# Microbial energy metabolism

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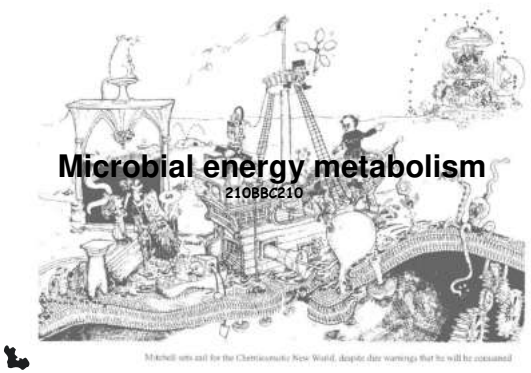

Mitchell sits sail for the Chemiosmotic New World, despite dire warnings that he will be consumed

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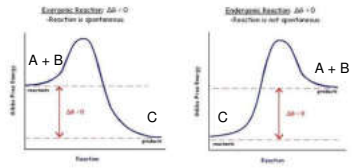
## A bit of chemistry
## Free energy change

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 $\rightarrow$, EXErgonic

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




## Free energy change

$\Delta G^o$ = 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)

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

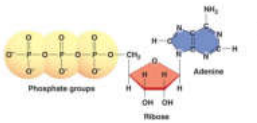
However, if [A]×[B] >>[C], real $\Delta G<0$ and reaction will go from left to right $\rightarrow$

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

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

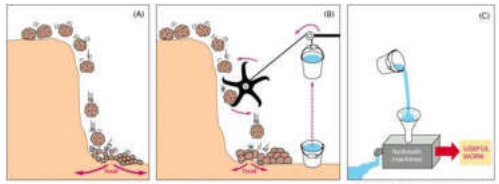
Enzymes accelerate the attainment of equilibrium, but not shift it or reverse reaction. Direction of the reaction is defined by $\Delta G$. Some of the biological reactions have $\Delta G^o>0$, but due to the concentration component (in logarithm) $\Delta G<0$.

## 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 + $P_i$ + *energy*

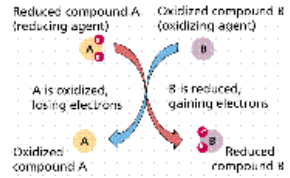



Burning (oxidation) of glucose to $CO_2$ and $H_2O$ produces heat only

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)




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

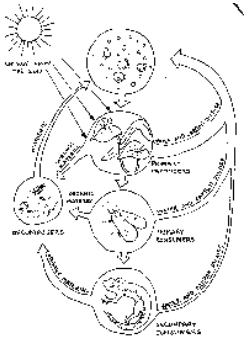
$A_{red} \rightarrow A_{ox} + 2e^-$

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

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

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

## 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

Animals →

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

← Plants

## Oxidative phosphorylation
History

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

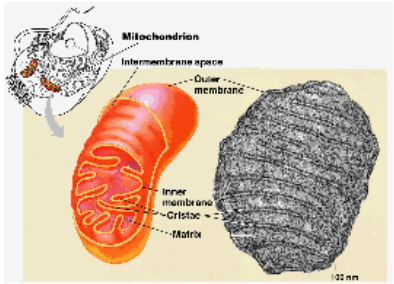
***Warburg*** vs ***Thunberg*** and ***Keilin*** - respiratory enzyme vs dehydrogenase

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

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

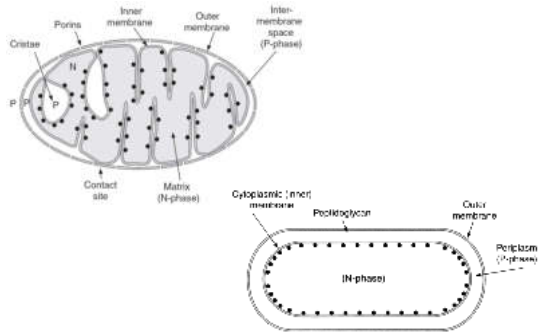
***Piter Mitchell*** – energy transduction in membranes Nobel Prize 1978

## Mitochondria – respiring organelle




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

## Membranes of mitochondria and bacteria

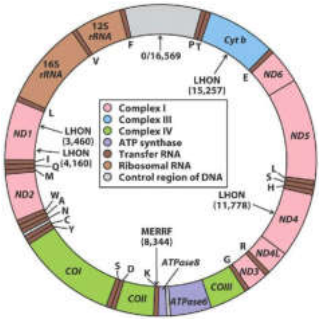



## What are mitochondria?

- 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

Cytoplasm

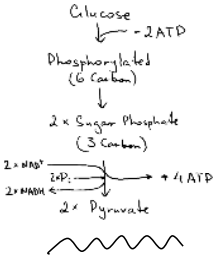
- Oxidative phosphorylation
  - Inner mitochondrial membrane

Plasma membrane

⇒ **Compartmentalisation**

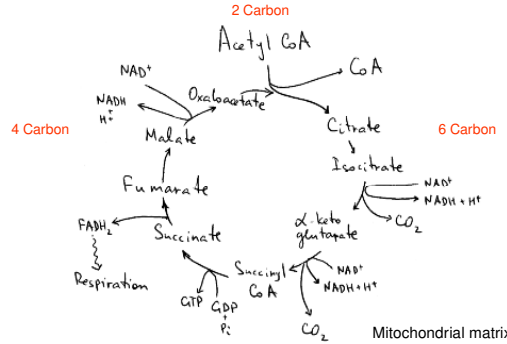
# Glycolysis

Cytoplasm



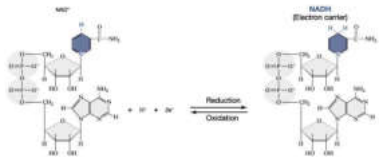

Mitochondrial matrix

# Krebs cycle (TCA cycle)




Mitochondrial matrix

# Electron transferring coenzyme




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

**After glycolysis and TCA cycle**

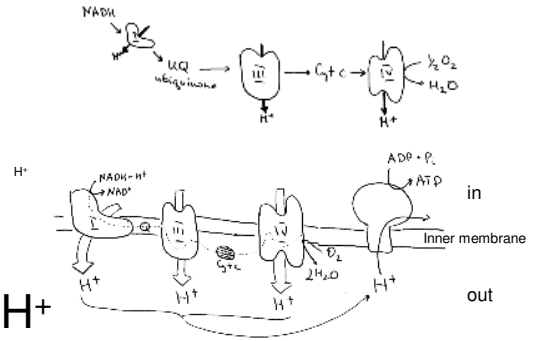
Not much ATP formed
Lots of reduced coenzymes
Per glucose molecule:
- 10 NADH
- 2 $FADH_2$ (!!!)

**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

## Electron transport chain and ATP synthase

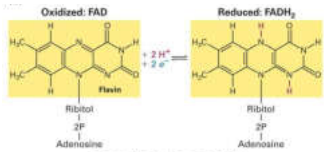



## Redox centres

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

## Flavin

Complex I
Complex II = succinate dehydrogenase from Krebs cycle




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

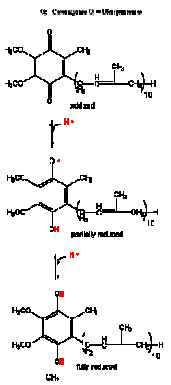
Usually serves as intermediate of electron transfer between 2e- donor and 1e- acceptor
No free flavins in a cell !!!

## 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



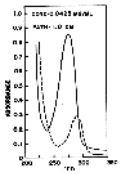
2 electrons + 2H+

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

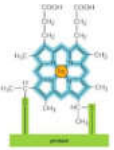
Proton-translocating Q-cycle in complex III

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

Menaquinone and rhodoquinone in some bacteria and plastoquinone in chloroplasts



## Cytochromes



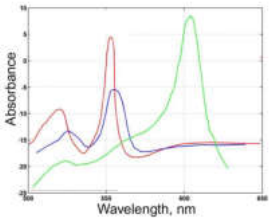
Cytochrome *c*

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

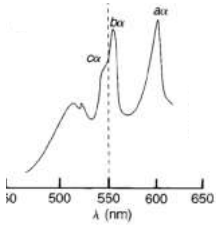
Protein part and haem part containing Fe ion
Cytochromes *a*, *b*, *c* … + number
As separate proteins (e.g. cytochrome c) or as subunits of enzymatic complexes (Complex II, III, IV)

## Respiratory chain cytochromes

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




Reduced-oxidised spectra of mitochondrial membranes

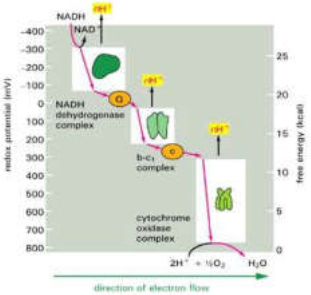



Easy to observe reduction/oxidation as change in optical spectra

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

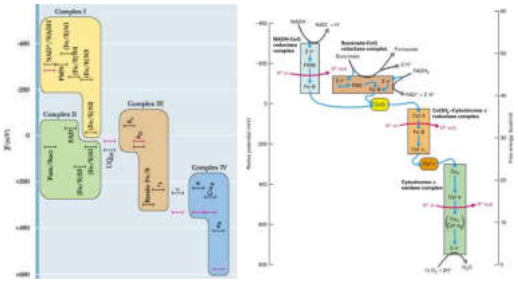
Hugo Theorell Nobel prize 1955
Redox enzymes and biological oxidation

## Electron transport chain

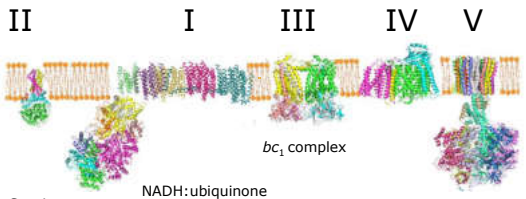



Values of redox potential of respiratory chain components fall downstream from NADH to oxygen.

## Electron transport chain



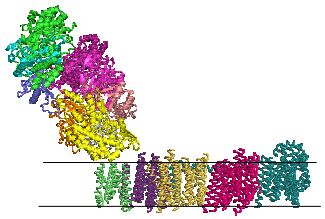
## Structure of respiratory chain



## 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

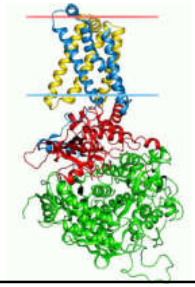
Tightly-bound semiquinones as intermediates of electron transfer

Classical inhibitors:

Rotenone, piericidine, $MPP^+$

## Complex II
## Succinate dehydrogenase of TCA cycle

Succinate + UQ ↔ Fumarate + $UQH_2$



Mammalian enzyme: 4 subunits

Bacterial enzyme: 4 subunits

Flavin = FAD

Cytochrome *b*

Three FeS clusters

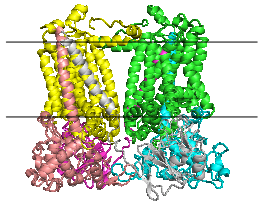
Classical inhibitors:

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

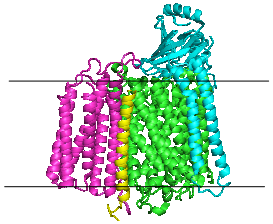
Classical inhibitors:

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

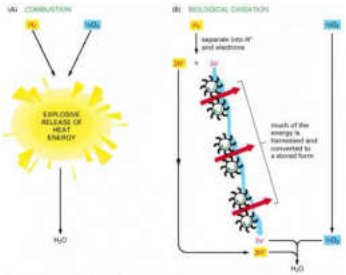
Cytochrome *a*, $a_3$,

Two copper $Cu_A$ $Cu_B$ centers

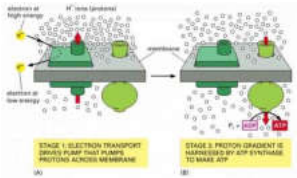
Classical inhibitors:

Cyanide, carbon monoxide, nitric oxide

## Oxidative phosphorylation



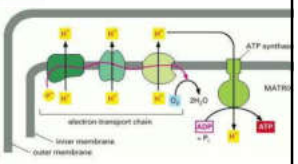
## Chemiosmotic theory



Peter Mitchell, Nobel Prize 1978

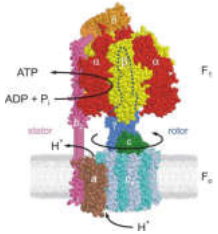
Electron transfer along electron transport chain is coupled with proton translocation at complexes I, II and IV.

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



## ATP synthase

Nobel prize 1997 John Walker

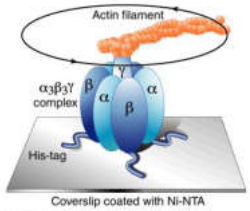



Bacterial enzyme: 8 subunits

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

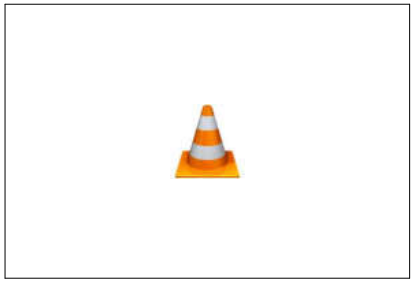
~10H$^+$ per 3 molecules of ATP

## ATP synthase

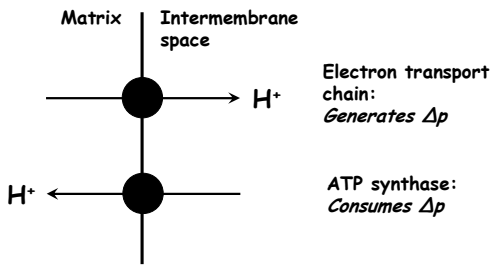



Yoshida Lab http://www.res.titech.ac.jp/~seibutu/
Kinosita Lab http://www.k2.phys.waseda.ac.jp/Movies.html

## ATP-synthase



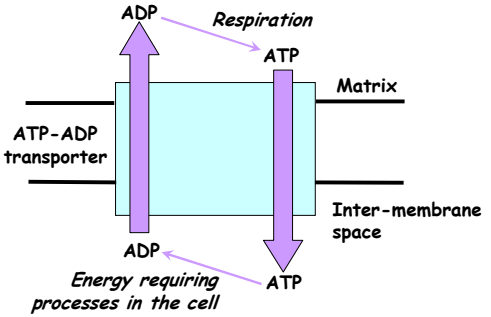
## ATP synthesis in mitochondria




## 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!)



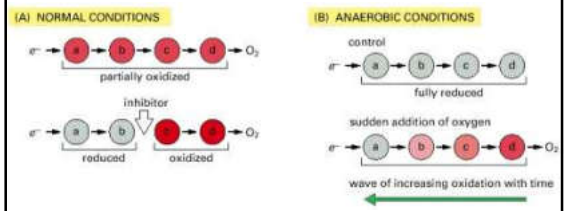
## Mitochondrial respiration

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

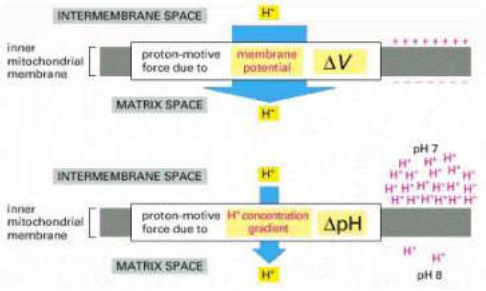
## How to study electron transport chain?



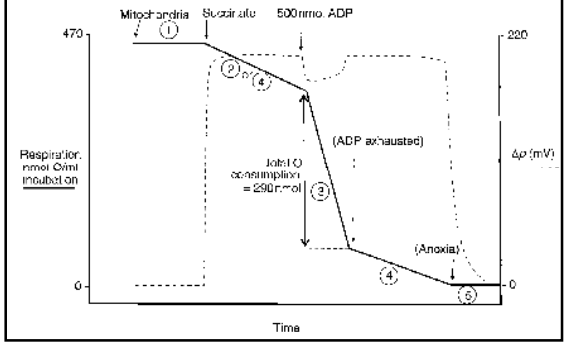

## Oxidative phosphorylation inhibitors

- 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

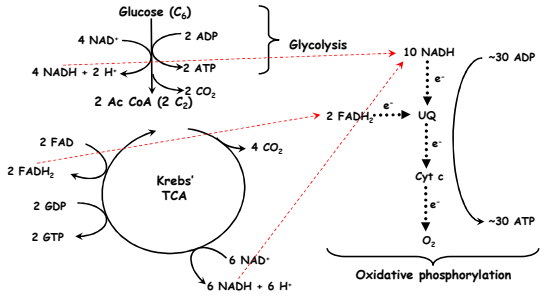
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

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

Oxygen:
Required at the end of oxidative phosphorylation

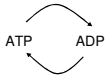
Glucose:
Chemical energy from food

## Aerobic respiration




## Aerobic respiration

(in mammals!)




Inner mitochondrial membrane ~1400m$^2$

We consume ~380 *l* of oxygen per day

ATP turnover ~60 kg/day

$3 \times 10^{23}$ $H^+$ per second through ATP synthase

90% ATP is synthesised during oxidative phosphorylation

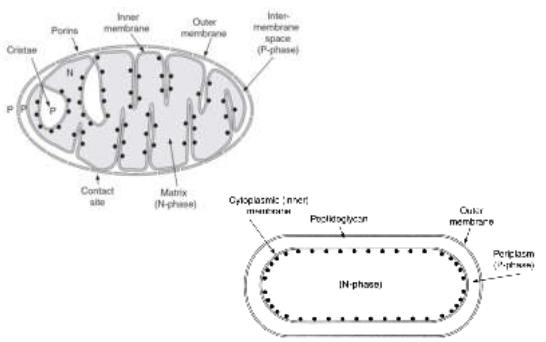
## Bacterial energy metabolism

Live in various environment

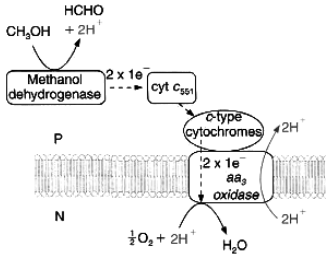
Able metabolise different substrates

Can adopt to the changing environment

## Membranes of mitochondria and bacteria




## What to do if food is scarce?



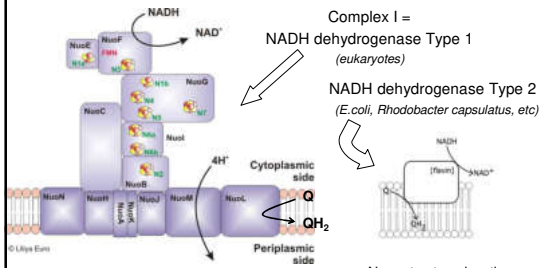

Some bacteria can grow on low carbon substrates like methanol as its sole source of energy. In *P. denitrificans* methanol is oxidised in periplasm and electrons are transferred directly to cytochrome oxidase

## 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

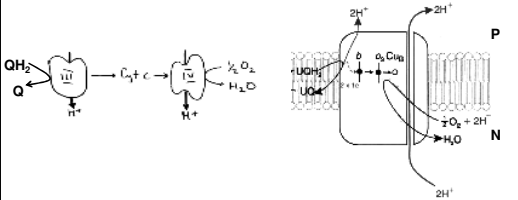
No proton translocation
(not even a membrane protein)

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

## Bypassing

$bc_1$ (III) and cytochrome *c* oxidase (IV) together (*in mitochondria*)

$bo_3$ oxidase (quinol oxidase)
*(E.coli, Burkholderia phymatum)*

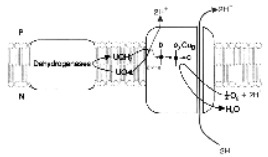



$2QH_2 + O_2 + 4H^+_{in} \leftrightarrow 2Q + 2H_2O + 4H^+_{out}$

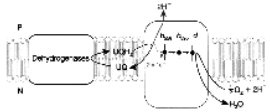
Direct oxidation of quinol by oxygen, bypassing $bc_1$ complex and cyt *c* + translocation of protons (but with lower efficiency)

## Bypassing and simplification

$bo_3$ oxidase (quinol oxidase)
*(E.coli, Burkholderia phymatum)*



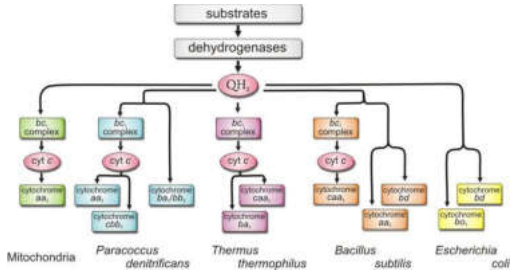
*bd* oxidase (quinol oxidase)
(no $H^+$ translocation)
*(E.coli, Klebsiella pneumoniae, Mycobacterium tuberculosis )*



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

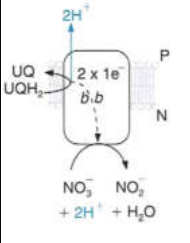
Direct oxidation of quinol by oxygen, bypassing $bc_1$ complex and cyt *c* + but no (!!!) translocation of protons

## Scheme of Respiratory Chains of mitochondria and bacteria

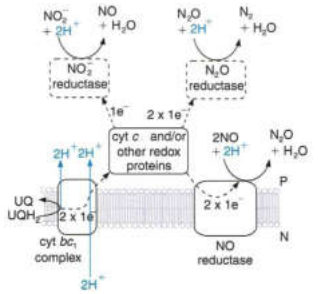



## What to do if the oxygen is absent?

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

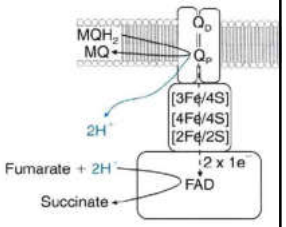
↓

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

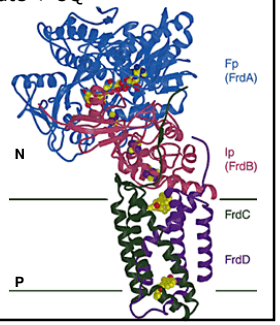
Fumarate + $UQH_2$ ↔ Succinate + UQ

Very similar to mitochondrial succinate dehydrogenase

4 subunits

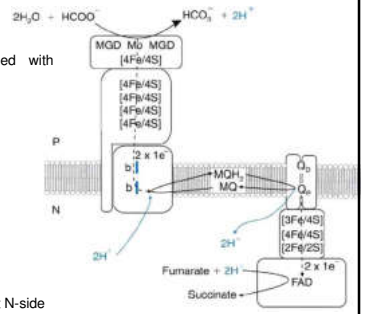
Flavin – FAD

3 FeS clusters




## How to generate proton gradient without actual proton translocation?

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

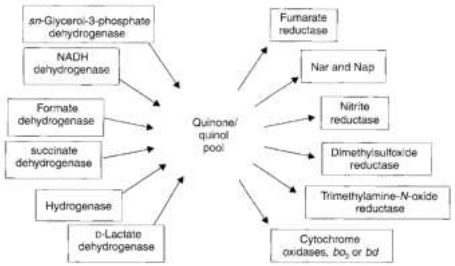



Net reaction:

release $2H^+$ at P-side

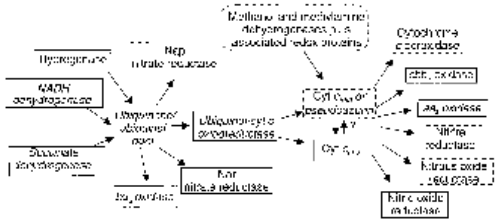
consumption of $2H^+$ at N-side

## *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



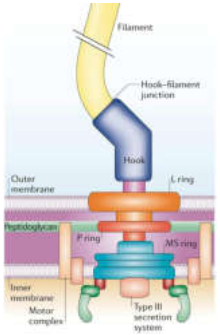
The components in italics are constitutive. The other components are induced at appropriate growth conditions and are unlikely to be all present at once. Continuous boxes indicate integral membrane components; dashed lines represent periplasmic components. NADH dehydrogenase, succinate dehydrogenase, 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.

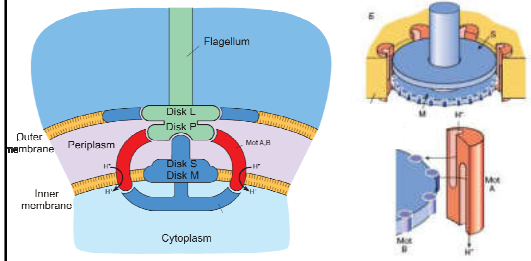
Sodium bioenergetics: *Halophilic bacteria, Vibrio cholerae, Yersinia pestis*

## Flagellum = whip




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

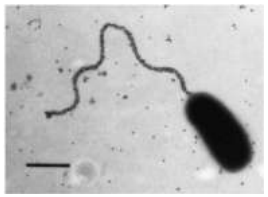
## How does it work?



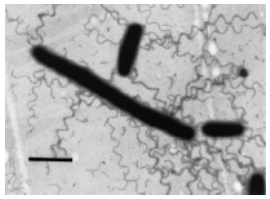

## Using two fuels

*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

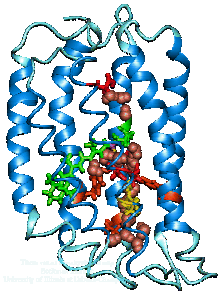


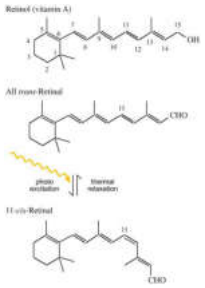
Proton motive force



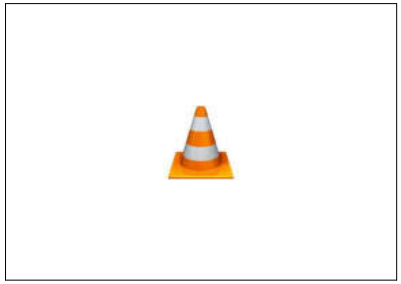
## How to use light ?

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

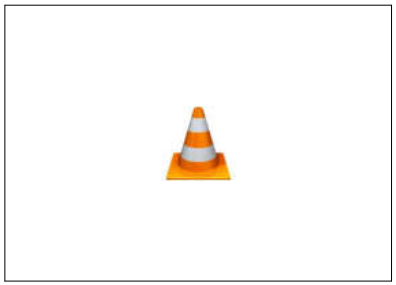




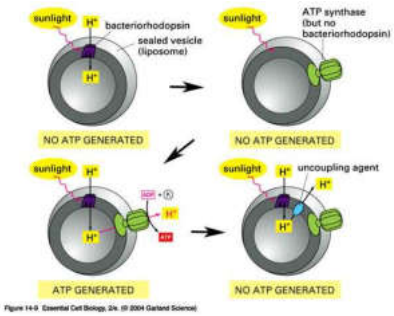
## Bacteriorhodopsin



## Bacteriorhodopsin



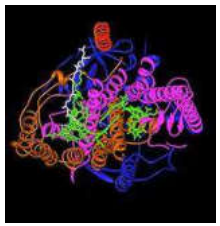
## Proof of chemiosmosis




Photosynthesis in bacteria and light phase in chloroplasts

## Photosynthesis in bacteria

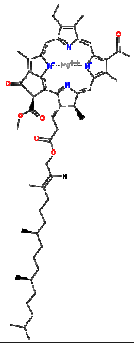
No mitochondria, no chloroplasts ⇒ everything is located in the same membrane!



Hartmut Michel Nobel prize 1988
Structure of photosynthetic reaction centre

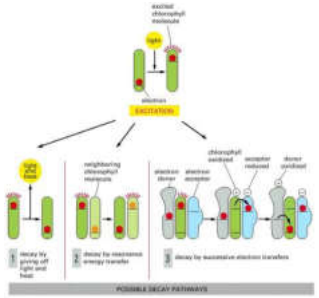
## Bacteriochlorophyll and carotenoids

*(in light harvesting complexes)*



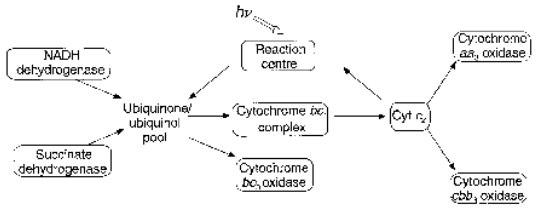
$P^* \xrightarrow{-e^-} P^+ \xrightarrow{+e^-} P$

Carotenoids

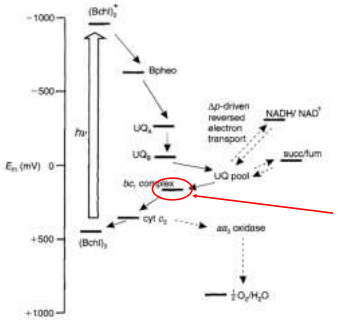


The light energy absorbed by an isolated pigment molecule is completely released as light and heat by process 1. In photosynthesis, by contrast, pigment undergo process 2 in the antenna complex and process 3 in the reaction center.

## Light driven cyclic electron transfer
*(Rh. Sphaeroides)*

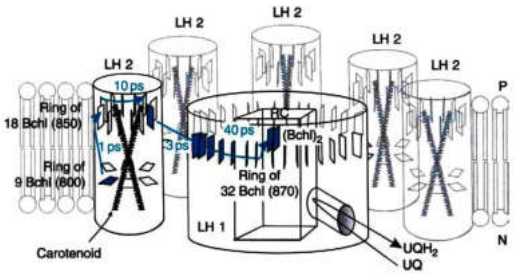



## Electron transfer in *Rh. Sphaeroides*



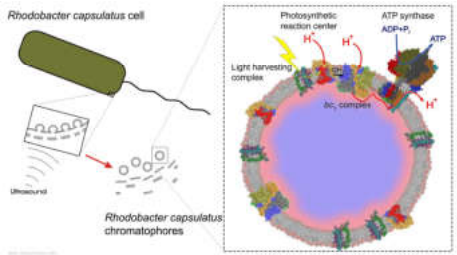

H⁺-translocation by complex III

## 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

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

Photosynthetic organisms

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

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

Animals

Oxidation of food to obtain energy

Animals

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

Plants

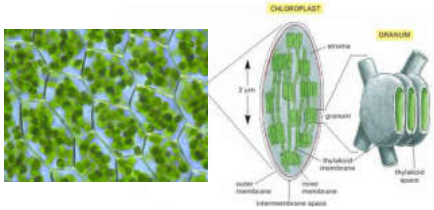
## Photosynthesis

- 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**

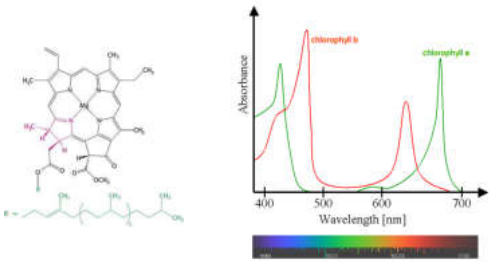
## Photosynthesis

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

## Photosynthesis takes place in chloroplasts




## Chlorophyll

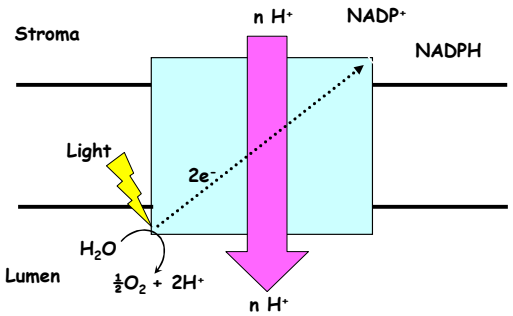



Like heme, chlorophyll *a* is a cyclic tetrapyrrole. One of the pyrrole rings (shown in red) is reduced. A phytol chain (green) is connected by an ester linkage. Magnesium ion binds at the center of the structure.

## 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

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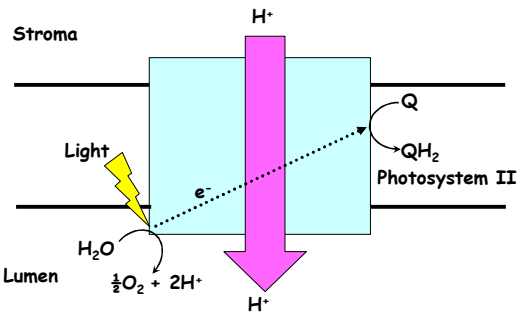

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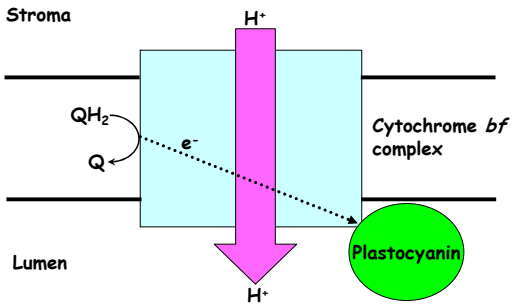


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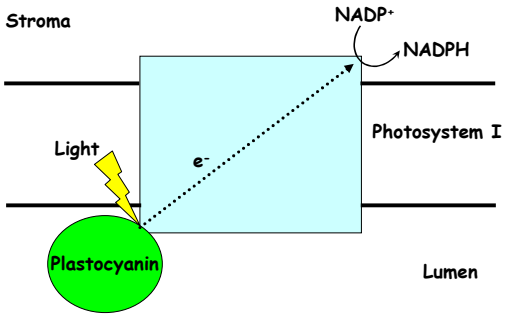


## Photosynthesis: Light reactions

- **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:
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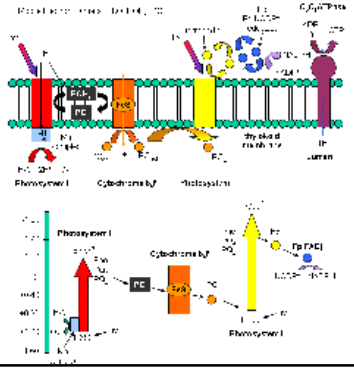
## Photosynthesis: Light reactions



**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/