Mitochondria, Antioxidants and Aging

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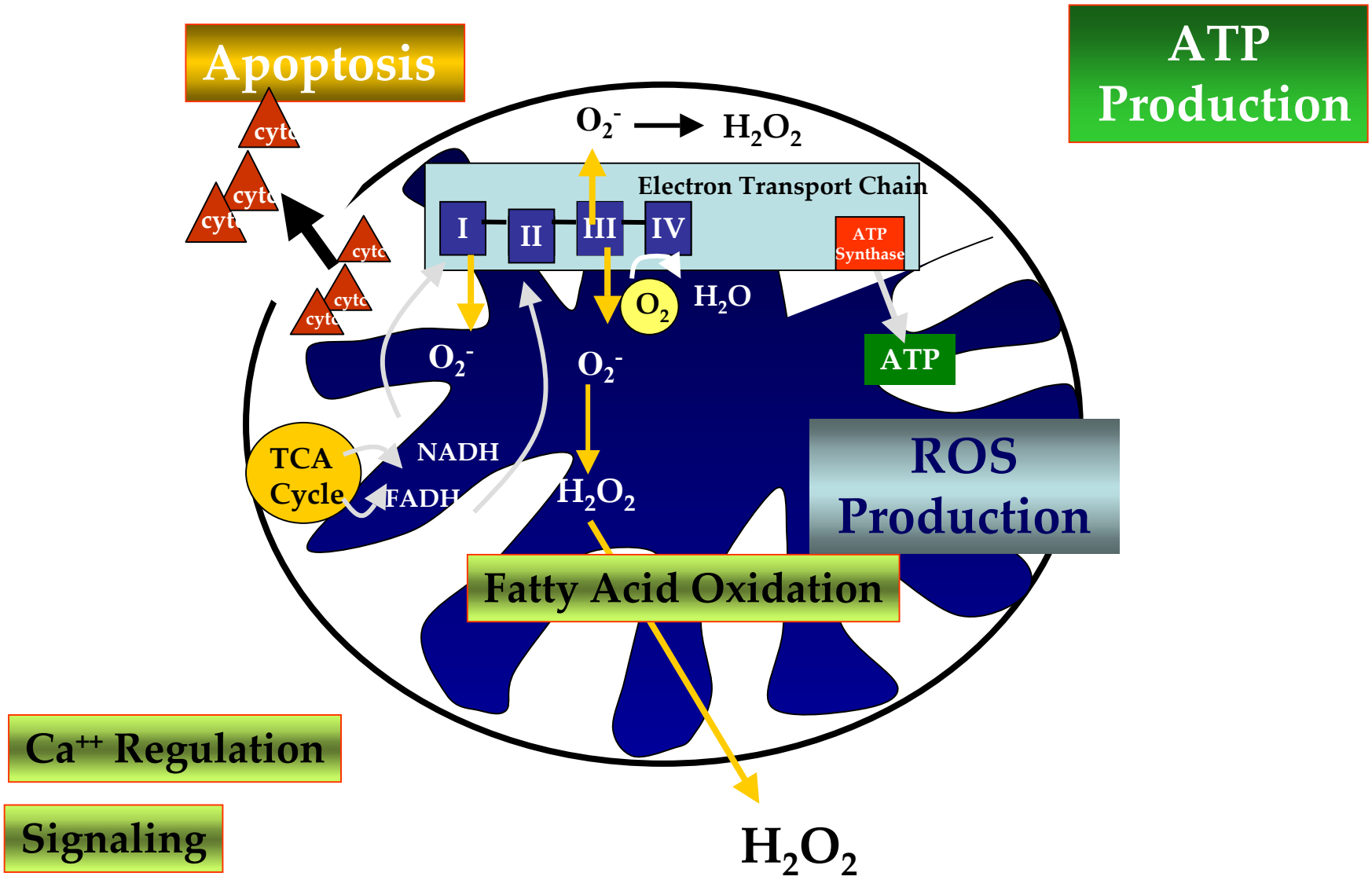
Review of the Role of Mitochondria in Aging

I. Mitochondrial Function and Oxidative Stress

II. Mitochondrial Theory of Aging

III. Studies Manipulating Mitochondrial Oxidative Stress in Animal Models-what can we learn?


Apoptosis
ATP Production
cyt c
cyt c
cyt c
cyt c
cyt c
$O_2^- \rightarrow H_2O_2$
Electron Transport Chain
I
II
III
IV
ATP Synthase
O_2
H_2O
O_2^-
O_2^-
ATP
TCA Cycle
NADH
FADH
H_2O_2
ROS Production
Fatty Acid Oxidation
H_2O_2
Ca^{++} Regulation
Signaling

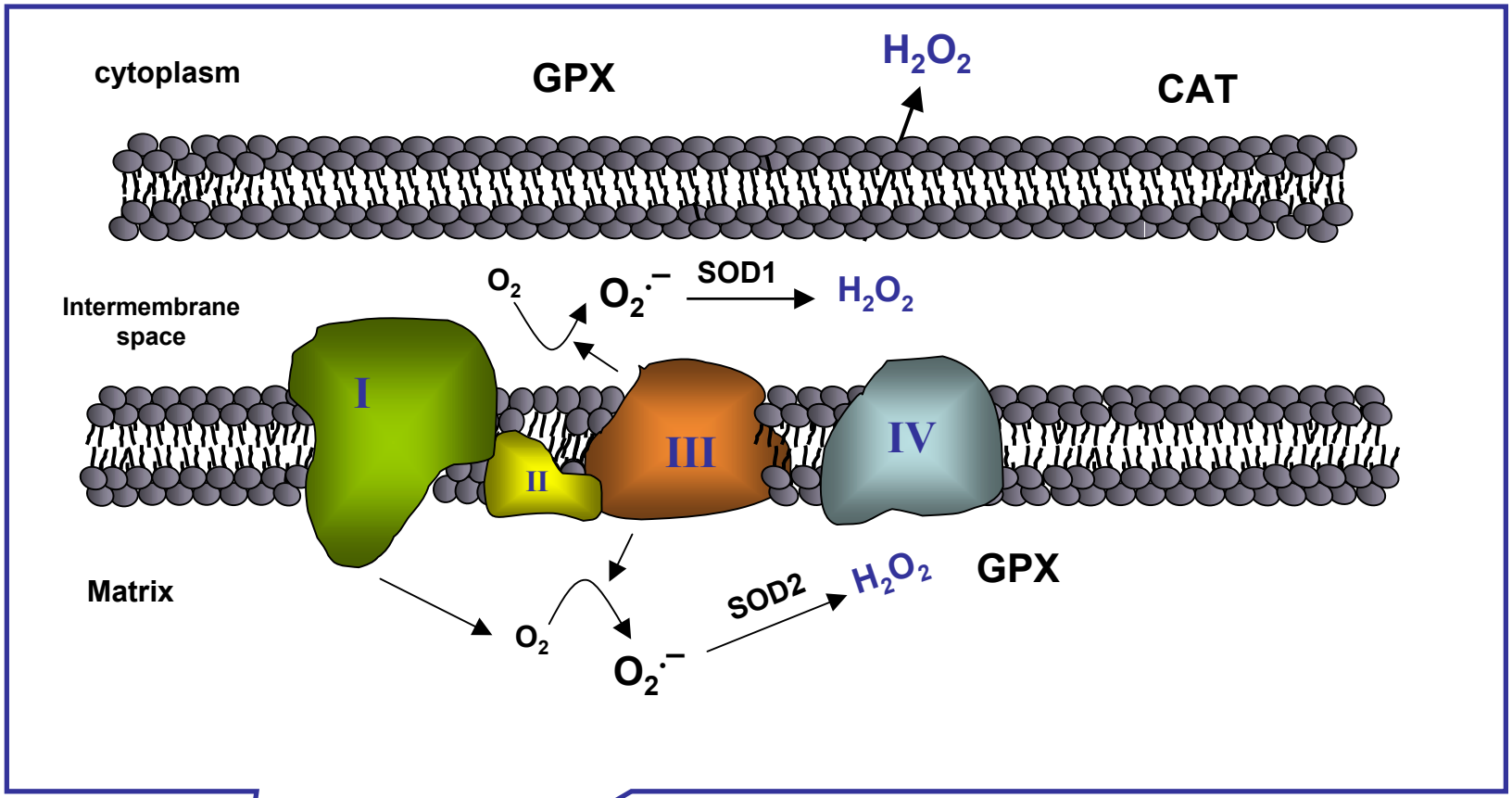
Sources of Reactive Oxygen Species

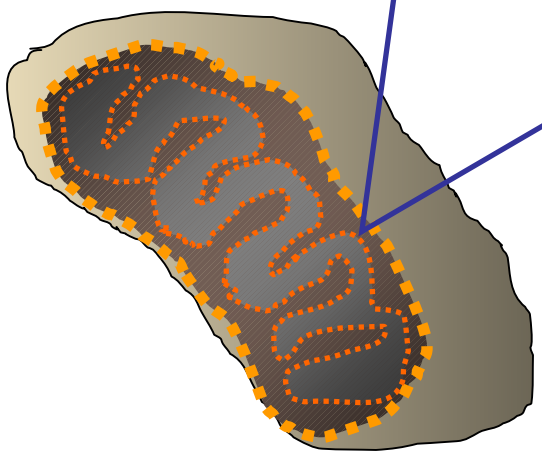
Non mitochondrial:

- NADPH Oxidases
- Microsomal cytochrome P-450
- Cyclooxygenases
- Monoamine oxidases
- Peroxisomal β oxidation of fatty acids
- Phagocytes

>90% is mitochondrial

electron transport chain contains several redox centers that may leak electrons to oxygen

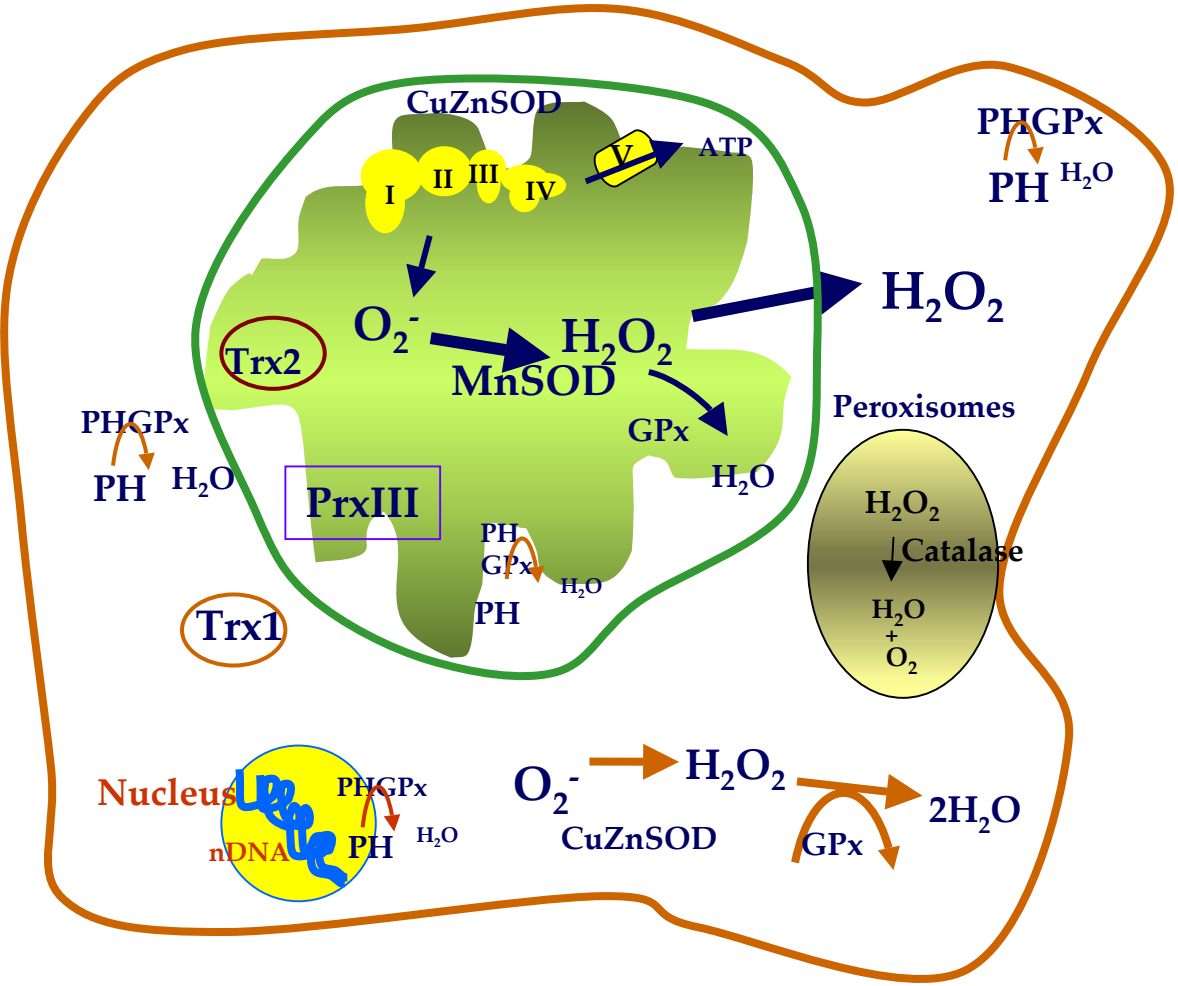




Mitochondrial ROS generation

ROS production is at Complex I and Complex III

Mitochondrial Antioxidant Defense:

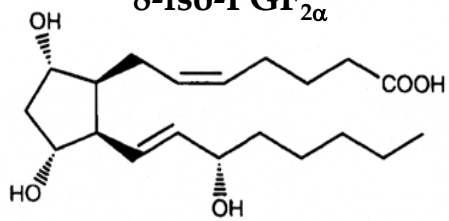
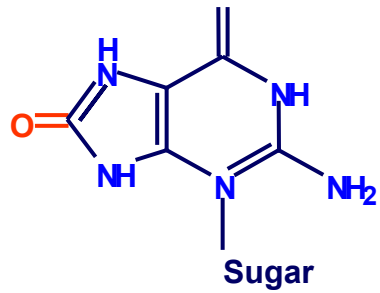
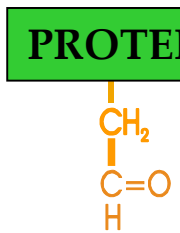


Enzyme	Target
MnSOD	O_2^-
CuZnSOD	O_2^-
Gpx1	H_2O_2
Gpx4	lipid peroxides
PrxIII	H_2O_2
Trx2	$Pr-S_2$

Non-enzymatic:

Ascorbate
Glutathione (GSH)
Tocopherols

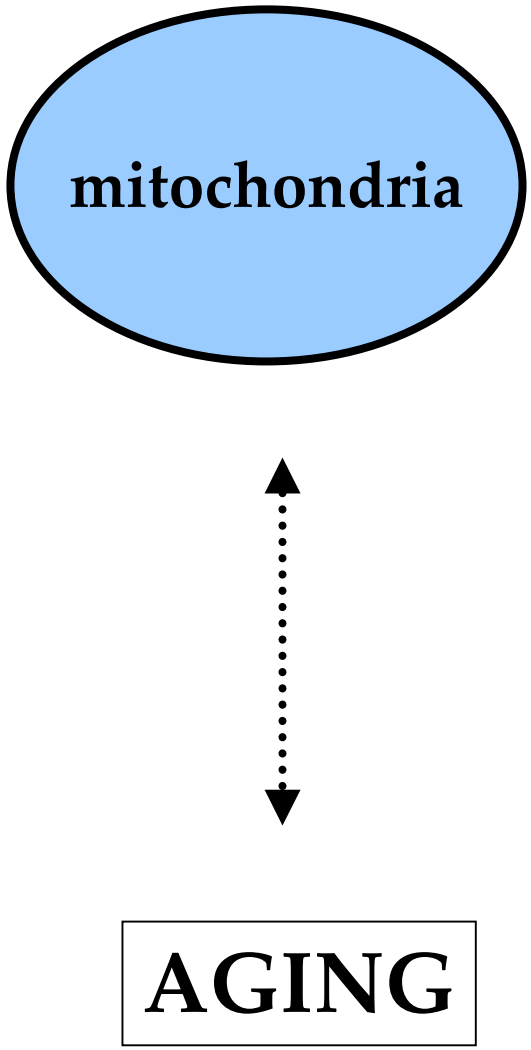
Consequences of mitochondrial oxidative stress

		Marker
Oxidative Damage to Lipids	**Membrane peroxidation** **Decreased membrane fluidity**	**F_2-Isoprostanes** **8-iso-$PGF_{2\alpha}$** 
Oxidative Damage to DNA	**Mutations** **Deletions**	**oxo8dG** 
Oxidative Damage to Proteins	**Oxidation of sulfhydryl groups** **Reactions with aldehydes** **Protein aggregation**	**Protein carbonyls** 

The problem:

Mitochondria are required for energy production

Mitochondria produce potentially harmful reactive oxygen species



What is the link?

Some Current Theories of Aging:

- **Rate of living theory**
  - Energy consumption is limiting for longevity
- **Evolutionary Theories**
  - Aging is a programmed event
    - Mutation accumulation
    - Antagonistic Pleiotropy
- **Genomic Instability**
- **Cell Senescence/Telomere Shortening**
- **GH/IGF-1 axis**
- **Free Radical Theory**

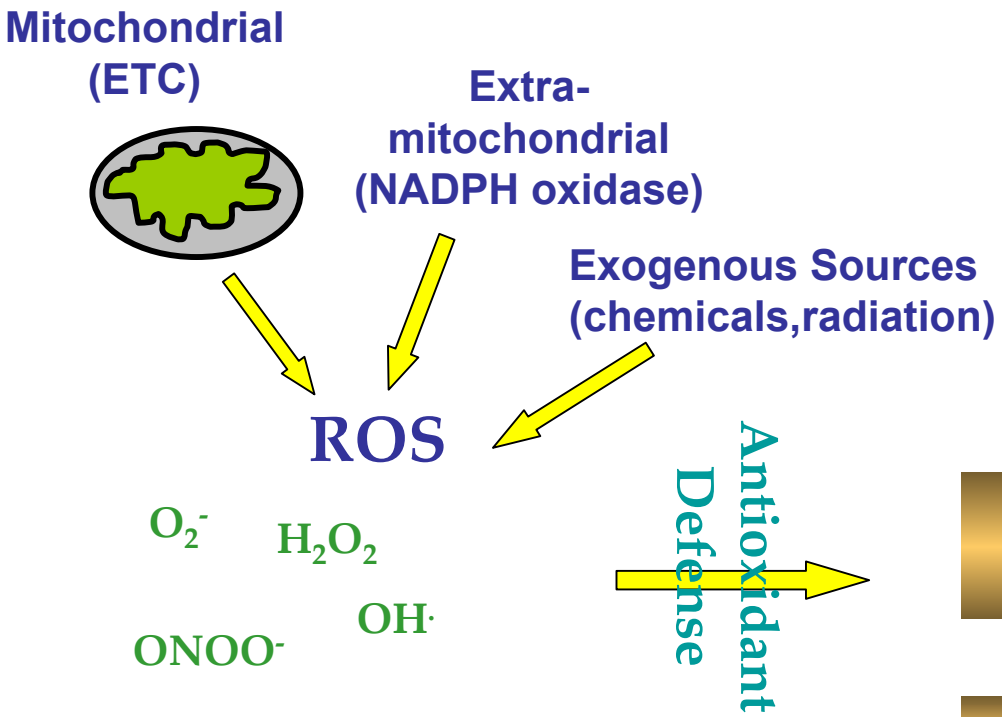
Free Radical Theory of Aging

"Aging: a theory based on free radical and radiation chemistry"
1956, D. Harman *J.Gerontology 11(3):298-300*

A single common process, modified by genetics and environmental factors, is responsible for the aging and death of all living things.

"Aging and the degenerative diseases associated with it are attributed basically to the deleterious side attacks of free radicals on cell constituents and on the connective tissues…….. The free radicals probably arise largely through reactions involving molecular oxygen catalyzed in the cell by oxidative enzymes and in the connective tissues by traces of metals such as iron, cobalt, and manganese."

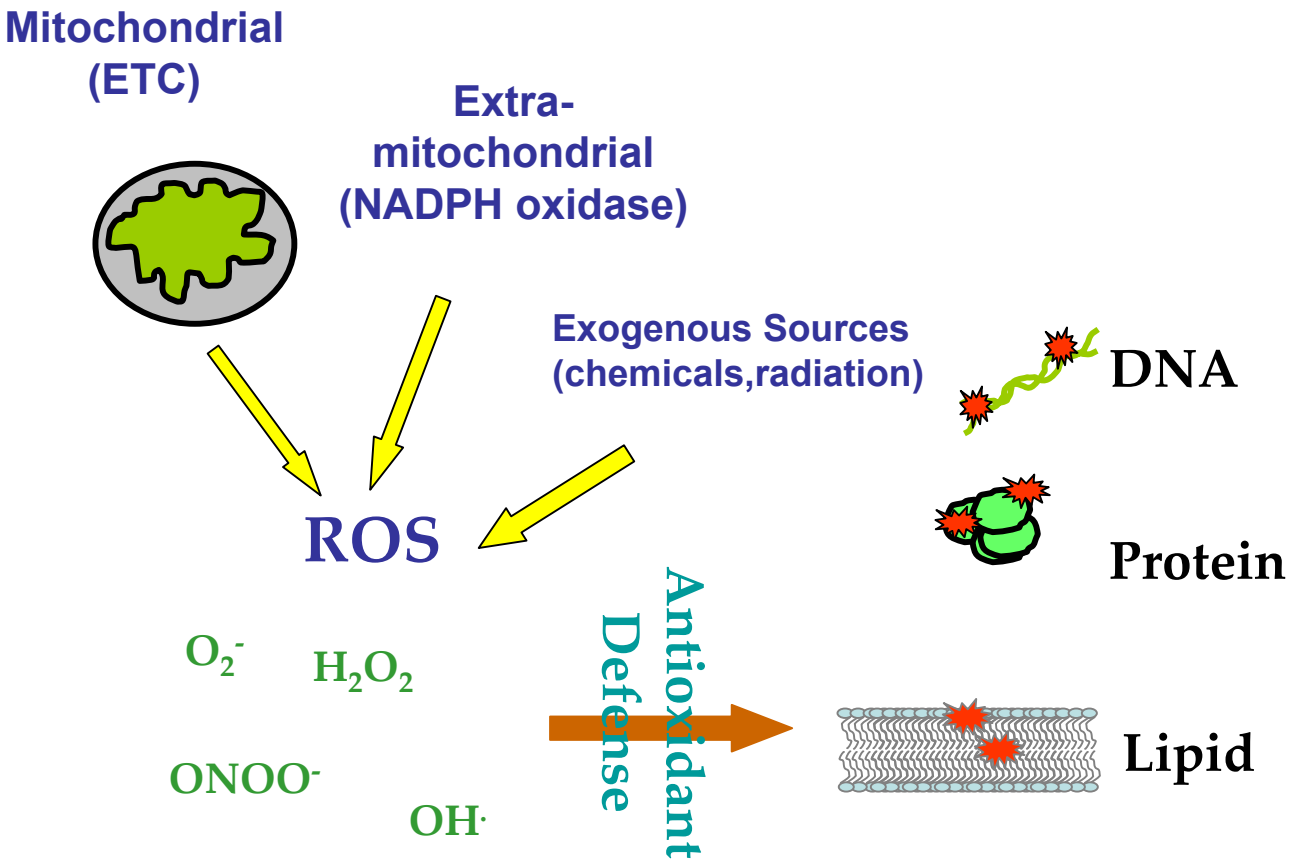
Oxidative Stress Theory of Aging



Imbalance between pro-oxidants and antioxidants.

Chronic state of oxidative stress

Oxidative Stress Theory of Aging



Steady-state accumulation of oxidative damage that increases during aging

Progressive loss in efficiency of cellular processes

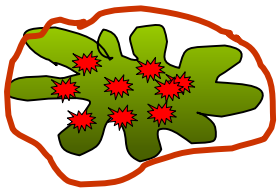
Mitochondria are major source of ROS

Mitochondrial Theory of Aging

Harman,D. 1972 "The biologic clock: the mitochondria?"
J. Am Ger Society 20:145-57

Free radicals escaping from the respiratory chain …would be expected to produce deleterious effects mainly in the mitochondria
…Are these effects mediated in part by mitochondrial DNA functions?

Mitochondria Theory of Aging

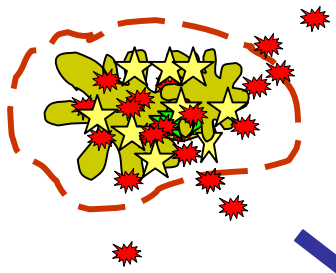


Increased Production of ROS

-Alterations in ETC function
-mtDNA deletions/mutations

Damaged mitochondria produce more ROS

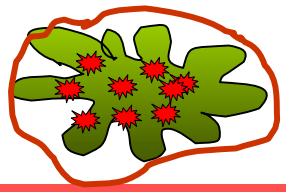
Accumulation of oxidative damage with age



Decreased ATP
Cell Death/Apoptosis
Functional Decline

Aging

Mitochondria Theory of Aging



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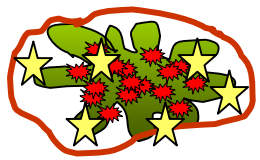
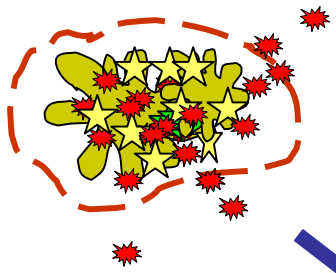




Decreased ATP
Cell Death/Apoptosis
Functional Decline

Aging

Early studies supported an increase in ROS with age

Housefly flight muscle
(submitochondrial particles)
Sohal and Sohal (1991)
Mech of Aging and Dev. 187-202.

Mouse kidney, heart and brain mitochondria
(submitochondrial particles)
Sohal et al (1994)
Mech.Ageing Dev.121-133

Rat vastus lateralis or soleus muscle
(homogenates/isolated mitochondria)
Bejma and .Ji (1999)
J.Appl.Physiol. 465-470

Other studies show no increase…..

Rat Muscle

Capel et al.
Mech. Aging Dev., (2004) 367-373.

Soleus ↑
Tibialis Anterior ↔

Drew et al
Am.J.Physiol
Regul.Integr.Comp Physiol, (2003) R474-R480

Gastrocnemius ↔

Rat Heart

Hansford et al
J.Bioenerg.Biomembr., (1997) 89-95

↔

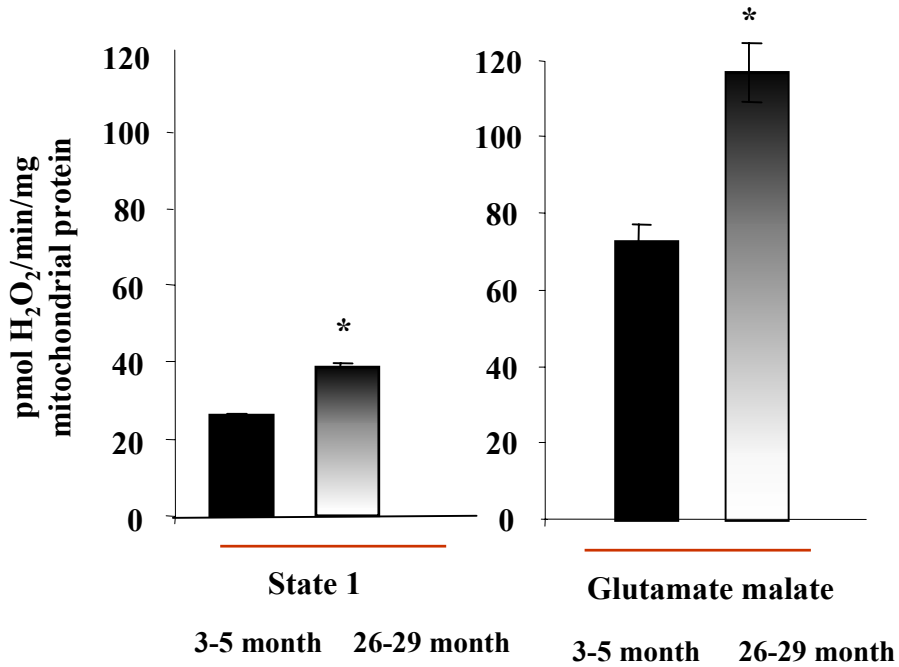
Rat Liver

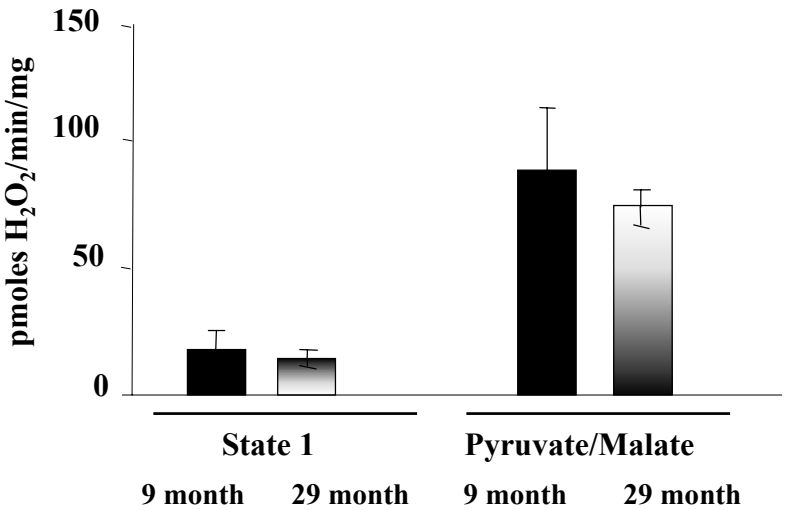
Lopez-Torres et al.
Free Radic.Biol.Med., (2002) 882-889

↔

Increase may be tissue specific?.....

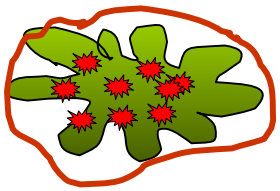
Mouse hindlimb
Skeletal muscle

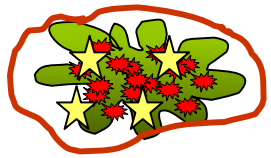




Mouse heart

Mitochondria Theory of Aging





-Alterations in ETC function

-mtDNA deletions

Increased Production of ROS

Some tissues?

Aging and ETC function

	Muscle Tissue	Complex I	Complex II	Complex III	Complex IV	Complex V
Trounce et al., (1989) *Lancet* (8639):637-9	Human muscle	↓	↓		↓	
Boffoli et al. (1994) *BBA* 1226:73-82	Human muscle	↓	↓	↔	↓	
Sugiyama et al. (1993) *Biochem Mol Biol Int* 30:937-44	Rat muscle	↓	↔	↔	↓	
Muller-Hocker et al. (1990) *J Neurol Sci* 100:14-21	Human muscle		↔		↓	
Desai et al. (1996) *Arch Bioc Biophys* 333:145-151	B6C3F1 mice	↓	↔	↓	↓	
Muller-Hocker et al. (1996) *Mech Aging Dev* 86:197-213	monkeys			↓	↓	↓
Kwong et al. (2000) *Arch Bioc Biophys* 373: 16-22	C57Bl6 mice		↓	↓	↓	

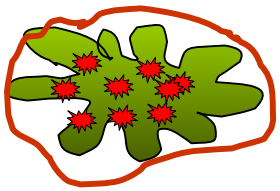
More recent studies in humans, mice

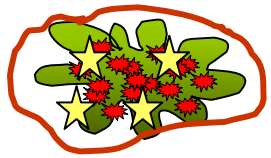
Barrientos 1996
Rasmussne 2003
Chretien 1998,
Kwong and Sohal 2000

No consistent change

Current evidence suggests that there is no consistent overall defect in the ETC with age.

Mitochondria Theory of Aging





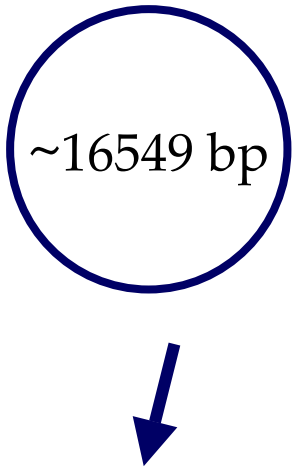
-Alterations in ETC function (?)

-mtDNA mutations/deletions

Increased Production of ROS

Some tissues?

MtDNA



-Double-stranded, circular molecule

-2 to 10 copies in each mitochondrion
> 1,000 in each cell

mtDNA encodes for 37 genes:
22tRNA
13 mitochondrial peptides (ETC subunits)
16S and 26S rRNA

(Remaining > 67 ETC subunits are nuclear encoded)

mtDNA is more sensitive to oxidative damage than nuclear DNA

Estimated at 1/8000 bases for mtDNA vs 1/130,000 for nDNA
(Richter et al 1988 PNAS)

-high metabolism

-proximity to generation of ROS

-lack of histones

-repair systems less efficient

mtDNA Deletions

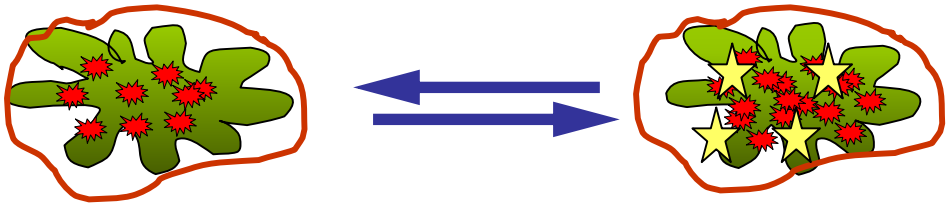
- **Tissues that turn-over more slowly (skeletal muscle, heart) have more mtDNA deletions than more rapidly dividing tissues (liver)**

- **Deletions increase with age in muscle heart and brain (identified using PCR)**

- **Common deletion mtDNA4977 increases by a factor of 10,000 in muscle during a normal human lifespan reaching 0.1% of total muscle mtDNA by 84 yrs**

- **Evidence indicates focal accumulation of deletions in some tissues**

Mitochondrial Theory of Aging



Alterations in ETC function (?)

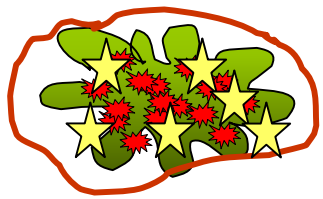
mtDNA mutations/deletions

YES

Increased Production of ROS

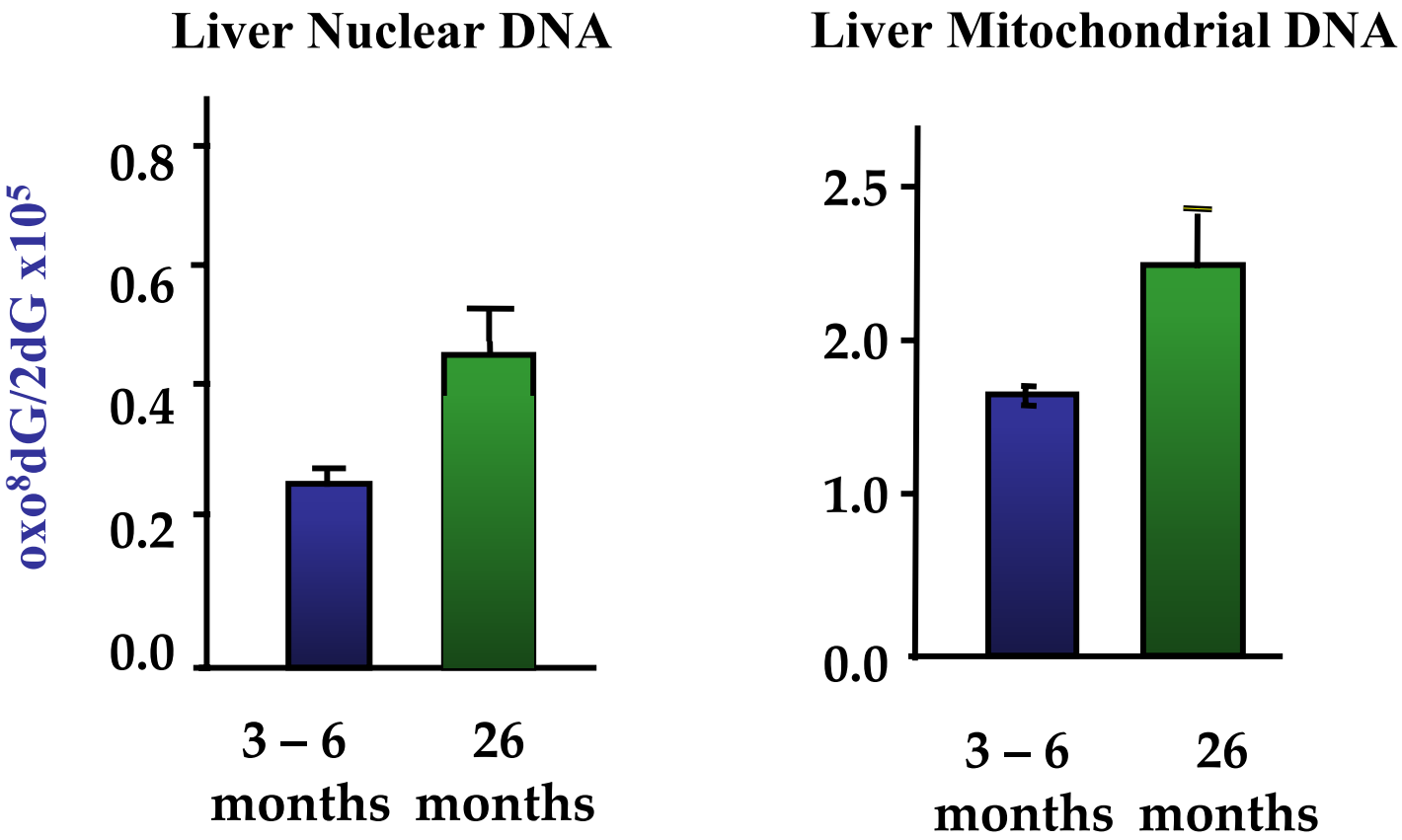
Some tissues?

Damaged mitochondria produce more ROS



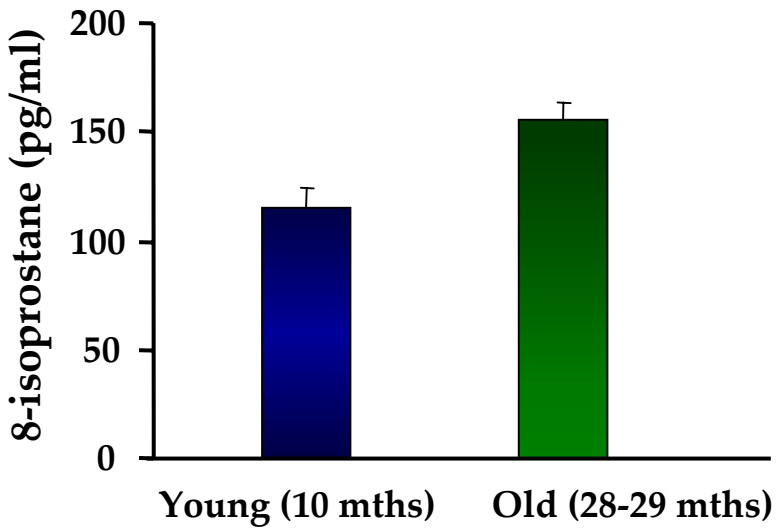
Accumulation of oxidative damage with age (mitochondrial and non-mitochondrial)

Age related changes in mitochondrial DNA oxidative damage



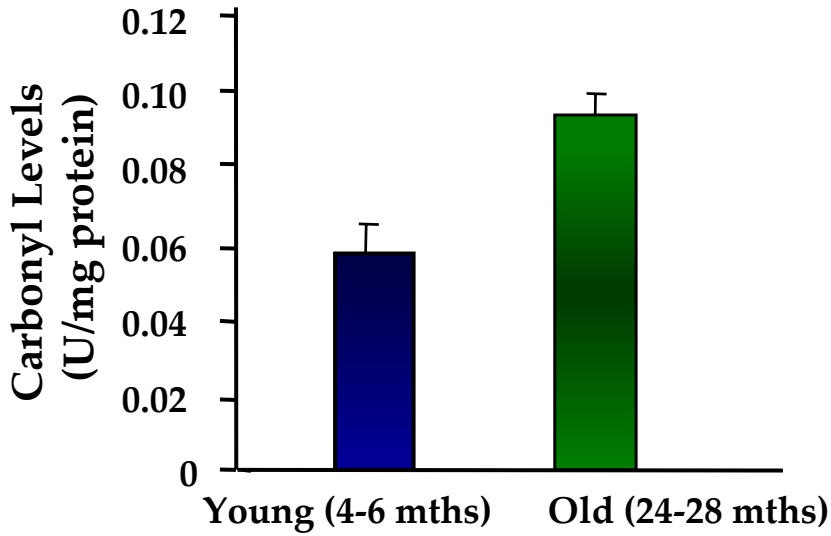
Van Remmen et al., Physiol. Genomics, 2003

Plasma Free Isoprostanes



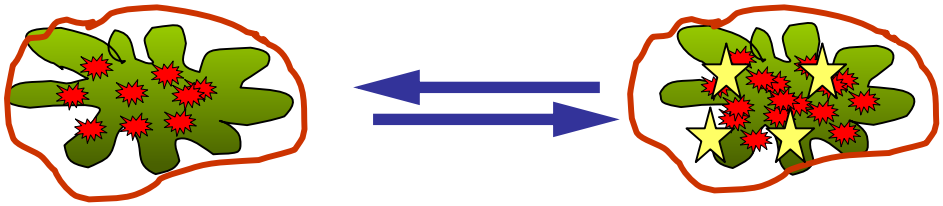
Jack Roberts, Vanderbilt University

Carbonyl Groups in Liver Protein



Asish Chaudhuri, San Antonio

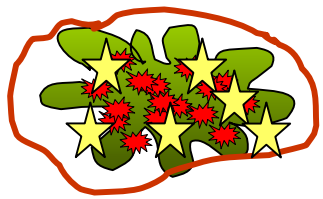
Mitochondrial Theory of Aging



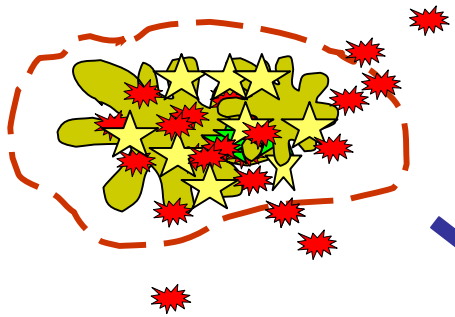
Alterations in ETC function (?)
mtDNA mutations/deletions
YES

Increased Production of ROS
Some tissues?

Damaged mitochondria
produce more ROS



Accumulation of oxidative damage with age (mitochondrial and non-mitochondrial)
YES



Decreased ATP
Cell Death
Functional Decline
?

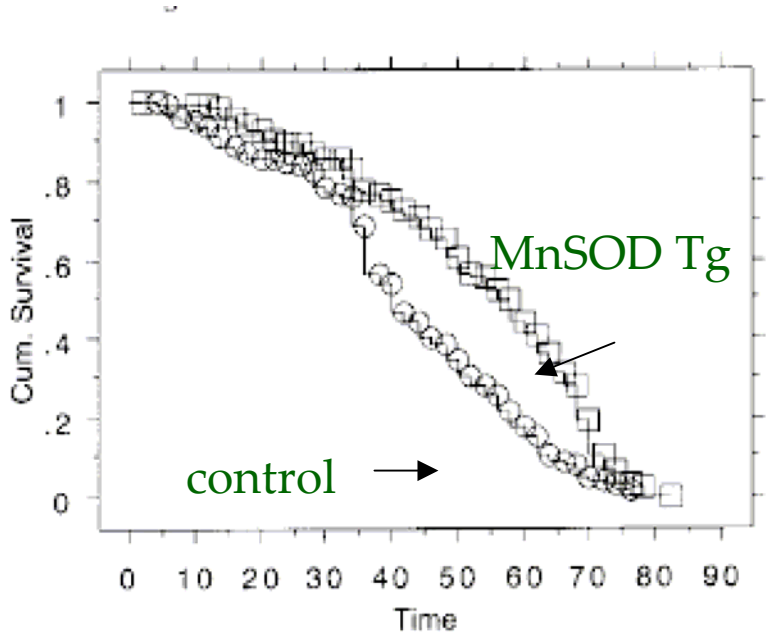
Aging

Effects of Manipulating Mitochondrial Oxidative Stress in Animal Models

Altered expression of MnSOD in *Drosophila*

Sun et al., Genetics. (2002) 161(2):661-72.

Induced overexpression of mitochondrial Mn-SOD extends the life span of adult Drosophila melanogaster.



Mean life span increased by an average of 16%

Another study finds no increase in lifespan in MnSOD Tg flies

(Orr et al., 2003 JBC)

Increased Expression of Mitochondrial Uncoupling Protein 2

Targeted expression of the human uncoupling protein 2 (hUCP2) to adult neurons extends life span in the fly.

Alterations in Mitochondrial Function and Oxidative Stress

- decrease in ROS production
- decrease in oxidative damage
- increased resistance to paraquat

Increase in lifespan

Fridell et al., Cell Metab. 2005 1(2):145-52

Increased expression of Mitochondrial genes	Effect on Lifespan
MnSOD	↑
UCP2	↑

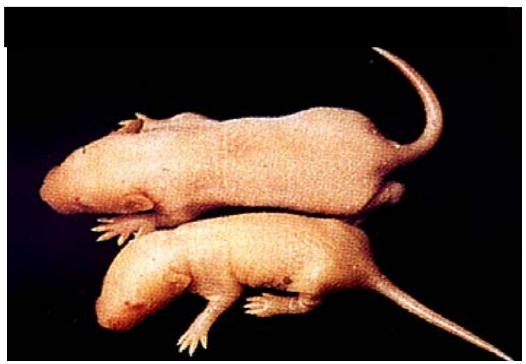
Seem to be consistent with the theory…..

What can we learn about mitochondrial theory of aging from mouse models?

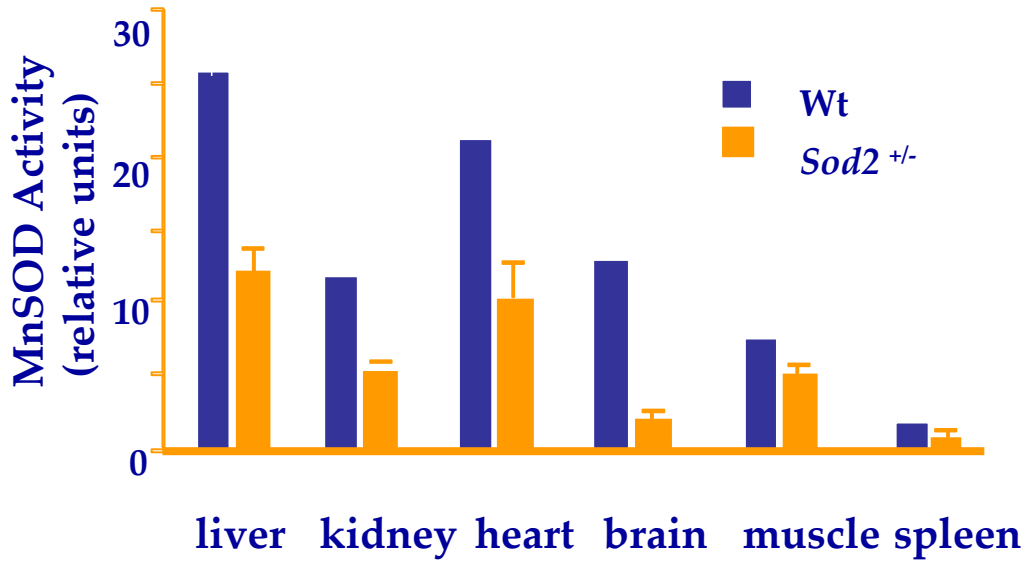
- MnSOD-mitochondrial antioxidant
- mtDNA polymerase mutant mice
- Mitochondrial targeting of catalase

MnSod (*Sod2*$^{+/-}$) Knockout mice

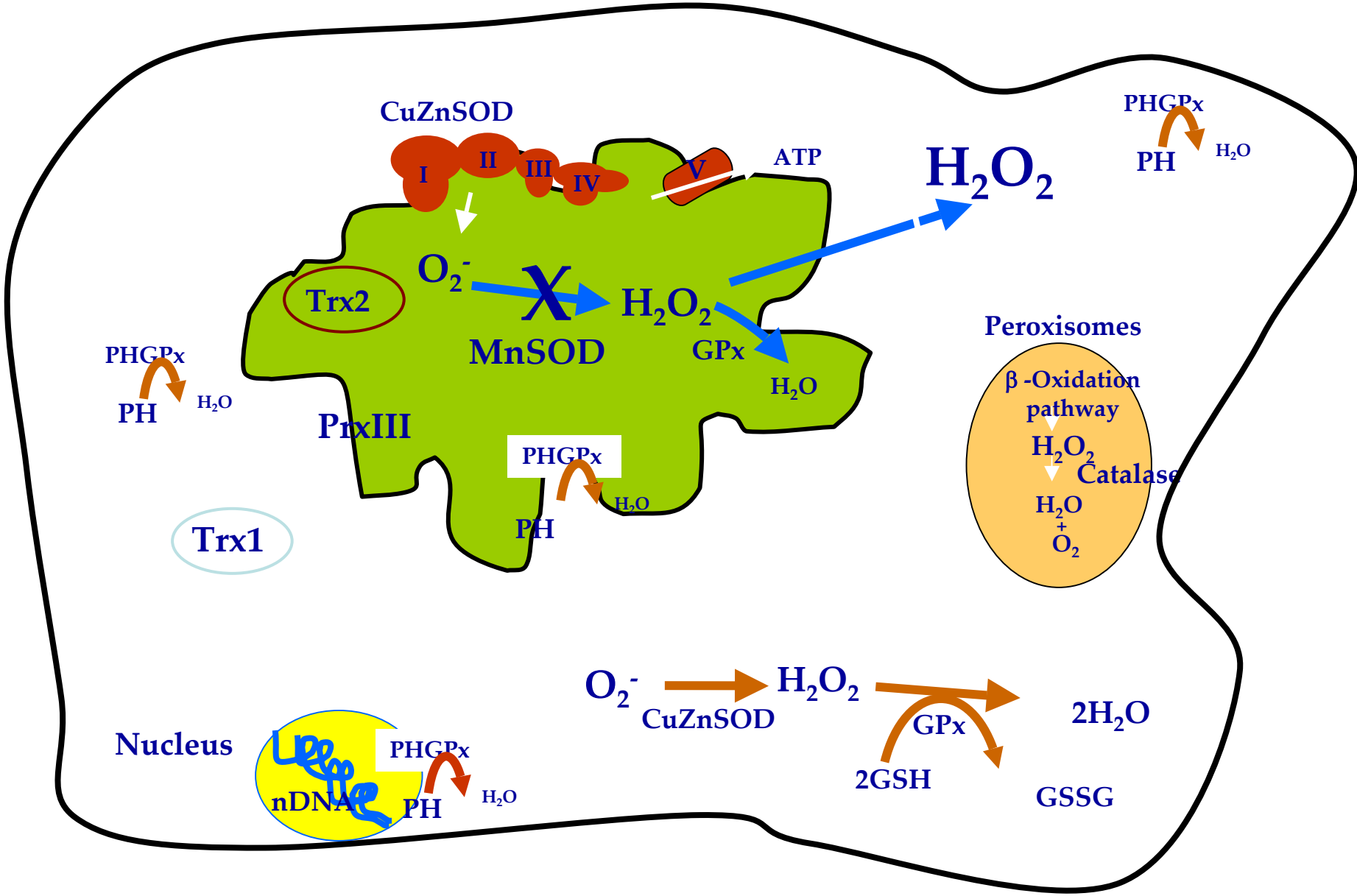
- Mitochondrial antioxidant enzyme ($O_2^- + O_2^- \rightarrow H_2O_2$)
- Homozygous mutant is lethal (survival is less than 2 weeks)
- 50% decrease in MnSOD activity in all tissues studied
- No compensation by other major antioxidant enzymes



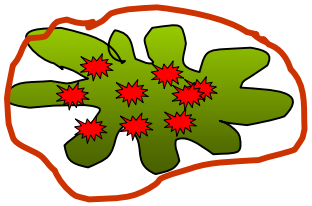
(Li et al. (1995) Nature Genetics.

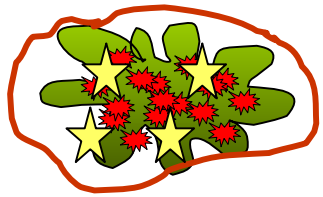


Sod2$^{+/-}$ Knockout Mice



Mitochondrial Theory of Aging



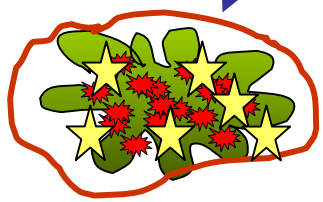


Alterations in ETC function
mtDNA mutations/deletions

Increased Production of ROS

$Sod2^{+/-}$ mice
(Reduced Antioxidant Protection)

↑↑ Accumulation of oxidative damage with age

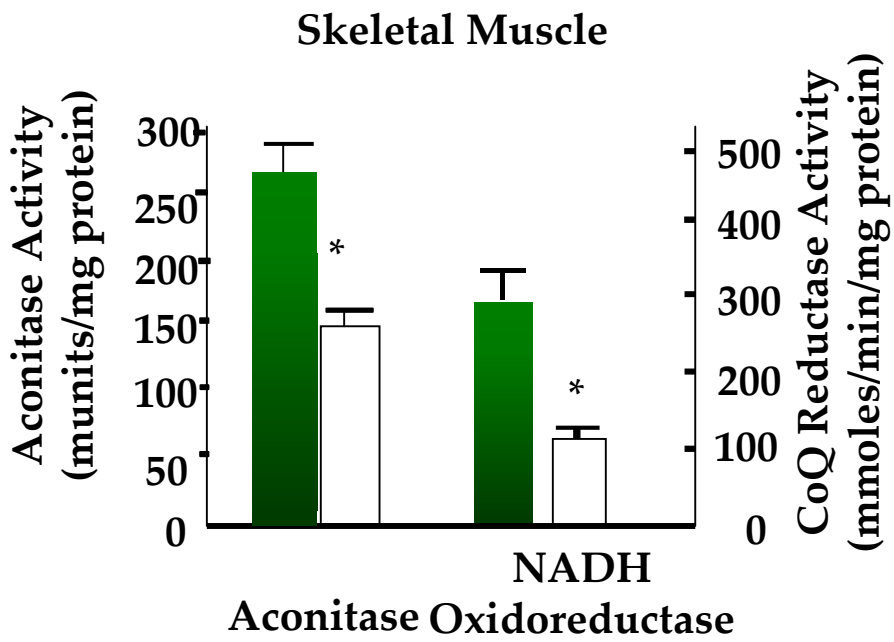


Decreased ATP
Cell Death
Functional Decline

Increased Aging

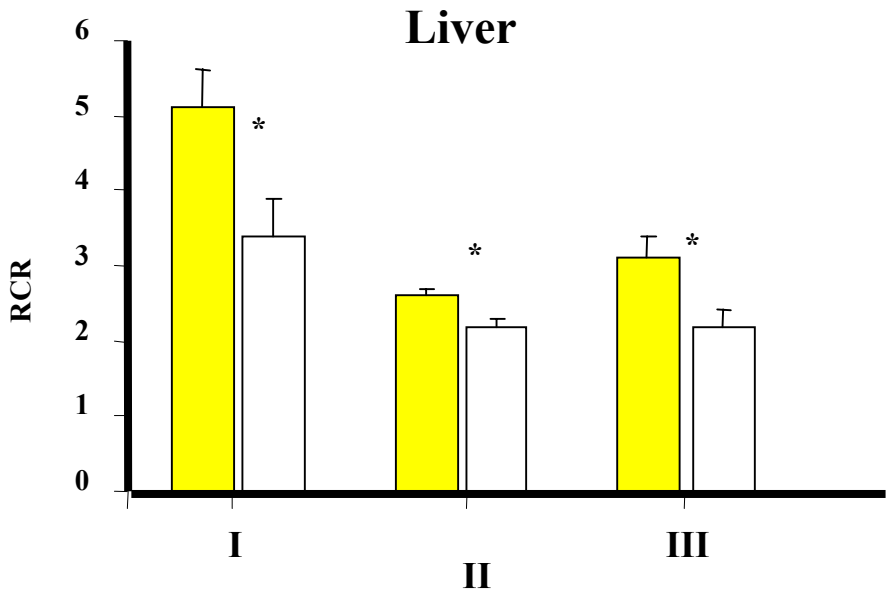
MnSOD Heterozygous Knockout Mice

Oxidative damage to specific mitochondrial proteins
aconitase
NADH oxidoreductase



MnSOD Heterozygous Knockout Mice

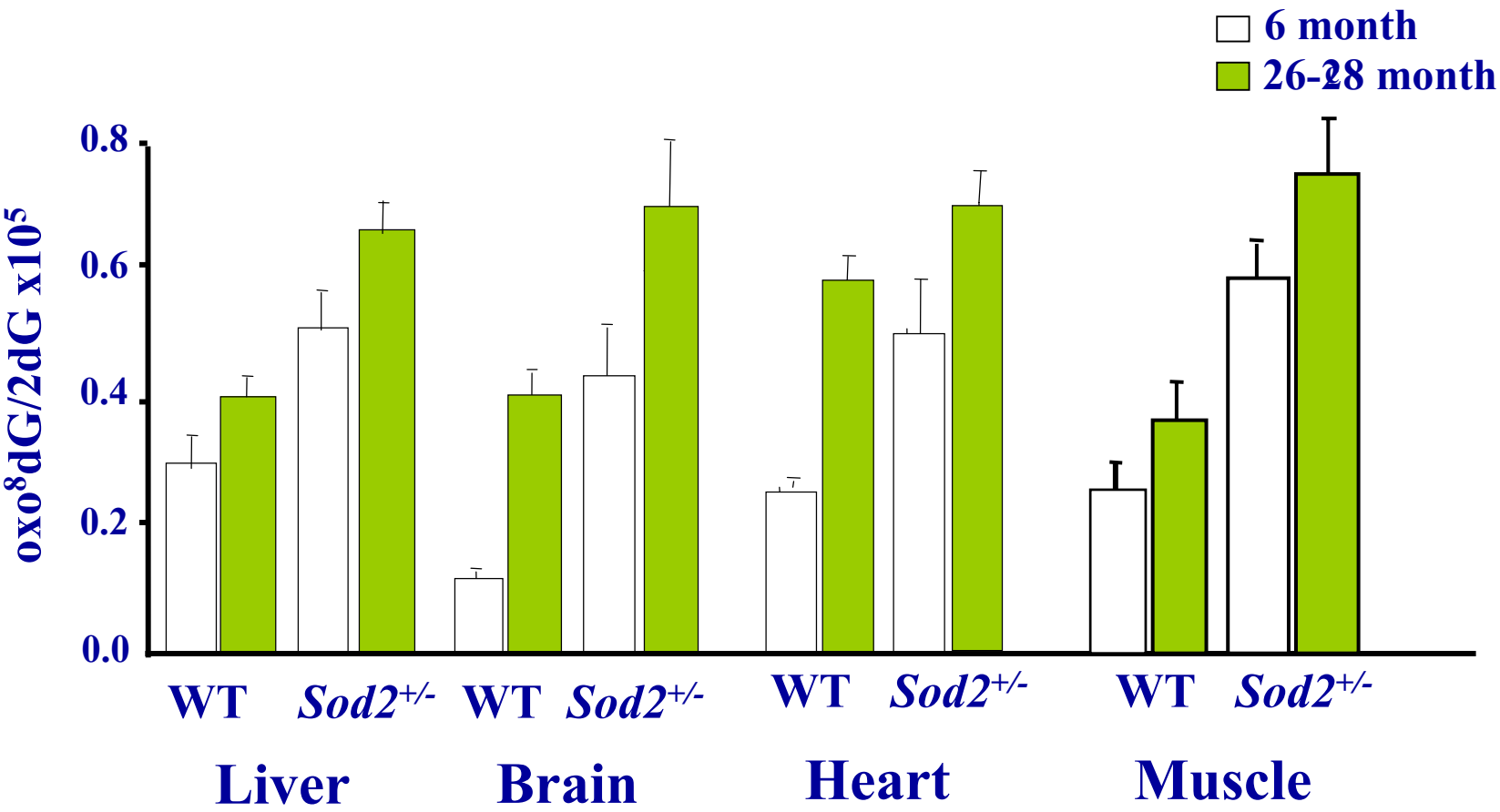
Altered Mitochondrial Function /Decreased activities of ETCs



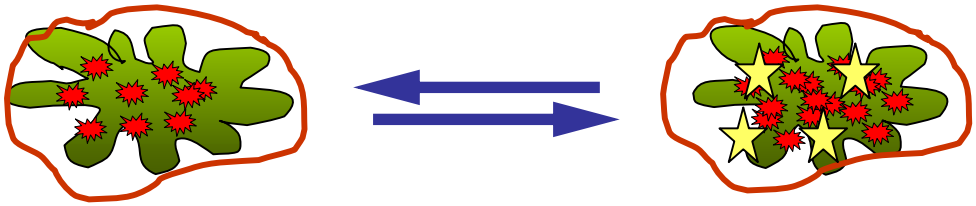
MnSOD Heterozygous Knockout Mice

Increased oxidative damage to DNA

Nuclear DNA



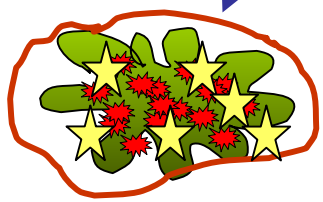
Mitochondrial Theory of Aging



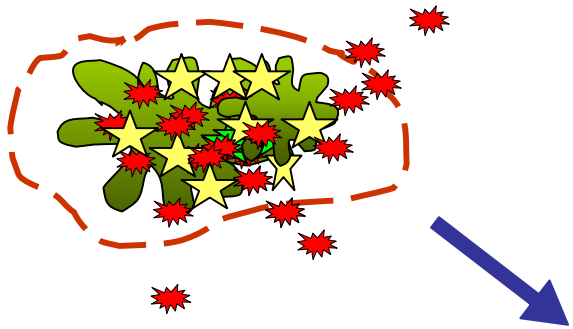
Alterations in ETC function
mtDNA mutations/deletions

Increased Production of ROS

Sod2$^{+/-}$ mice
(Reduced Antioxidant Protection)

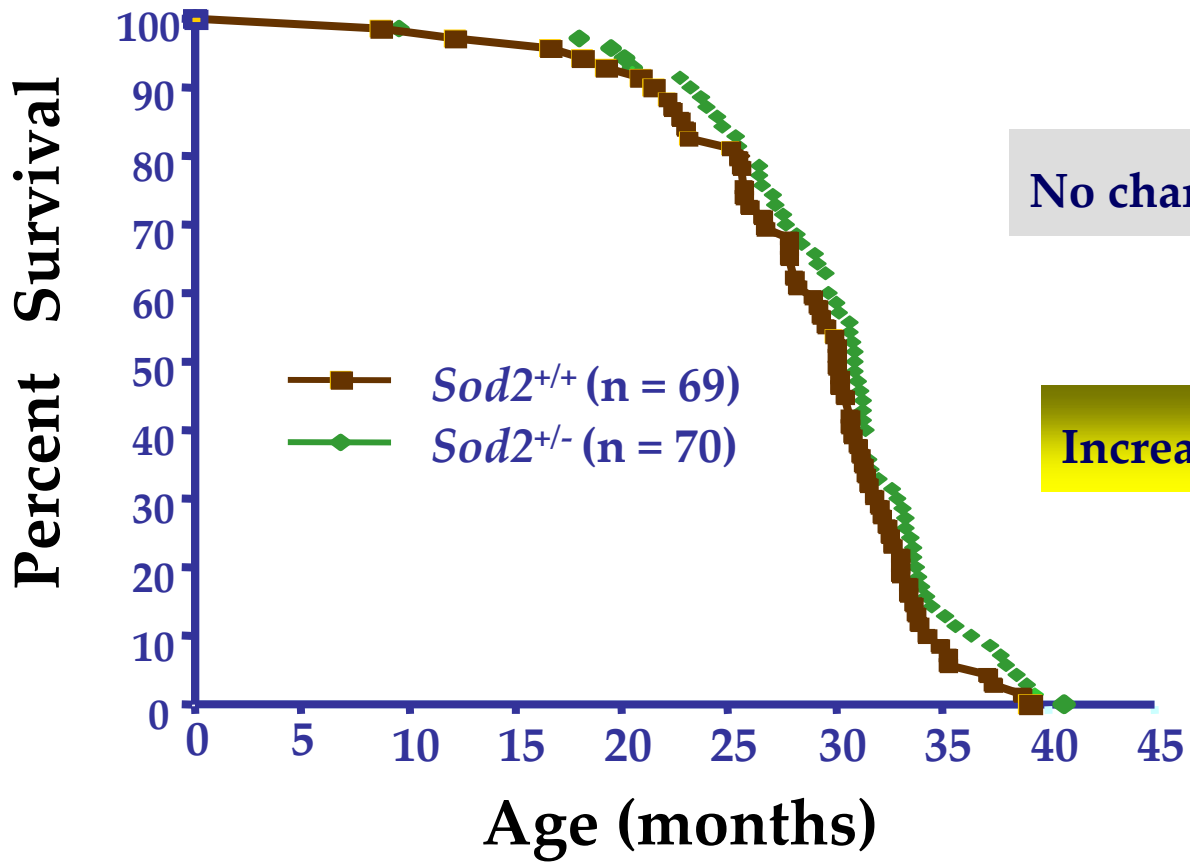


↑↑ Accumulation of oxidative damage with age



Aging

MnSOD Heterozygous Knockout Mice



No change in survival

Increase in Cancer

Premature ageing in mice expressing defective mitochondrial DNA polymerase

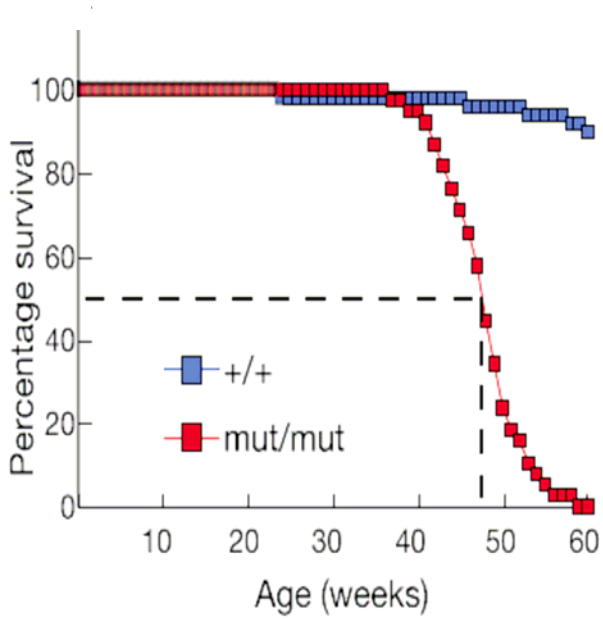
Nature, May 27, 2004, Trifunovic *et al*

Homozygous knock-in mice for a proof reading deficient PolgA-mtDNA polymerase

- **3-5 fold increase in point mutations and deletions in mtDNA**
- **Normal appearance until ~6 months (25 weeks)**
  -Weight loss, kyphosis, alopecia, decreased fat,osteoporosis
- **Decreased ETC activity and ATP production in heart**

mtDNA mutations -- Shortened lifespan

- Median lifespan, ~48 weeks, or 336 days
- All died by 61 weeks, or ~430 days



a link between mtDNA mutations and ageing phenotypes in mammals?

PolgA-mtDNA polymerase mutants

Nature, May 27, 2004, Trifunovic *et al*

Mitochondrial DNA Mutations, Oxidative Stress, and Apoptosis in Mammalian Aging

Kujoth et al Science 259, 2005

-Targeted mutation of PolgA Mitochondrial DNA polymerase
-Residue substitution in the exonuclease domain that impairs proof reading ability

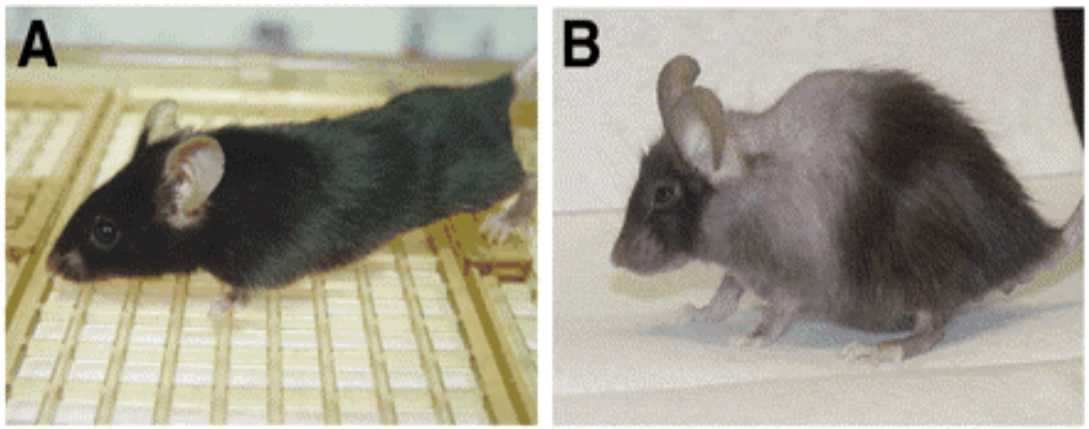
- increased mutations in mtDNA

- No increase in oxidative damage

- Apoptotic markers were increased during aging [increased caspase 3 activity]

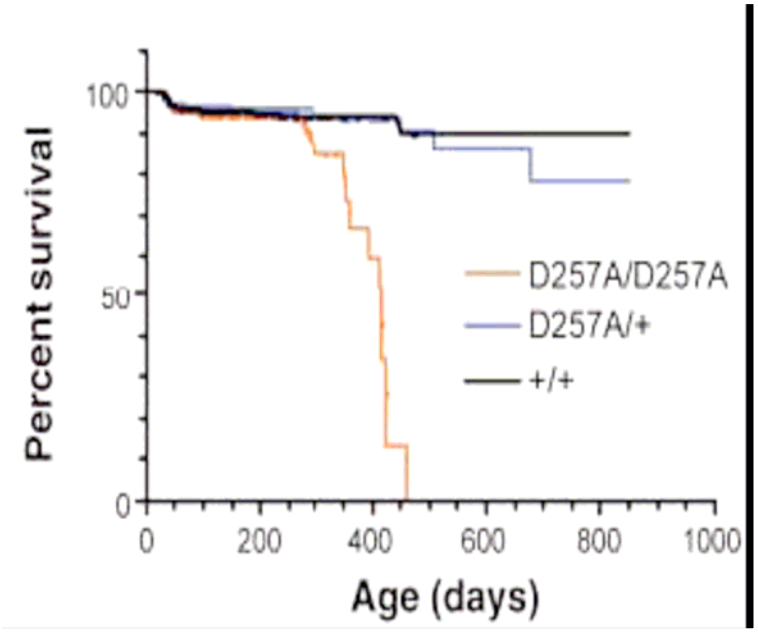
Phenotype shows age-associated characteristics

-Indistinguishable in young age
-Phenotype evident at ~9 months



- Hair loss, graying, and kyphosis.
- Thymic involution
- Testicular atrophy associated with the depletion of spermatogonia
- Loss of bone mass
- Loss of intestinal crypts
- Decrease in circulating red blood cells
- Weight loss
- Hearing loss
- Loss of muscle mass

Kujoth et al Science 259, 2005



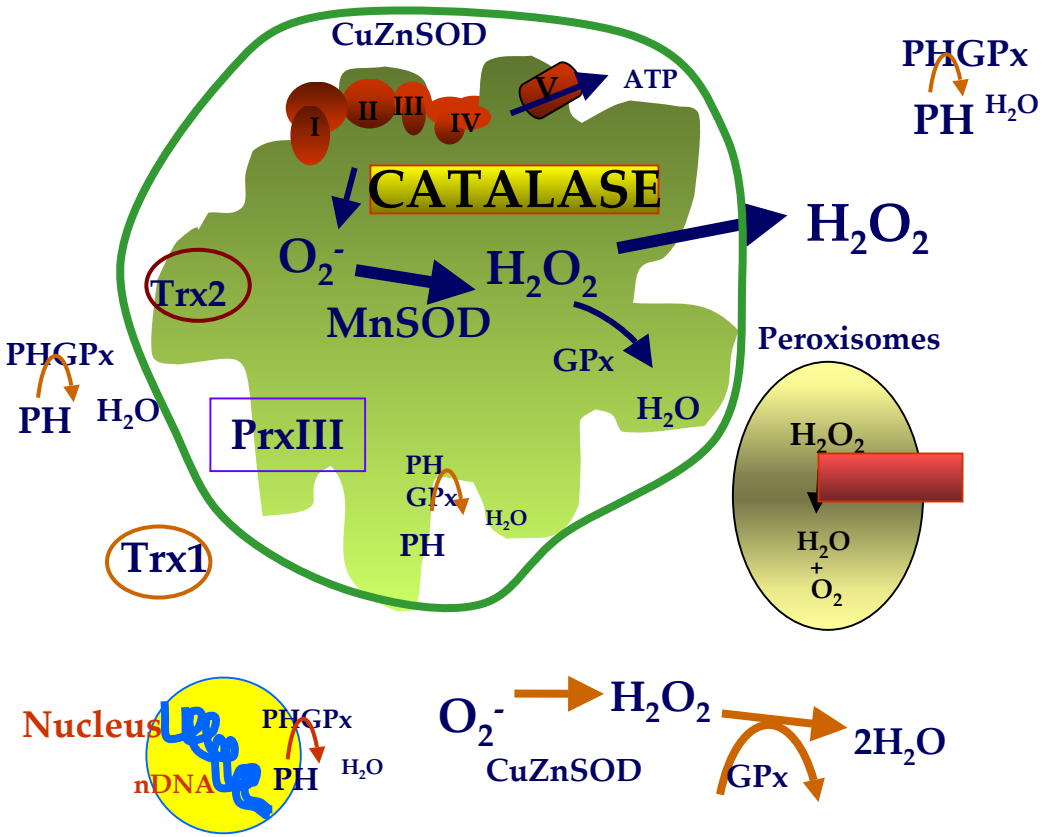
- **Maximal survival, 460 days.**
- **Median survival, 416 days**
- **Wildtype, both are > 850 days**

Are mtDNA mutations linked to apoptosis and aging?

Mitochondrial DNA polymerase mutant

Kujoth et al Science 259, 2005

Extension of Murine Life Span by Overexpression of Catalase Targeted to Mitochondria

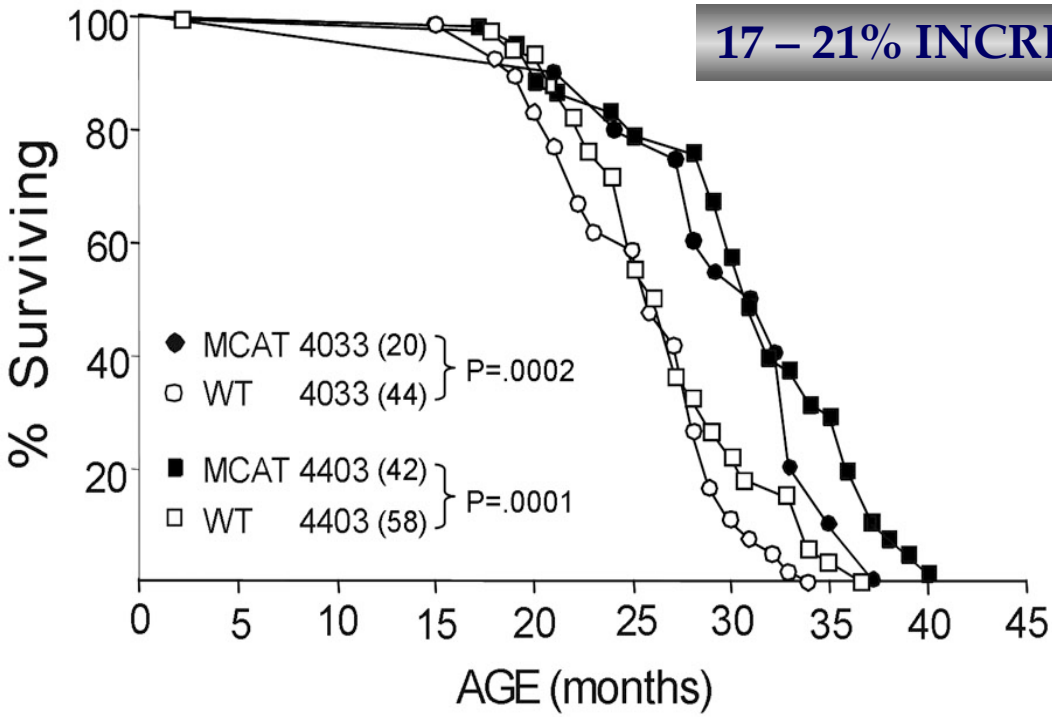


Increased expression in heart muscle and brain

Increased over 50x in heart mitochondria

Science, 2005- Schriner et al.

17 – 21% INCREASE IN LIFESPAN



Both median and maximum lifespan were increased in MCAT animals

Science, 2005- Schriner et al.

- **Cardiac pathology and cataract development were delayed**
- **H_2O_2 production was reduced**
- **Oxidative damage was reduced (aconitase activity, 8-OHdG)**
- **The development of mitochondrial deletions was reduced.**

Support for the free radical theory of aging and the importance of mitochondria ??

	Lifespan Effect on Lifespan
▪50% reduction in MnSOD	
▪Increased mtDNA mutations	
▪Mitochondrial targeting of catalase	

Do mitochondria play a key role in aging??

Evidence to date would suggest that mitochondria and oxidative stress in general certainly play a role in age related disease, but a direct link between mitochondria and aging has not been definitively established