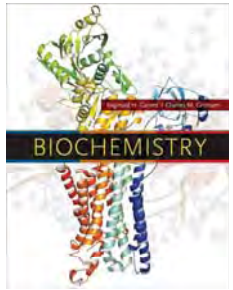
# Chapter 20

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## Electron transport and oxidative phosphorylation

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## Before the class

- Do you know….
  - What is reduction potential?
  - How electron transferred between two molecule?
  - What materials could pass the membrane of mitochondria?
  - What is the relationship of mitochondria function and cell fate?





## Outline

- Part 1
  - Where in the cell do electron transport and oxidative phosphorylation occur?
  - What are reduction potentials, and how are they used to account for free energy changes in redox reactions?
- Part 2
  - How is the electron-transport chain organized?
  - What are the thermodynamic implications of chemiosmotic coupling?
- Part 3
  - How does a proton gradient drive the synthesis of ATP?
  - What is the P/O ratio for mitochondrial oxidative phosphorylation?
  - How are the electrons of cytosolic NADH fed into electron tansport?
  - How do mitochondria mediate apoptosis?



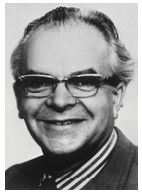


## Introduction

- Oxidative phosphorylation is the final stage of cellular respiration, in which the energy of oxidation drives the ATP synthesis.
- Oxidative phosphorylation involves the reduction of $O_2$ to $H_2O$ with electrons donated by NADH and $FADH_2$.
- Oxidative phosphorylation takes place at inner membrane of mitochondria

# The chemiosmotic theory

- A great unifying principles of 20th century biology
- Transmembrane difference in proton concentration are the reservoir of the energy extracted from biological oxidation reactions.
- The chemical energy is used to synthesize ATP in mitochondria and chloroplast.

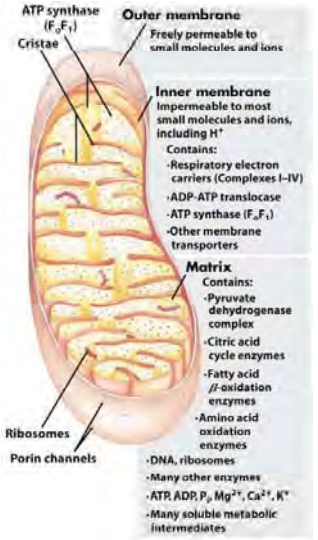


**Peter Mitchell**
The Nobel Prize
in Chemistry 1978



# Mitochondria

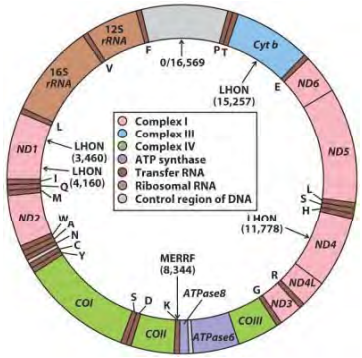
- Outer membrane: 30~40% lipid (rich in PI), 60~70% protein.
- Inner membrane: 80% protein; fatty acid highly unsaturated (cardiolipin and diphophatidylglycerol are abundant, but no cholesterol)
- Cristae: provide large surface area.
- Not anything could go inside mitochondria (Porin <10kDa)






# Mitochondria DNA

- **A typical cell : hundreds or thousands of mitochondria**
- **A mitochondria: five copies of a circular double-stranded DNA molecule.**
- **The human mitochondrial chromosome (16,569bp) contains 37 genes, including 13 that encode subunits of proteins of the respiratory chain.**
- **About 900 different mitochondrial proteins are encoded by nuclear genes, synthesized on cytoplasmic ribosomes, and imported as well as assembled within mitochondria.**



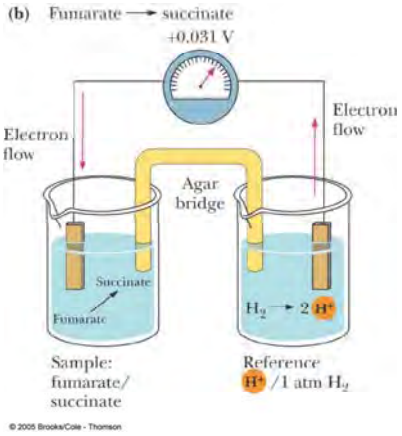



# Before we jump into electron transport……

Why electrons are transported?

# Reduction potential

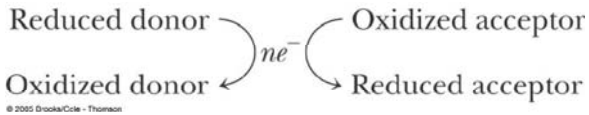
- Measure:
  - Reference: $H_2$ (1 atm); $H^+$ (1M)
  - Sample:redox couple (both 1M)
- Meaning:
  - If electron flow to sample half cell, reduction occur spontaneously
  - This reaction has a positive reduction potential.








# Free energy and reduction potential



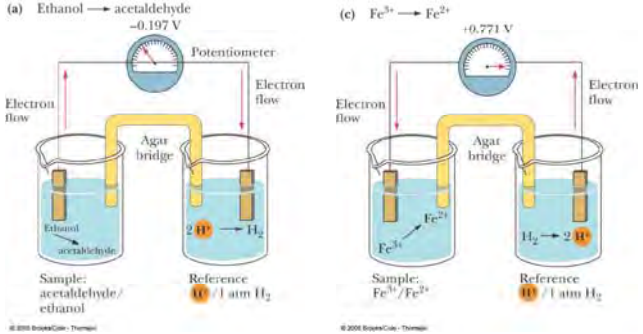

- $\Delta G^{o\prime} = - n\ F\ \Delta E_0'$
- F (Faraday's constant; 96,485 J/V)
- $\Delta E_0'$ = the difference in reduction potential between donor and acceptor.





# Exercise

- Which sample half-cell will spontaneously reduction?








**Reducing Power**

**(easy to be oxidized & Good e⁻donor)**

Table 20.1
Standard Reduction Potentials for Several Biological Reduction Half-Reactions

| Reduction Half-Reaction | $\mathscr{E}_0'$ (V) |
|---|---|
| $\frac{1}{2}O_2 + 2\,H^+ + 2\,e^- \longrightarrow H_2O$ | 0.816 |
| $Fe^{3+} + e^- \longrightarrow Fe^{2+}$ | 0.771 |
| Photosystem P700 | 0.430 |
| $NO_3^- + 2\,H^+ + 2\,e^- \longrightarrow NO_2^- + H_2O$ | 0.421 |
| Cytochrome $f$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $f$ ($Fe^{2+}$) | 0.365 |
| Cytochrome $a_3$($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $a_3$($Fe^{2+}$) | 0.350 |
| Cytochrome $a$($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $a$($Fe^{2+}$) | 0.290 |
| Rieske Fe-S($Fe^{3+}$) + $e^- \longrightarrow$ Rieske Fe-S($Fe^{2+}$) | 0.280 |
| Cytochrome $c$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $c$ ($Fe^{2+}$) | 0.254 |
| Cytochrome $c_1$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $c_1$ ($Fe^{2+}$) | 0.220 |
| UQH · + $H^+$ + $e^- \longrightarrow UQH_2$ (UQ = coenzyme Q) | 0.190 |
| UQ + 2 $H^+$ + 2 $e^- \longrightarrow UQH_2$ | 0.060 |
| Cytochrome $b_H$($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $b_H$($Fe^{2+}$) | 0.050 |
| Fumarate + 2 $H^+$ + 2 $e^- \longrightarrow$ succinate | 0.031 |
| UQ + $H^+$ + $e^- \longrightarrow$ UQH · | 0.030 |
| Cytochrome $b_L$($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $b_L$ ($Fe^{2+}$) | 0.020 |
| [FAD] + 2 $H^+$ + 2 $e^- \longrightarrow$ [$FADH_2$] | 0.003–0.091* |
| Cytochrome $b_L$($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $b_L$($Fe^{2+}$) | −0.100 |
| Oxaloacetate + 2 $H^+$ + 2 $e^- \longrightarrow$ malate | −0.166 |
| Pyruvate + 2 $H^+$ + 2 $e^- \longrightarrow$ lactate | −0.185 |
| Acetaldehyde + 2 $H^+$ + 2 $e^- \longrightarrow$ ethanol | −0.197 |
| FMN + 2 $H^+$ + 2 $e^- \longrightarrow$ $FMNH_2$ | −0.219 |
| FAD + 2 $H^+$ + 2 $e^- \longrightarrow$ $FADH_2$ | −0.219 |
| Glutathione (oxidized) + 2 $H^+$ + 2 $e^- \longrightarrow$ 2 glutathione (reduced) | −0.230 |
| Lipoic acid + 2 $H^+$ + 2 $e^- \longrightarrow$ dihydrolipoic acid | −0.290 |
| 1,3-Bisphosphoglycerate + 2 $H^+$ + 2 $e^- \longrightarrow$ glyceraldehyde-3-phosphate + $P_i$ | −0.290 |
| $NAD^+$ + 2 $H^+$ + 2 $e^- \longrightarrow$ NADH + $H^+$ | −0.320 |
| $NADP^+$ + 2 $H^+$ + 2 $e^- \longrightarrow$ NADPH + $H^+$ | −0.320 |
| Lipoyl dehydrogenase [FAD] + 2 $H^+$ + 2 $e^- \longrightarrow$ lipoyl dehydrogenase [$FADH_2$] | −0.340 |
| α-Ketoglutarate + $CO_2$ + 2 $H^+$ + 2 $e^- \longrightarrow$ isocitrate | −0.380 |
| 2 $H^+$ + 2 $e^- \longrightarrow H_2$ | 0.421 |
| Ferredoxin (spinach) ($Fe^{3+}$) + $e^- \longrightarrow$ ferredoxin (spinach) ($Fe^{2+}$) | −0.430 |
| Succinate + $CO_2$ + 2 $H^+$ + 2 $e^- \longrightarrow$ α-ketoglutarate + $H_2O$ | −0.670 |



**Reducing Power**

**(Good Oxidant, & e⁻acceptor)**

# A real example

- $NAD^+$ + isocitrate -------> NADH + $H^+$ + α-ketoglutarate + $CO_2$
- $NAD^+$ + 2 $H^+$ + 2$e^-$ -------> NADH + $H^+$
  Eo' = -0.32 V
- α-ketoglutarate + $CO_2$ + 2 $H^+$ + 2$e^-$ -------> isocitrate
  Eo' = -0.38 V
- Δ Eo' = Eo' (acceptor) – Eo' (donor) = - 0.32 V – (-0.38 V) = +0.06V
- Δ G°' = - (2) (96.485 kJ/V·mol)(0.06 V) = -11.58 kJ/mol
  Spontaneous reaction!





# More about Reduction Potential

- Concentration effect!

$$Ox + ne^- \rightleftharpoons Red$$

$$E = E_0' + (RT/nF) \ln \frac{[Ox]}{[Red]}$$

- E value can be varied by the environment (Ex. FAD)





# The next question….

- What kinds of substrates need to be oxidized?
- Electron carriers: NADH, $FADH_2$, CoQ, cytochromes, Fe-S protein, protein-bound copper
- What is the final oxidant?
- $O_2$

  ½ $O_2$ + $e^-$ +2$H^+$ ----> H2O





# 20.3 How Is the Electron Transport Chain Organized?

- Four protein complexes in the inner mitochondrial membrane
- A lipid soluble coenzyme (UQ, CoQ) and a water soluble protein (cyt *c*) shuttle between protein complexes
- Electrons generally fall in energy through the chain - from complexes I and II to complex IV

# Electron carriers in oxidative phosphorylation

- The action of dehydrogenases collect electrons from catabolic pathways and funnels them into universal electron acceptors such as NAD+, NADP+, FMN, FAD

**TABLE 19–1 Some Important Reactions Catalyzed by NAD(P)H-Linked Dehydrogenases**

| Reaction* | Location† |
|---|---|
| **NAD-linked** | |
| α-Ketoglutarate + CoA + $NAD^+$ ⇌ succinyl-CoA + $CO_2$ + NADH + $H^+$ | M |
| L-Malate + $NAD^+$ ⇌ oxaloacetate + NADH + $H^+$ | M and C |
| Pyruvate + CoA + $NAD^+$ ⇌ acetyl-CoA + $CO_2$ + NADH + $H^+$ | M |
| Glyceraldehyde 3-phosphate + $P_i$ + $NAD^+$ ⇌ 1,3-bisphosphoglycerate + NADH + $H^+$ | C |
| Lactate + $NAD^+$ ⇌ pyruvate + NADH + $H^+$ | C |
| β-Hydroxyacyl-CoA + $NAD^+$ ⇌ β-ketoacyl-CoA + NADH + $H^+$ | M |
| **NADP-linked** | |
| Glucose 6-phosphate + $NADP^+$ ⇌ 6-phosphogluconate + NADPH + $H^+$ | C |
| **NAD- or NADP-linked** | |
| L-Glutamate + $H_2O$ + $NAD(P)^+$ ⇌ α-ketoglutarate + $NH_4^+$ + NAD(P)H | M |
| Isocitrate + $NAD(P)^+$ ⇌ α-ketoglutarate + $CO_2$ + NAD(P)H + $H^+$ | M and C |

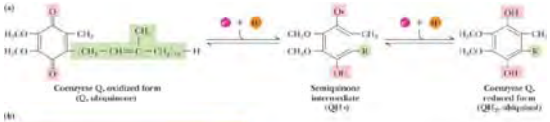
*These reactions and their enzymes are discussed in Chapters 14 through 18.
†M designates mitochondria; C, cytosol.

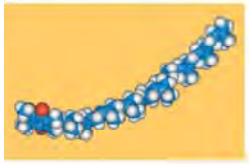




# New electron transporter #1: Ubiquinone

- *aka Coenzyme Q, CoQ or UQ (Q10)*
- Either one- or two- electron transfer





lipid-soluble





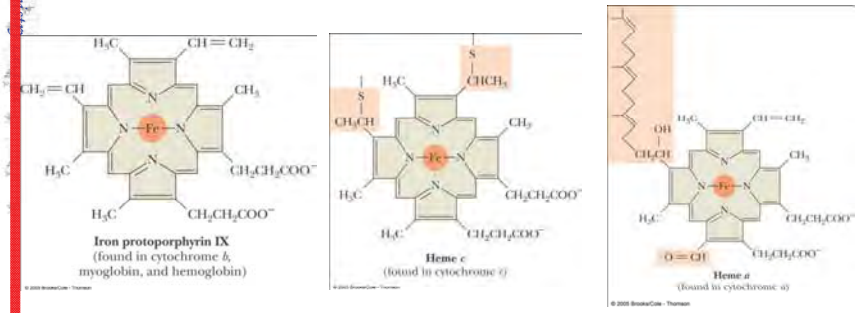
# New electron transporter #2: Cytochrome

- Mitochondria contain cytochromes a, b and c. Each has a characteristic light-absorption spectrum.
- The heme cofactors of a and b are tightly but not covalently bound to their associated protein.
- The hemes of c-type cytochromes are covalently attached through Cys residue.
- Cyt a, cyt b and some of the cyt c are integral proteins of inner mitochondrial membrane.
- The cytochrome c of mitochondria is a soluble protein that associates through electrostatic interaction with the outer surface of the inner membrane.



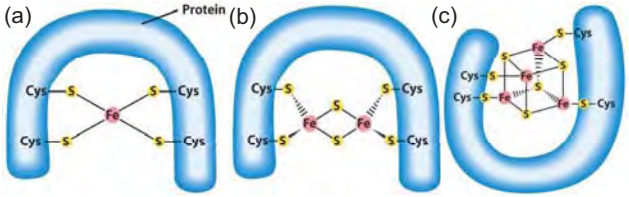


# Different heme groups



# New electron transporter #3: The iron-sulfur proteins

- The iron-sulfur (Fe-S) proteins contain Fe atom coordinated to four Cys-SH groups.
- Reiske iron-sulfur proteins contain one Fe atom coordinated to two His residues rather than two Cys residues.
- All Fe-S proteins participate in one-electron transfers in which one iron atom of the iron-sulfur cluster is oxidized or reduced.








# Electron flow

- The order of carriers is NADH→Q→cyt b →cyt c1→cyt c→cyt a→cyt a3→$O_2$

**TABLE 19-2** Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers

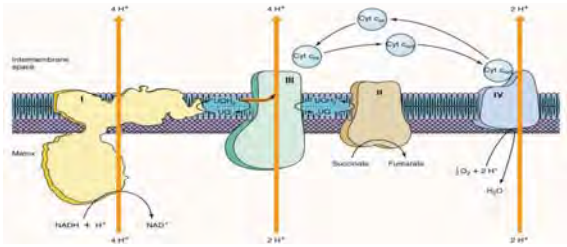
| *Redox reaction (half-reaction)* | $E'^{0}$ *(V)* |
|---|---|
| $2H^+ + 2e^- \longrightarrow H_2$ | −0.414 |
| $NAD^+ + H^+ + 2e^- \longrightarrow NADH$ | −0.320 |
| $NADP^+ + H^+ + 2e^- \longrightarrow NADPH$ | −0.324 |
| NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$) | −0.30 |
| Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol | 0.045 |
| Cytochrome *b* ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome *b* ($Fe^{2+}$) | 0.077 |
| Cytochrome $c_1$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $c_1$ ($Fe^{2+}$) | 0.22 |
| Cytochrome *c* ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome *c* ($Fe^{2+}$) | 0.254 |
| Cytochrome *a* ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome *a* ($Fe^{2+}$) | 0.29 |
| Cytochrome $a_3$ ($Fe^{3+}$) + $e^- \longrightarrow$ cytochrome $a_3$ ($Fe^{2+}$) | 0.35 |
| $\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$ | 0.8166 |





# Four parts of electron-transport chain

- Complex I: NADH-coenzyme Q reductase
- Complex II: succinate-coenzyme Q reductase
- Complex III: coenzyme Q-cytochrome c reductase
- Complex IV: cytochrome c oxidase

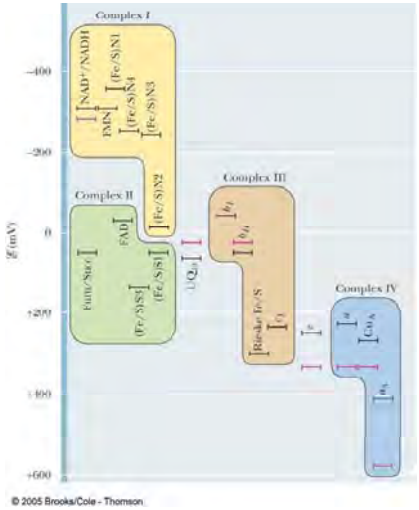






# Reduction potential of complexes

- Complex I & II transfer $e^-$ to $UQ_{10}$
- Complex III transfer $e^-$ to cytochrome c



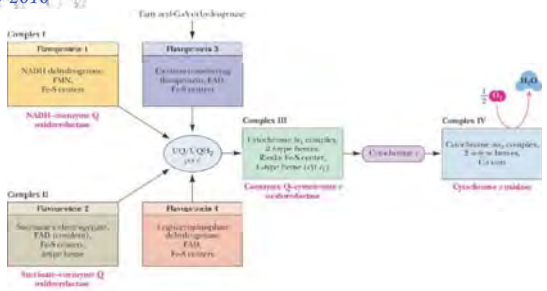


**TABLE 20.2 Protein Complexes of the Mitochondrial Electron-Transport Chain**

| Complex | Mass (kD) | Subunits | Prosthetic Group | Binding Site for: |
|---|---|---|---|---|
| NADH–UQ reductase | 980 | ≥45 | FMN<br>Fe-S | NADH (matrix side)<br>UQ (lipid core) |
| Succinate–UQ reductase | 140 | 4 | FAD<br>Fe-S | Succinate (matrix side)<br>UQ (lipid core) |
| UQ–Cyt *c* reductase | 250 | 9–10 | Heme $b_L$<br>Heme $b_H$<br>Heme $c_1$<br>Fe-S | Cyt *c* (intermembrane space side) |
| Cytochrome *c* | 13 | 1 | Heme *c* | Cyt $c_1$<br>Cyt *a* |
| Cytochrome *c* oxidase | 162 | 13 | Heme *a*<br>Heme $a_3$<br>$Cu_A$<br>$Cu_B$ | Cyt *c* (intermembrane space side) |





# End of Part 1

- You should know…
  - How to define the reduction potential of one compound?
  - How to tell a Redox reaction spontaneous or not?
  - How many complexes in electron transport?
  - What are mobile electron transporters?
  - What is the last electron receiver?





# Complex I

- Complex I, NADH dehydrogenase, is also called NADH: ubiquinone oxidoreductase.
- The complex catalyzes two coupled processes:
  - NADH + $H^+$ + Q→$NAD^+$ + $QH_2$ (an exergonic transfer)
  - the movement of four proton from matrix to the intermembrane space (an endogonic transfer).
  - Therefore, the catalysis of complex is vectorial with a proton pump driver by the energy of electron transfer.
  - Overall: NADH + $5H_N^+$ + Q→$NAD^+$ + $QH_2$ + $4H_P^+$.
- Ubiquinol ($QH_2$) diffuses in the inner memebrane from complex O to complex III, where it is oxidized to Q in a process that also involves the outward movement of $H^+$.





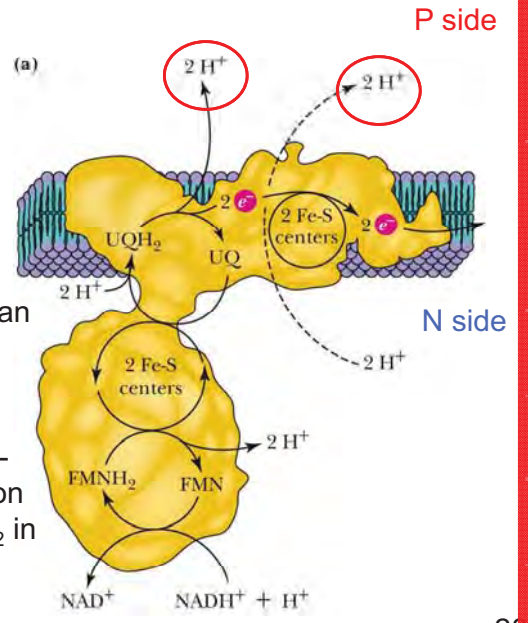



Figure 20.6 Structural organization of mammalian Complex I, based on electron microscopy, showing functional relationships within the L-shaped complex. Electron flow from NADH to $UQH_2$ in the membrane pool is indicated.

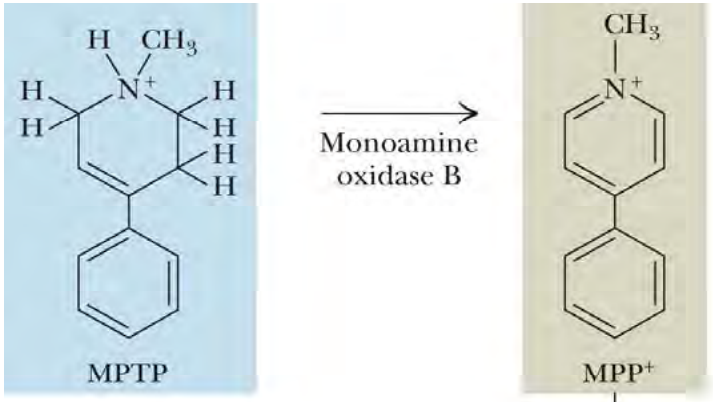
## Solving a Medical Mystery Revolutionized Our Treatment of Parkinson's Disease

- Cases of paralysis among illegal drug users in 1982 was traced to synthetic heroin that contained MPTP as a contaminant
  - MPTP is converted rapidly in the brain to $MPP^+$
  - $MPP^+$ is a potent inhibitor of mitochondrial Complex I
- Such inhibition occurs especially in regions of the brain that deteriorate in Parkinson's disease
- Treatment of the paralysis victims with L-Dopa restored normal movement
- J. William Langston also use L-Dopa to treat Parkinson's disease!





## Complex I Transports Protons From the Matrix to the Cytosol




- MPTP is converted in the brain to $MPP^+$
- $MPP^+$ is a potent inhibitor of mitochondrial Complex I





## Complex II Oxidizes Succinate and Reduces Coenzyme Q

- Succinate-CoQ Reductase aka succinate dehydrogenase (from TCA cycle) aka flavoprotein 2 ($FP_2$) - FAD covalently bound
- four subunits, including 2 Fe-S proteins
- Three types of Fe-S cluster:

  4Fe-4S, 3Fe-4S, 2Fe-2S
- Path: succinate $\rightarrow FADH_2 \rightarrow 2Fe^{2+} \rightarrow UQH_2$
- Net reaction:

  succinate + UQ $\rightarrow$ fumarate + $UQH_2$



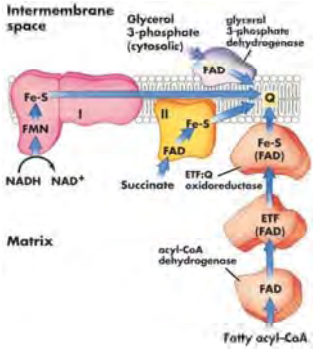


## Complex II and other UQH2 producer

- Complex II is succinate dehydrogenase, the only membrane-bound enzyme in TCA cycle.
- Acyl-CoA dehydrogenase involves transfer of electrons from fatty acyl-CoA to FAD of the dehydrogenase, and then to electron-transferring flavoprotein (ETF), which in turn passes its electrons to ETF-ubiquinone oxidoreductase.
- Glycerol 3-phosphate degydrogenase is a flavoprotein located on the outer surface of inner mitochondrial membrane. It channels electrons into the respiratory chain by reducing quinone. Its role is important in shuttling reducing equivalent from cytosolic NADH into mitochondrial matrix.

# Flavinprotein complexes

- 4 kinds of flavinprotein feed $e^-$ to $UQ_{10}$

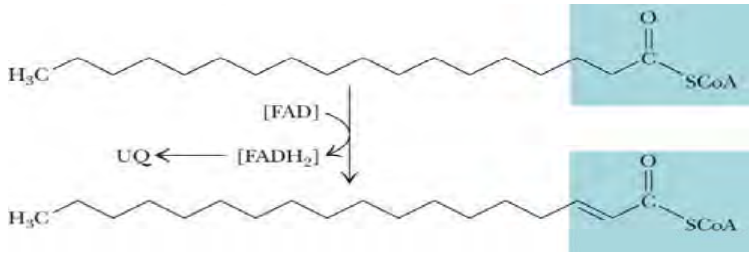







# Fatty-Acyl-CoA Dehydrogenases Also Supply Electrons to UQ

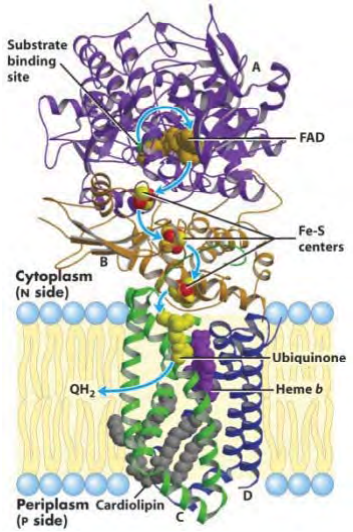
Figure 20.8 The fatty acyl-CoA dehydrogenase reaction, emphasizing that the reaction involves reduction of enzyme-bound FAD (indicated by brackets).




The fatty acyl-CoA dehydrogenases are three soluble matrix enzymes involved in fatty acid oxidation (See also Chapter 23).







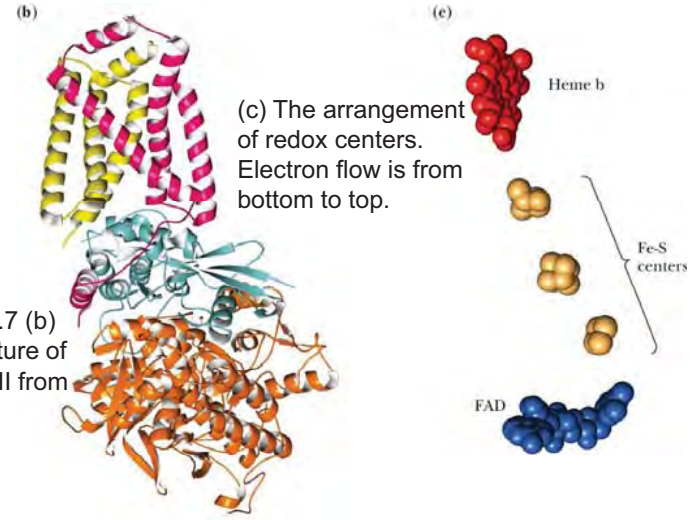

# Complex II

- Aka flavoprotein 2 ($FP_2$)
- 3 Fe-S clusters (4Fe-4S; 3Fe-4S; 2Fe-2S)
- Net reduction potential is too small (0.029 V) to drive the transport of proton (membrane potential 0.15 V)





# The Structure of Complex II




(c) The arrangement of redox centers. Electron flow is from bottom to top.

Figure 20.7 (b) The structure of Complex II from pig heart.

## Complex III Mediates Electron Transport from Coenzyme Q to Cytochrome *c*

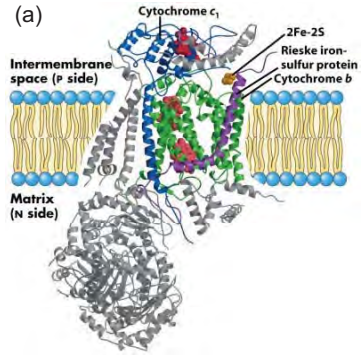
- *UQ-Cytochrome c Reductase*
- CoQ (UQ) passes electrons to cyt *c* (and pumps $H^+$) in a unique redox cycle known as the Q cycle
- The principal transmembrane protein in complex III is the *b* cytochrome - with hemes $b_L$ and $b_H$
- Cytochromes, like Fe in Fe-S clusters, are one-electron transfer agents
- Remember!
  - $UQH_2$ is a lipid-soluble electron carrier
  - cyt *c* is a water-soluble electron carrier





## Structure of Complex III

- aka cytochrome bc1 complex, ubiquinone: cytochrome c oxidoreductase (UQ-cyt c reductase),
- couples the transfer of electrons from ubiquinol (QH2) to cyt c with the vectorial transport of proton from matrix to intermembrane space.




Heme b (H & L) & Heme c1

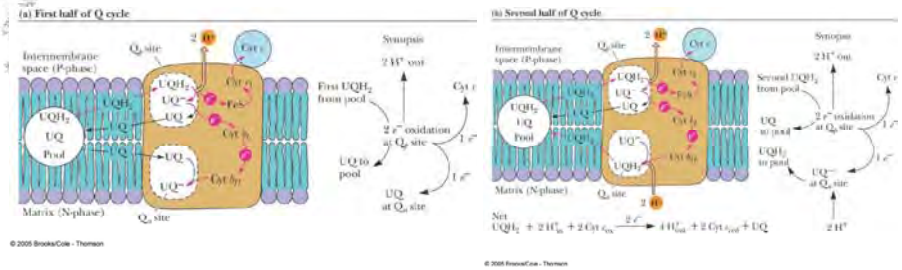




## The Q cycle

- $QH_2$ + cyt c1(oxi)→•$Q^-$ + $2H_P^+$ +cyt c1(red)
- $QH_2$ + •$Q^-$ + $2H_N^+$ + cyt c1(oxi) →$QH_2$+$2H_P^+$ + Q + cyt c1(red)
- Net equation:

  $QH_2 + 2\text{cyt c1(oxi)} + 2H_N^+ \rightarrow Q + 2\text{ cyt c1(red)} + 4H_P^+$

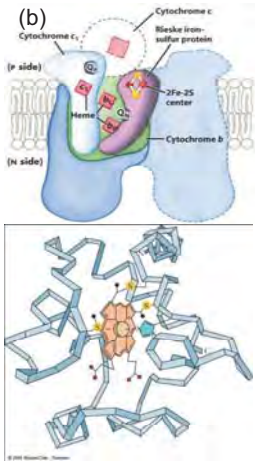







## Cytochrome c

- A mobile electron carrier
- A soluble protein of intermembrane space, accepts an electron from complex III and move to complex IV to donate the electron to a binuclear copper center.



## Detection of the redox state of Cytochrome c by absorption spectrum

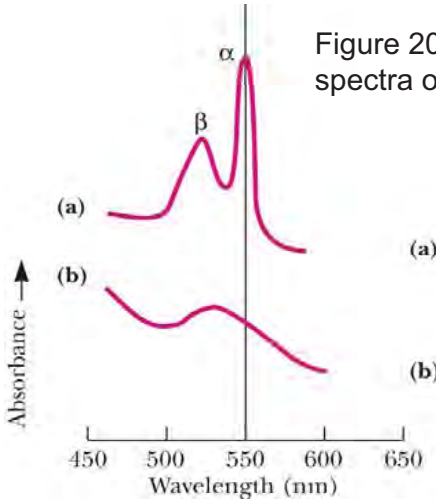



Figure 20.9 Visible absorption spectra of cytochrome *c*.

(a) Cytochrome *c* reduced spectrum

(b) Cytochrome *c* oxidized spectrum





## Complex IV Transfers Electrons from Cytochrome *c* to Reduce Oxygen on the Matrix Side

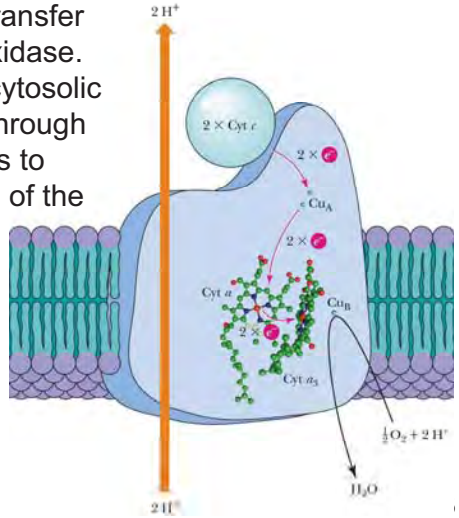
- *Cytochrome c Oxidase*
- Electrons from cyt *c* are used in a four-electron reduction of $O_2$ to produce $2H_2O$
- Oxygen is thus the terminal acceptor of electrons in the electron transport pathway
- Cytochrome *c* oxidase utilizes 2 hemes (*a* and $a_3$) and 2 copper sites
- Complex IV also transports $H^+$ across the inner mitochondrial membrane
- Four $H^+$ participate in $O_2$ reduction and four $H^+$ are transported in each catalytic cycle





## Complex IV Transfers Electrons from Cytochrome *c* to Reduce Oxygen on the N Side

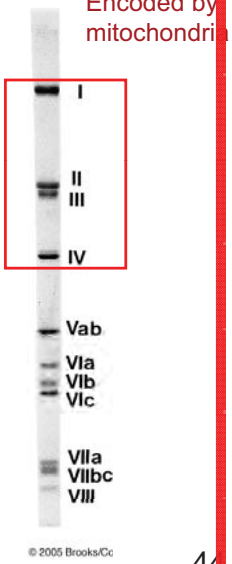
Figure 20.16 The electron-transfer pathway for cytochrome *c* oxidase. Cytochrome *c* binds on the cytosolic face, transferring electrons through the copper and heme centers to reduce $O_2$ on the matrix side of the membrane.



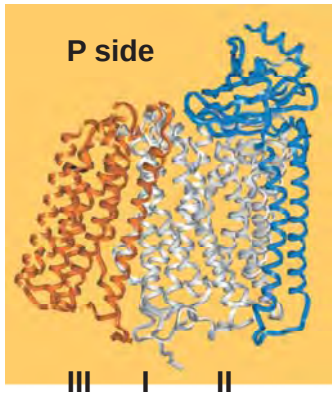


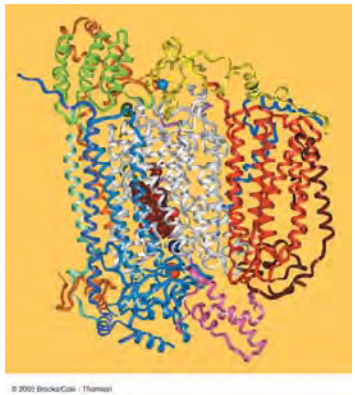

## Complex IV

- cytochrome oxidase, carriers electrons from cyt c to $O_2$, reducing it to $H_2O$.
- Three subunits are critical to the electron transfer



# Structure of complex IV

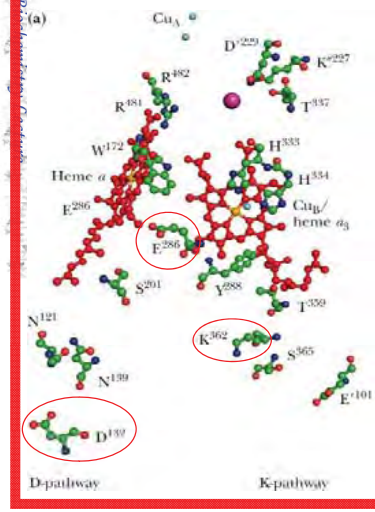


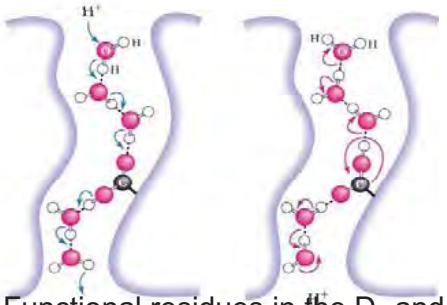
**3 subunit + regulatory proteins**



# Figure 20.18 The proton channels of cytochrome *c* oxidase from *R. spheroides*




(b) Protons are presumed to "hop" along arrays of water molecules



(a) Functional residues in the D- and K-pathways are indicated. The D- and K-pathways converge at the $Cu_B$/heme $a_3$ center.





# Net reaction in complex IV

- For every four electrons passing through complex IV, the enzyme consumes four $H^+$ from matrix in converting $O_2$ to $H_2O$. The energy of this redox reaction is used to pump one proton outward into the intermembrane space for each electron that passes through. Overall:
  4 cyt c(red) + $8H_N^+$ + $O_2 \rightarrow$ 4 cyt c(oxi) + $4H_P^+$ + $2H_2O$





# The Four Electron-Transport Complexes are Independent

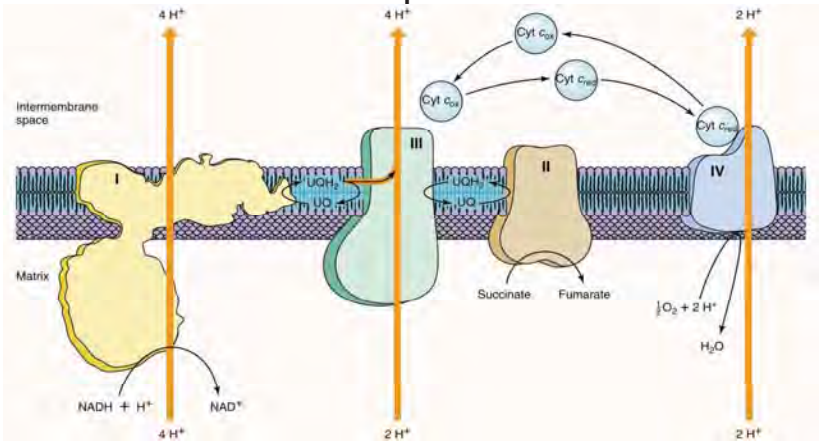



Figure 20.19 A model for the electron-transport pathway in the mitochondrial inner membrane. UQ/UQH2 and cyt c are mobile carrioers and transfer electrons between the complexes.

## Electron Transfer Energy Stored in a Proton Gradient: The Mitchell Hypothesis

- Many biochemists squandered careers searching for the elusive "high energy intermediate"
- Peter Mitchell proposed a novel idea - a proton gradient across the inner membrane could be used to drive ATP synthesis
- The proton gradient is created by the proteins of the electron-transport pathway
- Mitchell was ridiculed, but the **chemiosmotic hypothesis** eventually won him a Nobel prize
- Be able to calculate the $\Delta G$ for a proton gradient (Equation 20.24)





## Electron Transfer Energy Stored in a Proton Gradient: The Mitchell Hypothesis

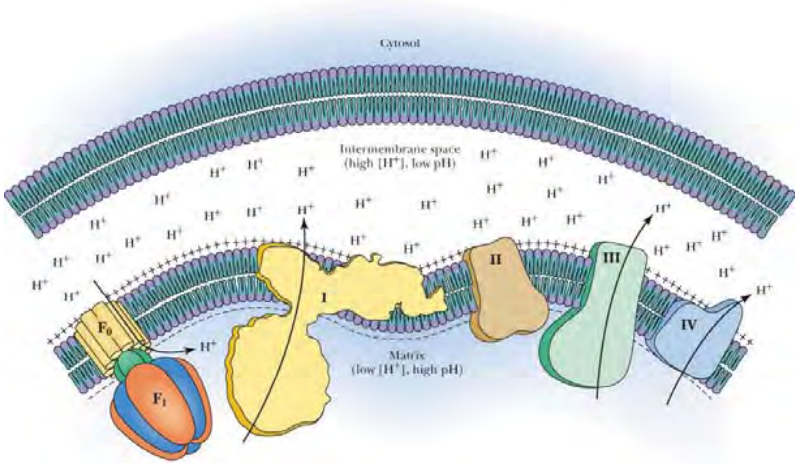



Figure 20.20 The proton and electrochemical gradients existing across the inner mitochondrial membrane.





## 20.4 What Are the Thermodynamic Implications of Chemiosmotic Coupling?

- How much energy can be stored in an electrochemical gradient?
- The free energy difference for protons across the inner mitochondrial membrane includes a term for the concentration difference and a term for the electrical potential:

$$\Delta G = RT \ln \frac{[c_2]}{[c_1]} + Z\mathcal{F}\Delta\Psi$$

- $c_1$ and $c_2$ are proton concentrations on the two sides of the membrane, Z is the proton charge, $\mathcal{F}$ is Faraday's constant, and $\Delta\psi$ is the potential difference across the membrane





## Mitchell's chemiosmotic hypothesis

- Proton gradient stored in the inner membrane could drive the synthesis of ATP.

$$H^+_{in} \rightarrow H^+_{out}$$

$$\Delta G = RT \ln \frac{[H^+_{out}]}{[H^+_{in}]} + ZF\Delta\Psi$$

**Potential effect** ($\Delta\Psi$=potential different across membrane)

**Concentration effect**

$\Delta G = 23.3$ kJ

# $H^+/2e^-$ Ration

- The energy of electron transfer is efficiently conserved in a proton gradient.
- $NADH+H^++1/2O_2 \rightarrow NAD^++H_2O$ $\Delta E'o=1.14V$
  $\Delta G'^o = -nF\Delta E'o = -2(96.5\ KJ/V)(1.14V)$
  $= -220\ KJ/mol\ of\ NADH$
- The oxidation of succinate to $O_2$
  $\Delta G'^o = -150\ KJ/mol\ of\ FADH_2$
- For each pair of electrons transferred to $O_2$, four protons are pumped out by complex I, four by complex III and two by complex IV

$$NADH+11H_N^++1/2\ O_2 \rightarrow NAD^++10H_P^++H_2O$$





# End of Part 2

- You should know…
  - What is P side? What is N side?
  - How many $H^+$ are pumped out in each complexes?
  - How many $H^+$ are pumped out when one NADH is fully oxidated?
  - What is the $H^+/2e^-$ Ration of $FADH_2$ oxidation?
  - What are the four kind of flavinproteins?
  - What is the Mitchell Hypothesis?





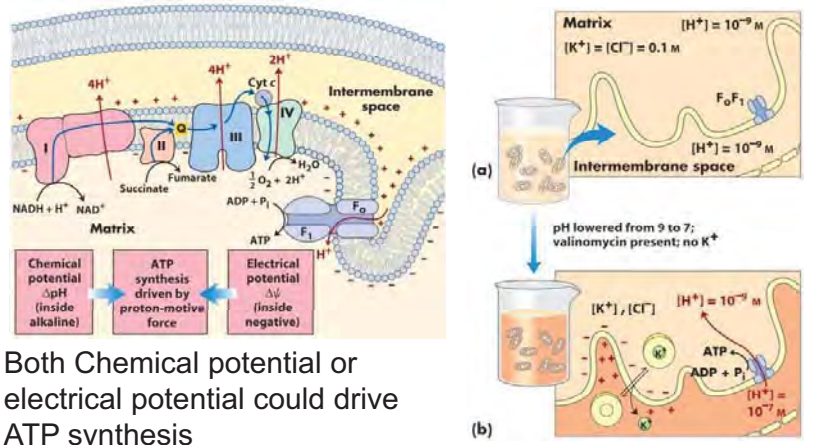
# 20.5 How Does a Proton Gradient Drive the Synthesis of ATP?

- *Proton diffusion through the **ATP synthase** drives ATP synthesis*
- The ATP synthase consists of two parts: $F_1$ and $F_0$ (latter was originally named "$F_o$" for its inhibition by oligomycin)
  - $F_1$ consists of five polypeptides: α, β,γ, δ, and ε
  - $F_0$ includes three hydrophobic subunits denoted *a*, *b* and *c*
  - $F_0$ forms the transmembrane pore or channel through which protons move to drive ATP synthesis
  - The *a* and *b* subunits comprise part of the **stator** and a ring of *c* subunits forms a **rotor**





# Evidence for the role of a proton gradient in ATP synthesis




Both Chemical potential or electrical potential could drive ATP synthesis

# ATP Synthase is Composed of $F_1$ and $F_0$

**TABLE 20.3** Yeast $F_1F_0$–ATP Synthase Subunit Organization

| Complex | Protein Subunit Function | Mass (kD) | Stoichiometry | |
|---|---|---|---|---|
| $F_1$ | $\alpha$ | 55.4 | 3 | Stator |
| | $\beta$ | 51.3 | 3 | Stator |
| | $\gamma$ | 30.6 | 1 | Rotor |
| | $\delta$ | 14.6 | 1 | Rotor† |
| | $\epsilon$ | 6.6 | 1 | Rotor |
| $F_0$ | $a$ | 27.9 | 1 | Stator |
| | $b$ | 23.3 | 1 | Stator |
| | $c$ | 7.8 | 10–15* | Rotor |
| | $d$ | 19.7 | 1 | Stator |
| | $h$ | 10.4 | 1 | Stator |
| | OSCP | 20.9 | 1 | Stator |



# The structure of ATP Synthase

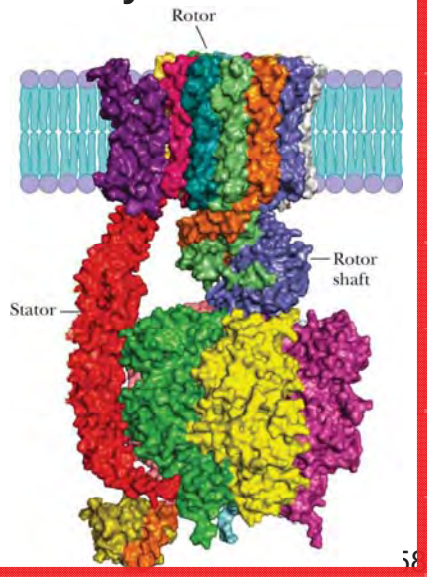



Figure 20.21 The ATP synthase, a rotating molecular motor. The *c*, γ and ε subunits constitute the rotating portion (the rotor) of the motor. The b, d and h subunits form a long, slender stalk that connects $F_0$ in the membrane and $F_1$. Flow of protons from the *a*-subunit through the *c*-subunits turns the rotor and drives the cycle of conformation changes in α and β that synthesize ATP.



# The Structure of Catalytic Sites of ATP Synthase

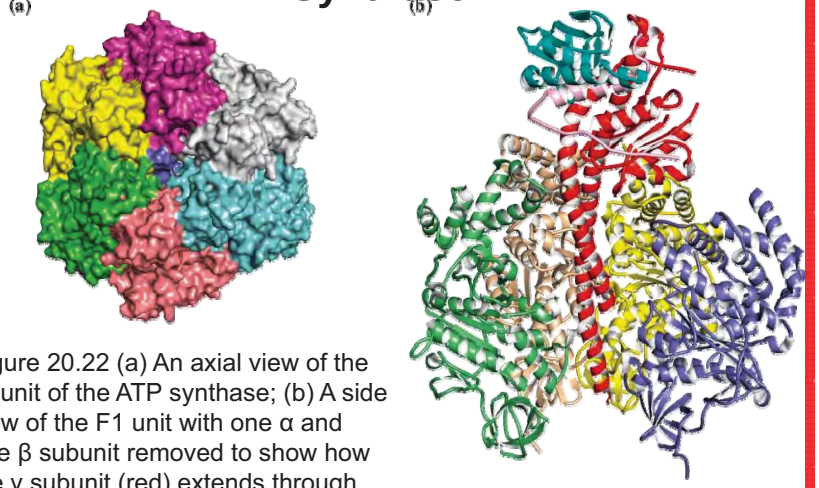



Figure 20.22 (a) An axial view of the $F_1$ unit of the ATP synthase; (b) A side view of the F1 unit with one α and one β subunit removed to show how the γ subunit (red) extends through the center of the hexamer.





# Boyer's $^{18}O$ Exchange Experiment Identified the Energy-Requiring Step

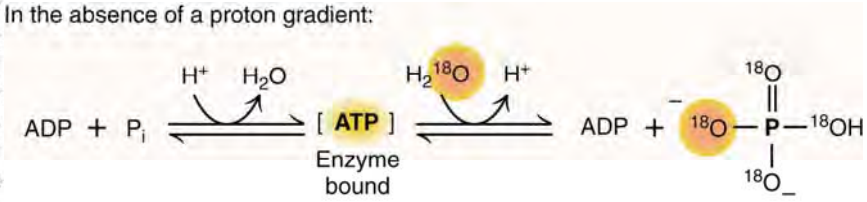



Figure 20.23 ATP-ADP exchange in the absence of a proton gradient. Exchange leads to incorporation of $^{18}O$ in phosphate as shown. Boyer's experiments showed that $^{18}O$ could be incorporated into all four positions of phosphate, demonstrating that the free energy change for ATP formation from enzyme-bound ADP + $P_i$ is close to zero.

## How F1 catalyzes ATP synthesis?

- Paul Boyer proposed that, at any instant:
  - the three β subunits of F1 exist in three different conformations
  - these different states represent the three steps of ATP synthesis
  - each site steps through the three conformations or states to make ATP
- In Boyer's **binding change mechanism**, the three catalytic sites thus cycle through the three intermediate states of ATP synthesis





## The Binding Change Mechanism

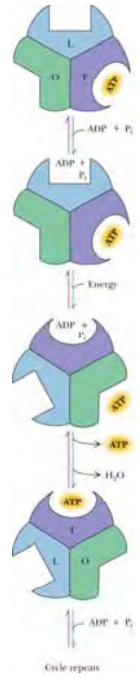


Figure 20.24 The binding change mechanism for ATP synthesis by ATP synthase. This model assumes that F1 has three interacting and conformationally distinct active sites: an open (O) conformation with almost no affinity for ligands, a loose (L) conformation with low affinity for ligands, and a tight (T) conformation with high affinity for ligands.





## The proton gradient drives the release of ATP from the enzyme surface

(1) In the absence of proton gradient, the newly synthesized ATP does not leave the enzyme surface.

(2) Release of ATP by the proton gradient

(3) For the continued synthesis of ATP, the enzyme must cycle between a form that binds ATP very tightly and a form that release ATP.

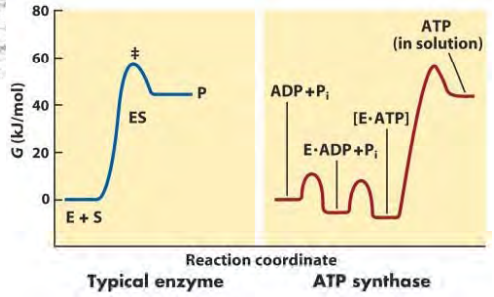
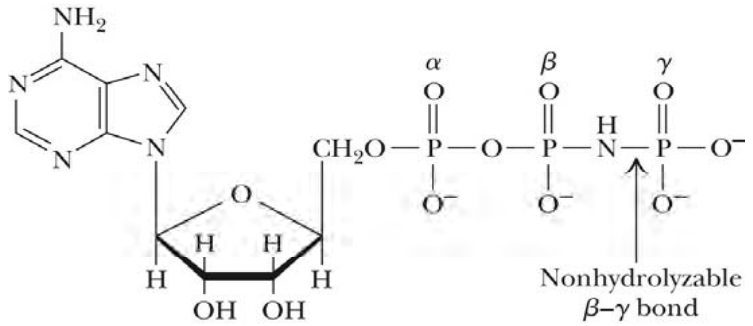



**FIGURE 19–22 Reaction coordinate diagrams for ATP synthase and for a more typical enzyme.** In a typical enzyme-catalyzed reaction (left), reaching the transition state (‡) between substrate and product is the major energy barrier to overcome. In the reaction catalyzed by ATP synthase (right), release of ATP from the enzyme, not formation of ATP, is the major energy barrier. The free-energy change for the formation of ATP from ADP and $P_i$ in aqueous solution is large and positive, but on the enzyme surface, the very tight binding of ATP provides sufficient binding energy to bring the free energy of the enzyme-bound ATP close to that of ADP + $P_i$, so the reaction is readily reversible. The equilibrium constant is near 1. The free energy required for the release of ATP is provided by the proton-motive force.





## John Walker Determined the Structure of the $F_1$ Portion of ATP Synthase

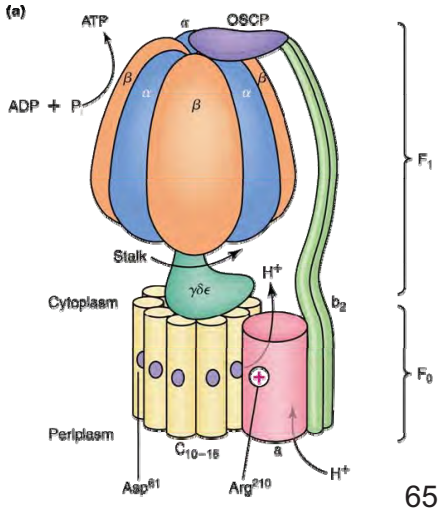



**β, γ-Imidoadenosine 5′-triphosphate (AMP-PNP)**

In Walker's crystal structure of $F_1$, one of the β subunits contains AMP-PNP, one contains ADP, and the third site is empty – the three states of Boyer's model!

# Proton Flow Through $F_0$ Drives Rotation of the Motor and Synthesis of ATP

Figure 20.25 (a) Protons entering the inlet half-channel in the α-subunit are transferred to binding sites on *c*-subunits. Rotation of the *c*-ring delivers protons to the outlet half-channel in the α-subunit. Flow of protons through the structure turns the rotor and drives the cycle of conformational changes in β that synthesize ATP.



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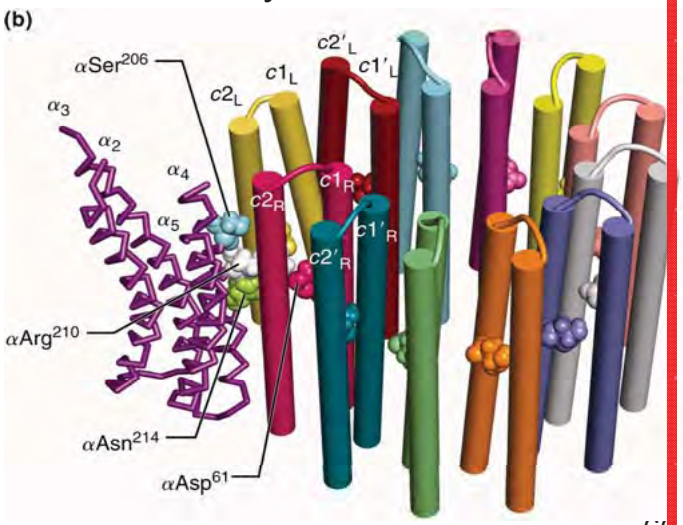
# Proton Flow Through $F_0$ Drives Rotation of the Motor and Synthesis of ATP

Figure 20.25 (b) $Arg^{210}$ on the a-subunit lies between the end of the inlet half-channel ($Asn^{214}$) and the end of the of the outlet half-channel ($Ser^{206}$).

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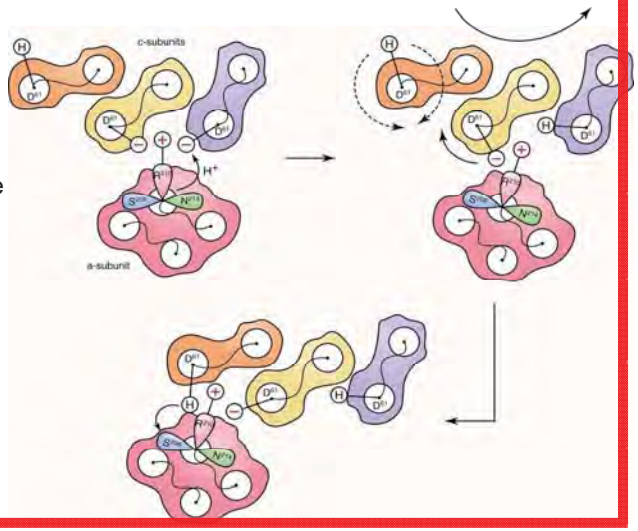
# Proton Flow Through $F_0$ Drives Rotation of the Motor and Synthesis of ATP

Figure 20.25 (c) A view looking down into the plane of the membrane. Transported protons flow from the inlet half-channel to $Asp^{61}$ residues on the c-ring, around the ring, and then into the outlet half-channel.

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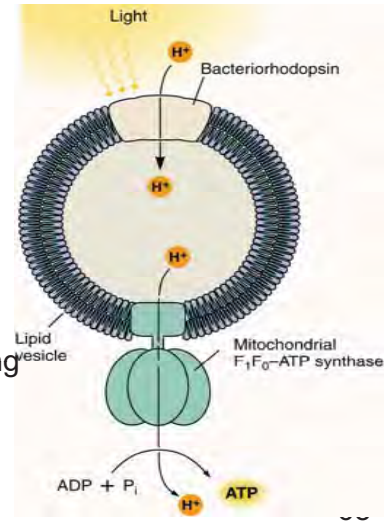
# Racker and Stoeckenius Confirmed the Mitchell Model in a Reconstitution Expt

Figure 20.26 The reconstituted vesicles containing ATP synthase and bacteriorhodopsin used by Stoeckenius and Racker to confirm the Mitchell chemiosmotic hypothesis.

Upon illumination, bacteriorhodopsin pumped protons into these vesicles, and the resulting proton gradient was sufficient to drive ATP synthesis by the ATP synthase.



# How to study the pathway?

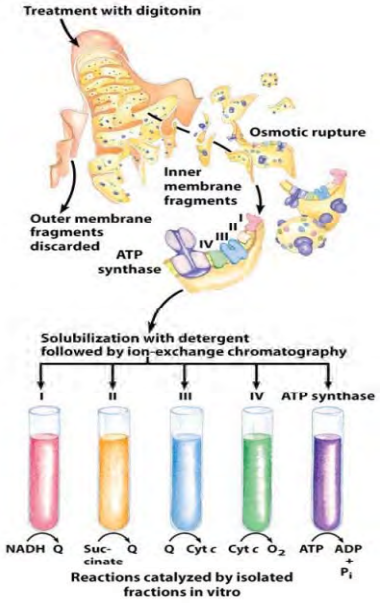


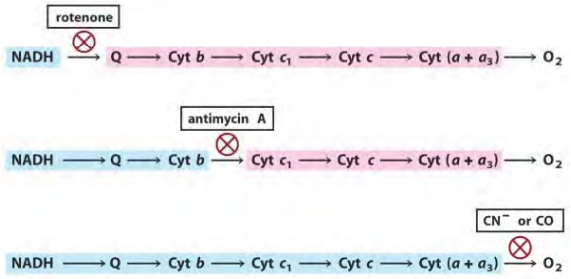

**FIGURE 19–7 Separation of functional complexes of the respiratory chain.** The outer mitochondrial membrane is first removed by treatment with the detergent digitonin. Fragments of inner membrane are then obtained by osmotic rupture of the mitochondria, and the fragments are gently dissolved in a second detergent. The resulting mixture of inner membrane proteins is resolved by ion-exchange chromatography into different complexes (I through IV) of the respiratory chain, each with its unique protein composition (see Table 19–3), and the enzyme ATP synthase (sometimes called Complex V). The isolated Complexes I through IV catalyze transfers between donors (NADH and succinate), intermediate carriers (Q and cytochrome *c*), and $O_2$, as shown. In vitro, isolated ATP synthase has only ATP-hydrolyzing (ATPase), not ATP-synthesizing, activity.





# How to study the pathway?

- By using inhibitors







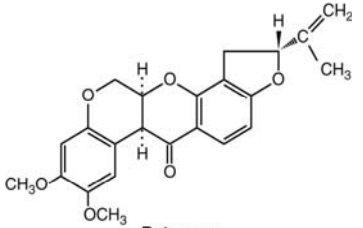

# Inhibitors of Oxidative Phosphorylation Reveal Insights About the Mechanism

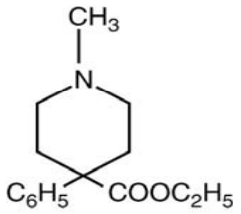
- Many details of electron transport and oxidative phosphorylation have been learned from studying the effects of inhibitors
- **Rotenone** inhibits Complex I - and helps natives of the Amazon rain forest catch fish
- (Natives have learned to beat the roots of certain trees along river banks, releasing rotenone, which paralyzes the fish, making them easy prey)
- **Cyanide**, **azide** and **CO** inhibit Complex IV, binding tightly to the ferric form ($Fe^{3+}$) of $a_3$
- **Oligomycin** is an ATP synthase inhibitor

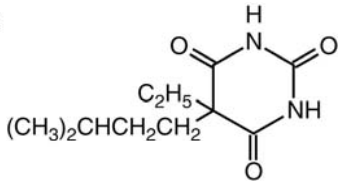




# Inhibitors of Complex I



## Inhibitors of Oxidative Phosphorylation Reveal Insights About the Mechanism

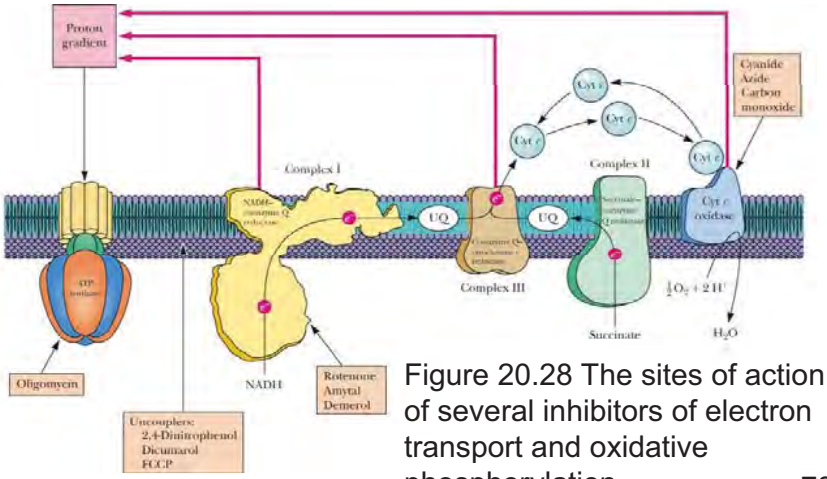



Figure 20.28 The sites of action of several inhibitors of electron transport and oxidative phosphorylation.





## Uncouplers Disrupt the Coupling of Electron Transport and ATP Synthase

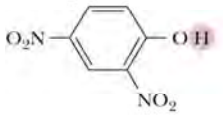
- Uncoupling $e^-$ transport and oxidative phosphorylation
- Uncouplers disrupt the tight coupling between electron transport and oxidative phosphorylation by dissipating the proton gradient
- Uncouplers are hydrophobic molecules with a dissociable proton
- They shuttle back and forth across the membrane, carrying protons to dissipate the gradient



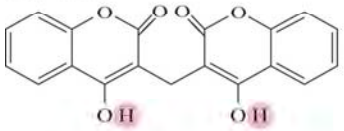


## Uncouplers Disrupt the Coupling of Electron Transport and ATP Synthase

**Dinitrophenol**



**Dicumarol**



**Carbonyl cyanide-*p*-trifluoro-methoxyphenyl hydrazone**
—best known as **FCCP**; for **F**luoro **C**arbonyl **C**yanide **P**henylhydrazone

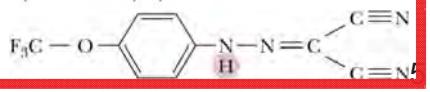
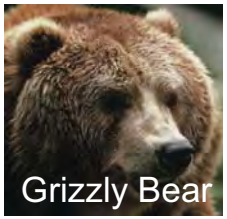


Figure 20.29 Structures of several uncouplers, molecules that dissipate the proton gradient across the inner mitochondrial membrane and thereby destroy the tight coupling between electron transport and the ATP synthase reaction.



## Endogenous Uncouplers

- Hibernating Animals Generate Heat by Uncoupling Oxidative Phosphorylation
- Brown fat → thermogenin or uncoupling protein 1 (UCP1)
- Some Plants Use Uncoupled Proton Transport to Raise the Temperature of Floral Spikes


Grizzly Bear


Skunk Cabbage

## ATP-ADP Translocase Mediates the Movement of ATP & ADP Across the Mitochondrial Membrane

*ATP must be transported out of the mitochondria*

- ATP out, ADP in - through a "translocase"
- ATP movement out is favored because the cytosol is "+" relative to the "-" matrix
- But ATP out and ADP in is net movement of a negative charge out - equivalent to a $H^+$ going in
- So every ATP transported out costs one $H^+$
- One ATP synthesis costs about 3 $H^+$
- Thus, making and exporting 1 ATP = $4H^+$





## ATP-ADP Translocase

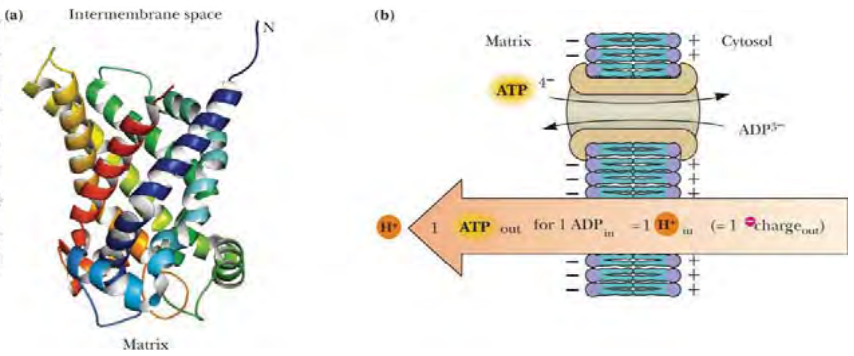



Figure 20.30 (a) The bovine ATP-ADP translocase. (b) Outward transport of ATP (via the ATP-ADP translocase is favored by the membrane electrochemical potential.





## 20.6 - What Is the P/O Ratio for Mitochondrial Electron Transport and Oxidative Phosphorylation?

*How many ATP can be made per electron pair sent through the chain?*

- The $e^-$ transport chain yields 10 $H^+$ pumped out per electron pair from NADH to oxygen
- 4 $H^+$ flow back into matrix per ATP to cytosol
- 10/4 = 2.5 for electrons entering as NADH
- For electrons entering as succinate ($FADH_2$), about 6 $H^+$ pumped per electron pair to oxygen
- 6/4 = 1.5 for electrons entering as succinate





## 20.7 – How Are the Electrons of Cytosolic NADH Fed into Electron Transport?

*Most NADH used in electron transport is cytosolic and NADH doesn't cross the inner mitochondrial membrane*

- What to do?
- 2 "Shuttle systems" effect electron movement without actually carrying NADH
  - Glycerophosphate shuttle stores electrons in glycerol-3-P, which transfers electrons to FAD
  - Malate-aspartate shuttle uses malate to carry electrons across the membrane

## The Glycerophosphate Shuttle Ensures Efficient Use of Cytosolic NADH

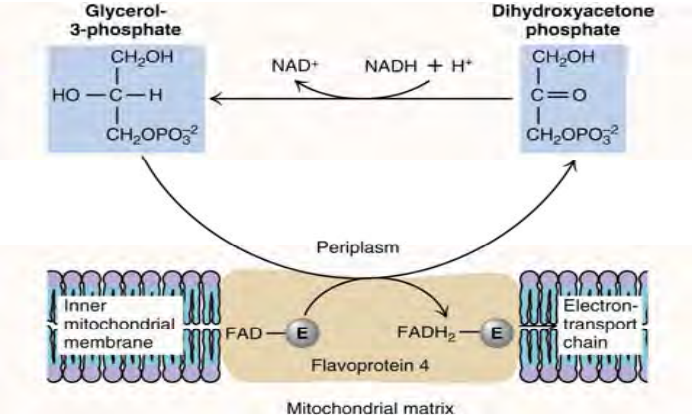



Figure 20.31 The glycerophosphate shuttle couples cytosolic oxidation of NADH with mitochondrial reduction of [FAD].





## The Malate-Aspartate Shuttle is Reversible

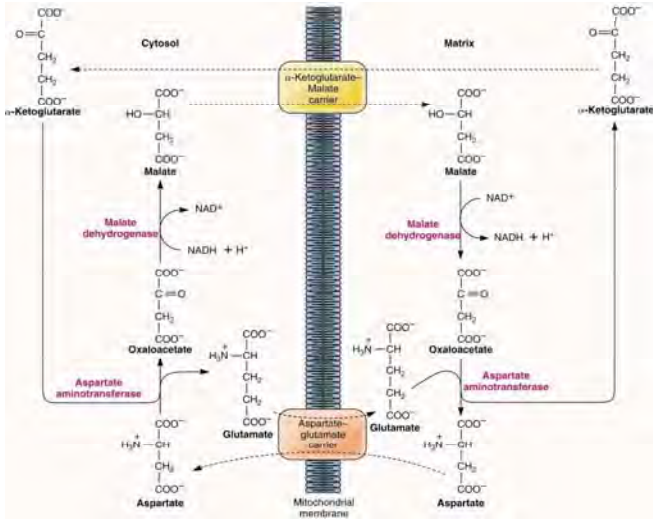



Figure 20.32 The malate-aspartate shuttle.





## The Net Yield of ATP from Glucose Oxidation Depends on the Shuttle Used

- 30 ATP per glucose if glycerol-3-P shuttle used
- 32 ATP per glucose if malate-Asp shuttle used
- In bacteria - no mitochondria - no extra $H^+$ used to export ATP to cytosol, so:
  - 10/3 = ~3ATP/NADH
  - 6/3 = ~ 2ATP/$FADH_2$



## The Net Yield of ATP from Glucose Oxidation Depends on the Shuttle Used

**TABLE 20.4** Yield of ATP from Glucose Oxidation

| Pathway | ATP Yield per Glucose: Glycerol-Phosphate Shuttle | ATP Yield per Glucose: Malate-Aspartate Shuttle |
|---|---|---|
| *Glycolysis: glucose to pyruvate (cytosol)* | | |
| Phosphorylation of glucose | −1 | −1 |
| Phosphorylation of fructose-6-phosphate | −1 | −1 |
| Dephosphorylation of 2 molecules of 1,3-BPG | +2 | +2 |
| Dephosphorylation of 2 molecules of PEP | +2 | +2 |
| Oxidation of 2 molecules of glyceraldehyde-3-phosphate yields 2 NADH | | |
| *Pyruvate conversion to acetyl-CoA (mitochondria)* | | |
| 2 NADH | | |
| *Citric acid cycle (mitochondria)* | | |
| 2 molecules of GTP from 2 molecules of succinyl-CoA | +2 | +2 |
| Oxidation of 2 molecules each of isocitrate, α-ketoglutarate, and malate yields 6 NADH | | |
| Oxidation of 2 molecules of succinate yields 2 [$FADH_2$] | | |
| *Oxidative phosphorylation (mitochondria)* | | |
| 2 NADH from glycolysis yield 1.5 ATPs each if NADH is oxidized by glycerol–phosphate shuttle; 2.5 ATP by malate–aspartate shuttle | +3 | +5 |
| Oxidative decarboxylation of 2 pyruvate to 2 acetyl-CoA: 2 NADH produce 2.5 ATPs each | +5 | +5 |
| 2 [$FADH_2$] from each citric acid cycle produce 1.5 ATPs each | +3 | +3 |
| 6 NADH from citric acid cycle produce 2.5 ATPs each | +15 | +15 |
| *Net Yield* | 30 | 32 |

# 20.8 How Do Mitochondria Mediate Apoptosis?

- Mitochondria play a significant role in **apoptosis**, the programmed death of cells
- Mitochondria do this in part, by partitioning some of the apoptotic activator molecules, e.g., cytochrome *c*
  - Oxidation of bound cardiolipins releases cytochrome *c* from the inner membrane
  - Opening of pores in the outer membrane releases cytochrome c from the mitochondria
  - Binding of cytochrome c to Apaf-1 in the cytosol leads to assembly of apoptosomes, thus triggering the events of apoptosis





## Apoptosis Step 1: Release of Cyt c

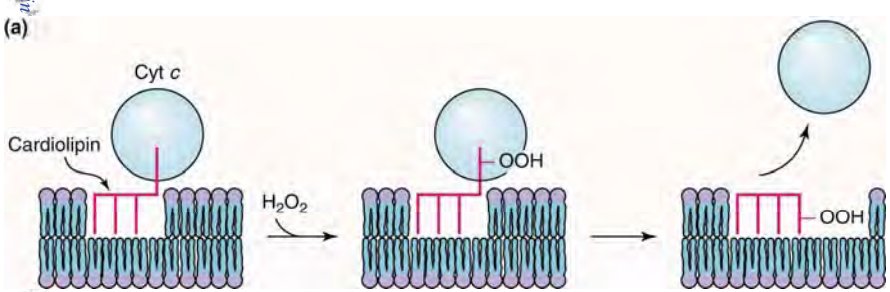



Figure 20.33 (a) Cytochrome c is anchored at the inner mitochondrial membrane by association with cardiolipin. The peroxidase activity of cytochrome c oxidizes a cardiolipin lipid chain, releasing cytochrome c from the membrane.





## Apoptosis Step 2: Opening of pores

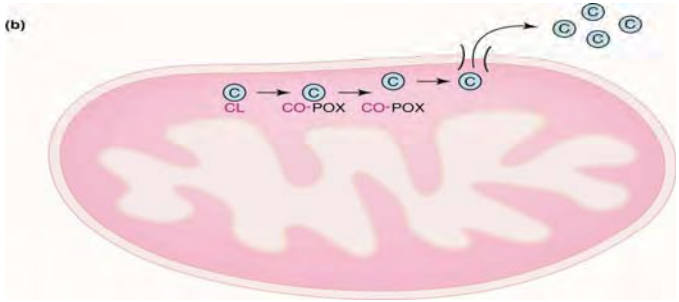



Figure 20.33 The opening of pores in the outer membrane, induced by a variety of triggering agents, releases cytochrome c to the cytosol, where it initiates the events of apoptosis.





## Apoptosis Step 3: Apoptosome formation

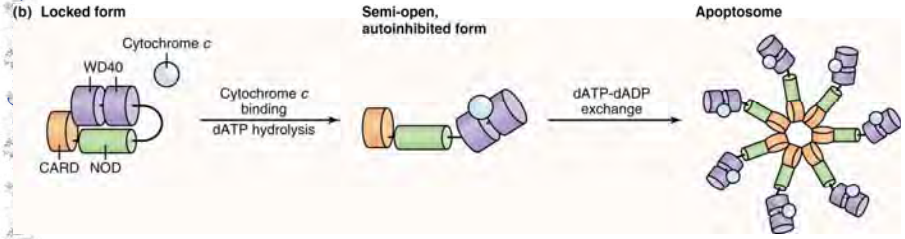
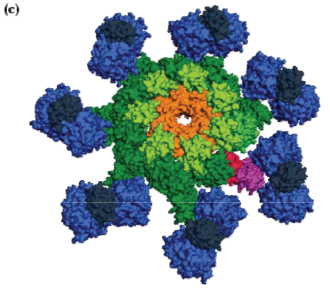



Figure 20.34 Binding of cytochrome c to the WD40 domains and ATP hydrolysis unlocks Apaf-1 to form the semi-open conformation. Nucleotide exchange leads to oligomerization and apoptosome formation.



# End of Part 3

- You should know…
  - What is the mechanism of $FoF_1$-ATP synthase?
  - What is the Boyer's binding change mechanism?
  - How many $H^+$ influx could produce 1 ATP?
  - What is the P/O ratio of NADH and $FADH_2$?
  - How cytosolic NADH are tranported into mitochondria?
  - How mitochondria triggered apoptosis?





# End of the class

- You should have learned…
  - The reduction potential of the electron transporters
  - The flow of electron between complexes
  - How metabolites and NADH are transported into mitochondria
  - The Mitchell's chemiosmotic hypothesis
  - How to calculate the amount of energy transformed into ATP.
  - How ATP synthase works?
  - How mitochondria triggers apoptosis?