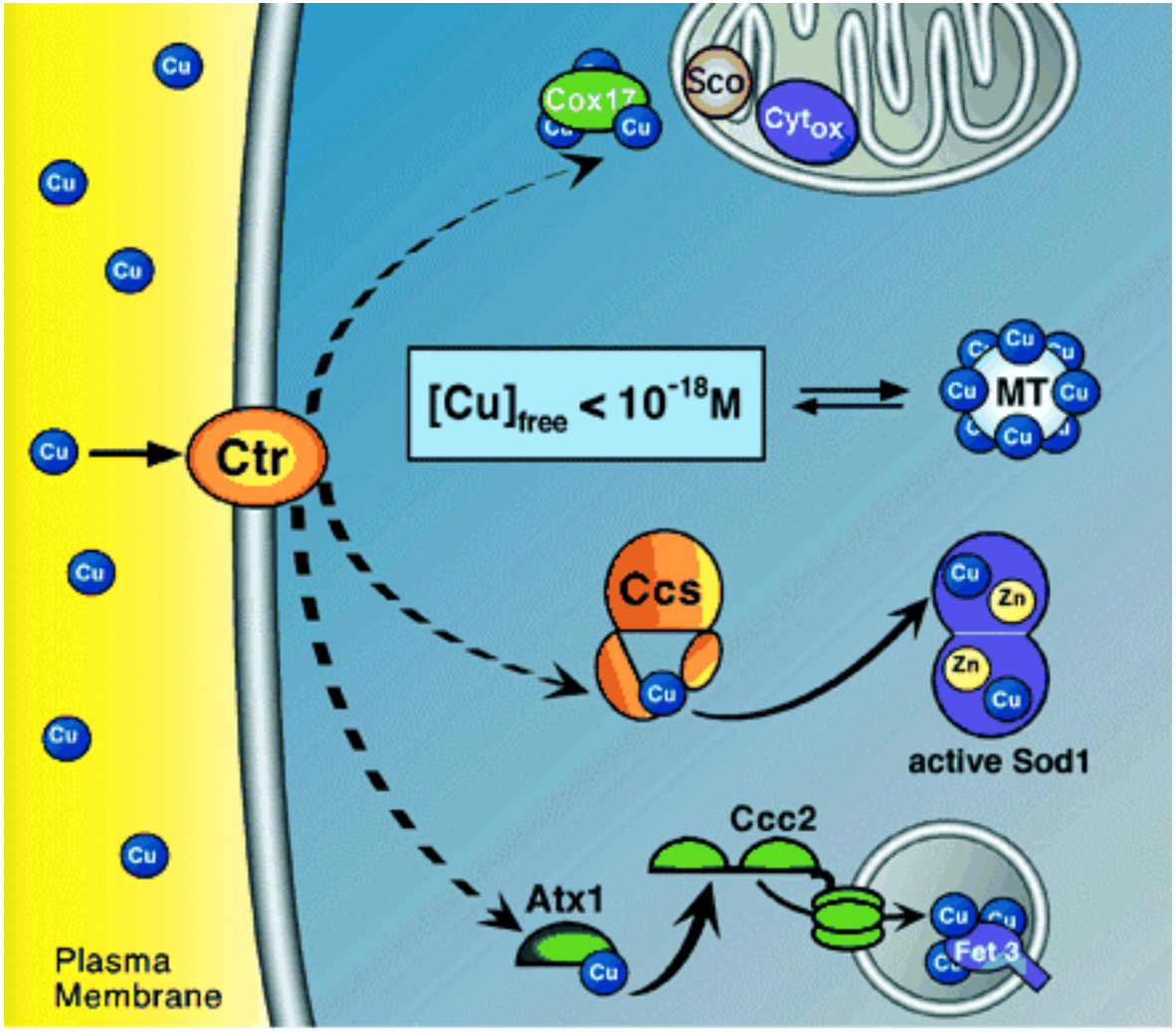
SOD1:
Disulfide formation between C57 and C146





A rare cytosolic disulfide

Copper chaperones: escort proteins for metals



From O'Halloran and Culotta, *J. Biol. Chem.* 2000

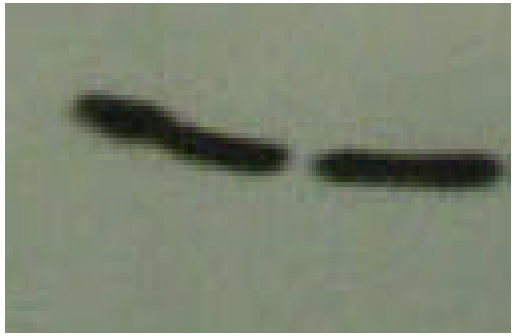
Yeast cells lacking CCS have no SOD1 activity

Wild type ***CCS-***

Mn SOD2

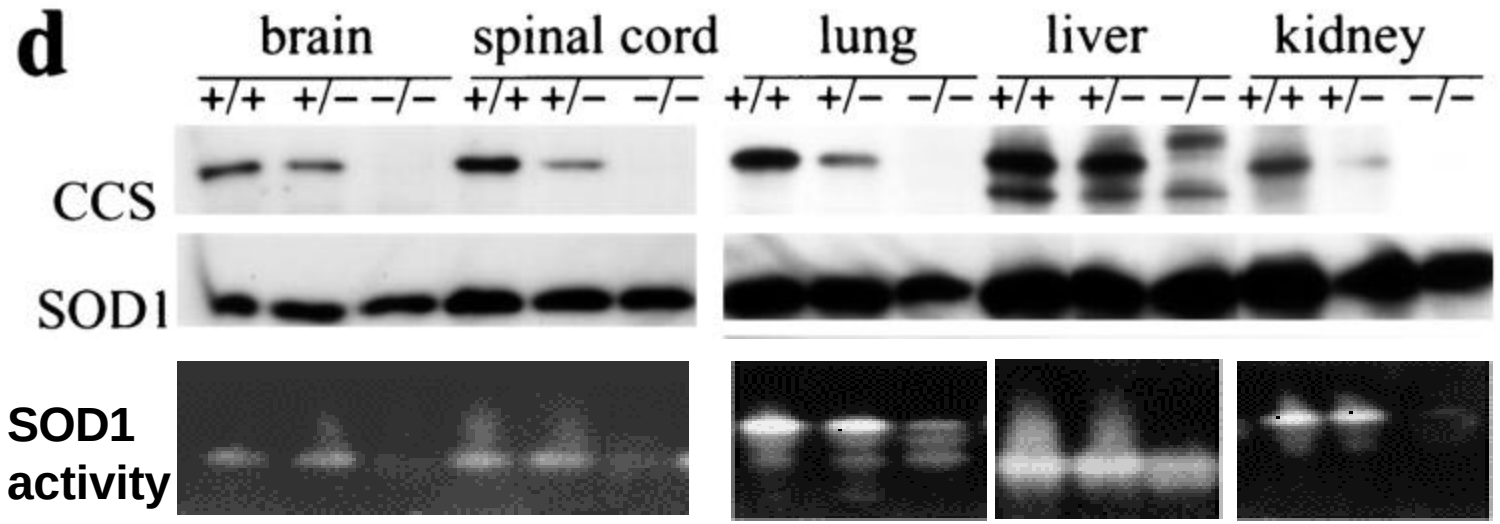
Cu/Zn SOD1

Native gel assay for SOD activity



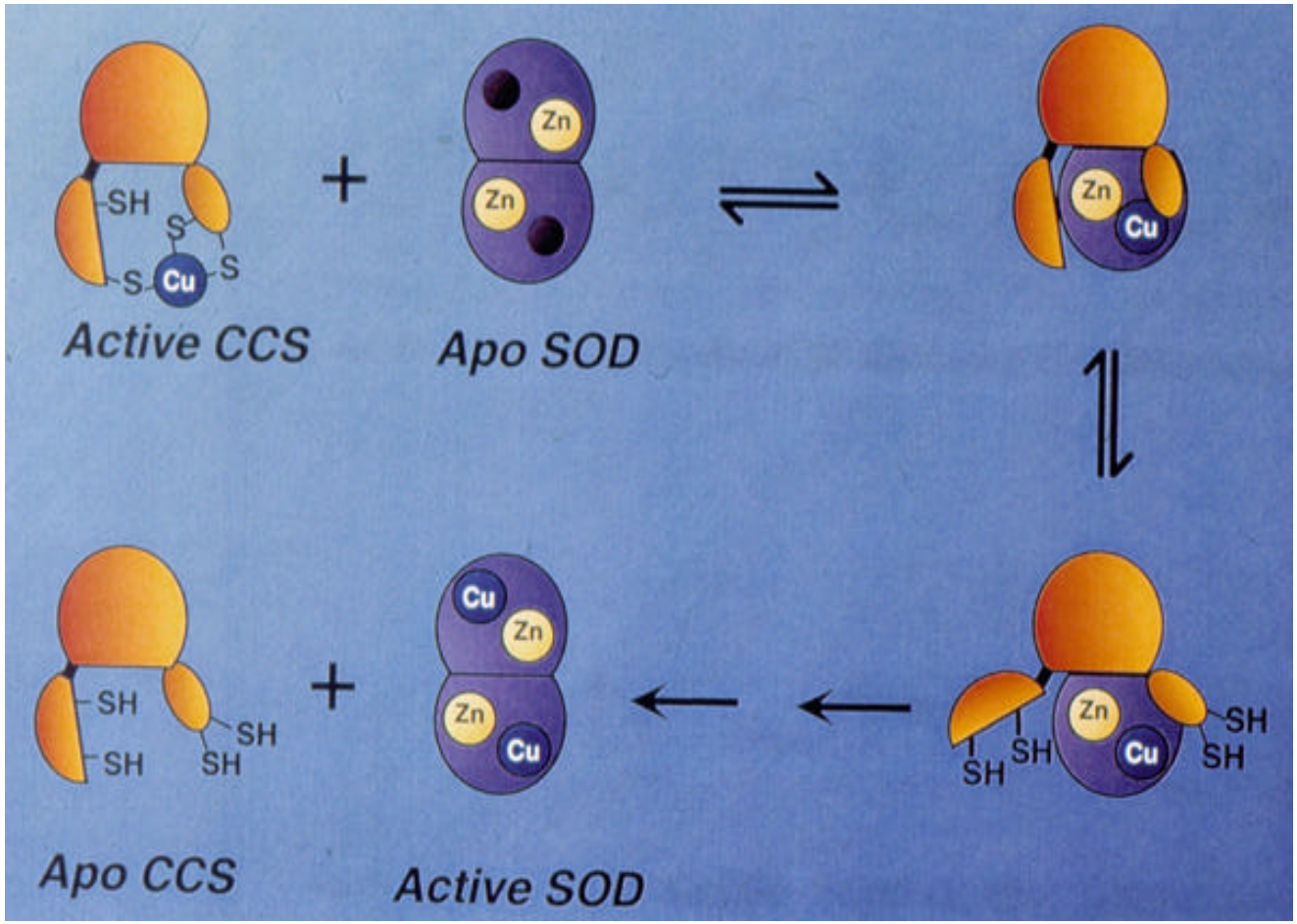
Western for SOD1 protein

Mammalian SOD1 has low activity without CCS



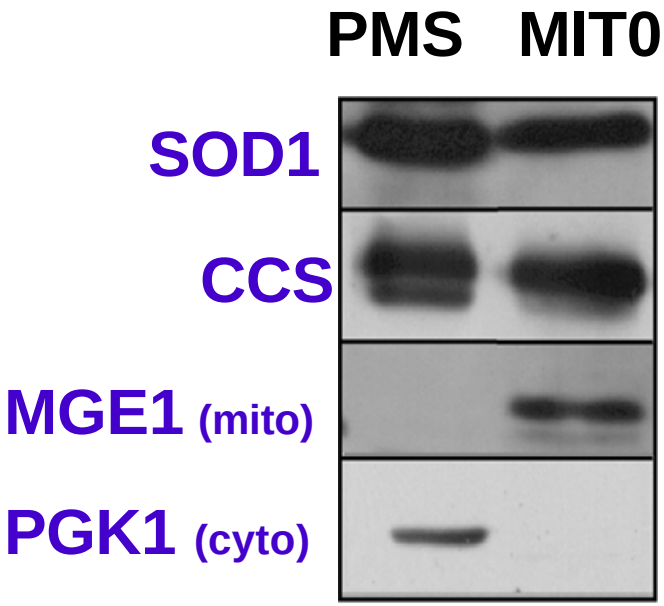
From Wong et al. Proc. Natl. Acad Sci 2000

Copper activation of SOD1 via CCS



From O'Halloran and Culotta 2000

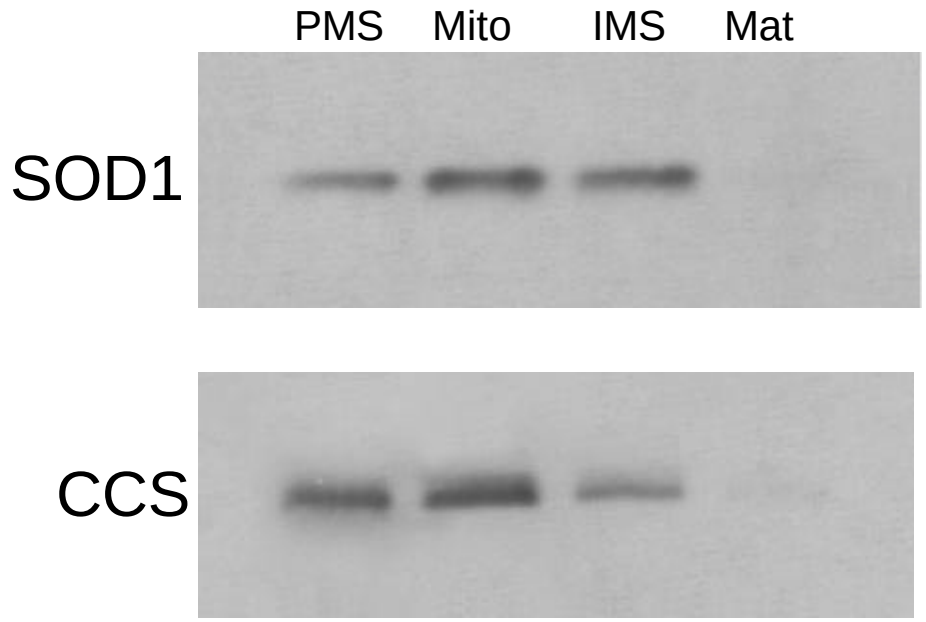
A fraction of SOD1 and its copper chaperone CCS localize to the mitochondria of yeast



PMS = post mitochondrial spnt; largely cytosol

From Sturtz et al., JBC 2001

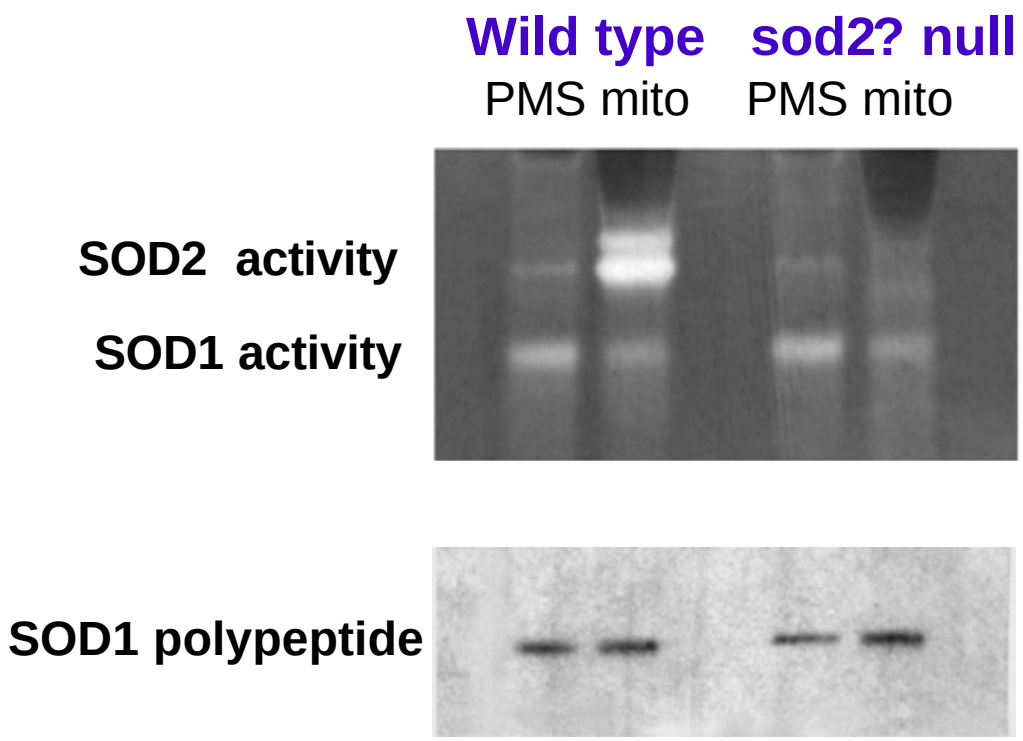
SOD1 and CCS localize to the intermembrane space of mitochondria



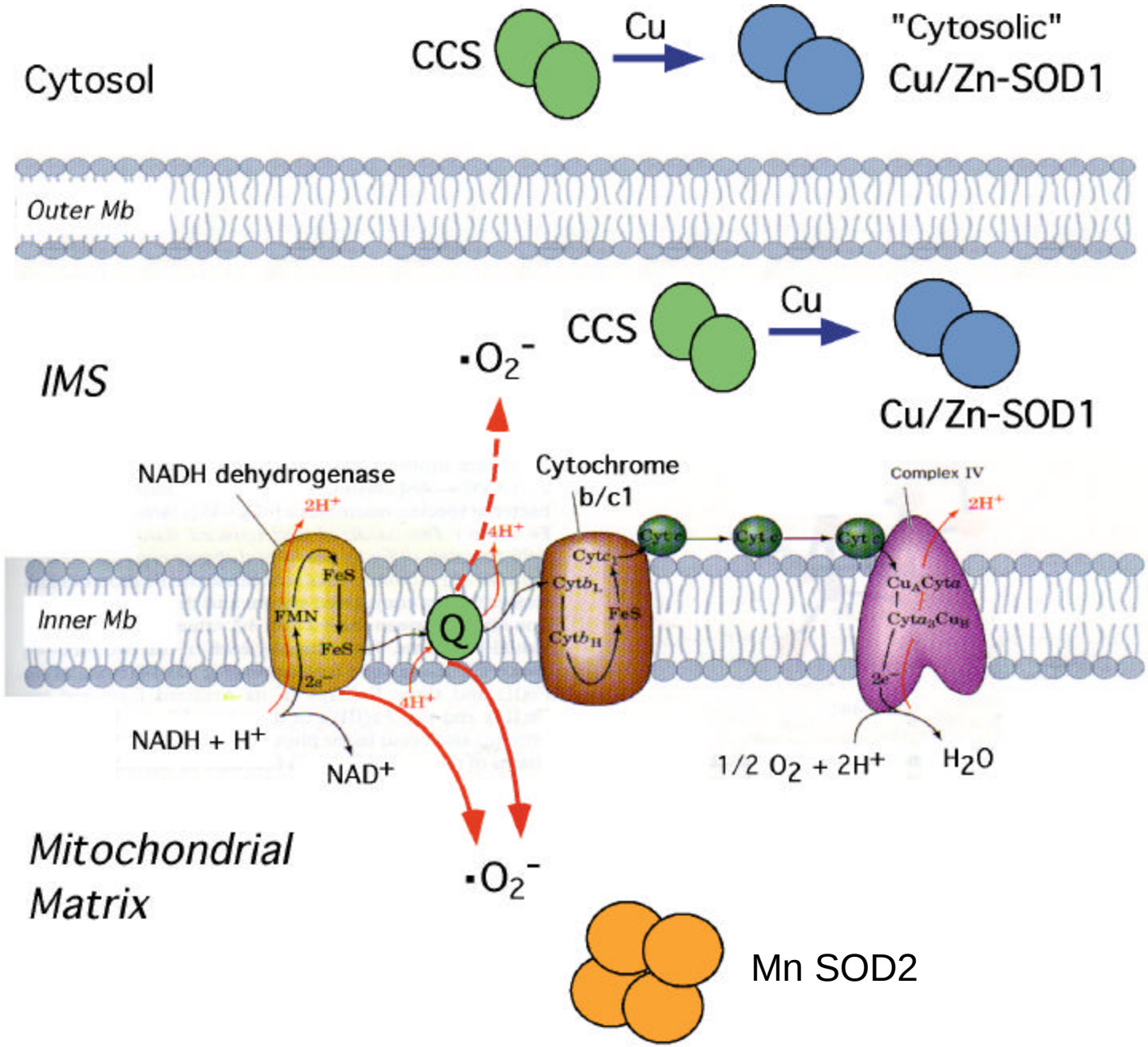
PMS = post-mitochondrial supernatant
Mito = mitochondria
IMS = intermembrane space
Mat = matrix

Sturtz, et al. *JBC* 2001

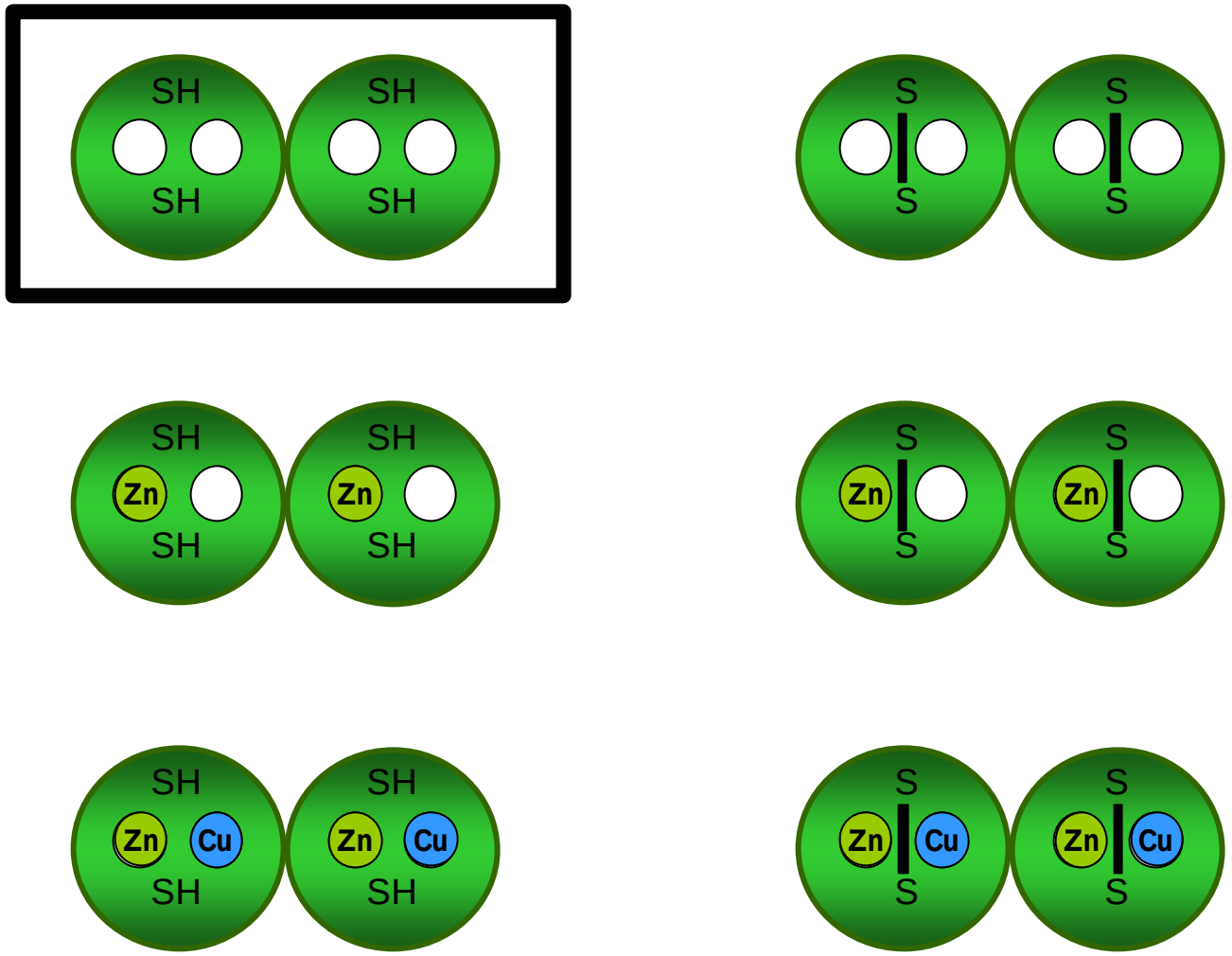
SOD1 in the mitochondria shows activity



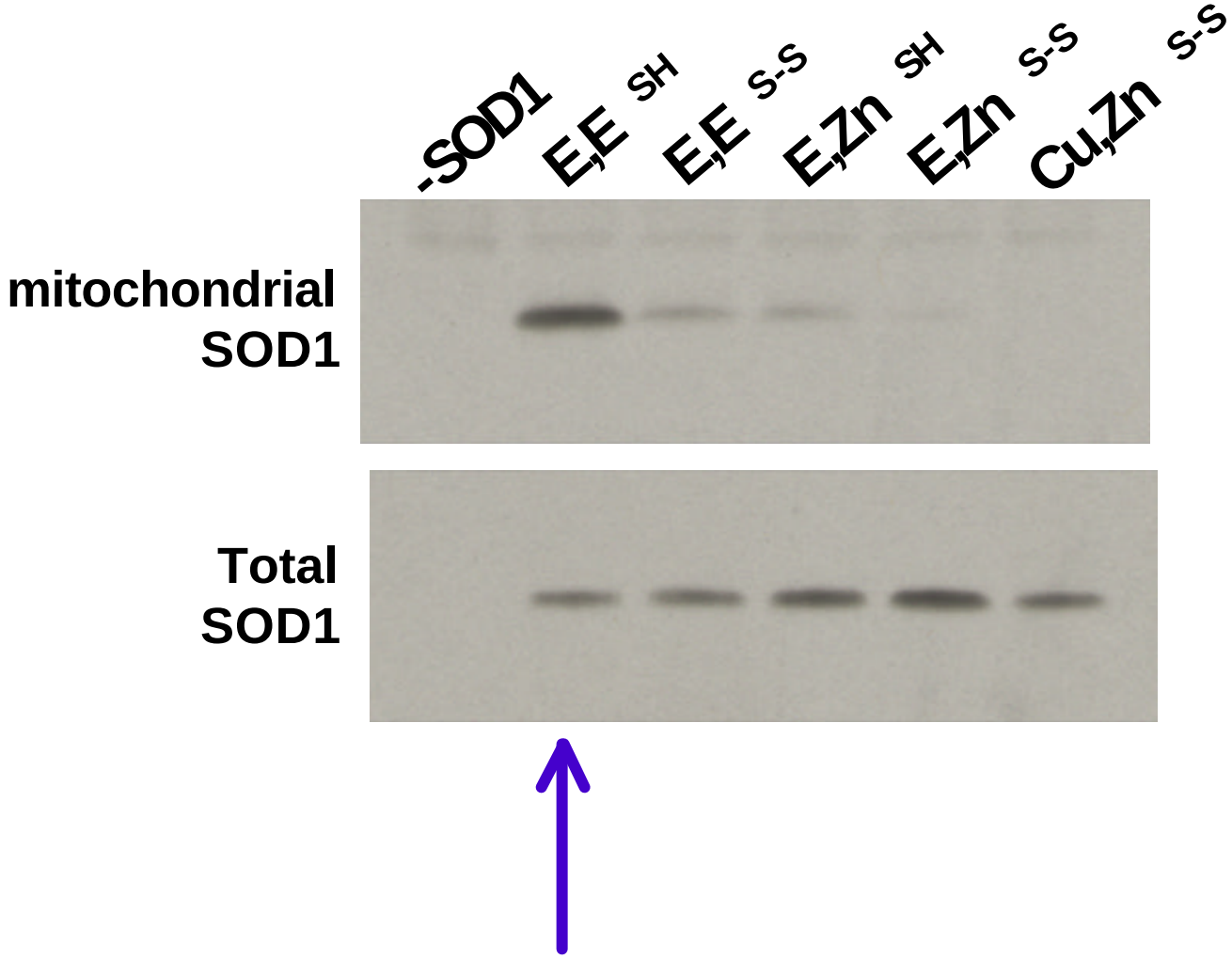
From Sturtz et al., JBC 2001


Cytosol
CCS
Cu
"Cytosolic"
Cu/Zn-SOD1
Outer Mb
IMS
CCS
Cu
Cu/Zn-SOD1
$\cdot O_2^-$
NADH dehydrogenase
Cytochrome
b/c1
Complex IV
2H+
4H+
FeS
FMN
FeS
Q
Cyt c_1
Cyt b_L
Cyt b_H
FeS
Cyt c
Cyt c
Cyt c
Cu_A Cyt a
Cyt a_3 Cu_B
2e−
Inner Mb
NADH + H^+
NAD^+
1/2 O_2 + 2H^+
H_2O
Mitochondrial
Matrix
$\cdot O_2^-$
Mn SOD2

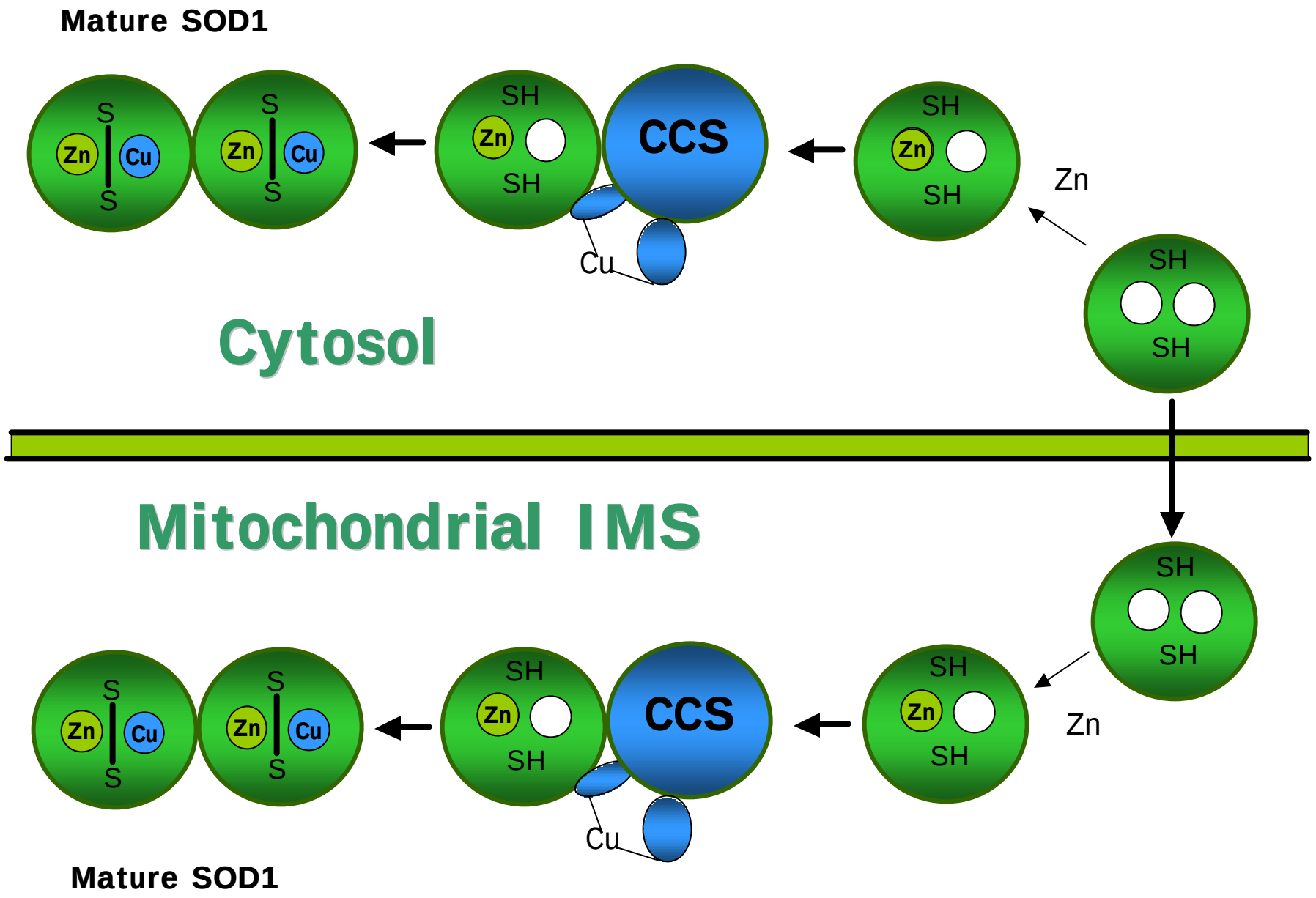
What is the form of SOD1 that enters mitochondria?



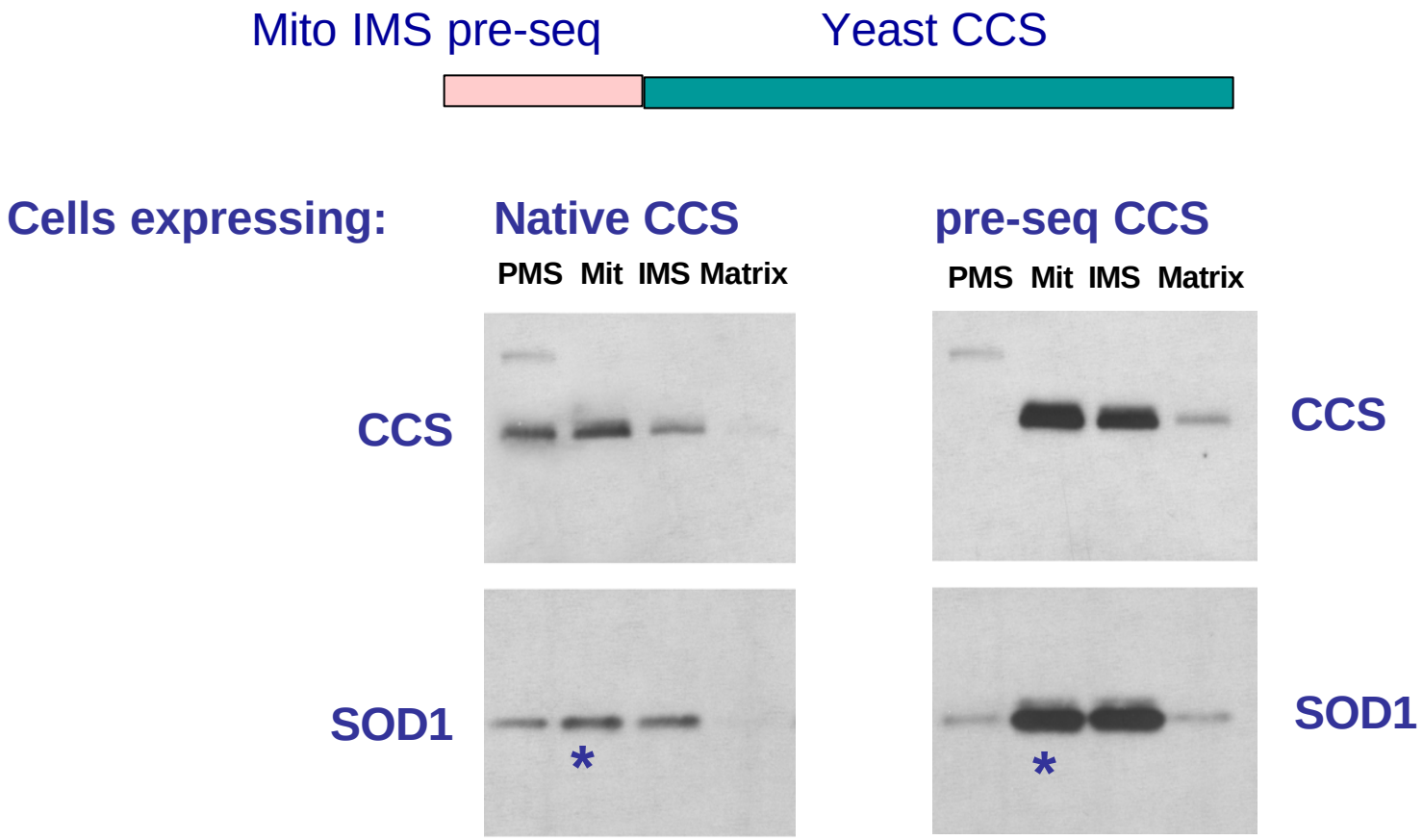
Apo, reduced SOD1 is taken up most efficiently by mitochondria during *in vitro* import



Field, et al. *JBC* 2003


Mature SOD1
S
Zn
Cu
S
S
Zn
Cu
S
SH
Zn
SH
CCS
Cu
SH
Zn
SH
Zn
SH
SH
Cytosol
Mitochondrial IMS
SH
SH
SH
Zn
SH
Zn
CCS
SH
Zn
SH
Cu
S
Zn
Cu
S
S
Zn
Cu
S
Mature SOD1

SOD1 appears to “follow” CCS into mitochondria

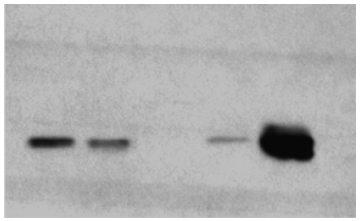


From Sturtz et al., JBC 2001

High levels of SOD1 activity in cells with high mitochondrial CCS

Cells expressing: Native CCS preseq-CCS

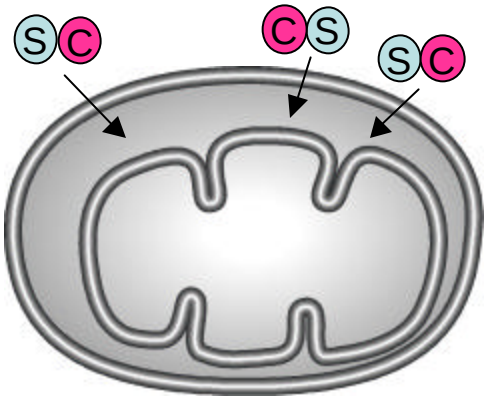
P M P M



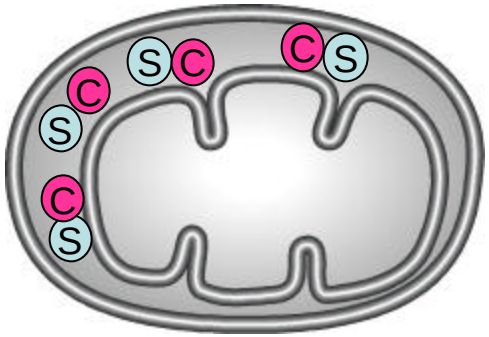
SOD1 protein

SOD1 activity

How does CCS affect mitochondrial accumulation of SOD1?



Co-import?



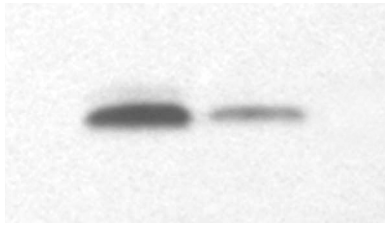
Mitochondrial retention?

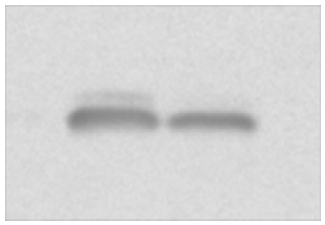
CCS helps to retain SOD1 in the mitochondria

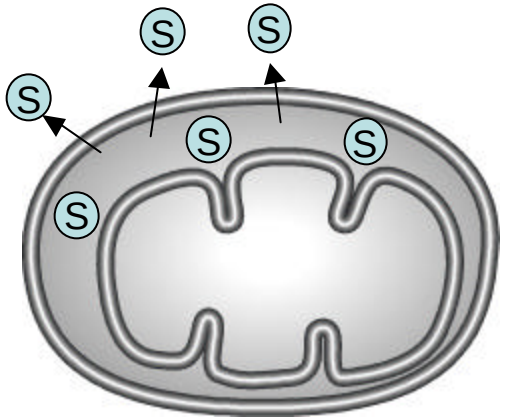
No mitochondrial CCS ***Abundant mitochondrial CCS***

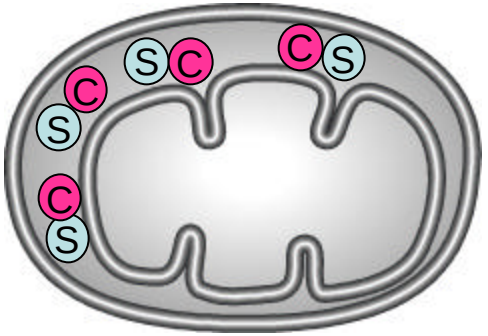
Total Retained Total Retained

Mitochondrial SOD1





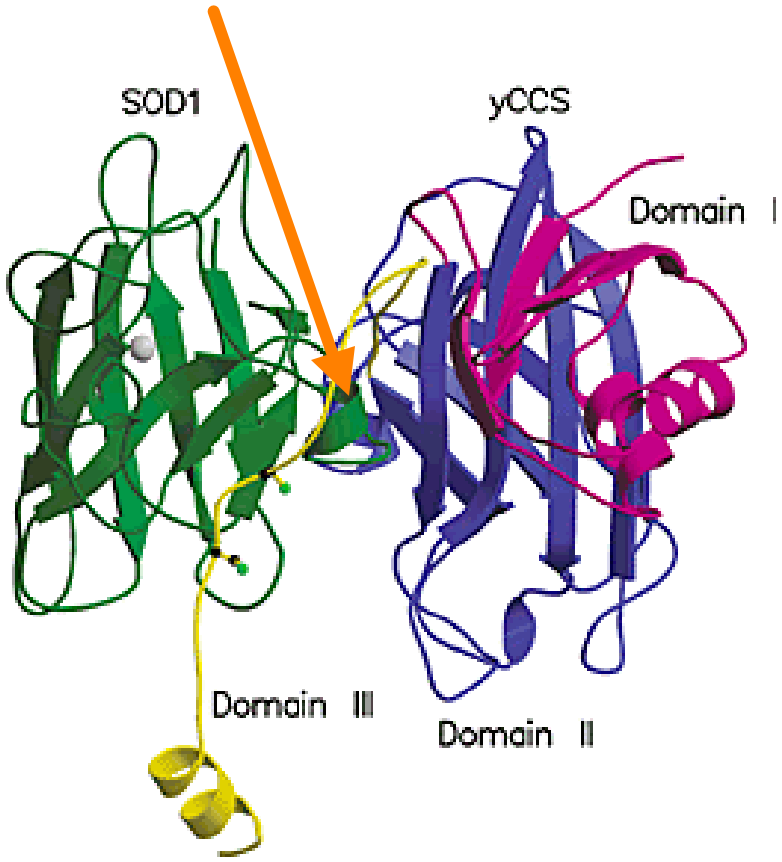




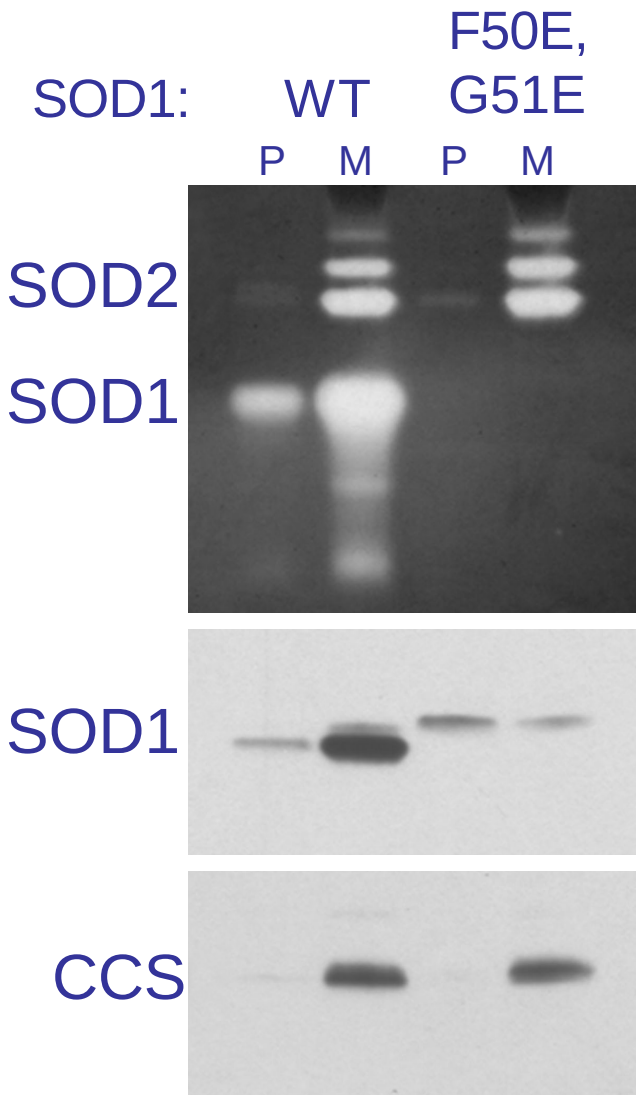
Field, et al. *JBC* 2003

Disrupting SOD1-CCS interactions

SOD1 FG50,51EE (disrupts dimer interface)



From Lamb et al., Nat. Struc. Biol. 2001



Disrupting the dimerization interface between SOD1 and CCS prevents mitochondrial accumulation of SOD1

Field, et al. *JBC* 2003

Stationary Phase Survival:
A marker of chronological lifespan and oxidative damage

Chronological life span in yeast: time a non-dividing cell will survive in stationary phase

Markers of oxidative damage and mitochondrial ROS increase with time in stationary phase

Stationary phase survival is strongly influenced by anti-oxidant enzyme activity

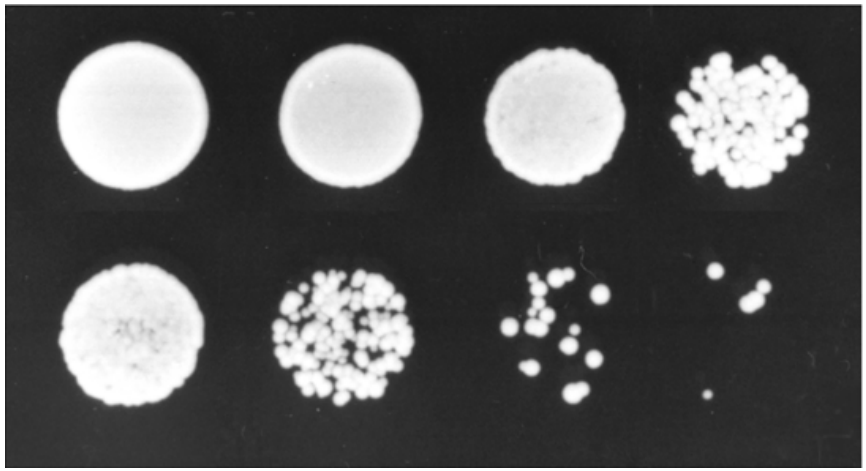
From the labs of V. Longo, J. Valentine, E. Gralla and T. Bilinski

sod1? yeast exhibit a shortened lifespan during stationary phase

Day 2

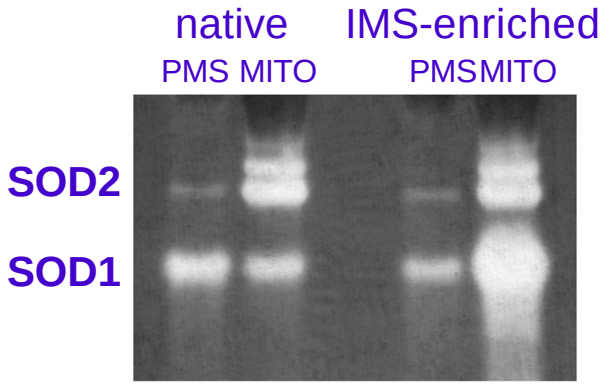
WT

sod1?



Sturtz, et al. *JBC* 2001

IMS SOD1 prolongs lifespan during stationary phase

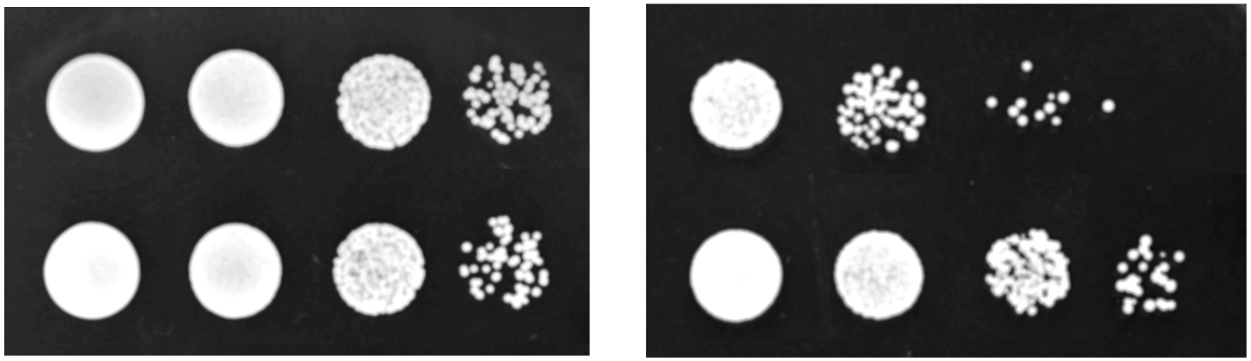


Day 2

Day 5

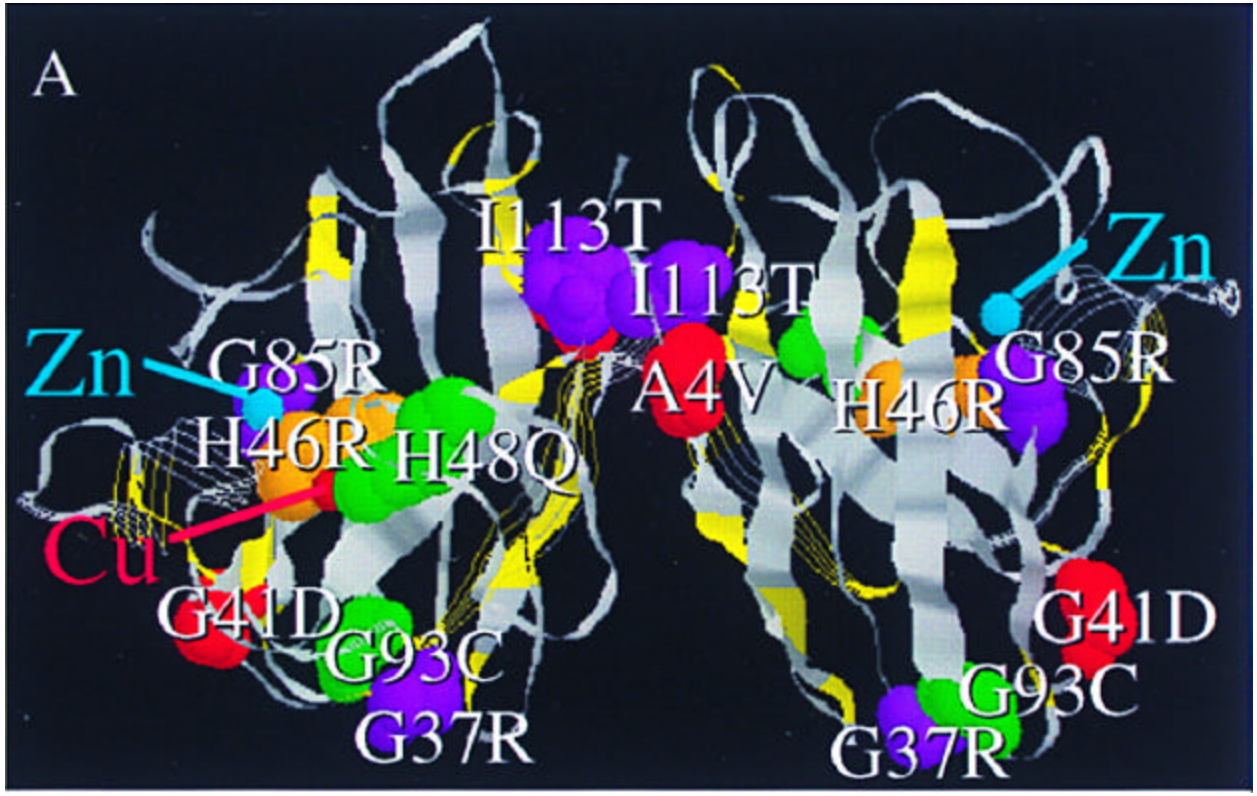
native SOD1

IMS-enriched SOD1

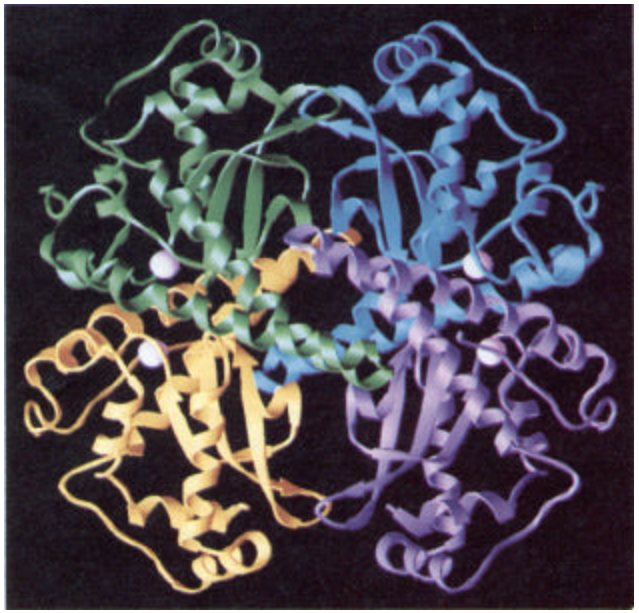


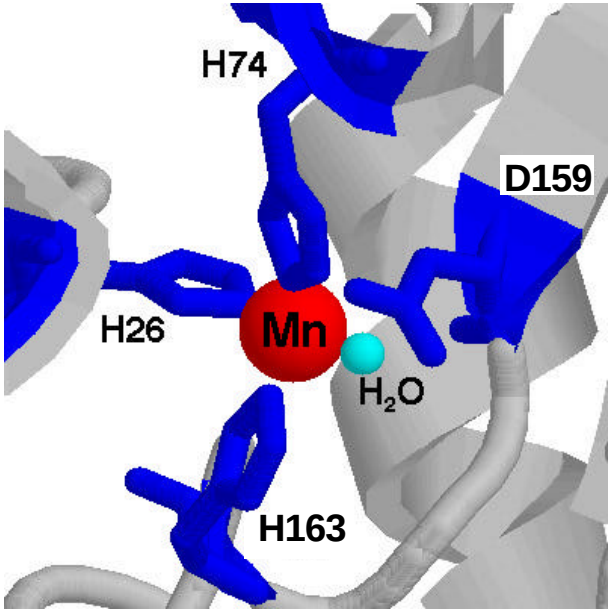
Sturtz, et al. *JBC* 2001

Human SOD1 mutations and familial ALS (Lou Gehrigs disease)



Eukaryotic mitochondrial SOD2 - a homotetramer





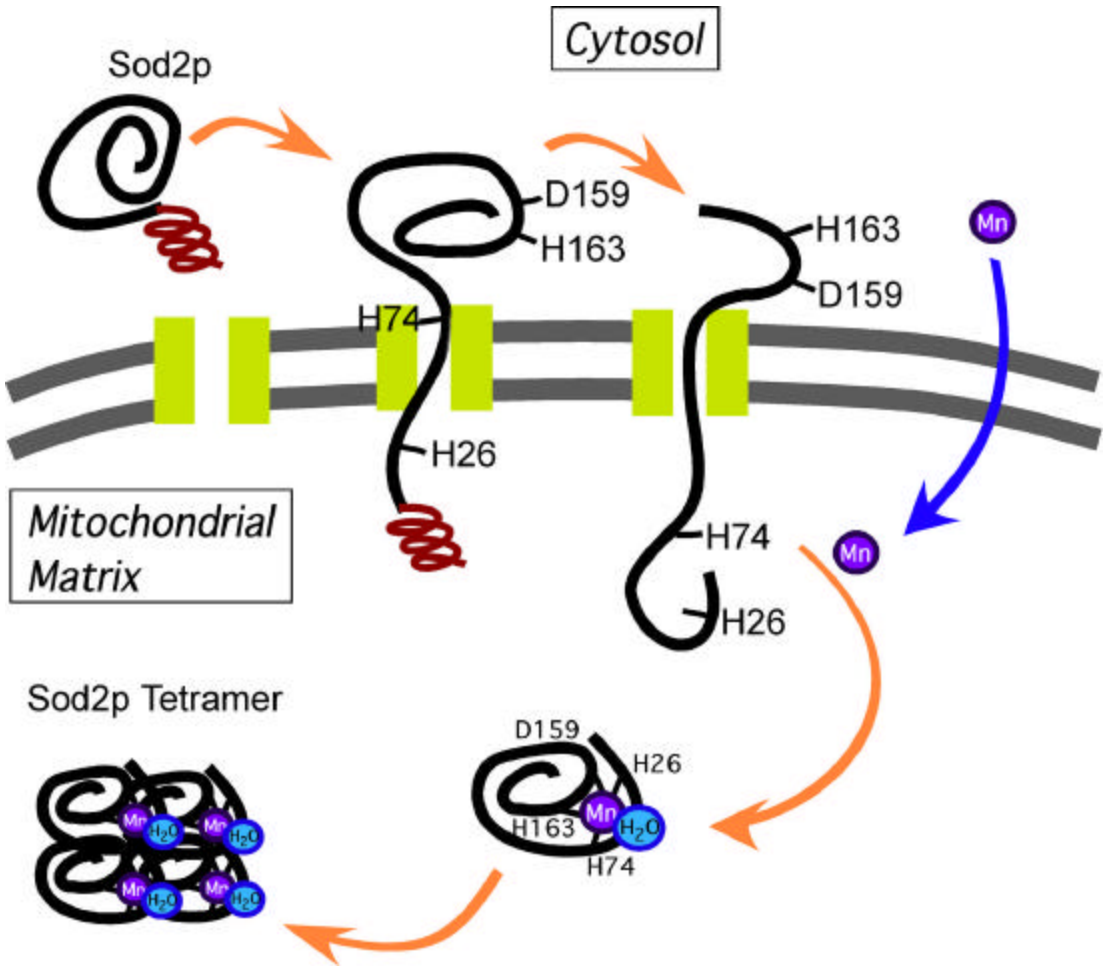
From Borgstahl et al.,
Cell 1992

1) $O_2^{?-} + Mn^{+++} \longrightarrow Mn^{++} + O_2$

2) $O_2^{?-} + Mn^{++} + 2H^+ \longrightarrow Mn^{+++} + H_2O_2$

SOD2 must acquire its Mn in the mitochondria

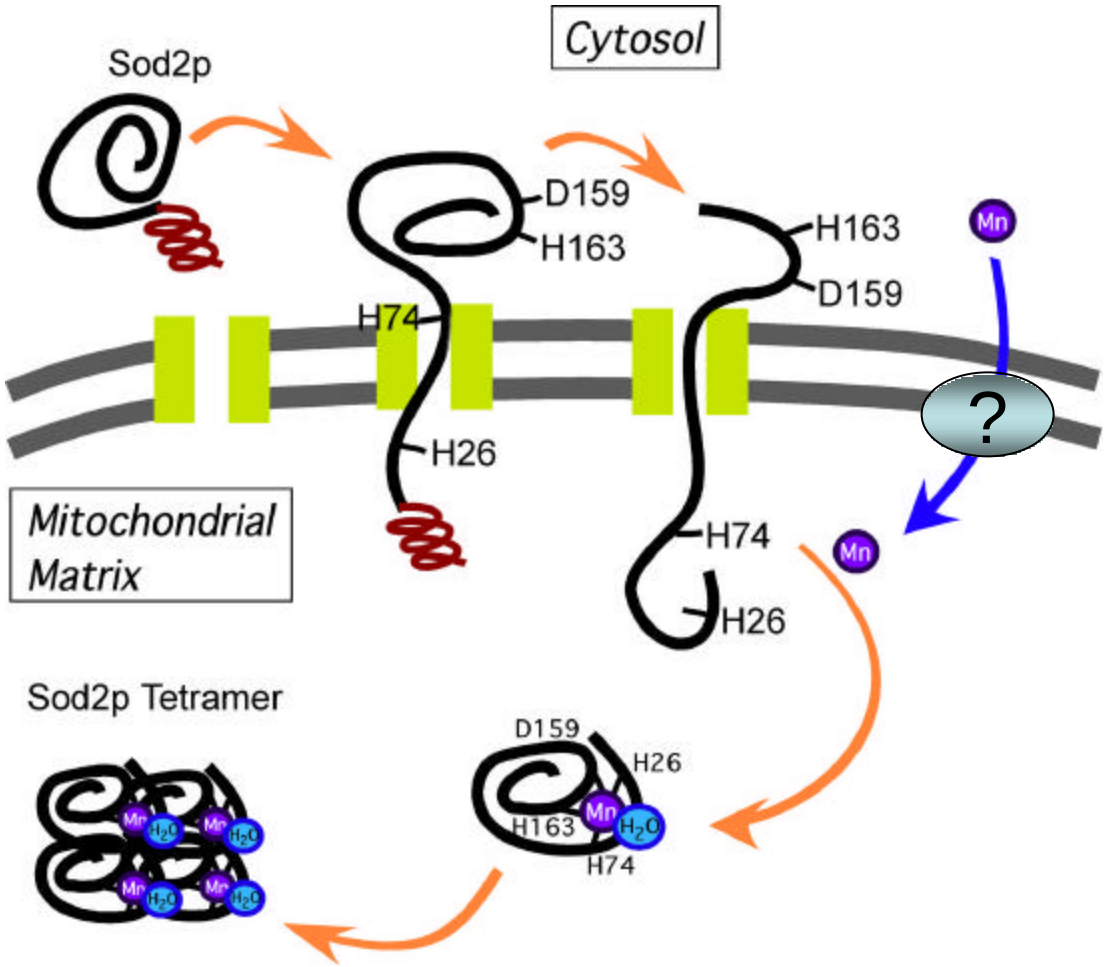
Synthesized in cytosol



Activated in mitochondria

Mn insertion and mitochondrial import of SOD1 appear coupled

Newly synthesized SOD2

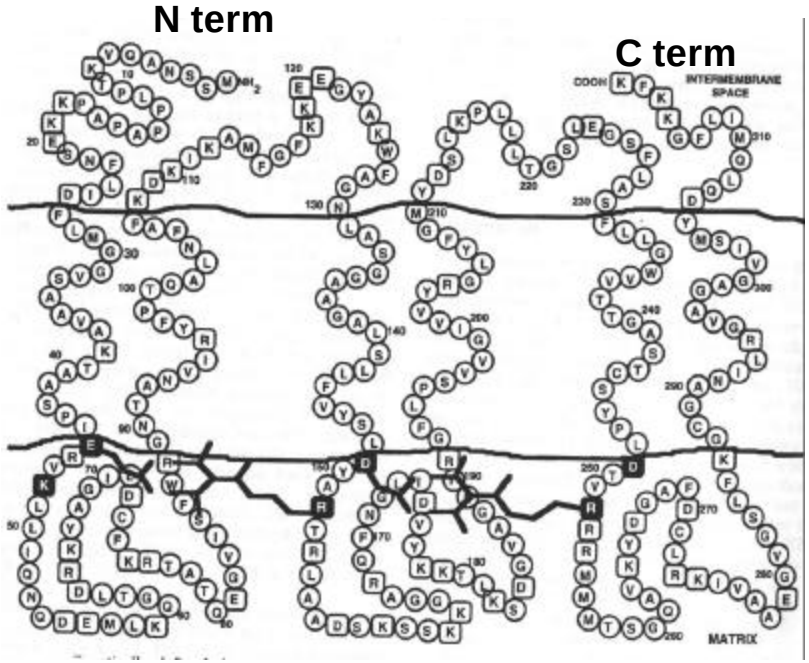


Possible mitochondrial transporters for manganese:

The MCF (mito carrier family) transporters

Intermembrane space

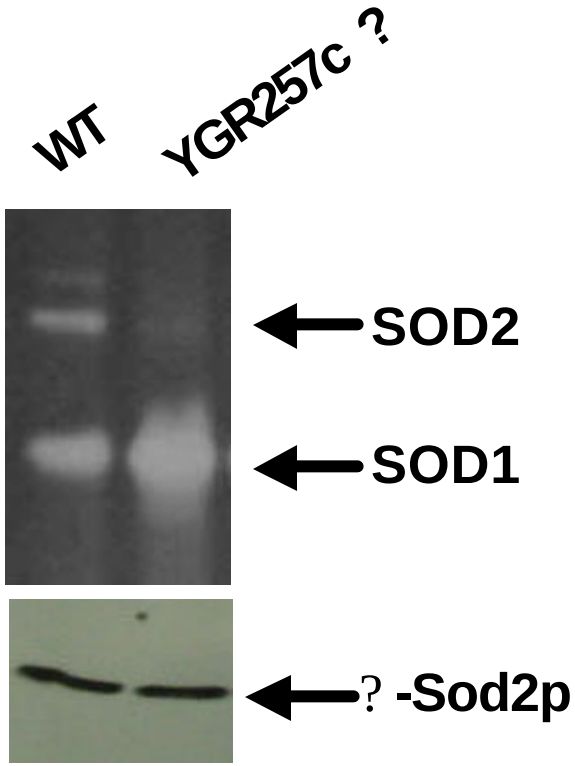
Mitochondrial matrix



Mitochondrial inner membrane

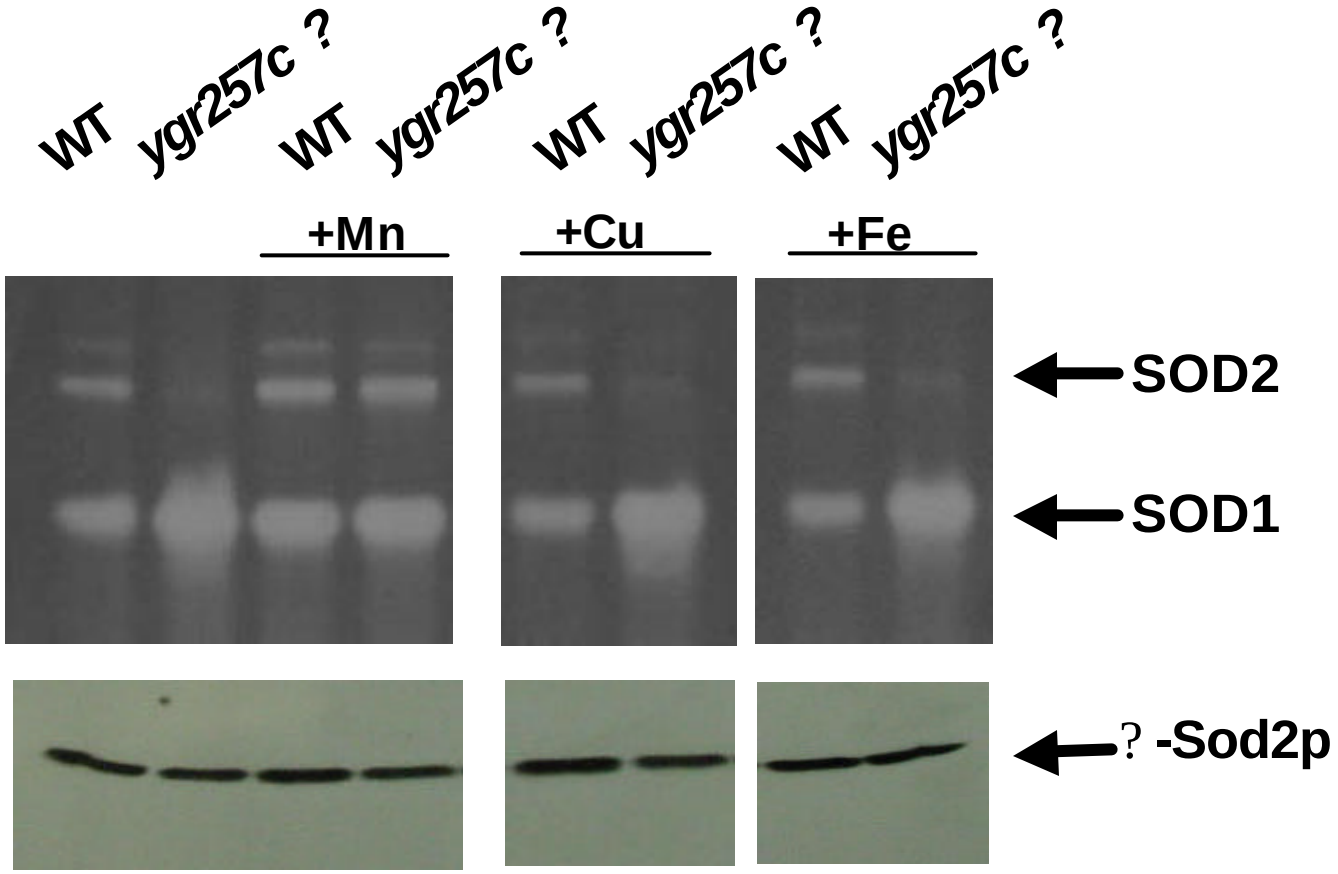
YGR257c

= an unknown member of MCF family of mito transporters



- YGR257c deletion leads to virtual inactivation of SOD2.
- SOD1 activity increases probably due to compensatory mechanism.

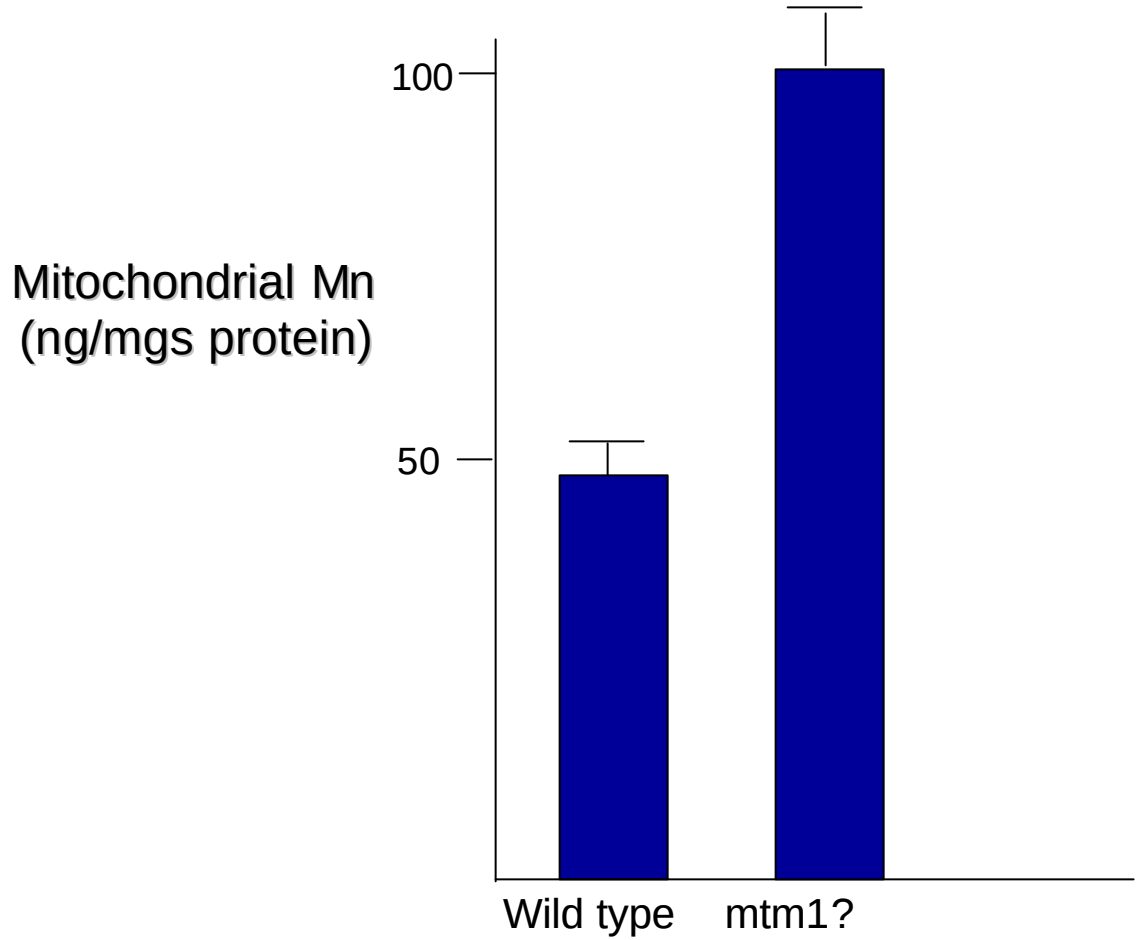
Supplementation of Mn (but not other metals) fully restores SOD2 activity

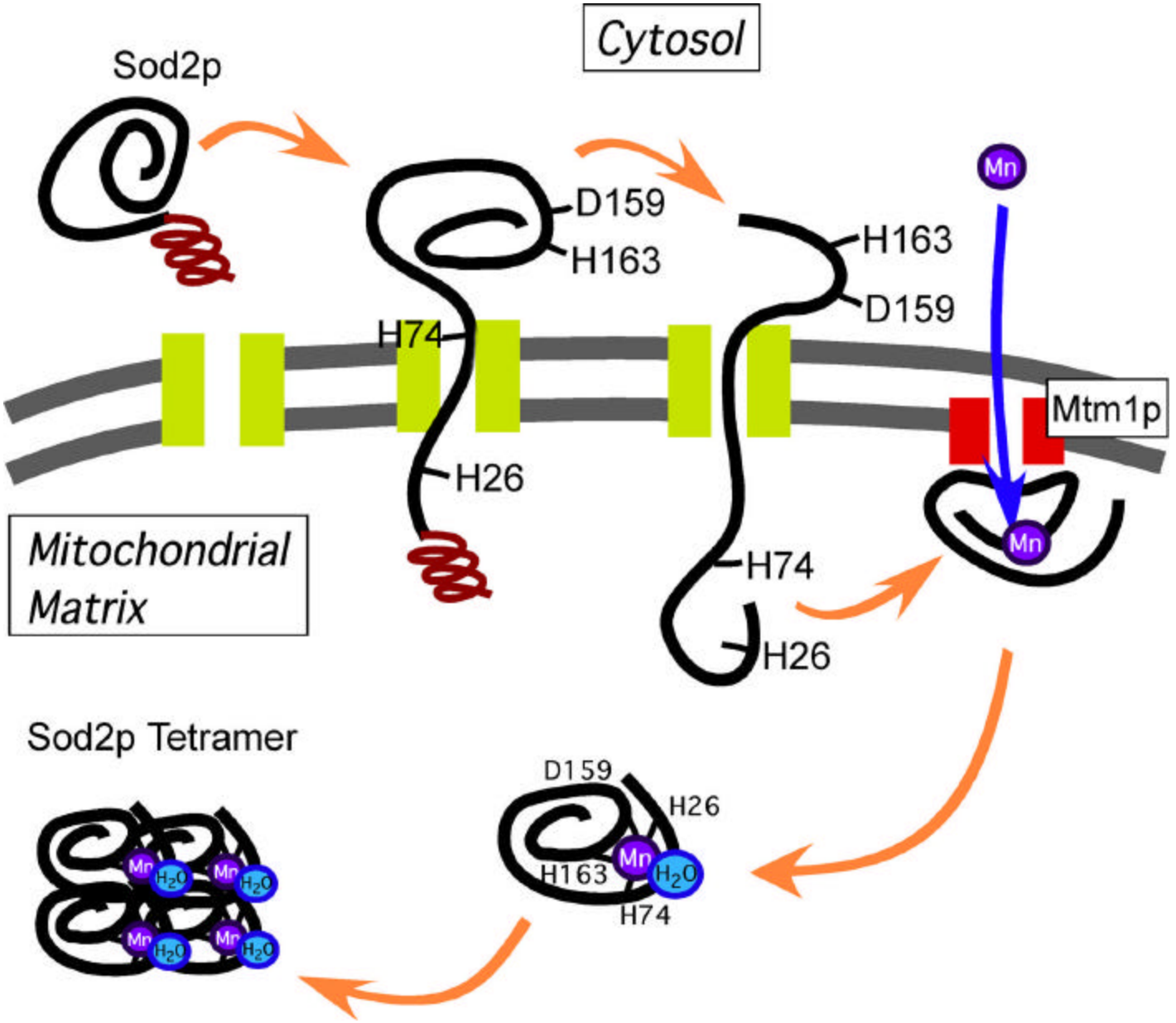


The MCF family of *S. cerevisiae*

Gene	Loci	Function
MIR1	YJR077C	phosphate
AAC1	YMR056C	ADP/ATP
AAC2	YBL030C	ADP/ATP
AAC3	YBR085W	ADP/ATP
ARG11	YOR130C	ornithine
CAC1	YOR100C	carnitine
CTP1	YBR291C	citrate
ACR1	YJR095W	dicarboxylate
OAC1	YKL120W	oxaloacetate
DIC1	YLR348C	dicarboxylate
FLX1	YIL134W	flavin
LEU5	YHR002W	acetylCoA
MRS3	YJL133W	Mg++, Fe++?
MRS4	YKR052C	Mg++, Fe++?
RIM2	YBR192W	Fe++, cysteine?
YMC1	YPR058W	?
YMC2	YBR104W	?
PET8	YNL003C	?
YHM1	YDL198C	?
-	YMR241W	??
-	YPR011C	??
-	YHR002W	??
-	YNL083W	??
-	YGR096W	??
-	YOR222W	??
-	YPL134C	??
-	YEL006W	??
-	YIL006W	??
-	YPR128C	??
-	YER053C	??
-	YER053C	??
-	YFR045W	??
-	YPR021C	??
-	YMR166C	??
MTM1	YGR257C	Mito transporter for Mn
-	YDL119C	??
-	YDR470C	??

Mitochondrial manganese




Cytosol
Sod2p
D159
H163
H74
H26
H163
D159
Mn
Mtm1p
Mn
H74
H26
Mitochondrial Matrix
Sod2p Tetramer
D159
H26
Mn
H163
H_2O
H74

Human homologue to MTM1