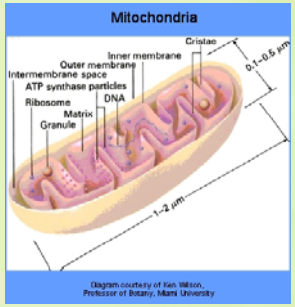
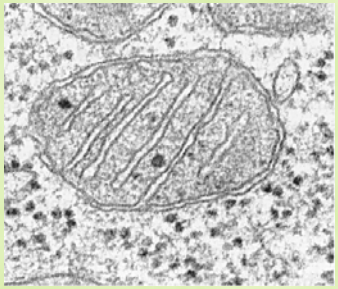
Mitochondrial free radical theory of aging





A&S300-002 Jim Lund

Mechanistic theory: How animals age

- OR, What biological process is responsible for aging?
- Complexity of the aging phenotype has led to many theories that focus on particular aspects of the phenotype.
- These theories don't necessarily compete with one another.

The challenge

- Finding theories that account for the many aspects of the phenotype.
- Needed: theory that relates changes in one biological process to another--that incorporates many different aspects of the aging phenotype and relates them to common underlying mechanisms.

Mechanistic aging theory

- Theories get evaluated on:
  - Empirical validity (local evaluation)
  - Breadth of phenotypes explained by the theory.
- Example: replicative senescence model of aging.
  - Doesn't explain aging in post-reproductive tissues like the brain, or animals like the nematode worm

Mechanistic aging theories

- There are a host of mechanistic theories, one review counted 200+.
- Typically researchers studying one aspect of aging would focus on an underlying aspect that seemed responsible for aging in that tissue, and then theorize that this process is responsible for other aspects of aging.
- Most theories were soon discarded--they **didn't** have much explanatory power.

Overview of mechanistic theories

- DNA damage and DNA repair
  - Loss of repair effciency with age leads to somatic mutation with effects described above.
- Mitochondrial free radical theory
  - Damage to mitochondria and cellular proteins from free radicals generated in mitochondria causes cell aging.
- Altered proteins
  - Accumulation of damaged protein in cells causes cellular processes to work poorly.

Mitochondrial free radical theory of aging

- Oxidative damage theory
  - Proposed by Denham Harman, 1956.
- Mitochondrial free radical theory
  - First proposed in 1972 by Harman, further refined and developed in 1980 by Jaime Miquel.

Oxidative damage

- 95% of a cell's energy is produced in the mitochondria.
- Most O_2 is utilized in the mitochondria.

O_2 is required for animal life, but O_2 is damaging--high concentrations are toxic to most plants and animals.

Oxidative damage

- Pure O_2 damages human lungs--long enough exposure permanently damages the aveoli.

Why is O_2 toxic?

The damaging effects are due primarily to damage caused by **free radicals**.

Formation of AGEs occur at much slower rates.

Free radicals

- Free radical: a chemical with an odd number of electrons.
- Chemicals with an unpaired electron are highly reactive, readily combine with other molecules.
- Most chemical reactions in a cell are well controlled--require specific starting conditions or enzymes. But free radicals are thermodynamically unstable and can react with most molecules and break most covalent bonds.

Free radicals

- Normal bond: ':' represents a pair of elections
  Breaking a bond: **A:B -> $A:^-$ + B^+**
  Products are ions.
- Free radical formation:
  A:B -> A· + B·
  Products each have an unpaired electron!

Free radical breakdown of **H_2O**:

H:O:H -> ·OH + H·

Forms **Hydroxyl radical and hydrogen radical.**

Stages of free radical reactions

Initiation, Propagation, and Termination

Initiation

- Oxygen (O_2) is reduced in mitochondria in one electron steps. Oxygen with an unpaired electron often escapes as: **O_2·, called superoxide radical.**
- 2–3% of the oxygen atoms taken up by mitochondria escape as free radicals!
- **O_2· quickly reacts with H_2O_2:**
  O_2· + H_2O_2 -> OH + OH- + O_2·

Propagation

- Free radicals can propagate indefinitely:
  R· + O_2 -> ROO·
  ROO· -> ROOH + R·

Stages of free radical reactions

Initiation, Propagation, and Termination

Termination

$R\cdot + R\cdot \rightarrow R{:}R$
$R\cdot + ROO\cdot \rightarrow ROOR$
$2\ ROO\cdot \rightarrow ROOR + O_2$
Antioxidant H + $ROO\cdot$ -> Antioxidant· + ROOH

Termination occurs when free radicals react with other free radicals or antioxidant molecules.

Cellular free radical defense

Compartmentalization:

- Most oxidative metabolism and free radical production occurs at the inner mitochondrial membrane.

Protective enzymes

- Several: SOD, catalase, glutathione peroxidase.
  $2H^+ + O_2\cdot + O_2\cdot$ --SOD-> $O_2 + H_2O_2$
  $H_2O_2 + H_2O_2$ --CAT-> $H_2O + O_2$

- Concentrated in the mitochondria.

Antioxidant molecules

Evidence for the oxidative damage theory

- Correlation between species-specific levels of anti-oxidant defenses and lifetime energy expenditure (Cutler, 1984).
- Correlations stronger between mitochondrial (MnSOD) than cytoplasmic (CuSOD, ZnSOD) defense levels.

Free radical scavenging systems

Table 10.6 **Nature and Distribution of Cellular Defenses against Free Radicals**

Type	Location
Nonradical decomposition	
Catalase	Cytoplasm, mitochondrial matrix space
Glutathione	Cytoplasm, mitochondrial matrix space
Glutathione peroxidase	Cytoplasm
Glutathione-S-transferase	Cytoplasm
Quenching of active oxygen	
MnSOD	Mitochondrial inner membrane and matrix space
CuZnSOD	Extracellular, cytoplasm
Lipophilic agents	Cytoplasmic and mitochondrial membranes
Vitamin E, carotenoids, flavonoids, ubiquinol, etc.	
Hydrophilic agents	Plasma, sera
Vitamin C, uric acid, bilirubin, albumin, etc.	

Source: from Lippman (1983) and Camougrand and Rigoulet (2001).

Evidence for the oxidative damage theory

- Comparison of mammals and birds:
  - Rats (4 yr lifespan) and pigeons (35 yrs).
  - Pigeon mitochondria leak only 30% of the free radicals than those from rat.
  - (Herrero and Barja, 1997).
- Antioxidant EUK-134:
  - Fed to *C. elegans*, increased mean and maximum lifespan 44%
  - Fed to *mev-1*, lifespan only 60% of wt, restores lifespan to same as wt.
  - (Giblin et al., 2003)

Activity in houseflies experiment

- Raised houseflies in either:
  - Large chamber, could fly (high activity)
  - Low chamber, flies only walk (low activity)
  - Low activity animals had longer mean and max lifespan, lower rate of lipofuscin formation.
- Catalase activity high in young flies, decreases with age.
- Peroxide levels (a measure of lipid oxidation) low in young flies, increase with age.

Sohal and Donato, 1978.

Testing the oxidative damage theory

1. Construct long-lived and short-lived animals and then assay their antioxidant defense levels.
2. Construct animals with genetically altered levels of antioxidant defense enzymes and then test for lifespan.

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Effect of altered levels of SOD and catalase in fly

- See Orr and Sohal, 1994
  - http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=8108730&query_hl=24&itool=pubmed_docsum
- See Phillips et al., 2000
  - http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11113599&query_hl=21&itool=pubmed_docsum

Metabolic rate declines with age

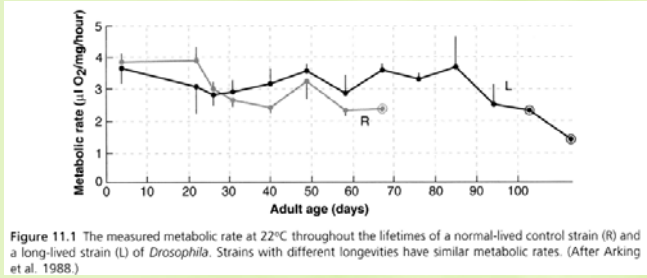

Figure 11.1 The measured metabolic rate at 22°C throughout the lifetimes of a normal-lived control strain (R) and a long-lived strain (L) of *Drosophila*. Strains with different longevities have similar metabolic rates. (After Arking et al. 1988.)

Biology of Aging, R. Arking, 3rd ed.

Focus on mitochondria

- Damage to mitochondrial genome!
- Impaired mitochondrial gene expression.
- Inability of mitochondria to replicate, divide, further reducing energy production, etc.
- Damaged mitochondria replicate faster than intact mitochondria.

Mitochondrial damage

- Young samples: intact mitochondrial DNA
- Old samples: most mitochondrial DNA has deletion.
- Damage accumulates exponentially.
- Observed in a wide range of animals, from *C. elegans* to humans.

8-oxodG/10^5dG in **nuclear** DNA

See Barja and Herrero, 2000

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=10657987&query_hl=25&itool=pubmed_docsum

8-oxodG/10^5dG in **mitochondiral** DNA

See Barja and Herrero, 2000

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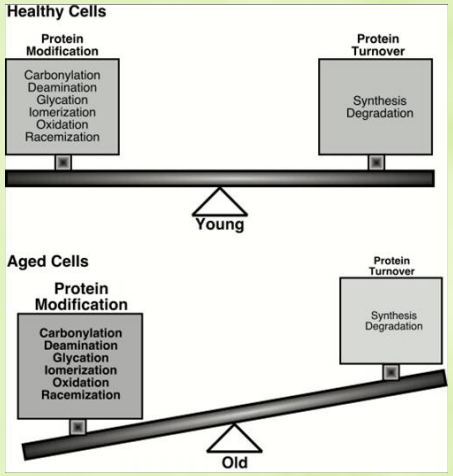
Heart Brain

Altered protein theory

- Changes to proteins impairs cellular process in a progressive manner until subvital levels.
- Initial evidence observed:
  - Catalytic activity of many enzymes decreases 25-50% in older animals.

Altered protein theory



Altered protein theory

Reduced protein function due to several types of post-translational changes:

- Denaturation of proteins (can be heated/cooled to refold restore function).
- Covalent modifications:
  - protein carbonyl levels higher in old animals
  - Other protein modifications.
  - In an old animal, oxidized protein is 30-50% of total protein (Berlett and Stadtman, 1997).

Altered protein theory

Protein turnover slows down as animals age:

- Protein synthesis rate is reduced.
- Cytoplasmic protein degradation pathway activity is reduced.
- This increases protein half-life (the time proteins exist) and increases total protein damage levels.