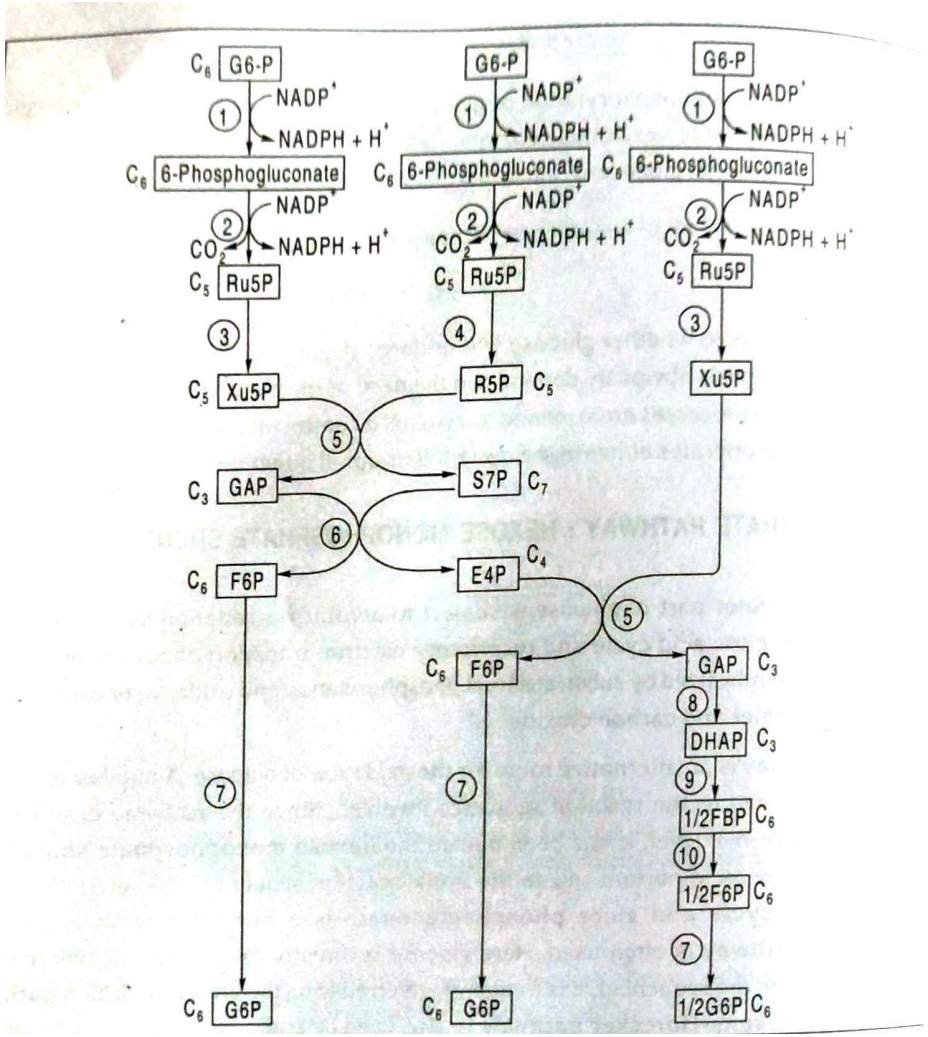
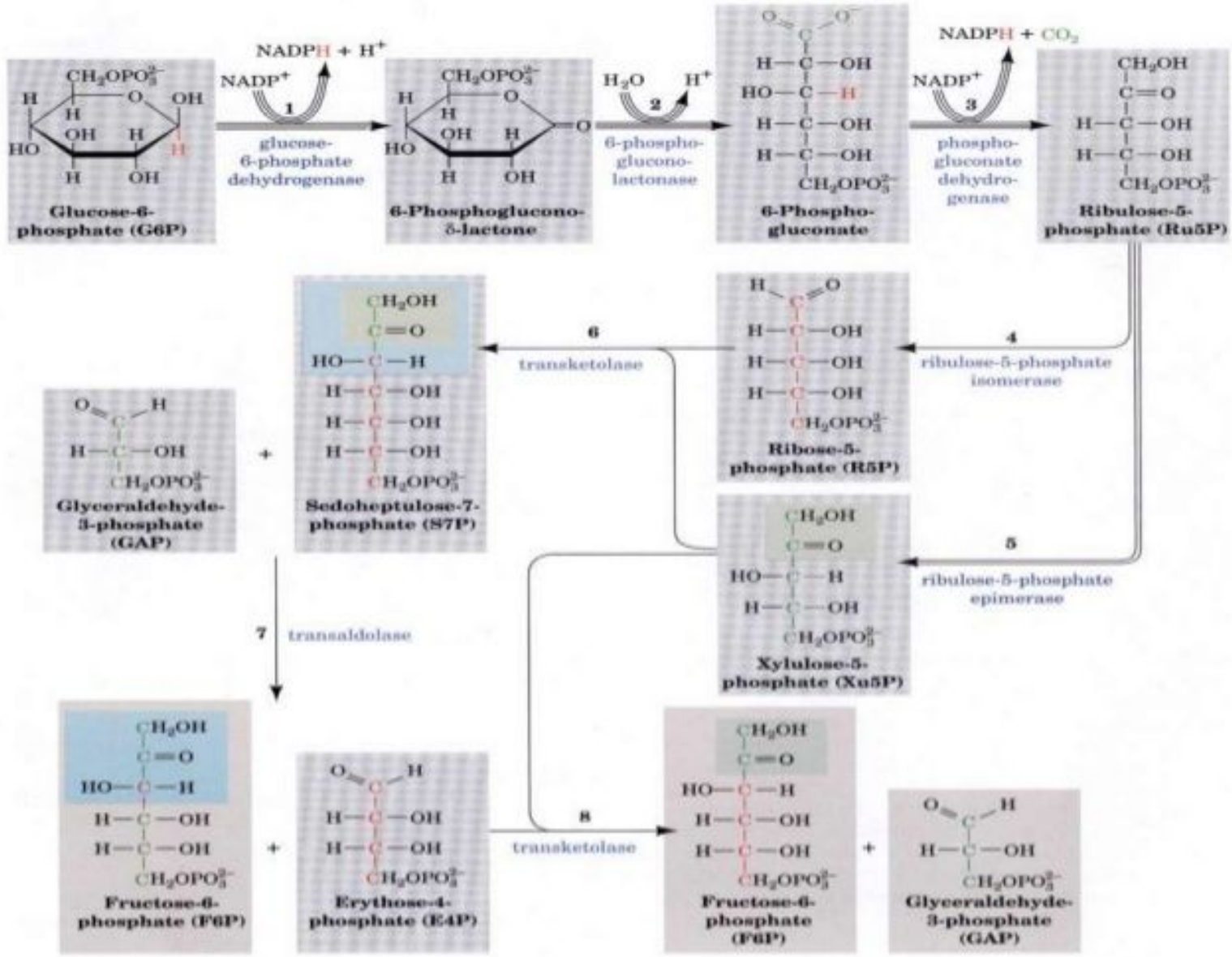
Respiration

Oxidative Pentose Phosphate pathway

- **Oxidative pentose phosphate pathway** is an alternative pathway to oxidise glucose in the cytosol, other than glycolytic pathway
- It is also known as **hexose monophosphate shunt** as it diverge from glycolysis at G-6-P level
- It is also termed phosphogluconate pathway as phosphogluconate is a key intermadiate
- The first two reactions of this series represent the oxidative events of this pathway where 6 carbon G-6-P is converted to 5 carbon RUMP with loss of CO_2 and generation of NADPH
- The remaining reactions of the pathway convert Ribulose-5-P to the glycolytic intermediates such as glyceraldehyde-3-P and Fructose-6-P
- Though PPP plays very little role in total carbon flux of plant tissues its presence is important during transition from meristematic to more differentiated state
- PPP differs from glycolysis in utilising $NADP^+$ and not NAD^+ as the hydrogen acceptor
- PPP is characterised by CO_2 production while it is not produced at all in glycolytic pahway

- Oxidation of glucose takes place in two phases-oxidative phase and non-oxidative phase
- At first phase G-6-P is oxidised at carbon 1 by NADP dependent dehydrogenase to phosphogluconolactone which is either spontaneously or enzymatically is converted to phosphogluconic acid
- During the non-oxidative phase the ribulose-5-P, developed from phosphogluconate, is further metabolised by a series of reactions involving interconversions of 3-,4-, 5-, 6- and 7-carbon monosaccharides
- Ribulose 5-P is converted to ribose 5-P by isomerase while epimerase convert it to xylulose 5-P
- Enzyme transketolase, with coenzyme thiamine pyrophosphate and Mg2+ ions, convert the above two 5 carbon components into glyceraldehyde-3-P and sedoheptulase-7-P
- Next step is nediated by trans aldolase that convert G-3-P and S-7-P into fructose-6-P and erythrose-4-Pnext another transketolase mediated reaction occurs that E-4-P and Xu-5-P to F-6-P and GAP
- GAP my either enter glycolysis or regenerate a molecule of G-6-P




Glucose-6-phosphate (G6P)
NADPH + H+
NADP+
1
glucose-6-phosphate dehydrogenase
6-Phosphogluconо-δ-lactone
H2O
H+
2
6-phospho-gluconо-lactonase
6-Phospho-gluconate
NADPH + CO2
NADP+
3
phospho-gluconate dehydro-genase
Ribulose-5-phosphate (Ru5P)
4
ribulose-5-phosphate isomerase
Ribose-5-phosphate (R5P)
5
ribulose-5-phosphate epimerase
Xylulose-5-phosphate (Xu5P)
6
transketolase
Glyceraldehyde-3-phosphate (GAP)
Sedoheptulose-7-phosphate (S7P)
7
transaldolase
Fructose-6-phosphate (F6P)
Erythose-4-phosphate (E4P)
8
transketolase
Fructose-6-phosphate (F6P)
Glyceraldehyde-3-phosphate (GAP)

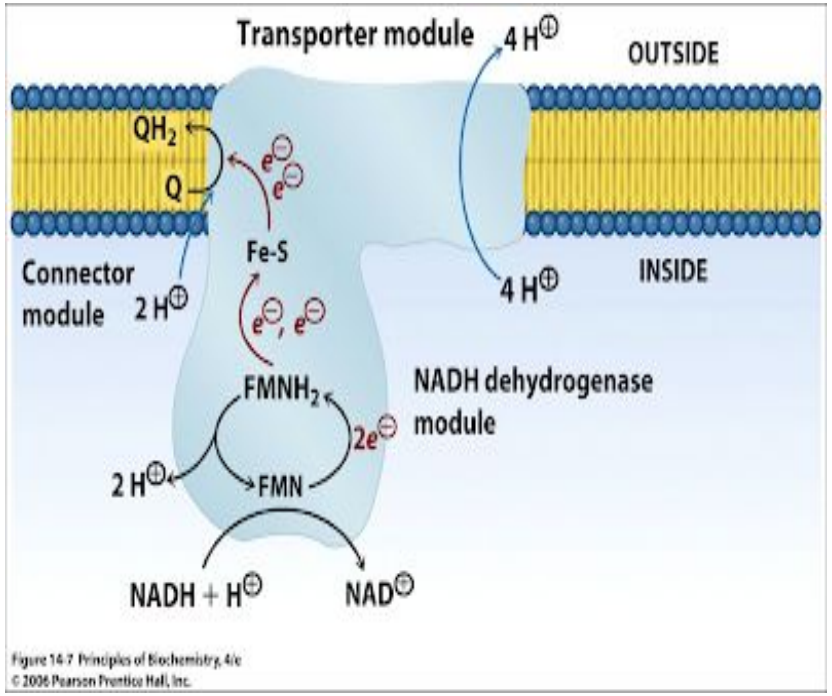
Significance of Pentose Phosphate Pathway

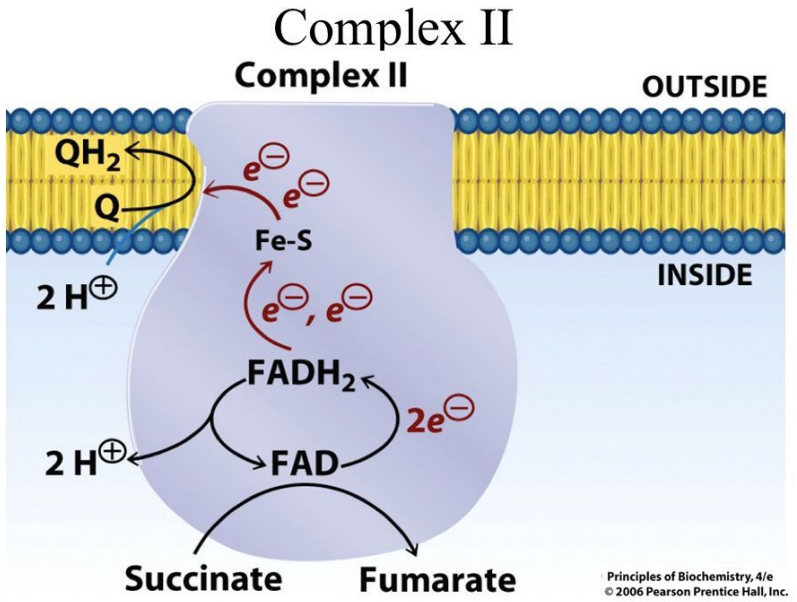
- The primary purpose of PPP is to generate reducing power in the extra mitochondrial cytoplasm in the form of NADPH, which is supposed to drive reduction steps associated with various biosynthetic reactions of cytosol
- Most of the NADPH produced can provide the reducing power for the synthesis of a series of compounds in cytosol like fatty acids, mevalonic acid and steroids that facilitate the conversion of pyruvate to OAA by malic enzymes
- As the NADH dehydrogenase present in the inner mitochondrial membrane is also capable of oxidising NADPH, some of it may be utilised to reduce O_2 and generate ATP
- During the early stage of greening, the oxidative pentose phosphate pathway is thought to be involved in generating Calvin cycle intermediates
- This process converts hexoses into pentoses (Ribose-5-P), which is a precursor of the riboses and deoxyriboses in the synthesis of DNA and RNA
- Another intermediate in this pathway, the 4 carbon erythrose-4-P, combines with the PEP in the initial reactions to produce plant phenolic compounds like aromatic amino acids, precursors of lignin, flavonoids and phytoalexins

Mitochondrial Electron Transport System

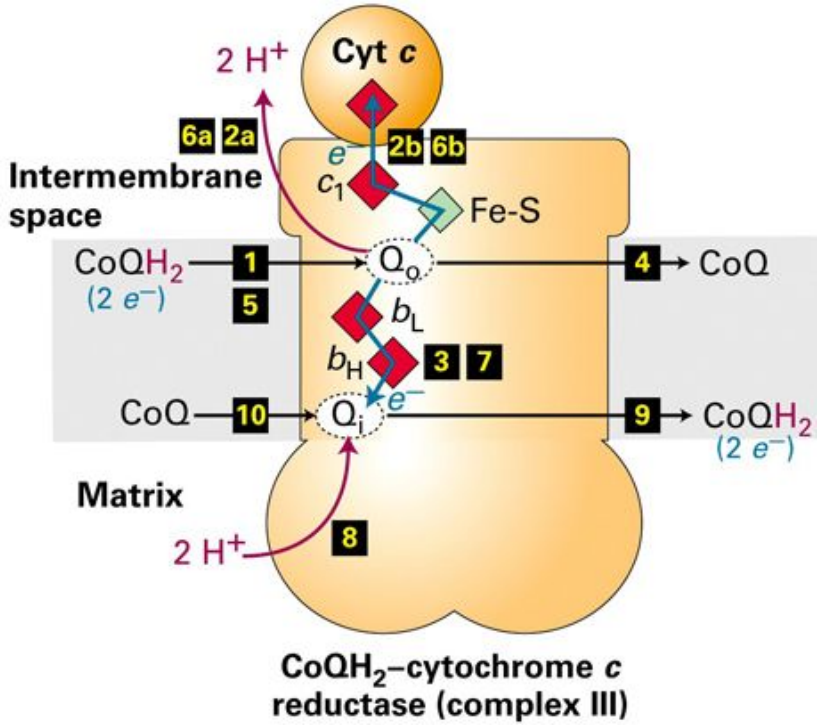
- The high energy electrons captured during TCA cycle in the form of NADH and $FADH_2$should be converted to ATP as that is the form of energy used by cells
- This conversion is an O_2 dependent process occurring in the inner mitochondrial membrane and involving series of electron carriers called **electron transport chain**
- ETC is composed of electron transport proteins, organised into a series of four multiprotein complexes (complex I-IV) located in the inner mitochondrial membrane
- For each molecule of glucose oxidised through glycolysis and TCA cycle, two molecules of NADH are generated in cytosol, 8 molecules of NADH and 2 molecules of $FADH_2$ appear in the mitochondrial matrix
- These reduced compounds must be reoxidised for uninterrupted occurrence of respiratory process
- The electron transport chain catalyses electron transfer from NADH and $FADH_2$ to oxygen, which is the final electron acceptor
- The equation for 2-electron transfer is as follows:
- $\mathrm{NADH} + \mathrm{H}^{+} + \frac{1}{2}\mathrm{O}^{2} = \mathrm{NAD}^{+} + \mathrm{H_2O}$
- Thus the role of electron transport chain is to oxidise NADH and $FADH_2$ and in the process utilise some of the free energy released to generate proton gradient across the mitochondrial membrane

- Electrons from NADH, generated in the matrix during TCA cycle are oxidised by complex I (NADH dehydrogenase)
- The electron carriers in complex I include a tightly bound cofactor (FMN, flavin mononucleotide) and several iron sulphur proteins
- The Fe-S proteins can be distinguished on the basis of their redox potential and their electron spin resonance (ESR)
- Complex I transfer electrons to Ubiquinone, a p-benzoquinone molecule that lies within the inner membrane and is not tightly associated with any protein
- The TCA cycle enzyme succinate dehydrogenase is the component of complex II
- Electrons derived from the oxidation of succinate are transferred via the $FADH_2$ and a group of three Fe-S proteins into the ubiquinone pool



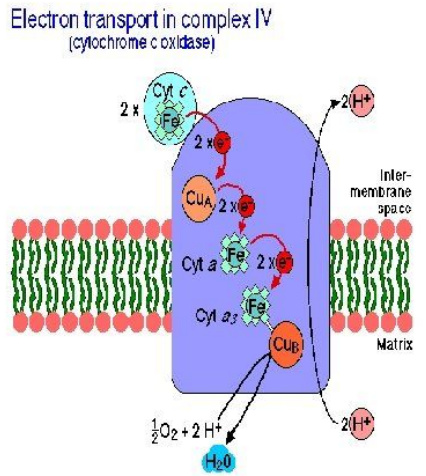


- Complex III acts as a ubiquinone- cytochrome C oxidoreductase
- It oxidises reduced ubiquinone and transfer electron to cytochrome C
- Electron transfer takes place through an Fe-S centre, two b type cytochromes (b_{565} and b_{560}) and a membrane bound cytochrome C_1
- Cytochrome C is the only protein in the electron transport chain that is not an integral membrane protein and serves as the mobile carrier to transfer electrons between complex III and IV
- Complex IV represent the cytochrome C oxidase
- It contains two copper centres (Cu_A and Cu_B) and cytochrome a and a_3
- Complex IV brings about the four electron reduction of O_2 to 2 molecules of H_2O
- An additional carrier named ubiquinone (Q) is present in the respiratory chain
- Q acts as a mobile component of the respiratory chin and collect electrons from fixed flavoproteins and transfers them to the fixed cytochromes



CoQH$_2$–cytochrome *c* reductase (complex III)

Complex IV



- **Cytochrome c oxidase**
- Transfers e^- from cytochrome c to O_2.

$\frac{1}{2} O_2 + 2 H^+ + 2 e^- \rightarrow H_2O$

The Q cycle funnels electrons from a two-electron carrier to a one-electron carrier and pumps protons

- QH2 passes two electrons to complex III, but the electrons acceptor cytochrome *c* can accept one electron

⇨ **Q cycle**

- Two QH_2 molecules bind to the complex consecutively, each giving up two electrons and two H^+

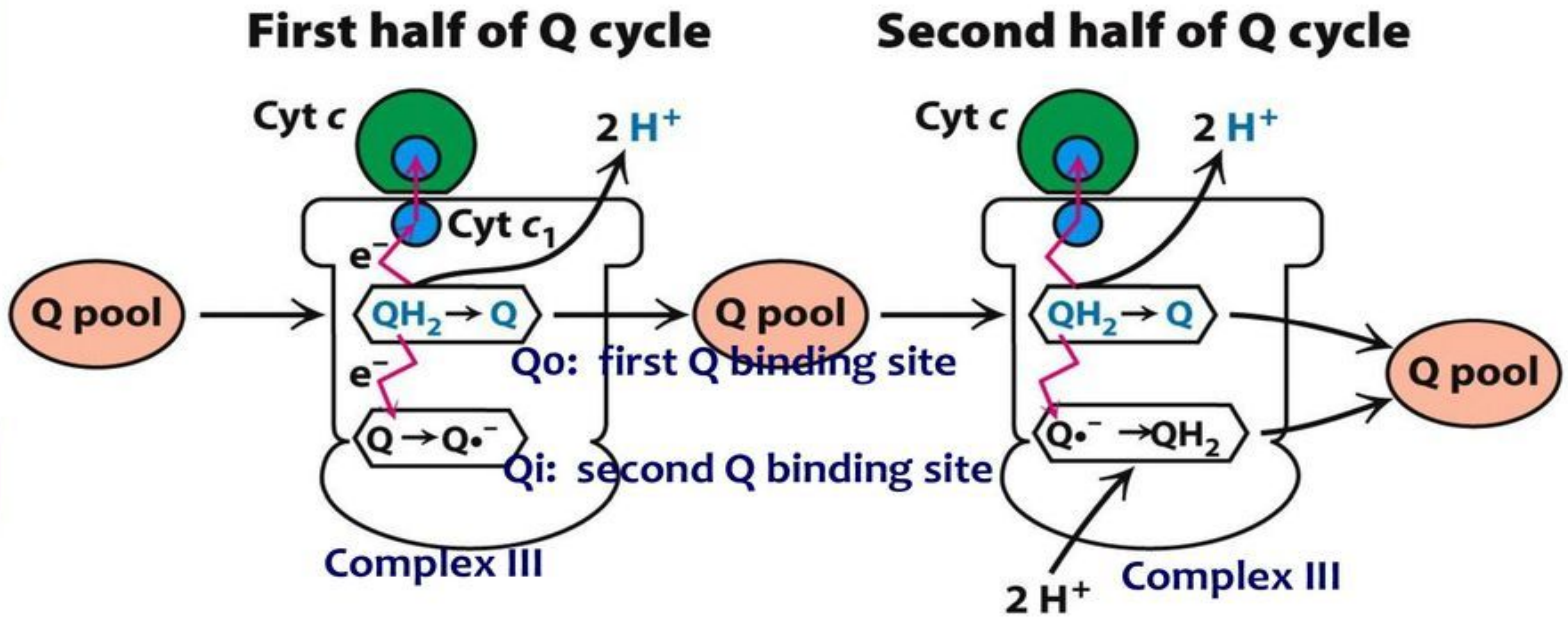
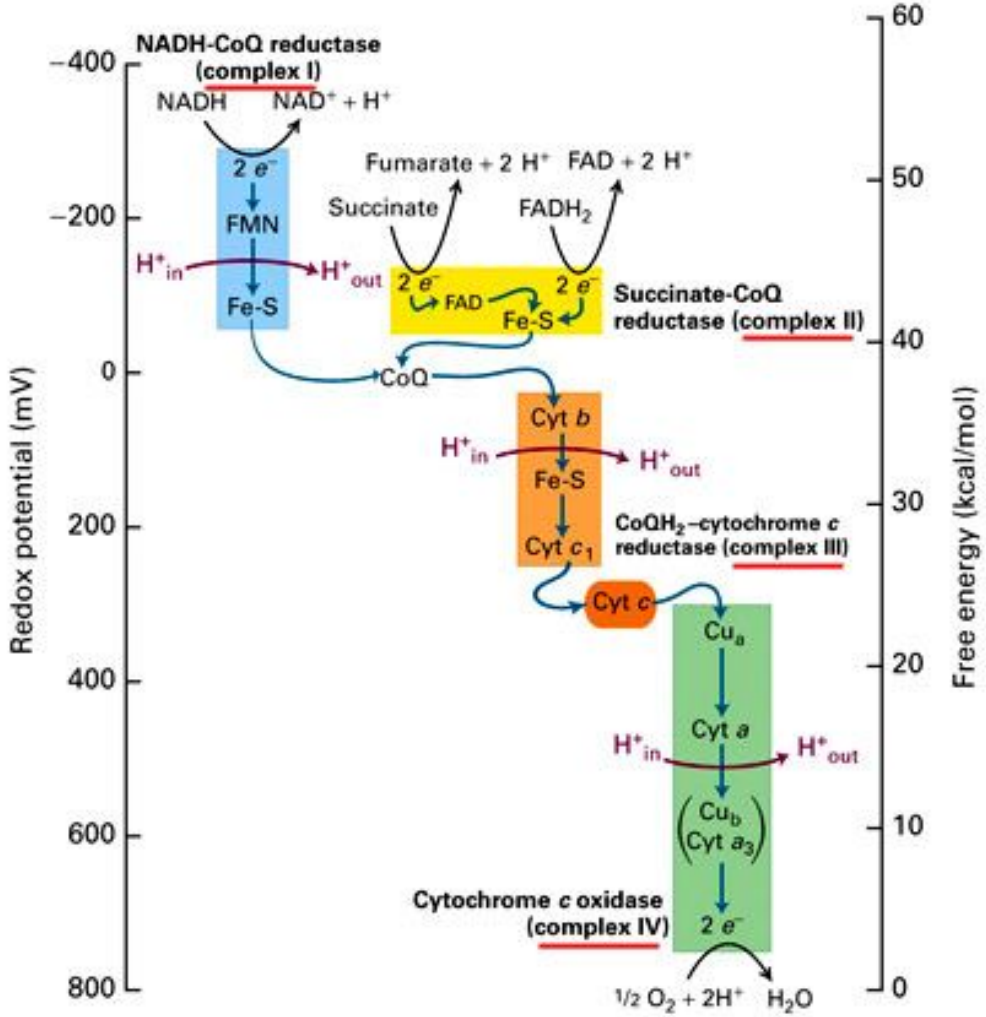


Figure 18.12
Biochemistry, Seventh Edition

Fig 18.12 Q-cycle


NADH-CoQ reductase
(complex I)
NADH
NAD+ + H+
2 e−
FMN
Fe-S
H+in
H+out
Fumarate + 2 H+
FAD + 2 H+
Succinate
FADH2
2 e−
FAD
Fe-S
2 e−
Succinate-CoQ
reductase (complex II)
CoQ
Cyt b
Fe-S
Cyt c1
H+in
H+out
CoQH2–cytochrome c
reductase (complex III)
Cyt c
Cua
Cyt a
H+in
H+out
Cub
Cyt a3
Cytochrome c oxidase
(complex IV)
2 e−
1/2 O2 + 2H+
H2O
Redox potential (mV)
−400
−200
0
200
400
600
800
Free energy (kcal/mol)
60
50
40
30
20
10
0

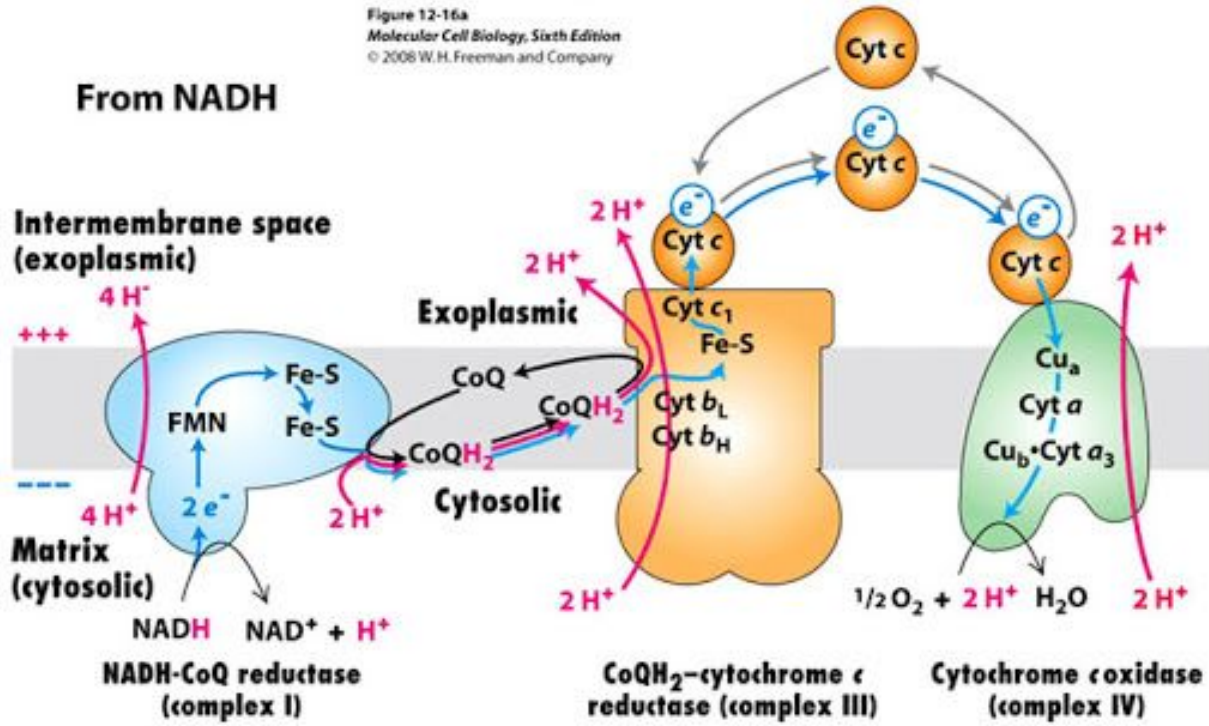


Figure 12-16a
Molecular Cell Biology, Sixth Edition
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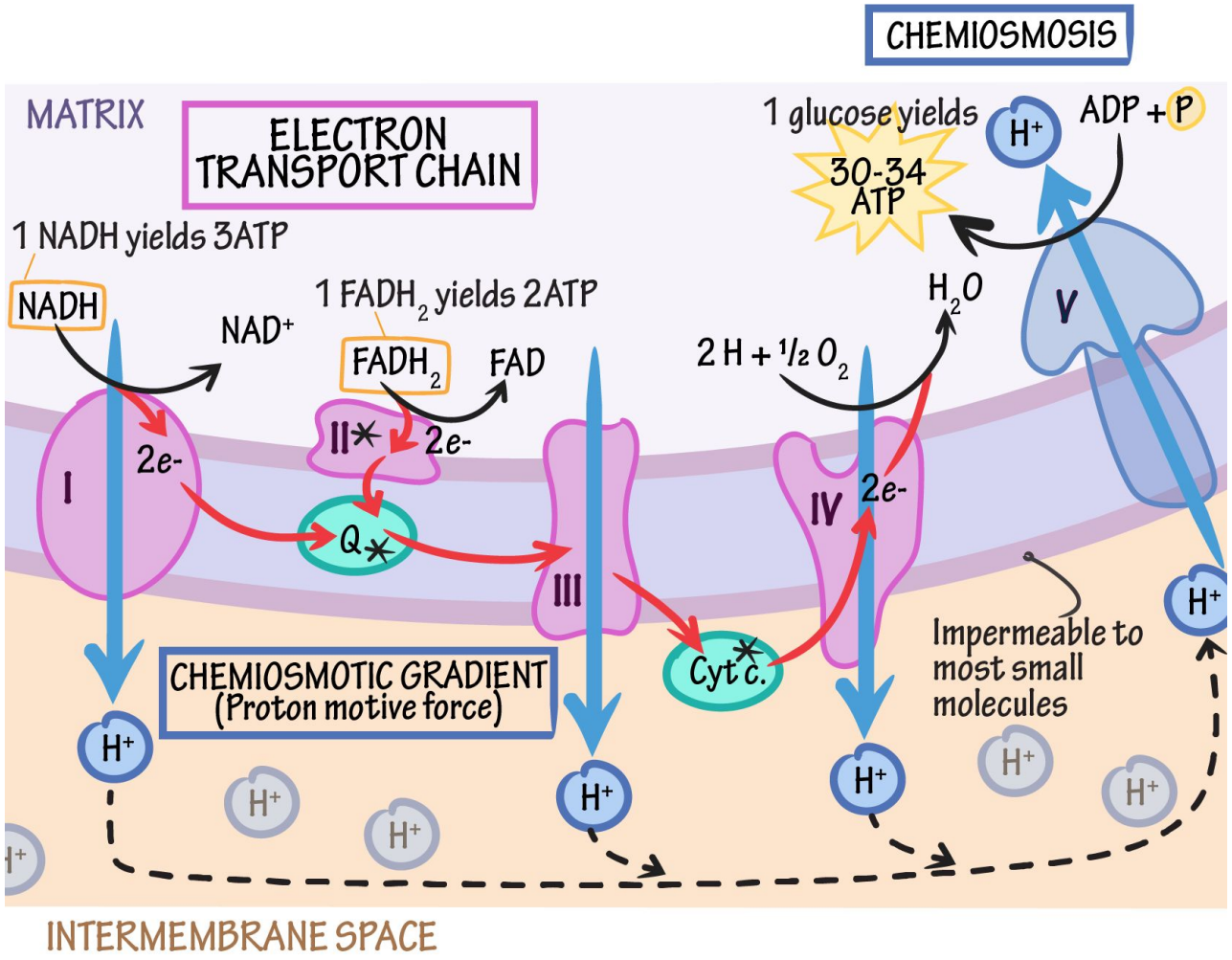
From NADH
Intermembrane space
(exoplasmic)
4 H+
+++
Exoplasmic
Cyt c
Cyt c
Cyt c
Cyt c1
Fe-S
Cytb L
Cyt bH
Cua
Cyt a
Cub•Cyt a3
2 H+
2 H+
2 H+
2 H+
2 H+
Fe-S
FMN
Fe-S
CoQ
CoQH2
CoQH2
2 e−
4 H+
2 H+
Cytosolic
Matrix
(cytosolic)
NADH
NAD+ + H+
1/2 O2 + 2 H+
H2O
NADH-CoQ reductase
(complex I)
CoQH2–cytochrome c
reductase (complex III)
Cytochrome c oxidase
(complex IV)

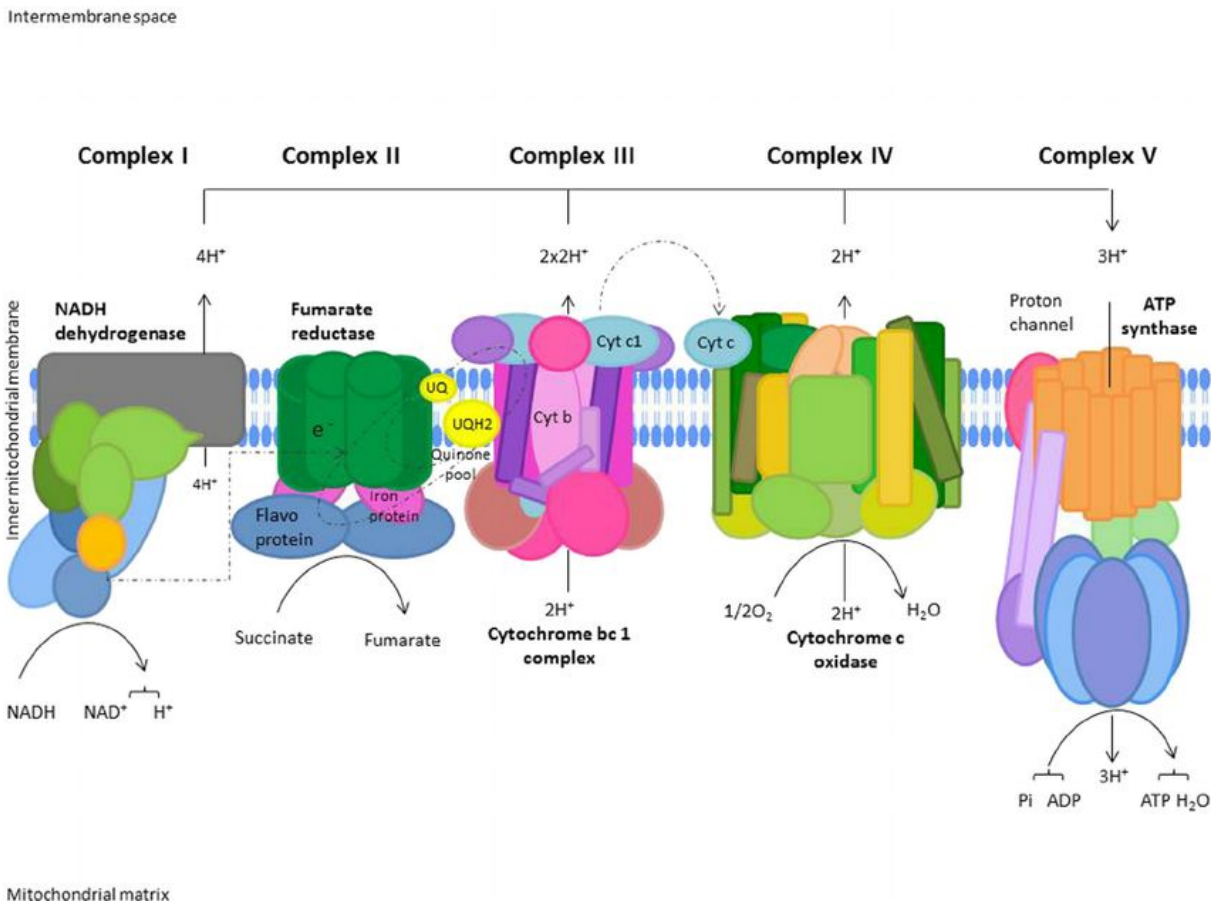
Oxidative Phosphorylation

- Transfer of electrons to oxygen via complex I-IV is coupled with synthesis of ATP
- Number of ATP synthesised depends on the nature of the electron donor
- One molecule of ATP synthesise when 3 protons flow through ATP synthase
- An additional proton is consumed in transporting ATP from the matrix to the cytosol
- Hence, about 2.5 molecules of cytosolic ATP are generated as a result of the electron flow from NADH to O_2
- Cytosolic NADH or $FADH_2$ yield about 1.5 molecules of ATP per electron pair



Oxidative Phosphorylation

- Flow of electron from NADH to O_2 is an exergonic process, while the synthesis of ATP coupled to electron flow is an endergonic process
- The enzyme complex responsible for ATP synthesis is termed as ATPase/ F_1-F_0 ATPase/ complex V
- The most accepted hypothesis regarding ATP synthesis is the chemiosmotic hypothesis proposed by Peter Mitchell (1961)
- Transfer of electrons through respiratory chain leads to the pumping of protons from the matrix to the cytosolic side of the inner mitochondrial membrane
- The proton motive force thus generated across the membrane drives the ATP synthesis
- Thus oxidation and phosphorylation is coupled in the process



- ATP synthase s a large, complex membrane embedded enzyme that looks like a ball on a stick
- It is also termed as the world's smallest **rotary motor**
- The ball, or the F_1 subunit protrudes into the mitochondrial matrix and contains the catalytic activity
- It consists of three types of polypeptide chains –α_3, β_3, γ, δ and ε
- The α and β make up the bulk of the F_1 and remain arranged alternatively in a hexameric ring
- Both of them binds nucleotides but only βsubunit take part in catalysis
- The central stalk consists of two proteins-the long α-helical coil of γ subunit and the ε subunit
- The γ subunit extends into the centre of $\alpha_3\beta_3$ hexamer

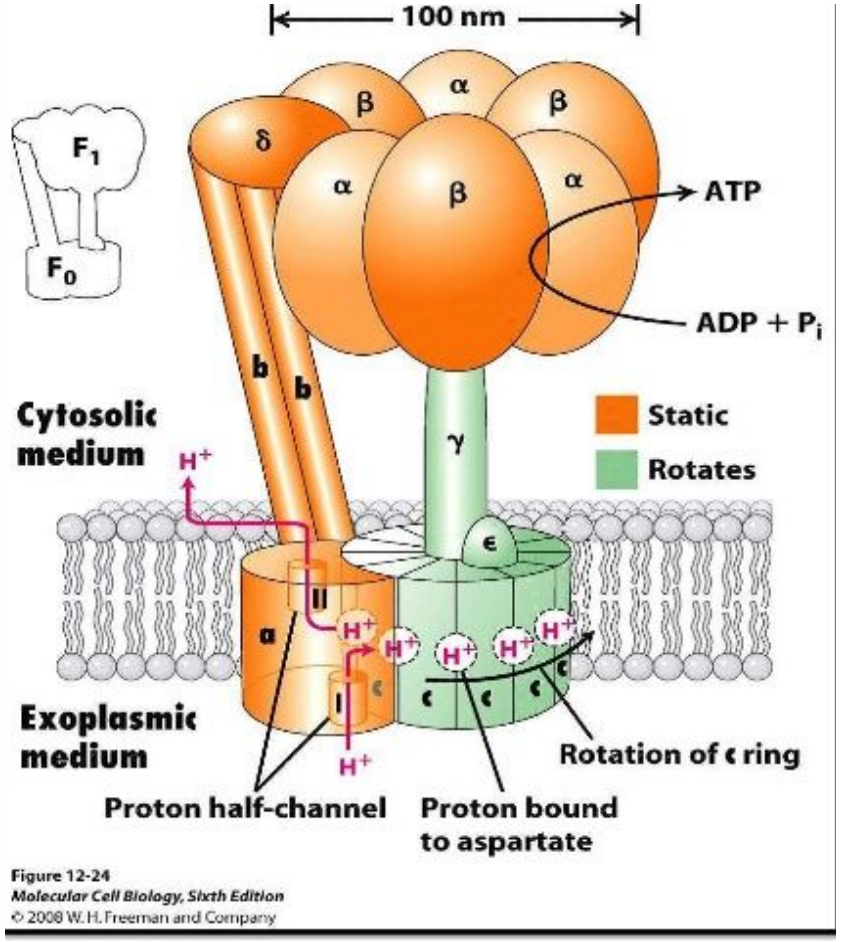


Figure 12-24
Molecular Cell Biology, Sixth Edition

- The F_0 subunit is a hydrophobic segment that spans the inner mitochondrial membrane
- It contains the proton channel consisting of a ring of 10-14 c subunits that remain embedded in the matrix
- A single a subunit binds to the outside of the ring
- The proton channel depends on both the a and c subunits
- The F0 and F1 subunit remain connected by an exterior column consisting of one a subunit, 2 b subunits and the δ subunit
- The functional enzyme consists of two components- a moving unit (**rotor**) composed of c ring and γε subunit and a stationary unit (**stator**) composed of the remainder of the molecule
- ATP synthase has diverse functions- in hydrolytic mode it catalyses the hydrolysis of ATP into ADP and Pi, while in reverse reaction the dehydration of ADP and Pi into ATP and water occurs
- It has been observed that enzyme bound ATP forms readily in absence of a proton-motive force too
- Thus the role of proton gradient is not in synthesis of ATP but to release it from the synthase
- Based on this fact Paul Boyer (1997) proposed the binding-change mechanism for proton driven ATP synthesis

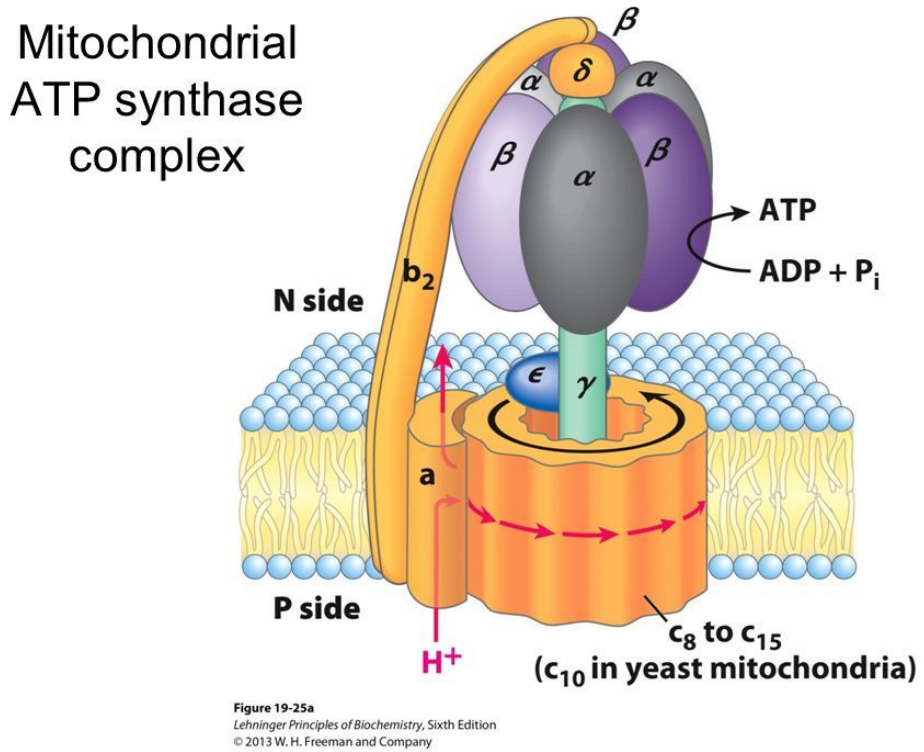


Figure 19-25a
Lehninger Principles of Biochemistry, Sixth Edition
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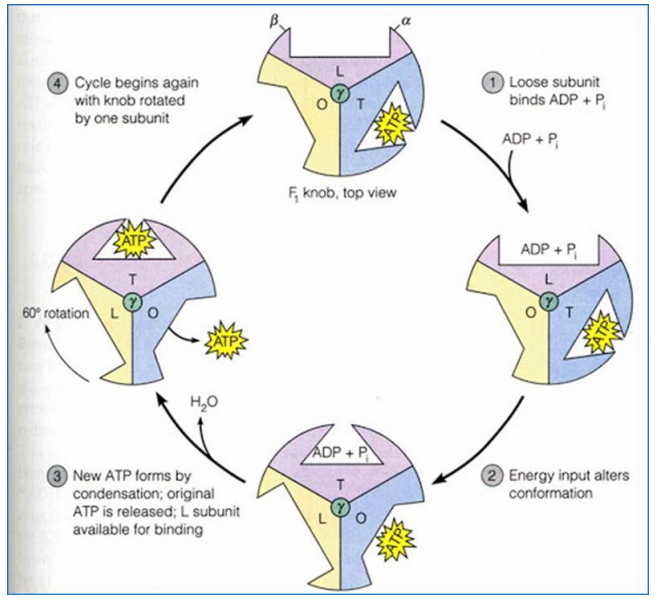
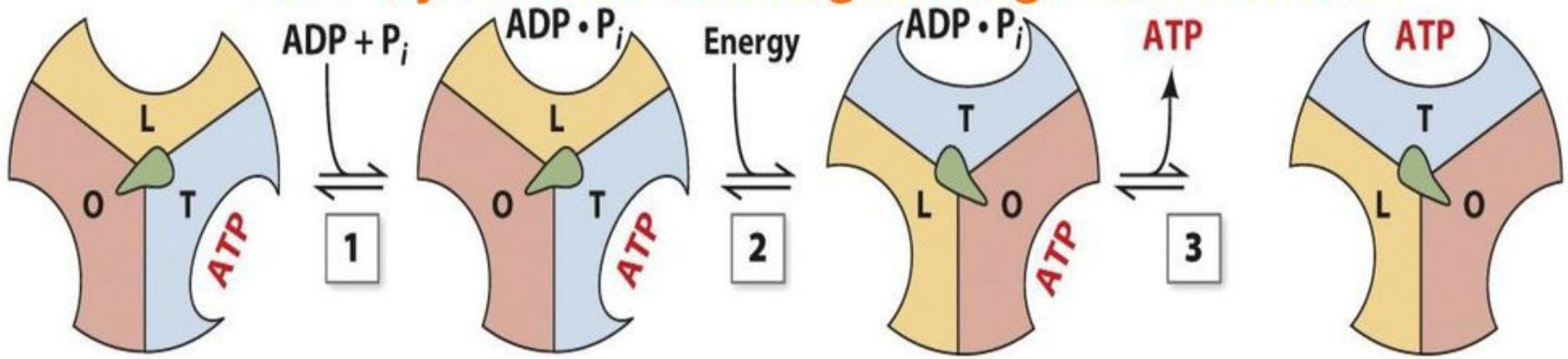
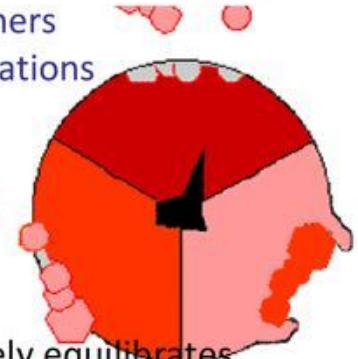


ATP Synthase: Binding Change Mechanism



In the so-called "binding change" mechanism, each of the three $\alpha\beta$ catalytic protomers of the $\alpha_3\beta_3$ subunits of F1 component is envisioned to adopt three distinct conformations designated O, L and T that are in equilibrium exchange with each other:

O → catalytically-inactive / low affinity for substrates (ADP and Pi)
L → catalytically-inactive / moderate affinity for substrates (ADP and Pi)
T → catalytically-active / high affinity for substrates (ADP and Pi)



(1) In the absence of the spinning action of the rotor ($c_{12}\gamma\varepsilon$), the $\alpha\beta$ protomer largely equilibrates between the O and L states—it cannot synthesize ATP from ADP and Pi—with the latter being able to accommodate ADP/Pi with moderate affinity

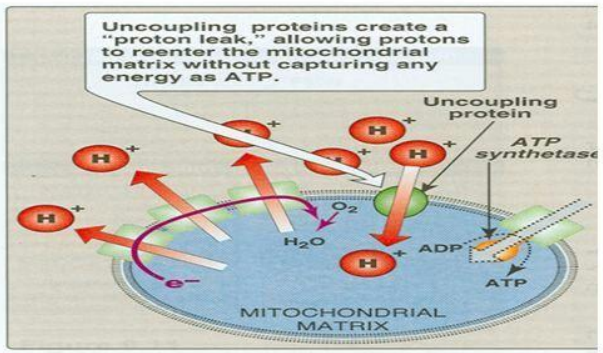
(2) Upon the spinning action of the rotor ($c_{12}\gamma\varepsilon$), the free energy released shifts the conformational equilibrium of the $\alpha\beta$ protomers from the L state to the catalytically-active T conformation, enabling it to "stick" together ADP and Pi to generate ATP

(3) Upon the synthesis of ATP, the T state undergoes conformational change to O state (with low affinity for substrates), thereby releasing ATP and enabling the $\alpha\beta$ protomer to return to its initial state in order to undergo another catalytic cycle

Uncouplers

- Compounds that can uncouple or delink the electron transport chain from oxidative phosphorylation, such compounds are known as Uncouplers.

- These increase the permeability of IMM to protons (H+).
- Thus an Uncoupler allows ETC but blocks the establishment of proton gradient across the IMM.



- The result is that ATP synthesis does not occur.
- The energy linked with the transport of electrons is dissipated as heat.

Chemical Uncouplers

- Chemical Uncouplers

1. 2,4-dinitrophenol (has been extensively studied).
2. Dinitrocresol.
3. Pentachlorophenol
4. Tri fluoro carbonyl cyanide phenyl hydra zone (FCCP).
5. Aspirin (high doses)

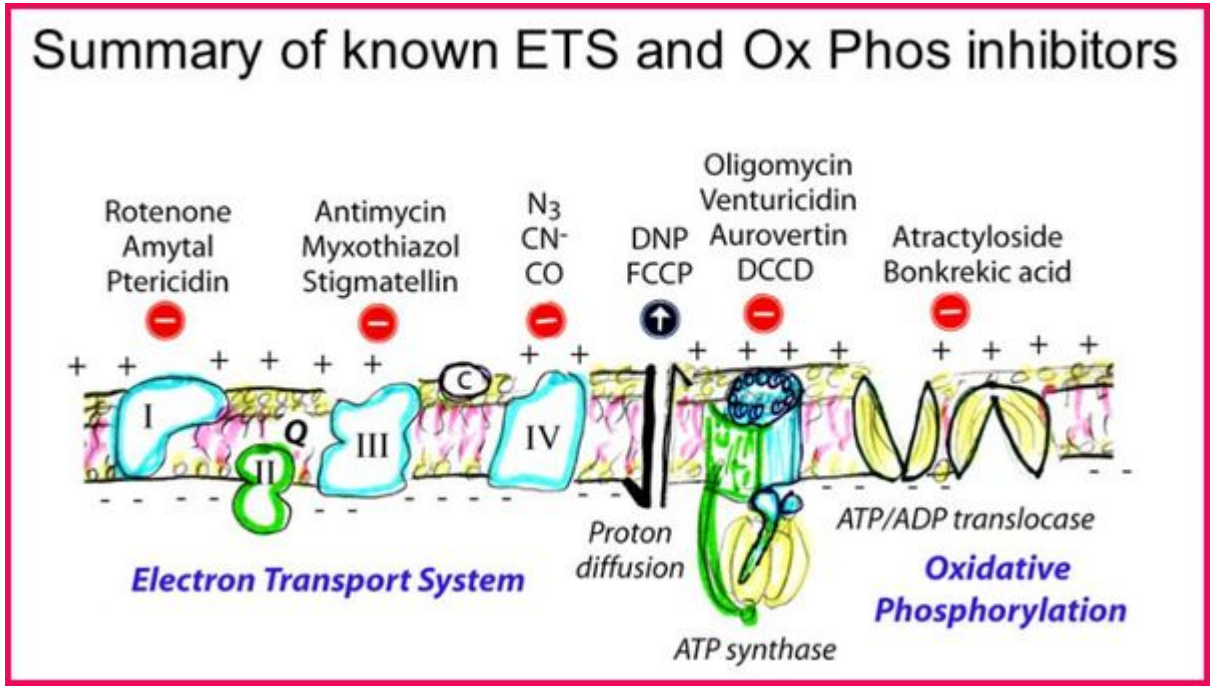
- Physiological Uncouplers

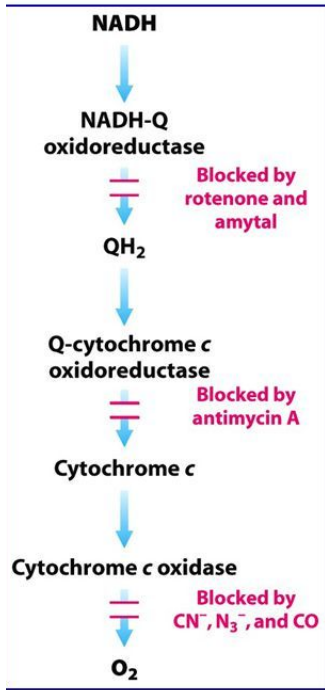
1. Thyroid hormones.
2. Long chain fatty acids.
3. Unconjugated Bilirubin.

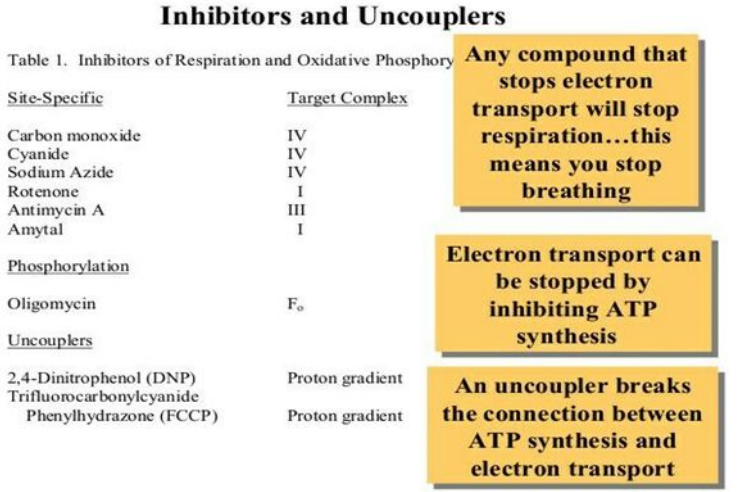
- These act as Uncouplers only at high concentration.

Uncouplers

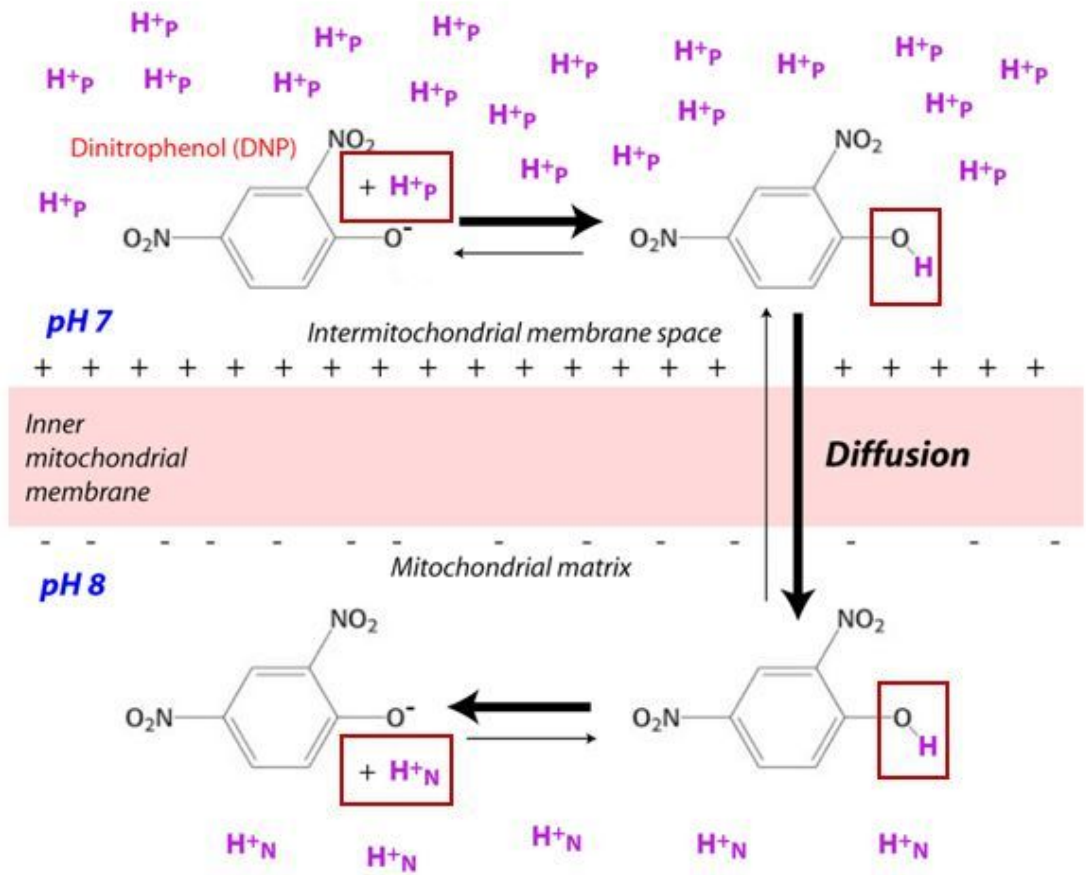
- Oxidative phosphorylation remains coupled with the electron transport chain
- There are toxic compounds that can uncouple this
- Specific inhibitors of electron transport can also ultimately inhibit ATP synthesis as the proton motive force is no longer generated
- **Rotenone** and **Amytal** block electron transfer in NADH-Q-oxidoreductase and thereby prevent utilisation of NADH as a substrate
- The electron flow resulting from oxidation of succinate remain unimpaired, as it directly enters through QH_2
- **Antimycin** blocks electron flow in cytochrome –C oxidoreductase
- Electron flow in cytochrome C oxidase can be blocked by **cyanide** (CN^-), **azide** (N^{3-})and **carbon monoxide** (CO)
- Cyanide and azide can react with the ferric form of a_3, while CO inhibits the ferrous form
- ATP synthesis can also be inhibited by oligomycin and dicyclohexilcarbodiimide (DCCD) that prevent the influx of proton through ATP synthase







Dinitrophenol (DNP) dissipates the proton gradient by carrying H^+ across the inner mitochondrial membrane through simple diffussion-mediated transport



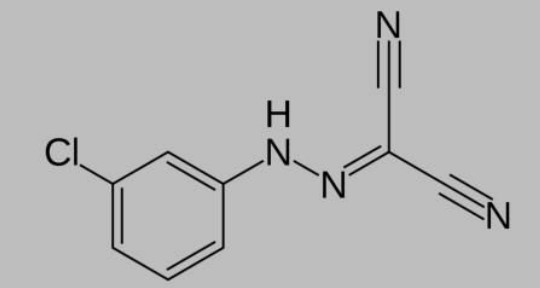
The result is that **carbohydrate and lipid stores are depleted** in an attempt to make up for the low energy charge in cells resulting from decreased ATP synthesis; **DNP short-circuits the proton circuit**.

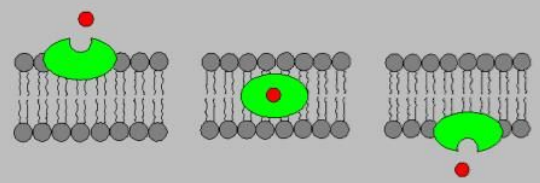
- CCCP enters intact mitochondria in the protonated form, discharging the pH gradient
- Then promptly leaves as anion, destroying the membrane potential
- The process is repeated several times so that a tiny amount of CCCP can catalyse large number of protons, and short-circuit the respiratory chain

What is CCCP?

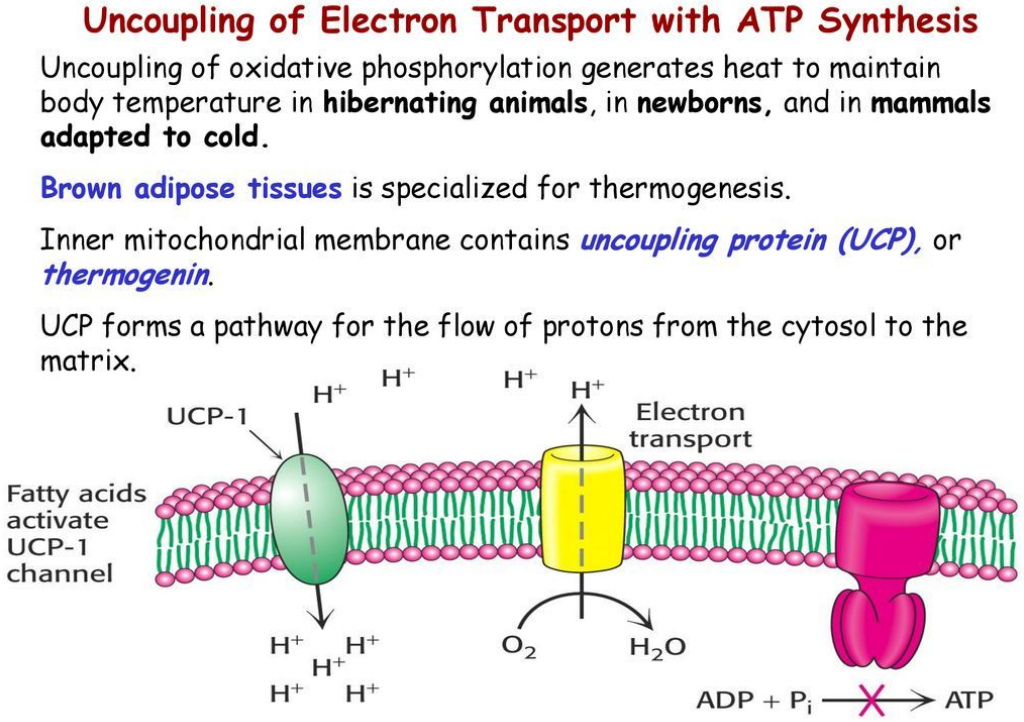
Carbonyl cyanide m-chlorophenyl hydrazone:

A chemical inhibitor of oxidative phosphorylation, destroys the proton gradient across the membrane.



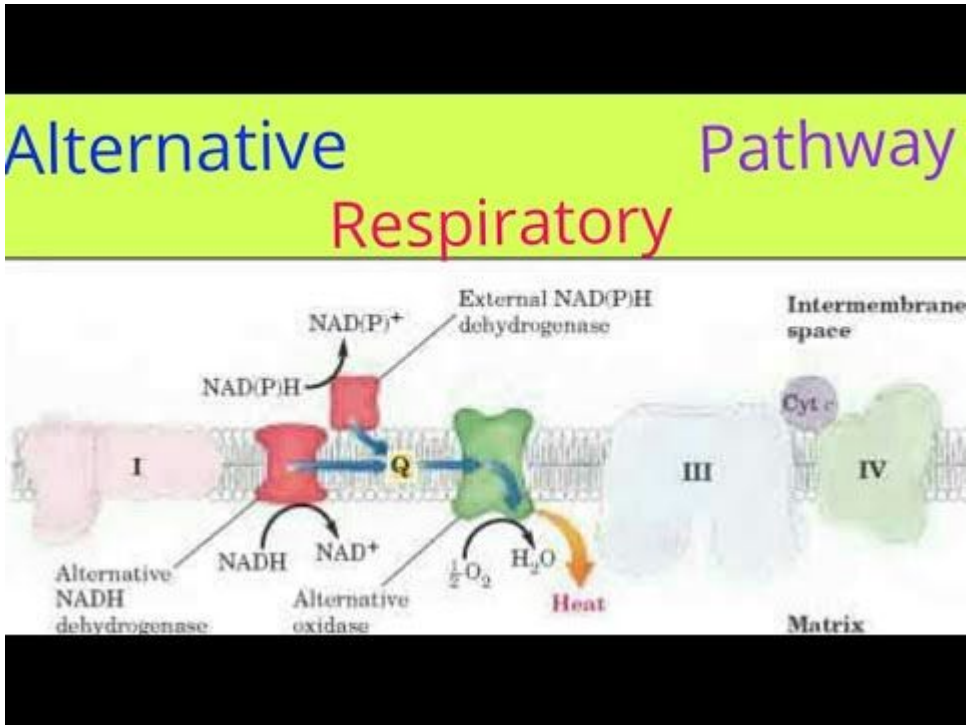


- Uncoupling of oxidative phosphorylation is a means to generate heat (thermogenesis) to maintain body temperature in hibernating animals, new-born babies and mammals adapted to cold
- Plants like slunk cabbage use such mechanism to heat its floral spikes, increasing the evaporation of odoriferous molecules to attract insects for pollination
- Brown adipose tissue is rich in mitochondria
- UCP-1 is a dimer of 33 kd subunits that resemble ATP-ADP translocase
- UCP uncouples oxidative phosphorylation from electron transport and thus energy from PMF is dissipated as heat rather than forming ATP
- This pathway is activated by free fatty acids liberated from triglycerols in response to hormonal signals
- UCP-2 and UCP-3 may also play role in energy homeostasis
- ATP-ADP translocase is specifically inhibited by very low concentration of atractyloside (plant glycoside) or bonkrekic acid
- Atractyloside binds to translocase when the nucleotide site faces cytosol and bonkrekic acid binds when it faces mitochondrial matrix



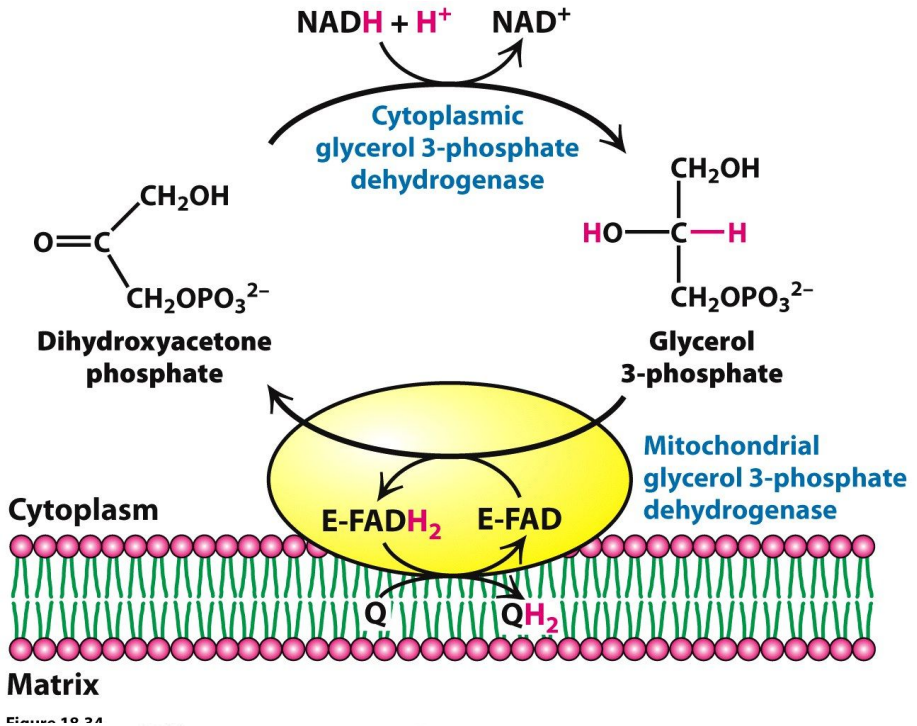
Cyanide Resistant Respiration

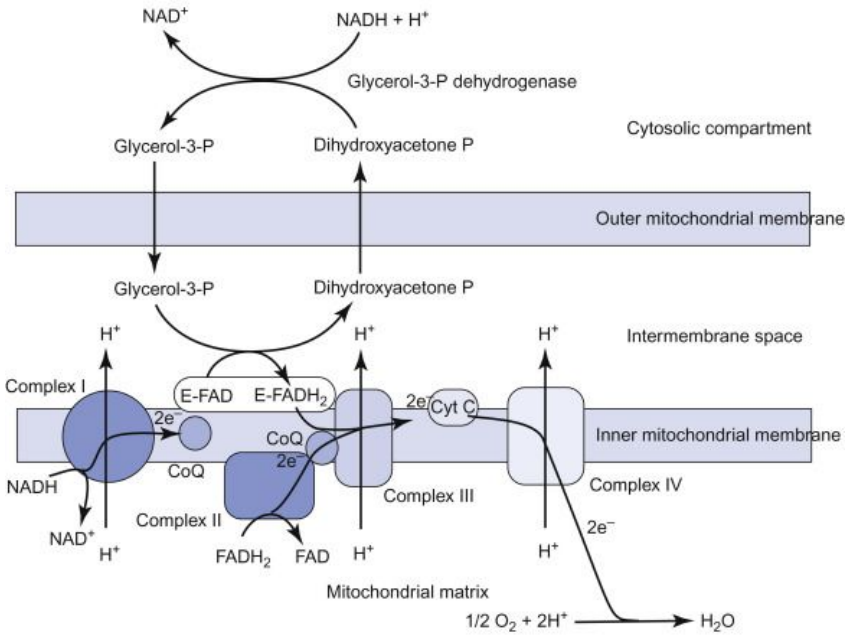
- Cyanide insensitive respiration was first observed in *Sauromatum* spadix
- It was observed by Bendall and Bonner that cyanide and antimycin-A insensitive respiration was mediated by a second oxidase known as cyanide resistant oxidase or alternate oxidase
- Alternate oxidase catalyses a 4-electron reduction of O_2 to water and two sites of energy conservation (complex III and IV) are by-passed
- Thus no ATP is formed and free energy is lost as heat
- Thus, it is also called as **thermogenic respiration**
- Alternate oxidase can be specifically inhibited by **salicylhydroxamic acid (SHAM)** and n-propylgallate
- Heat released during such respiration volatilises amines and indoles, giving rise to a putrid odour that attracts insects
- It serves as an energy overflow capacity, oxidising respiratory substrates that accumulate in excess
- It plays role in various stresses (chilling, draught) that can inhibit mitochondrial respiration
- It also prevents the generation of deleterious ROS radicals by draining off electrons from ETC



Oxidation of Cytosolic NADH

- The glycolytic pathway generates NADH I the cytosol and NAD^+ must be regenerated for glycolysis to continue
- Mitochondrial membrane is impermeable to both NADH and NAD^+
- It is assumed that electrons are carried across the mitochondrial membrane
- **Glycerol-3-phosphate shuttle** is one of the common pathway for such transport
- The reactions of the pathway are catalysed by the cytosolic enzyme glycerol 3-phosphate dehydrogenase and its mitochondrial membrane bound isozyme
- An electron pair from G-3-P is transferred to FAD prosthetic group of the enzyme, which ultimately enter the respiratory chain through QH_2
- Reoxidation of FADH2 via ETC yields two ATP molecules
- Thus, the cost of this shuttle is one ATP per cytosolic NADH oxidised
- Higher plant cells and active tissues like flight muscles of insects operate glycerol phosphate shuttle





- In mammalian tissue, particularly heart and liver, electrons from cytosolic NADH are brought into mitochondria by **malate-aspartate shuttle**
- The reactions are mediated by two membrane carriers and four enzymes
- In contrast with G-3-P shuttle, it is readily reversible
- It operates only if the NADH/NAD+ ratio is higher in the cytosol than in the mitochondria
- It also facilitates the exchange of key intermediates between mitochondria and cytosol
- It transports aspartate to cytosol, in contrast to G3-P shuttle where DHAP is generated at cytosol

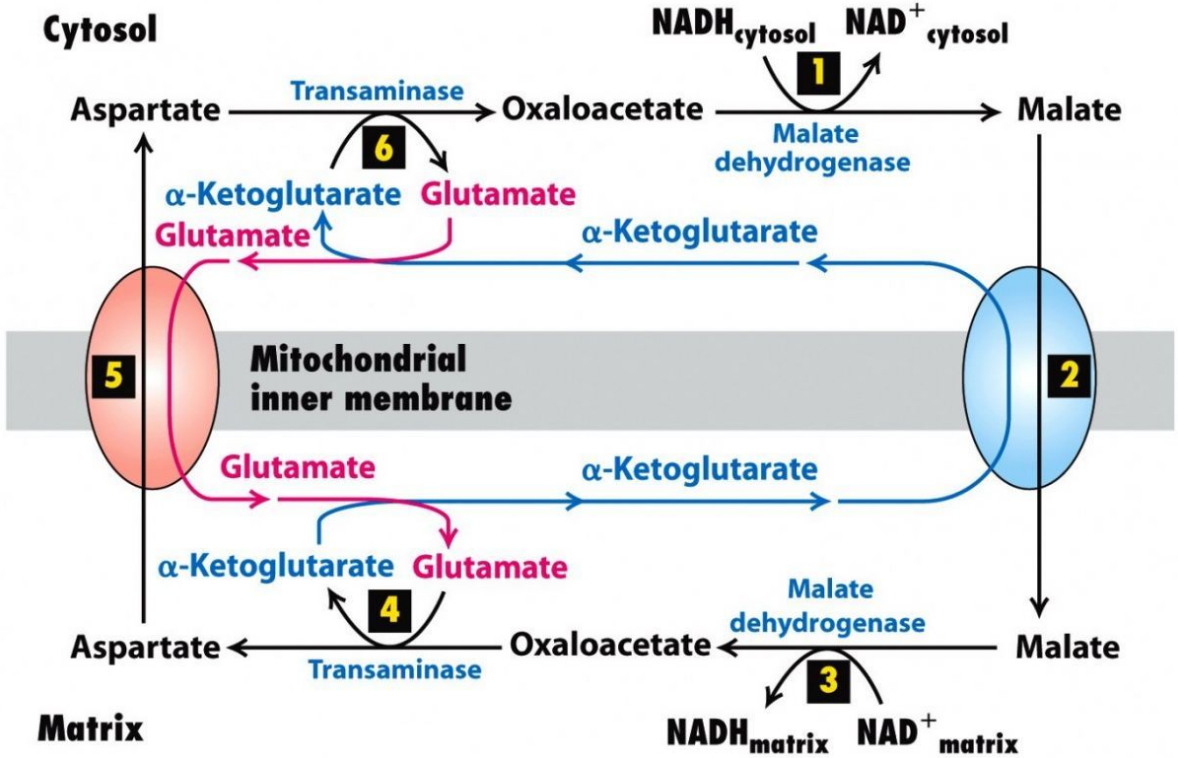


Figure 12-11
Molecular Cell Biology, Sixth Edition

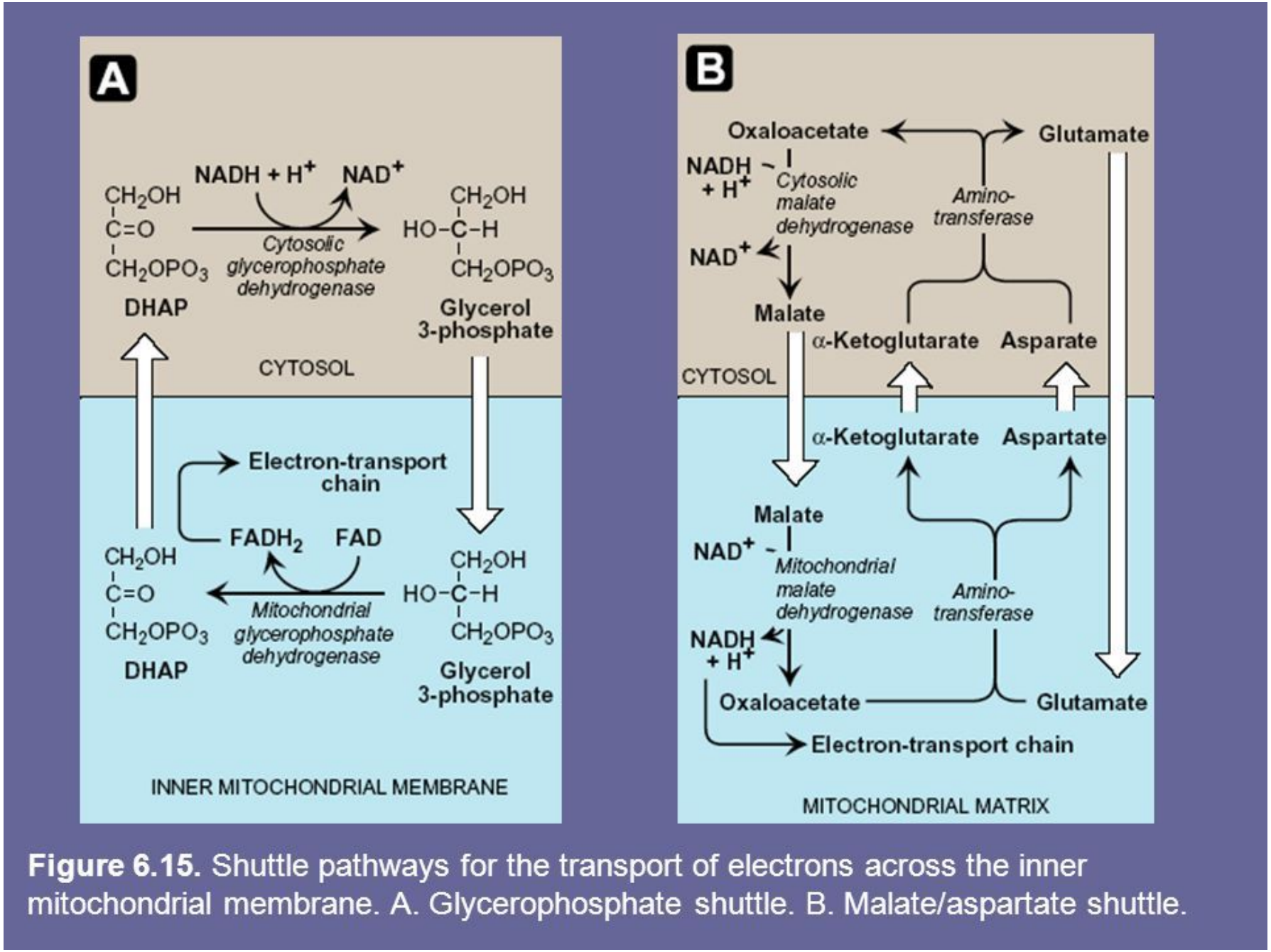


Figure 6.15. Shuttle pathways for the transport of electrons across the inner mitochondrial membrane. A. Glycerophosphate shuttle. B. Malate/aspartate shuttle.

- ATP generated through oxidative phosphorylation cannot diffuse freely across the inner mitochondrial membrane
- A specific transport protein, **ATP-ADP translocase/Adenine Nucleotide Translocase (ANT**) overcomes this permeability barrier
- The flow of ATP and ADP are coupled
- ANT is a dimer of identical 30 kd subunits, contains single nucleotide binding site that alternately faces the matrix and cytosol
- In the presence of a positive membrane potential the rate of binding site eversion from the matrix to cytosol is more rapid for ATP as it has one more negative charge
- The translocase evert to the matrix side only if the open cytosolic side is bound with ADP
- Thus entry of ADP into matrix is precisely coupled to the exit of ATP
-

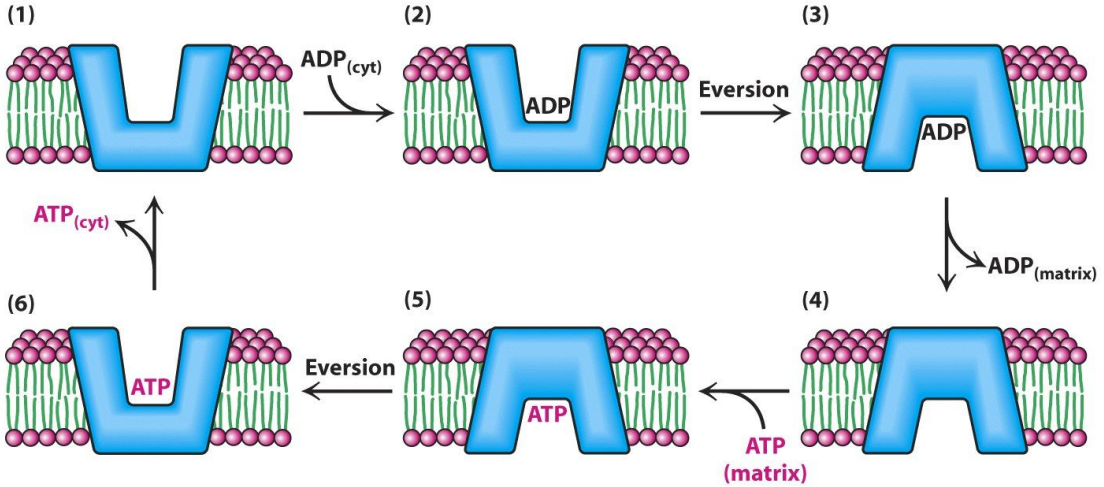
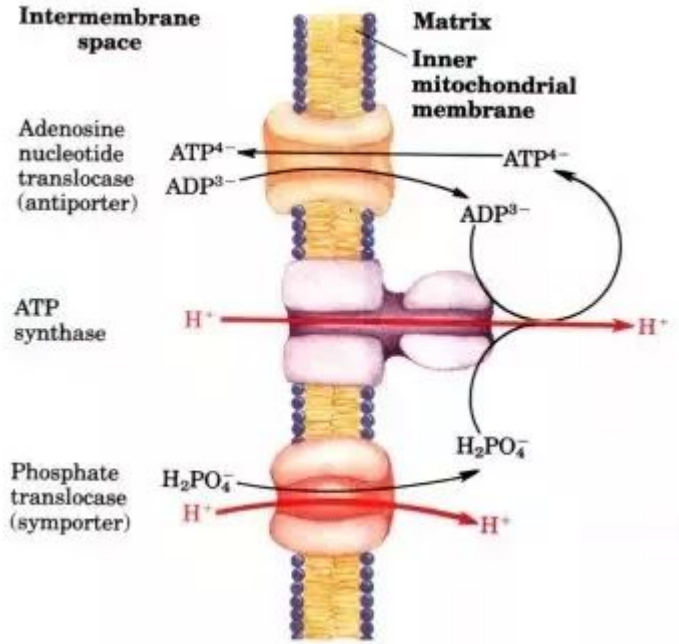


Figure 18-37
Biochemistry, Sixth Edition



- The total yield of ATP from the complete oxidation of glucose will be 36 when only the G-3-P shuttle is operating
- 2 ATPs via glycolysis, 2ATPs via TCA cycle (substrate level phosphorylation) and 32 ATPs via ETC (oxidative phosphorylation)
- As prokaryotes lack mitochondria this shuttle is absent there
- Cytosolic NADH is oxidised directly via ETC occurring in the cytosol
- Hence 3 ATPs will be formed per NADH
- Hence in prokaryotes 38 molecules of ATP is expected to generate on full oxidation of glucose