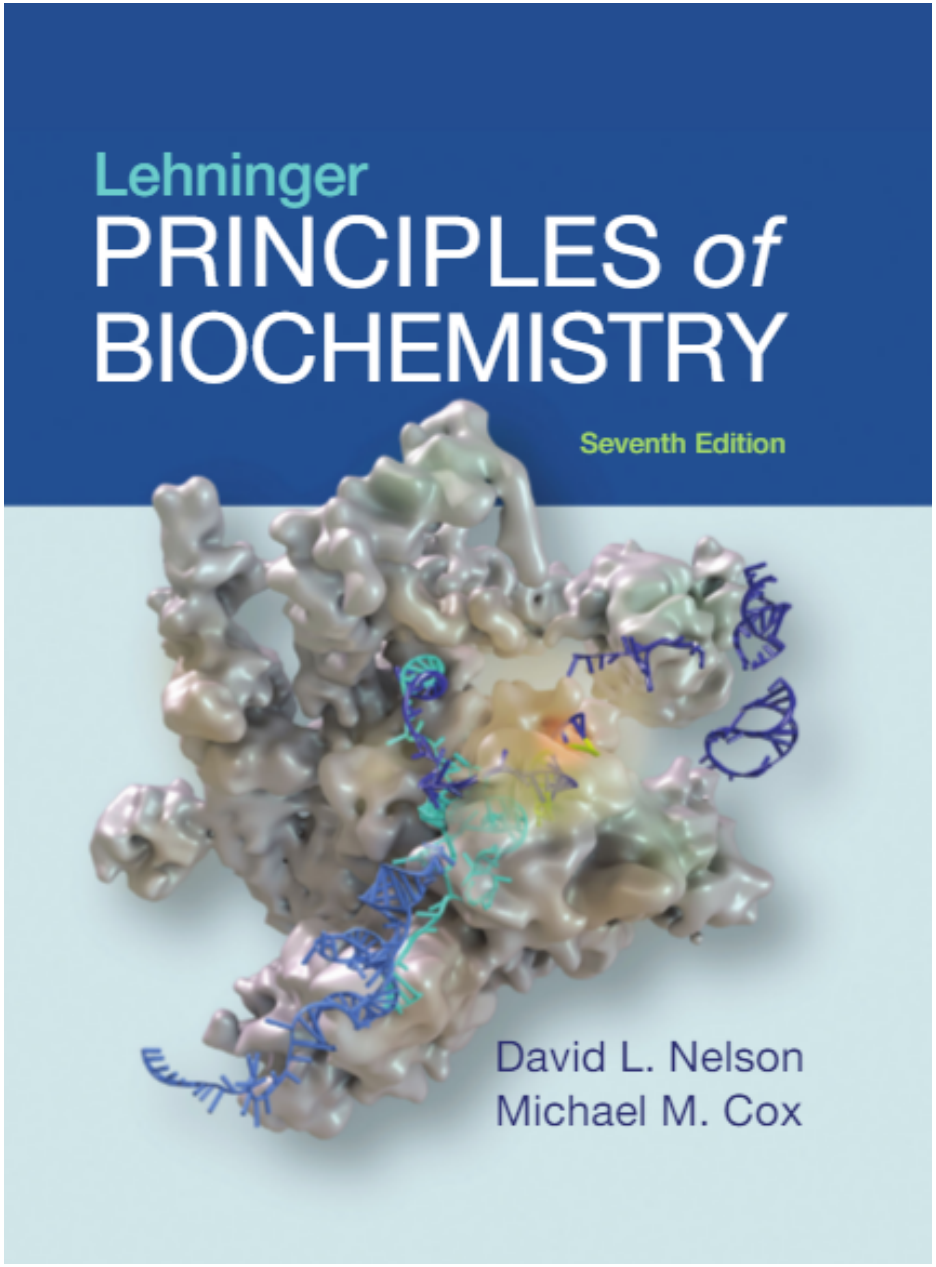
19| Oxidative Phosphorylation

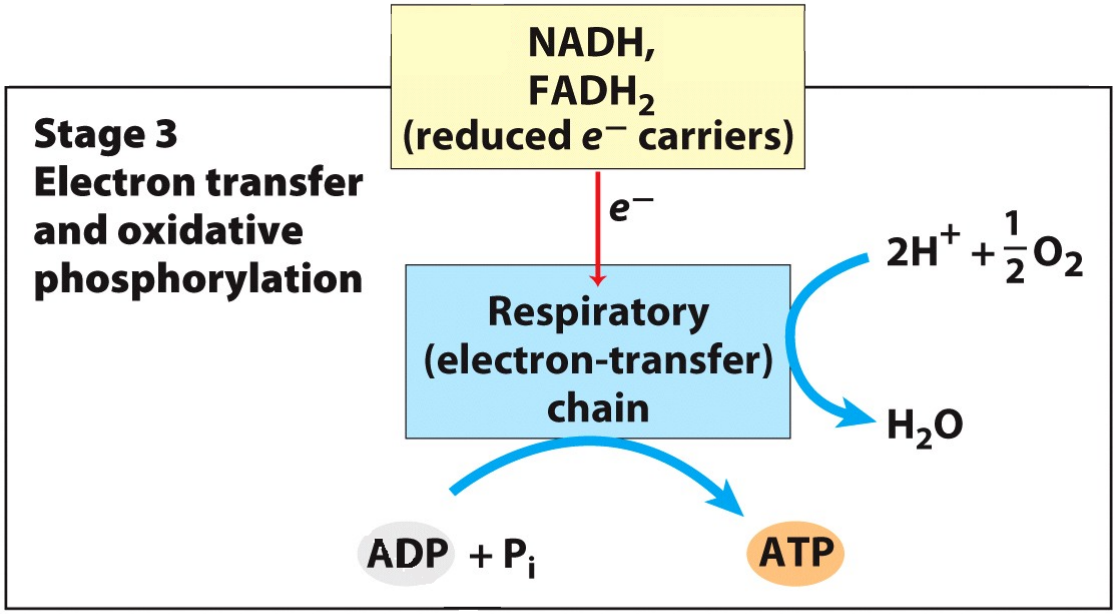


Energy from reduced fuels is used to synthesize ATP in animals

- Carbohydrates, lipids, and amino acids are the main reduced fuels for the cell
- Their oxidative steps *converge* in the final stage of cellular respiration
- Electrons from reduced fuels are transferred to reduced cofactors NADH or $FADH_2$
- In **oxidative phosphorylation**, energy from NADH and $FADH_2$ are used to make ATP

Oxidative Phosphorylation

- Electrons from the reduced cofactors NADH and $FADH_2$ are passed to proteins in the respiratory chain
- In eukaryotes, oxygen is the ultimate electron acceptor for these electrons
- Energy of oxidation is used to phosphorylate ADP



Photophosphorylation

- In photosynthetic organisms light causes charge separation between a pair of chlorophyll molecules
- Energy of the oxidized and reduced chlorophyll molecules is used to drive synthesis of ATP
- Water is the source of electrons that are passed via a chain of protein transporters to the ultimate electron acceptor, $NADP^+$
- Oxygen is the byproduct of water oxidation
- *Both processes:*

1. Involve the flow of e^-s through a chain
2. Coupled to an endergonic "uphill" transport of protons
3. Flow back of protons provides energy for making ATP

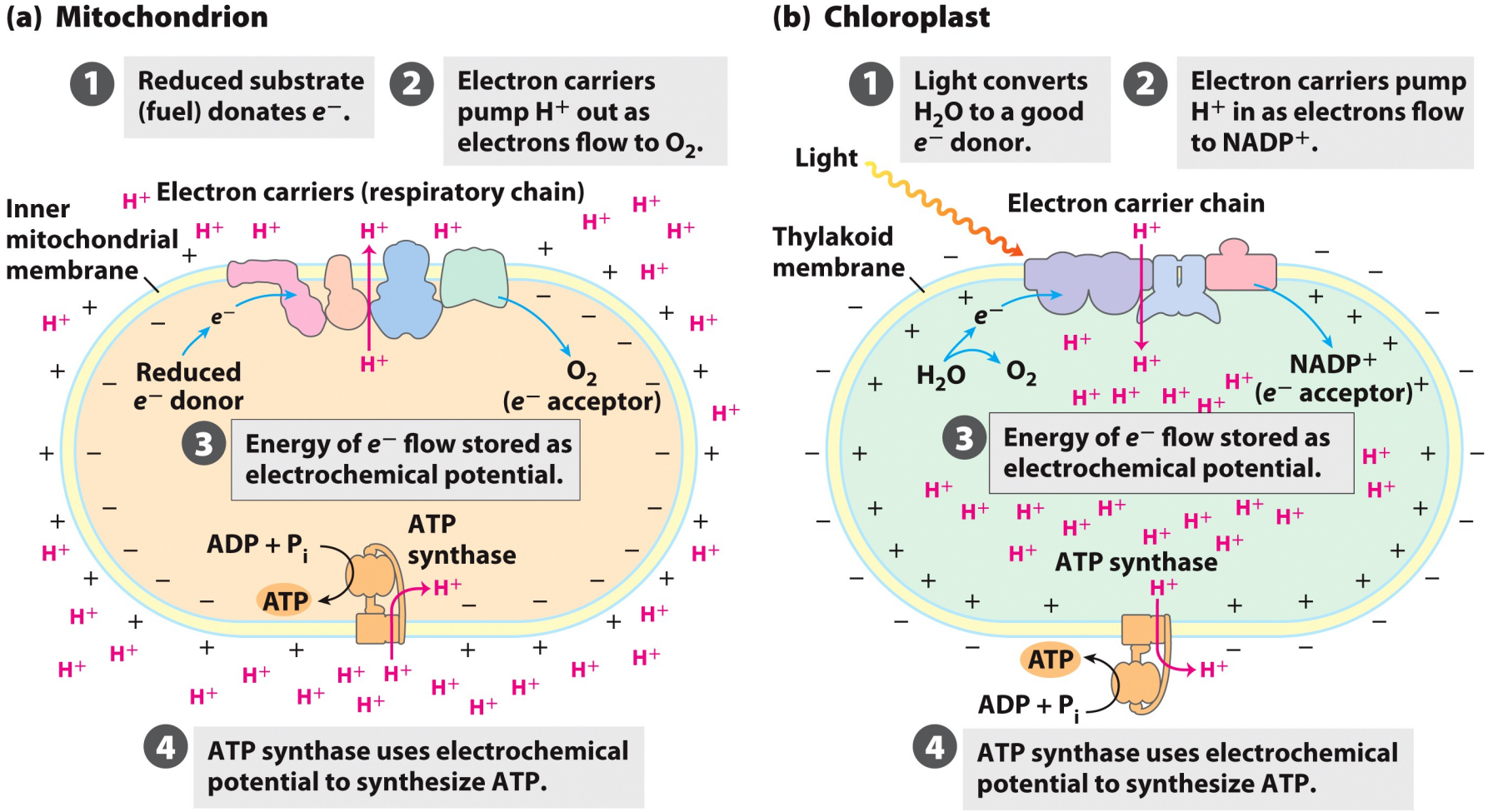
Chemiosmotic Theory

- ADP + P_i → ATP is Highly Thermodynamically Unfavorable
- How do we make it possible?
- Peter Mitchell proposed the ***chemiosmotic*** theory (Noble prize in chemistry, 1978)
- Phosphorylation of ADP is not a result of a direct reaction between ADP and some high-energy phosphate carrier (substrate-level phosphorylation)
- Energy needed to phosphorylate ADP is provided by the flow of protons down the electrochemical gradient
- The energy released by electron transport is used to transport protons against the electrochemical gradient

Chemiosmotic energy coupling requires membranes

- The proton gradient needed for ATP synthesis can be stably established across a membrane that is impermeable to ions
  - Plasma membrane in bacteria
  - Inner membrane in mitochondria
  - Thylakoid membrane in chloroplasts
- Membrane must contain proteins that couple the "downhill" flow of electrons in the electron-transfer chain with the "uphill" flow of protons across the membrane
- Membrane must contain a protein that couples the "downhill" flow of protons to the phosphorylation of ADP (oxidative phosphorylation)

Chemiosmotic Theory



e⁻s move through a chain spontaneously, driven by the ***high reduction potential*** of O_2 and the low reduction potentials of the reduced substrates

Flow of Protons: Mitochondria, Chloroplasts, Bacteria

- According to endosymbiotic theory, mitochondria and chloroplasts arose from entrapped bacteria
- Bacterial cytosol became mitochondrial matrix and chloroplast stroma

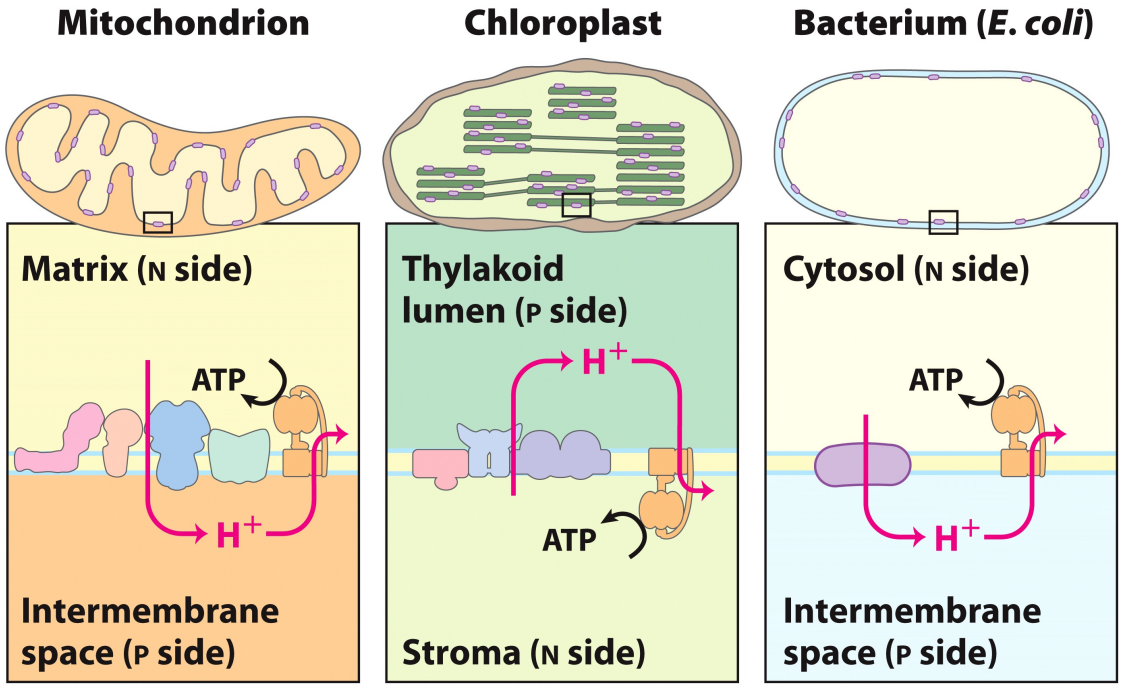


Figure 19-66
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Mitochondrial function(s)

Defects in mito function have serious medical consequences:

- Neurodegenerative diseases
- Cancer
- Diabetes
- Obesity

ATP production is not the only function of mito

- Thermogenesis
- Steroid synthesis
- Apoptosis

Like bacteria, mito divide by fission

Structure of a Mitochondrion

Double membrane leads to four distinct compartments:

1.Outer Membrane:

- Relatively porous membrane allows passage of metabolites
- Permeable to solutes <5000 Da

2.Intermembrane Space (IMS):

- similar environment to cytosol
- higher proton concentration (lower pH)

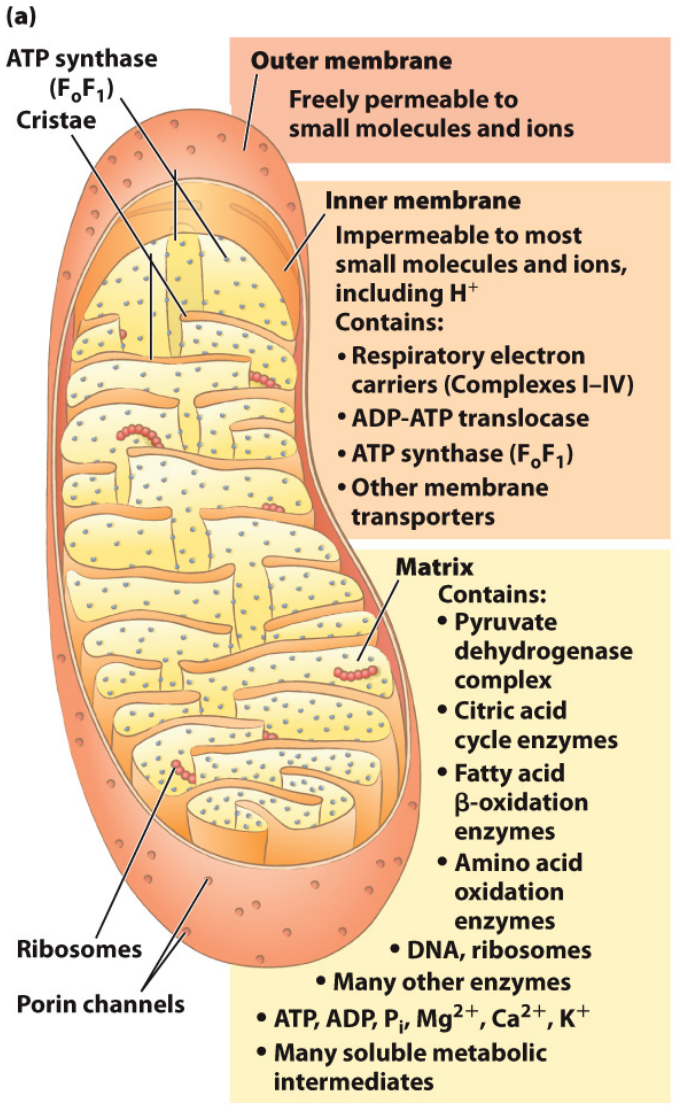
3.Inner Membrane

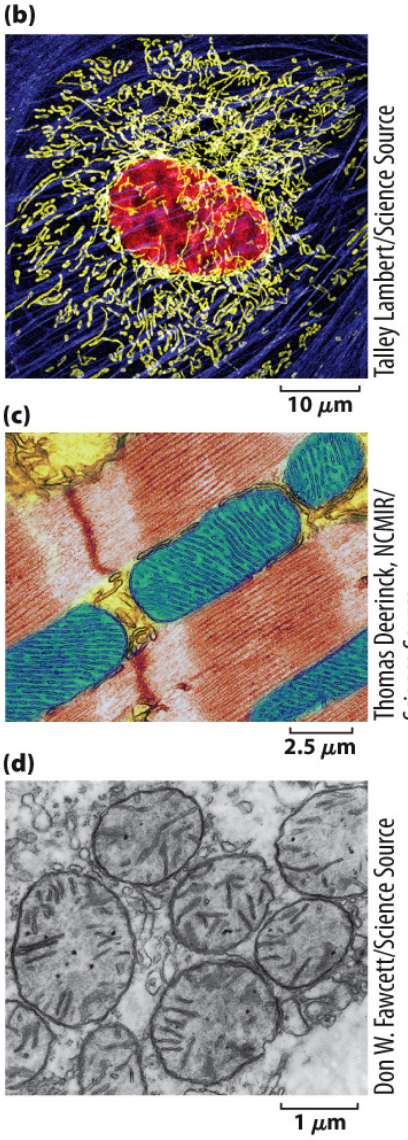
- Relatively impermeable, with proton gradient across it
- Location of electron transport chain complexes
- Convolutions called *Cristae* serve to increase the surface area (tissues with high demand for aerobic respiration contain thousands of mito and their cristae are more densely packed)

4.Matrix

- Location of the citric acid cycle and parts of lipid and amino acid metabolism *(all fuel oxidation pathways except glycolysis)*
- Lower proton concentration (higher pH)

Structure of a Mitochondrion





A typical animal cell has 1000s of mito. Bovine artery endothelial cell: actin (blue) DNA (red), and mito (yellow)

Heart muscle mito (blue) have more profuse cristae (much larger area of inner membrane, with more than three times as many sets of respiratory chains as liver mito (grey)… ***why???***

Figure 19-2
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Electron-transport chain complexes contain a series of electron carriers

- **Nicotinamide nucleotide-linked dehydrogenases** use NAD^+ or $NADP^+$ (NAD^+ in catabolism and NADPH in anabolism)

- Remove 2 e^-s and hydrogen atom from their substrates ($:H^-$ to NAD^+ and H^+)

- Each complex contains multiple redox centers consisting of:
  - **Flavin Mononucleotide (FMN)** or **Flavin Adenine Dinucleotide (FAD)**
    - Initial electron acceptors for Complex I and Complex II
    - Can carry two electrons by transferring *one at a time*
  - **Cytochromes *a, b*** or ***c***
  - **Iron-sulfur clusters**

Cytochromes

- One electron carriers
- *a, b* or *c* differ by ring additions (light absorption)
- Iron coordinating porphyrin ring derivatives (tightly but not covalently bound in *a* and *b* but covalent in *c*)

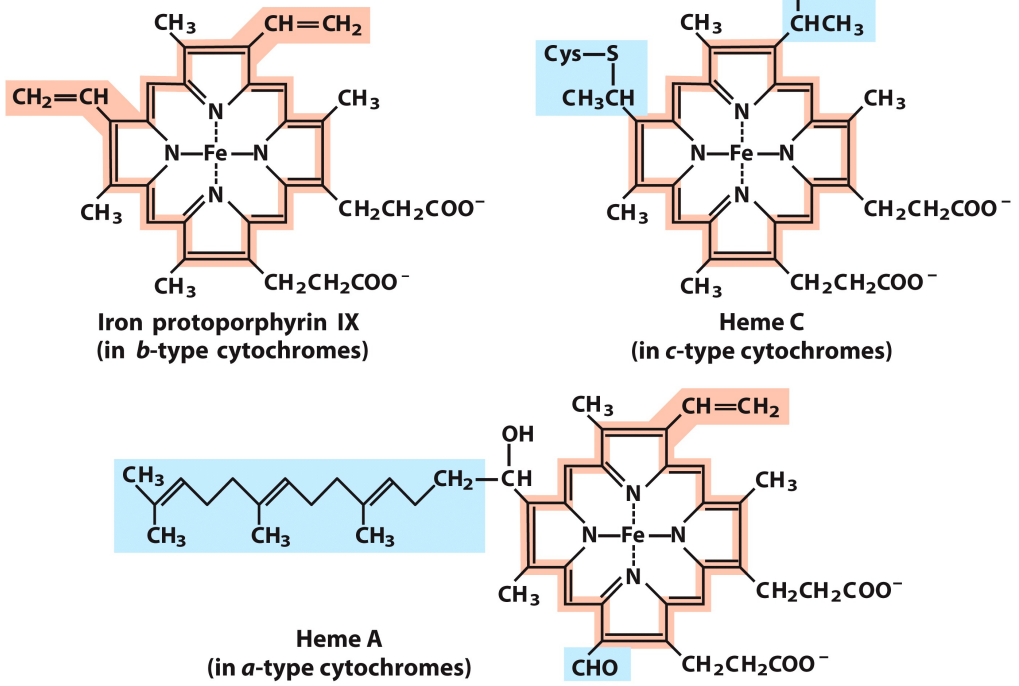

Figure 19-4a
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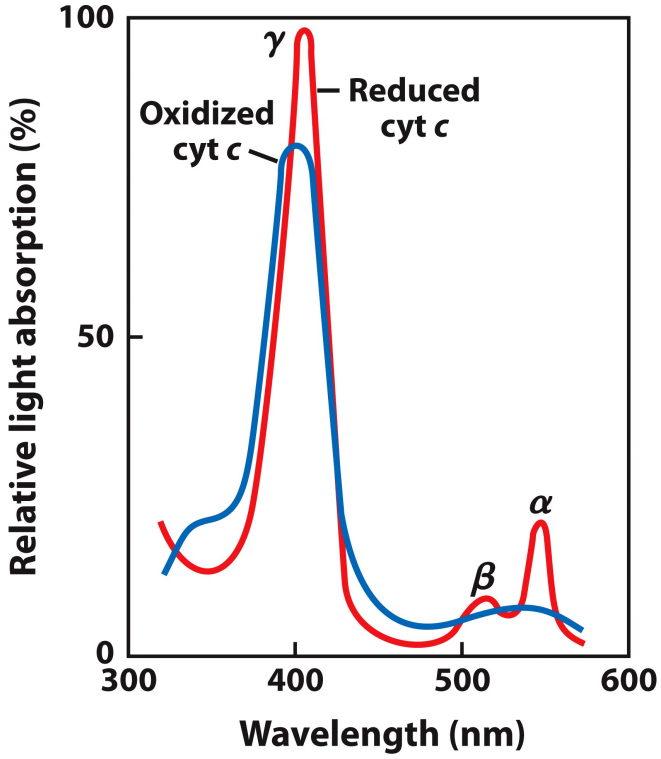

Figure 19-4b
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Iron-Sulfur Clusters

- One electron carriers
- Coordination by cysteines in the protein
- Containing equal number of iron and sulfur atoms (such as 2Fe-2S, 4Fe-4S) or unequal numbers (such as 3Fe-4S, 1Fe-0S)
- Rieske Fe-S proteins – 1 Fe is coordinated to two His instead of 2 Cys)
- At least 8 Fe-S proteins function in mitochondrial ETC

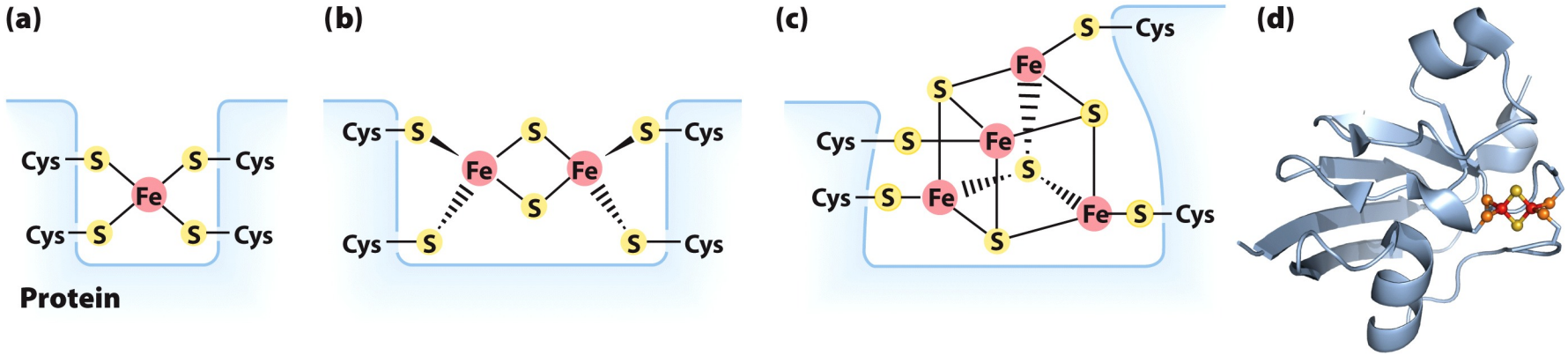

Figure 19-5
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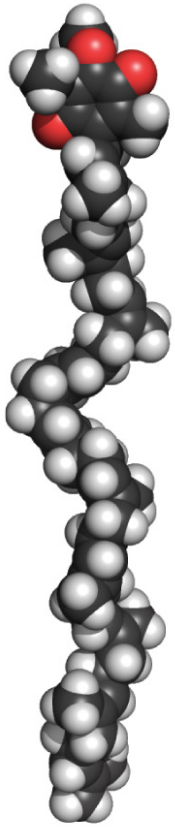
Coenzyme Q or Ubiquinone

- Ubiquinone (Q) is a lipid-soluble conjugated dicarbonyl compound that readily accepts electrons
- Upon accepting two electrons, it picks up two protons to produce an alcohol, ubiquinol (QH_2)
- Ubiquinol can freely diffuse in the membrane, carrying electrons with protons from one side of the membrane to another side
- Coenzyme Q is a mobile electron carrier transporting electrons from Complexes I and II to Complex III



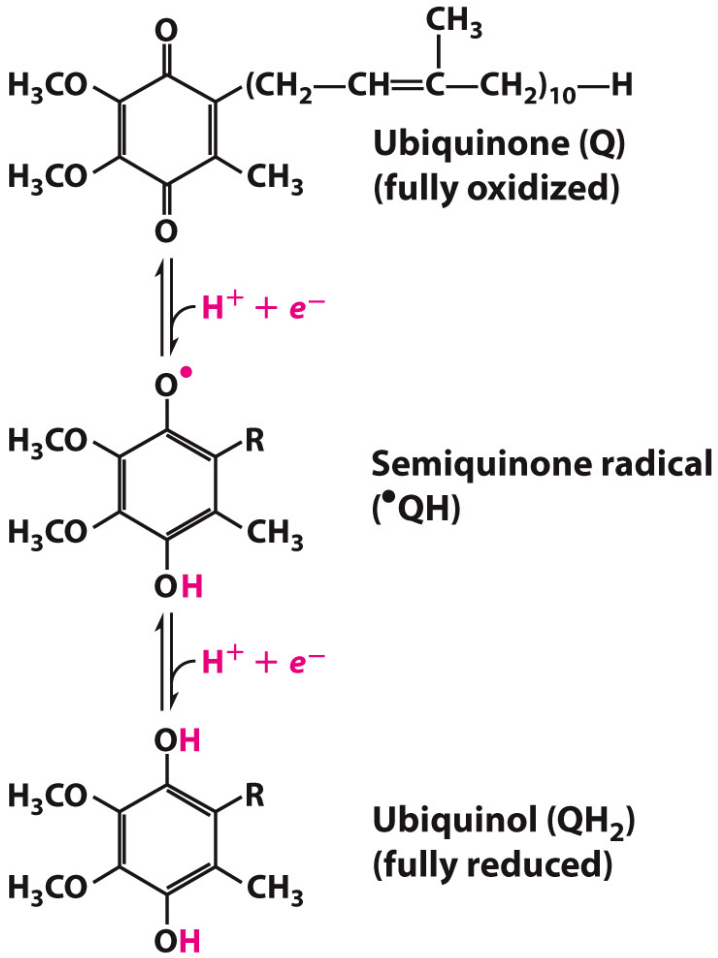


Figure 19-3
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Free Energy of Electron Transport

Reduction Potential (E)

$$\Delta E'^{o} = E'^{o}_{(e^- \text{ acceptor})} - E'^{o}_{(e^- \text{ donor})}$$

$$\mathbf{\Delta G'^{o} = -nF\Delta E'^{o}}$$

For negative ΔG need positive ΔE

$$E_{(\text{acceptor})} > E_{(\text{donor})}$$

Electrons are transferred from lower (more negative) to higher (more positive) reduction potential.

Free Energy released is used to pump protons, storing this energy as the electrochemical gradient

***Recall**: reduction potential is the relative tendency of a given chemical species to accept electrons in a redox reaction (the higher the reduction potential the more oxidized the species)*

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

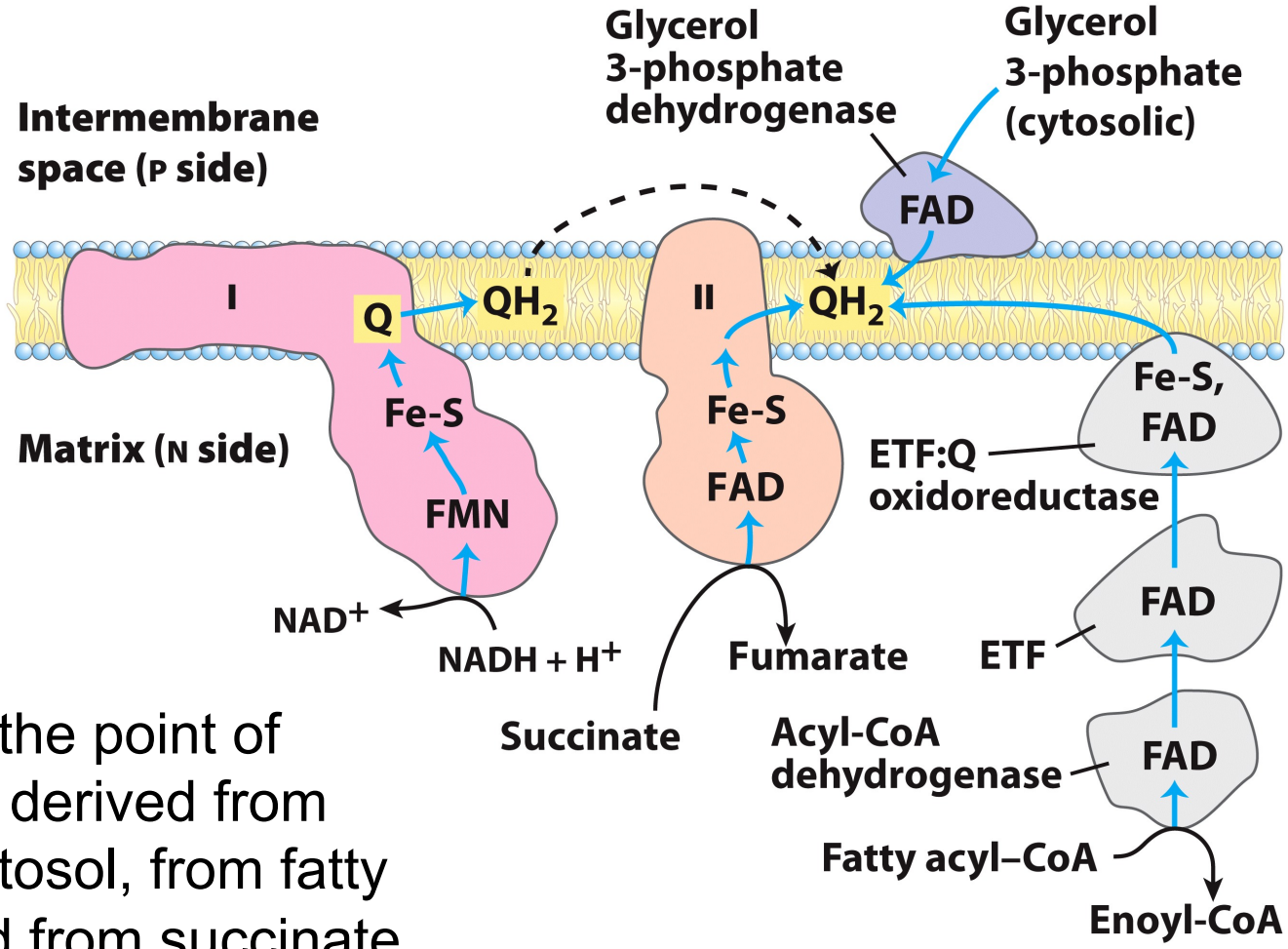
Redox reaction (half-reaction)	$E'°$ (V)
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ 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

We would expect the carriers to function in order of increasing reduction potential (e^-s flow spontaneously):

NADH → Q → cyt *b* → cyt c_1 → cyt *c* → cyt *a* → cyt a_3 → O_2

Not necessarily the same as the order of the ***actual*** reduction potential, but this sequence was confirmed by other experiments

Flow of Electrons from Biological Fuels into the Electron-Transport Chain



Ubiquinone (Q) is the point of entry for electrons derived from reactions in the cytosol, from fatty acid oxidation, and from succinate oxidation (in the citric acid cycle).

Electron carriers function in multienzyme complexes

TABLE 19-3 The Protein Components of the Mitochondrial Respiratory Chain

Enzyme complex/protein	Mass (kDa)	Number of subunits[a]	Prosthetic group(s)
I NADH dehydrogenase	850	45 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone: cytochrome *c* oxidoreductase[b]	250	11	Hemes, Fe-S
Cytochrome *c*[c]	13	1	Heme
IV Cytochrome oxidase[b]	204	13 (3–4)	Hemes; Cu_A, Cu_B

[a]Number of subunits in the bacterial complexes in parentheses.
[b]Mass and subunit data are for the monomeric form.
[c]Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

NADH dehydrogenase (Complex I)

- One of the largest macro-molecular assemblies in the mammalian cell
- Over 40 different polypeptide chains, encoded by both nuclear and mitochondrial genes
- NADH binding site in the matrix side
- Non-covalently bound flavin mononucleotide (FMN) accepts two electrons from NADH
- Several iron-sulfur centers pass one electron at a time toward the ubiquinone binding site

NADH:Ubiquinone Oxidoreducase Is a Proton Pump

- Transfer of two electrons from NADH to ubiquinone is accompanied by a transfer of protons from the matrix (N) to the intermembrane space (P) . A vectorial proton pump (in one direction only)
- Experiments suggest that about four protons are transported per one NADH.

$$NADH + Q + 5H^+_N = NAD^+ + QH_2 + 4\ H^+_P$$

P = positive (IMS); N = negative (matrix)

- Reduced coenzyme Q picks up two protons.
- Protons are transported by *proton wires*.
  - a series of amino acids that undergo protonation and deprotonation to get a net transfer of a proton from one side of a membrane to another

Complex I

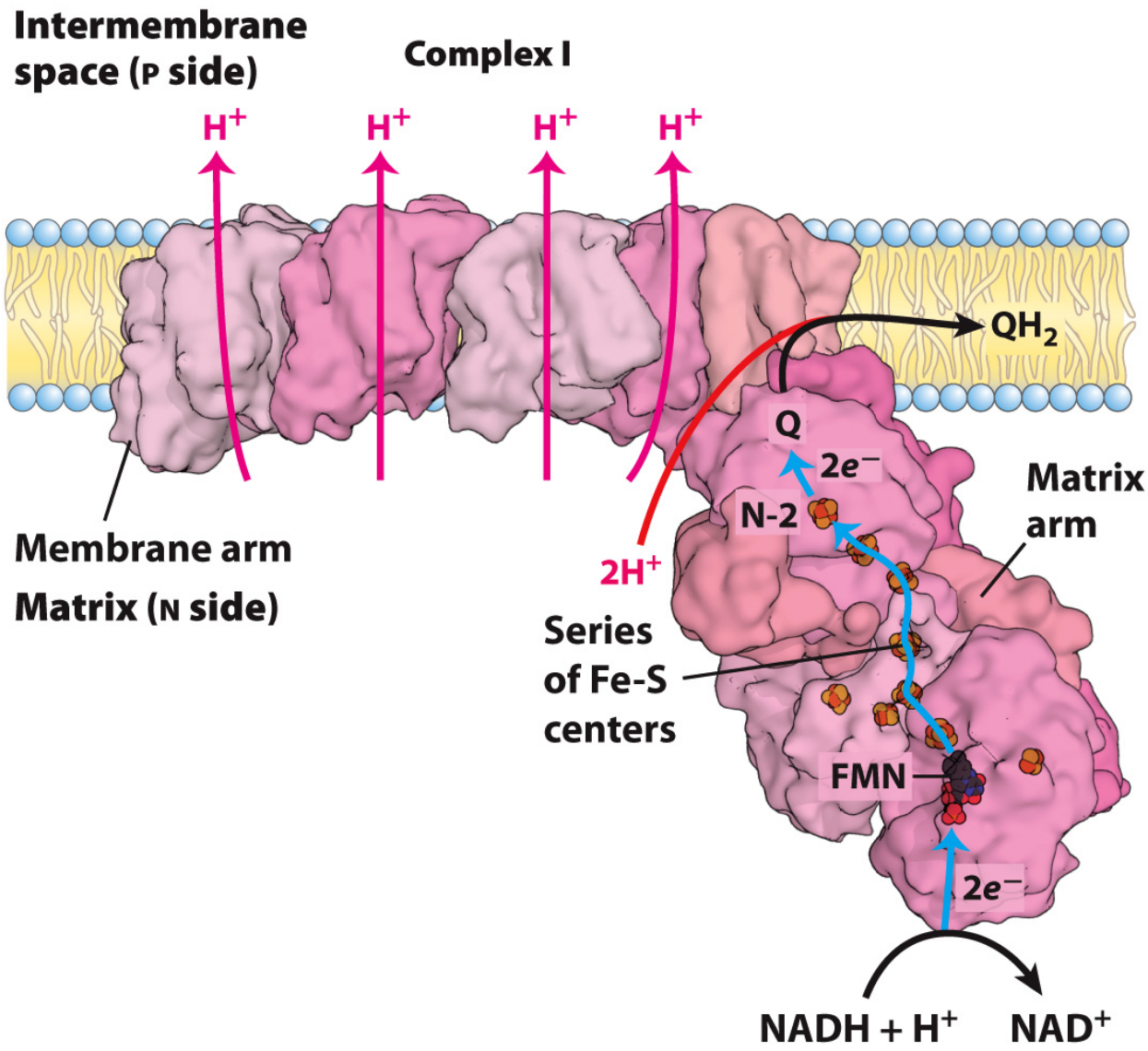


Figure 19-8
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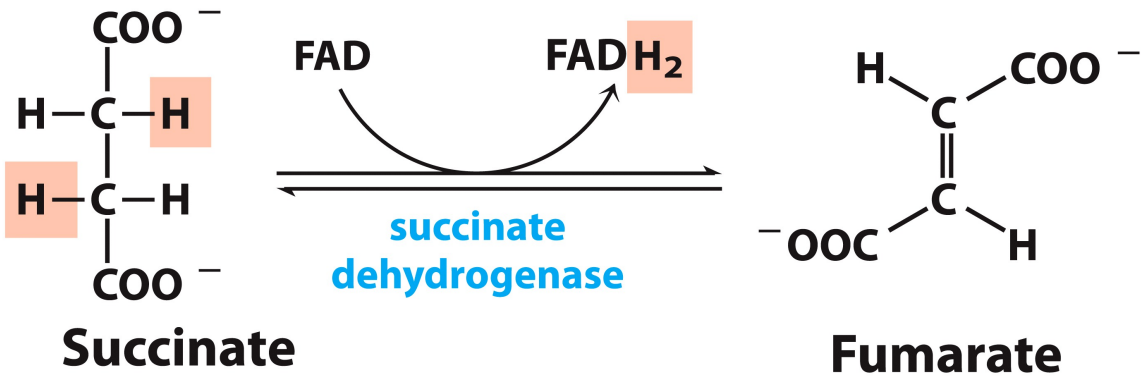
Succinate Dehydrogenase (Complex II)

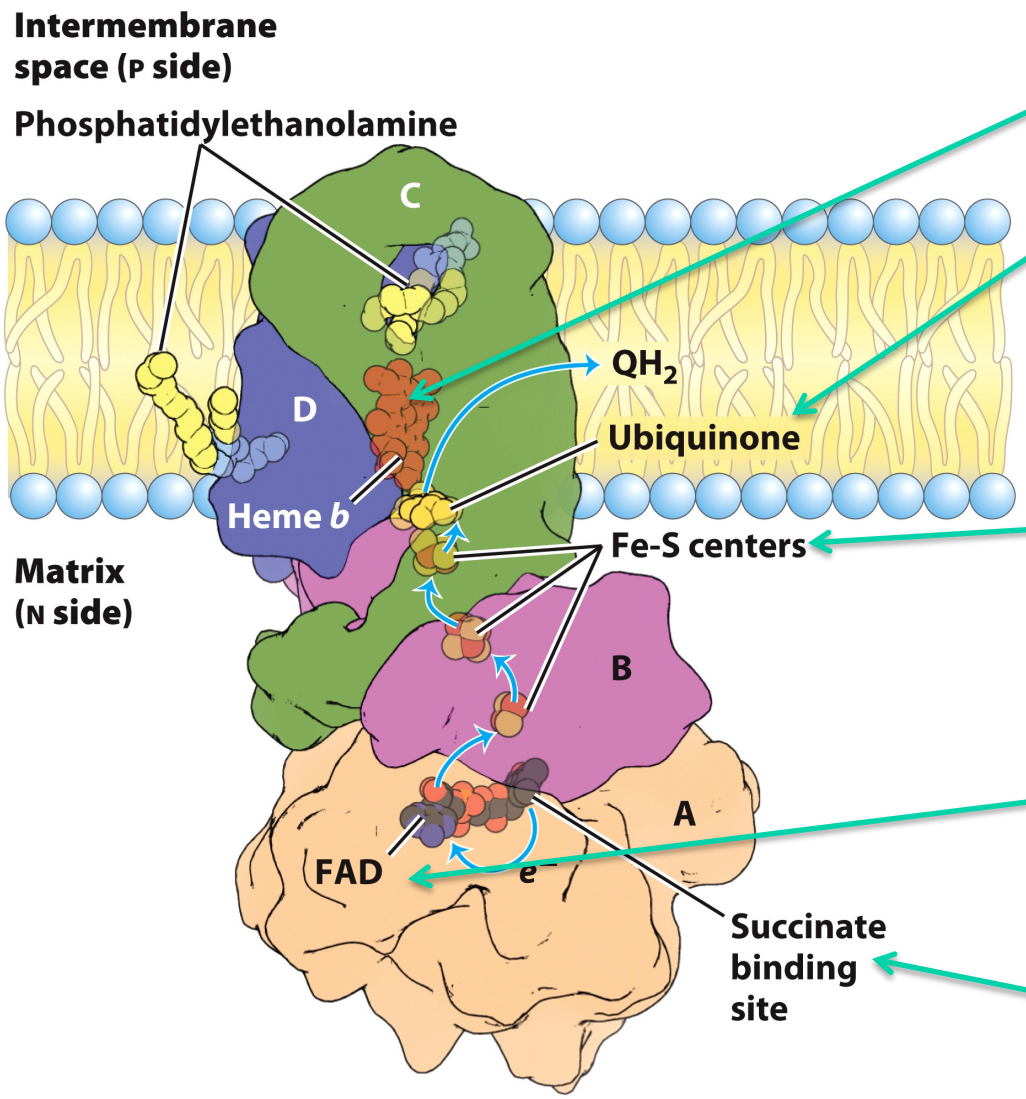
- Smaller and simpler than complex I
- FAD accepts two electrons from succinate
- Electrons are passed, one at a time, via iron-sulfur centers to ubiquinone, which becomes reduced QH_2
- Does *not* transport protons



Succinate **Fumarate**

$\Delta G'° = 0$ kJ/mol

Complex II



Heme *b*

Q binding site

C and D (integral proteins)

3 2Fe-2S

Bound FAD

Succinate binding site

A and B (matrix)

Figure 19-10
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Ubiquinone:Cytochrome *c* Oxidoreductase, (Complex III)

- Uses two electrons from QH_2 to reduce two molecules of cytochrome *c*
- Additionally contains iron-sulfur clusters, cytochrome *b*'s, and cytochrome *c*'s
- The Q cycle results in four additional protons being transported to the IMS

Complex III

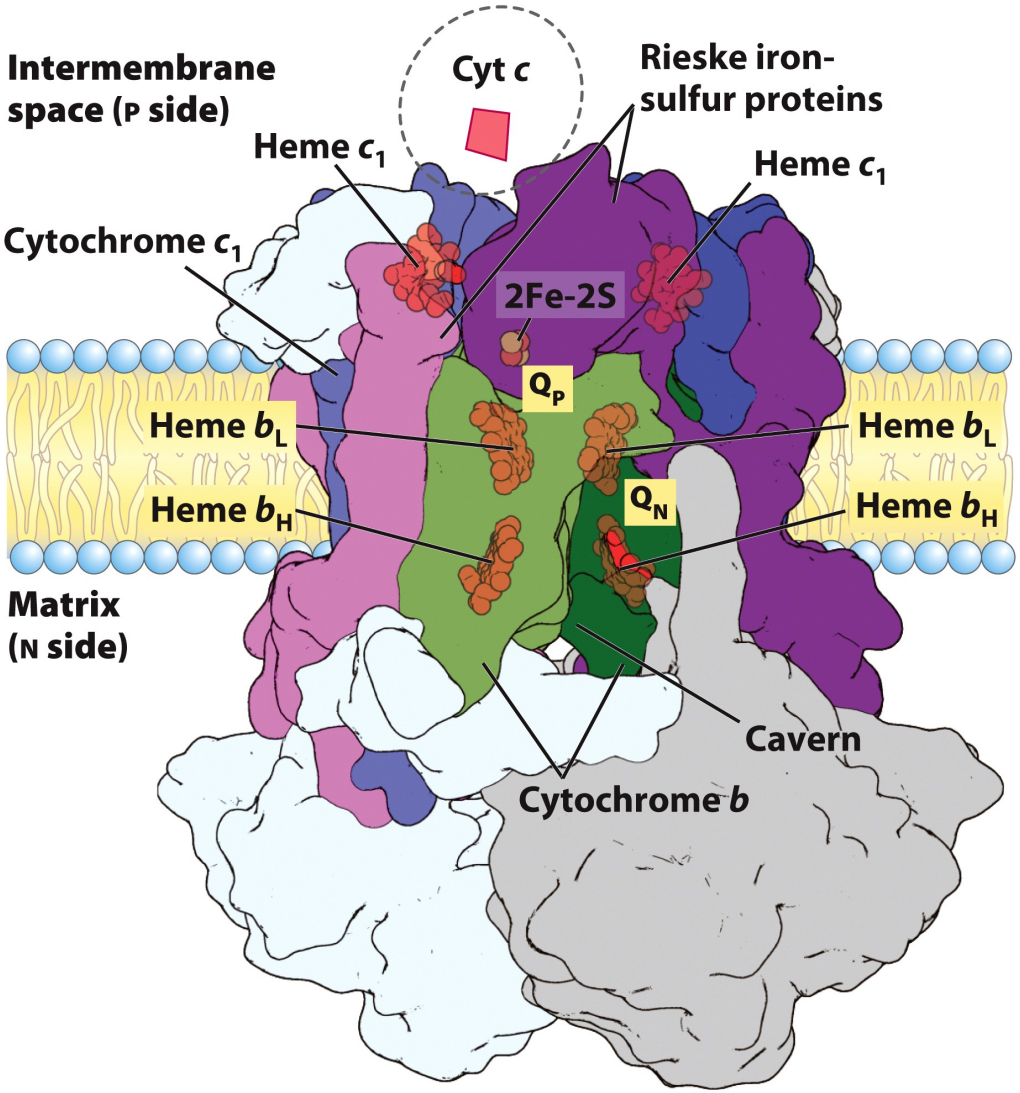

Figure 19-11
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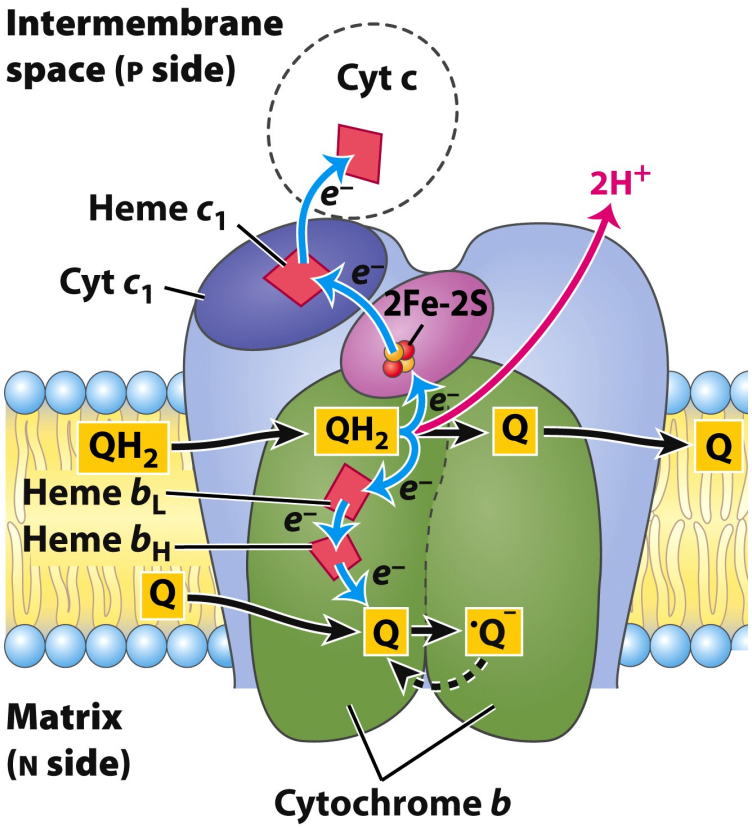
The Q Cycle

- Experimentally, four protons are transported across the membrane per two electrons that reach cyt *c*
- Two of the four protons come from QH_2
- The Q cycle provides a good model that explains how two additional protons are picked up from the matrix
- Two molecules of QH_2 become oxidized, releasing protons into the IMS
- One molecule becomes re-reduced, thus a net transfer of four protons per reduced Coenzyme Q
- Bifurcation of electrons

The Q Cycle: Cycle 1



$$QH_2 + Q + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow Q + {}^{\cdot}Q^- + 2H_P^+ + \text{cyt } c_1 \text{ (reduced)}$$

Figure 19-12
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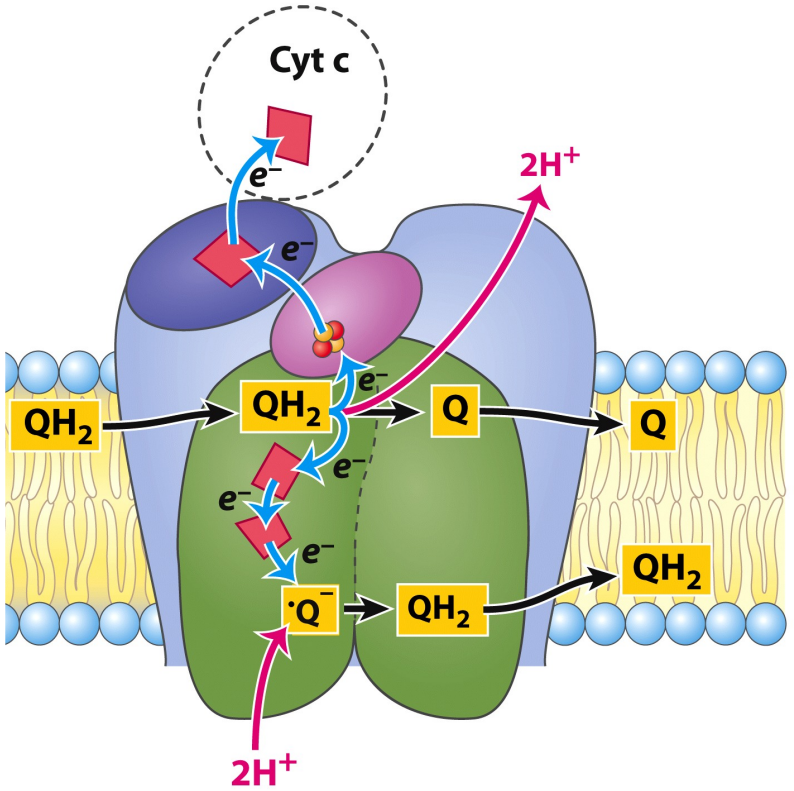
The Q Cycle: Cycle 2



$$QH_2 + Q + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow Q + {}^{\cdot}Q^- + 2H_P^+ + \text{cyt } c_1 \text{ (reduced)}$$

$$QH_2 + {}^{\cdot}Q^- + 2H_N^+ + \text{cyt } c_1 \text{ (oxidized)} \longrightarrow Q + 2H_P^+ + QH_2 + \text{cyt } c_1 \text{ (reduced)}$$

$$\text{Net equation: } QH_2 + 2 \text{ cyt } c_1 \text{ (oxidized)} + 2H_N^+ \longrightarrow Q + 2 \text{ cyt } c_1 \text{ (reduced)} + 4H_P^+$$

Figure 19-12
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Cytochrome *c*

- The second mobile electron carrier
- A soluble heme-containing protein in the intermembrane space
- Heme iron can be either ferric (Fe^{3+}, oxidized) or ferrous (Fe^{2+}, reduced)
- Cytochrome *c* carries a single electron from the cytochrome bc_1 complex to cytochrome oxidase (to a binuclear copper center)

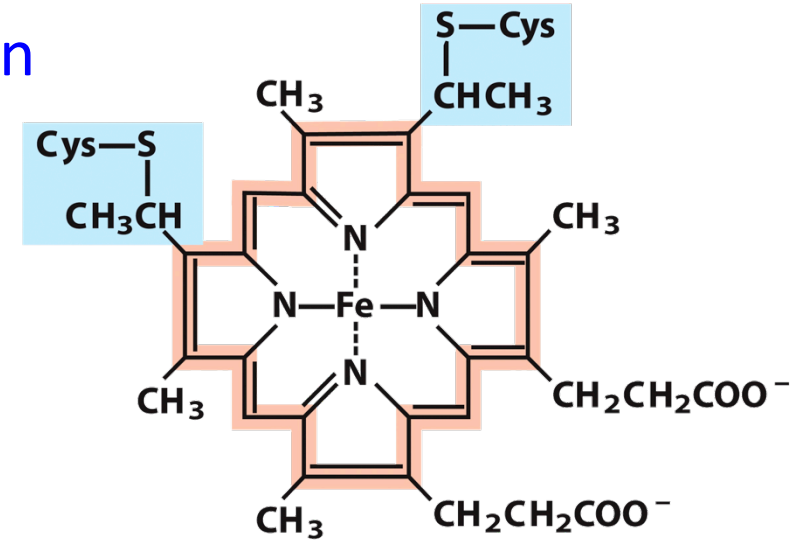

Figure 19-4a part 2
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Cytochrome Oxidase (Complex IV)

- Mammalian cytochrome oxidase is a membrane protein with 13 subunits
- Contains two heme groups: *a* and a_3
- Contains copper ions
  - Cu_A: two ions that accept electrons from cyt *c*
  - Cu_B: bonded to heme a_3 forming a binuclear center that transfers four electrons to oxygen

Cytochrome oxidase passes electrons to O_2

- Four electrons are used to reduce one oxygen molecule into two water molecules *(coming from 4 cyt c molecules)*
- Four protons are picked up from the matrix in this process
- Four additional protons are passed from the matrix to the intermembrane space

Electron flow through Complex IV

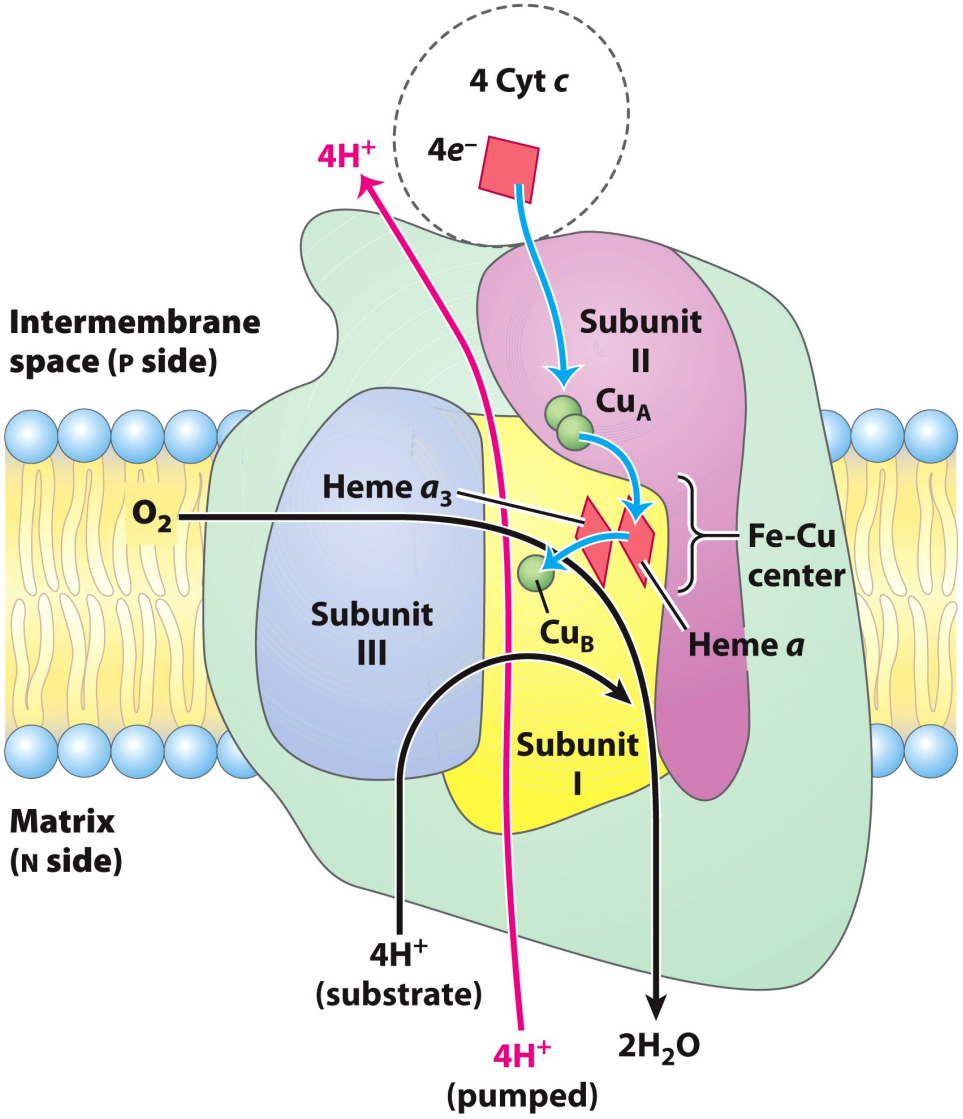

Figure 19-14
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Summary of the Electron Flow in the Respiratory Chain

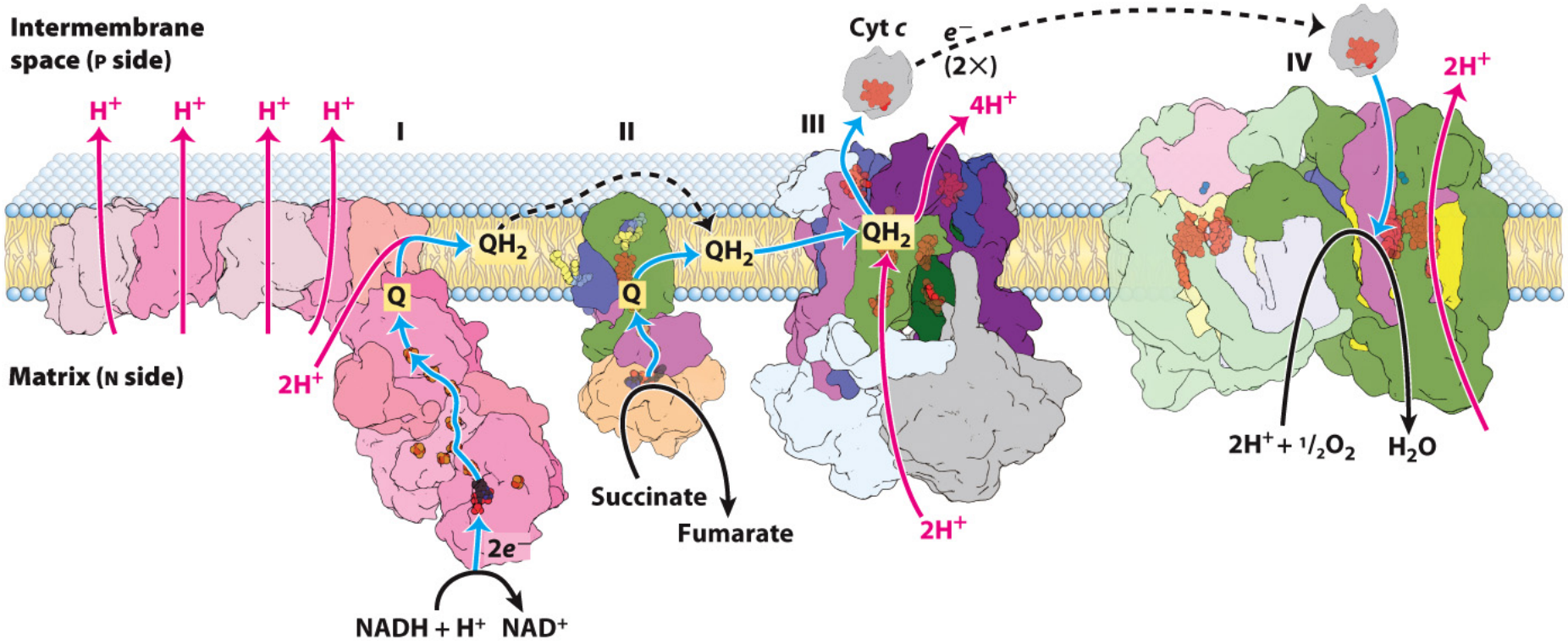

Figure 19-16
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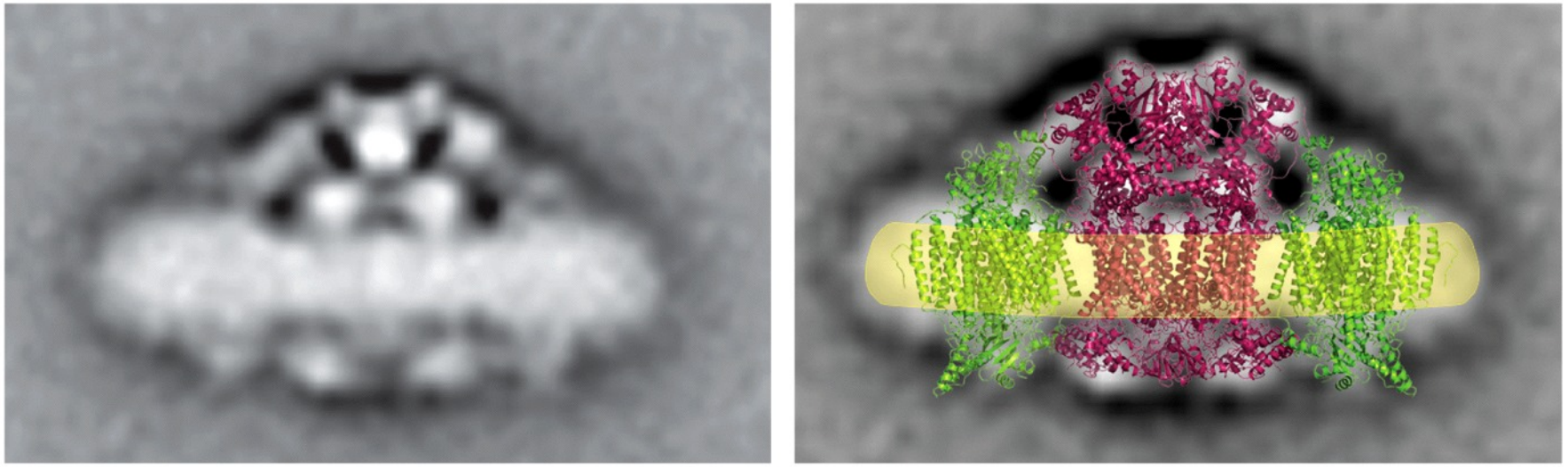
Multiple complexes associate together to form a respirasome

Substrate channeling → efficiency



(a) (b)

Figure 19-15
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Summary of Electron Transport

- Complex I → Complex IV

$1NADH + 11H^+_{(N)} + ½O_2 \longrightarrow NAD^+ + 10H^+_{(P)} + H_2O$

- Complex II → Complex IV

$FADH_2 + 6H^+_{(N)} + ½O_2 \longrightarrow FAD + 6H^+_{(P)} + H_2O$

Difference in number of protons transported reflects the amount of synthesized ATP.

Energy of electron transfer is efficiently conserved in a proton gradient

$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	-0.320
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.8166

$NADH + H^+ + ½\ O_2 \rightarrow NAD^+ + H_2O$ *(Net)*

$\Delta E'^o = E'^o_{(e^- \text{ acceptor})} - E'^o_{(e^- \text{ donor})} = 0.816 - (-0.32) = 1.14$ V

$\Delta G'^o = - nF\Delta E'^o = - 2 \times 96.5 \times 1.14 = - 220$ kJ/mol of NADH

Succinate to fumarate oxidation yields ~ – 150 kJ/mol

Much of this energy is used to pump protons **(proton-motive force)**

Proton-Motive Force

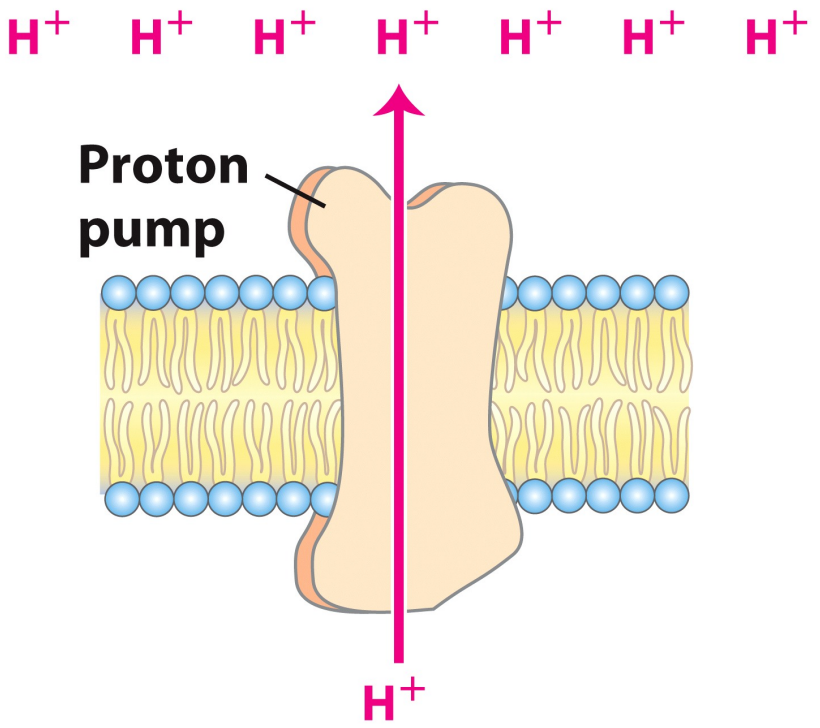
- 2 components:

1. Concentration gradient (of protons)
2. Electrical gradient (+ and – ions are segregated)

- The proteins in the electron-transport chain created the electrochemical proton gradient by one of three means:
  - Actively transport protons across the membrane
    - Complex I and Complex IV
  - Chemically remove protons from the matrix
    - Reduction of CoQ and reduction of oxygen
  - Release protons into the intermembrane space
    - Oxidation of QH_2

Proton-Motive Force

P side
$[H^+]_P = C_2$



N side
$[H^+]_N = C_1$

OH^- OH^- OH^- OH^- OH^- OH^- OH^-

$$\Delta G = RT \ln (C_2/C_1) + Z \ \Delta\mathcal{F}\psi$$
$$= 2.3RT\,\Delta\text{pH} + \mathcal{F}\Delta\psi$$

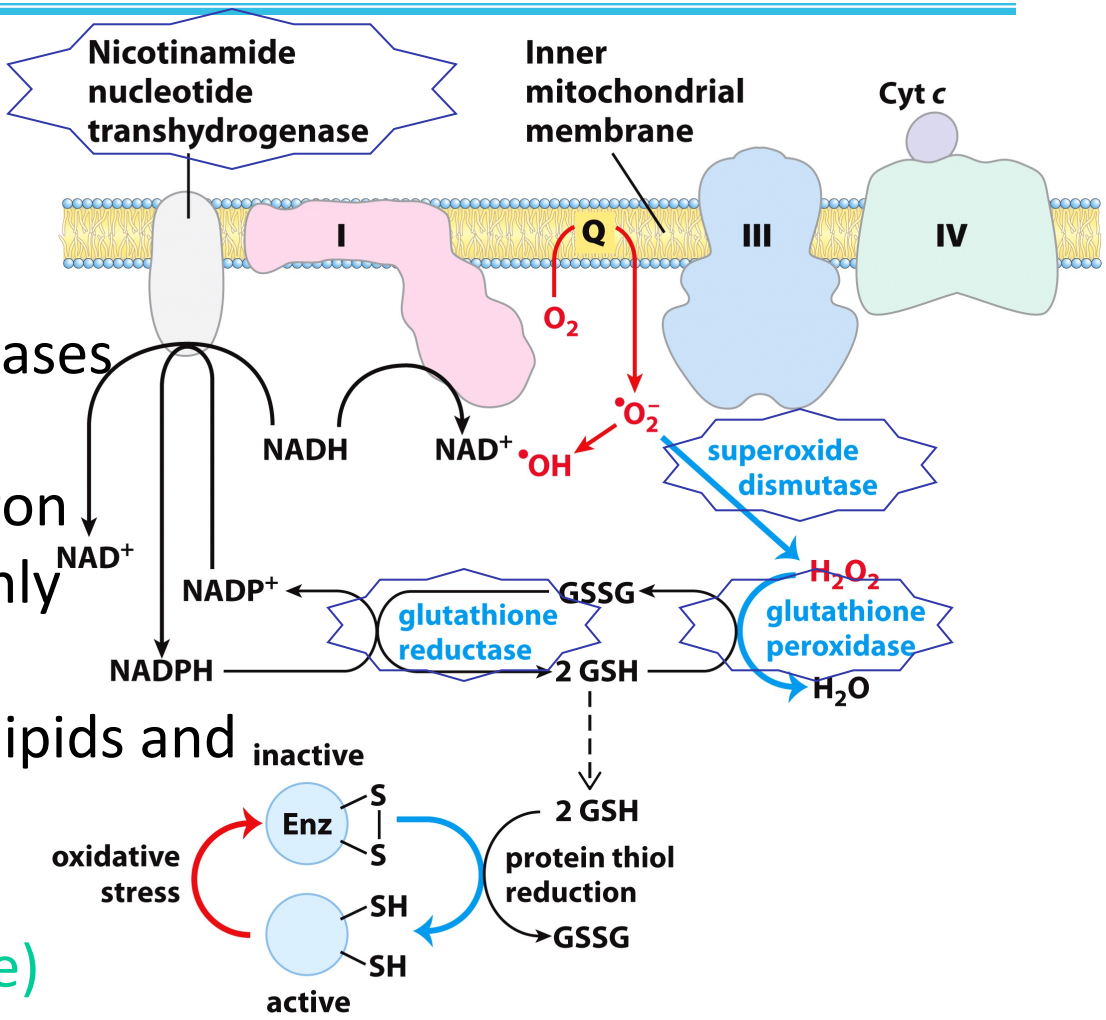
In actively respiring mito: $\Delta\psi$ ~0.15 V and the matrix is 0.75x more alkaline

ΔG = (5.7x0.75) + (96.5x0.15) = 19 kJ/mol

Since 2 e^-s from NADH leads to pumping of **10 protons** ➔ *roughly 190 kJ of the 220 kJ released by NADH oxidation is conserved in the proton gradient!*

Reactive oxygen species (ROS) can damage biological macromolecules

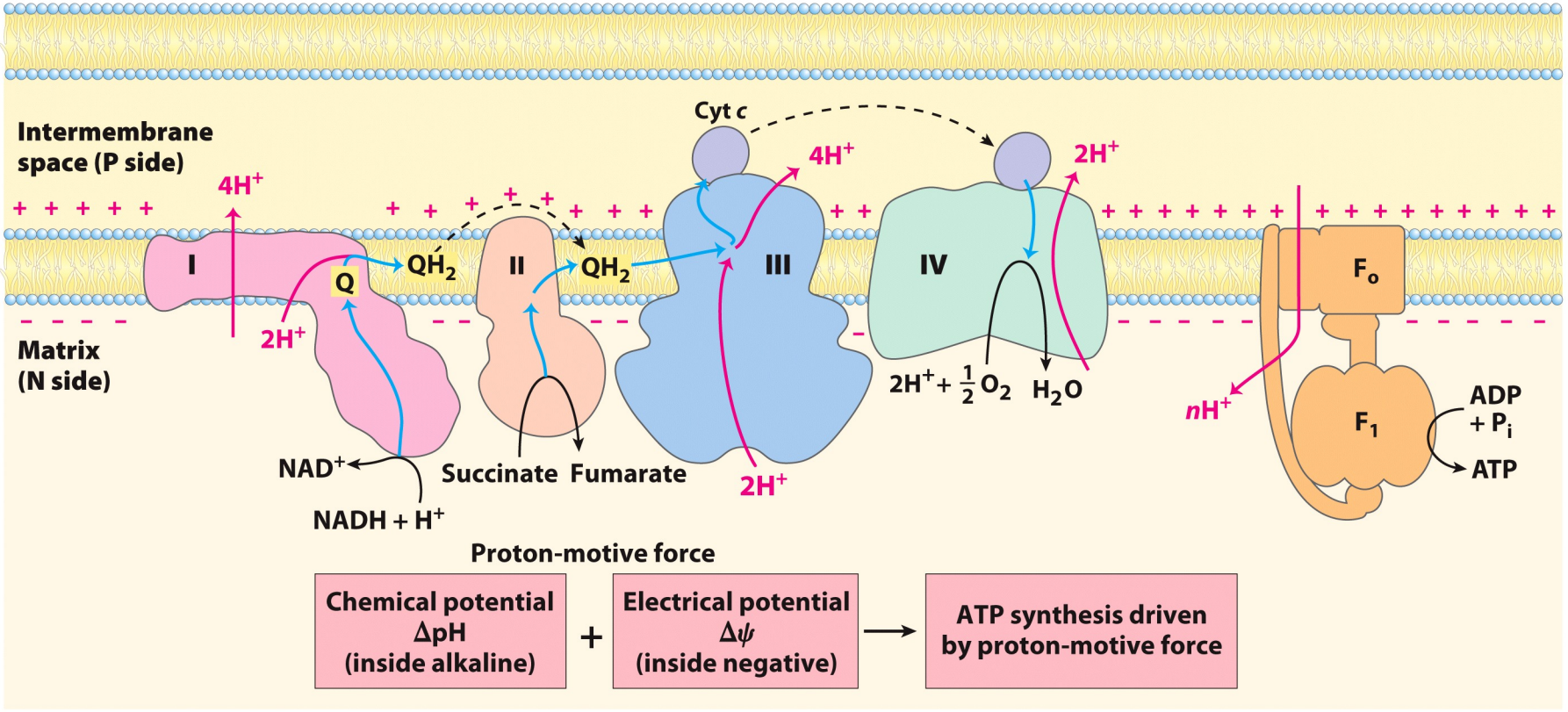
When the rate of e^- entry into the RC and the rate of e^- transfer through the chain are mismatched ➔ superoxide radical ($\bullet O_2^-$) production increases (partially reduced ubiquinone radical ($\bullet Q^-$) donates an electron to O_2) ➔ formation of the highly reactive hydroxyl free radical ($\bullet OH$) ➔ damaging enzymes, lipids and DNA. To prevent: *superoxide dismutase* & *glutathione peroxidase* (glutathione shuttle)



Because ubiquinone is naturally "leaky" and facilitates partial reduction of non-Complex III targets.

Chemiosmotic Model for ATP Synthesis

- Electron transport sets up a proton-motive force
- Energy of proton-motive force (~190 kJ) drives synthesis of ATP (requires 52 kJ) see worked example 13-2



$$ADP + P_i + nH^+_P \rightarrow ATP + H_2O + nH^+_N$$

Figure 19-19
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Inhibitors of the Electron Transport Chain Disrupt Oxidative Phosphorylation

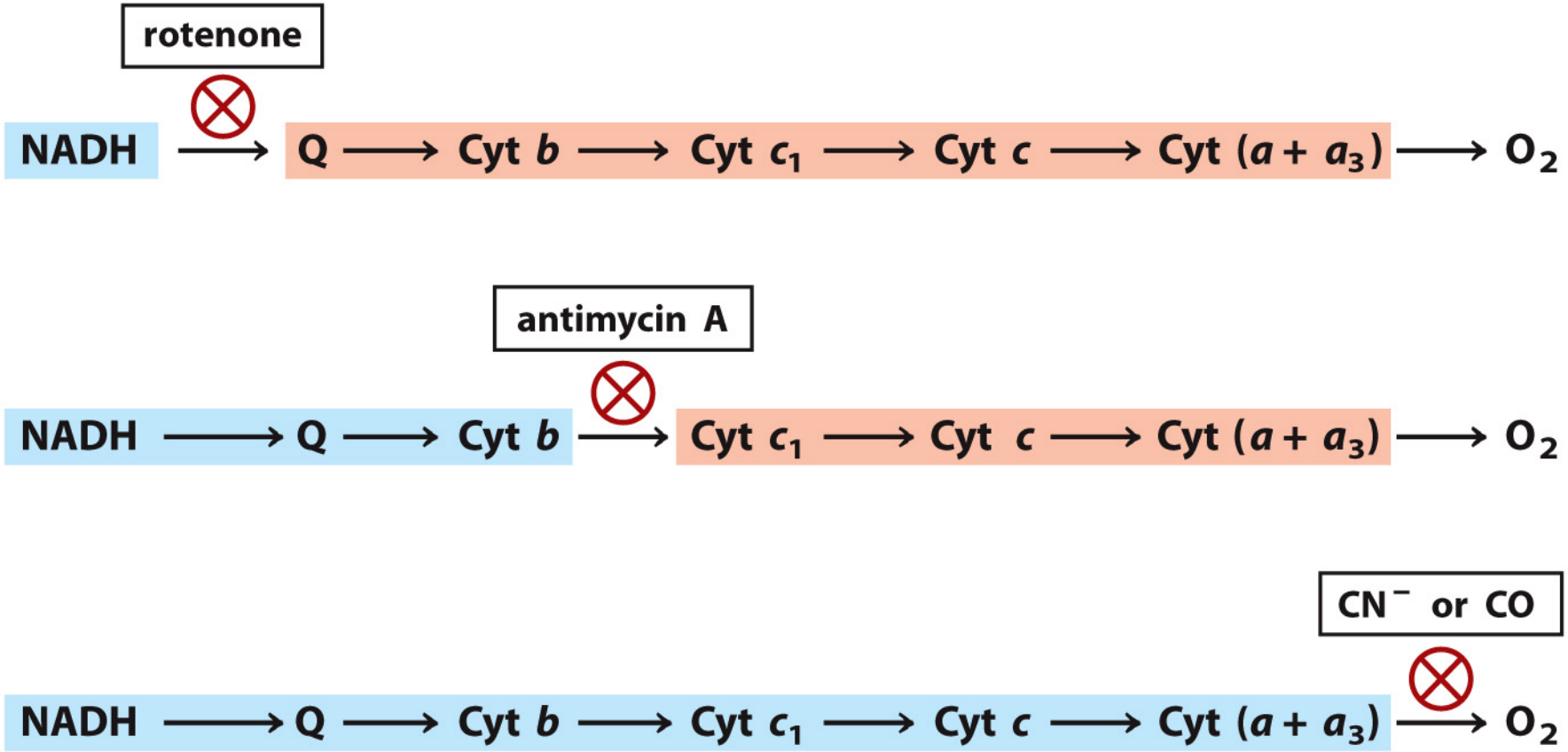

Figure 19-6
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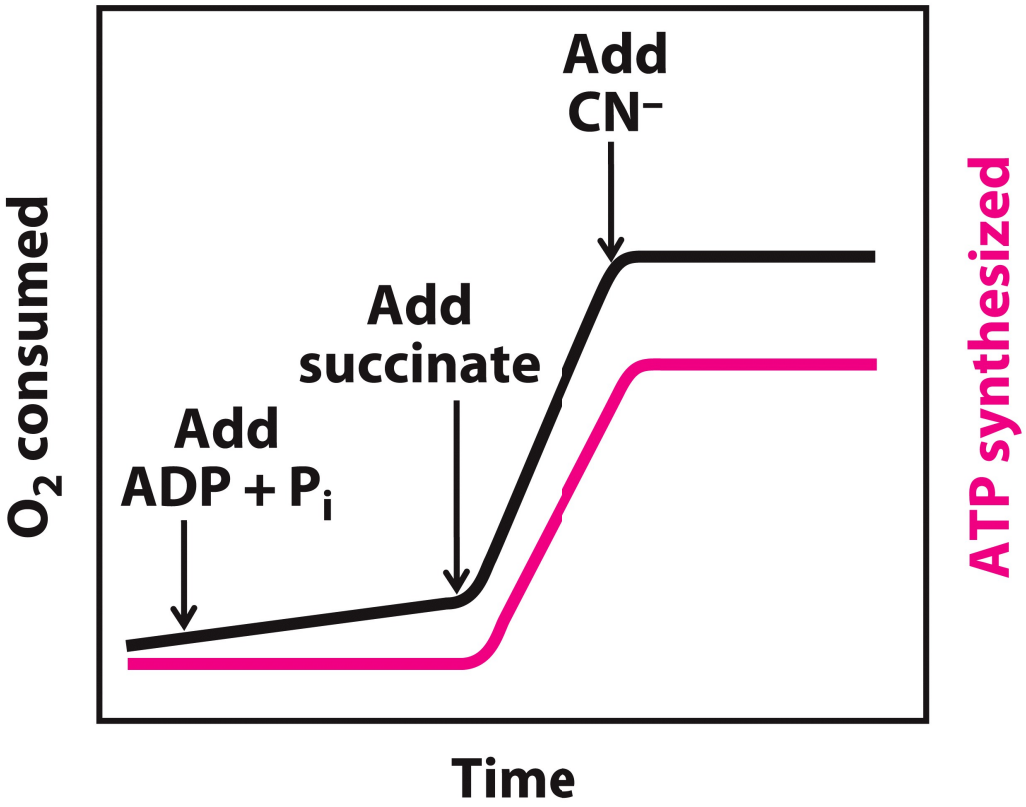
Coupling

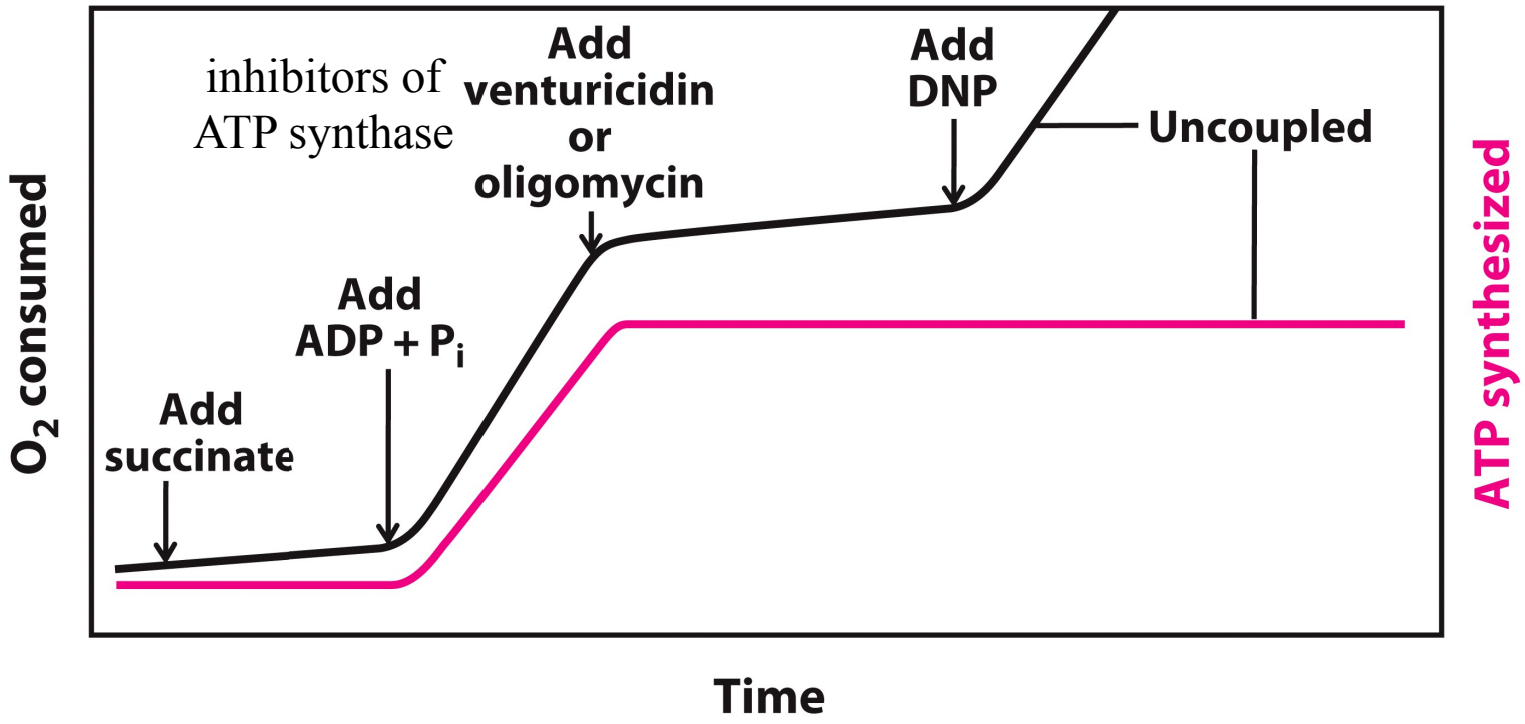
- O_2 consumption and ATP synthesis depends on the presence of **ADP + Pi** and an **oxidizable substrate**
- Blocking the passage of e^-s to O_2 will inhibit ATP production



Addition of cyanide (CN^-), which blocks electron transfer between cytochrome oxidase (Complex IV) and O_2, ***inhibits both*** *respiration and ATP synthesis.*

Coupling

- If ADP is not available succinate cannot be oxidized
- Inhibiting ATP synthesis will inhibit e^- transfer to O_2
- Chemical uncouplers of ATP synthesis from e^- transport dissipate proton gradients (weak hydrophobic acids)



Mitochondrial ATP Synthase Complex

- Mitochondrial ATP synthase (complex V) is an F-type ATPase
- Contains two functional units:
  - F_1
    - Peripheral membrane protein complex in the matrix
    - On its own catalyzes the hydrolysis of ATP
  - F_o
    - Integral membrane complex, a channel
    - Oligomycin-sensitive
    - Transports protons from IMS to matrix, dissipating the proton gradient
    - Energy transferred to F_1 to catalyze phosphorylation of ADP

Mitochondrial ATP Synthase Complex

- On the enzyme surface, ADP + P_i ←→ ATP + H_2O is readily reversible with $\Delta G' \sim 0$!! Why?
- The enzyme stabilizes ATP much more than ADP, more tightly bound ($K_{d(ATP)} < 10^{-12}$ M; $K_{d(ADP)} \sim 10^{-5}$ M)
- Binding energy of ~ 40 kJ/mol drives the synthesis of ATP

- If no proton gradient is present, ATP **cannot** leave the enzyme surface
- *To continually synthesize ATP the enzyme cycles between a conformation that binds ATP very tightly (to drive synthesis) and a conformation that releases ATP*

The F_1 catalyzes ADP + $P_i \rightleftharpoons$ ATP

- 9 subunits $\alpha_3\beta_3\gamma\delta\varepsilon$
- The head is a hexamer arranged in three $\alpha\beta$ dimers
- β has the catalytic activity and can exist in three different conformations (γ binds only one of the 3 β)
  - ***Open:*** empty
  - ***Loose:*** binding ADP and P_i
  - ***Tight:*** catalyzes ATP formation and binds product

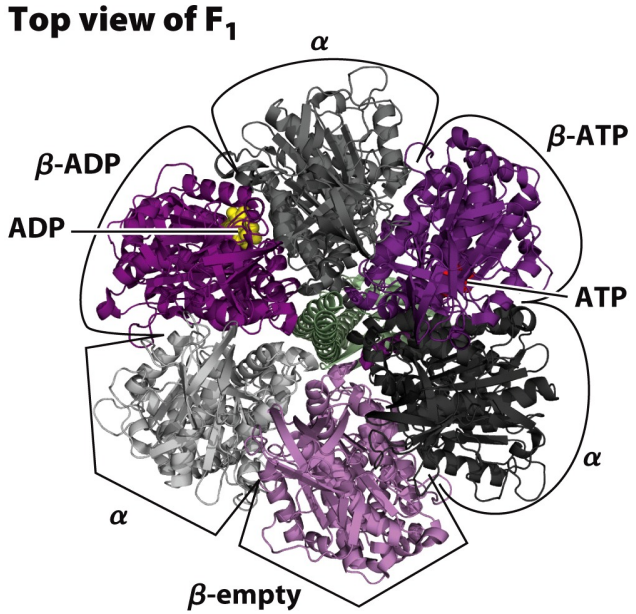


Figure 19-25b
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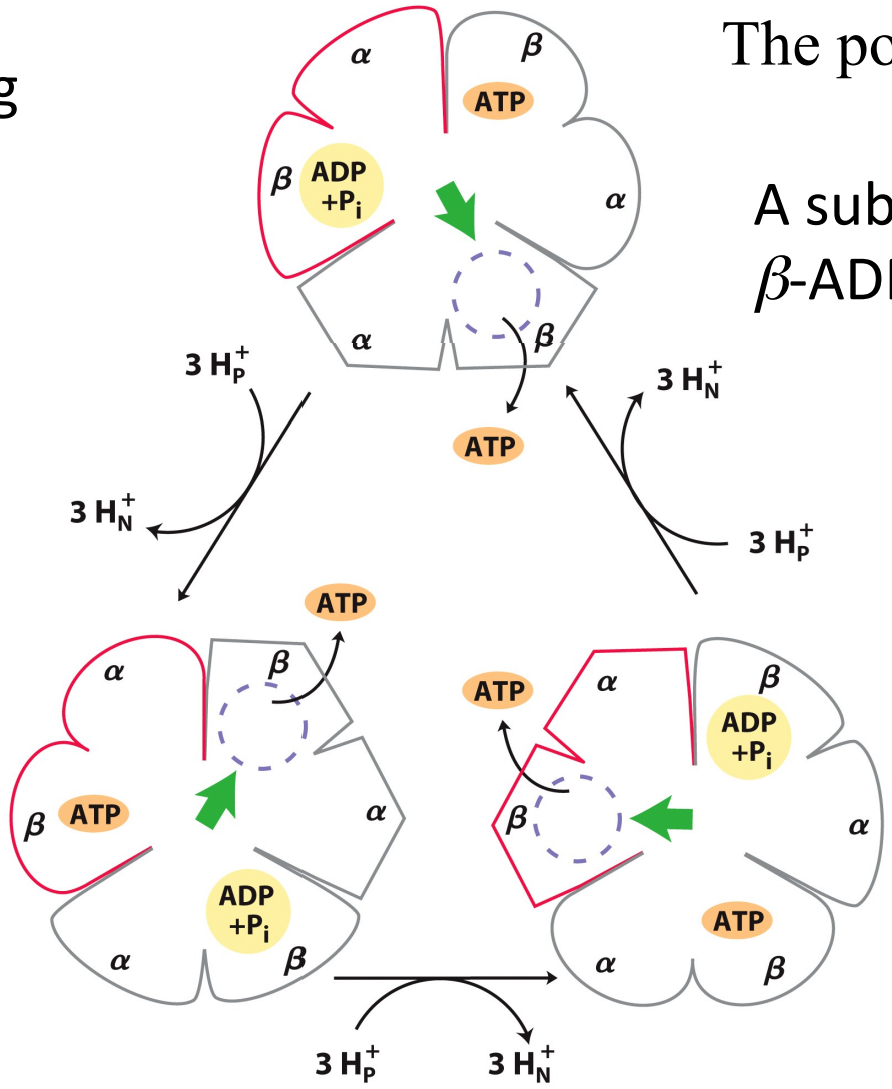
Binding-Change Model (rotational catalysis)

The 3 active sites take turn catalyzing the reaction driven by proton entering

It changes conformation to β-ATP, stabilizing ATP on enzyme surface



The position of γ

A subunit starts with β-ADP conformation

Subunit changes to β-empty which is a very low affinity conformation

Coupling Proton Translocation to ATP Synthesis

- Proton translocation causes a rotation of the F_o subunit and the central shaft γ
- This causes a conformational change within all the three $\alpha\beta$ pairs
- The conformational change in one of the three pairs promotes condensation of ADP and P_i into ATP

Evidence of Rotation

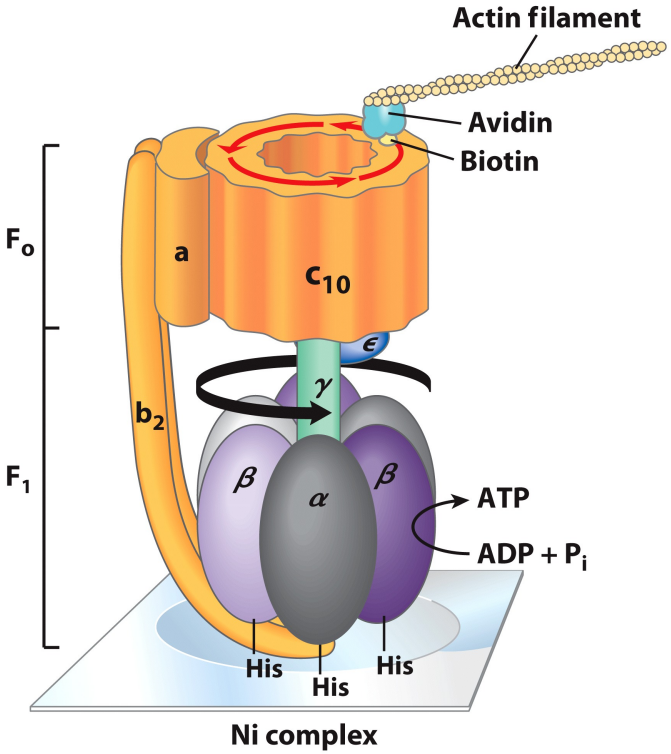


Figure 19-27a
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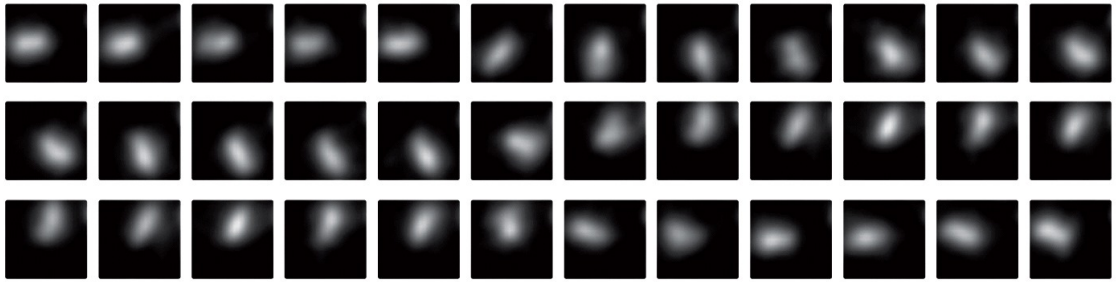

Figure 19-27b
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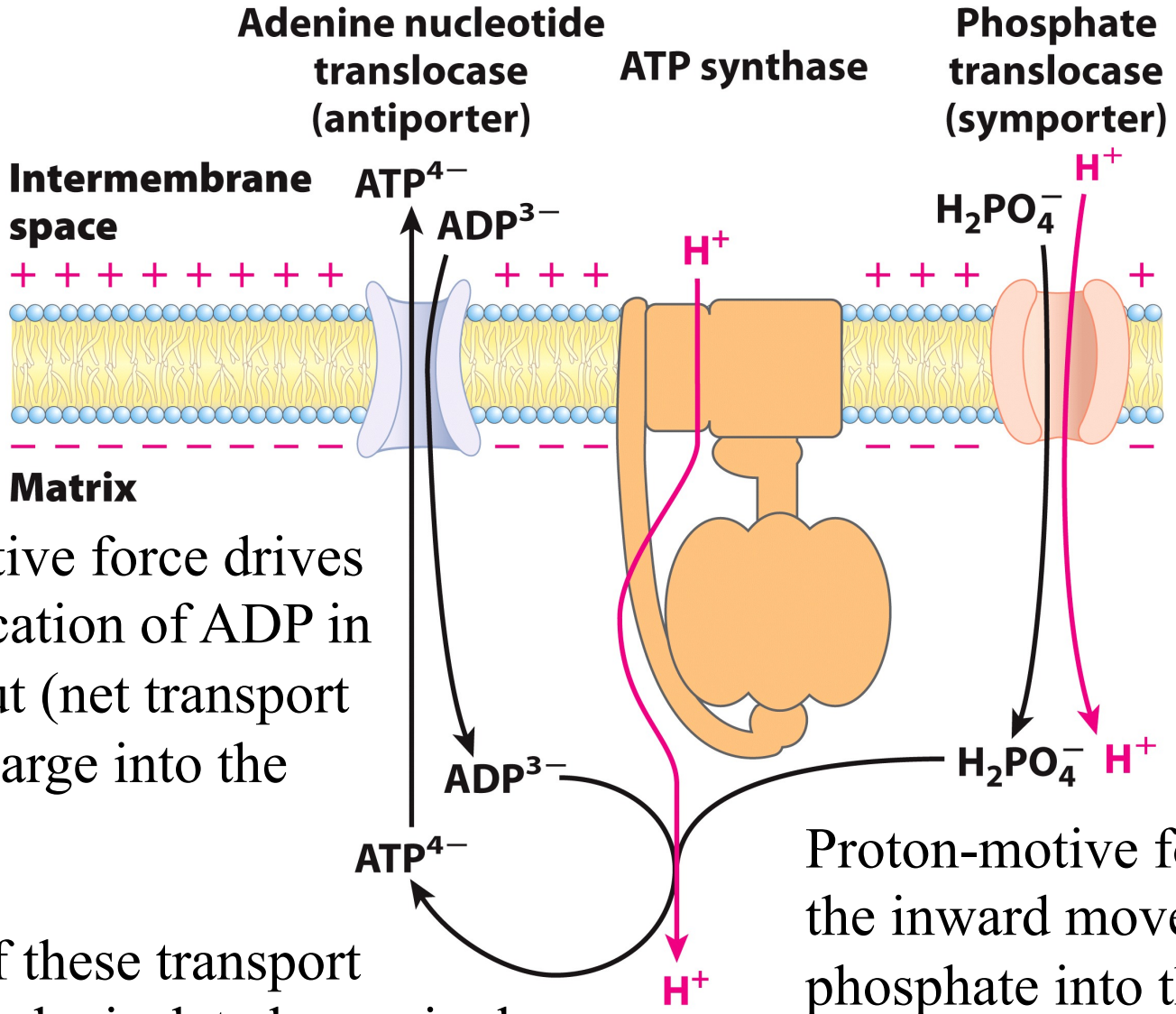
Stoichiometry of O_2 consumption and ATP Synthesis

- xADP + $x$$P_i$ + ½ O_2 + H^+ + NADH → xATP + H_2O + NAD^+
- x (**P/O ratio**) = number of ATP molecules synthesized per ½ O_2 (thought to be an integer)
- Switched the question to how many protons are pumped outward and how many protons must flow back in to make ATP
- 10 H^+ (from NADH) and 6 H^+ (from succinate) are pumped out per electron pair
- 4 H^+ are needed to flow back to make 1 ATP
  (3 to turn the F_o and 1 to transport P_i, ATP and ADP) → proton-based P/O ratios are:
  2.5 ATP/NADH and 1.5 ATP/succinate

Transport of ADP and P_i into the Matrix



Proton-motive force drives the translocation of ADP in and ATP out (net transport of 1 –ve charge into the +ve IMS

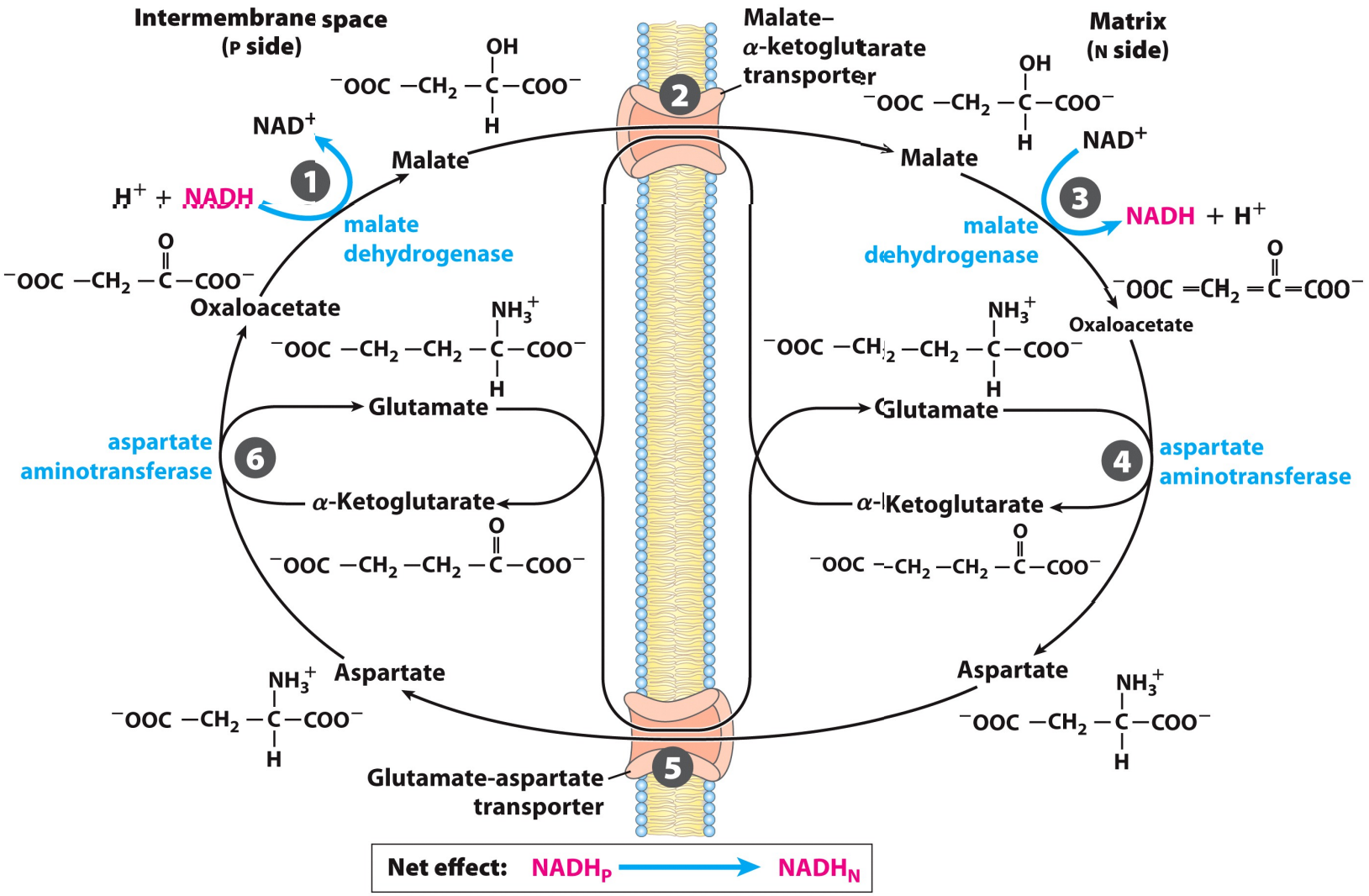
Proton-motive force drives the inward movement of phosphate into the matrix

All three of these transport systems can be isolated as a single membrane-bound complex (ATP synthasome)

Net Production of ATP by Oxidation of Glucose (and Other Fuels) Varies

- In prokaryotic systems, organelles do not segregate machinery, so all electron carriers can easily feed directly into the electron-transport chain.
- In eukaryotic systems, organellar segregation prevents NADH from the cytosol from directly entering the electron-transport chain at Complex I.
  - NAD^+ pools are kept segregated and cannot directly cross the mitochondrial inner membrane.
  - Two methods are used to feed the electrons from NADH from the cytosol into the mitochondria:
    - malate-aspartate shuttle
    - glycerol-3-phosphate shuttle

Malate-Aspartate Shuttle



In liver, kidney and heart mitochondria

Glycerol-3-Phosphate Shuttle

No pumping of H^+ from complex I (NADH from glycolysis has a P/O ratio of 1.5)

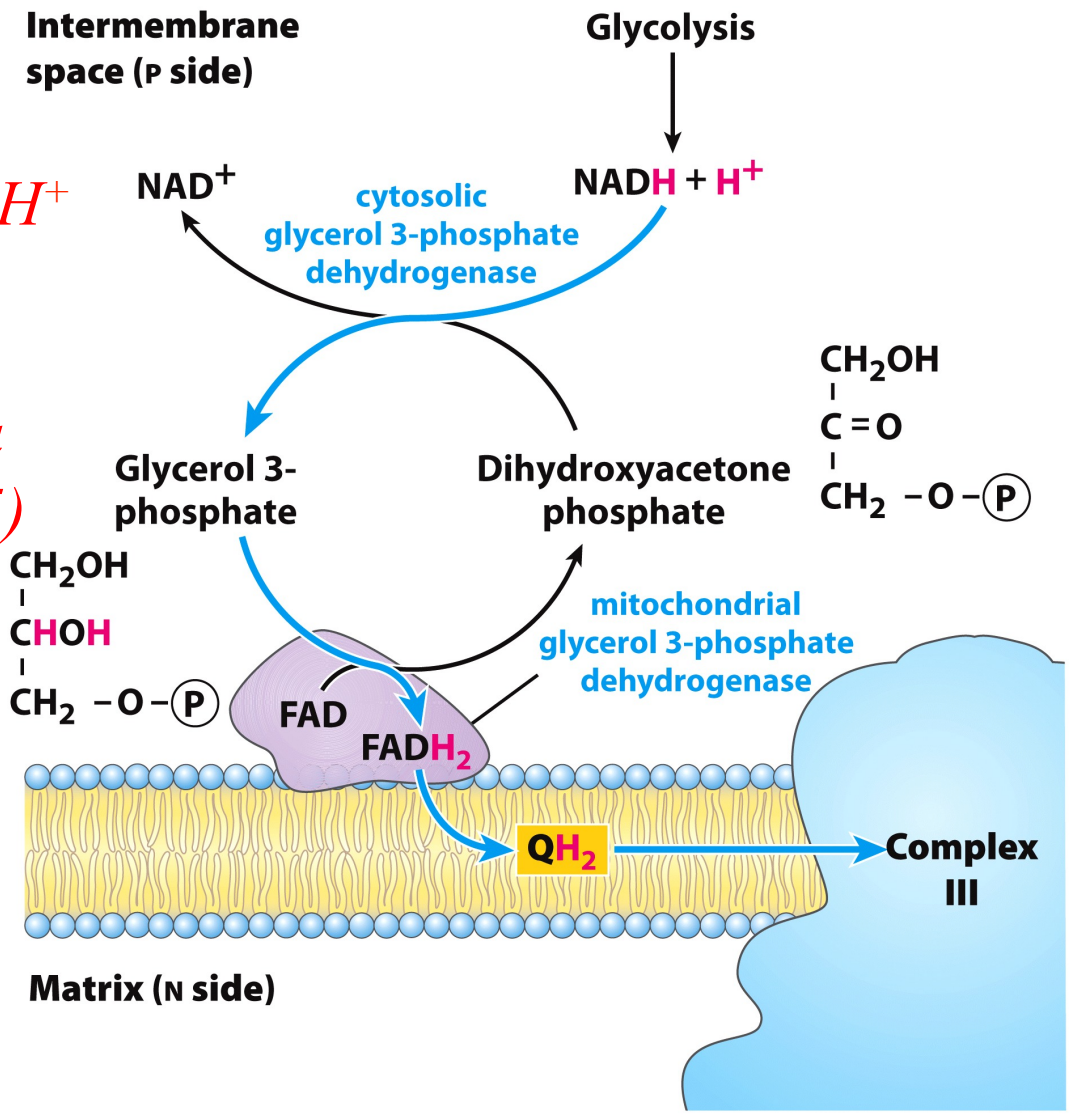


Figure 19-32
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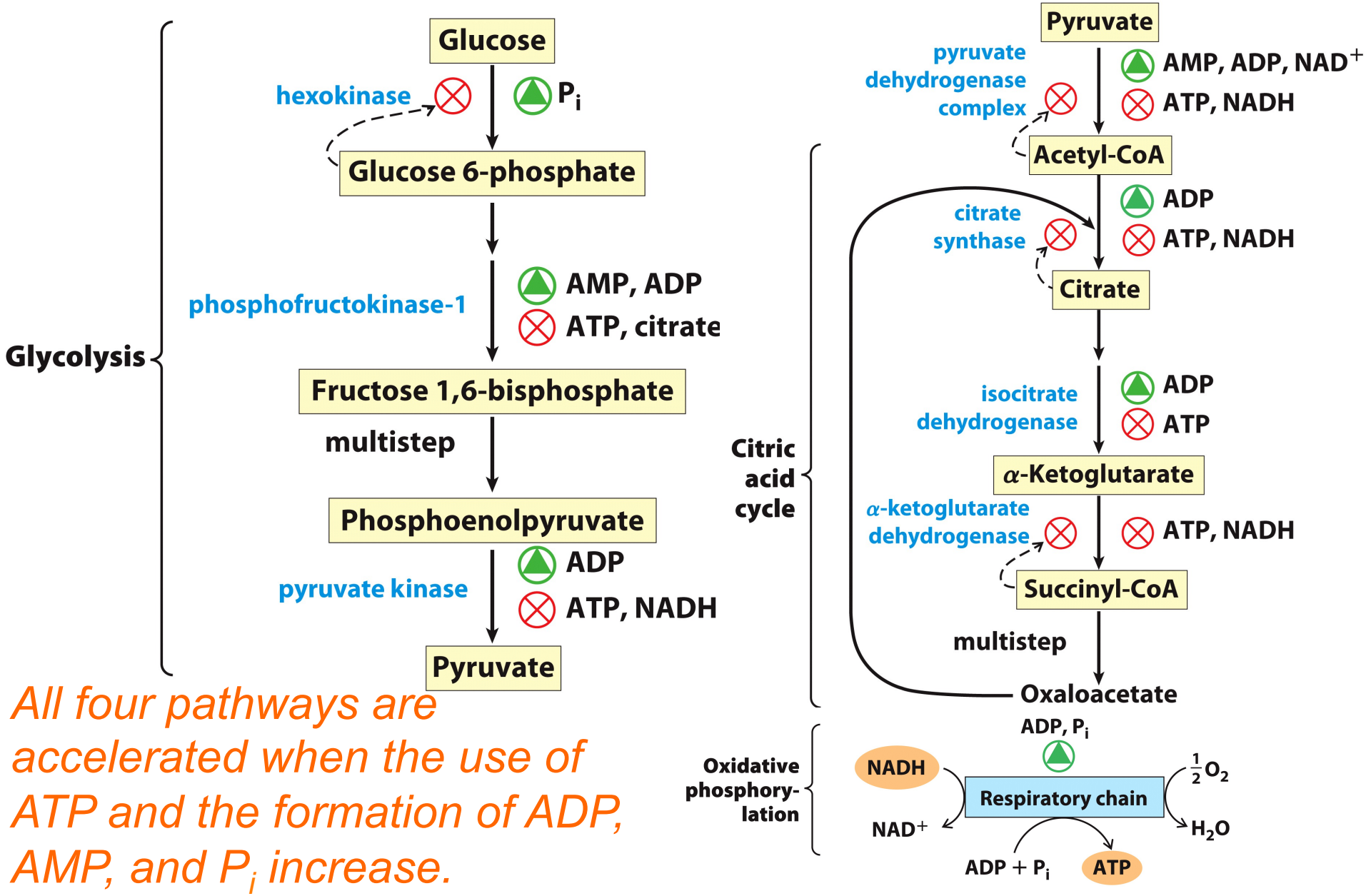
In brain and skeletal muscles

Regulation of Oxidative Phosphorylation

- Primarily regulated by substrate availability
  - **Acceptor control ratio** – maximal rate of ADP-induced O_2 consumption/basal rate (without ADP) ~ >10 in many cells
  - **Mass action ratio** – [ATP]/[ADP][P_i] is normally very high. When the rate of energy-requiring processes ↑, mass action ratio↓ →↑ADP available for OxPhos → respiration rate↑
  - *ATP is formed only as fast as it's used in energy-requiring activities*
- Inhibitor of F_1 (IF_1)
  - Prevents hydrolysis of ATP during low oxygen
  - Binds to 2 ATP synthases and inhibits their ATPase activities
  - Only active at lower pH, encountered when electron transport is slowed (i.e., low oxygen). *Recall lactic acid fermentation!*
- Inhibition of OxPhos leads to accumulation of NADH
  - Causes feedback inhibition cascade up to PFK-1 in glycolysis

Regulation of ATP-producing pathways



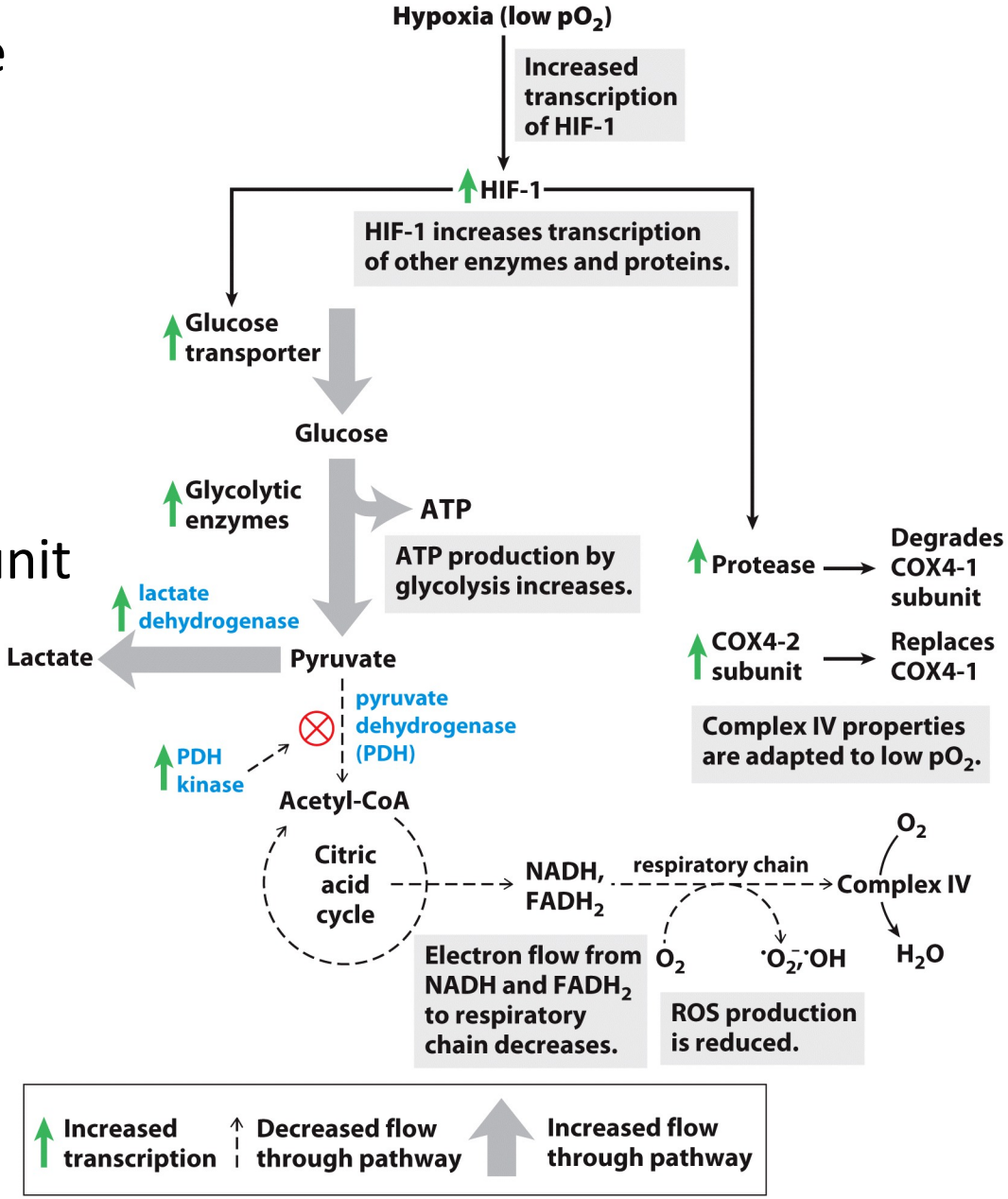
All four pathways are accelerated when the use of ATP and the formation of ADP, AMP, and P_i increase.

HIF

- Hypoxic cells ➔ Imbalance between e^- input and e^- transfer to O_2 ➔ ↑ROS

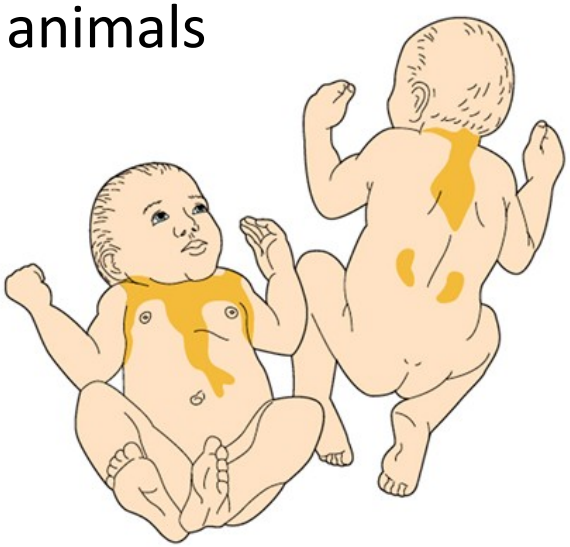
- Countered by:

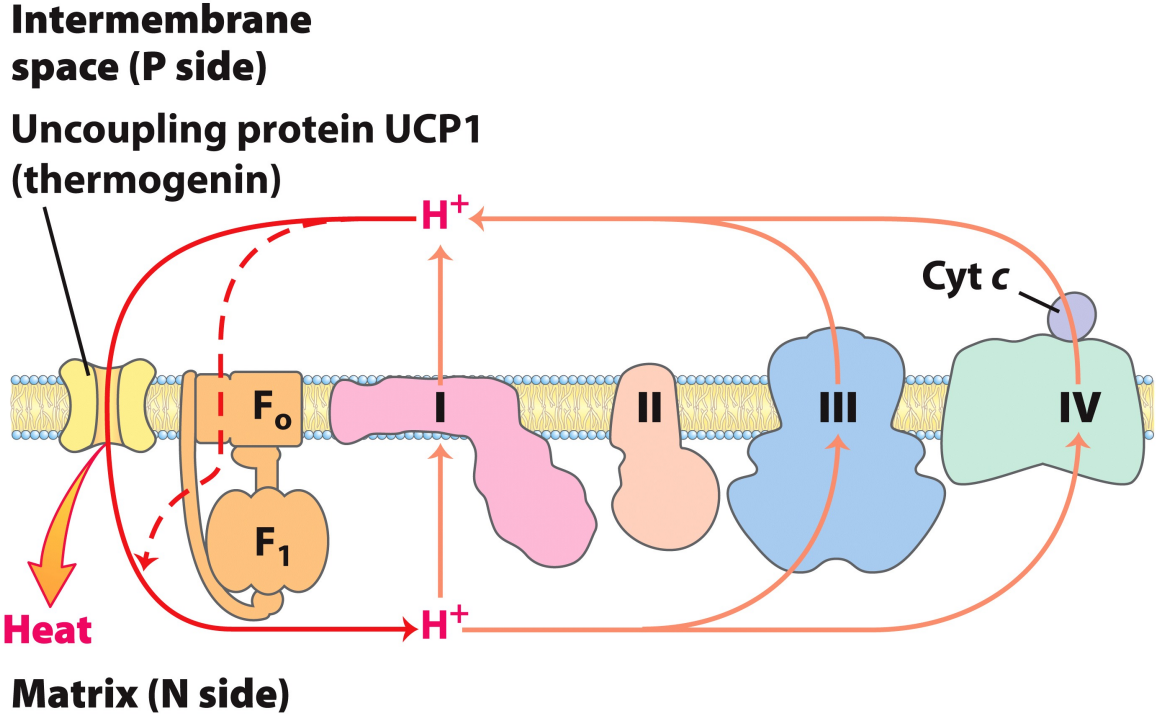
1. Increase in glycolysis
2. Inactivation of PDH
3. Replacement of COX subunit



Brown Adipose Tissue has uncoupled mito

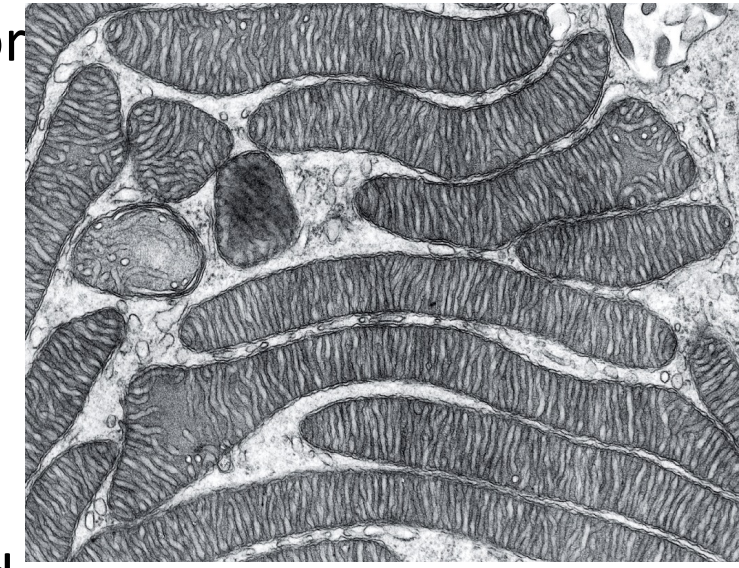
- In newborn mammals, BAT serves as heat-generating tissue
- Large number of mito ➔ large number of cytochromes ➔ looks brown
- BAT mito have an uncoupling protein in their inner membrane (**thermogenin**) which is a proton channel
- Path for protons to the matrix without passing through F_oF_1 complex ➔ short-circuiting of protons ➔ energy is not conserved as ATP by lost as heat
- Also in hibernating animals





Steroidogenesis

- Steroids are synthesized from cholesterol in a series of hydroxylations catalyzed by **cytochrome P-450**
- $R\text{-}H + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$
- Steroidogenic cells (e.g. adrenal glands) are packed with specialized mitochondria for steroid synthesis ↘



- P-450 are also found in ER, responsible for metabolism of **xenobiotics**
- Hydroxylation → more water soluble → more excretion in urine
- Many prescription drugs are substrates for P-450 → P-450 activity limits the drugs' lifetime and efficacy
- Humans differ in their P-450 contents and activities in their cells → an individual's genetics and personal history could have a say in determining therapeutic drug dose or form

Mitochondrial damage initiates apoptosis

- Apoptosis – Individual cells die for the benefit of the organism
- Initiated by external signals or internal events
- Early consequence of death signals in the increase in MOM permeability to proteins
- ***What causes this permeability? (My Ph.D. research ☺)***
- **Cytochrome *c*** (and others) is released into the cytosol
- 7 molecules of cyt *c* form an **apoptosome** with 7 **Apaf-1**
- Allows the docking and activation of procaspase-9
- Cleaves procaspase-9 (inactive) to **caspase-9** (active) which cleaves and activates procaspase-3 and 7 (into **caspase-3** and **caspase-7**) which is an executioner caspase (breaks down the macromolecular contents of cells)
- Caspase cascade

- Cytochrome *c* is another moonlightling protein

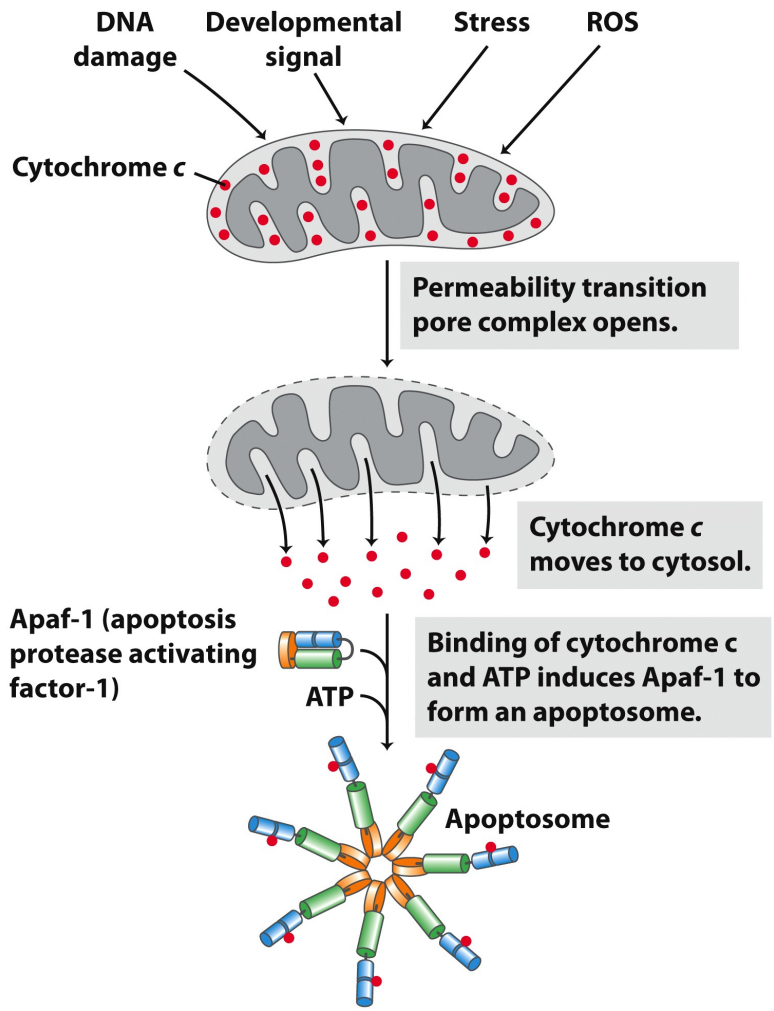


Figure 19-39 part 1
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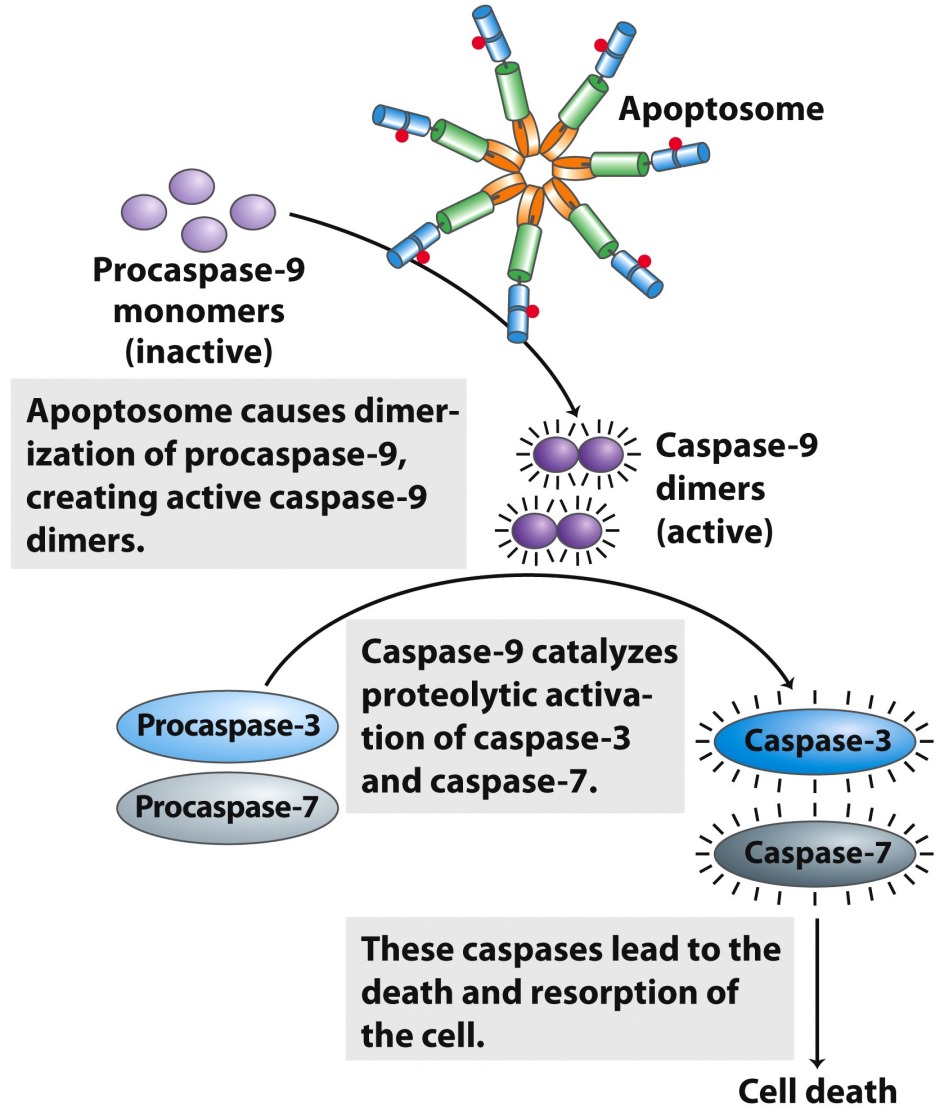

Figure 19-39 part 2
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Mitochondrial genes

- Circular double stranded mtDNA
- Each mito has ~ 5 copies
- Human mt genome contains 37 genes:
  13 encode subunits of respiratory chain proteins
  24 encode for tRNA and rRNA
- The majority of mito's 1100 proteins are encoded by nuclear genes and translated on cytosolic ribosomes

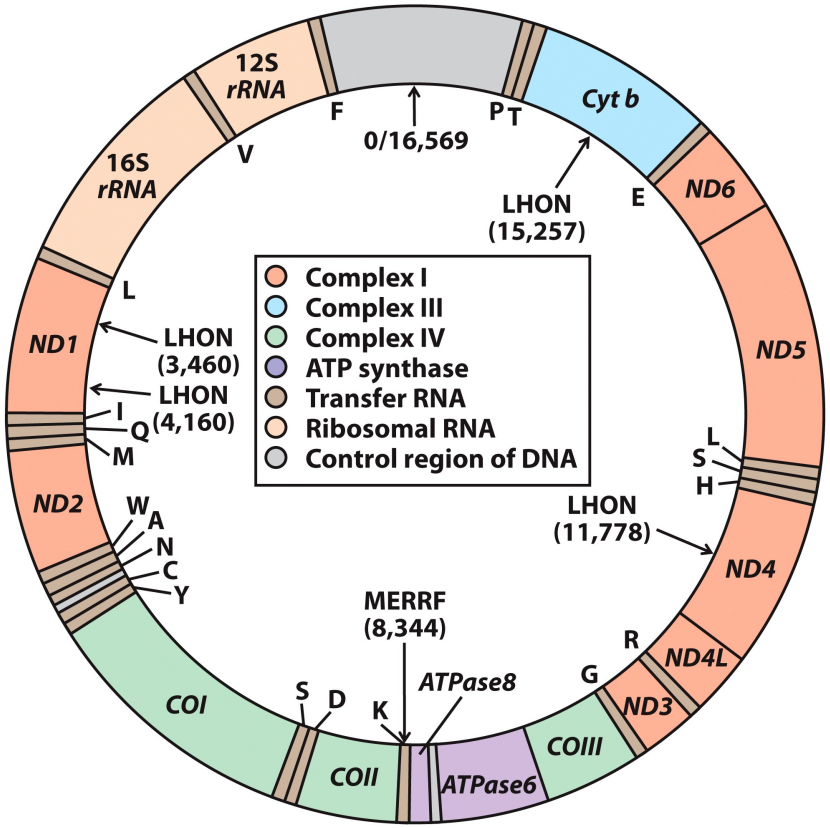

Figure 19-40a
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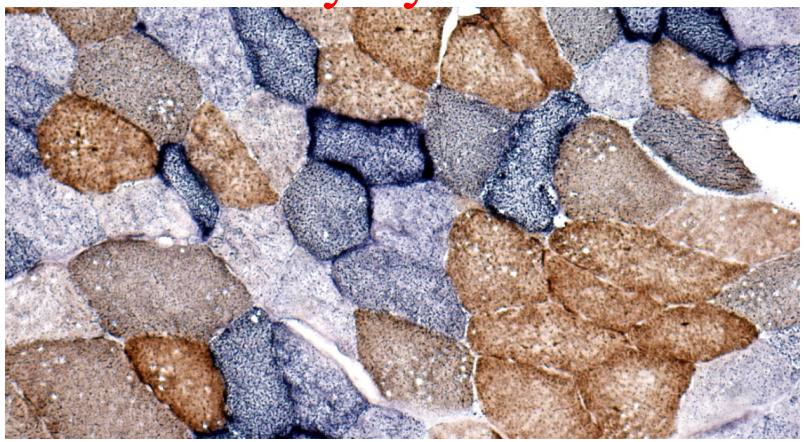
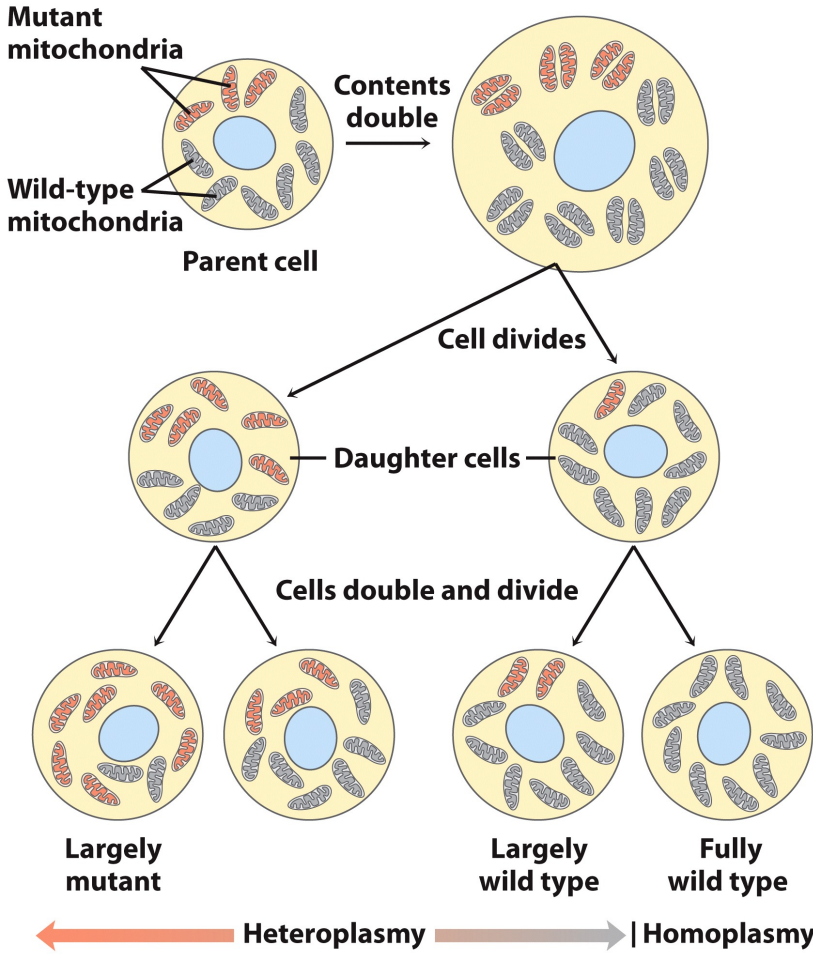
Mutations in mtDNA accumulate

- Mito are exposed the most to ROS
- mtDNA replication and repair are less effective than nuclear DNA replication ➔ *Defects in mtDNA occur over time*
- Animals inherit their mito from mothers
- 10^5-10^6 mito/egg and 10^2-10^3 mito/ sperm. Also eggs target sperm mito for degradation
- **Heteroplasmy** and **homoplasmy**

Different cells in the same tissue are affected differently by mito mutation



wt cells – blue
Mutant COX – brown



Mutations in mtDNA cause disease

- **Mitochondrial encephalomyopathies**
- affect brain and skeletal muscles
- **Leber's hereditary optic neuropathy (LHON)** affects the central nervous system (leads to loss of vision)
- Point mutation in mitochondrial gene ND4 → mito partially defective in electron transfer through complex I
- Mito can produce ATP from complex II but apparently cannot supply enough ATP to support the very active metabolism of neurons → damage to optic nerve → blindness

- **Diabetes**
- Defective OxPhos in pancreatic β cells blocks insulin secretion
- In normal β cells, glc is taken in and oxidized to raise [ATP] above threshold. ATP blocks K^+ channel → depolarization of membrane → opening of voltage-gated Ca^{2+} channels → Ca^{2+} influx into cytoplasm leads to the release of insulin into blood

Mutations in the Mitochondrial Genome Result in Disorders

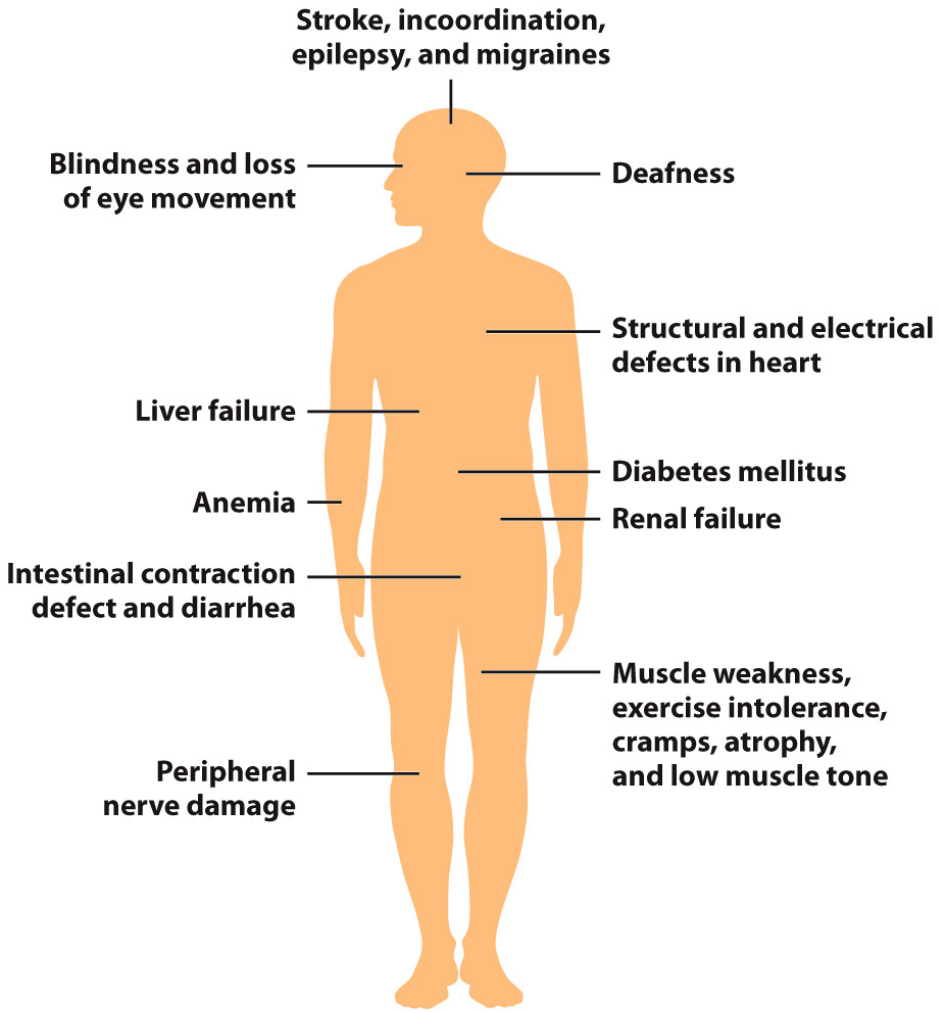


Figure 19-43
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Mitochondrial Mutations Result in a Rare form of Diabetes

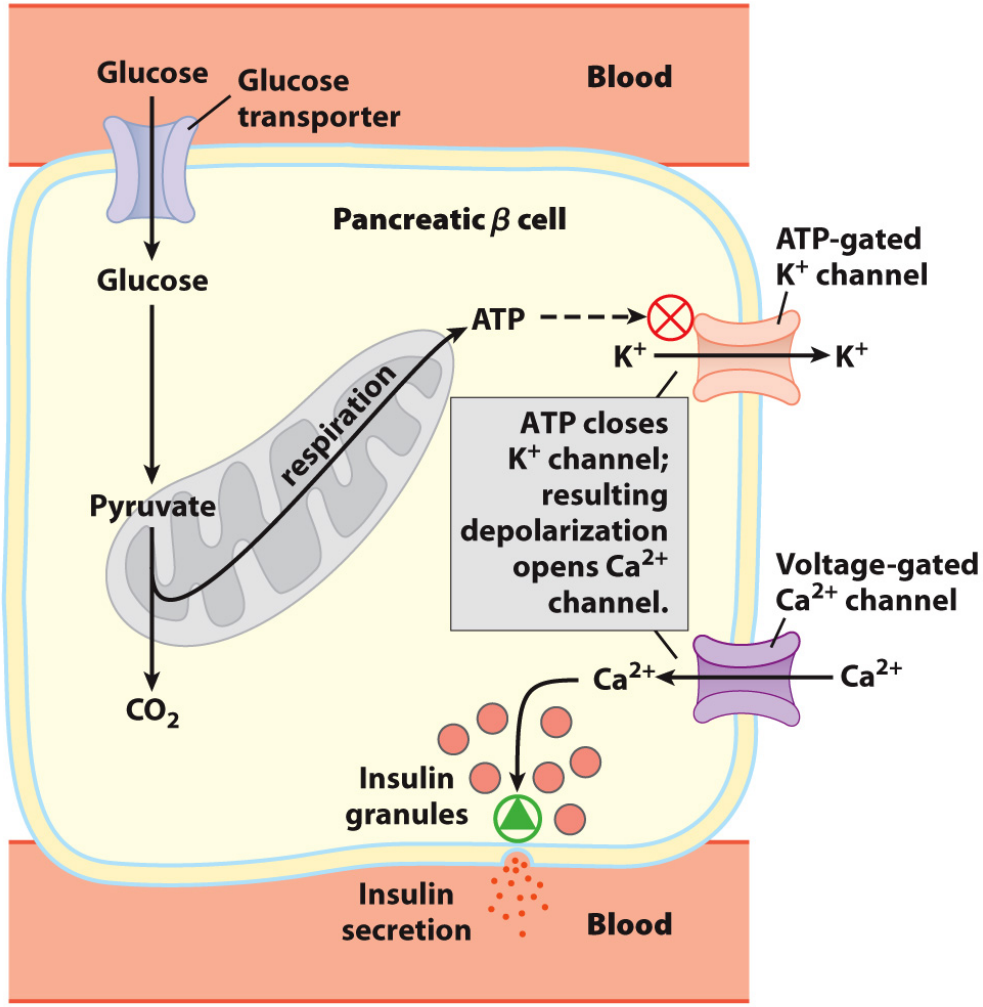

Figure 19-45
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- Defects in oxidative phosphorylation result in low [ATP] in the cell.
  - Insulin cannot be released from the cell.

Question 6 (Take home exam)
Due: NEXT WEEK (jstiban@birzeit.edu)

- **Please solve questions:**

1. **6 (uncouplers)**
2. **17 (ATP turnover)**
3. **22 (alanine)**
4. **24 (diabetes)**

For written answers, I prefer to have them typed in Word. I can accept the assignment in one file sent to my email. For answers that require solving mathematically, you can either type them or write them down and scan them.