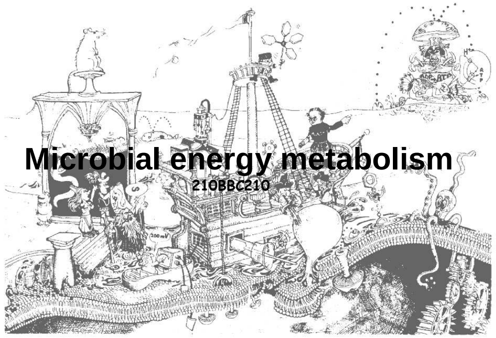

Microbial energy metabolism

21OBBC210

Mitchell sets sail for the Chemiosmotic New World, despite dire warnings that he will be consumed

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Contact Details

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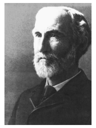
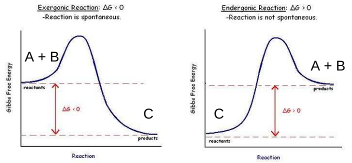
A bit of chemistry
Free energy change



Gibb's Free Energy change is a concept which allows to predict if a reaction is thermodynamically favorable

$A + B \leftrightarrow C$ $\Delta G<0$ – spontaneous reaction →, EXErgonic

$\Delta G>0$ – no reaction, ENDErgonic



Free energy change

ΔG^o = change of free energy of reaction at standard state conditions at 1M concentration of reactants. But in reality concentrations may vary!

$A + B \leftrightarrow C$ $\Delta G^o>0$ – no reaction if we mix A, B and C at concentrations of 1M ([A]=[B]=[C]=1M)

$$\Delta G = \Delta G^0 + \ln\frac{[C]}{[A]\times[B]}$$

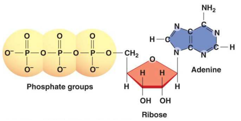
However, if [A]×[B] >>[C], real $\Delta G<0$ and reaction will go from left to right →

In particular case of standard conditions $\Delta G=\Delta G^o$ because

$$\Delta G = \Delta G^0 + \ln\frac{1}{1\times1} = \Delta G^0 + 0$$

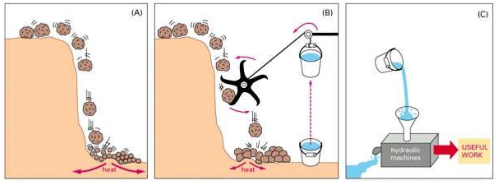
Enzymes accelerate the attainment of equilibrium, but not shift it or reverse reaction. Direction of the reaction is defined by ΔG. Some of the biological reactions have $\Delta G^o>0$, but due to the concentration component (in logarithm) $\Delta G<0$.

Adenosine triphosphate (ATP)



ATP is regarded as a universal source of energy occurring in all cell types. In animals it is produced during the degradation of foodstuff.

$$ATP \rightarrow ADP + P_i + \textit{energy}$$

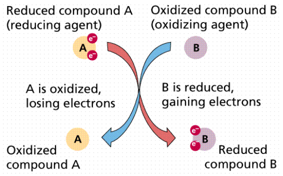


Burning (oxidation) of glucose to CO_2 and H_2O produces heat only

Cells can store energy during glucose oxidation can in form of ATP

ATP can be used to drive any cellular processes

Redox reactions
(reduction-oxidation reactions)



$A_{red} + B_{ox} \rightarrow A_{ox} + B_{red}$

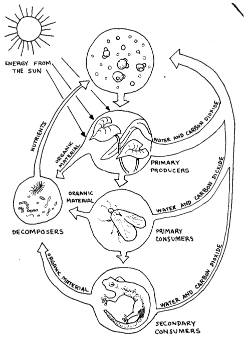
$A_{red} \rightarrow A_{ox} + 2e^-$
$B_{ox} + 2e^- \rightarrow B_{red}$

Midpoint redox potential (E^o) is a tendency of A_{red} to donate electrons

Electrons transferred from A_{red} to B_{ox} if
$E^o_{A^{ox}/A^{red}} < E^o_{B^{ox}/B^{red}}$

Photosynthesis and Respiration

(energy conversion)



Reduction of oxygen

$CO_2 + H_2O \overset{+h\nu}{\leftrightarrow} \{CH_2O\} + O_2$

Photosynthetic organisms

Capture of solar energy to use it for reduction of carbon compounds

$\{CH_2O\} + O_2 \leftrightarrow CO_2 + H_2O$ + energy

Animals or microorganisms

Oxidation of food to obtain energy

$$\xrightarrow{\text{Animals}}$$

$$O_2 + 4e^- + 4H^+ \leftrightarrow 2H_2O$$

$$\xleftarrow[\text{Plants}]{}$$

Oxidative phosphorylation

History

W. A. Engelhardt, 1936-39 - measured inorganic and organic phosphate content definition of oxidative phosphorylation

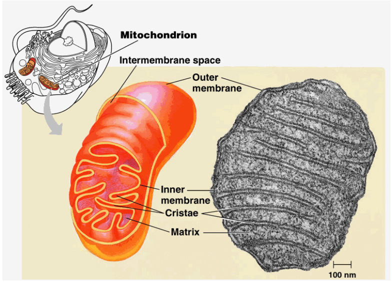
Warburg vs ***Thunberg*** and ***Keilin*** - respiratory enzyme vs dehydrogenase

Albert Lehninger – 1948 – mitochondria are the site of energy metabolism

David Green – 50s, isolation and reconstitution of electron transport chain

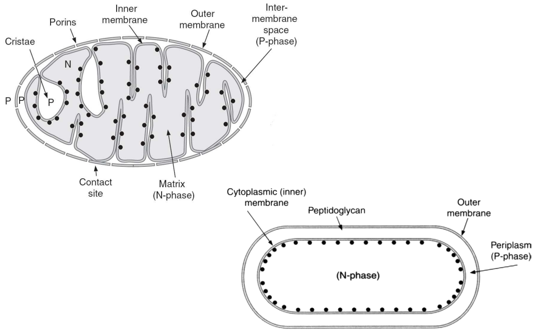
Piter Mitchell – energy transduction in membranes Nobel Prize 1978

Mitochondria – respiring organelle



Also contain its own DNA and its own transcription/translational system

Membranes of mitochondria and bacteria

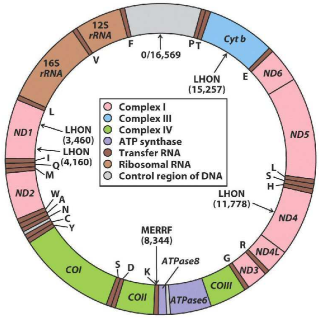


What are mitochondria?

- An intracellular organelle.
- There are 100 to 1000s of mitochondria/cell.
- All mitochondria come from the mother.
- Mitochondria have their own DNA.
- Major functions of mitochondria:
  - – Makes energy in the form of ATP.

Endosymbiotic theory

Mitochondrial DNA



Double stranded, circular

- No introns, 80 - 93% coding gene
- No repeats
- Lack histone and DNA repair mechanism ⇒ damage, mutations (free radicals)
- 37 gene: 22 tRNA, 2 rRNA & 13 protein

NUCLEOID
A dynamic complex that consists of several copies of mitochondrial DNA and key maintenance proteins within the organelle.

POLYCISTRONIC
A form of gene organization that results in transcription of an mRNA that codes for multiple gene products, each of which is independently translated from the mRNA.

Locations

Bacteria

- Glycolysis
  - Cytoplasm
- Krebs' TCA
  - Mitochondrial matrix
- Oxidative phosphorylation
  - Inner mitochondrial membrane

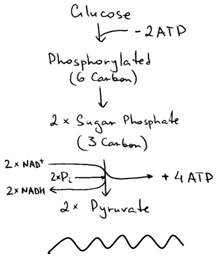
Cytoplasm

Plasma membrane

⇒ **Compartmentalisation**

Glycolysis

Cytoplasm



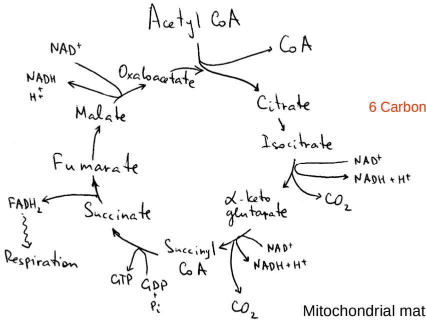
Mitochondrial matrix

Krebs cycle (TCA cycle)

2 Carbon

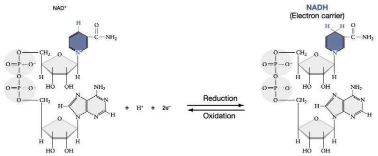
4 Carbon

6 Carbon



Mitochondrial matrix

Electron transferring coenzyme



$NAD^+ + 2e^- + H^+ \leftrightarrow NADH$

After glycolysis and TCA cycle

Not much ATP formed

Lots of reduced coenzymes

Per glucose molecule:

10 NADH

2 $FADH_2$ (!!!)

At the same time:

Reoxidation of NADH releases energy

Requires oxygen as oxidant

This energy can be used for ATP synthesis

Respiratory chain couples processes of oxidation and ATP synthesis

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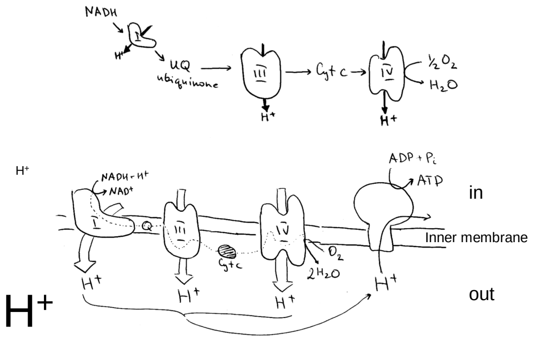
Electron transport chain and ATP synthase

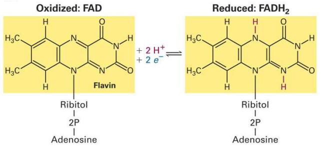


Redox centres

- Flavin
- Iron- sulphur centres (FeS-centres)
- Ubiquinone
- Cytochromes

Flavin

Complex I
Complex II = succinate dehydrogenase from Krebs cycle

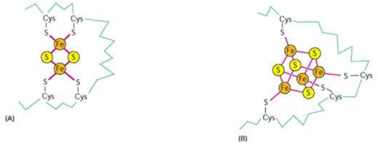


$FAD + 2e^- + 2H^+ \leftrightarrow FADH_2$

Usually serves as intermediate of electron transfer between $2e^-$ donor and 1e- acceptor
No free flavins in a cell !!!

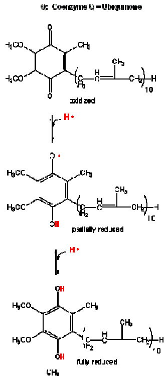
FeS clusters

Complex I
Complex II = succinate dehydrogenase from Krebs cycle
Complex III = bc_1 complex



Always transfer one electron at the time
Electron is delocalised

Ubiquinone



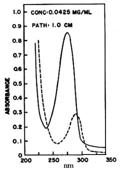
2 electrons + $2H^+$

Membrane mobile redox carrier linking Complexes I and II with Complex III

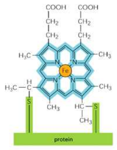
Proton-translocating Q-cycle in complex III

Different n for different species (*n*=6-10)

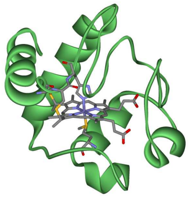
Menaquinone and rhodoquinone in some bacteria and plastoquinone in chloroplasts



Cytochromes



Cytochrome *c*

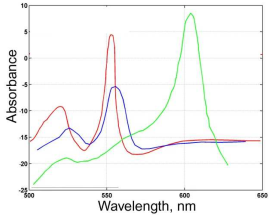


Cyt *c* (Fe^{3+}) + e^- ↔ Cyt *c* (Fe^{2+})

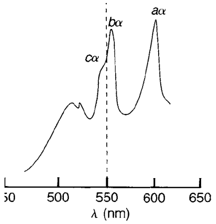
Protein part and haem part containing Fe ion
Cytochromes *a*, *b*, *c* … + number
As separate proteins (e.g. cytochrome c) or as subunits of enzymatic complexes (Complex II, III, IV)

Respiratory chain cytochromes

Reduced-oxidised spectra
------- a ------- b -------c



Reduced-oxidised spectra of mitochondrial membranes



Easy to observe reduction/oxidation as change in optical spectra

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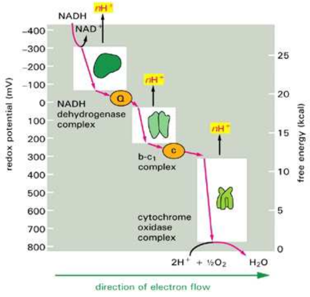
Otto Warburg Nobel Prize 1931
Nature and mode of action of the respiratory enzymes

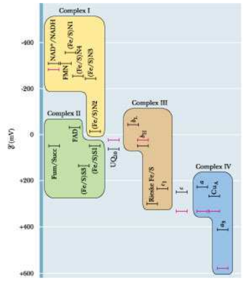
Hugo Theorell Nobel prize 1955
Redox enzymes and biological oxidation

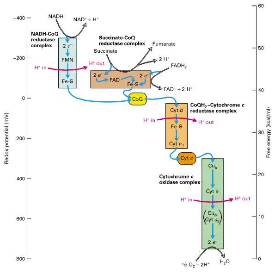
Electron transport chain



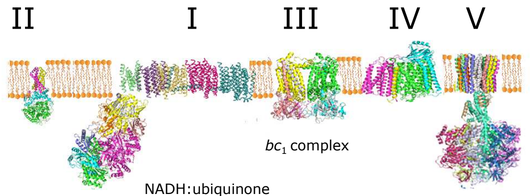
Values of redox potential of respiratory chain components fall downstream from NADH to oxygen.

Electron transport chain





Structure of respiratory chain



bc$_1$ complex

Succinate dehydrogenase

NADH:ubiquinone oxidoreductase

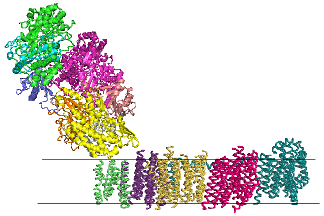
Cytochrome *c* oxidase

ATP synthase

Complex I (NADH:ubiquinone oxidoreductase)

$NADH + H^+ + 4H^+_{in} + UQ \leftrightarrow$

$\leftrightarrow NAD^+ + UQH_2 + 4H^+_{out}$



Mammalian enzyme: 45 subunits

Bacterial enzyme: 14 subunits

Flavin = FMN

8 FeS clusters

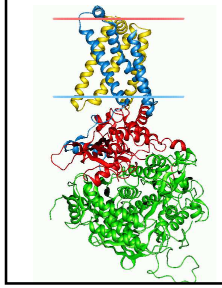
Tightly-bound semiquinones as intermediates of electron transfer

Classical inhibitors:

Rotenone, piericidine, MPP^+

Complex II
Succinate dehydrogenase of TCA cycle

$Succinate + UQ \leftrightarrow Fumarate + UQH_2$



Mammalian enzyme: 4 subunits

Bacterial enzyme: 4 subunits

Flavin = FAD

Cytochrome *b*

Three FeS clusters

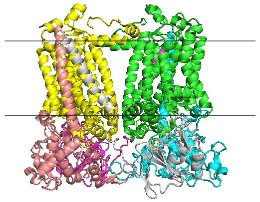
Classical inhibitors:

Malonate, Oxaloacetate

Complex III
bc$_1$ complex

$UQH_2 + 2Cyt\ c\ (Fe^{2+}) + 2H^+_{in} \leftrightarrow$

$\leftrightarrow UQ + 2Cyt\ c\ (Fe^{3+}) + 4H^+_{out}$



Mammalian enzyme: 11 subunits

Bacterial enzyme: 3 (+1) subunits

Cytochromes c_1, b_L and b_H

Rieske protein (2Fe2S cluster)

Tightly-bound semiquinones as intermediates of electron transfer

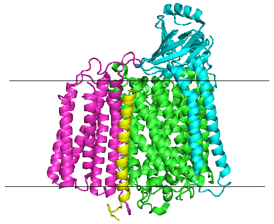
Classical inhibitors:

Antimycin A, myxothiazol

Complex IV
Cytochrome *c* oxidase

$4Cyt\ c\ (Fe^{3+}) + 4H^+_{in} + 4H^+_{in} + O_2 \leftrightarrow$

$\leftrightarrow 2Cyt\ c\ (Fe^{2+}) + 2H_2O + 4H^+_{out}$



Mammalian enzyme: 13 subunits

Bacterial enzyme: 2-3 subunits

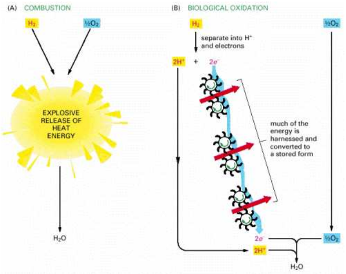
Cytochrome *a*, a_3,

Two copper Cu_A Cu_B centers

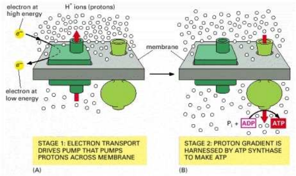
Classical inhibitors:

Cyanide, carbon monoxide, nitric oxide

Oxidative phosphorylation



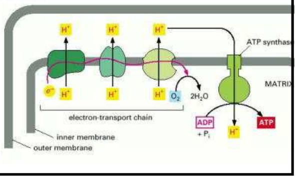
Chemiosmotic theory



Peter Mitchell, Nobel Prize 1978

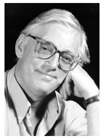
Electron transfer along electron transport chain is coupled with proton translocation at complexes I, II and IV.

The difference in concentration of protons can drive special molecular motor – ATP synthase.



ATP synthase

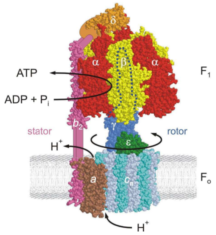
Nobel prize 1997 John Walker



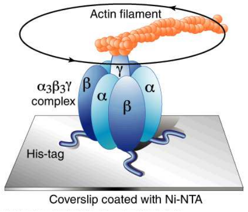
<u>Bacterial enzyme</u>: 8 subunits

Proton flow through F_o part is coupled with ATP synthesis in the F_1 part

~10H^+ per 3 molecules of ATP



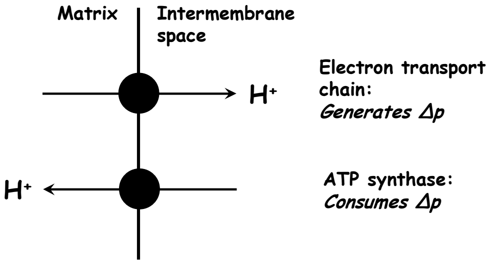
ATP synthase



Yoshida Lab http://www.res.titech.ac.jp/~seibutu/
Kinosita Lab http://www.k2.phys.waseda.ac.jp/Movies.html

ATP-synthase

ATP synthesis in mitochondria



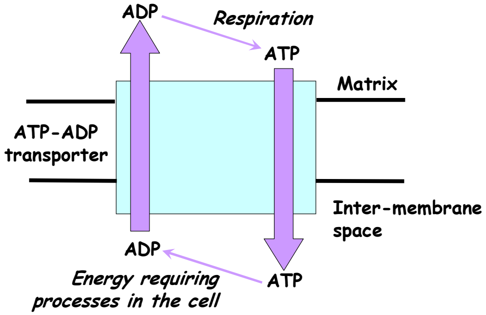
Electron transport chain:
Generates Δp

ATP synthase:
Consumes Δp

ATP synthesis

ATP synthesis in mitochondrial matrix
Needs to be transported out of mitochondria
Requires ATP-ADP transporter
Integral membrane protein
ATP and ADP transport coupled

ATP transport
(in mitochondria!)



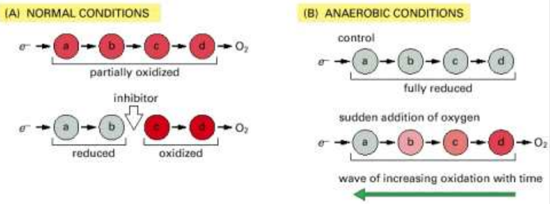
Mitochondrial respiration

History: Isolated mitochondria + substrates + oxygen

Some compounds block oxygen consumption – respiration inhibitors

Some compounds stop ATP synthesis but not respiration, they break the link between respiration and ATP synthesis – uncouplers

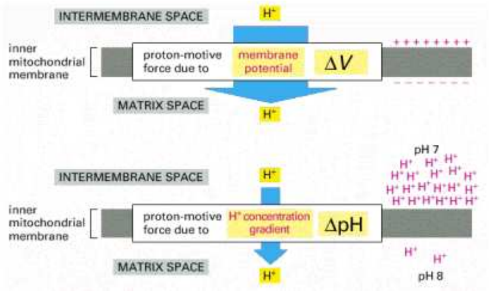
How to study electron transport chain?



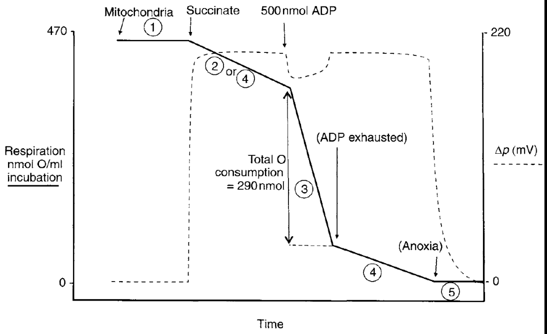
Oxidative phosphorylation inhibitors

- I – Rotenone
  - Ubiquinone-like structure
- II – Oxaloacetate
  - Succinate-like structure
- III - Antimycin A
  - Fungicide and insecticide
- **IV - Cyanide (CN^-), azide (N_3^-), carbon monoxide (CO), nitric oxide (NO)**
  - Similar electronic structures to O_2

Protonmotive force



Mitochondrial respiration



Oxidative phosphorylation

Respiratory control ratio

$H^+/2e^-$ stoichiometry of respiratory chain complexes

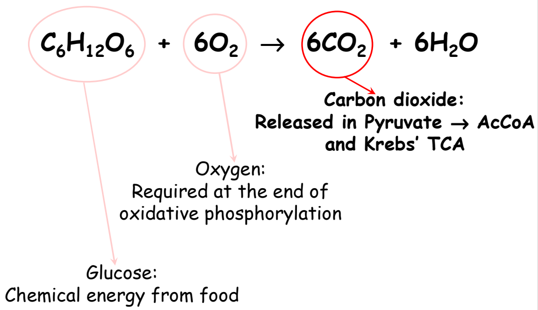
H^+/ATP stoichiometry of ATP synthase

ADP/O ratio – how much ADP can be converted to ATP per molecule of oxygen

Reversibility of reactions = reverse electron transfer

Reactive oxygen species generation

Aerobic respiration



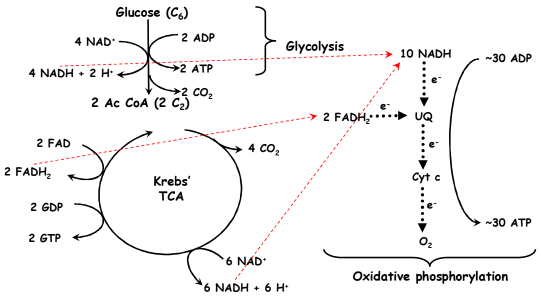
$C_6H_{12}O_6$ + $6O_2$ → $6CO_2$ + $6H_2O$

Carbon dioxide:
Released in Pyruvate → AcCoA
and Krebs' TCA

Oxygen:
Required at the end of
oxidative phosphorylation

Glucose:
Chemical energy from food

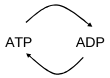
Aerobic respiration



Glucose (C_6)
4 NAD⁺
2 ADP
4 NADH + 2 H⁺
2 ATP
$2\ CO_2$
2 Ac CoA (2 C_2)
Glycolysis
10 NADH
2 $FADH_2$
UQ
Cyt c
O_2
e^-
~30 ADP
~30 ATP
2 FAD
2 $FADH_2$
Krebs' TCA
$4\ CO_2$
2 GDP
2 GTP
6 NAD⁺
6 NADH + 6 H⁺
Oxidative phosphorylation

Aerobic respiration

(in mammals!)



Inner mitochondrial membrane ~1400m^2

We consume ~380 *l* of oxygen per day

ATP turnover ~60 kg/day

3 × 10^{23} H^+ per second through ATP synthase

90% ATP is synthesised during oxidative phosphorylation

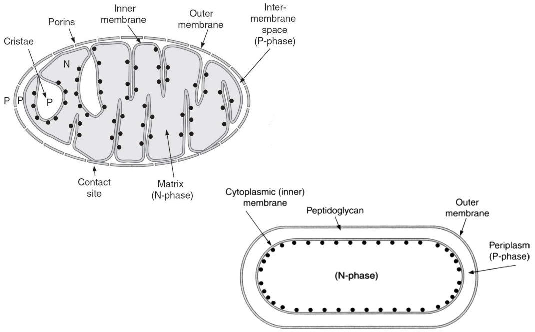
Bacterial energy metabolism

Live in various environment

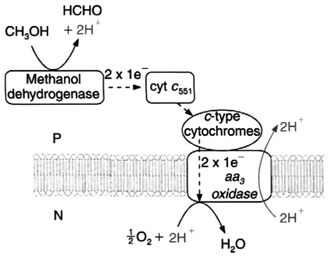
Able metabolise different substrates

Can adopt to the changing environment

Membranes of mitochondria and bacteria



What to do if food is scarce?



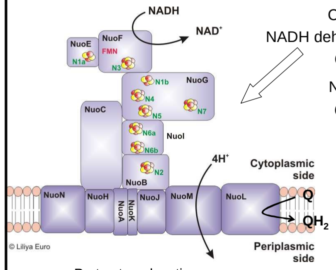
Some bacteria can grow on low carbon substrates like methanol as its sole source of energy. In *P. denitrificans* methanol is oxidised in periplasm and electrons are transferred directly to cytochrome oxidase

Bacterial energy metabolism

In many bacteria efficiency of respiration (ATP:O ratio) is lower than in mitochondria

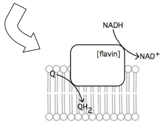
- More simple machinery of H^+/e^- transport
- Bypassing
- Shortening or branching of the chain

Simplification



Complex I =
NADH dehydrogenase Type 1
(eukaryotes)

NADH dehydrogenase Type 2
(E.coli, Rhodobacter capsulatus, etc)



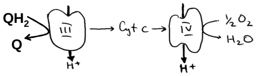
Proton translocation

No proton translocation
(not even a membrane protein)

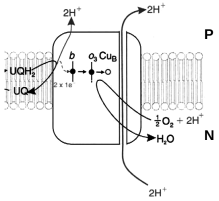
$$NADH + H^+ + Q \leftrightarrow NAD^+ + QH_2$$

Bypassing

bc$_1$ (III) and cytochrome *c* oxidase (IV) together (*in mitochondria*)



bo$_3$ oxidase (quinol oxidase)
(E.coli, Burkholderia phymatum)

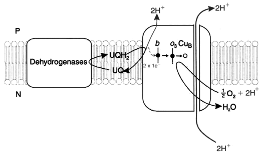


$$2QH_2 + O_2 + 4H^+_{in} \leftrightarrow 2Q + 2H_2O + 4H^+_{out}$$

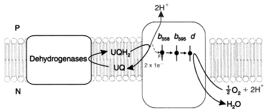
Direct oxidation of quinol by oxygen, bypassing *bc*$_1$ complex and cyt *c* + translocation of protons (but with lower efficiency)

Bypassing and simplification

bo$_3$ oxidase (quinol oxidase)
(E.coli, Burkholderia phymatum)



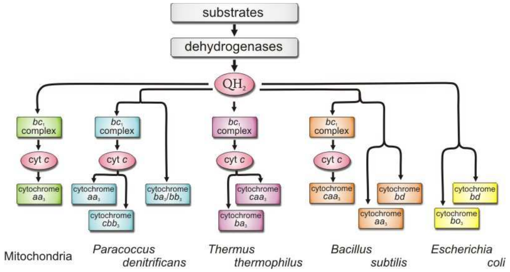
bd oxidase (quinol oxidase)
(no H$^+$ translocation)
(E.coli, Klebsiella pneumoniae, Mycobacterium tuberculosis)



$$2QH_2 + O_2 \leftrightarrow 2Q + 2H_2O$$

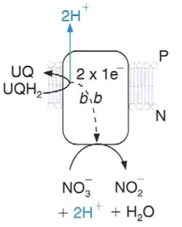
Direct oxidation of quinol by oxygen, bypassing *bc*$_1$ complex and cyt *c* + but no (!!!) translocation of protons

Scheme of Respiratory Chains of mitochondria and bacteria

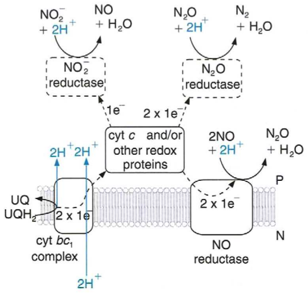


What to do if the oxygen is absent?

What would be the terminal electron acceptor or where electrons should end up?



P. denitrificans nitrate reductase system



$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

What to do if the oxygen is absent?

What would be the terminal electron acceptor or where electrons should end up?

NADH E_o= -320mV

↓

NADH Dehydrogenase Type 1 ⇨ up to $4H^+/2e^-$ translocated

↓

Menaquinone E_o= -74mV

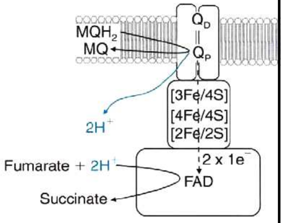
↓

Fumarate reductase Cyt *b*, FeS, FAD

↓

Fumarate E_o= +33mV

Fumarate reduction in *E.coli*



Why use menaquinone instead of ubiquinone?

Fumarate reductase from E.coli

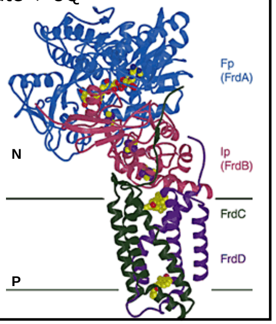
$\text{Fumarate} + UQH_2 \leftrightarrow \text{Succinate} + UQ$

Very similar to mitochondrial succinate dehydrogenase

4 subunits

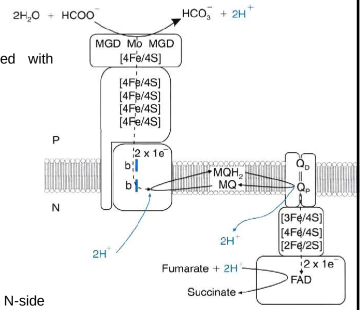
Flavin – FAD

3 FeS clusters



How to generate proton gradient without actual proton translocation?

Oxidation of formiate coupled with fumarate reduction in *E.coli*

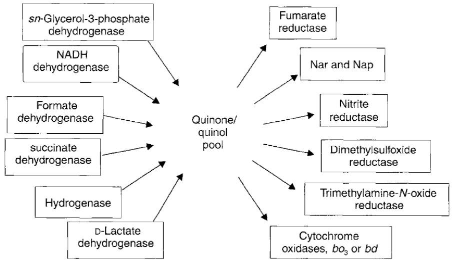


Net reaction:

release *2H*⁺ at P-side

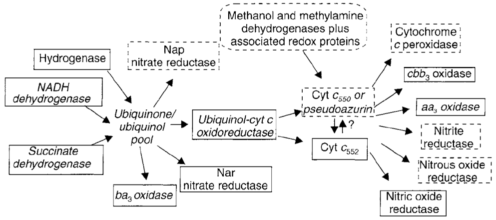
consumption of *2H*⁺ at N-side

E. coli respiratory chain



The components present depend on the growth conditions. Menaquinone replaces ubiquinone under anaerobic conditions. Many of the enzymes (e.g. nitrite reductase) have their active sites at the periplasm.

P. denitrificans respiratory chain



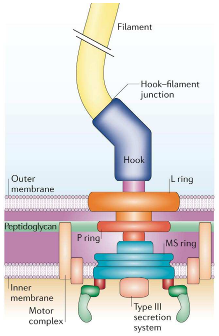
The components in italics are constitutive. The other components are induced at appropriate growth conditions and are unlikely to be all present at once. Continuous boxes indicate integral membrane components; dashed lines represent periplasmic components. NADH dehydrogenase, succinate dehydrogenase, ubiquinol cytochrome *c* oxidoreductase and aa_3 oxidase correspond to mitochondrial complexes I-IV.

What to do if it is very alkaline outside or too much salt?

Ions other than H⁺ can be used. In certain bacteria gradient of Na⁺ is created by special enzymes and can be used by special Na⁺ translocating ATP synthase for ATP synthesis.

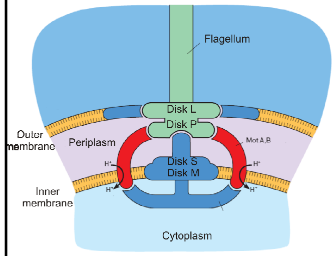
Sodium bioenergetics: *Halophilic bacteria, Vibrio cholerae, Yersinia pestis*

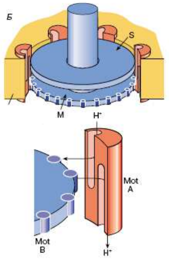
Flagellum = whip

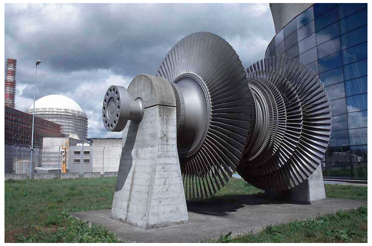


Energy of proton gradient can be converted in to mechanical movement (*E.coli, Helicobacter pylori, Salmonella typhimurium, Halobacterium*)
$\sim 10^3$ rpm = 300 Hz = 300 revolutions per second

How does it work?



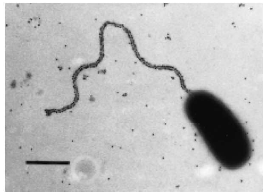




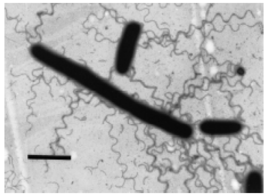
Using two fuels

Vibrio parahaemolyticus possesses two types of flagella. The swimmer cell moves fast in a liquid environments, with a single polar flagellum powered by the Na^+ motive force,. The swarmer cell, propelled by many lateral flagella powered by H^+ gradient and can move slowly through highly viscous environments. 15 000 rev per second on Na^{++}

Sodium motive force

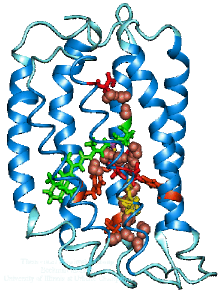


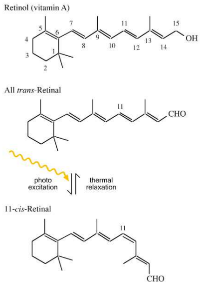
Proton motive force



How to use light ?

Bacteriorhodopsin is a protein from halobacteria. It uses light energy it to move protons across the membrane.





Bacteriorhodopsin

Bacteriorhodopsin

Proof of chemiosmosis

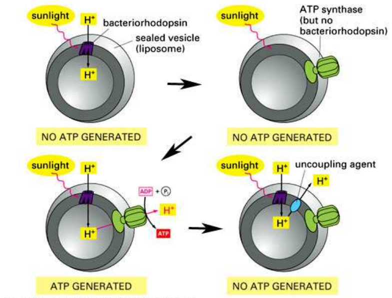
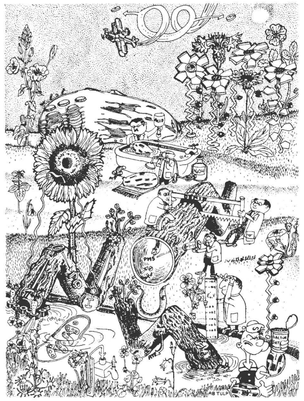


Figure 14-9 Essential Cell Biology, 2/e. (© 2004 Garland Science)



Photosynthesis in bacteria and light phase in chloroplasts

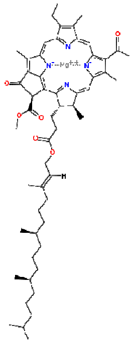
Photosynthesis in bacteria

No mitochondria, no chloroplasts ⇒ everything is located in the same membrane!

Hartmut Michel Nobel prize 1988
Structure of photosynthetic reaction centre

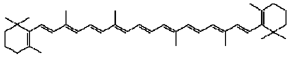
Bacteriochlorophyll and carotenoids

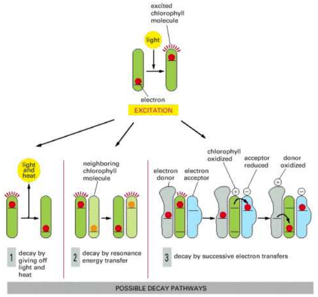
(in light harvesting complexes)



$$P^* \xrightarrow{-e^-} P^+ \xrightarrow{+e^-} P$$

Carotenoids

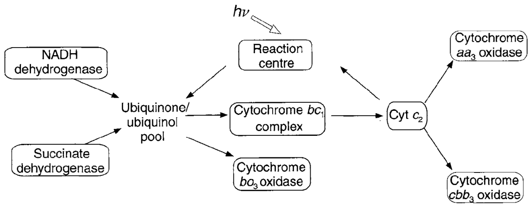




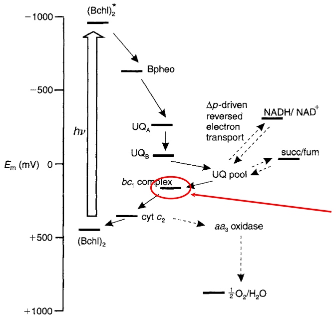
The light energy absorbed by an isolated pigment molecule is completely released as light and heat by process 1. In photosynthesis, by contrast, pigment undergo process 2 in the antenna complex and process 3 in the reaction center.

Light driven cyclic electron transfer

(Rh. Sphaeroides)

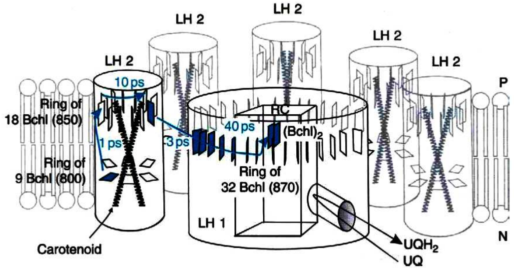


Electron transfer in *Rh. Sphaeroides*



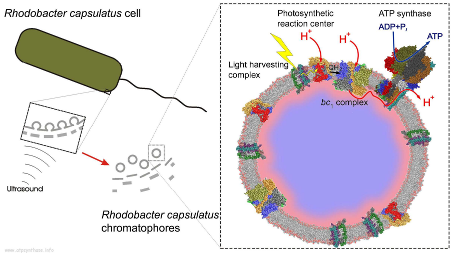
H^+-translocation by complex III

Organisation of light harvesting complexes



LH2=antennae absorb light at other λ than Bchl and also provides high rate of electron delivery to RC

Light driven electron transfer takes place in chromatophores



Greenplants and algae
Oxygen and carbohydrate formation

$CO_2 + H_2O \overset{+h\nu}{\leftrightarrow} \{CH_2O\} + O_2$	**$\{CH_2O\} + O_2 \leftrightarrow CO_2 + H_2O$ + energy**
Photosynthetic organisms	Animals
Capture of solar energy to use it for reduction of carbon compounds	Oxidation of food to obtain energy

$$\xrightarrow{\text{Animals}}$$

$$O_2 + 4e^- + 4H^+ \leftrightarrow 2H_2O$$

$$\xleftarrow[\text{Plants}]{}$$

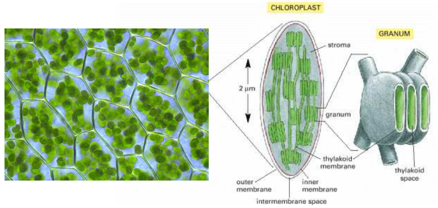
Photosynthesis

- Light reactions:
  - Need light to occur
  - Capture of light energy
  - Generation of pmf and reducing power (NADPH)
- **Dark reactions:**
  - Occur in light and dark
  - **Carbohydrate synthesis**

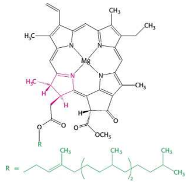
Photosynthesis

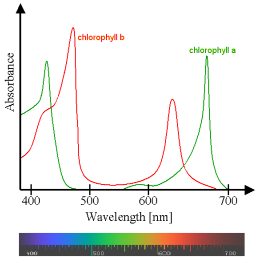
- $6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$
- Occurs in specialised organelles – chloroplasts
- **Light captured by chlorophyll**
  - Porphyrin
  - Contains Mg^{2+}
  - **Green**

Photosynthesis takes place in chloroplasts



Chlorophyll



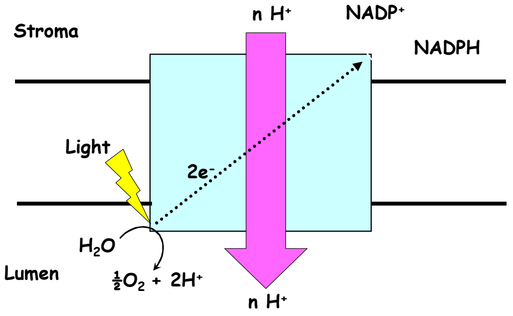


Like heme, chlorophyll *a* is a cyclic tetrapyrrole. One of the pyrrole rings (shown in red) is reduced. A phytol chain (green) is connected by an ester linkage. Magnesium ion binds at the center of the structure.

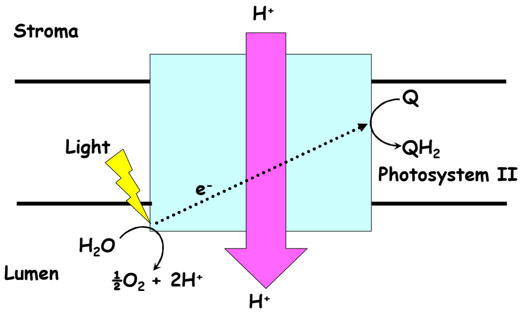
Photosynthesis: Light reactions

- Two light absorbing stages:
  - Photosystem II
  - Photosystem I
- Electron transport chains – several complexes of proteins
- **Soluble carriers:**
  - Plastoquinone (Q), lipid soluble
  - **Plastocyanin, water soluble**

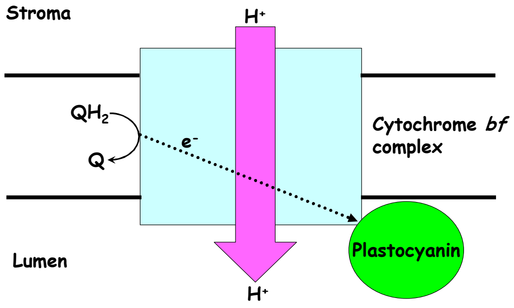
Photosynthesis: Light reactions



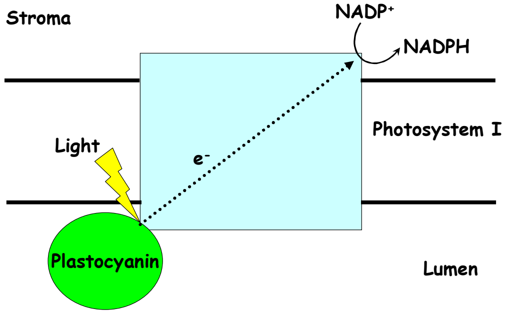
Photosynthesis: Light reactions



Photosynthesis: Light reactions



Photosynthesis: Light reactions



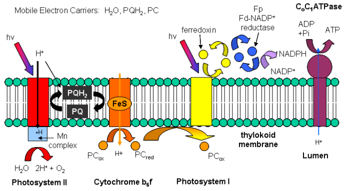
Photosynthesis: Light reactions

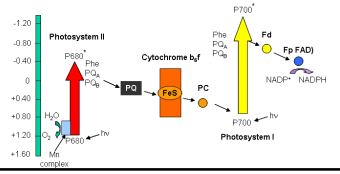
- **Products:**
  - Oxygen - released, essential for most life on earth
  - Proton motive force - used for ATP synthesis
  - **NADPH – used in biosynthesis, the Calvin cycle**

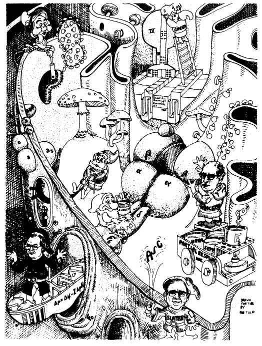
Photosynthesis: Light reactions

- Two light absorbing stages:
  - Photosystem II
  - Photosystem I
- Electron transport chains – several complexes of proteins
- **Soluble carriers:**
  - Quinone (Q), lipid soluble
  - **Plastocyanin, water soluble**

Photosynthesis: Light reactions







Some of the senior figures in the history of oxidative phosphorylation in action. Racker assembles the cold-labile F_1-ATP synthase (negatively stained with phosphotungstate), while Mitchell juggles protons and charges, Slater attempts to grasp the elusive squiggle (the non-existent chemical intermediate that was anticipated before the advent of the chemiosmotic theory), and Boyer induces a conformational strain which has proved to be more important in the ATP synthase itself rather than in coupling electron transport to ATP synthesis

Mitochondria related websites:
Mitochondria Research
http://www.mitochondrial.net/

Complex I home page
http://www.scripps.edu/mem/ci/

Joel Weiner Complex II related webpage
http://www.biochem.ualberta.ca/weinerlab/FrdABCD.htm

The *bc*1 complex home page
http://www.life.illinois.edu/crofts/bc-complex_site/

The Cytochrome Oxidase home page
http://www-bioc.rice.edu/~graham/CcO.html

Boris Feniouk ATP synthase home page
http://www.atpsynthase.info/

Antony Crofts bioenergetics course
http://www.life.illinois.edu/crofts/bioph354/

Bioenergetics course Leeds University
http://www.bmb.leeds.ac.uk/illingworth/oxphos/