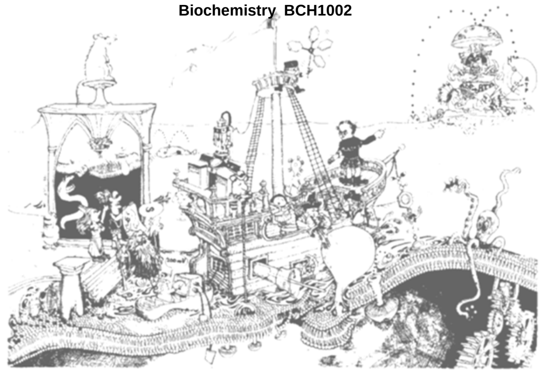
Energy metabolism

Biochemistry BCH1002



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

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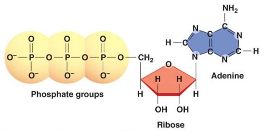
Lectures Outline

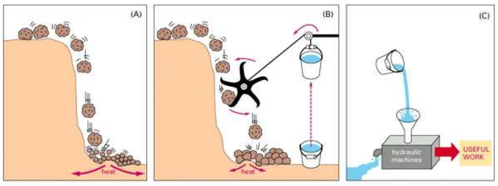
1. Oxidative phosphorylation
2. Chemiosmotic theory
3. Photosynthesis (light phase)

A bit of chemistry



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

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

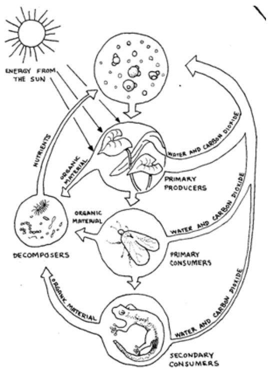


Burning (oxidation) of glucose to CO_2 and H_2O produces heat only

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

ATP can be used to drive any cellular processes

Photosynthesis and Respiration
(energy conversion)



Reduction of oxygen

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

Photosynthetic organisms

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

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

Animals

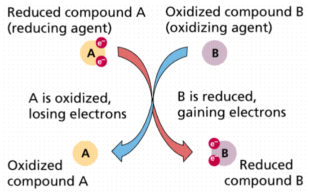
Oxidation of food to obtain energy

Animals →

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

← Plants

Redox reactions
(reduction-oxidation reactions)



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

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

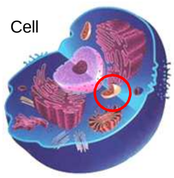
Midpoint redox potential (E^o) is a tendency of A_{red} to donate electrons

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

Where is it happening?

Adult consumes around 380 liters of O_2 each day
(top athletes can sustain 10 fold greater rate)

Cell

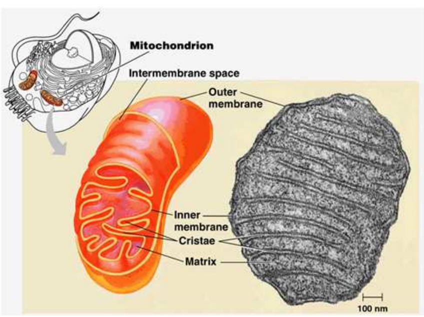


Mitochondria

95-99% of oxygen we breathe in is consumed by mitochondria

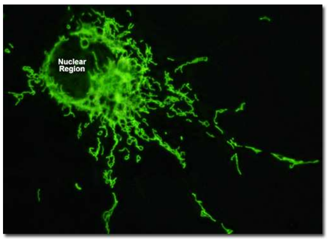
~10[15] mitochondria in our body ~ 10% of the body weight

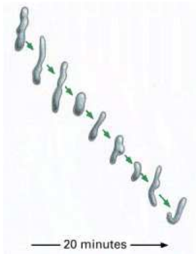
Mitochondria – respiring organelle



Contain its own DNA and its own transcription/translational system ⇒ probably are result of a symbiosis

Mitochondrial plasticity and mitochondrial network





Tracking mitochondria in live cell

Rhodamin-like dye

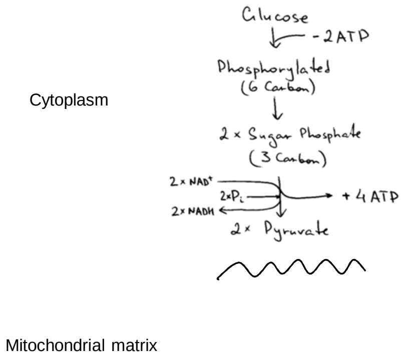
Green fluorescent protein

Locations

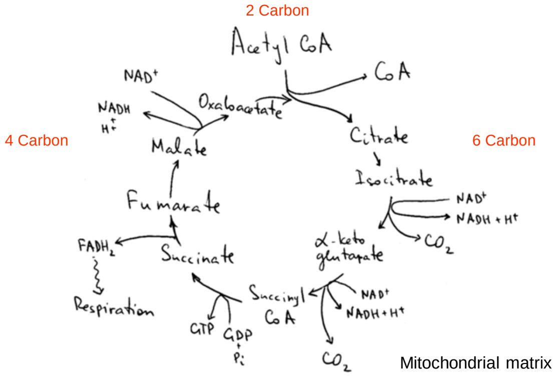
- Glycolysis
  - Cytoplasm
- Krebs' TCA
  - Mitochondrial matrix
- Oxidative phosphorylation
  - Inner mitochondrial membrane

⇒ **Compartmentalisation**

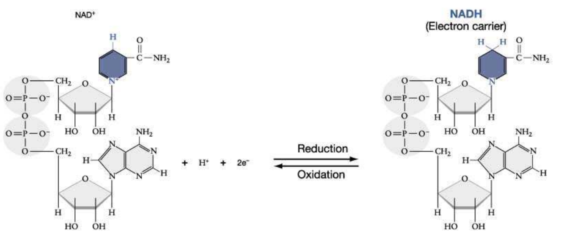
Glycolysis



Krebs cycle (TCA cycle)



Electron transferring coenzyme



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

After glycolysis and TCA cycle

Not much ATP formed

Per glucose molecule:

10 NADH (+ do not forget about succinate!!)

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

Oxidative Phosphorylation

Oxidative phosphorylation

History

W. A. Engelgardt, 1936-39 - measured inorganic and organic phosphate content
Definition of term "oxidative phosphorylation"

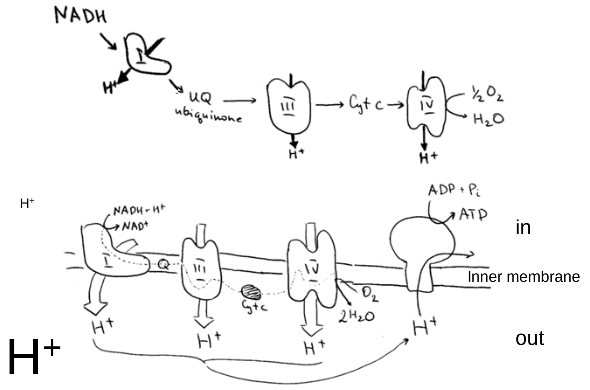
Warburg vs ***Thunberg*** and ***Keilin*** - respiratory enzyme vs dehydrogenase

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

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

Peter Mitchell – energy transduction in membranes Nobel Prize 1978

Electron transport chain and ATP synthase



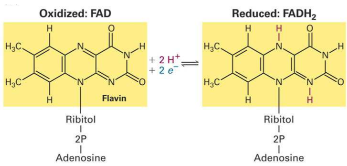
Electron transport chain and ATP synthase

Redox centres

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

Flavin

Complex I
Complex II = succinate dehydrogenase from Krebs cycle

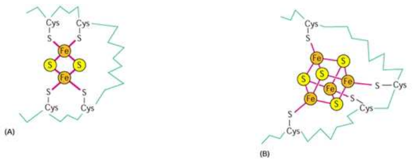


FAD + 2e$^-$ +2H$^+$ $\leftrightarrow$ FADH$_2$

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

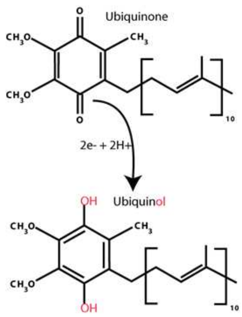
FeS clusters

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$^+$

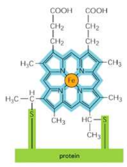
Membrane mobile redox carrier linking Complexes I and II with Complex III

Proton-translocating Q-cycle in complex III

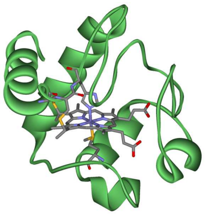
Different n for different species

Menaquinone and rhodoquinone in some bacteria and plastoquinone in chloroplasts

Cytochromes



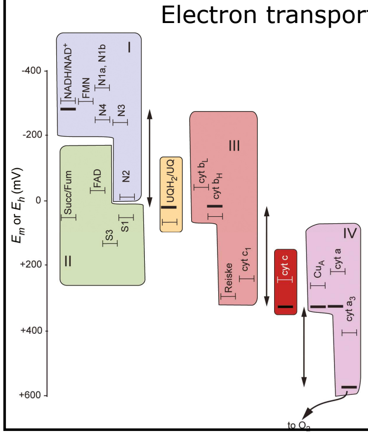
Cytochrome *c*



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)

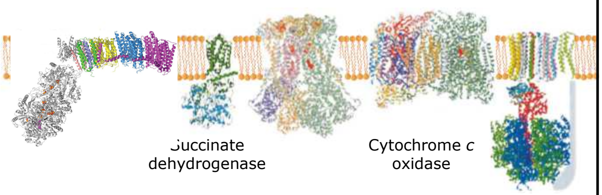
Electron transport chain



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

Structure of mitochondrial respiratory chain complexes

I II III IV V



Succinate dehydrogenase

Cytochrome *c* oxidase

NADH:ubiquinone oxidoreductase

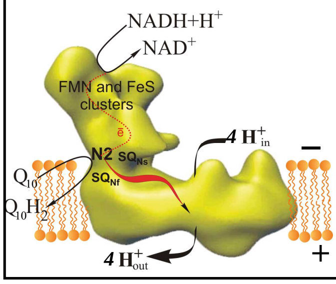
bc_1 complex

ATP synthase

Complex I (NADH:ubiquinone oxidoreductase)

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

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



Mammalian enzyme:

44 subunits

Flavin = FMN

8 FeS clusters

Tightly-bound semiquinones as intermediates of electron transfer

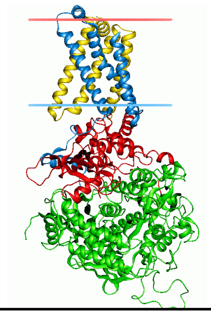
Bacterial enzyme:
14-16 subunits

Classical inhibitors:

Rotenone, piericidine, MPP^{+}

Complex II
Succinate dehydrogenase of TCA cycle

$Succinate + UQ \leftrightarrow Fumarate + UQH_2$



Mammalian enzyme:

4 subunits

Flavin = FAD

Cytochrome *b*

Three FeS clusters

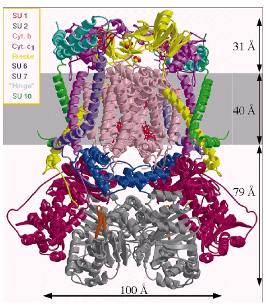
Classical inhibitors:

Malonate, Oxaloacetate

Complex III
bc$_1$ complex

$UQH_2 + 2Cyt\ c\ (Fe^{3+}) + 2H^{+}_{in} \leftrightarrow$

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



Mammalian enzyme:

11 subunits

Cytochromes c_1, b_L and b_H

Rieske protein (2Fe2S cluster)

Tightly-bound semiquinones as intermediates of electron transfer

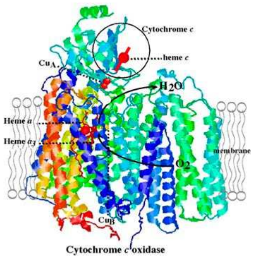
Bacterial enzyme:
4 subunits

Classical inhibitors:

Antimycin A, myxothiazol

Complex IV
Cytochrome *c* oxidase

$$4Cyt\ c\ (Fe^{2+}) + 4H^{+}_{in} + 4H^{+}_{in} + O_2 \leftrightarrow$$
$$\leftrightarrow 2Cyt\ c\ (Fe^{3+}) + 2H_2O + 4H^{+}_{out}$$



Mammalian enzyme:

13 subunits

Bacterial enzyme: 2-3 subunits

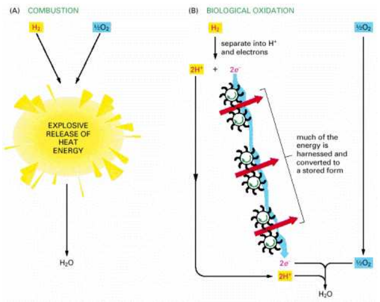
Cytochrome *a*, a_3,

Two copper Cu_A Cu_B centers

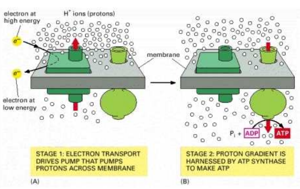
Classical inhibitors:

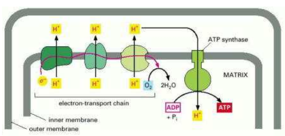
Cyanide, carbon monoxide, nitric oxide

Oxidative phosphorylation



Oxidative phosphorylation

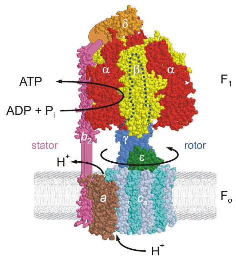




Electron transfer along electron transport chain is coupled with proton translocation at complexes I, II and IV.

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

ATP synthase



ADP + P_i + $nH^+_{out} \leftrightarrow$ ATP + nH^+_{in}

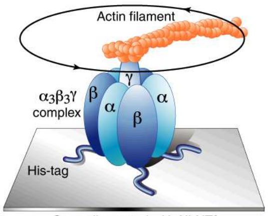
Proton flow through F_o part is coupled with ATP synthesis in the F_1 part

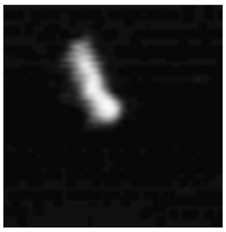
8-10H^+ per 3 molecules of ATP

Reversibility: proton flow from IM to matrix drives ATP-synthesis from ADP and P_i

ATP hydrolysis drives proton pumping from matrix to IM

ATP synthase

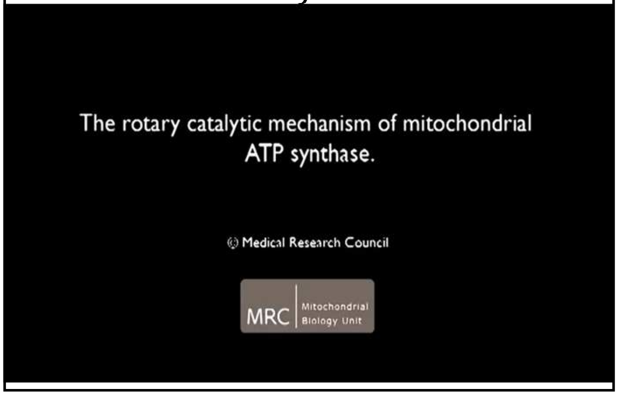




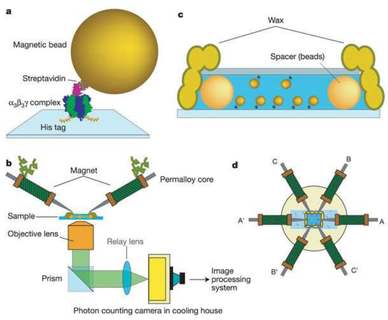
Yoshida Lab http://www.res.titech.ac.jp/~seibutu/
Kinosita Lab http://www.k2.phys.waseda.ac.jp/Movies.html

ATP synthase

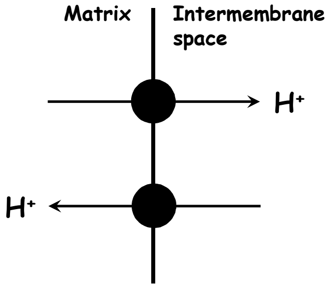
ATP-synthase



Mechanically driven ATP synthesis by F1-ATPase



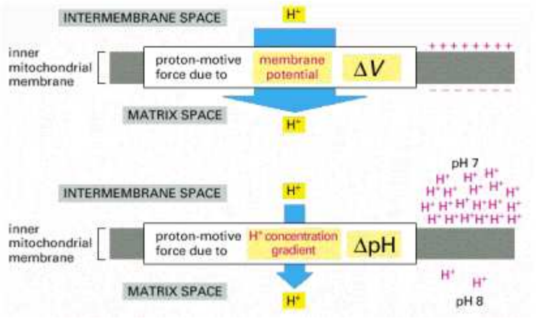
ATP synthesis in mitochondria



Electron transport chain:
Generates Δp

ATP synthase:
Consumes Δp

Protonmotive force



Proof of chemiosmosis

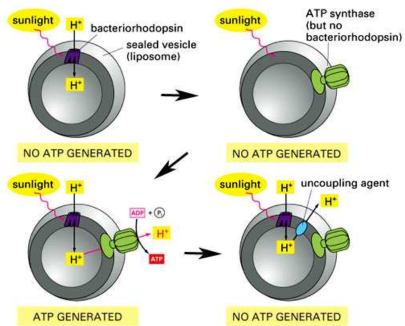


Figure 14-9 Essential Cell Biology, 2/e. (© 2004 Garland Science)

ATP synthesis

ATP synthesis in mitochondrial matrix

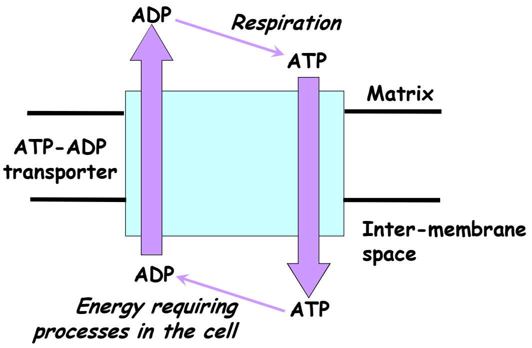
Needs to be transported out of mitochondria

Requires ATP-ADP transporter

Integral membrane protein

ATP and ADP transport coupled

ATP transport



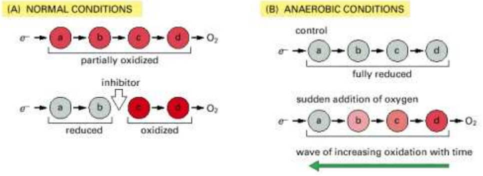
Mitochondrial respiration

History: Isolated mitochondria + substrates + oxygen

Some compounds block oxygen consumption – respiration inhibitors

Some compounds stop ATP synthesis but not respiration, they uncouples respiration and ATP synthesis – uncouplers

How to study electron transport chain?



Oxidative phosphorylation inhibitors

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

Aerobic respiration

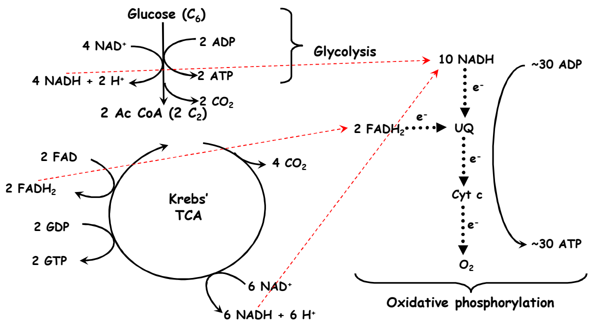
$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

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

$$Glucose + 6O_2 \rightarrow 6CO_2 + 6H_2O + energy$$

Glycolysis

$$Glucose + 2ADP + P_i \rightarrow 2\ Pyruvate + 2ATP$$

Krebs cycle

$$Pyruvate + CoA + NAD^+ \rightarrow Acetyl\text{-}CoA + CO_2 + NADH + H^+$$

$$Acetyl\text{-}CoA + 3NAD^+ + Q + GDP + P_i + 2H_2O \rightarrow 2\ CO_2 + 3NADH + QH_2 + GTP + 2H^+ + CoA$$

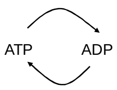
Oxidative phosphorylation

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightarrow H_2O + NAD^+ + \Delta p \qquad xADP + xP_i + \Delta p \rightarrow xATP$$

$$xADP + xP_i + \tfrac{1}{2}O_2 + H^+ + NADH \rightarrow xATP + H_2O + NAD^+$$

⇒6 molecules of O_2 per one glucose
⇒30 molecules of ATP is formed during aerobic metabolism of one glucose molecule

Aerobic respiration



Inner mitochondrial membrane ~1400m^2

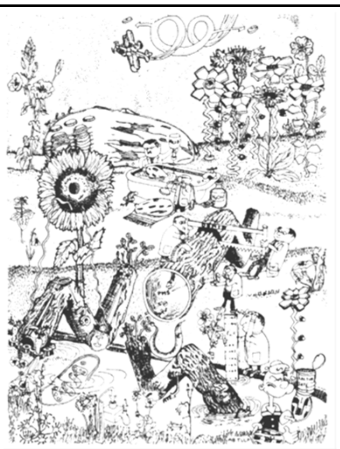
We consume ~380 *l* of oxygen per day

ATP turnover ~60 kg/day

NADH turnover in mitochondria ~85 kg/day

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

90% ATP is synthesised during oxidative phosphorylation



Photosynthesis

Light phase

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

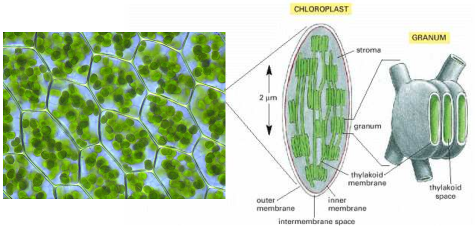
Photosynthesis

- Light reactions:
  - Need light to occur
  - Capture of light energy
  - Generation of pmf and reducing power ($NADPH_2$)
- **Dark reactions:**
  - Occur in light and dark
  - **Carbohydrate synthesis**

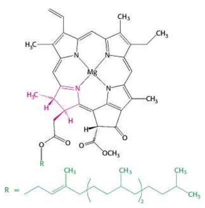
Photosynthesis

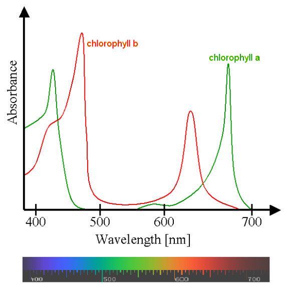
- $6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$
- Occurs in specialised organelles – chloroplasts
- **Light captured by chlorophyll**
  - Porphyrin
  - Contains Mg^{2+}
  - **Green**

Photosynthesis takes place in chloroplasts

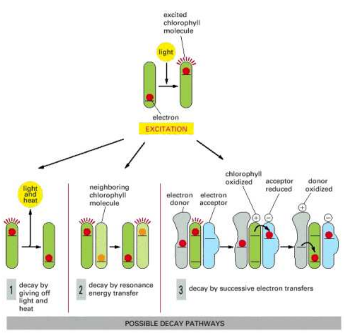


Chlorophyll





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.

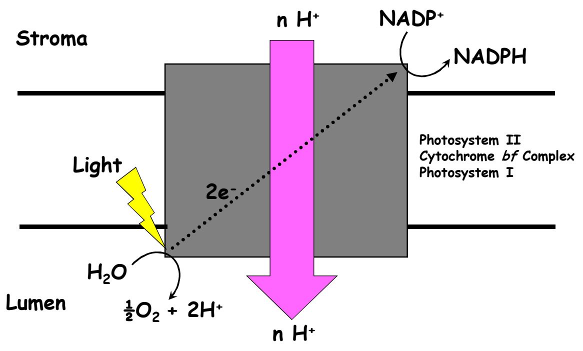


The light energy absorbed by an isolated chlorophyll molecule is completely released as light and heat by process 1. In photosynthesis, by contrast, chlorophylls undergo process 2 in the antenna complex and process 3 in the reaction center, as described in the text.

Photosynthesis: Light reactions

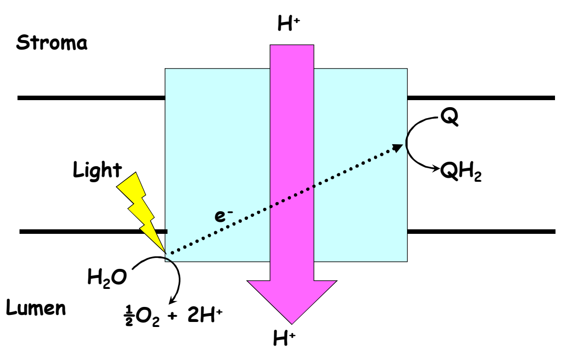
- Two light absorbing stages:
  - Photosystem II
  - Photosystem I
- Electron transport chains – several complexes of proteins
- **Soluble carriers:**
  - Plastoquinone (Q), lipid soluble
  - **Plastocyanin, water soluble**

Photosynthesis: Light reactions



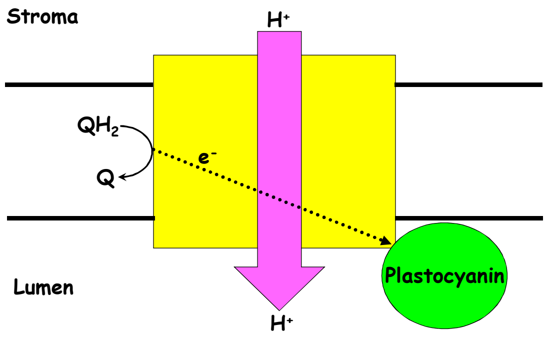
Photosynthesis: Light reactions

Photosystem II



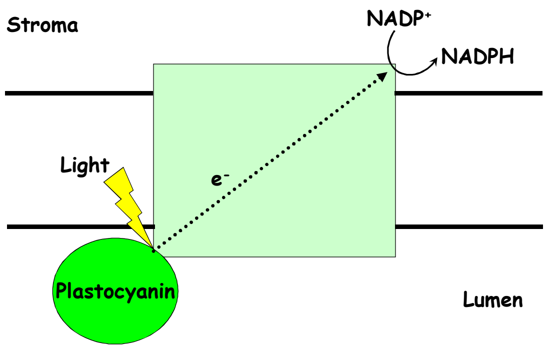
Photosynthesis: Light reactions

Cytochrome bf complex



Photosynthesis: Light reactions

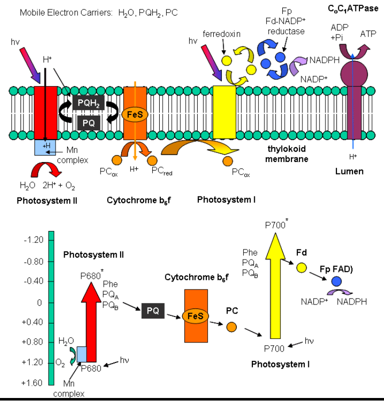
Photosystem I

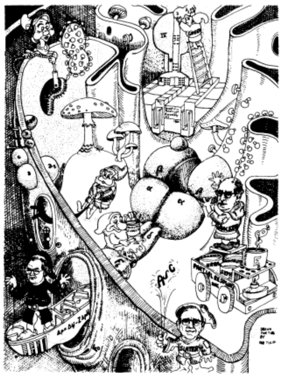


Photosynthesis: Light reactions

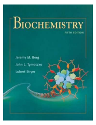
- **Products:**
  - Oxygen - released, essential for most life on earth
  - Proton motive force - used for ATP synthesis
  - **NADPH – used in biosynthesis, the Calvin cycle**

Photosynthesis: Light reactions



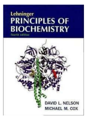


Some of the senior figures in the history of oxidative phosphorylation in action. Racker assembles the cold-labile F_1-ATP synthase (negatively stained with phosphotungstate), while Mitchell juggles protons and charges, Slater attempts to grasp the elusive squiggle (the non-existent chemical intermediate 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



Biochemistry

(Berg, Tymoczko & Stryer)



Principles in Biochemistry

Lehninger A

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/