

Oxidative Phosphorylation

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Reactive Oxygen Species (ROS)

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- Reactive Oxygen Species (ROS) is a phrase used to describe a number of reactive molecules and free radicals derived from molecular oxygen.
- These molecules, (produced as byproducts during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes and metal catalyzed oxidation), have the potential to cause a number of harmful events.
- It was originally thought that only phagocytic cells were responsible for ROS production as their part in host cell defense mechanisms.
- Recent work has demonstrated that ROS have a role in cell signaling, including; apoptosis; gene expression; and the activation of cell signaling cascades. It should be noted that ROS can serve as both intra- and intercellular messengers.

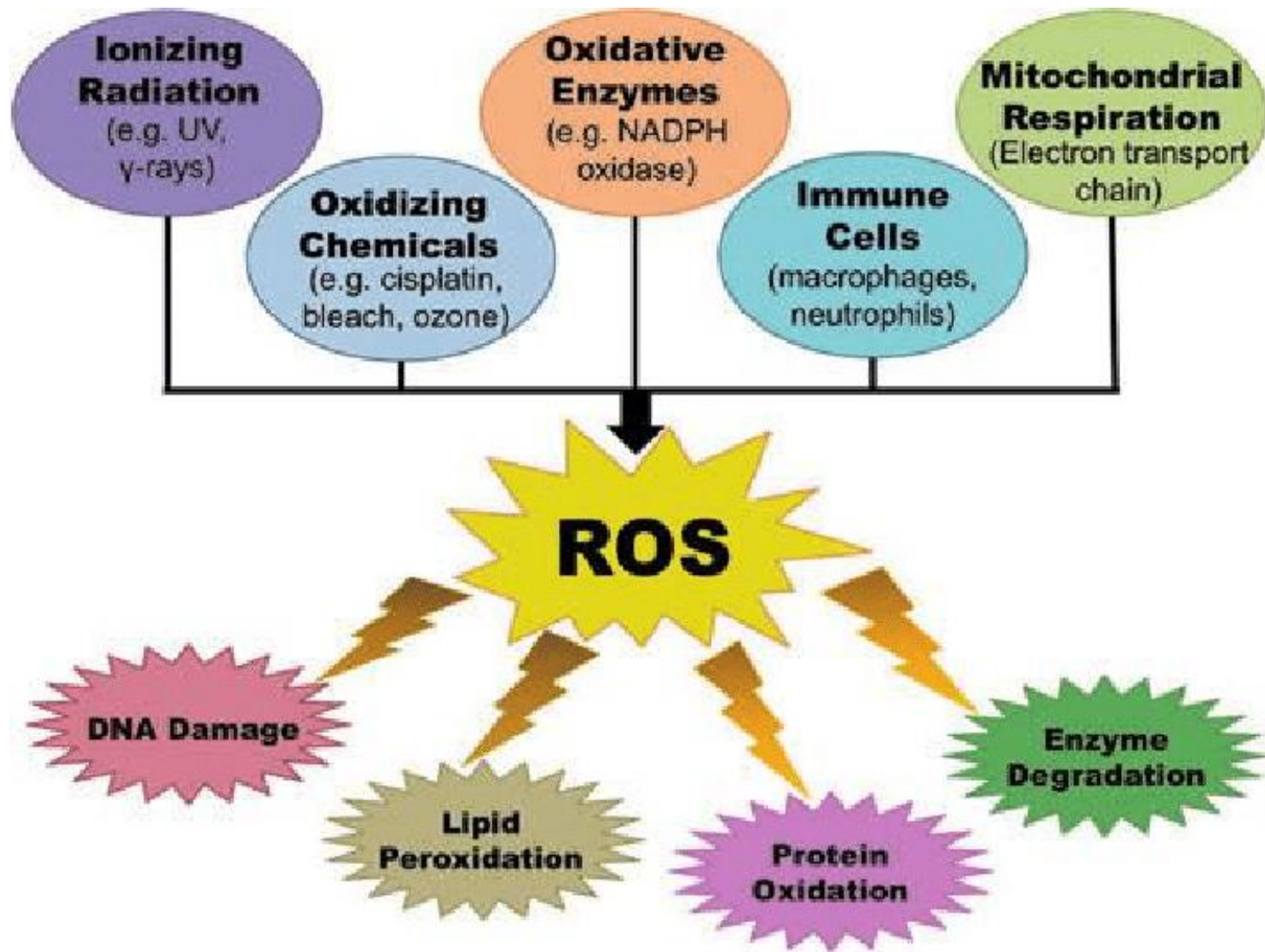
- Most reactive oxygen species are generated as by-products during mitochondrial electron transport and oxidative phosphorylation steps.
- Several steps in the path of oxygen reduction in mitochondria have the potential to produce highly reactive free radicals that can damage cells. The passage of electrons from QH_2 to Complex III and the passage of electrons from Complexes I and II to QH_2 involve the radical $\bullet\text{Q}_2$ as an intermediate.
- The $\bullet\text{Q}_2$ can, with a low probability, pass an electron to O_2 in the reaction.



The superoxide free radical thus generated is highly reactive; its formation also leads to production of the even more reactive hydroxyl free radical, $\bullet\text{OH}$.

Types of Reactive Oxygen Species

- Atomic oxygen has two unpaired electrons in separate orbits in its outer electron shell.
 - This electron structure makes oxygen susceptible to radical formation.
 - The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including: superoxide; hydrogen peroxide; hydroxyl radical; hydroxyl ion; and nitric oxide.
- Reactive oxygen species can wreak havoc, reacting with and damaging enzymes, membrane lipids, and nucleic acids.
 - In actively respiring mitochondria, 0.1% to as much as 4% of the O_2 used in respiration forms $\bullet O_2^-$ more than enough to have lethal effects unless the free radical is quickly disposed of.





Oxygen



Superoxide anion



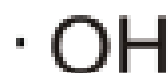
Peroxide



Hydrogen Peroxide



Hydroxyl radical



Hydroxyl ion



Electron structures of common reactive oxygen species. Each structure is provided with its name and chemical formula. The • designates an unpaired electron.

The Radicals

Superoxide ($\text{O}_2^{\bullet-}$)

Hydroxyl (OH^{\bullet})

Peroxyl, alkoxyl (RO_2^{\bullet} , RO^{\bullet})

Oxides of nitrogen (NO^{\bullet} , NO_2^{\bullet})

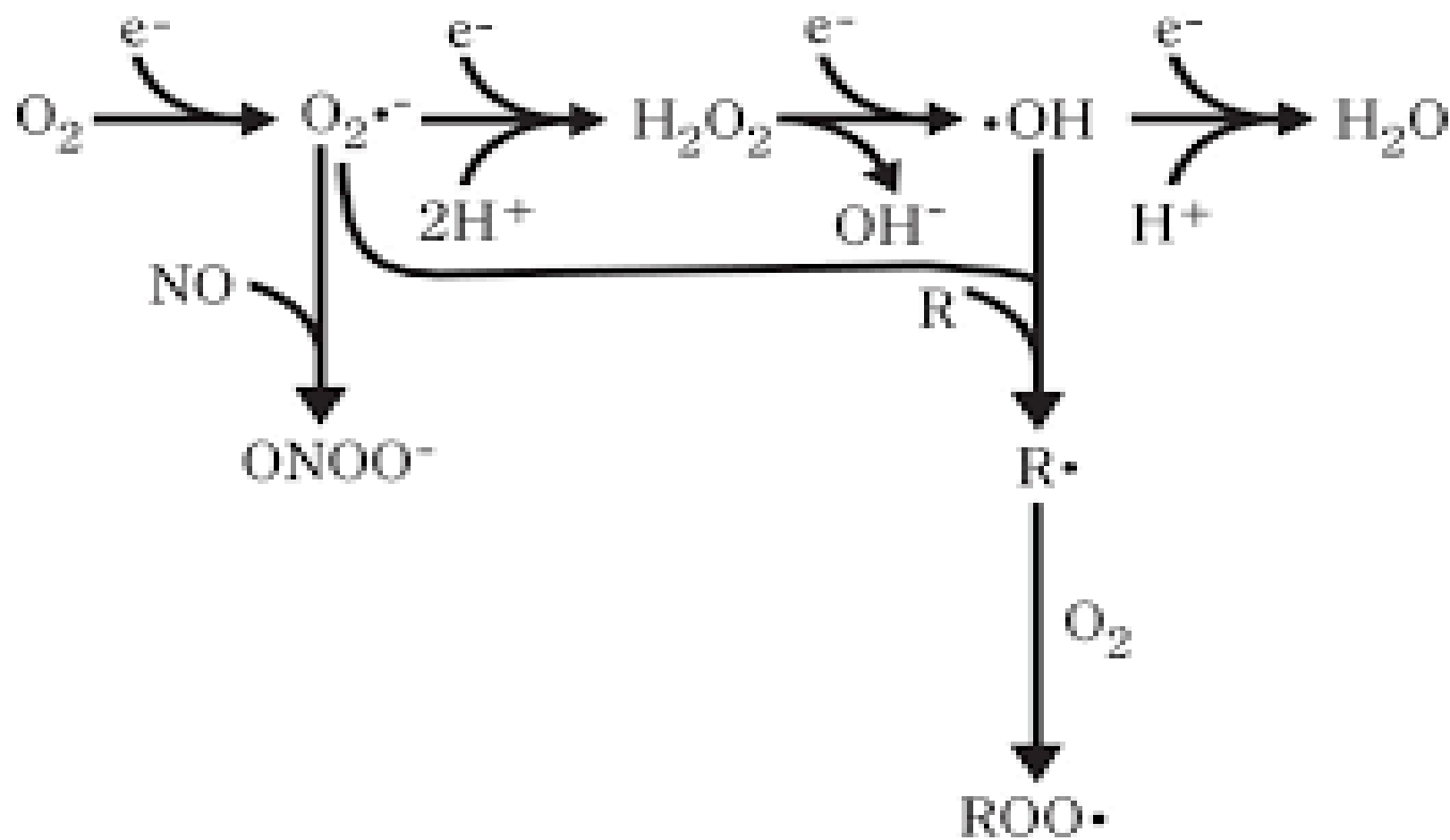
The Non radicals

Hydrogen peroxide (H_2O_2)

Hypochlorous acid (HOCl)

Ozone (O_3)

Singlet oxygen ($^1\text{O}_2$)



- Factors that slow the flow of electrons through the respiratory chain increase the formation of superoxide, perhaps by prolonging the lifetime of $\bullet\text{O}_2^-$ generated in the Q cycle.
- The formation of ROS is favored when two conditions are met:
 - (1) mitochondria are not making ATP (for lack of ADP or O_2) and therefore have a large proton-motive force and a high ratio of QH_2/Q , and
 - (2) there is a high NADH/NAD ratio in the matrix. In these situations, the mitochondrion is under oxidative stress—more electrons are available to enter the respiratory chain than can be immediately passed through to oxygen.

- When the rate of electron entry into the respiratory chain and the rate of electron transfer through the chain are mismatched, superoxide radical ($\bullet\text{O}_2^-$) production increases at Complexes I and III as the partially reduced ubiquinone radical ($\bullet\text{Q}_2$) donates an electron to O_2 .
- Superoxide acts on aconitase (Enzyme of TCA cycle), a 4Fe-4S protein, to release Fe^{2+} . In the presence of Fe^{2+} , the Fenton reaction leads to formation of the highly reactive hydroxyl free radical ($\bullet\text{OH}$).
- When the supply of electron donors (NADH) is matched with that of electron acceptors, there is less oxidative stress, and ROS production is much reduced.
- Although overproduction of ROS is clearly detrimental, *low levels of* ROS may be used by the cell as a signal reflecting the insufficient supply of oxygen (hypoxia), triggering metabolic adjustments.

Endogenous sources

Mitochondrial oxidative phosphorylation
Lipid peroxidation chain reactions
Fenton reaction
Haber-Weiss reaction
and etc. (see Table 1)

Exogenous sources

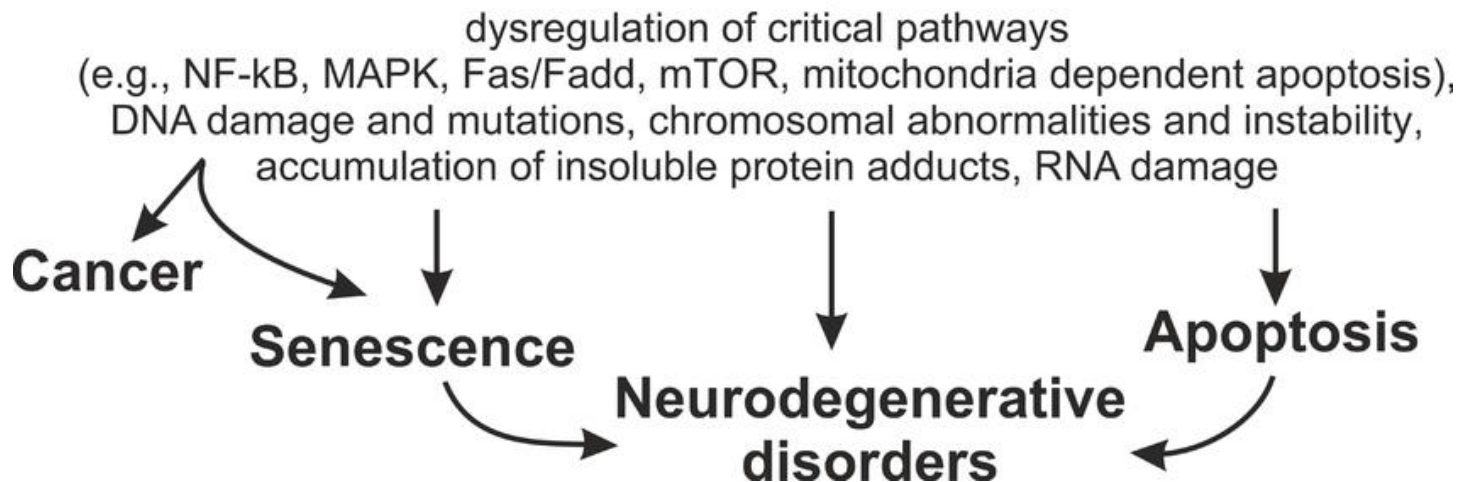
Radiation
Pathogenes
Chemicals
Pollutants
Inflammation
Food and nutrients
Smoking

Mitochondrial dysfunction

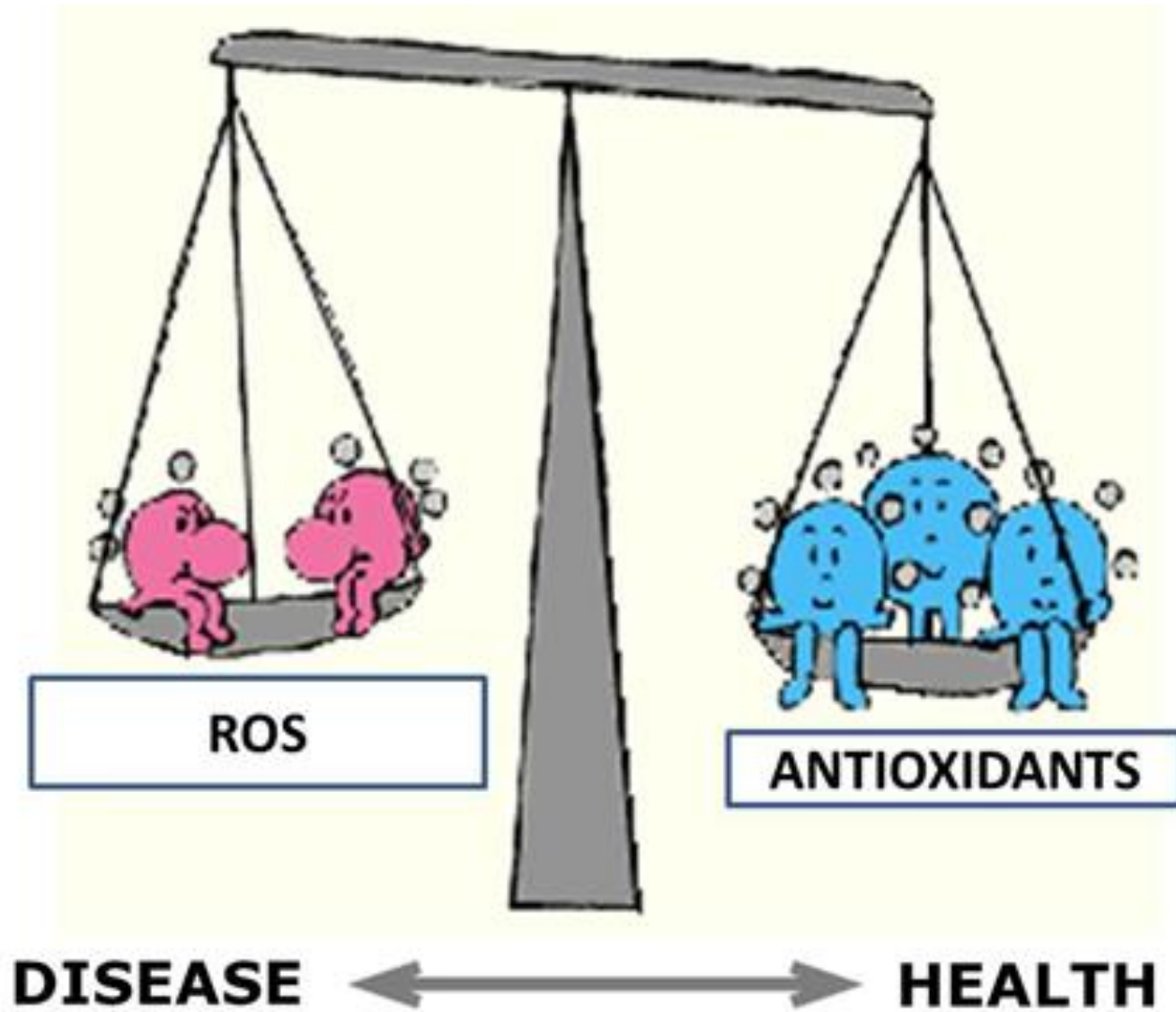


Oxidative stress

lipid peroxidation of membranes, nDNA and mtDNA oxidation,
modification of proteins, lipids and nucleic acids



Cellular defense against ROS



- Detoxification of reactive oxygen species is important for the survival of all aerobic life forms.
- As such a number of defense mechanisms have evolved to meet this need and provide a balance between production and removal of ROS.
- An imbalance toward the pro-oxidative state is often referred to as “Oxidative stress”.
- Cells have a variety of defense mechanisms to ameliorate the harmful effects of ROS.
- Superoxide dismutase (SOD) catalyzes the conversion of two superoxide anions into a molecule of hydrogen peroxide (H_2O_2) and oxygen (O_2).

- To prevent oxidative damage by $\bullet\text{O}_2^-$, cells have several forms of the enzyme **superoxide dismutase**, which catalyzes the reaction



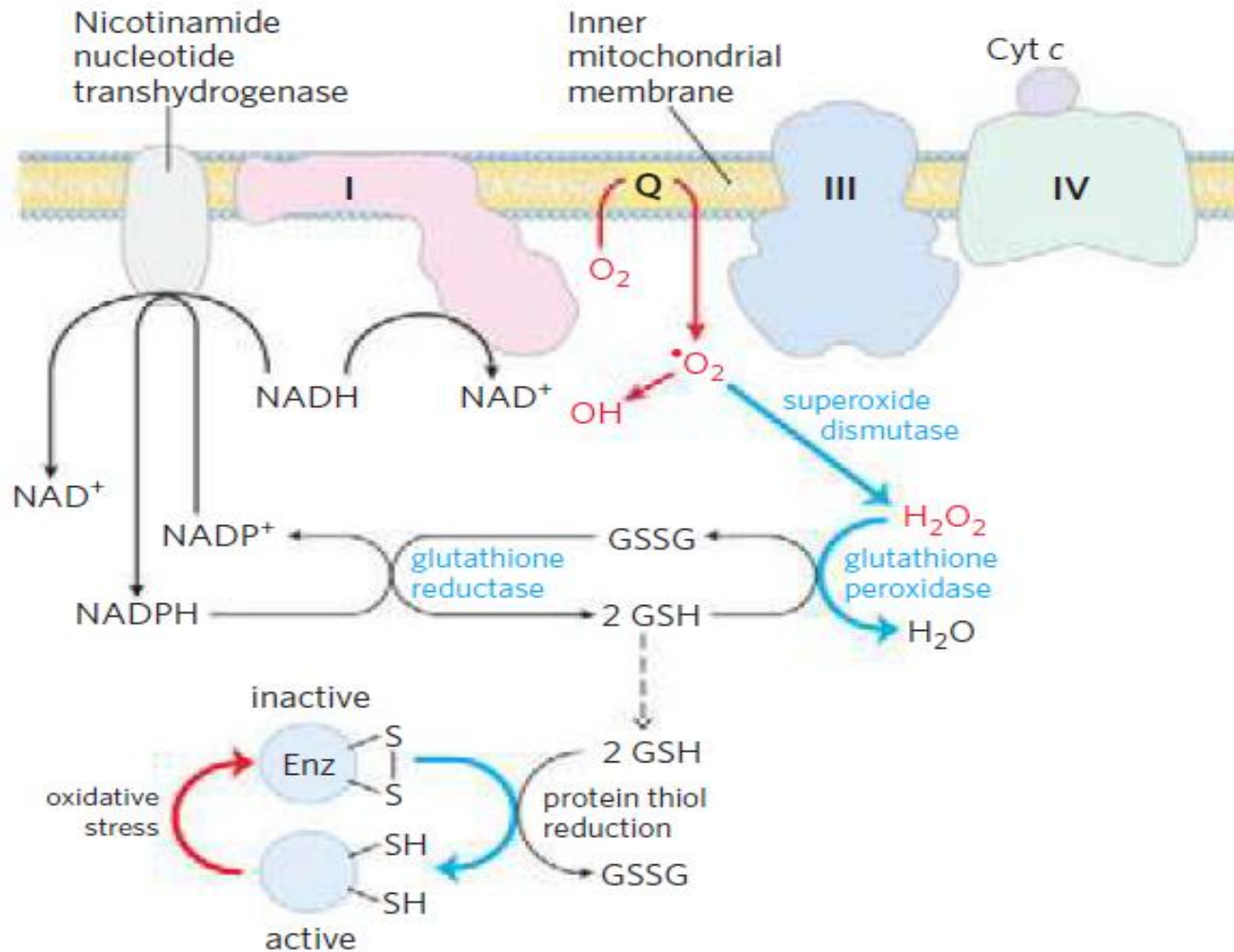
In the peroxisomes of eukaryotic cells, the enzyme catalase converts H_2O_2 to water and oxygen, and thus completes the detoxification initiated by SOD.

The hydrogen peroxide (H_2O_2) generated can also be rendered harmless by the action of **glutathione peroxidase**.



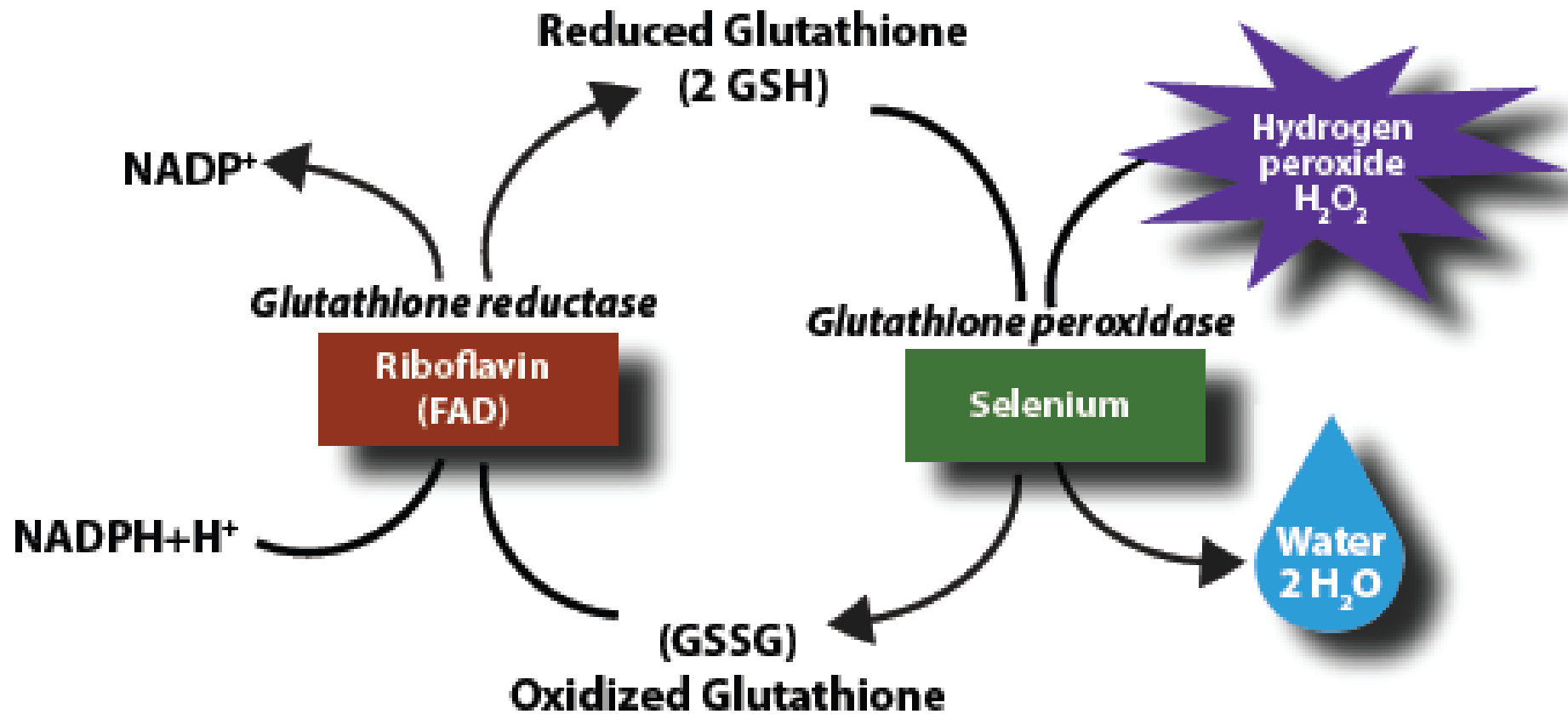
- Glutathione (selenium containing) may be the most important intra-cellular defense against the deleterious effects of reactive oxygen species. It provides an exposed sulfhydryl group, which serves as an abundant target for attack.
- Glutathione reductase recycles the oxidized glutathione to its reduced form, using electrons from the NADPH generated by nicotinamide nucleotide transhydrogenase (in the mitochondrion) or by the pentose phosphate pathway as in the cytosol.

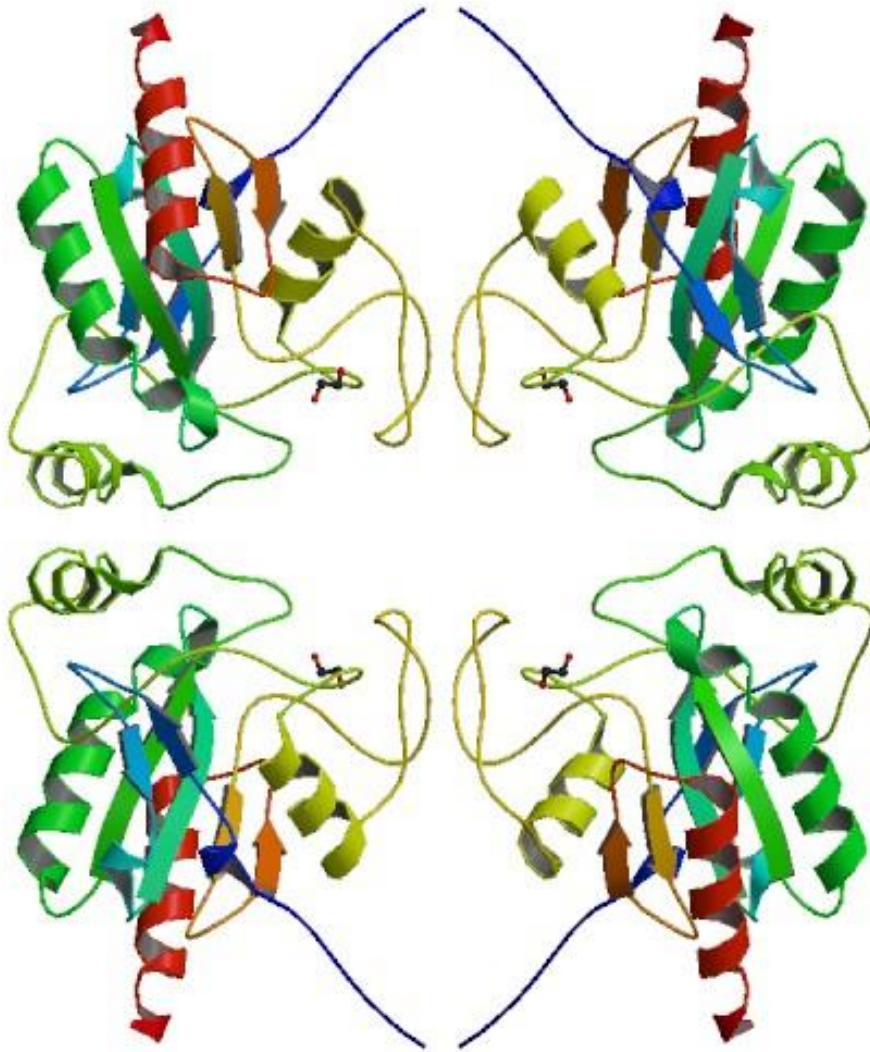
Schematic for destruction of ROS



Reactions that defend the cell against the damaging effects of superoxide. Reduced glutathione (GSH) donates electrons for the reduction of H_2O_2 and of the oxidized Cys residues of enzymes and other proteins, and GSH is regenerated from the oxidized form (GSSG) by reduction with NADPH.

- Reactions with ROS molecules oxidize glutathione, but the reduced form is regenerated in a redox by an NADPH dependent reductase.
- Reduced glutathione also serves to keep protein sulfhydryl groups in their reduced state, preventing some of the deleterious effects of oxidative stress.
- Nicotinamide nucleotide transhydrogenase is critical in this process: it produces the NADPH essential for glutathione reductase activity.





- Glutathione peroxidase is a tetramer consisting of 4 identical single polypeptide chains each with 178 amino acid residues .

Each monomer contains two parallel and two anti-parallel pleated β -sheets surrounded by four α -helices .

α helices 1, 2, and 4, exist on one side of the β sheet complex whereas $\alpha 4$ exists on the other side .

The catalytically active selenocysteine is located near the first turn of $\alpha 1$.

- Vitamin C or ascorbic acid is a water soluble molecule capable of reducing ROS. Vitamin C is one of the potent reducing agents and scavenger of free radicals in biological systems, working as a scavenger of oxidizing free radicals and harmful oxygen-derived species, such as hydroxyl radical, hydrogen peroxide (H_2O_2), and singlet oxygen.
- Vitamin E (α -tocopherol) is a lipid soluble molecule that has been suggested as playing a similar role in membranes.
- The ratio of the oxidized form of glutathione (GSSG) and the reduced form (GSH) is a dynamic indicator of the oxidative stress of an organism.

UNCOUPLERS

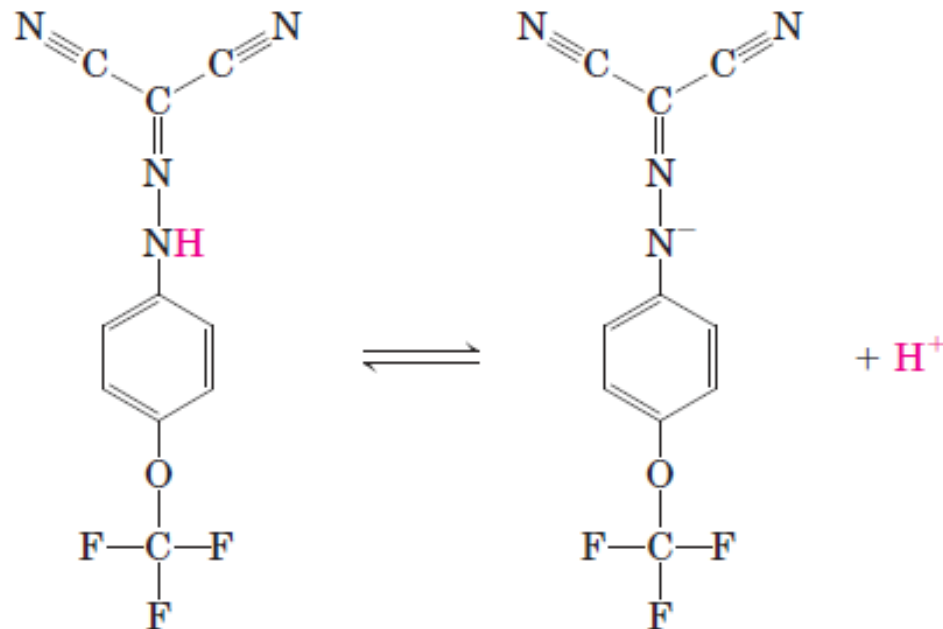
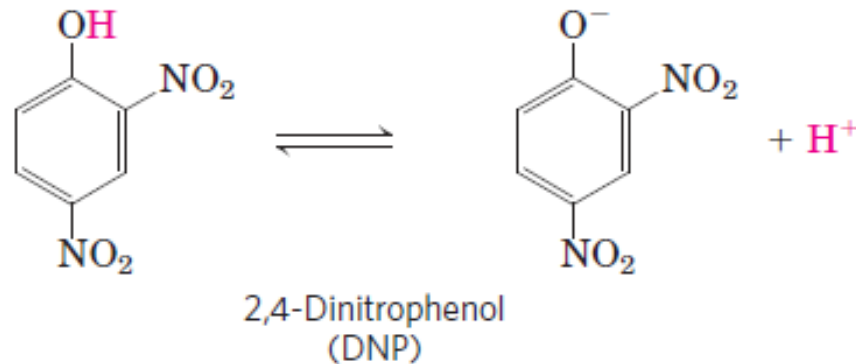
“Chemiosmotic coupling.”

- Coupling refers to the *obligate connection* between mitochondrial ATP synthesis and electron flow through the respiratory chain; neither of the two processes can proceed without the other.

- Certain conditions and reagents, can uncouple oxidation from phosphorylation. When intact mitochondria are disrupted by treatment with detergent or by physical shear, the resulting membrane fragments can still catalyze electron transfer from succinate or NADH to O_2 , but no ATP synthesis is coupled to this respiration.
- Certain chemical compounds cause uncoupling without disrupting mitochondrial structure. Chemical uncouplers include 2,4-dinitrophenol (DNP) and carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), weak acids with hydrophobic properties that permit them to diffuse readily across mitochondrial membranes.

Two chemical uncouplers of oxidative phosphorylation.

Both DNP and FCCP have a dissociable proton and are very hydrophobic. They carry protons across the inner mitochondrial membrane, dissipating the proton gradient. Both also uncouple photophosphorylation.

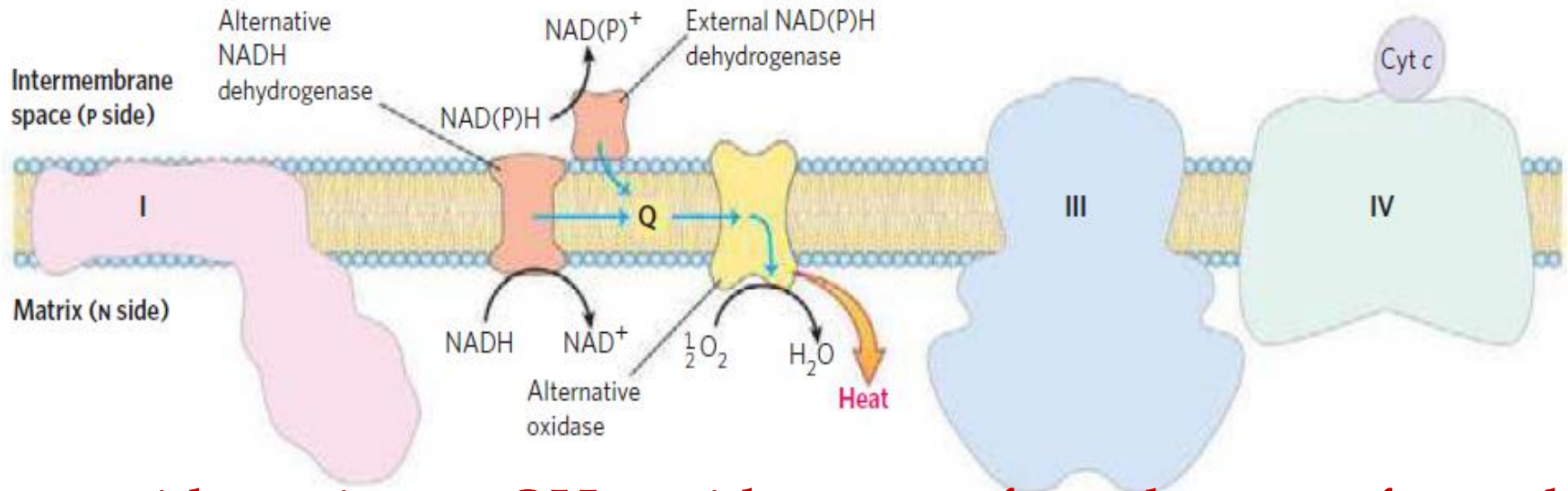


- After entering the matrix in the protonated form, they can release a proton, thus dissipating the proton gradient. Resonance stabilization delocalizes the charge on the anionic forms, making them sufficiently permeant to diffuse back across the membrane, where they can pick up a proton and repeat the process.
- Ionophores such as valinomycin allow inorganic ions to pass easily through membranes. Ionophores uncouple electron transfer from oxidative phosphorylation by dissipating the electrical contribution to the electrochemical gradient across the mitochondrial membrane.
- A prediction of the chemiosmotic theory is that, because the role of electron transfer in mitochondrial ATP synthesis is simply to pump protons to create the electrochemical potential of the proton-motive force, an artificially created proton gradient should be able to replace electron transfer in driving ATP synthesis. (if interested, read it in Leningher)

THERMOGENESIS

- One family of stinking plants is the Araceae, which includes philodendrons, arum lilies, and skunk cabbages.
- These plants have tiny flowers densely packed on an erect structure, the spadix, surrounded by a modified leaf, the spathe. The spadix releases odors of rotting flesh or dung. Before pollination the spadix also heats up, in some species to as much as 20 to 40 °C above the ambient temperature.
- **Heat production (thermogenesis)** helps evaporate odorant molecules for better dispersal, and because rotting flesh and dung are usually warm from the hyperactive metabolism of scavenging microbes, the heat itself might also attract insects.
- Eastern skunk cabbage which flowers in late winter or early spring when snow still covers the ground, thermogenesis allows the spadix to grow up through the snow.

Energy that might have been conserved as ATP is instead released as heat.



A cyanide resistant QH₂ oxidase transfers electrons from the ubiquinone pool directly to oxygen, bypassing the two proton-translocating steps of Complexes III and IV.

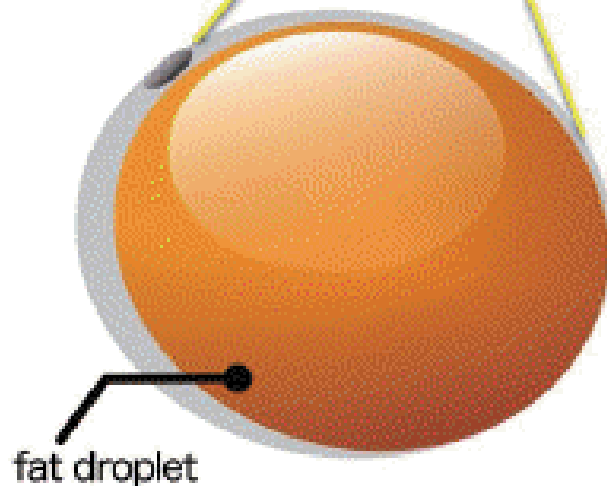
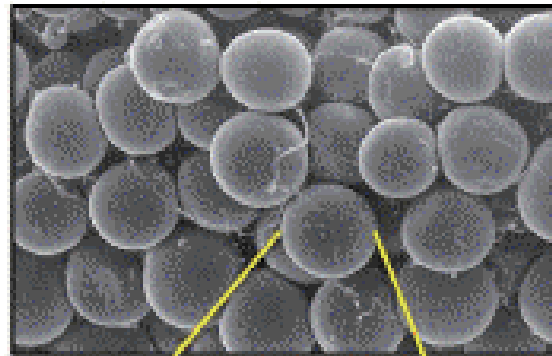
Plant mitochondria also have an **alternative NADH dehydrogenase**, insensitive to the Complex I inhibitor rotenone that transfers electrons from NADH in the matrix directly to ubiquinone, bypassing Complex I and its associated proton pumping.

- Plant mitochondria have yet another NADH dehydrogenase, on the external face of the inner membrane, that transfers electrons from NADPH or NADH in the intermembrane space to ubiquinone, again bypassing Complex I.
- Electrons enter the alternative respiratory pathway through the rotenone-insensitive NADH dehydrogenase, the external NADH dehydrogenase, or succinate dehydrogenase (Complex II), and pass to O_2 via the cyanide-resistant alternative oxidase, energy is not conserved as ATP but is released as heat.

Uncoupled Mitochondria in Brown Adipose Tissue Produce Heat

- Most newborn mammals, including humans, have a type of adipose tissue called **brown adipose tissue (BAT)** in which fuel oxidation serves, not to produce ATP, but to generate heat to keep the newborn warm.
- This specialized adipose tissue is brown because of the presence of large numbers of mitochondria and thus high concentrations of cytochromes, with heme groups that are strong absorbers of visible light.
- Hibernating animals also depend on the activity of uncoupled BAT mitochondria to generate heat during their long dormancy.

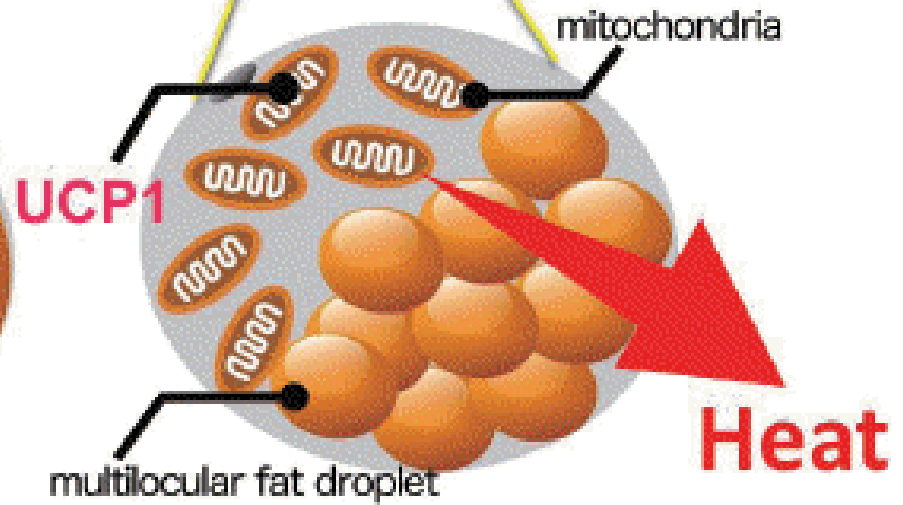
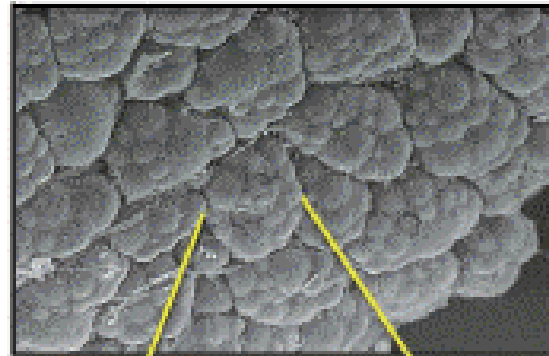
White adipose tissue (WAT)



energy storage/
release

White adipocyte

Brown adipose tissue (BAT)



fatty acid oxidation/
thermogenesis

Brown adipocyte

White adipose tissue

White adipocytes contain single large lipid droplet, few mitochondria

Secretes adipose derived hormones, that regulate insulin sensitivity and satiety

Stores excess energy as triglycerides, releases fatty acids during fasting periods

Brown adipose tissue

Brown adipocytes contain multiple small lipid droplets, rich in mitochondria

Densely vascularized and innervated by sympathetic nerve endings

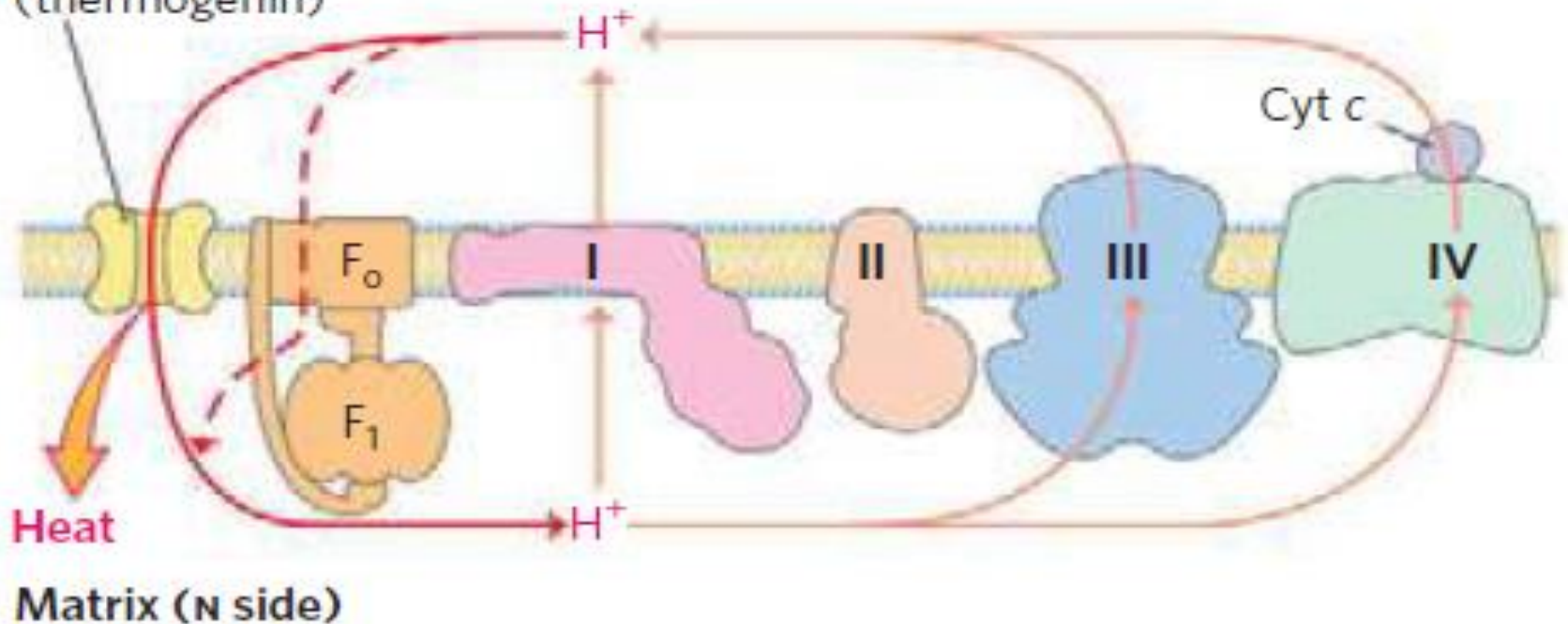
Expression of uncoupling protein 1

Dissipates chemical energy (mainly from fatty acids) to generate heat

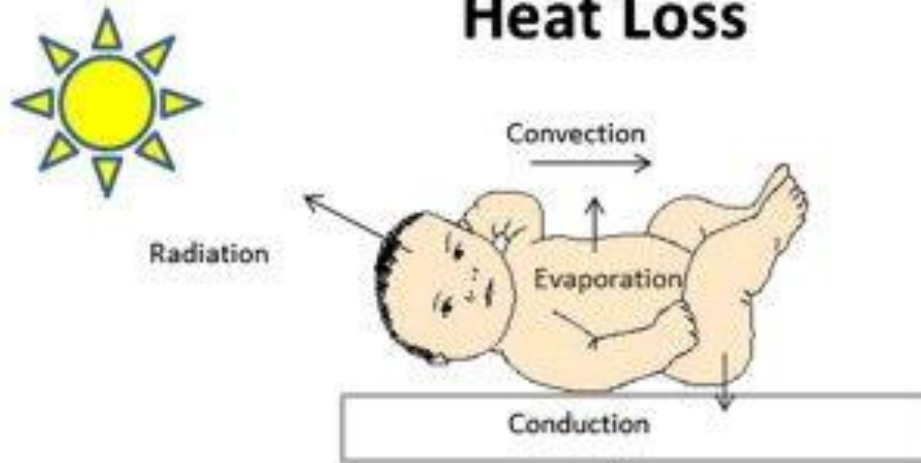
- **Thermogenin**, also called **uncoupling protein 1** (the **product** of the *UCP1* gene), provides a path for protons to return to the matrix without passing through the FoF1 complex

Intermembrane
space (P side)

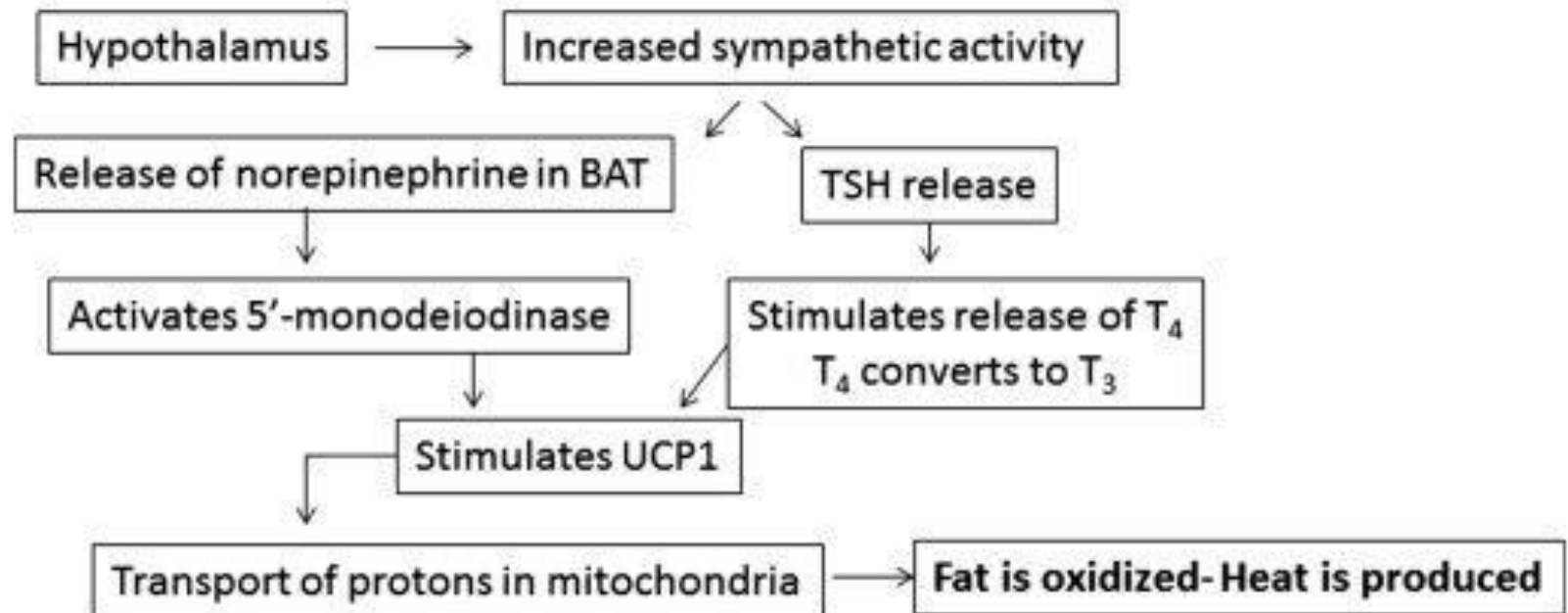
Uncoupling protein UCP1
(thermogenin)



Heat Loss



Non-shivering Thermogenesis



Thank you

MIGHTY CHONDRION

