OXIDATIVE PHOSPHORYLATION

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Electron transfer reactions

- Life on Earth is driven by electron transfer reactions.
- Catalyzed by a suite of enzymes that comprise the superfamily of oxidoreductases (Enzyme Classification EC1).
- Three-dimensional topologies of proteins change more slowly than sequences.
- The similarity between proximal cofactor-binding folds show that they are derived from a common ancestor.
- Two recurring folds were central to the origin of metabolism: ferredoxin and Rossmann-like folds.

Ferredoxin fold

- A common α+β protein fold with a signature βαββαβ secondary structure along its backbone.
- Structurally, the ferredoxin fold can be regarded as a long, symmetric hairpin that is wrapped once around
- Its two terminal β-strands hydrogen-bond to the central two β-strands, forming a four-stranded, antiparallel β-sheet covered on one side by two α-helices.

Ferrodoxin folds

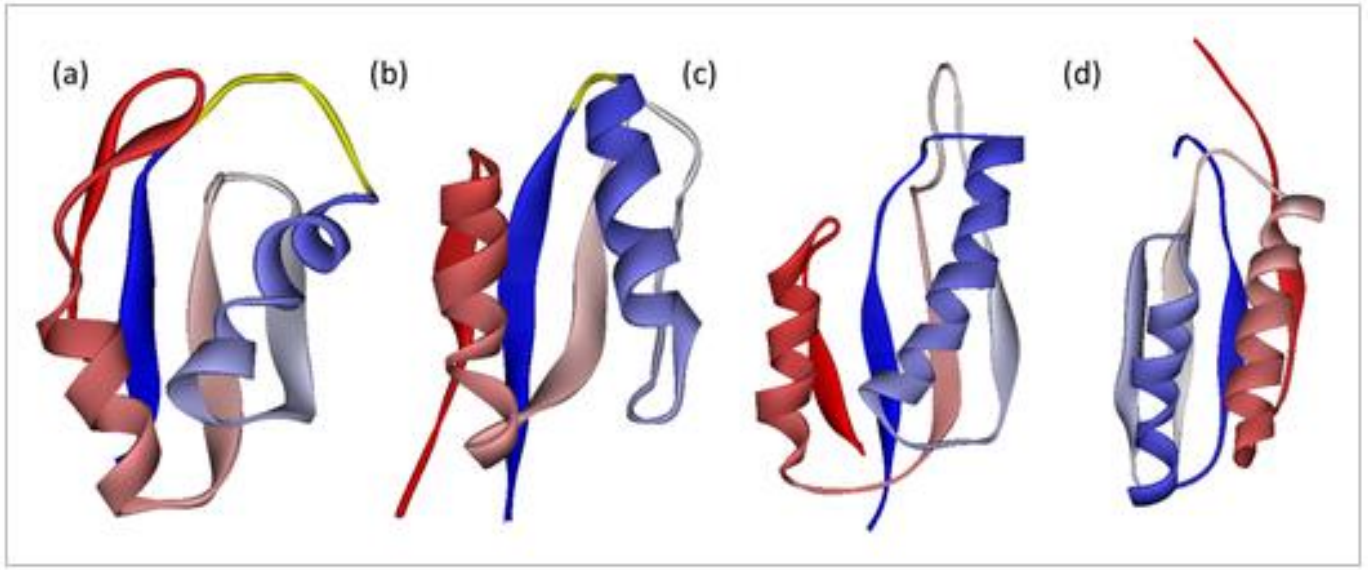


Figure 1

Open in figure viewer | PowerPoint

Native structures of (**a**) U1A, (**b**) ADA2h, (**c**) S6, and (**d**) S6_p54-55. Proteins are colored by gradation from blue to red with respect to N- to C-termini. Loops connecting β1 and α1 of U1A and ADA2h are colored yellow.

Sugita, M, Kikuchi, T, “Analyses of the folding properties of ferredoxin-like fold proteins by means of a coarse-grained Gō model: Relationship between the free energy profiles and folding cores,” Proteins 2014; 82(6):954-965 https://doi.org/10.1002/prot.24469
Accessed 03/20/2020

Ferredoxin

- Soluble, iron/sulfur (Fe_2S_2) protein
- Shuttles electrons from NADPH to P450 enzymes
- Resides either free in the mitochondrial matrix or is loosely bound to the mitochondrial membrane
- Genes:
- Ferredoxin 1 (FDX1 at 11q22)
- Induced by cAMP and P450scc
- Steroid, Vitamin D, bile acid synthesis
- Ferredoxin 2 (FDX2 or FDX1L at 19p13.2)
- FDX2 is essential for Heme A biosynthesis
- Lost in mitochondrial muscle myopathy

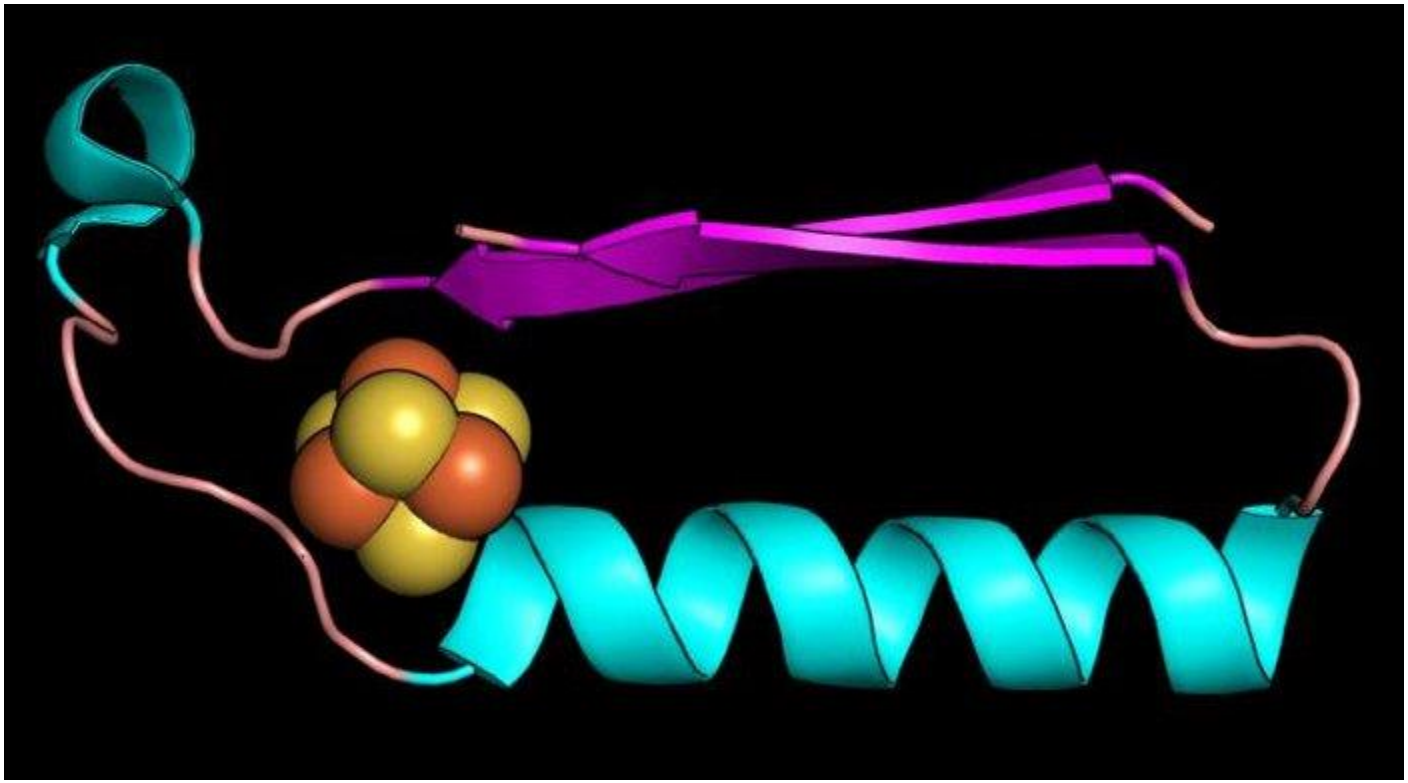
Rossman-like folds

- Found in nucleotide-binding proteins that utilize diphosphate-containing cofactors such as NAD+, NADP+, FAD.
- Involved in nucleotide and amino acid pathways as well as oxidoreductase metabolism
- The core of these protein structures included two sets of β-α-β-α-β units, forming a single parallel β-sheet flanked by α-helices on either side.
- A crossover between β-strands 3 and 4 that creates a natural cavity for binding nucleotides

Electron transfer reactions

- In turn, these two folds likely shared a common ancestor that, through duplication, recruitment, and diversification, evolved to facilitate electron transfer and catalysis at a very early stage in the origin of metabolism.

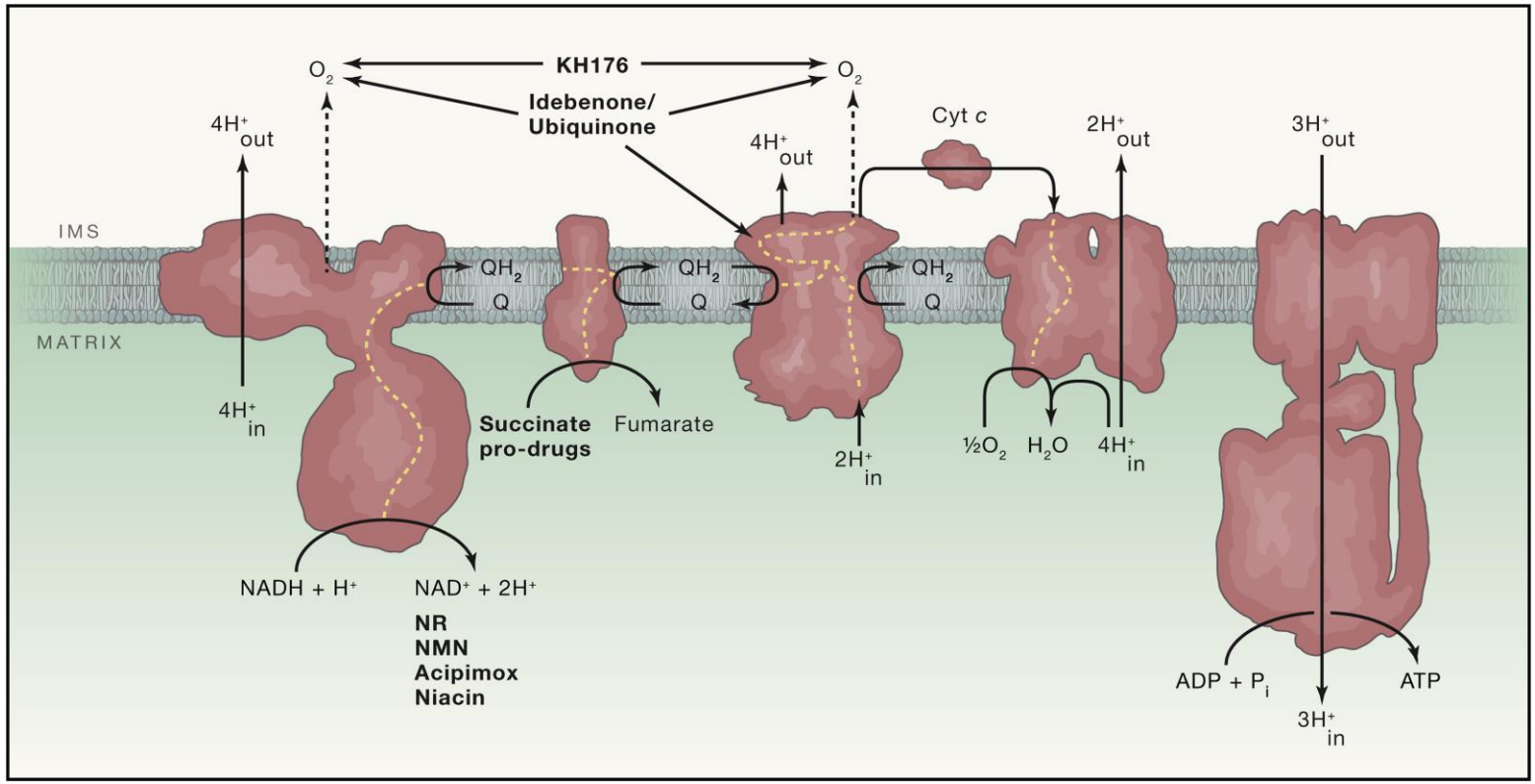
Archean protein



Raanan, H, Poudel, S, Pike, DH, Nanda, V, Falkowski, PG, “Small protein folds at the root of an ancient metabolic network,” PNAS Mar 2020, 201914982; DOI: 10.1073/pnas.1914982117 Accessed 03/22/2020

Oxidative phosphorylation

- Two tightly coupled processes in the mitochondrion:
- One, the passage of electrons through a chain of membrane-bound oxidation-reduction carriers and the concomitant pumping of protons across the membrane, generating a H^+ concentration gradient which "drives" the phosphorylation of ADP
- Two, the phosphorylation of ADP to ATP by the membrane-bound enzyme complex ATP synthase.

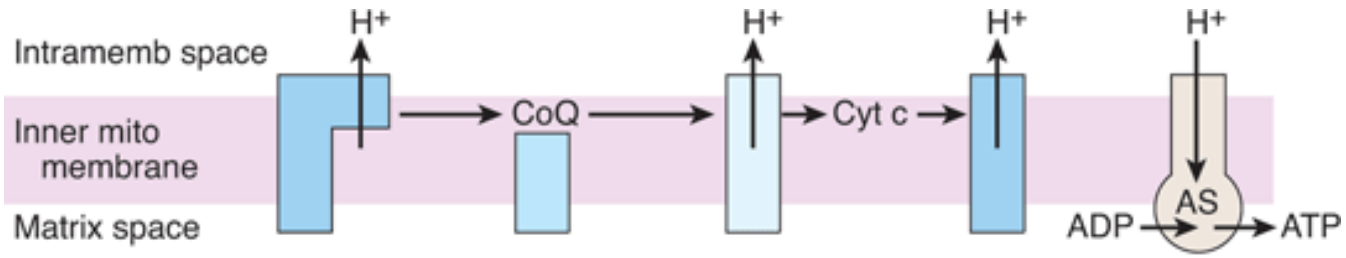


Cell DOI: (10.1016/j.cell.2020.02.051
Accessed 03/20/2020

Mitochondrion

- Responsible for the reduction of O_2 to H_2O with electrons donated by NADH and ubiquinol. ATP is generated.
- Outer membrane is highly permeable to small ions and small molecules.
- Inner membrane is impermeable (high cardiolipin content of membrane).
- The inner membrane contains the enzymes for electron transport and oxidation.
- The matrix contains dehydrogenases, Ca^{2+} and Mg^{+} granules, and double stranded circular DNA genome (inherited from the mother).

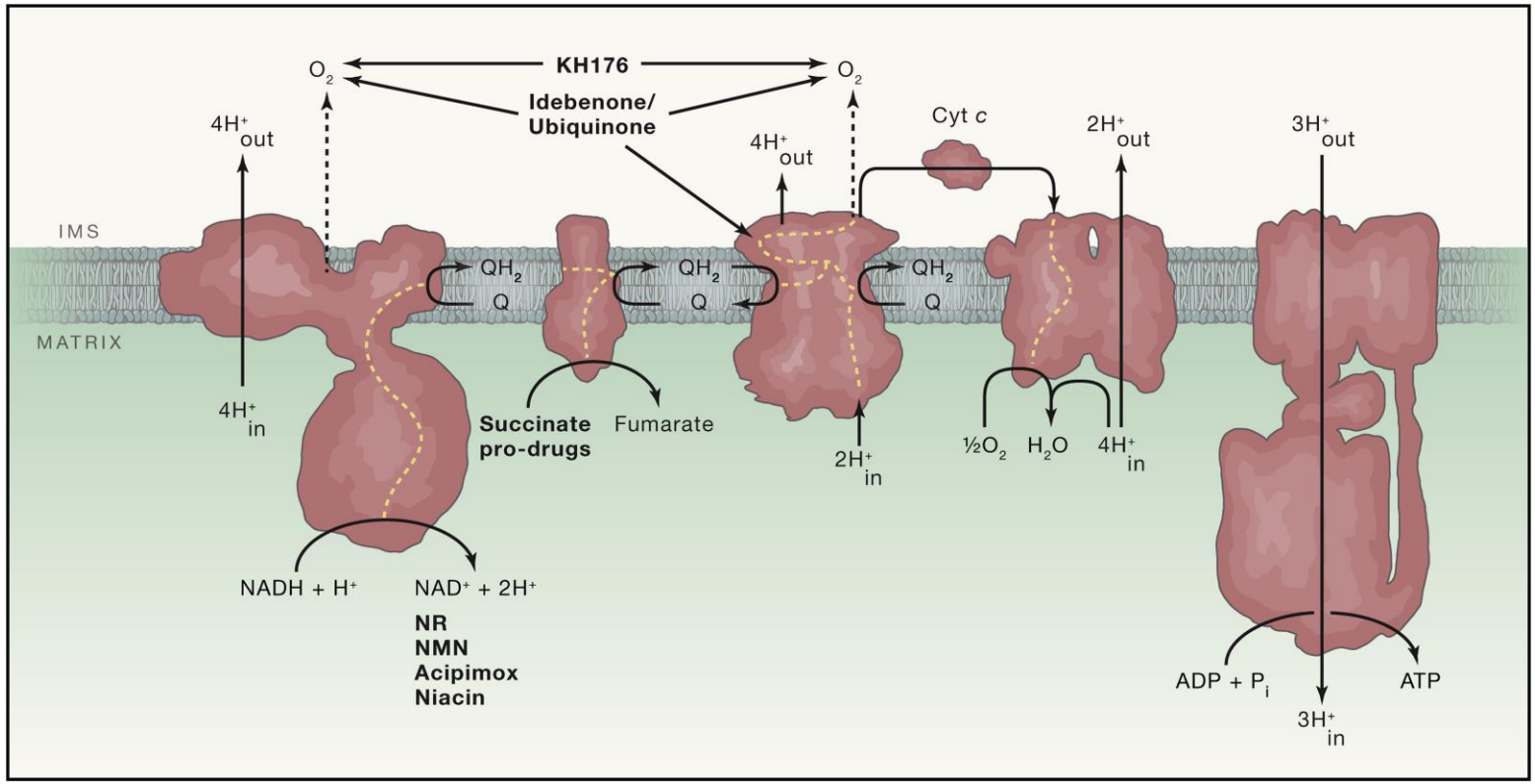
Oxidative phosphorylation



Complex	I	II	III	IV	V
Subunits from mDNA	7	0	1	3	2
Subunits from nDNA	39	4	10	10	14

Fig. 2-4
Accessed 07/01/2010

As enzyme complexes I through IV convert 2-carbon metabolic fragments to CO_2 and H_2O, protons (H^+) are pumped into the intermembrane space. The proteins diffuse back to the matrix space via complex V, ATP synthase (AS), in which ADP is converted to ATP. The enzyme complexes are made up of subunits coded by mitochondrial DNA (mDNA) and nuclear DNA (nDNA).



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Respiratory chain

- The respiratory chain consists of three protein complexes (I, III, IV) integrated into the inner mitochondrial membrane and two mobile complexes (ubiquinone, cytochrome C).
- Succinate dehydrogenase, part of the TCA cycle, participates also in the respiratory chain as complex II.
- Flavins are found in complexes I, II.
- Iron-Sulfur clusters are found in complexes I, II, IV.
- Heme groups are found in complexes II, III, IV.

Respiratory chain

- Protons are transported through complexes I, III, IV from the matrix into the inter-membrane space.
- NADH enters complex 1; FADH, complex II.
- Only ATP synthase allows protons to flow back into the matrix.
- This couples electron transport to ATP synthesis.
- 3 H^+ are channeled back and consumed during the process.

Respiratory chain

- Electrons are transported via ubiquinone and cytochrome C.
- Ubiquinone is freely mobile within the membrane as it is highly lipid soluble.
- Cytochrome C is water soluble and found on the inner surface of the membrane.

Respiratory chain

- In the oxidation of NADH by complex I, electrons pass by flavins and Iron-Sulfur clusters to ubiquinone.
- Electrons arising during the oxidation of succinate are passed to ubiquinone by succinate dehydrogenase.
- Electrons arising during the oxidation of acyl CoA, choline, or α-glyceriophosphate are passed to ubiquinone by enzyme bound FAD and the electron transporting flavin.

Respiratory chain

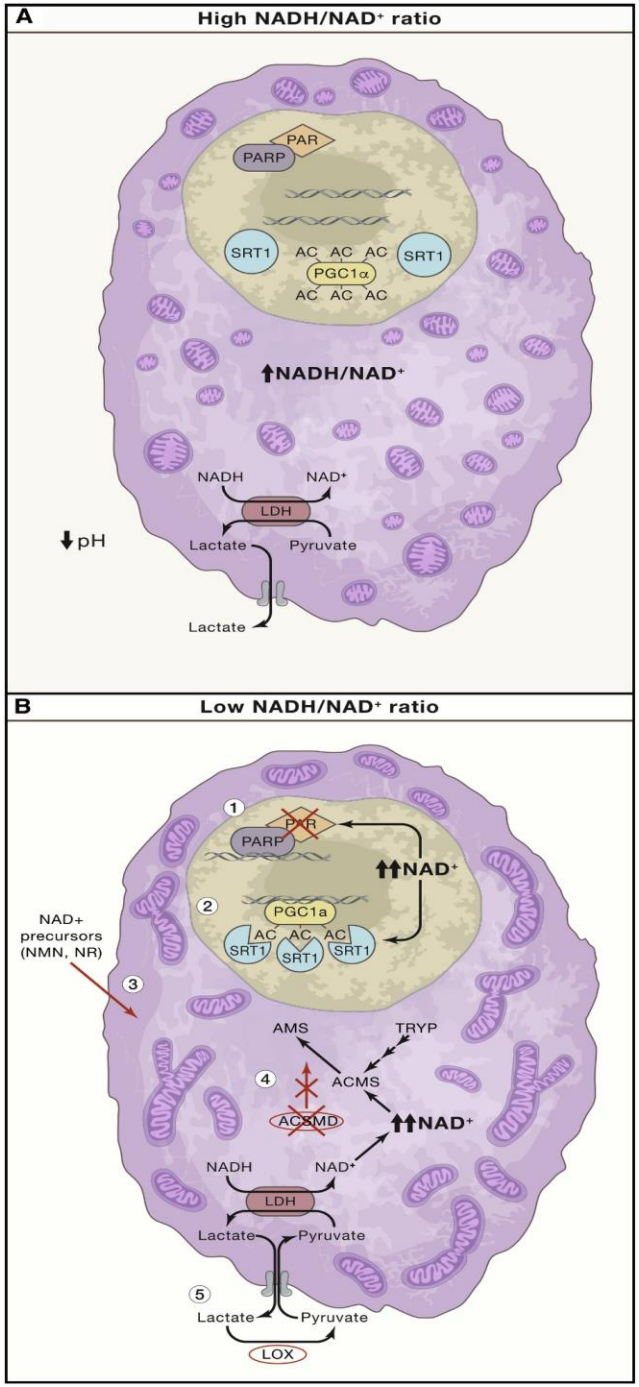
- Electrons are then transported to complex III by ubiquinol and, from complex III (ubiquinol-cytochrome C reductase) by cytocrome C.
- Cytochrome C transports electrons to complex IV, cytochrome oxidase.
- Cytochrome oxidase contains two Cu^{+} centers and two heme groups.
- Oxygen is then reduced by two electrons and water is produced by binding with a proton.
- Electron transfer is coupled to proton gradient generated in complexes I, III, IV.

Respiratory chain

- ATP is generation is driven through ATP synthase (complex V).
- NADH generates 3 ATP; FADH, 2.
- The respiratory chain produces most of the energy required during metabolism.
- Exergonic.
- Respiratory control ensures a constant supply of ATP.
- ATP consumption and synthesis are coupled via shared coenzymes.

Respiratory chain

- If little ADP is available, ATP synthase cannot reverse the proton gradient
- Therefore, electron transport is inhibited and NADH can no longer be re-oxidized to NAD+ and the TCA cycle is inhibited.


A
High NADH/NAD+ ratio
PAR
PARP
SRT1
AC AC AC
SRT1
PGC1α
AC AC AC
↑NADH/NAD+
NADH
NAD+
LDH
↓pH
Lactate
Pyruvate
Lactate
B
Low NADH/NAD+ ratio
1
PAR
PARP
↑↑NAD+
2
PGC1a
AC AC AC
SRT1 SRT1 SRT1
NAD+
precursors
(NMN, NR)
3
AMS
TRYP
4
ACMS
ACSMD
↑↑NAD+
NADH
NAD+
LDH
Lactate
Pyruvate
5
Lactate
Pyruvate
LOX

Respiratory chain

- Thermogenin, uncoupling protein 1, functionally separates oxidation from phosphorylation.
- H^+ pass from the intermembrane space back into the matrix without involving ATP synthetase.
- This is found in brown fat.
- In cold periods, norepinephrine activates hormone sensitive lipase which leads to free fatty acid production.
- H^+ is bound to the free fatty acids, passes the uncoupling protein in this form, and is released in the matrix.
- Heat alone is generated (as with thyroxine).

Respiratory chain

- CN^{3-}, CO, and H_2S inhibit complex IV.
- Antimycin inhibits complex III.
- Amytal and rotenone inhibit complex I.
- Arsenic (As^{3+}) can replace phosphates in ATP.