VIVEKANANDA COLLEGE
THAKURPUKUR
KOLKATA-700063

NAAC ACCREDITED ‘A’ GRADE

Topic	:	Oxidative phosphorylation (contd)
Course Title	:	Membrane Biology and Bioenergetics
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Unit	:	II
Semester	:	4
Name of the Teacher	:	Dr. Kakali Roy
Name of the Department	:	Biochemistry

Metabolite Transporters in Mitochondria

A mechanism for transport of metabolites or chemical groups across the mitochondrial membrane is called shuttle.

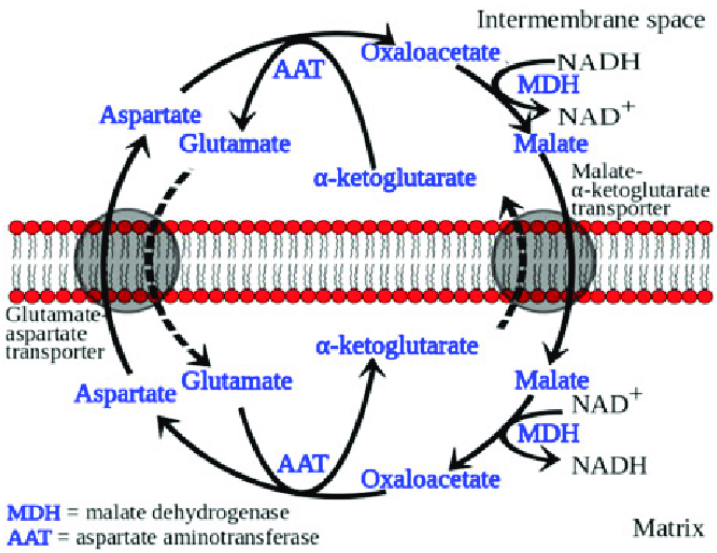
The **mitochondrial shuttles** are **systems** used to transport reducing agents across the inner **mitochondrial** membrane. NADH as well as NAD^+ cannot cross the membrane, but it can reduce another molecule like FAD and [QH_2] that can cross the membrane, so that its electrons can reach the electron transport chain.

There are two shuttle systems in human :

Malate aspartate shuttle and Glycerol 3-phosphate shuttle.

Malate Aspartate shuttle :

The **malate-aspartate shuttle** is a biochemical system for translocating electrons from cytosolic NADH produced during glycolysis across the semipermeable inner membrane of the mitochondrion for oxidative phosphorylation in the heart and liver. These electrons enter the electron transport chain of the mitochondria via reduction equivalents to generate ATP. The shuttle system is required because the mitochondrial inner membrane is impermeable to NADH, the primary reducing equivalent of the electron transport chain. To circumvent this, malate carries the reducing equivalents across the membrane.



The shuttle consists of four protein parts:

- Malate dehydrogenase (MDH) in the mitochondrial matrix and intermembrane space.
- Aspartate aminotransferase (AAT) in the mitochondrial matrix and intermembrane space.
- malate-α-ketoglutarate antiporter in the inner membrane.
- glutamate-aspartate antiporter in the inner membrane.

  - Electrons are transferred from NADH in the cytosol to oxaloacetate, forming malate which traverses inner mitochondrial membrane and is then re-oxidised by NAD^+ in the matrix to form NADH in a reaction catalysed by TCA cycle enzyme MDH.
  - The resulting oxaloacetate does not readily cross the inner mitochondrial membrane and so a transamination reaction is needed to form aspartate which can be transported to the cytosolic site.

- The net effect of malate-aspartate shuttle is purely **redox** : NADH in the cytosol is oxidised to NAD^+ and NAD^+ in the matrix is reduced to NADH. The NAD^+ in the cytosol can then be reduced again by another round of glycolysis and the NADH in the matrix can be used to pass electron to the ETC. So ATP can be synthesised.

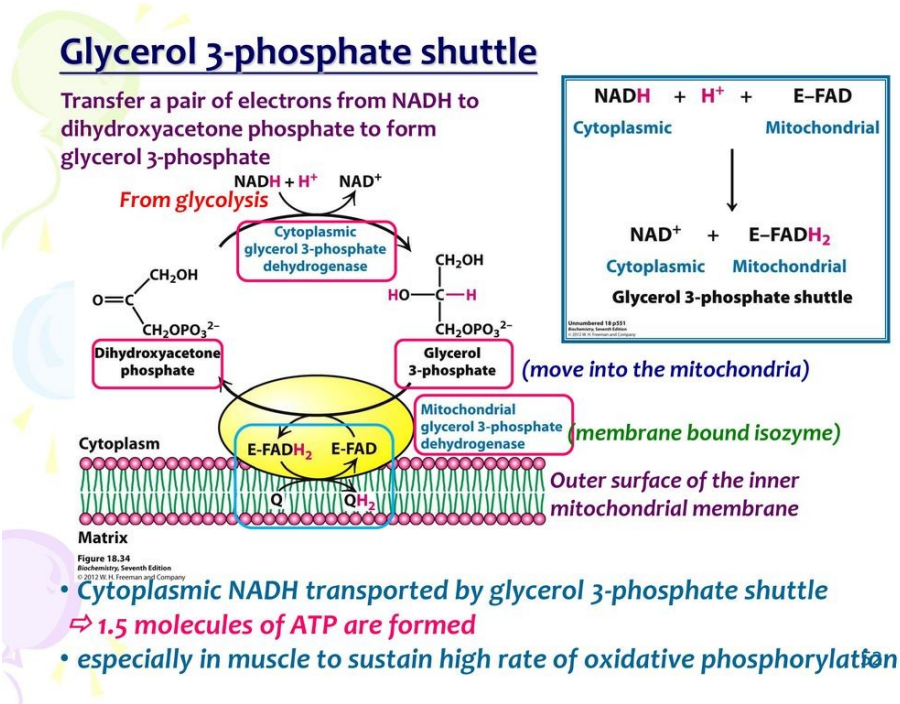
$$NADH + NAD^+ \leftrightarrow NAD^+ + NADH$$

(Cytosolic) (Mitochondrial) (Cytosolic) (mitochondrial)

Glycerol 3 phosphate Shuttle :

The glycerol-3-phosphate shuttle allows the NADH synthesized in the cytosol by glycolysis to contribute to the oxidative phosphorylation pathway in the mitochondria to generate ATP.

The glycolytic pathway generates NADH in the cytosol in oxidation of glyceraldehyde 3-phosphate and NAD^+ must be generated for glycolysis to continue. NADH cannot simply pass into mitochondria for oxidation by the respiratory chain because the inner mitochondrial membrane is impermeable to NADH, H^+ and NAD^+. So the electron from NADH are carried across the mitochondrial membrane by glycerol 3-phosphate shuttle.



- The 1st step in this shuttle is a transfer of a pair of electron from NADH to dihydroxyacetone phosphate (DHAP), a glycolytic intermediate, to form glycerol 3-phosphate catalysed by glycerol 3-P dehydrogenase in the cytosol. Glycerol 3-P is re oxidised to DHAP on the outer surface of the inner mitochondrial membrane by a membrane bound isozyme of glycerol 3-P dehydrogenase.

- In the 2nd step an electron pair from glycerol 3-P is transferred to FAD, a prosthetic group in this enzyme to form $FADH_2$ and regenerate DHAP.
- In step 3 the reduced Flavin transfers its electron to the electron carrier CoQ or ubiquinone which then enters the respiratory chain as QH_2.
- This shuttle is predominant in muscle (brown adipose tissue).

ROS production and antioxidant mechanism

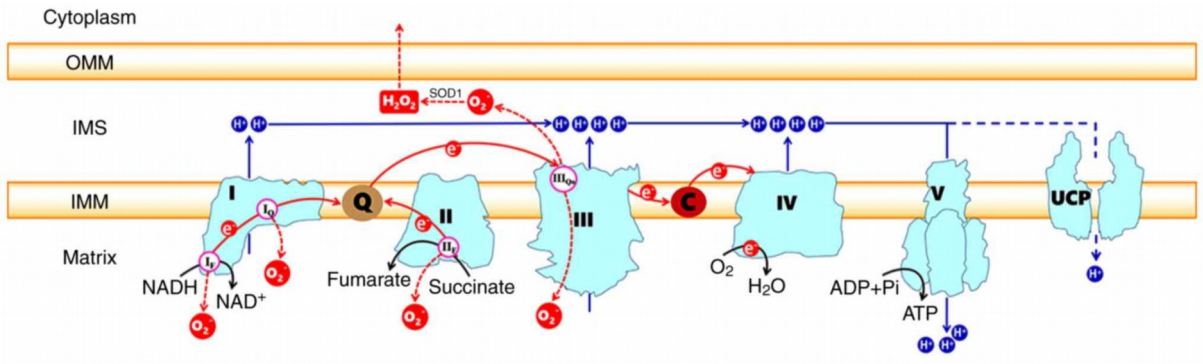
Mitochondrial **ROS** (mtROS or mROS) are **reactive oxygen species** (**ROS**) that are **produced** by mitochondria. Generation of mitochondrial **ROS** mainly takes place at the electron transport chain located on the inner mitochondrial membrane during the process of **oxidative phosphorylation** (OXPHOS).

Reactive oxygen species, such as superoxide, hydrogen peroxide, and hydroxyl radical production within most cells is the mitochondria. Within the mitochondria the primary reactive oxygen species produced is superoxide, most of which is converted to hydrogen peroxide by the action of superoxide dismutase. The production of superoxide by mitochondria has been localized to several enzymes of the electron transport chain, including Complexes I and III and glycerol-3-phosphate dehydrogenase.

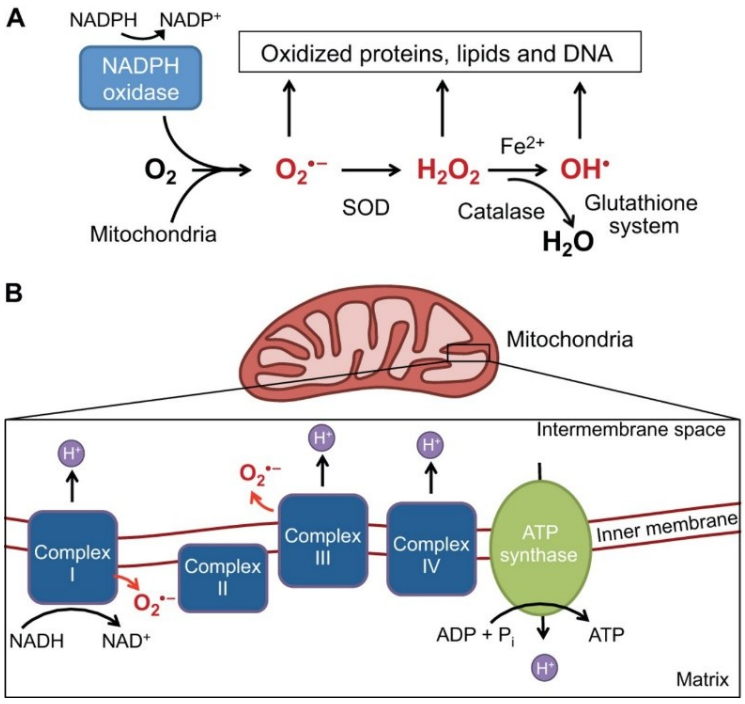
Under physiological conditions, 0.2-2% of the electrons in the ETC do not follow the normal transfer order but instead directly leak out of the ETC and interact with oxygen to produce superoxide or hydrogen peroxide. A total of 11 sites that produce superoxide (O_2^-) and/or hydrogen peroxide (H_2O_2) that are associated with substrate oxidation and the ETC have currently been identified in mammalian mitochondria. Sites O_F, P_F, B_F and A_F are in the 2-oxoacid dehydrogenase complexes, sites I_F and I_Q are in CI, site III_{Qo} is in CIII, and sites II_F, G_Q, E_F and D_Q are linked to the Q-dependent dehydrogenases in the QH_2/Q pool. The occurrence of numerous diseases and hypoxia are closely related to the increase of ROS production. CI and CIII, especially CI, are considered to be the main sites of ROS production in mitochondria.

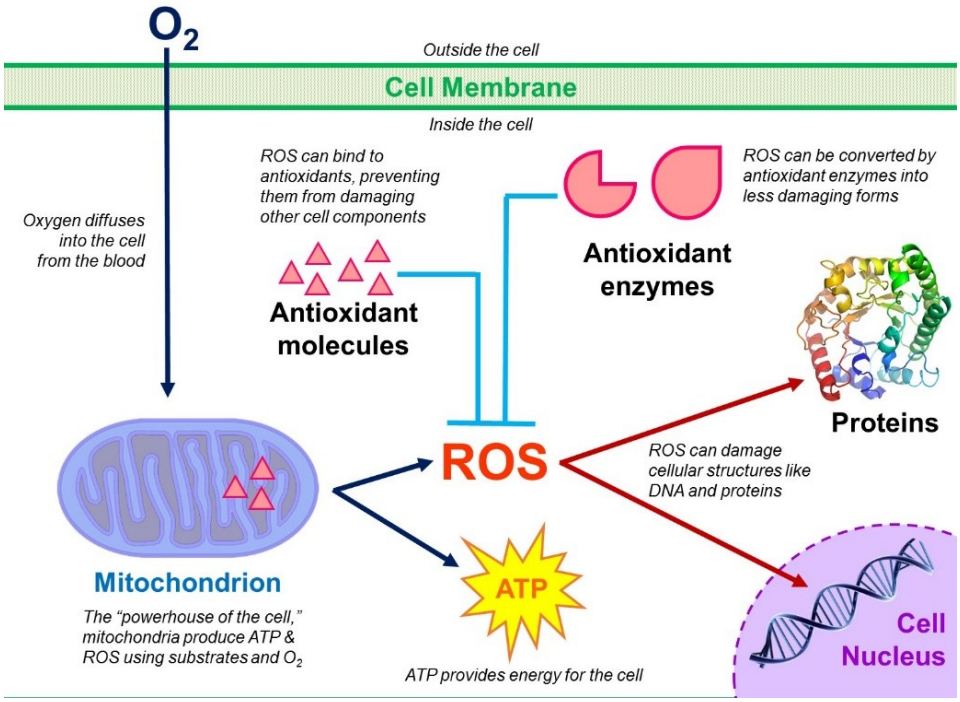
ROS can be generated in the matrix at both site I_F (FMN site) and site I_Q (CoQ binding site) during the transfer of electrons from NADH to CoQ in CI. Rotenone and piericidin are site I_Q inhibitors that interrupt the electron transfer to CoQ and increase ROS production at site I_F. CII produces ROS at site II_F, which is associated with succinate dehydrogenase. The level of ROS produced by site II_F under normal conditions is negligible, but the increases in ROS observed in CII mutation-related diseases are mainly derived from site II_F. The capacity of site II_F to produce ROS is closely related to the quantity of reduced flavoprotein, whose FAD is a potent site of electron leakage to generate ROS. ROS are exclusively produced in the matrix, because the flavoprotein is located on the matrix side of the inner mitochondrial

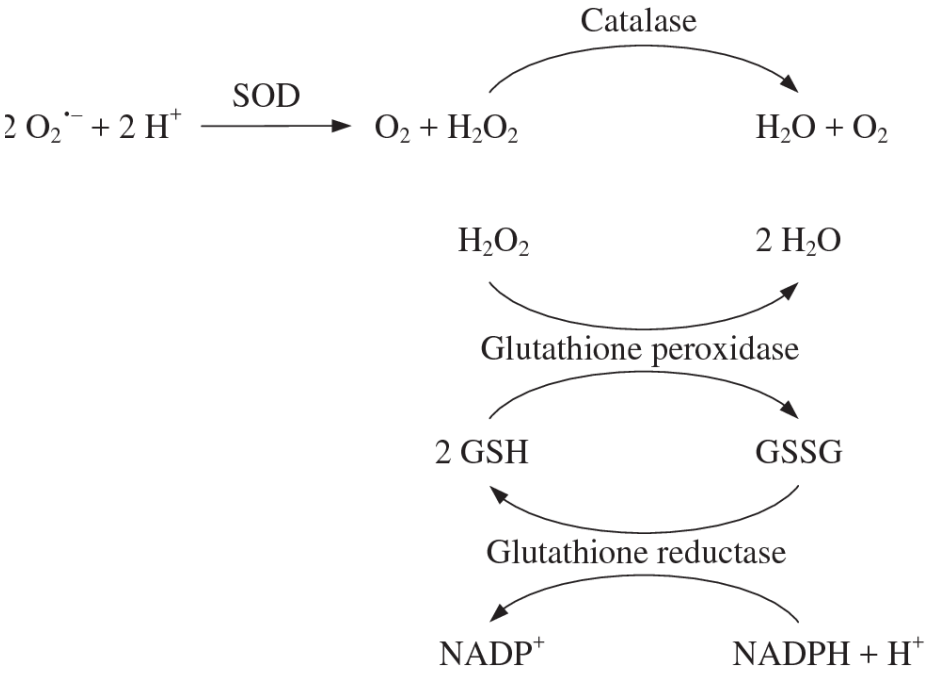
membrane . In addition, any contribution by site II_F can be dampened by the occupation of the CII flavoprotein site by dicarboxylic acids, particularly oxaloacetate, malate and succinate, which blocks the access of oxygen to site II_F, where it would form ROS.

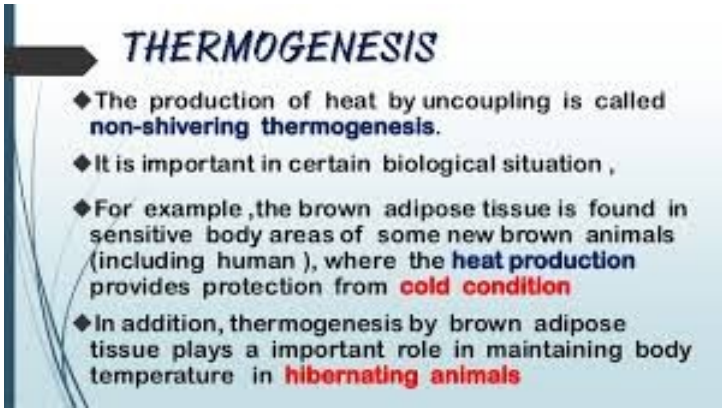


CIII produces small amounts of ROS, which could be overlooked compared to the ROS production of CI. CIII transfers electrons through the Q-cycle. In this process, ubisemiquinone (QH^-) of the Q_o site carrying a single electron can move freely in CIII, directly leaking the single electron to O_2, forming ROS through a nonenzymatic reaction. The formed ROS can be released into both the matrix and IMS (intermembrane space) despite the location of the Q_o site on the IMS side of the inner mitochondrial membrane. The O_2^- released into the IMS can be converted to the relatively more stable form of H_2O_2 by superoxide dismutase (SOD) enzymes. This permanent and stable oxidant molecule, which freely disperses through the outer membrane of mitochondria, acts as an intracellular signaling molecule, physiologically functioning via the direct modification of amino acids. However, supporting evidence demonstrates that O_2^-can permeate through the mitochondrial membrane into the cytosol through anion channels.









Thermogenesis is the process of heat production in organisms. It occurs in all warm-blooded animals.

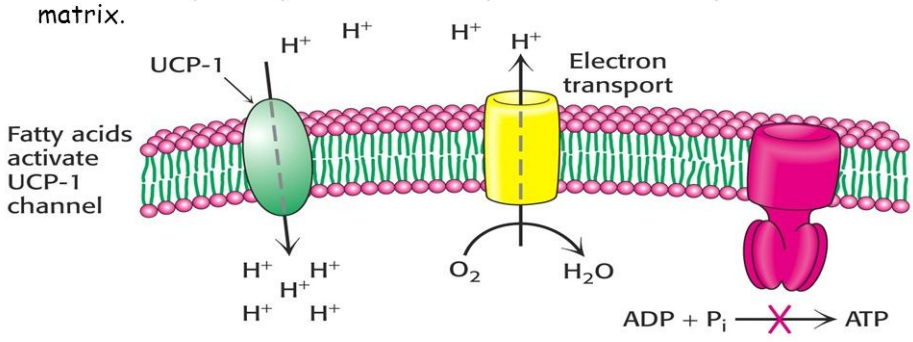
Uncoupling of Electron Transport with ATP Synthesis

Uncoupling of oxidative phosphorylation generates heat to maintain body temperature in **hibernating animals**, in **newborns**, and in **mammals adapted to cold**.

Brown adipose tissues is specialized for thermogenesis.

Inner mitochondrial membrane contains ***uncoupling protein (UCP),*** or ***thermogenin***.

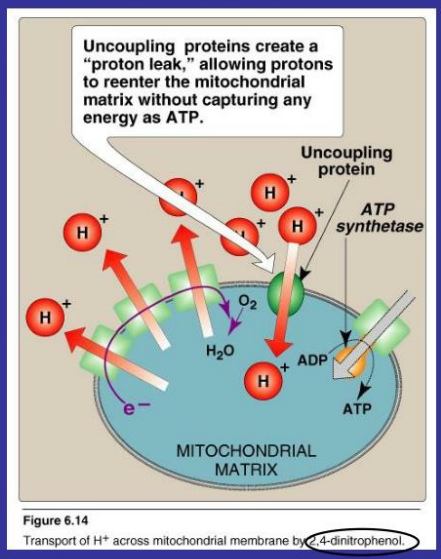
UCP forms a pathway for the flow of protons from the cytosol to the matrix.



Brown adipose tissue creates heat by thermogenesis

Thermogenin = uncoupling protein – UCP1

The energy is given off as heat



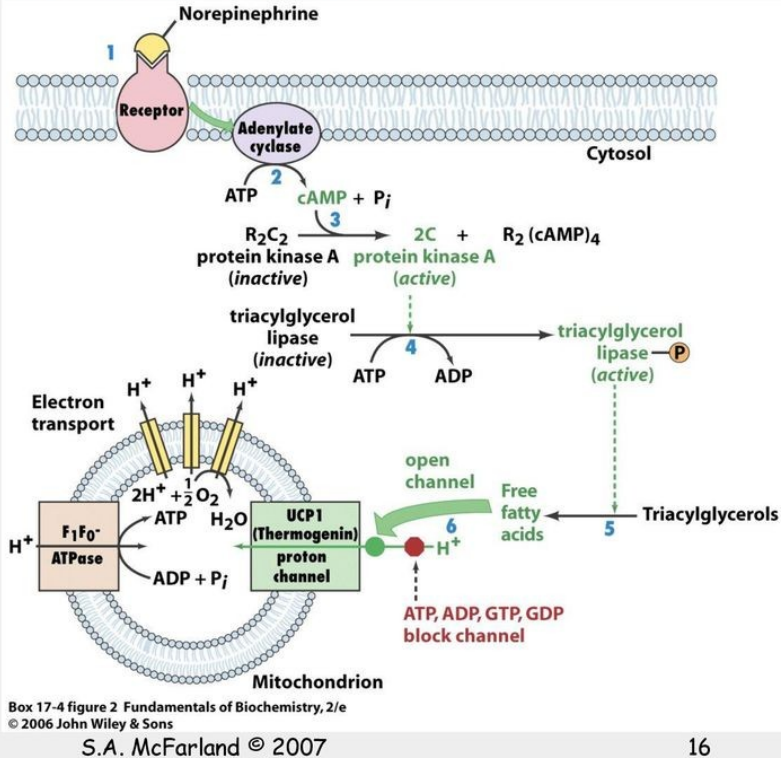
Mechanism is to ↑ FA oxidation which uncouples oxidation phosphorylation

Breaks down proton gradient

Uncoupling oxidative phosphorylation

Thermogenesis in brown fat is under hormonal control

- Norepinephrine induces production of 2nd messenger cAMP
- cAMP activates protein kinase A
- Protein kinase A activates TAG lipase by phosphorylation
- Activated lipase hydrolyzes TAGs to yield free fatty acids
- Free fatty acids counteract inhibitory effect of the purine nucleotides on UCP1
- Flow of protons through UCP1 dissipates the proton gradient across the inner mitochondrial membrane
- Substrate oxidation proceeds and generates heat, without the synthesis of ATP



Box 17-4 figure 2 Fundamentals of Biochemistry, 2/e
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