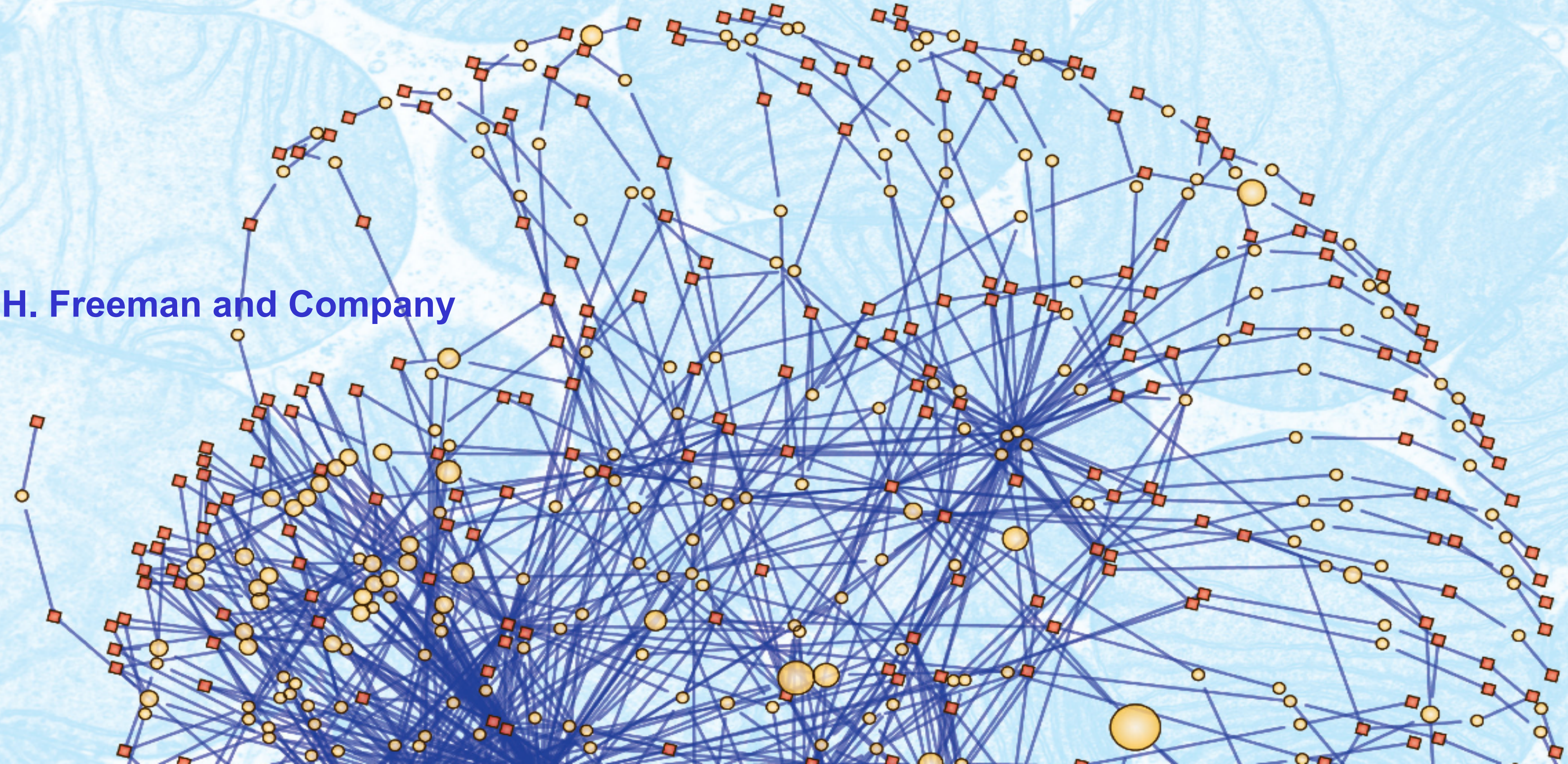
Lehninger

SIXTH EDITION

Principles of Biochemistry

David L. Nelson | Michael M. Cox

19| Oxidative Phosphorylation



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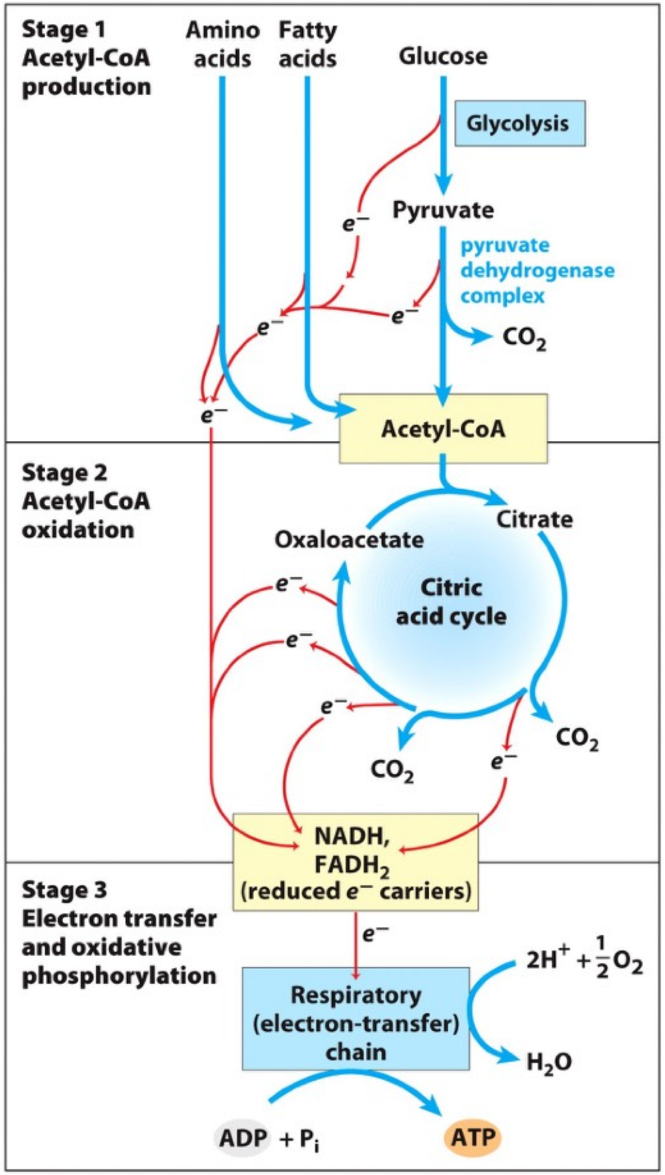
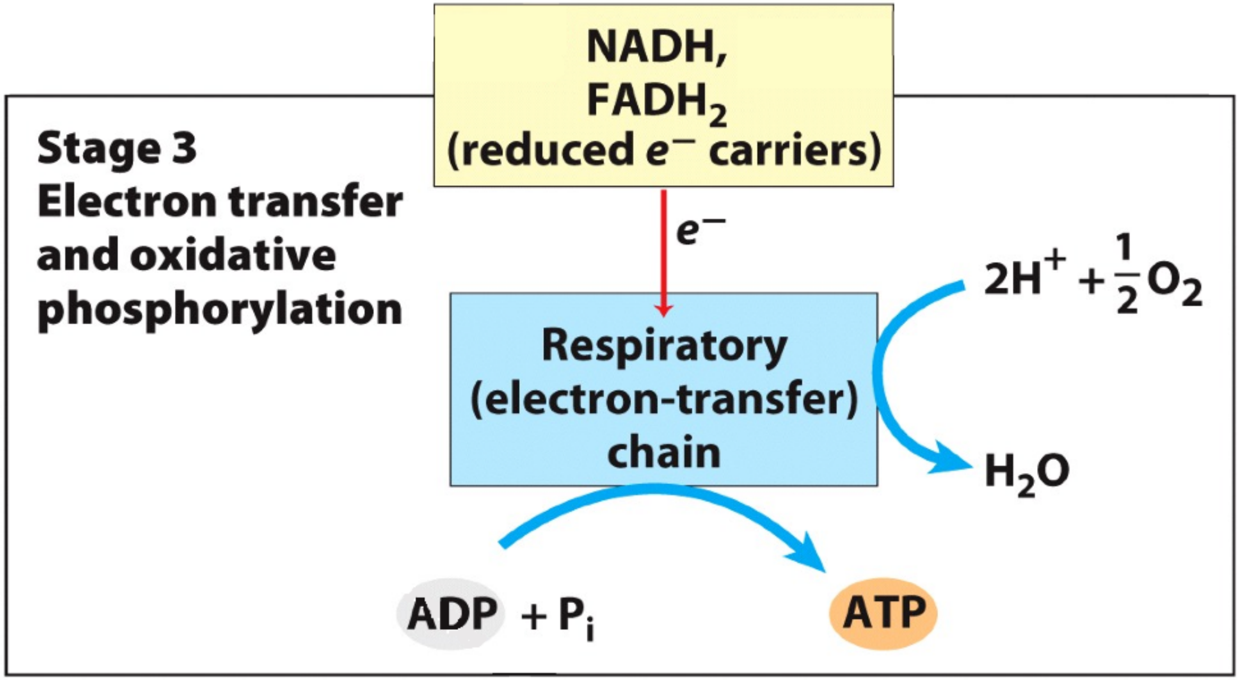
Energy Flow in Cellular Respiration

- Carbohydrates, lipids, and amino acids are the main reduced fuels for the cell
- 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



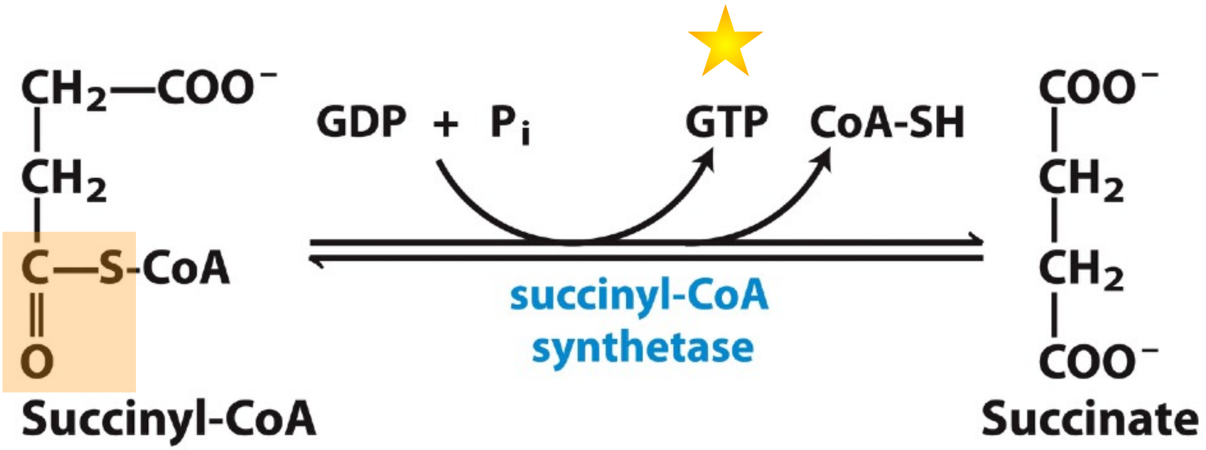
Oxidative Phosphorylation

- 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



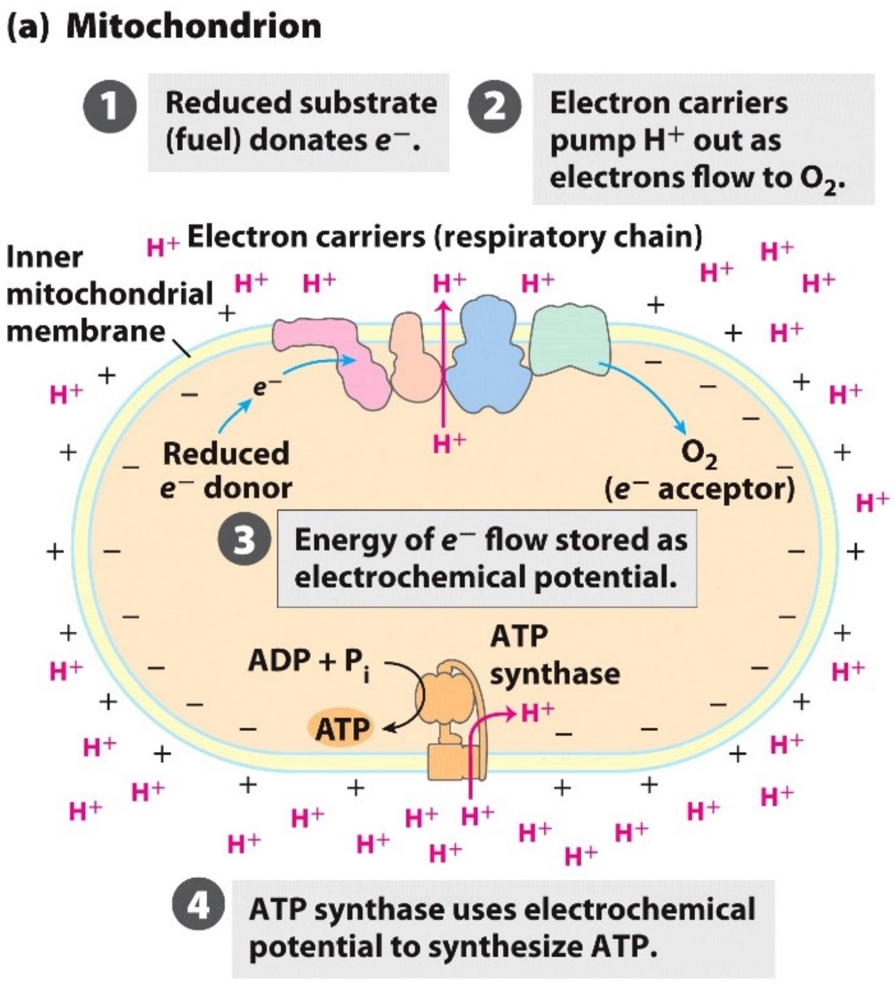
Chemiosmotic Theory

- Synthesis of ATP from ADP and P_i is highly thermodynamically unfavorable
- How do we make it possible?
  - Phosphorylation of ADP is NOT a result of a direct reaction between ADP and some high-energy phosphate carrier
  - 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 Theory

1. Electrons flow through a chain of membrane-bound carriers
    - From NADH to O_2
2. Electron flow is coupled to transport of protons
    - Electron flow is “downhill”
    - Proton transport is “uphill”
    - Free energy is conserved as trans-membrane electrochemical potential
3. Proton flow drives ATP synthesis
    - Protons flow back
    - Energy is used for ATP synthesis
    - Catalyzed by ATP synthase

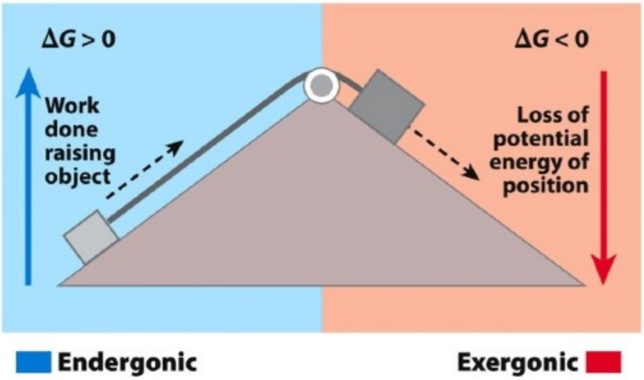

(a) Mitochondrion

① Reduced substrate (fuel) donates e^-.

② Electron carriers pump H^+ out as electrons flow to O_2.

③ Energy of e^- flow stored as electrochemical potential.

④ ATP synthase uses electrochemical potential to synthesize ATP.

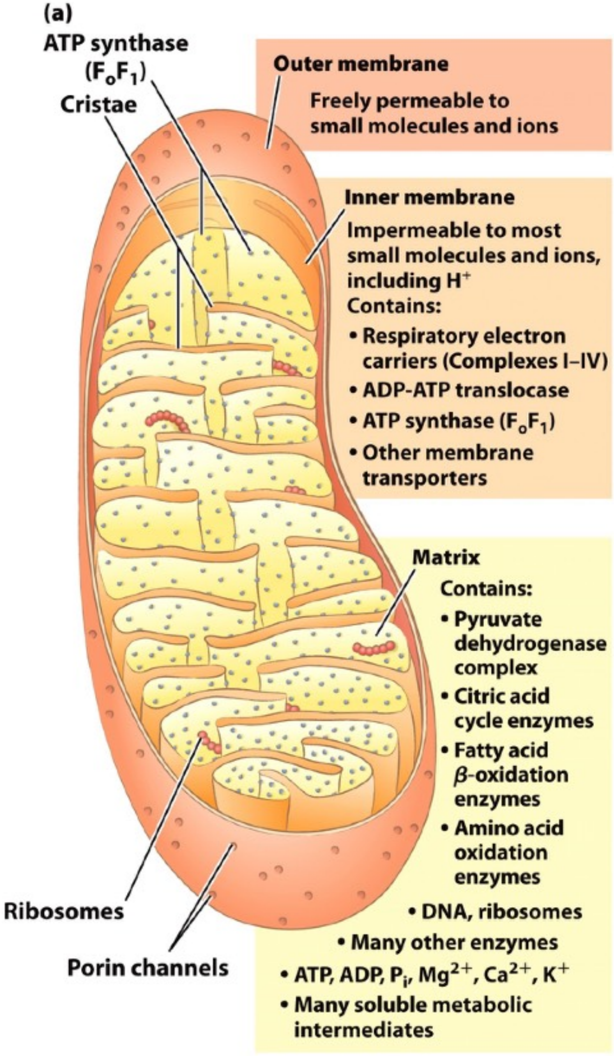


Week 13 Oxidative Phosphorylation

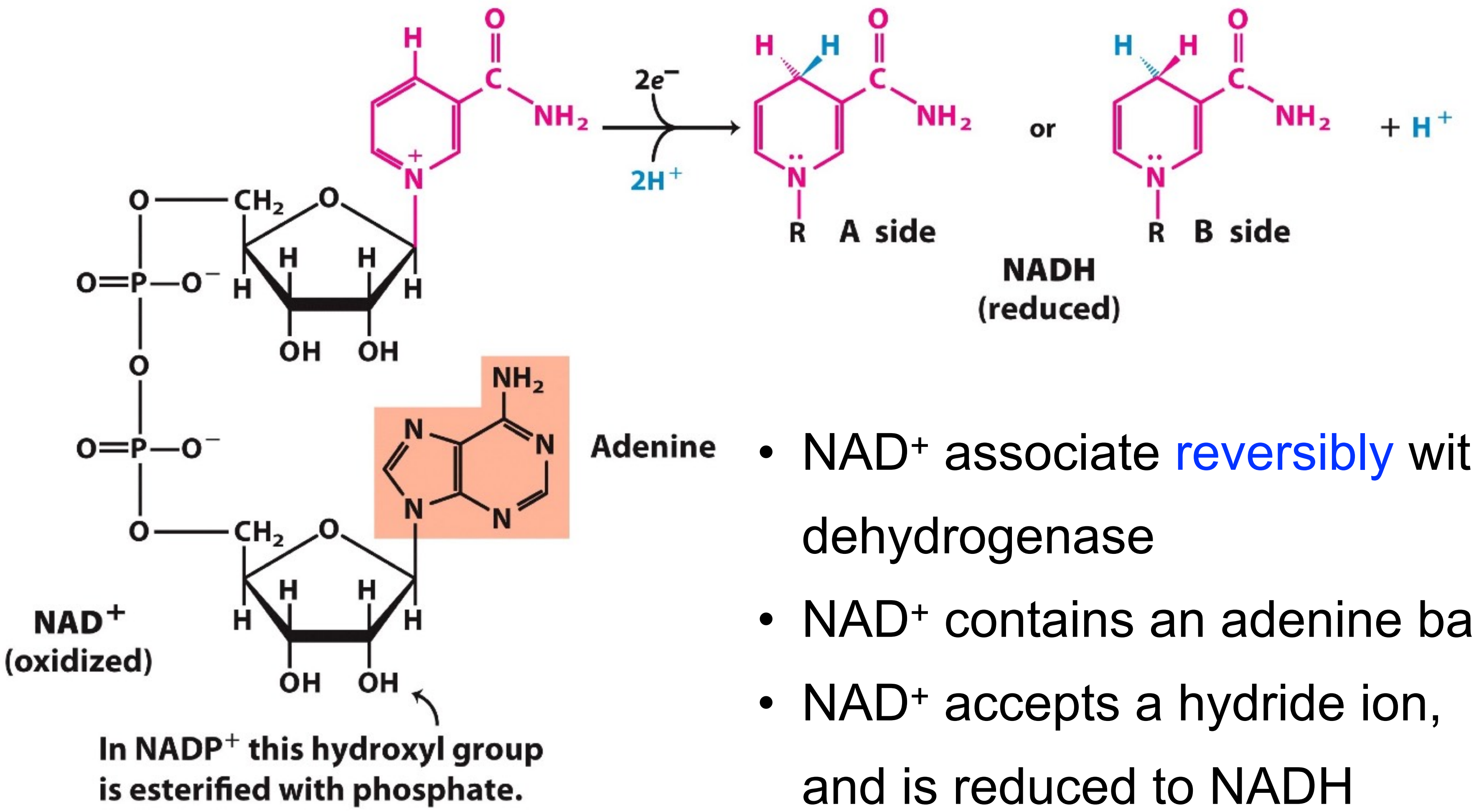
Structure of a Mitochondrion

Four distinct compartments:

1. Outer Membrane
   - Allows passage of metabolites
2. Inter-membrane Space
   - High H^+ 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
4. Matrix
   - Location of the citric acid cycle and parts of fatty acid and amino acid metabolism
   - Lower proton concentration (higher pH)



Universal Electron Acceptors

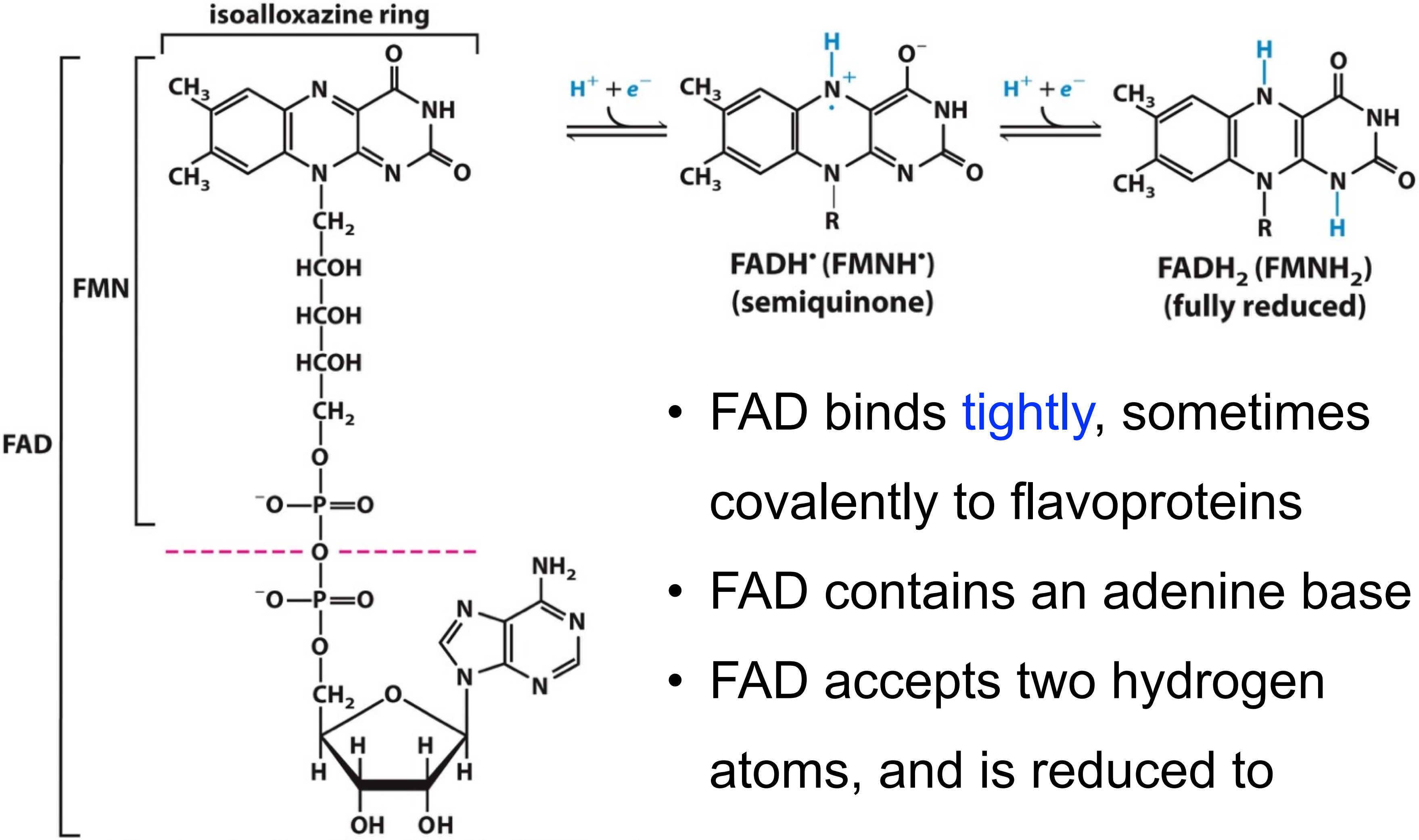


In NADP$^+$ this hydroxyl group is esterified with phosphate.

Nicotinamide adenine dinucleotide (NAD$^+$)

- NAD$^+$ associate reversibly with dehydrogenase
- NAD$^+$ contains an adenine base
- NAD$^+$ accepts a hydride ion, and is reduced to NADH

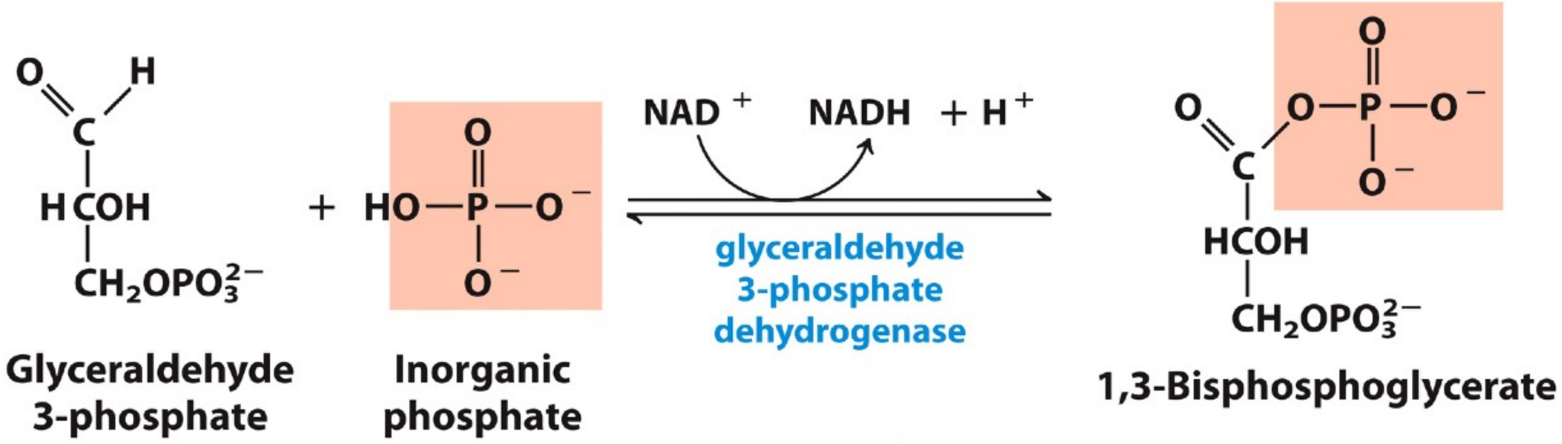
Universal Electron Acceptors



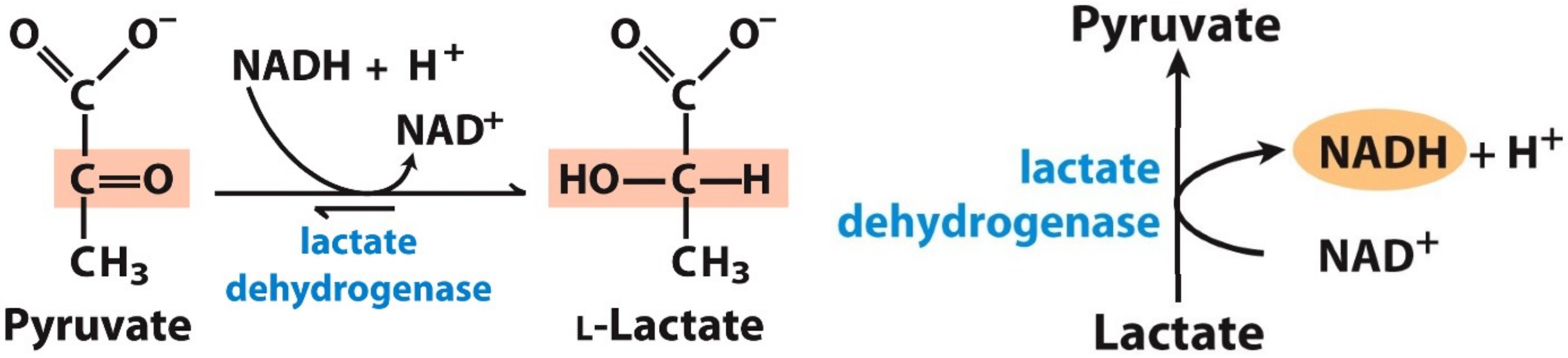
Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

- FAD binds tightly, sometimes covalently to flavoproteins
- FAD contains an adenine base
- FAD accepts two hydrogen atoms, and is reduced to $FADH_2$

NAD$^+$ As Electron Acceptor

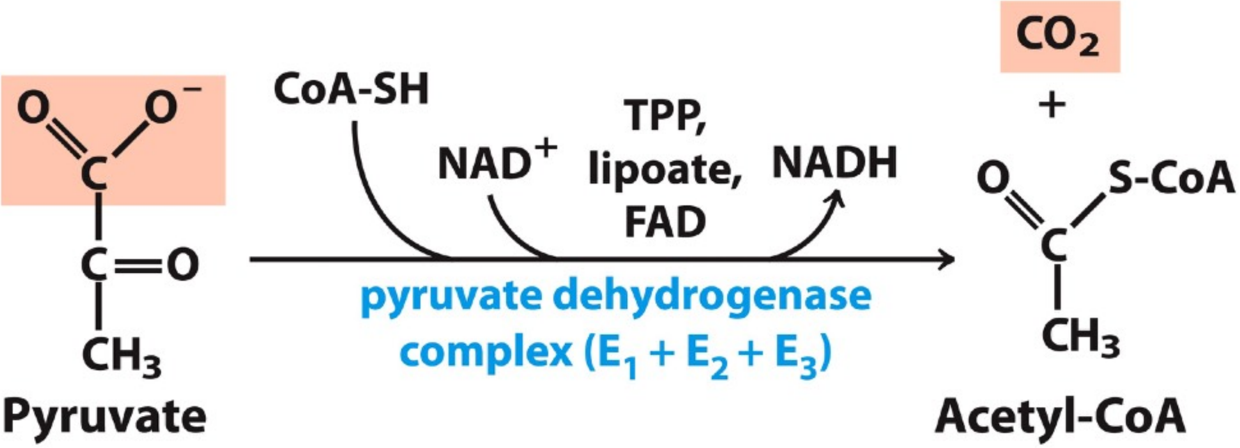


Step 6 in glycolysis. Oxidation of aldehyde to anhydride.

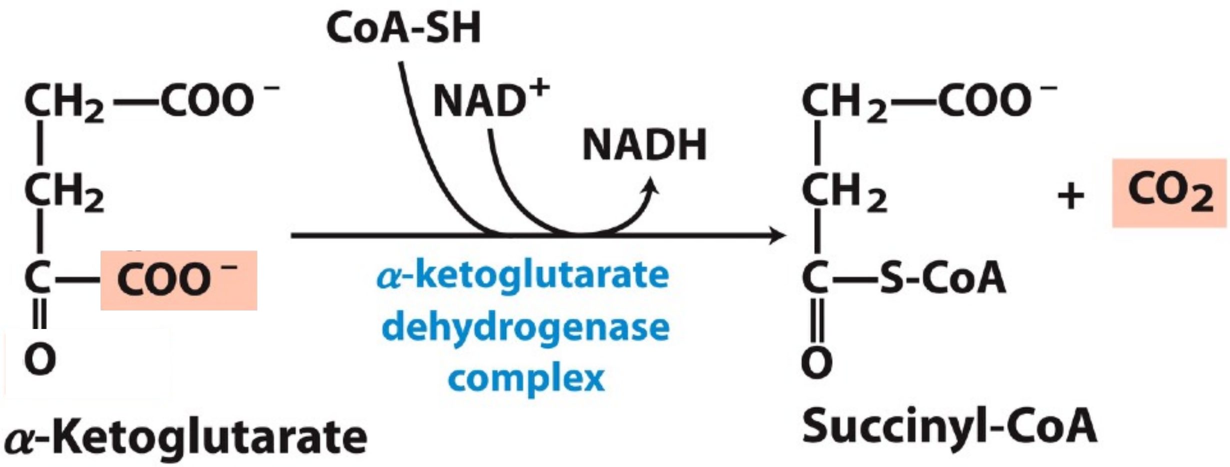


Step 1 in gluconeogenesis from lactate. Oxidation of alcohol to ketone.

NAD^+ As Electron Acceptor



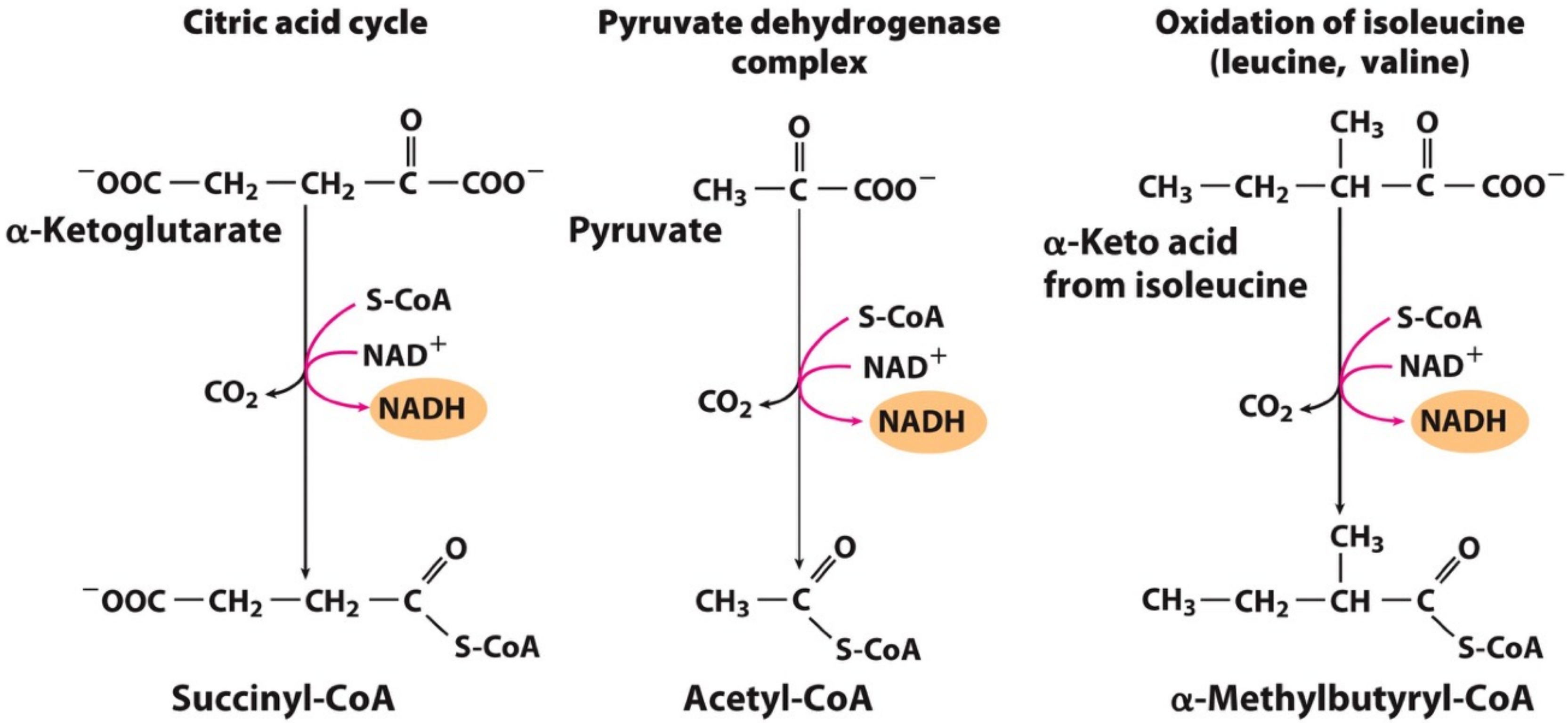
Production of acetyl-CoA from pyruvate. Oxidative decarboxylation.



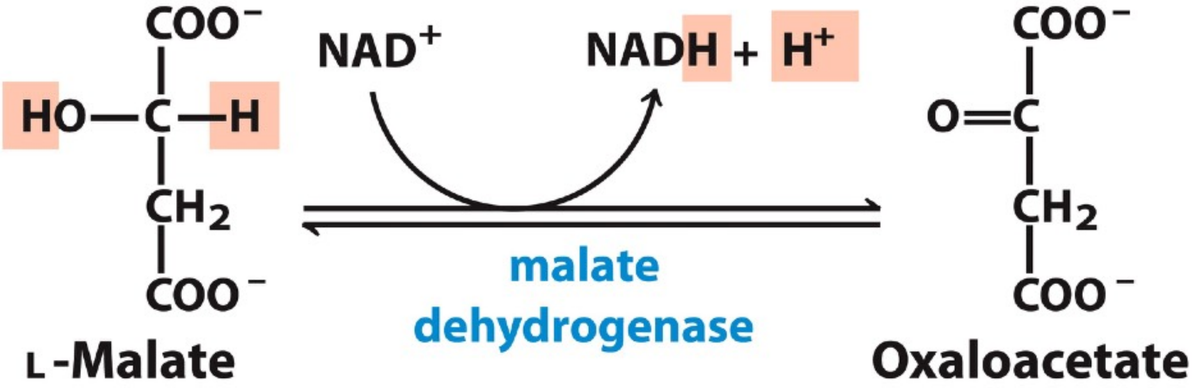
Step 4 in citric acid cycle. Oxidative decarboxylation.

NAD$^+$ As Electron Acceptor

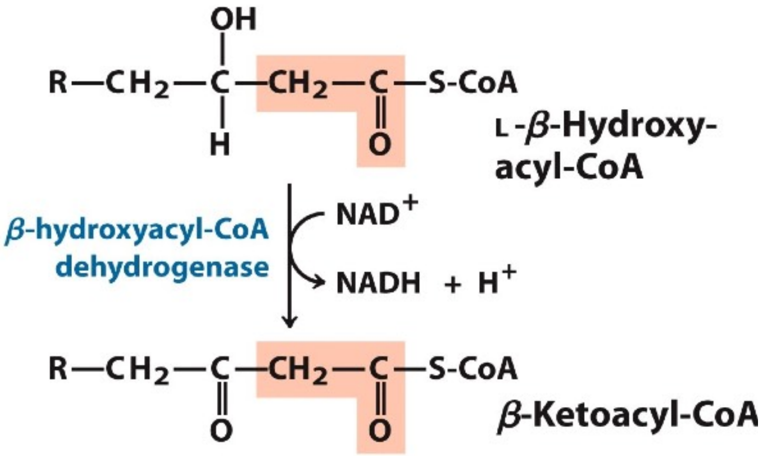
- Production of acetyl-CoA, which enters citric acid cycle
- Production of succinyl-CoA. Step 4 in citric acid cycle
- Production of acyl-CoA. Branched-chain amino acid oxidation



NAD+ As Electron Acceptor

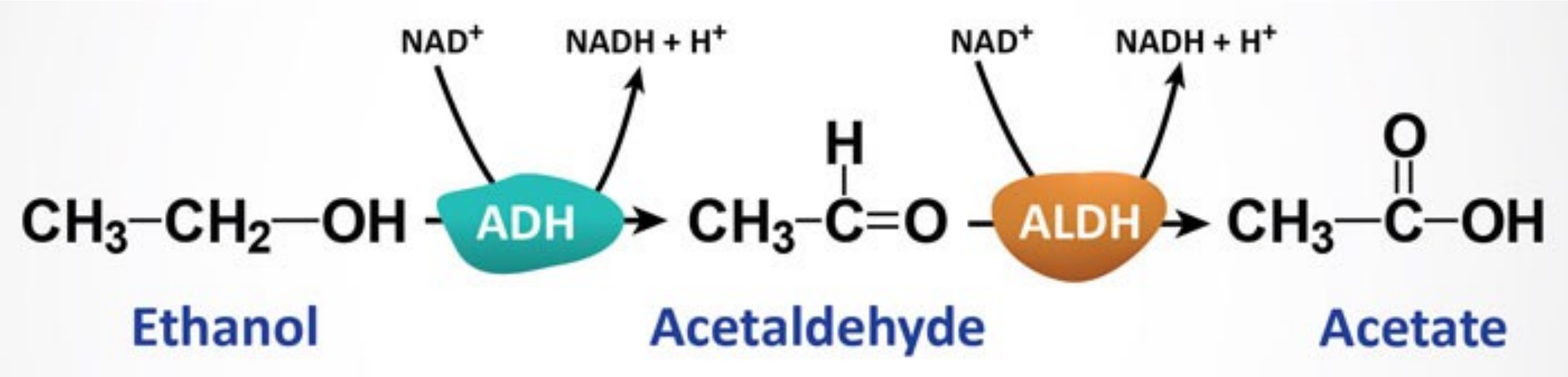


Step 8 in citric acid cycle. Oxidation of alcohol to ketone.



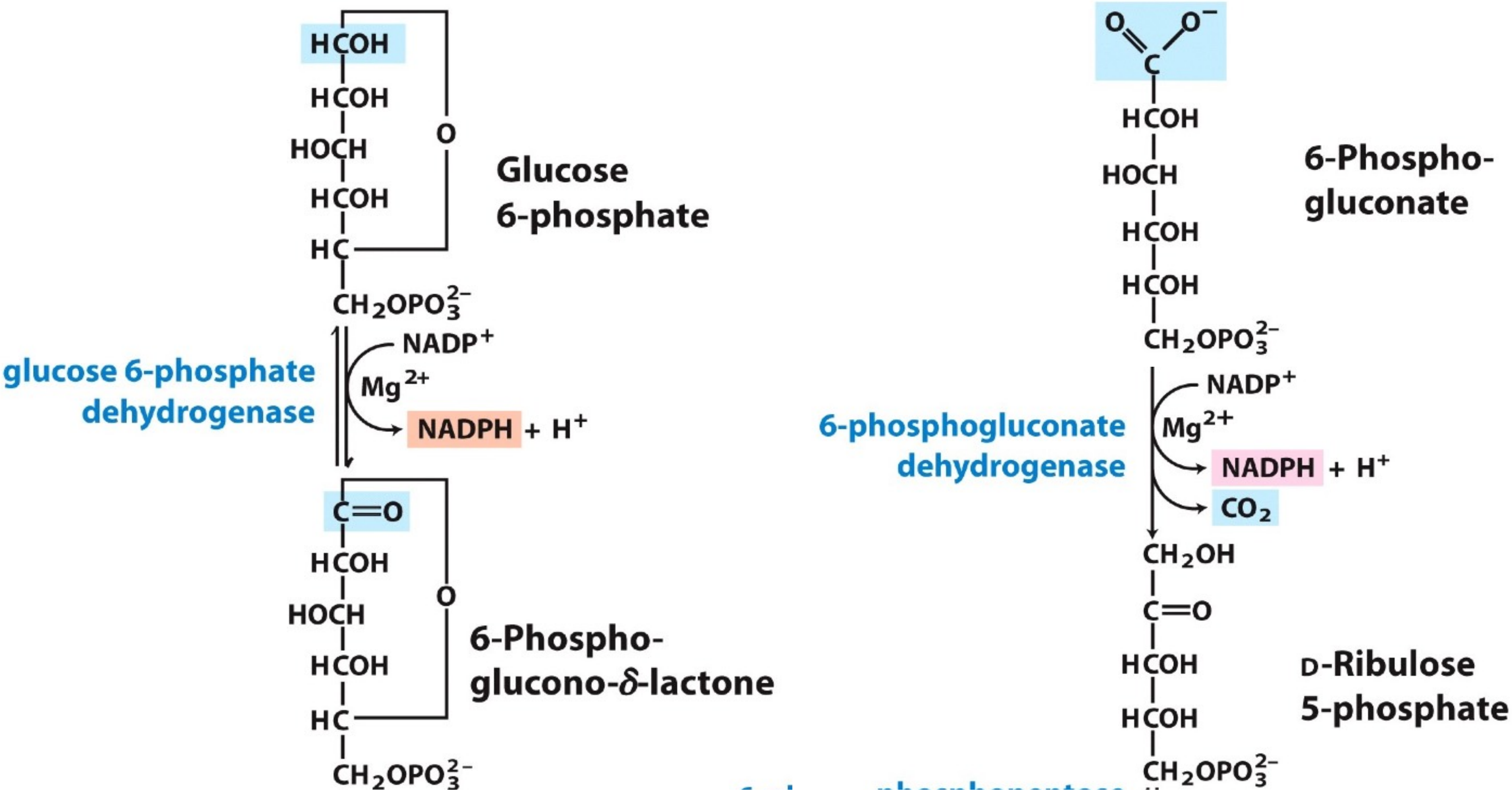
Step 3 in fatty acid β-oxidation. Oxidation of alcohol to ketone.

NAD^+ As Electron Acceptor



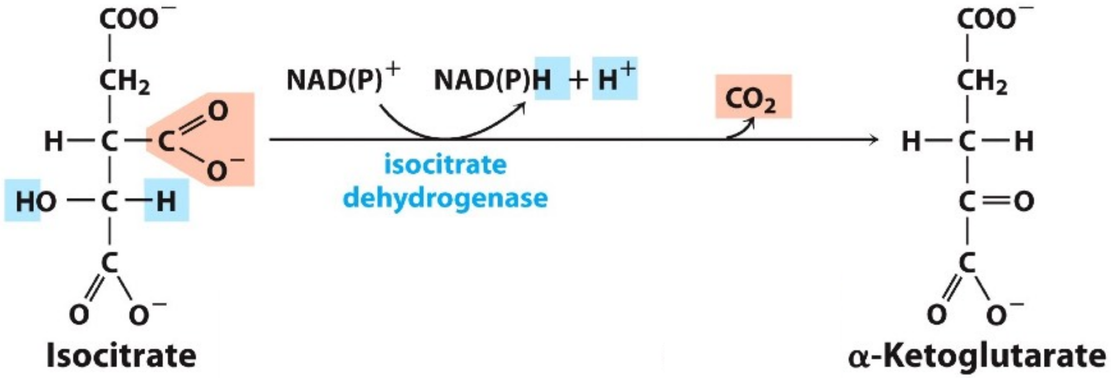
- Alcohol dehydrogenase.
  - Oxidation of alcohol to aldehyde.
- Acetaldehyde dehydrogenase.
  - Oxidation of aldehyde to carboxylic acid.

NADP⁺ As Electron Acceptor

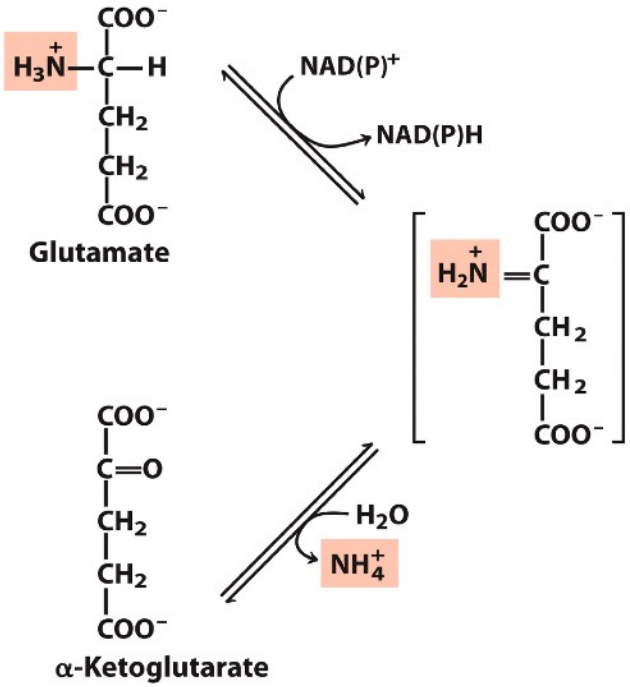


- Step 1 and step 3 in pentose phosphate pathway.
  - Step 1: oxidation of alcohol to ketone.
  - Step 3: oxidative decarboxylation.

NAD⁺ or NADP⁺ As Electron Acceptor

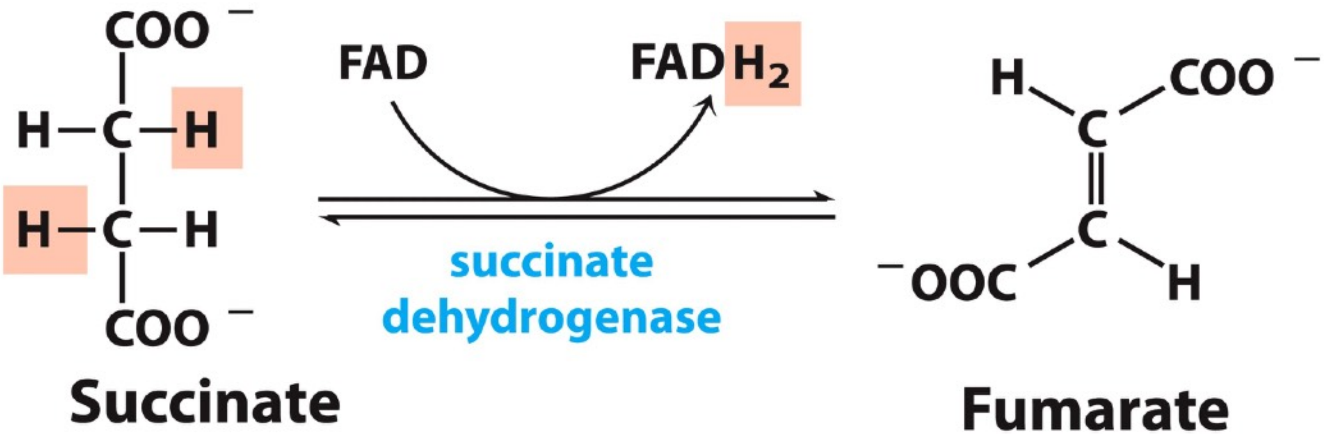


Step 3 in citric acid cycle. Oxidative decarboxylation.

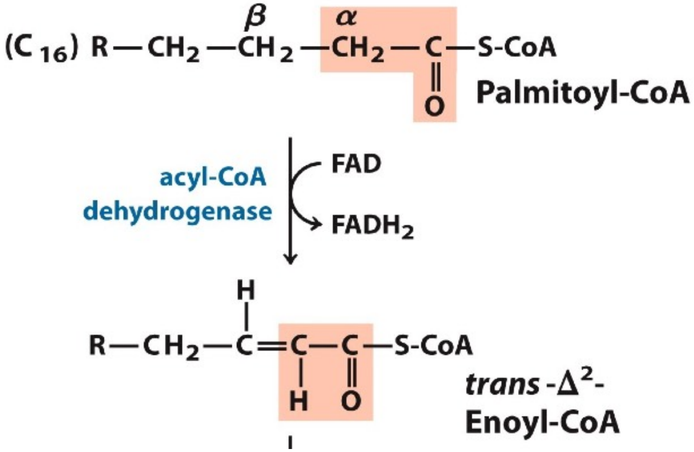


Oxidative deamination in amino acid catabolism.

FAD As Electron Acceptor



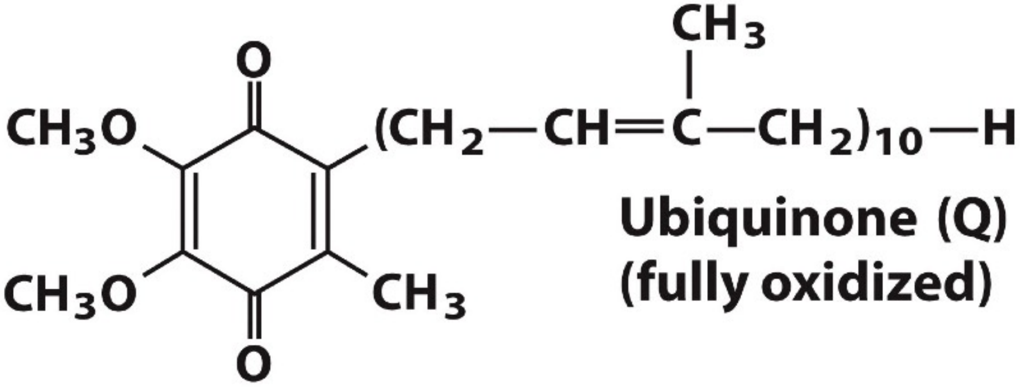
Step 6 in citric acid cycle. Oxidation of alkane to alkene.



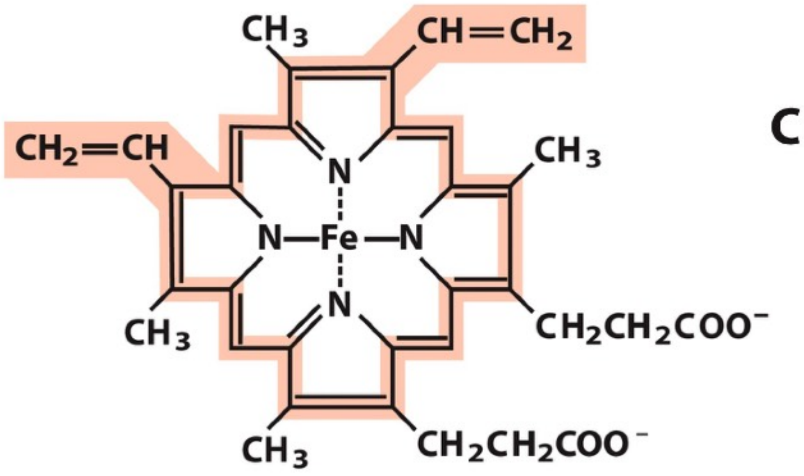
Step 1 in fatty acid β-oxidation. Oxidation of alkane to alkene.

A Series of Electron Carriers

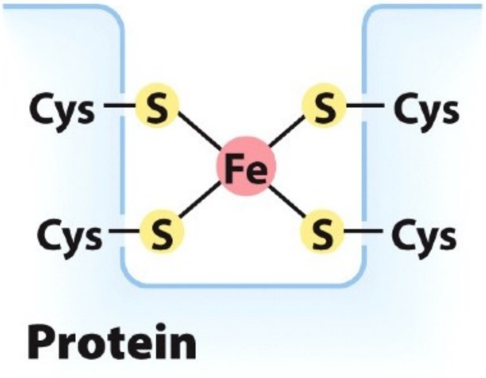
- In addition to NAD and flavoproteins, three other types of electron-carrying molecules function in respiratory chain.
  - Hydrophobic ubiquinone (coenzyme Q, or Q).
  - Iron-containing protein cytochrome.
  - Iron-sulfur protein.



Ubiquinone (Q)
(fully oxidized)

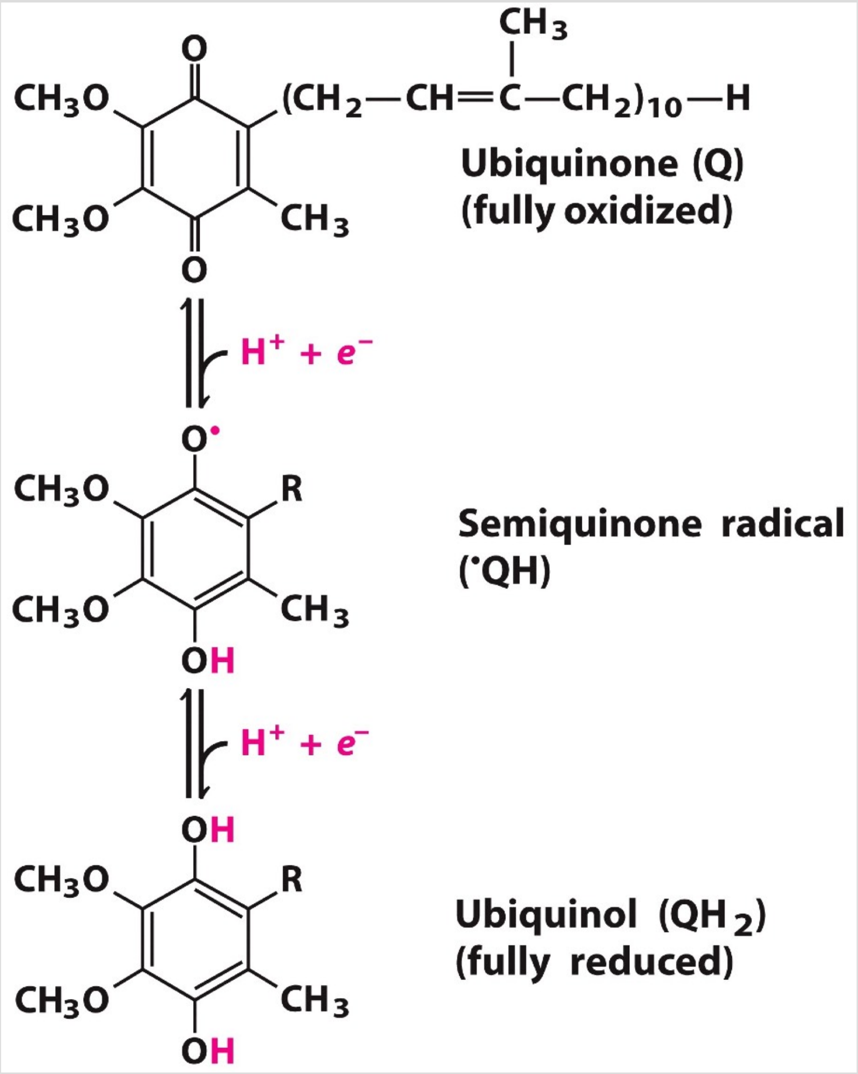


Iron protoporphyrin IX
(in *b*-type cytochromes)



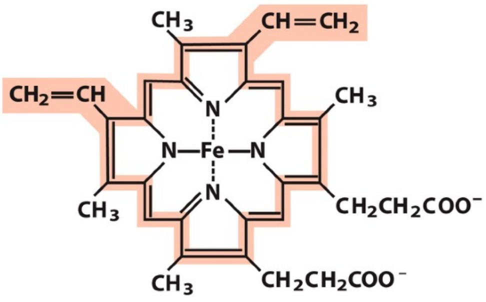
Ubiquinone (1 or 2 Electron Carrier)

- A dicarbonyl compound. Also called coenzyme Q, or simply Q.
- Can accept two electrons to form ubiquinol.
  - Ending with -one means “ketone”.
  - Ending with -ol means “alcohol”.
- Small and hydrophobic.
  - Freely diffusible across inner membrane of mitochondria.
  - Shuttle reducing equivalent.

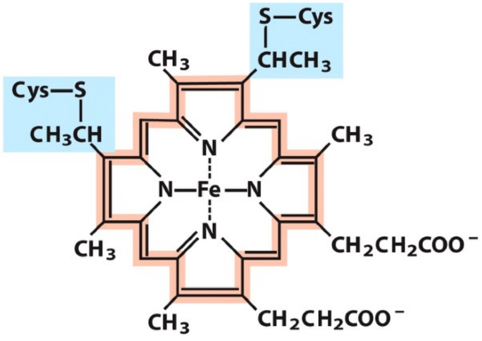


Cytochrome (1 Electron Carrier)

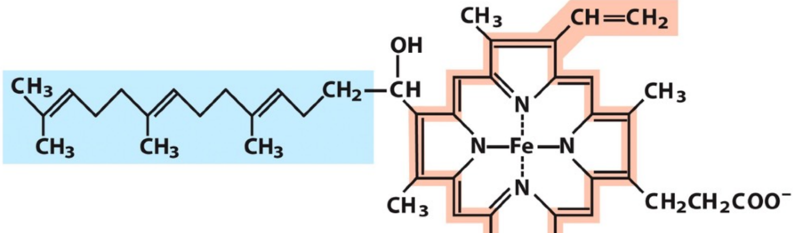
- Proteins with strong absorption of visible light.
  - Iron-containing heme prosthetic group.
- Three classes based on light-absorption spectrum (three peaks).
  - Cytochrome a. Longest wavelength band near 600 nm. Noncovalent.
  - Cytochrome b. Longest wavelength band near 560 nm. Noncovalent.
  - Cytochrome c. Longest wavelength band near 550 nm. Covalent.



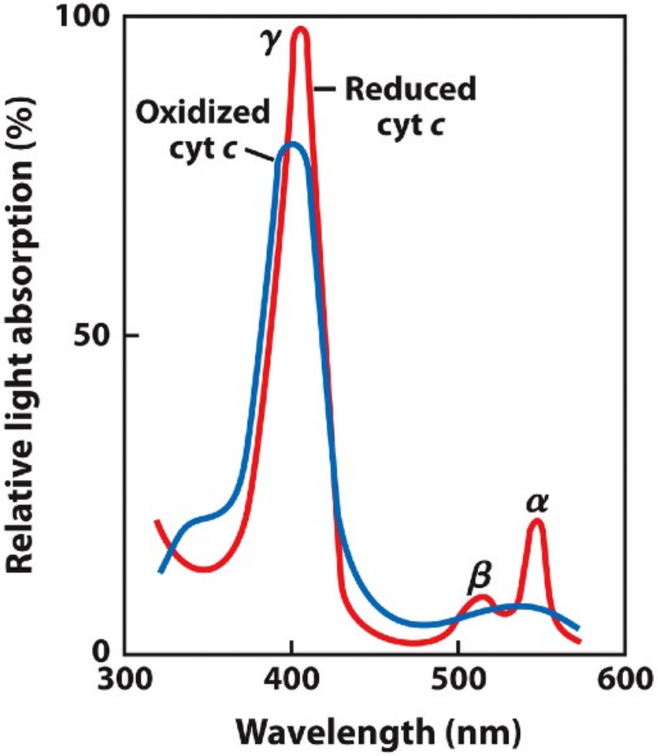
Iron protoporphyrin IX
(in *b*-type cytochromes)



Heme C
(in *c*-type cytochromes)

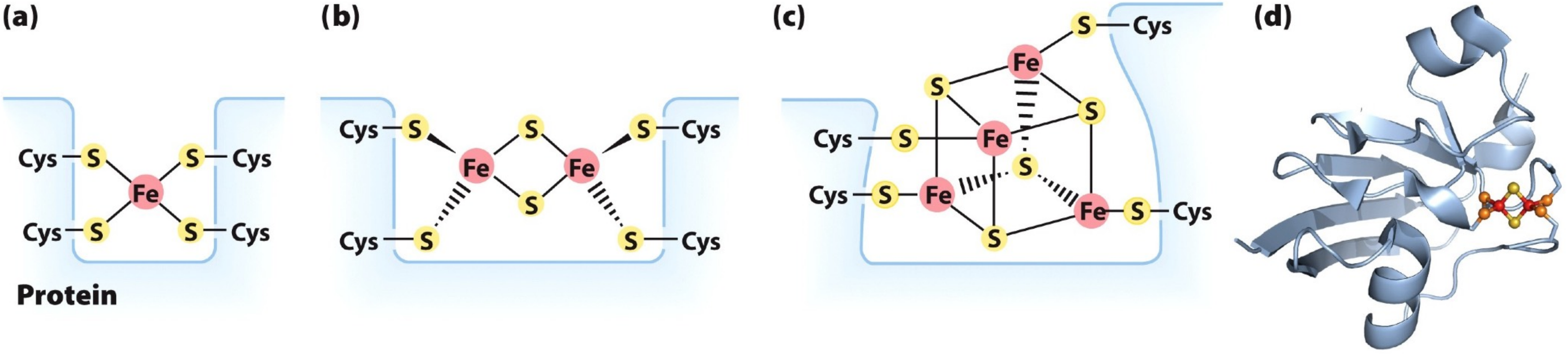


Heme A
(in *a*-type cytochromes)



Iron-Sulfur Cluster (1 Electron Carrier)

- Iron is associated with sulfur atoms.
    - Inorganic sulfur atoms.
    - Sulfur atoms of Cys residues in protein.
    - NOT associated with heme.
- Simple and complex iron-sulfur clusters.
    - One Fe surrounded by S atoms of four Cys residues.
    - 2Fe-2S and 4Fe-4S include both inorganic and Cys S atoms.
    - In these names, only inorganic S atoms are counted.



Electron Carriers in Multienzyme Complex

- Complex I.
  - Catalyzes electron transfer from NADH to ubiquinone.
- Complex II.
  - Catalyzes electron transfer from succinate to ubiquinone.
- Complex III.
  - Catalyzes electron transfer from ubiquinone to cytochrome c.
- Complex IV.
  - Catalyzes electron transfer from cytochrome c to O_2.

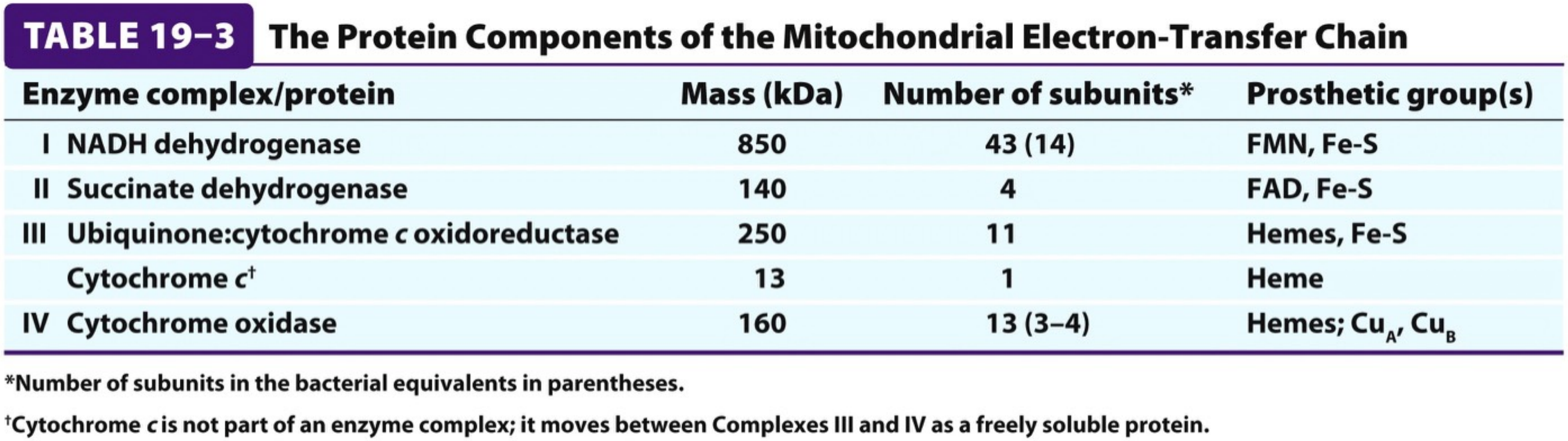


TABLE 19–3 The Protein Components of the Mitochondrial Electron-Transfer Chain

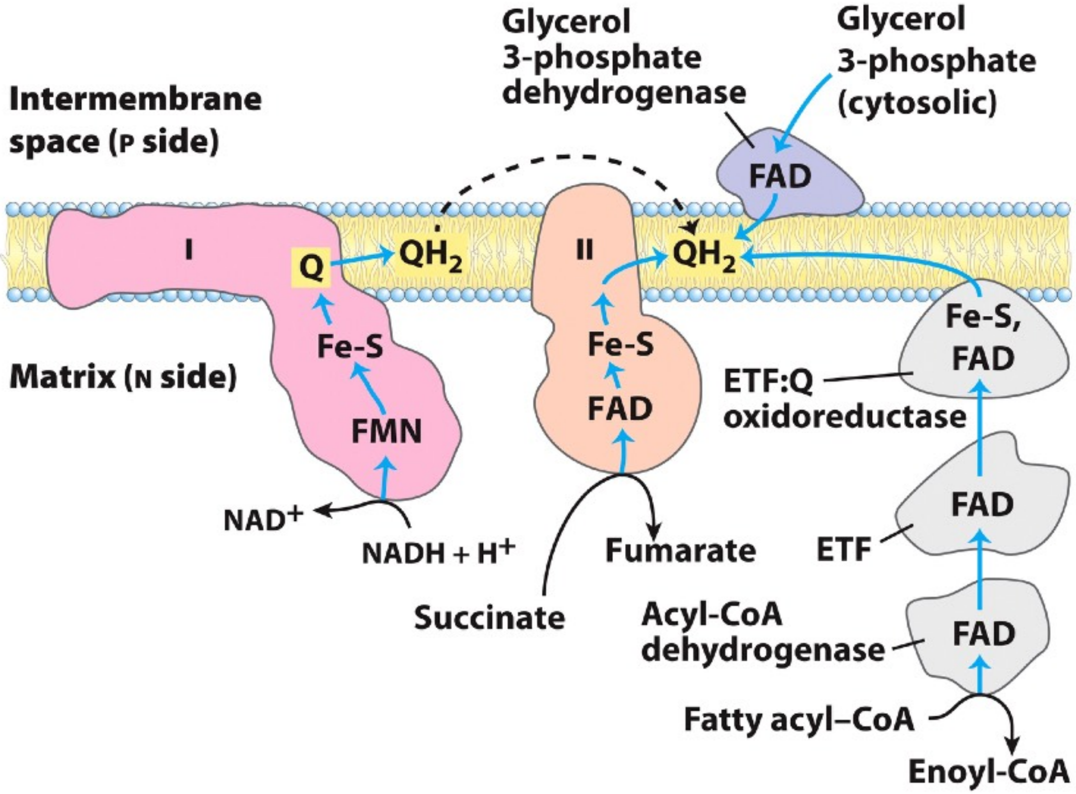
Enzyme complex/protein	Mass (kDa)	Number of subunits*	Prosthetic group(s)
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone:cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome *c*†	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu_A, Cu_B

*Number of subunits in the bacterial equivalents in parentheses.

†Cytochrome *c* is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

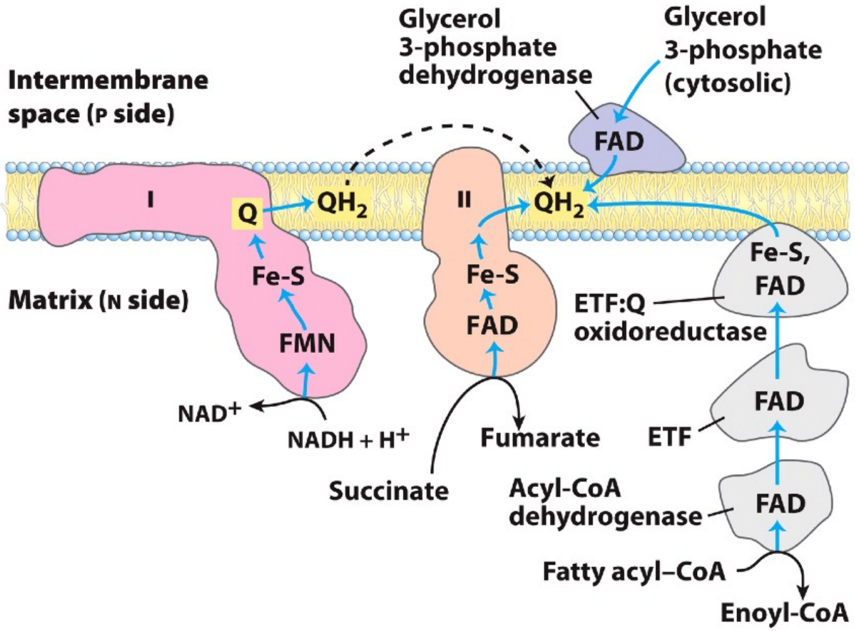
Electrons From Fuels To Coenzyme Q

- Ubiquinone is point of entry for electrons from reactions such as:
  - Succinate oxidation (step 6 in citric acid cycle, $FADH_2$ production).
  - Fatty acid oxidation (step 1, $FADH_2$ production).
  - Glycerol oxidation (step 2 in how glycerol enters glycolysis, NADH production).



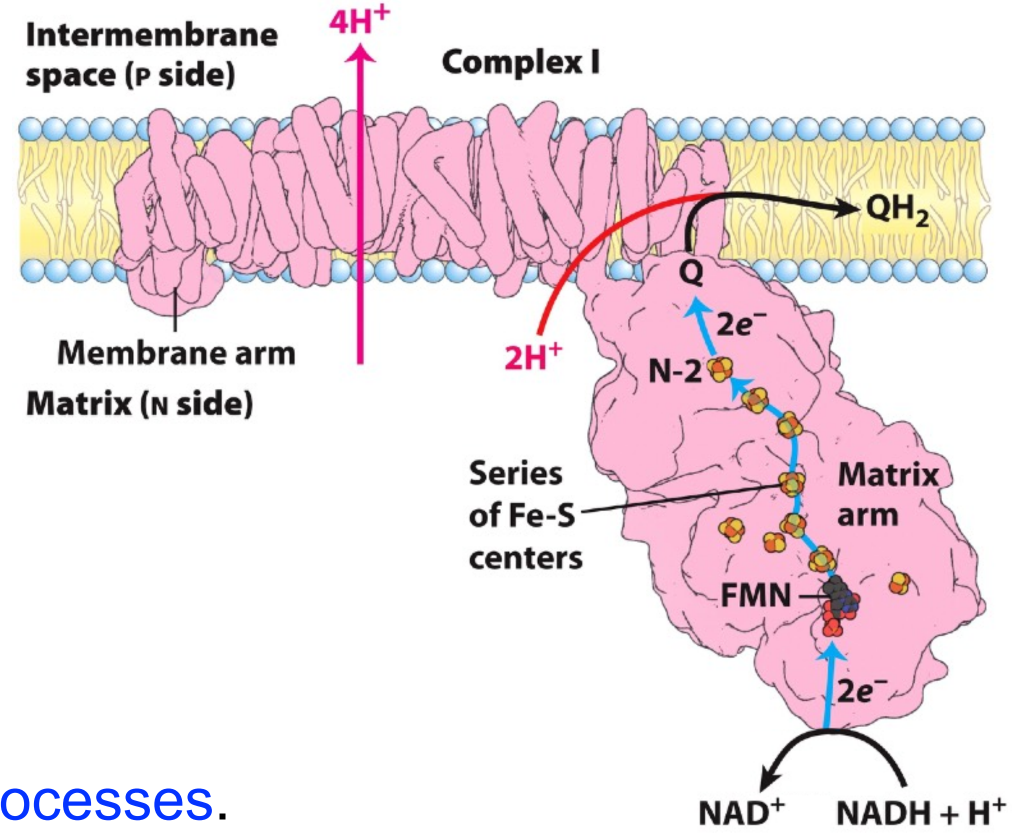
Electrons From Fuels To Coenzyme Q

- Major NADH binding site in matrix side.
  - Accept NADH generated from CAC and fatty acid oxidation in mitochondrion.
- Flavoproteins and Fe-S clusters pass electrons towards Q.
  - Accept NADH generated from glycerol 3-phosphate oxidation in cytosol.
  - Accept $FADH_2$ generated from succinate oxidation in mitochondrion.
  - Accept $FADH_2$ generated from fatty acid oxidation in mitochondrion.



Complex I: NADH To Ubiquinone

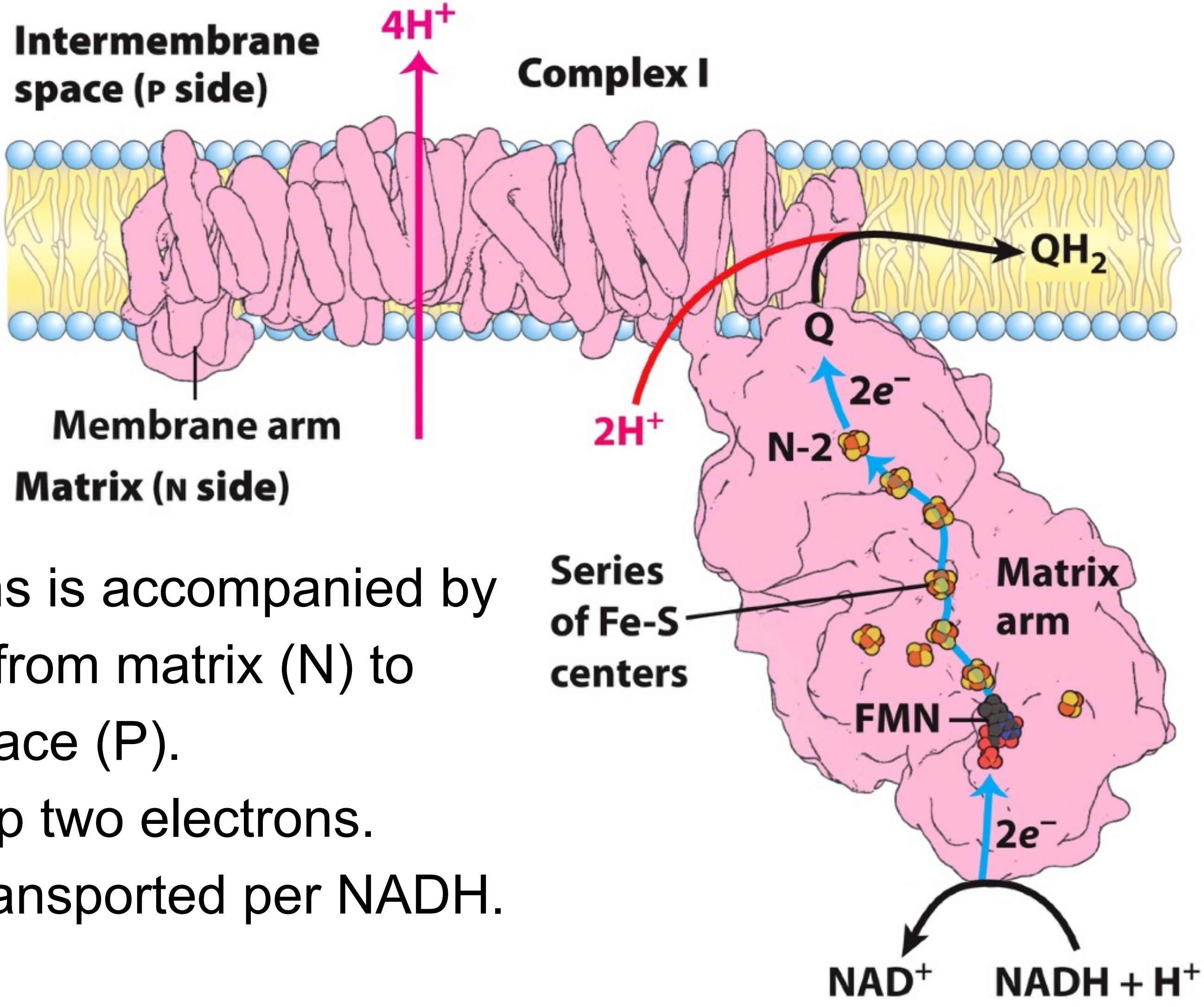
- Complex I is also called:
  - NADH dehydrogenase.
  - NADH:ubiquinone oxidoreductase.
- L-shaped.
  - One arm in membrane.
  - The other arm extends into matrix.



- Catalyzes following two processes.
  - $NADH + H^+ + Q \rightarrow NAD^+ + QH_2$.
  - Movement of 4 protons from matrix to inter membrane space.
  - Overall reaction: $NADH + 5H^+_N + Q \rightarrow NAD^+ + QH_2 + 4H^+_P$.

Complex I is A Proton Pump

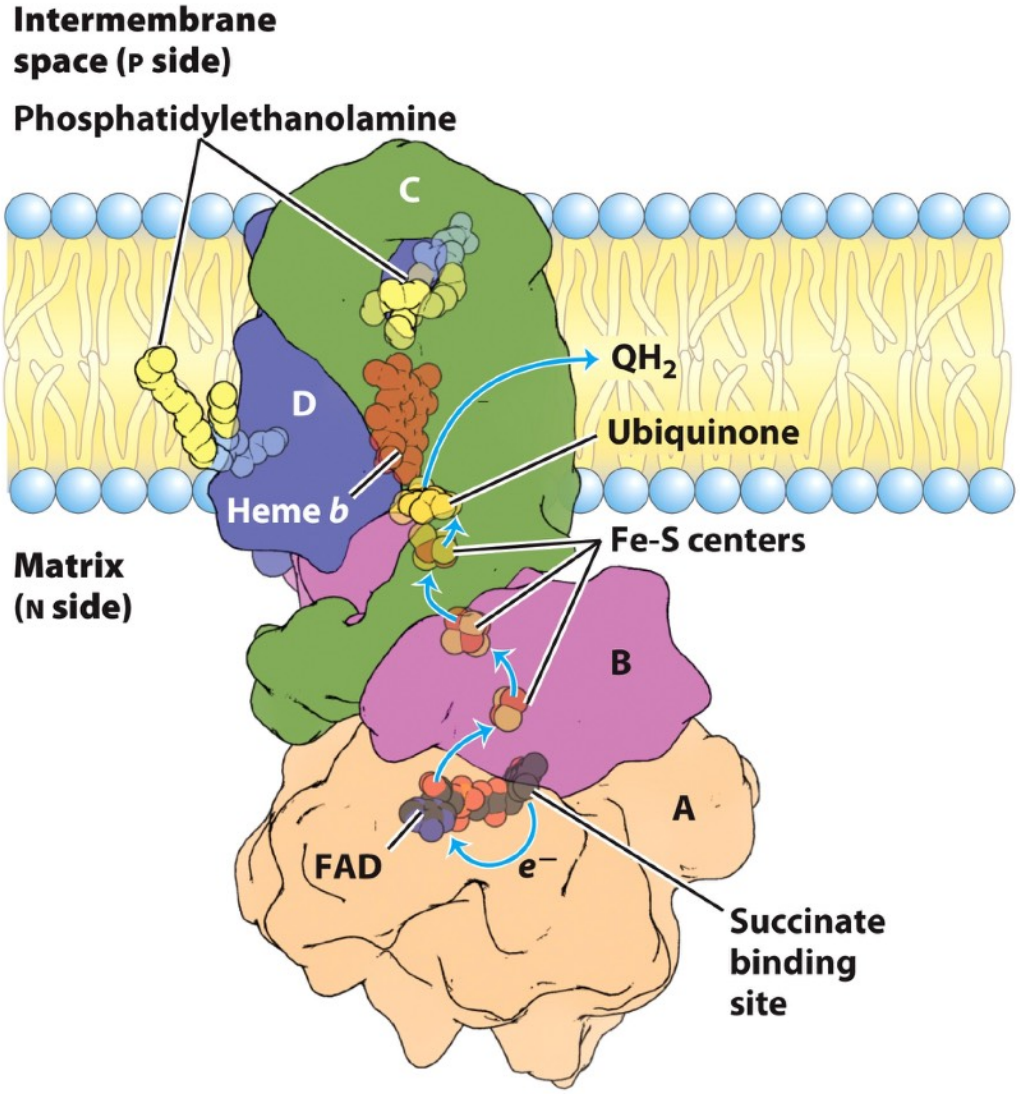
$$\mathrm{NADH} + \mathrm{Q} + 5\mathrm{H}^+_N \longrightarrow \mathrm{NAD}^+ + \mathrm{QH}_2 + 4\mathrm{H}^+_P$$



- Transfer of electrons is accompanied by transfer of protons from matrix (N) to inter-membrane space (P).
- Reduced Q picks up two electrons.
- Four protons are transported per NADH.

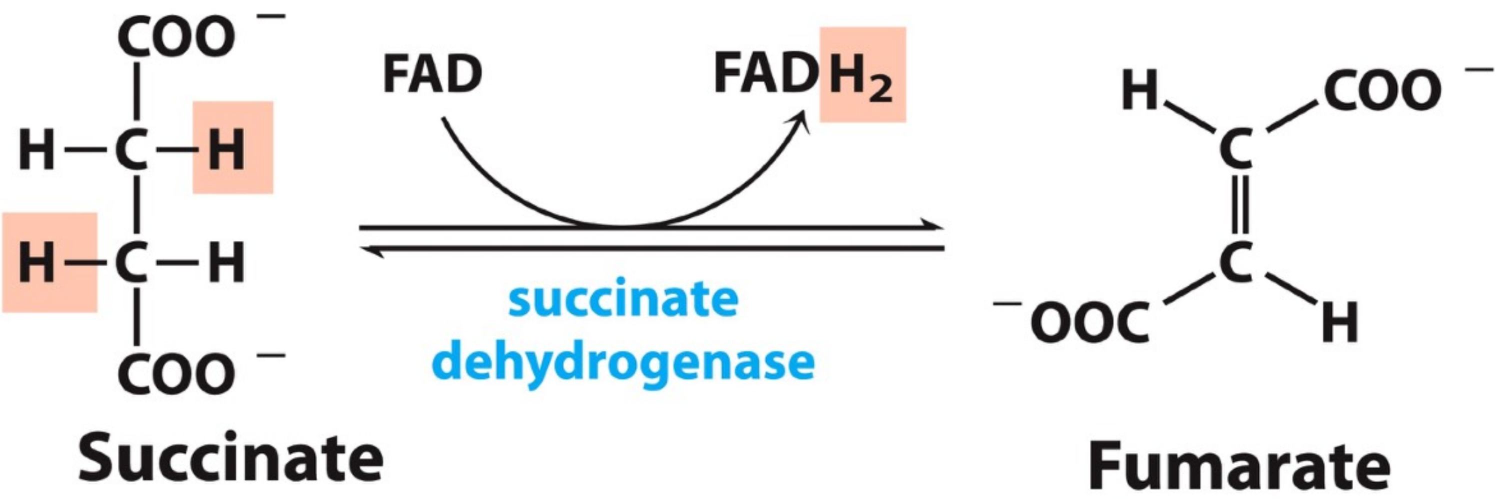
Complex II: Succinate to Ubiquinone

- Complex II.
  - Succinate dehydrogenase.
  - Step 6 enzyme in CAC.
  - Membrane-bound.
- Four subunits.
  - A and B extend into matrix.
  - C and D are integral membrane proteins.
- Five prosthetic groups.
  - FAD in A subunit.
  - 3 Fe-S clusters in B subunit.
  - Q bound to B subunit.



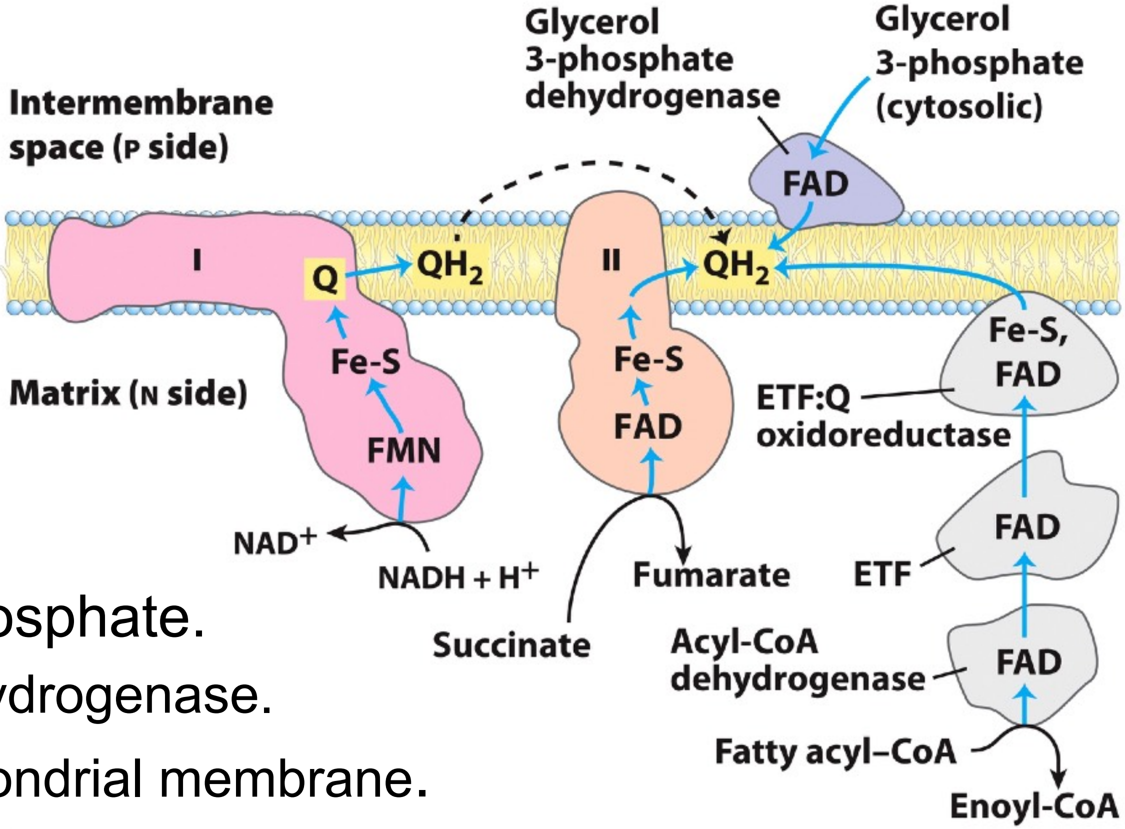
Complex II Does Not Transport Protons

- FAD accepts two electrons from succinate.
- Electrons are passed, via Fe-S clusters, to ubiquinone, which becomes reduced QH_2.
- Does not involve movement of protons like complex I.



Other Substrates Pass Electrons To Q

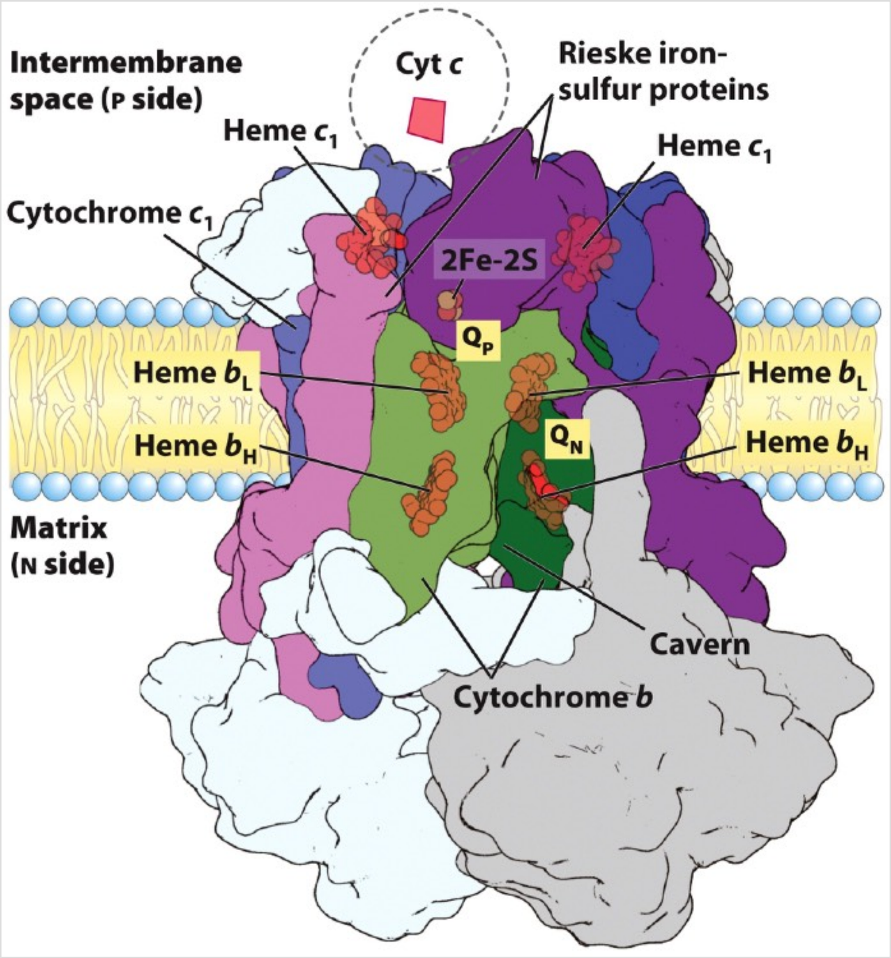
- 1st Step in β oxidation of fatty acyl-CoA.
  - Acyl-CoA dehydrogenase.
  - Electrons transfer from substrate to FAD.
  - Electrons transfer from $FADH_2$ to ubiquinone.



- Oxidation of glycerol 3-phosphate.
  - Glycerol 3-phosphate dehydrogenase.
  - Outer face of inner mitochondrial membrane.
  - Electrons transfer from substrate to NADH, and to respiratory chain by reducing Q.

Complex III: Ubiquinone to Cytochrome *c*

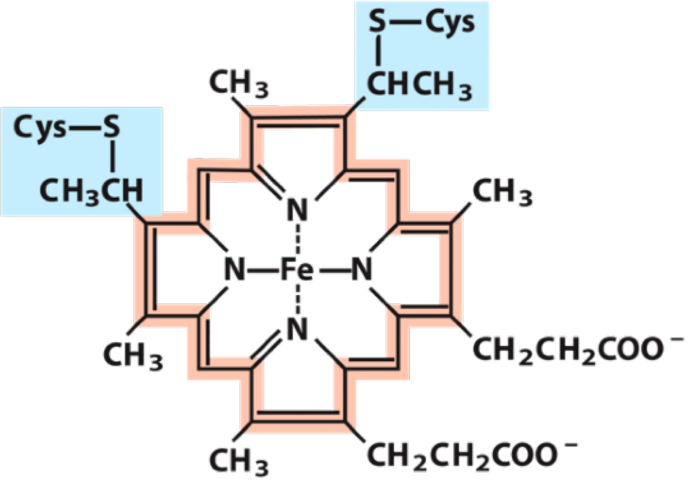
- Complex III.
  - Ubiquinone:cytochrome *c* oxidoreductase.
  - cytochrome bc_1 complex.
- Transfer of electrons from ubiquinone to cytochrome *c*.
  - QH_2 is oxidized to Q.
  - Two cytochrome *c* are reduced.
- Transport of protons from matrix to inter-membrane space.
  - Uptake of 2 protons on N side.
  - Release of 4 protons on P side.



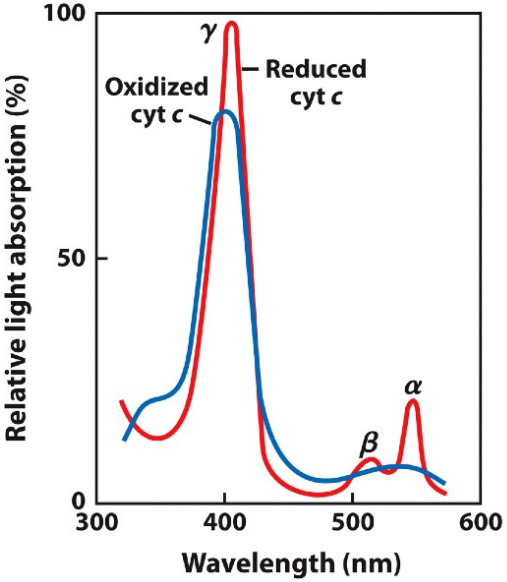
$$QH_2 + 2 \text{ cyt } c \text{ (oxidized)} + 2H^+_N \rightarrow Q + 2 \text{ cyt } c \text{ (reduced)} + 4H^+_P$$

Cytochrome *c*

- 2nd mobile electron carrier (1st one is ubiquinone).
- A soluble heme-containing protein in the inter-membrane space.
    - Iron can be either ferrous (Fe^{3+}, oxidized) or ferric (Fe^{2+}, reduced).
    - Carry a single electron from complex III to complex IV.
- Have intense red color due to strong absorption at 400 nm (blue).

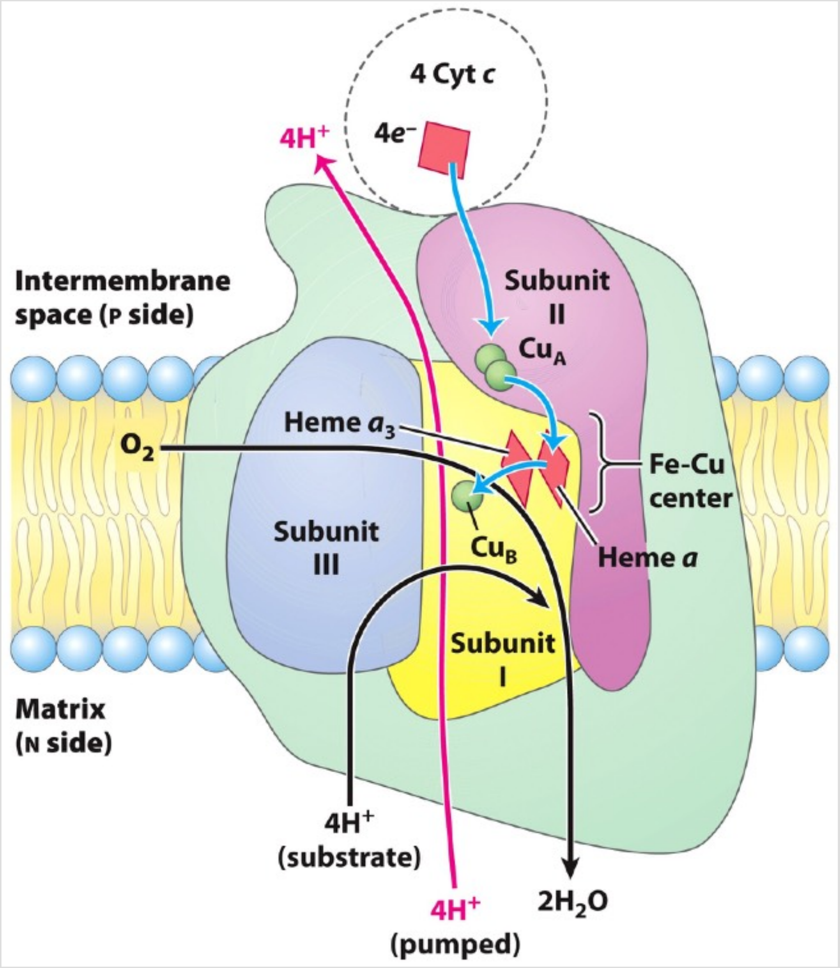


Heme C
(in *c*-type cytochromes)



Complex IV: Cytochrome *c* to O_2

- Complex IV.
  - Cytochrome oxidase.
- Transfer of electrons from cytochrome *c* to O_2.
  - 4 cytochrome *c* are oxidized.
  - 1 O_2 is reduced to 2 H_2O.
- Transport of protons from matrix to inter-membrane space for each O_2 reduced.
  - Uptake of 8 protons on N side.
  - Release of 4 protons on P side.



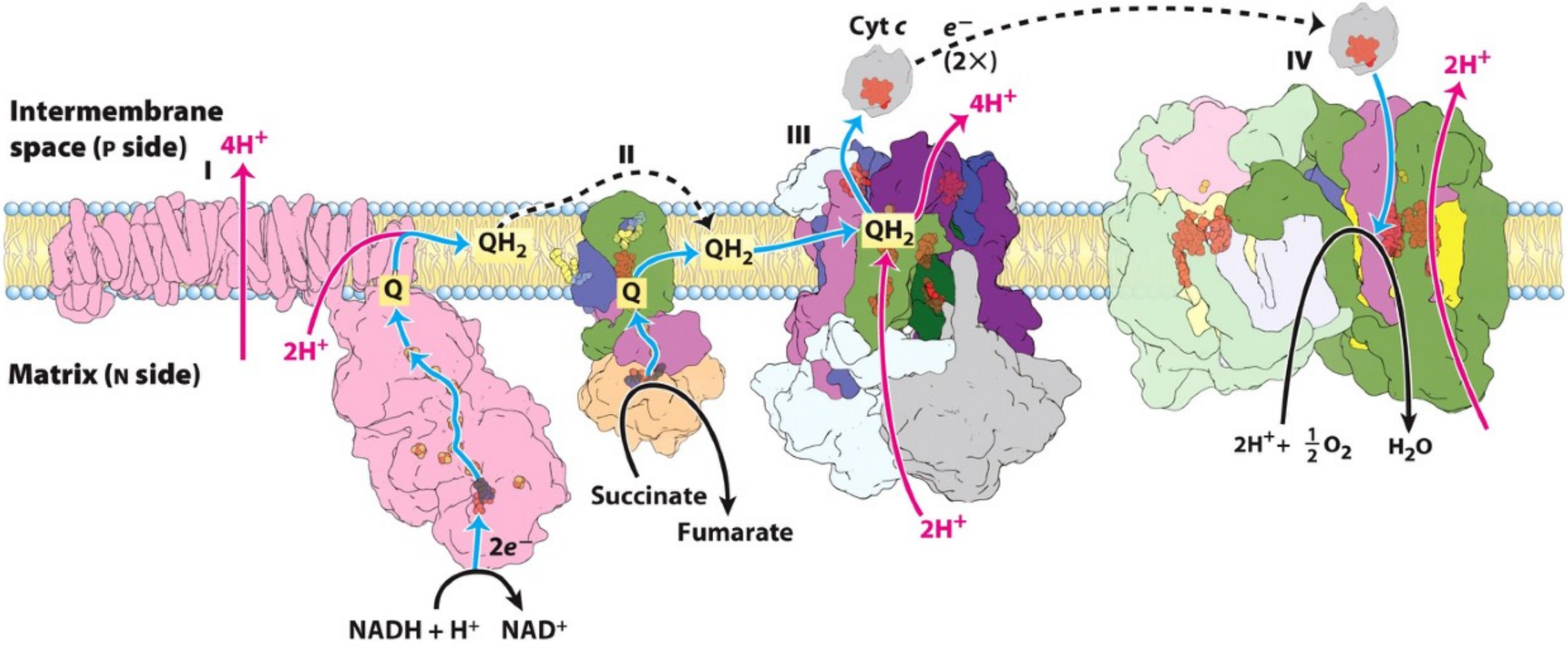
$$4\text{ cyt } c\text{ (reduced)} + O_2 + 8H^+_N \rightarrow 4\text{ cyt } c\text{ (oxidized)} + 2H_2O + 4H^+_P$$

Summary of Complex I - IV Reactions

- Complex I.
  - NADH dehydrogenase or NADH:ubiquinone oxidoreductase.
  - $NADH + Q + 5H^+_N \rightarrow NAD^+ + QH_2 + 4H^+_P$.
- Complex II.
  - Succinate dehydrogenase.
  - Succinate + Q -> fumarate + QH_2.
- Complex III.
  - Ubiquinone:cytochrome *c* oxidoreductase.
  - QH_2 + 2 cyt *c* (oxidized) + $2H^+_N$ -> Q + 2 cyt *c* (reduced) + $4H^+_P$.
- Complex IV.
  - Cytochrome oxidase.
  - 4 cyt *c* (reduced) + O_2 + $8H^+_N$ -> 4 cyt *c* (oxidized) + $2H_2O$ + $4H^+_P$.
- Overall reaction from Complex I, III and IV.
  - $NADH + \frac{1}{2}O_2 + 11H^+_N \rightarrow NAD^+ + H_2O + 10H^+_P$.
- Overall reaction from Complex II, III and IV.
  - $FADH_2 + \frac{1}{2}O_2 + 6H^+_N \rightarrow FAD + H_2O + 6H^+_P$.

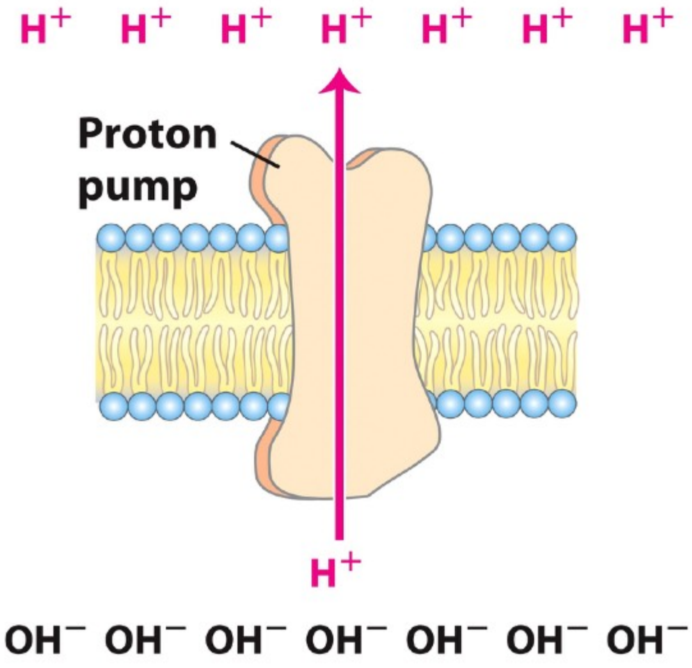
Summary of Electron Flow

- Complex I.
  - 2 electrons from NADH to Q. 4 protons released to IMS.
- Complex II.
  - 2 electrons from $FADH_2$ to Q. No protons released to IMS.
- Complex III.
  - 2 electrons from QH_2 to 2 cyt *c*. 4 protons released to IMS.
- Complex IV.
  - 2 electrons from 2 cyt *c* to ½ O_2. 2 protons released to IMS.



Energy Conserved in Proton Gradient

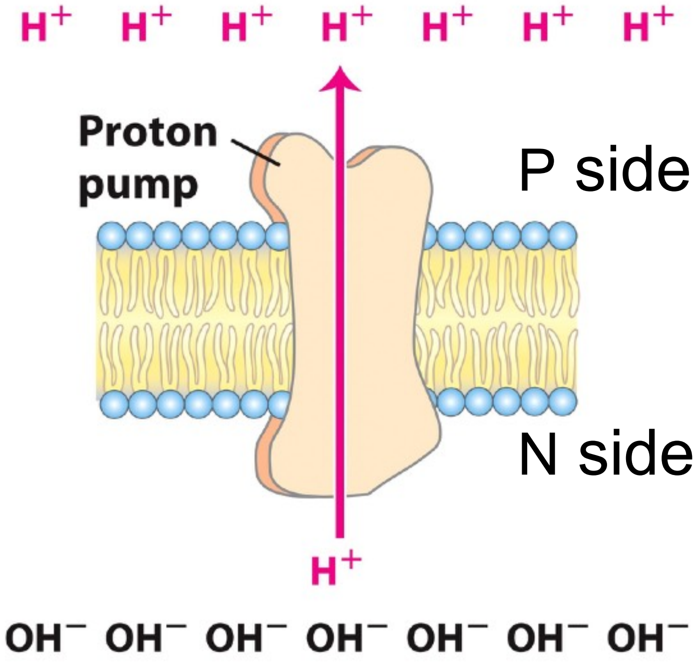
- Overall reaction (Complex I, III and IV).
  - NADH + ½O_2 + 11H^+_N -> NAD^+ + H_2O + 10H^+_P.
  - Net reaction of [NADH + ½O_2 + H^+_N -> NAD^+ + H_2O] is highly exergonic.
  - Energy is used to pump protons out of matrix.
    - 4 protons are pumped out by complex I.
    - 4 protons are pumped out by complex III.
    - 2 protons are pumped out by complex IV.
- Overall reaction (Complex II, III and IV).
  - $FADH_2$ + ½O_2 + 6H^+_N -> FAD + H_2O + 6H^+_P.



- One NADH yields 2.5 ATP
- One $FADH_2$ yields 1.5 ATP

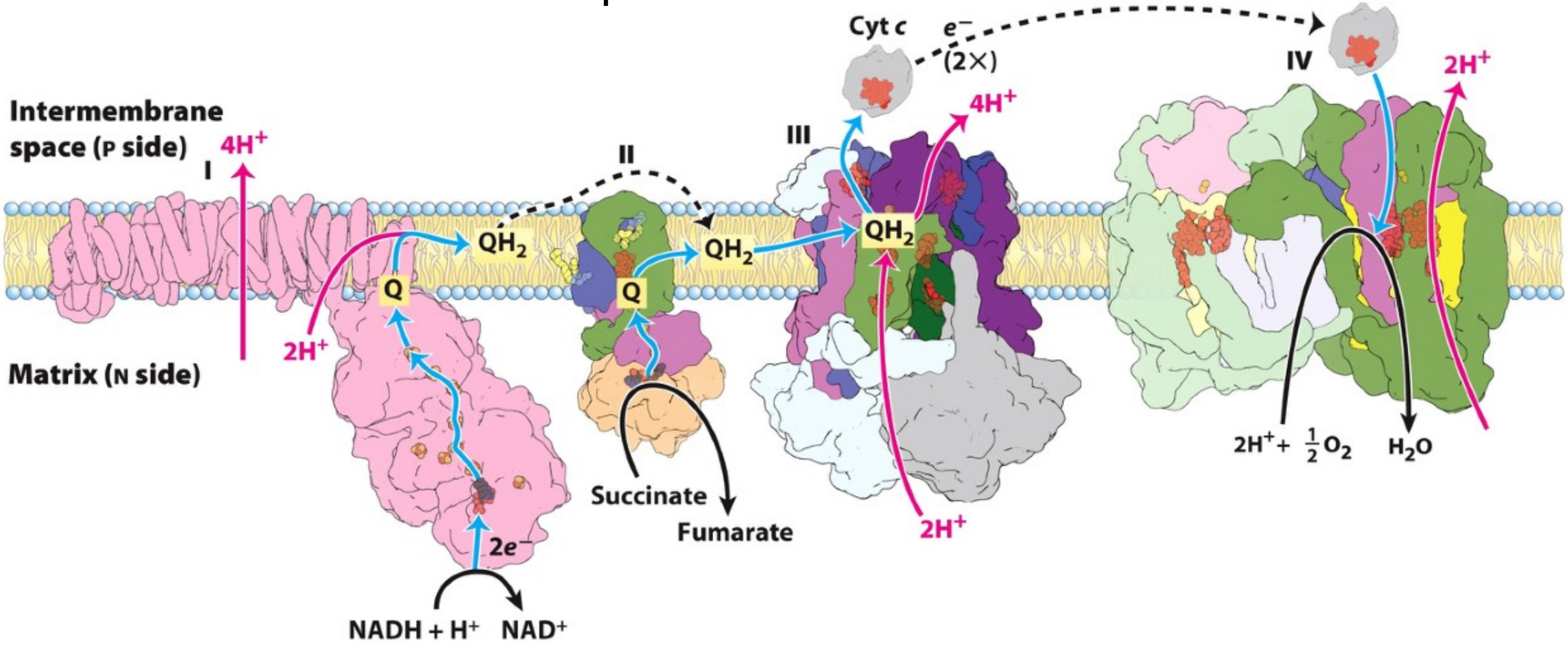
Proton-Motive Force

- Energy stored in proton gradient has two components.
  - Chemical potential energy.
    - Due to difference in concentration of a chemical species (H^+) across membrane.
  - Electrical potential energy.
    - Resulted from movement of protons across membrane without counterions.



How to Create A Proton Gradient

- Proteins in electron-transport chain created the electrochemical proton gradient by one of three means:
  - Actively transport protons across membrane.
    - Complex I and complex IV.
  - Chemically remove protons from the matrix.
    - Reduction of Q (complex I and III) and reduction of oxygen (complex IV).
  - Release protons into the inter-membrane space.
    - Oxidation of QH_2 in complex III.



Summary 19.1 Electron-Transfer Reactions

- In chemiosmotic theory, energy of electron transfer is conserved by pumping of protons across membrane, producing an electrochemical gradient.
- Electrons are passed from NADH to ubiquinone (1st mobile carrier) in complex I. Q passes electrons to cytochrome *c* (2nd mobile carrier) in complex III. Cytochrome *c* then passes electrons to O_2, reducing it to H_2O, in complex IV.
- Some electrons enter respiratory chain through alternative paths. Succinate is oxidized by complex II. Electrons from fatty acid oxidation also pass to Q.

Week 13 Oxidative Phosphorylation

19.1 Electron-Transfer Reactions in Mitochondria

19.2 ATP Synthesis

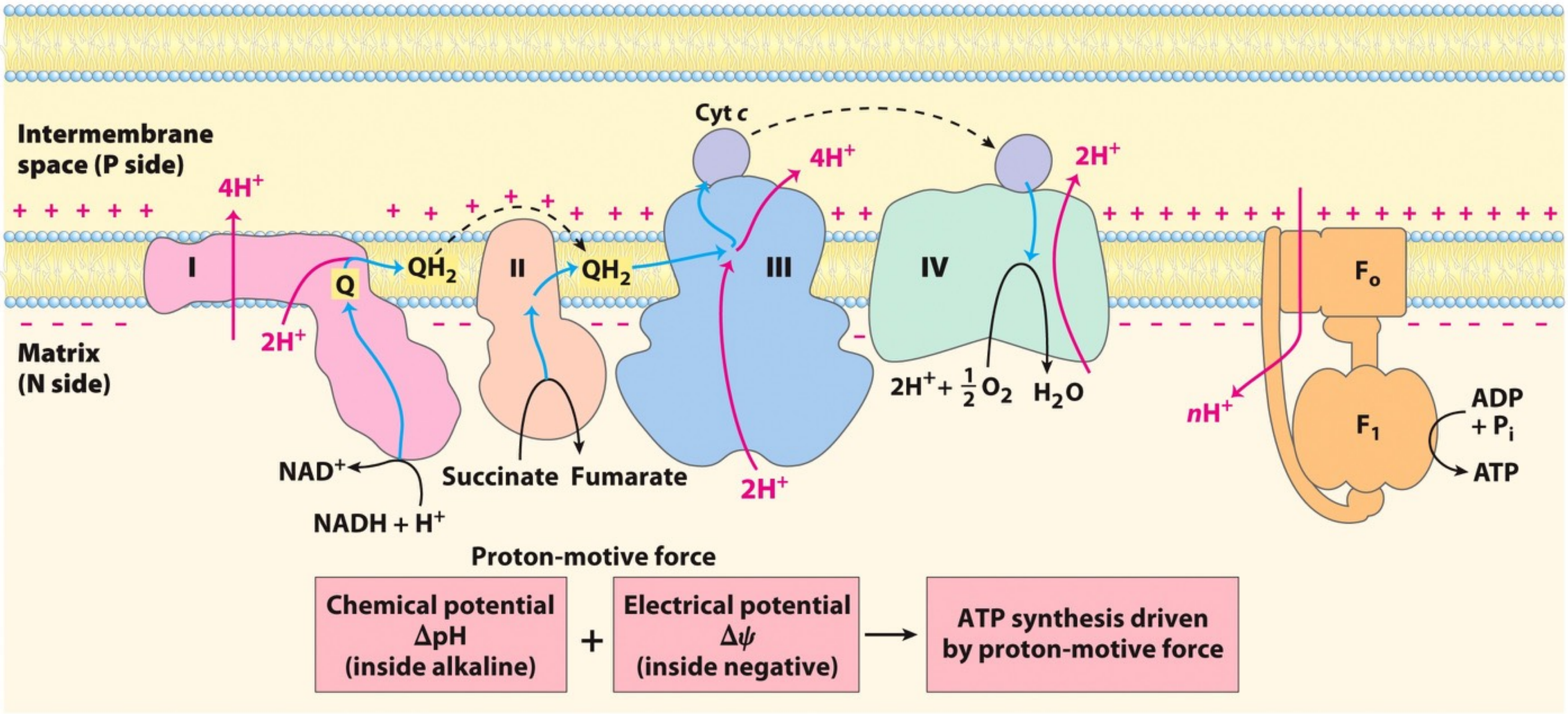
19.3 Regulation of Oxidative Phosphorylation

19.4 Mitochondria in Other Processes

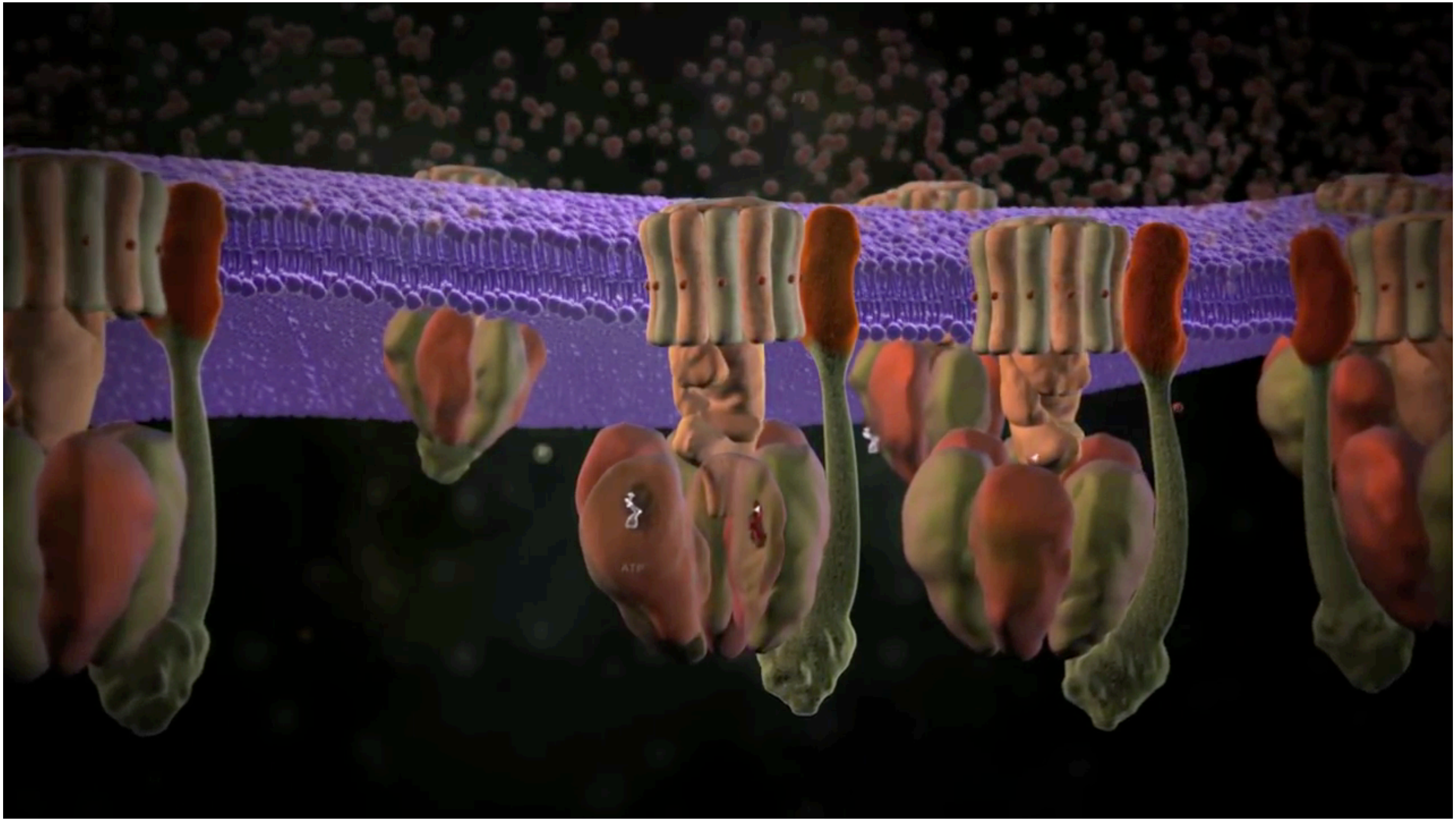
19.5 Mitochondrial Genes

Chemiosmotic Model for ATP Synthesis

- Electron transport sets up a proton-motive force.
- Energy of proton-motive force drives synthesis of ATP.

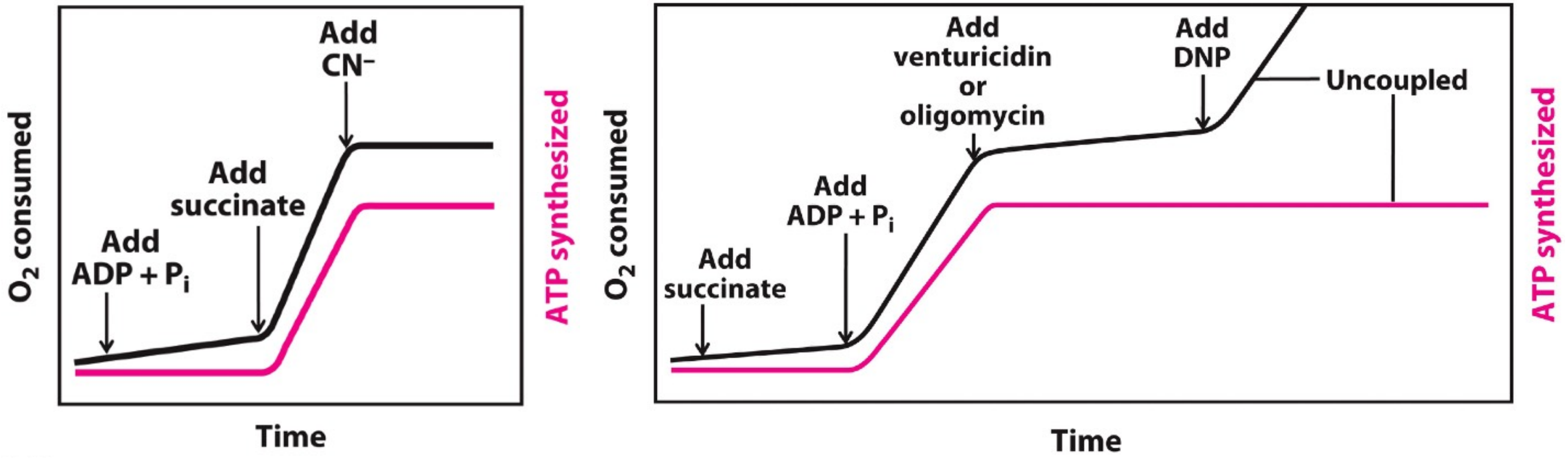


ATP Synthase: Power Plant of Cell



Electron Transport - ATP Synthesis

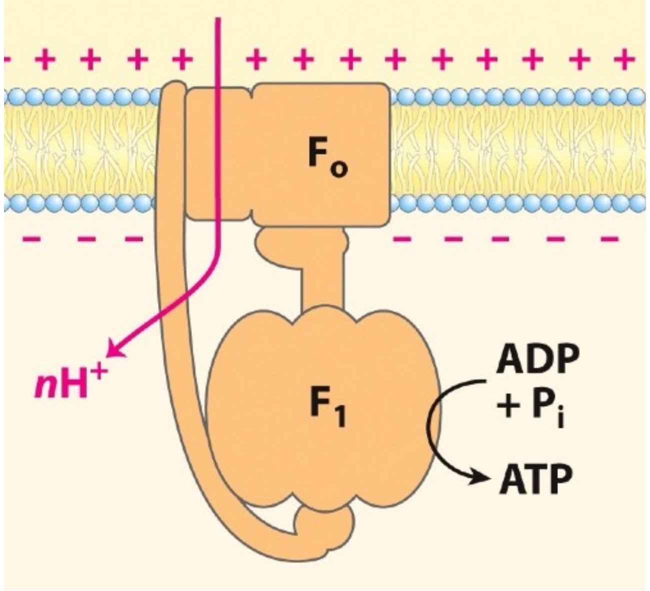
- ATP synthesis requires electron transport.
- Surprisingly, electron transport also requires ATP synthesis.



- ADP + P_i: substrate for ATP synthesis.
- Succinate: source of electrons.
- Cyanide (CN^-): block electron transfer between complex IV and O_2.
- Venturicidin/oligomycin: inhibit ATP synthase.
- DNP: a chemical uncoupler (equilibrate [H^+] across membrane).

Mitochondrial ATP Synthase Complex

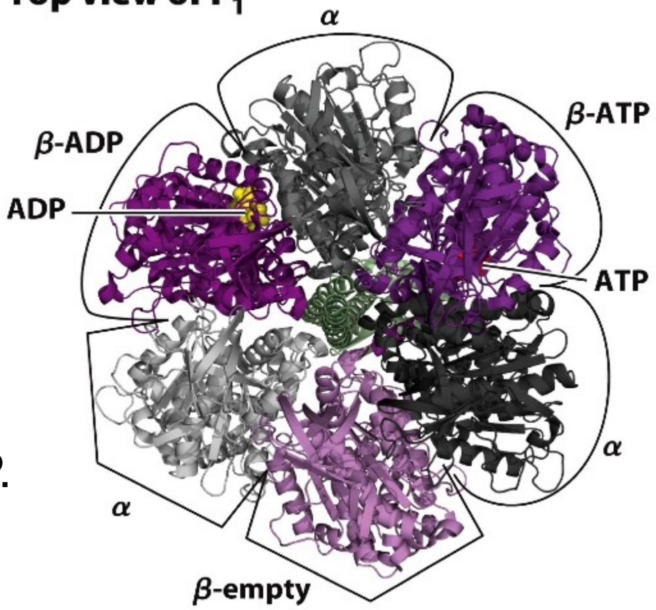
- Contain two functional units.
  - F_1 is a soluble protein complex in matrix.
    - First factor recognized as essential for oxidative phosphorylation.
    - Individually catalyze hydrolysis of ATP (also called F_1 ATPase).
  - F_o is an integral membrane protein complex.
    - Subscript letter o means it is oligomycin-sensitive.
    - Transports protons from IMS to matrix, dissipating the proton gradient.
    - Energy transferred to F_1 to catalyze phosphorylation of ADP.



F_1 catalyzes ADP + $P_i \rightleftharpoons$ ATP

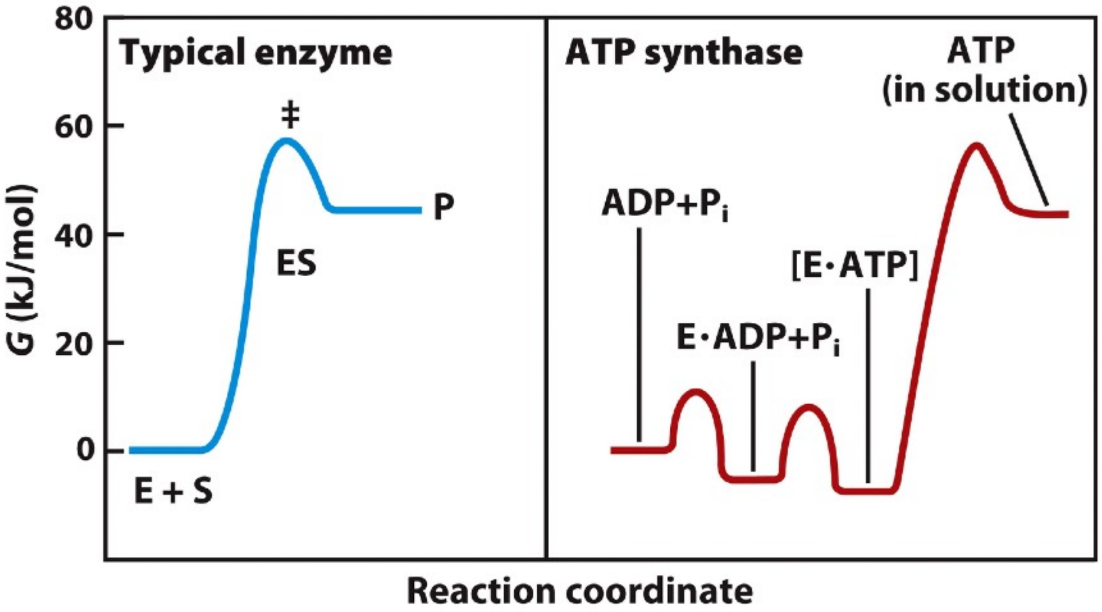
- When F_1 is gently removed from inner mitochondrial membrane.
  - F_1 catalyzes ATP hydrolysis.
  - The rest (respiratory chain + F_o) catalyzes electron transfer from NADH and O_2 but cannot produce a proton gradient.
    - F_o has a proton pore.
    - Protons leak as fast as they are pumped by electron transfer.
    - Without proton gradient, F_1-depleted vesicle cannot synthesize ATP.
- F_1 has 9 subunits of 5 types ($\alpha_3\beta_3\gamma\delta\varepsilon$)
  - F_1 catalyzes ATP hydrolysis.
  - Hexamer arranged in three $\alpha\beta$ dimers.
  - Dimers exist in three different conformations:
    - Open: empty
    - Loose: binds ADP and P_i
    - Tight: catalyzes ATP formation and binds ATP.

Top view of F_1



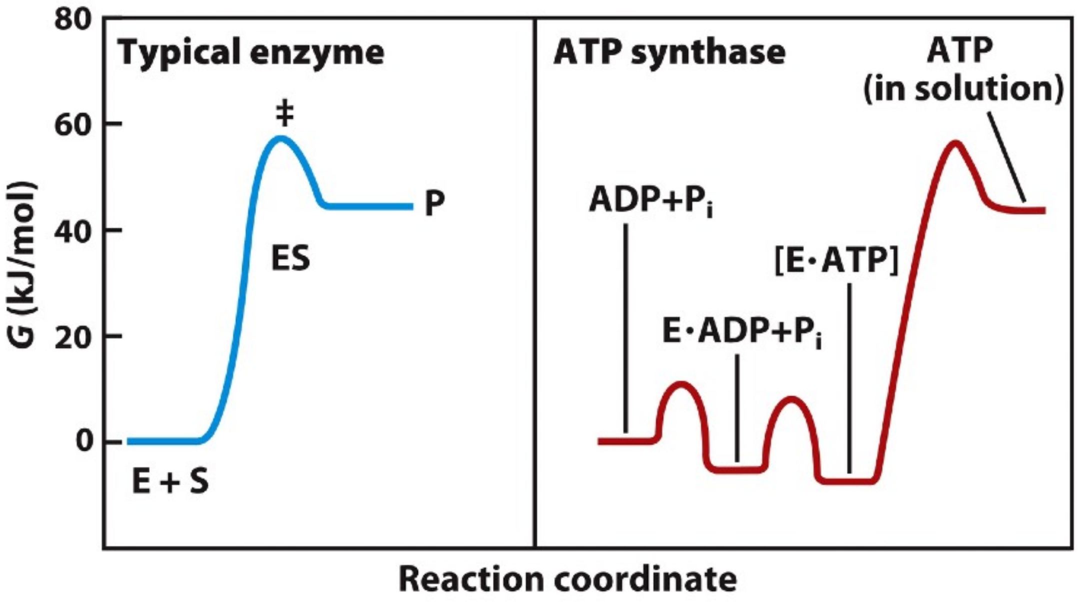
ATP is Stabilized on F_1 Surface

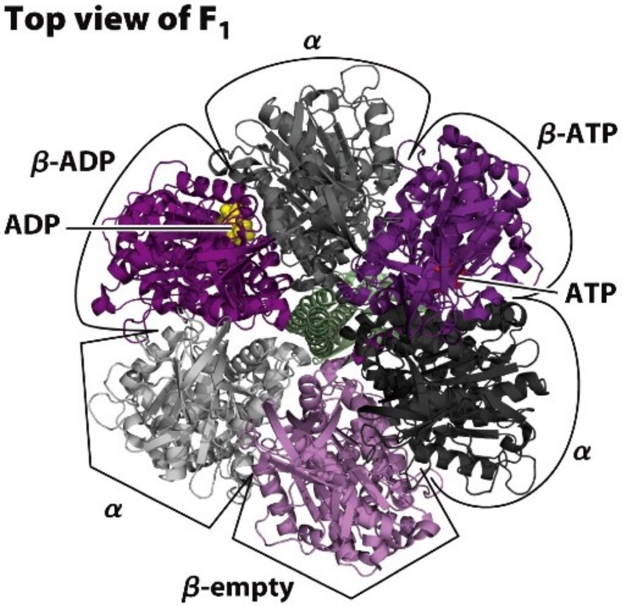
- On F_1 enzyme surface, reaction ADP + $P_i \rightleftharpoons$ ATP is reversible.
  - Free-energy change for ATP synthesis is close to zero.
  - Very different from hydrolysis of ATP free in solution.
  - ATP synthase stabilizes ATP relative to ADP + P_i by binding ATP more tightly, releasing enough energy to counter-balance cost of making ATP.
    - High affinity for ATP ($K_d \leq 10^{-12}$ M).
    - Much lower affinity for ADP ($K_d \approx 10^{-5}$ M).



Proton Gradient Drives Release of ATP

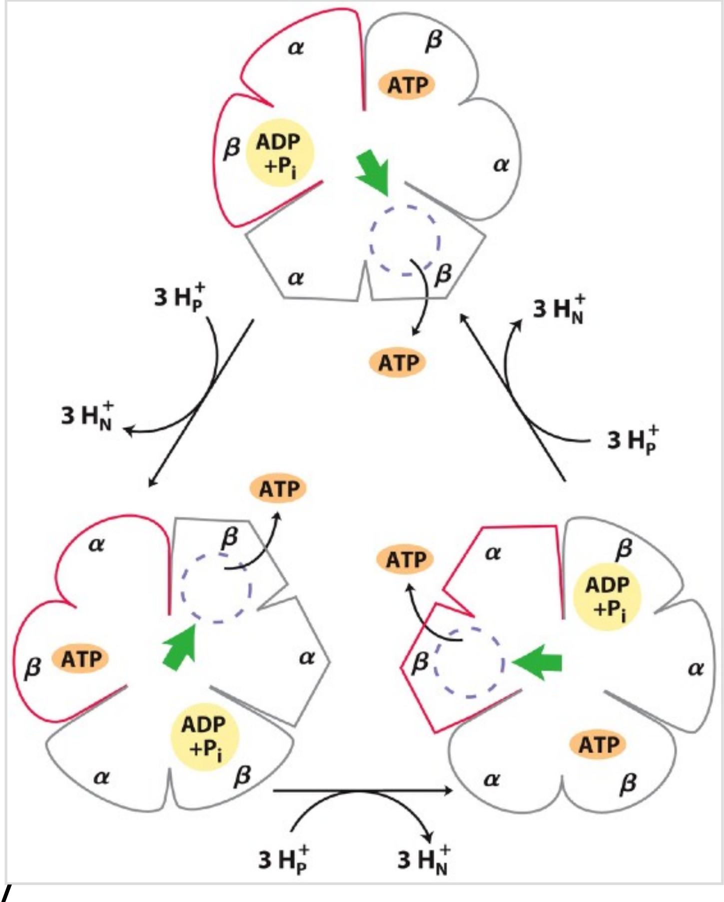
- In the absence of a proton gradient, ATP does not leave enzyme.
- Proton gradient causes enzyme to release newly-synthesized ATP.
  - Enzyme must cycle between at least two forms.
    - Binds ATP very tightly.
    - Releases ATP.





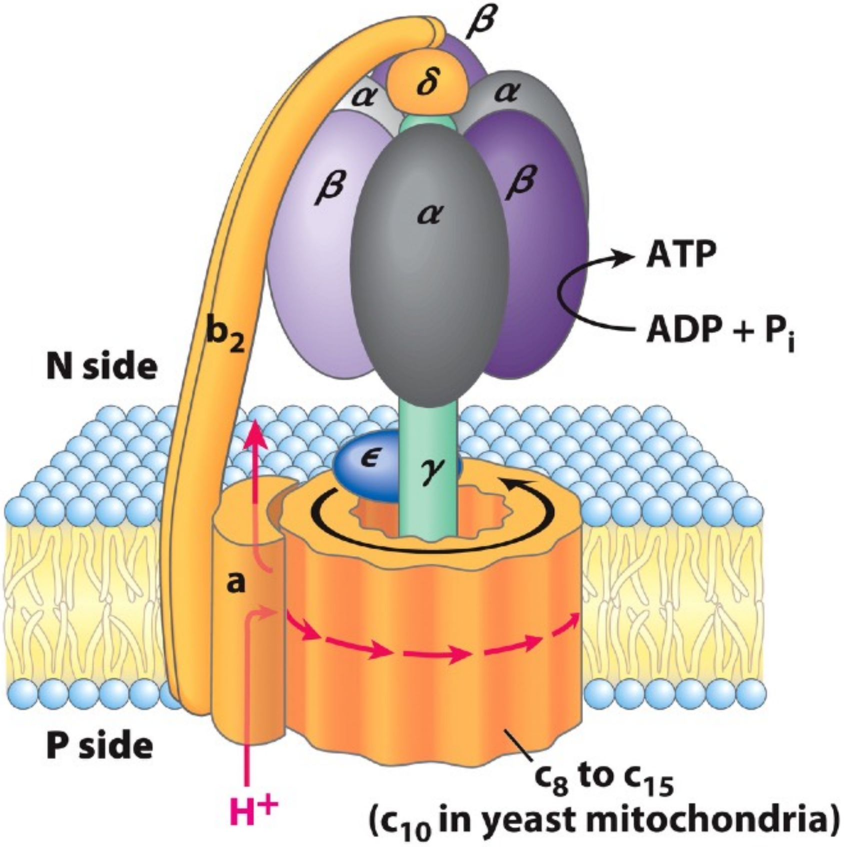
Binding-Change Model

- F_1 complex has 3 ADP/ATP-binding sites, one for each αβ dimer.
- Each site has 3 conformations.
  - β-ATP conformation binds ATP tightly.
  - β-empty conformation binds very loosely.
  - β-ADP conformation binds ADP loosely.
- Proton-motive force causes rotation of central γ subunit.
  - γ subunit contacts αβ dimer and produces cooperative conformational change.
    - β-ATP converted to β-empty. Release ATP.
    - β-empty converted to β-ADP. Bind ADP loosely.
      β-ADP converted to β-ATP. Promote ATP formation.



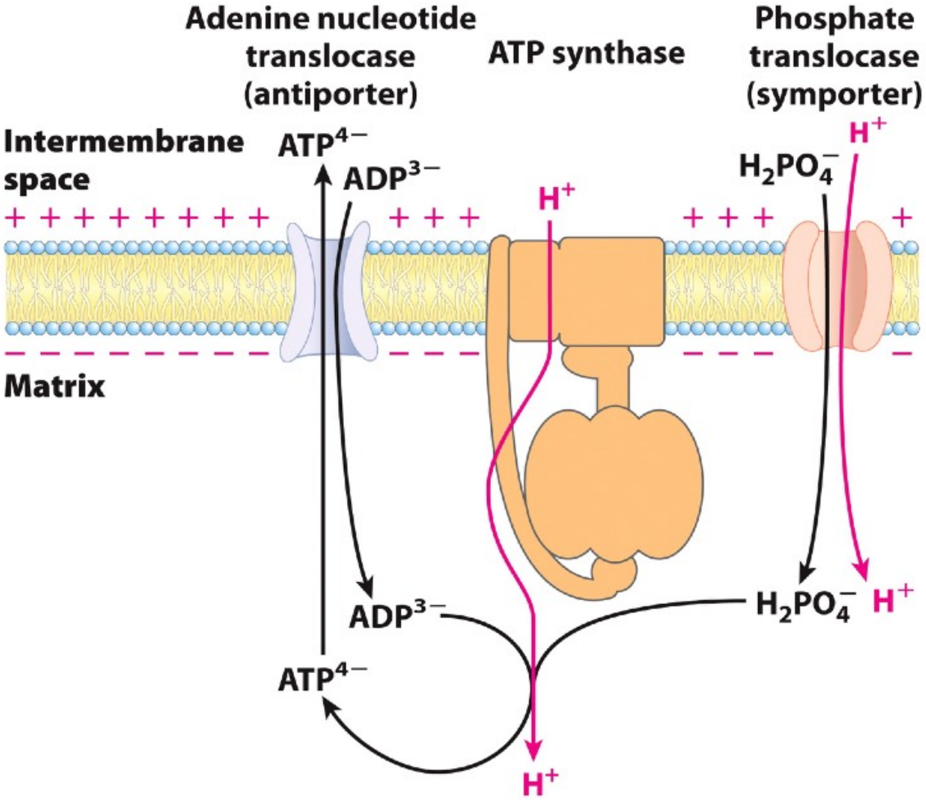
Proton Translocation - ATP Synthesis

- F_o is composed of 3 types of subunits, in the proportion of ab_2c_n
- The c subunits in this ring rotate together as a unit
  - F_1 γ subunit stands firmly on c ring
- Proton translocation causes rotation of c ring and γ subunit
  - γ subunit rotates and contacts $\alpha\beta$ dimer, causing a conformational change within all three $\alpha\beta$ dimers
  - Conformational change in one of the three pairs promotes condensation of ADP and P_i into ATP



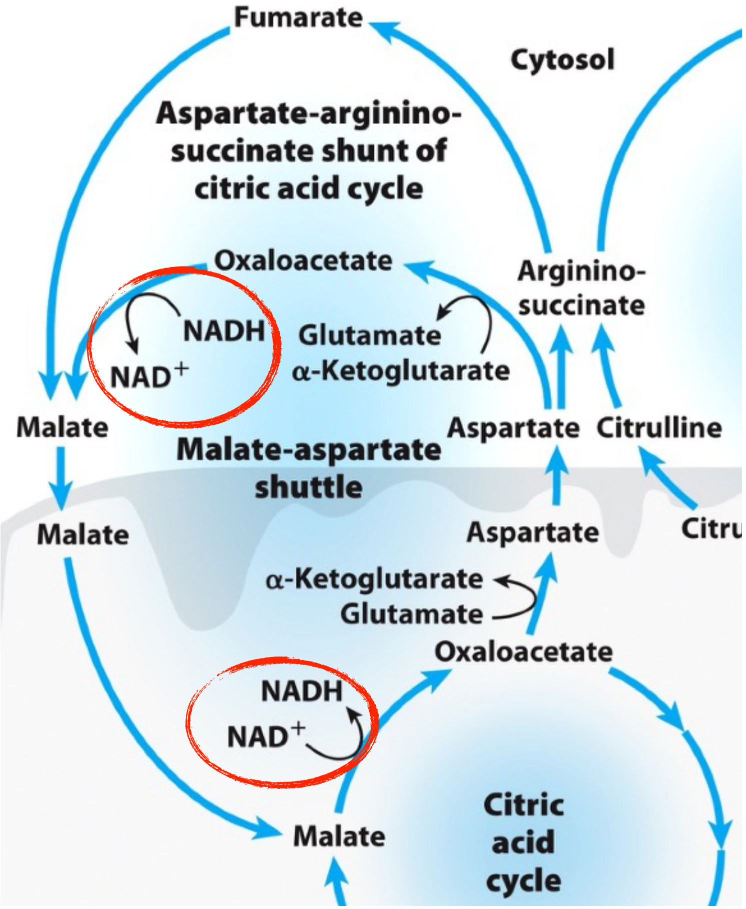
Transport of ADP and P_i into Matrix

- Adenine nucleotide translocase.
  - Transport ADP^{3-} from inter-membrane space to matrix.
  - Transport ATP^{4-} from matrix to inter-membrane space.
  - Net transport of a negative charge from inside to outside.
    - Favored by transmembrane proton gradient.
- Phosphate translocase.
  - Transport of $H_2PO_4^-$ and H^+ from inter-membrane space to matrix.
    - Favored by transmembrane proton gradient.
    - Some energy of electron transfer is consumed.



NADH Shuttle Systems

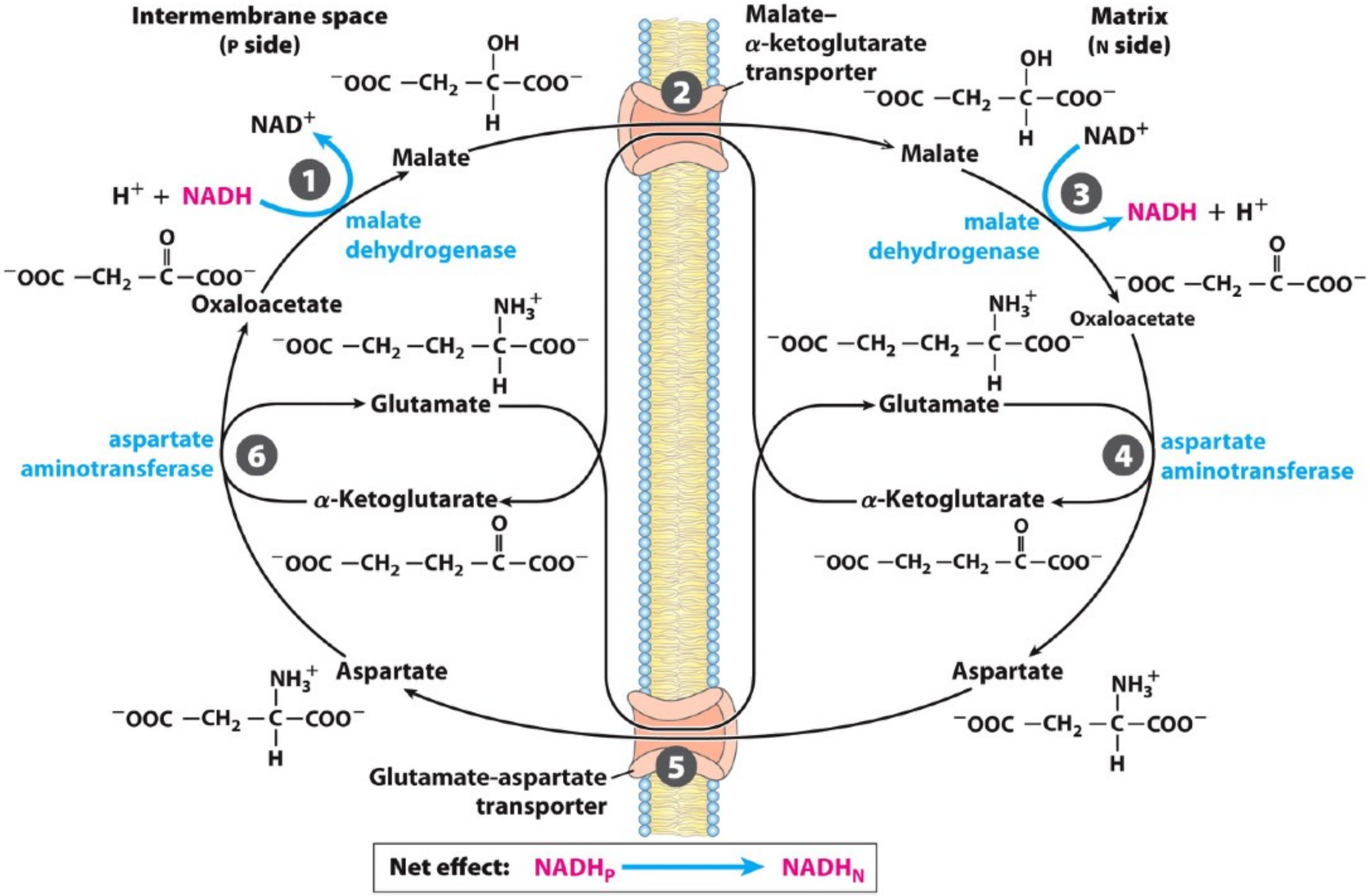
- Mitochondrial membrane NOT permeable to NADH.
- How can NADH generated by glycolysis in cytosol be transported to respiratory chain in mitochondria?
  - In cytosol.
    - Aspartate -> oxaloacetate (transamination).
    - Oxaloacetate -> malate (reduction, with NADH oxidized to NAD⁺).
    - Malate enters mitochondria.
  - In mitochondria.
    - Malate -> oxaloacetate (oxidation, with NAD⁺ reduced to NADH).
    - Oxaloacetate -> aspartate (transamination).
    - Aspartate enters cytosol.



Net Result: Cytosolic NADH becomes mitochondrial NADH.

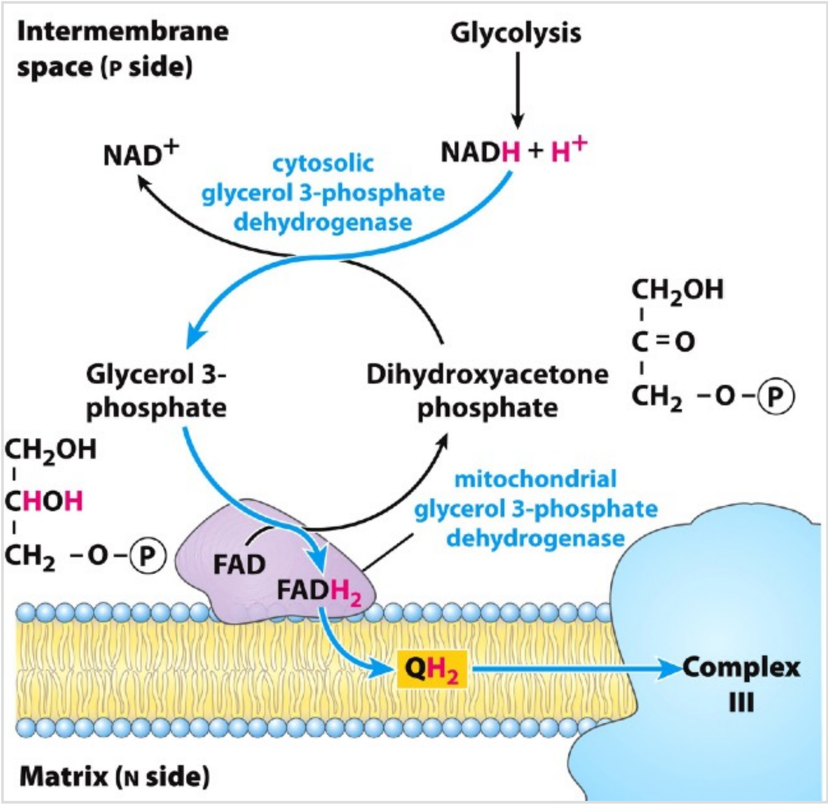
Malate-Aspartate Shuttle

- Function in liver, kidney, and heart
- Malate-α-ketoglutarate transporter
- Glutamate-aspartate transporter



Glycerol-3-Phosphate Shuttle

- Function in skeletal muscle and brain.
- Electrons are passed from NADH to Q and into complex III, not complex I.
- Provide only enough energy to synthesize 1.5 ATP molecules per NADH.



ATP Yields from Glucose and Fatty Acid

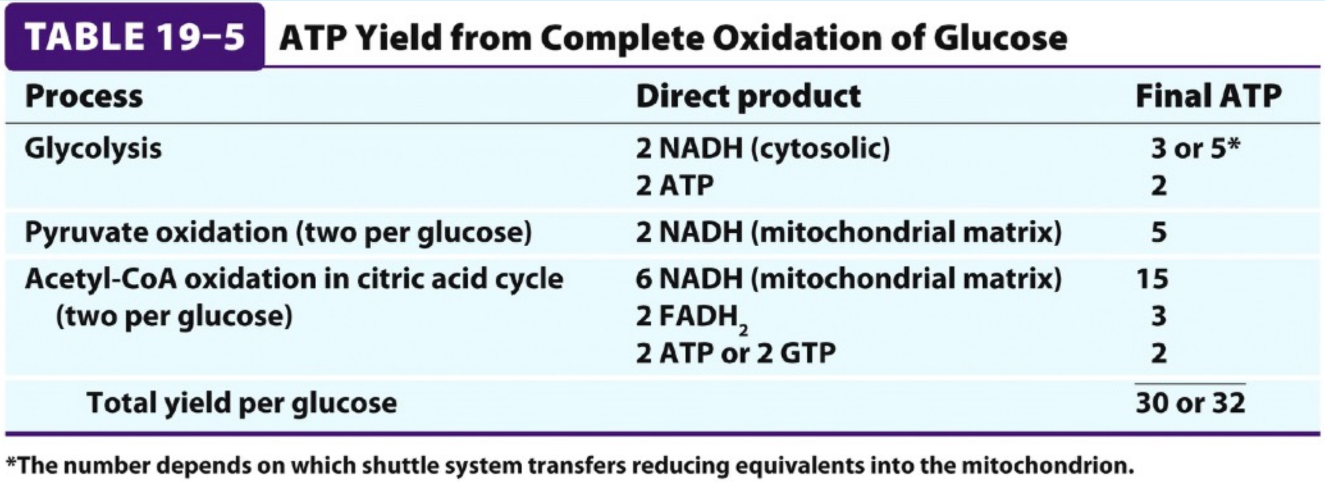


TABLE 19–5 ATP Yield from Complete Oxidation of Glucose

Process	Direct product	Final ATP
Glycolysis	2 NADH (cytosolic) 2 ATP	3 or 5* 2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix) 2 $FADH_2$ 2 ATP or 2 GTP	15 3 2
Total yield per glucose		30 or 32

*The number depends on which shuttle system transfers reducing equivalents into the mitochondrion.

Net gain per glucose is 30 or 32 ATP depending on which shuttle system is used.

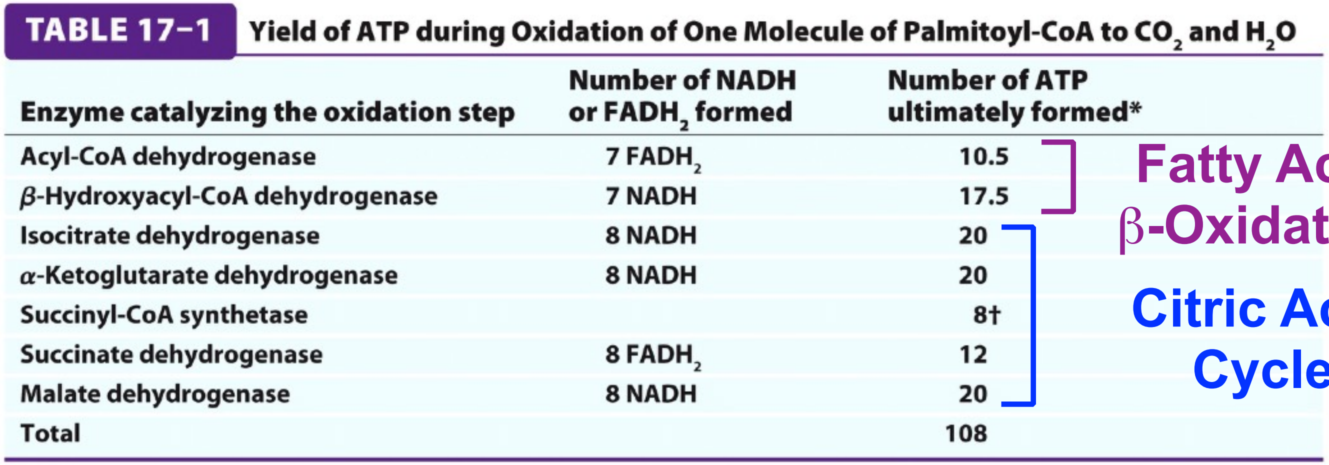


TABLE 17–1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2O

Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed*
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8†
Succinate dehydrogenase	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108

Fatty Acid β-Oxidation

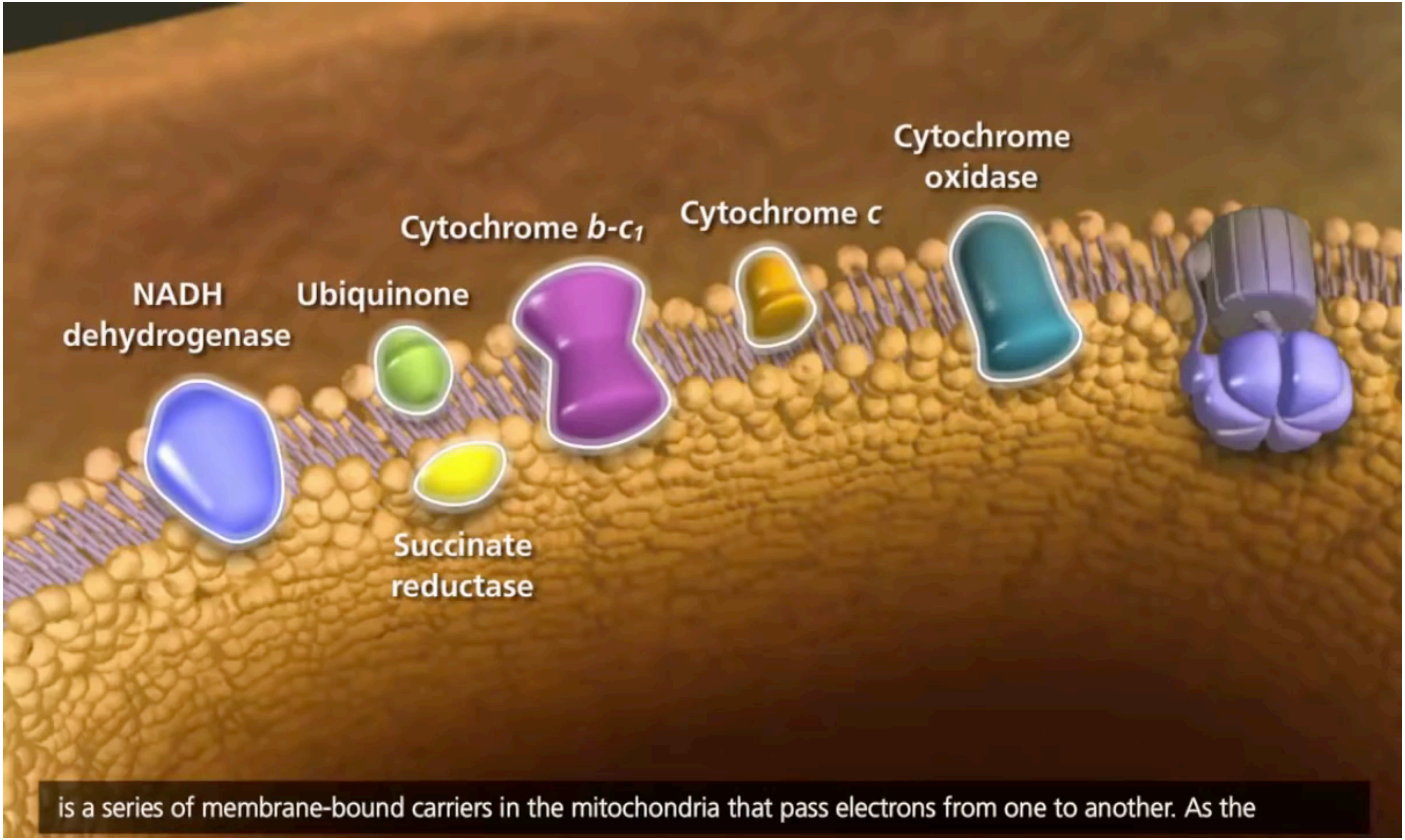
Citric Acid Cycle

Net gain per palmitate is 106 ATP because 2 ATPs are consumed in activation.

Summary 19.2 ATP Synthesis

- Proton gradient provides energy for ATP synthesis. Flow of protons through F_o causes β subunit in F_1 to cycle from [ADP-bound] to [ATP-bound] to [empty].
- ATP formation on ATP synthase requires little energy. Role of proton-motive force is to push ATP from its binding site on enzyme surface.
- 2.5 ATP molecules are generated per NADH when electrons enter at complex I. 1.5 ATP molecules are generated per $FADH_2$ when electrons enter at Q.
- NADH equivalents are moved from cytosol to mitochondrial matrix by one of two shuttle systems.

Summary of Cellular Respiration



is a series of membrane-bound carriers in the mitochondria that pass electrons from one to another. As the

Week 13 Oxidative Phosphorylation

19.1 Electron-Transfer Reactions in Mitochondria

19.2 ATP Synthesis

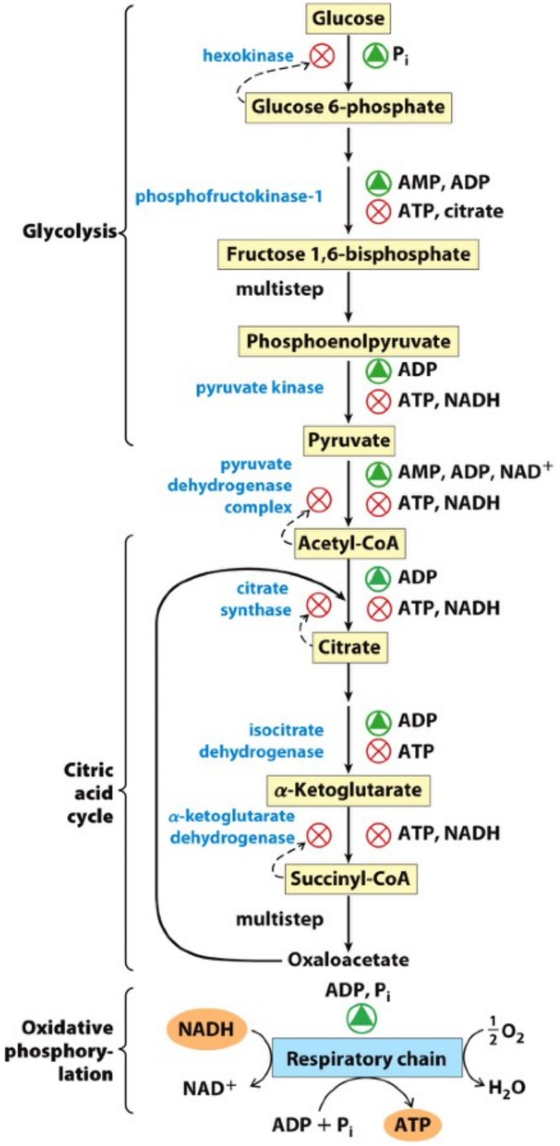
19.3 Regulation of Oxidative Phosphorylation

19.4 Mitochondria in Other Processes

19.5 Mitochondrial Genes

OxPhos Regulated by Energy Needs

- Similar regulation of following processes.
  - Glycolysis.
  - Citric acid cycle.
  - Oxidative phosphorylation.
- Regulated by energy status of cells.
  - ADP and AMP are stimulatory.
  - ATP and NADH are inhibitory.
- Rate of fuel oxidation very tightly regulated.
  - [ATP]/[ADP] ratio fluctuates only slightly.
  - ATP is formed only as fast as it is used in energy-requiring cellular activities.



Week 13 Oxidative Phosphorylation

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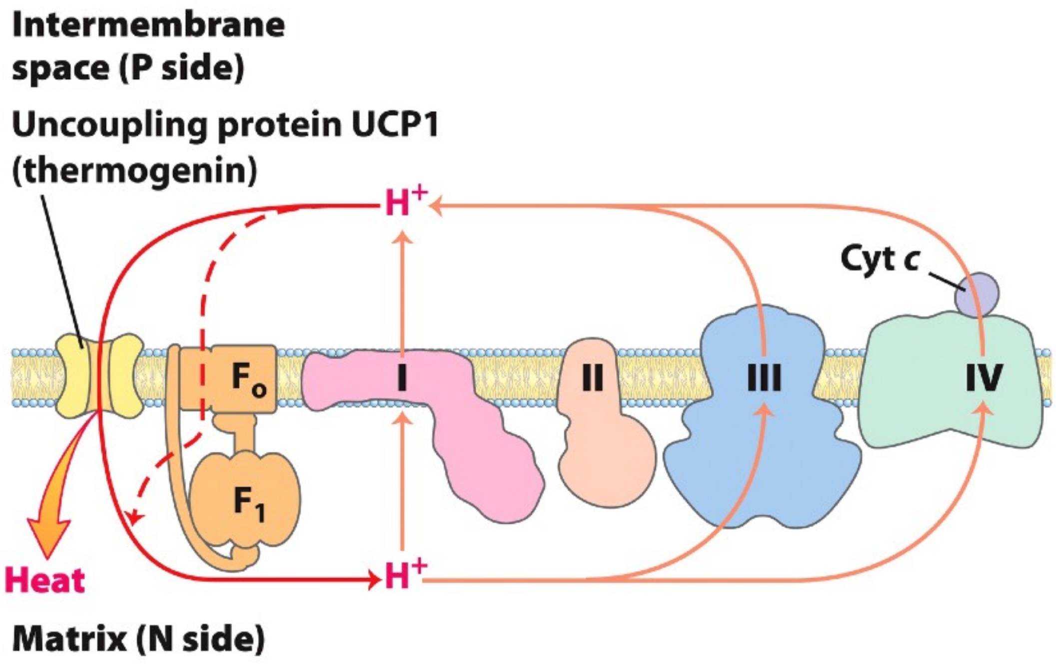
Uncoupled Mitochondria Produce Heat

- Newborn infants have brown adipose tissue.
  - Energy from fuel oxidation is NOT used for ATP synthesis.
  - Fuel oxidation serves to generate heat to keep newborn warm.
  - Brown color because of many mitochondria and thus high concentration of cytochromes (heme groups show strong absorption of visible light).
- Difference of mitochondria in brown adipose tissue.
  - Uncoupling protein **thermogenin** provides a path for protons to return.



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19.1 Electron-Transfer Reactions in Mitochondria

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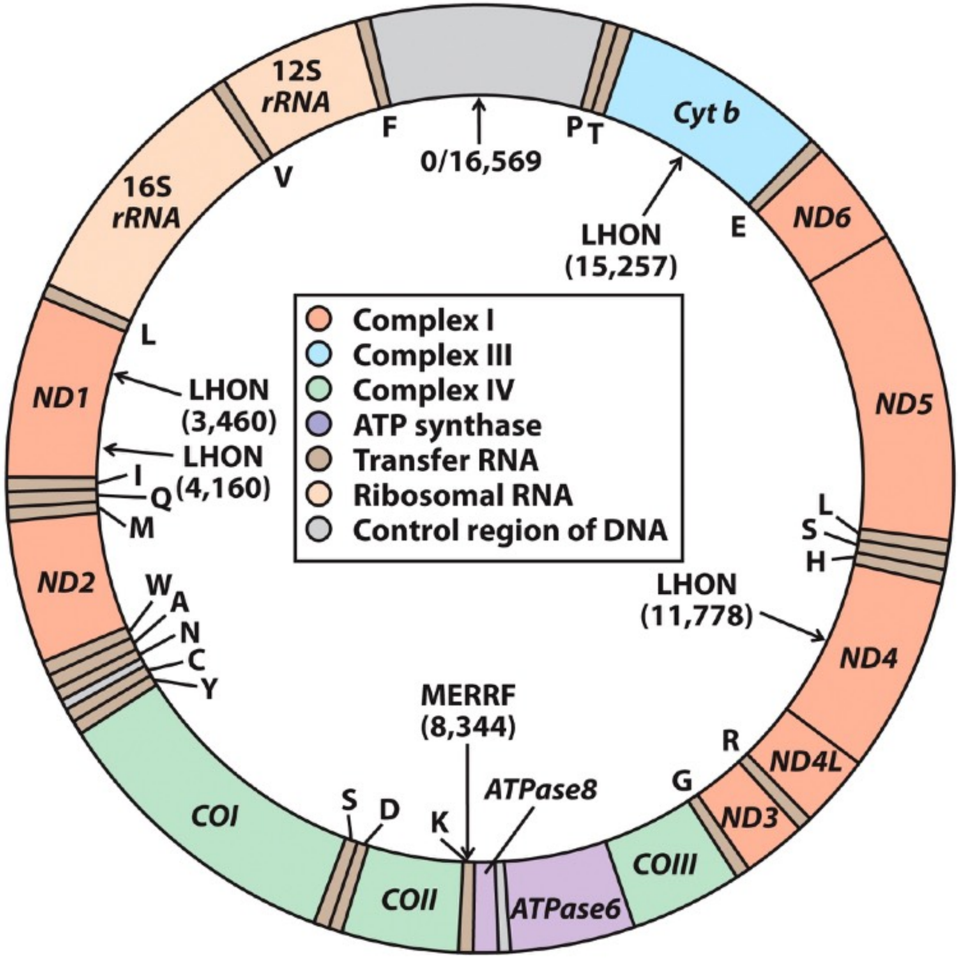
19.4 Mitochondria in Other Processes

19.5 Mitochondrial Genes

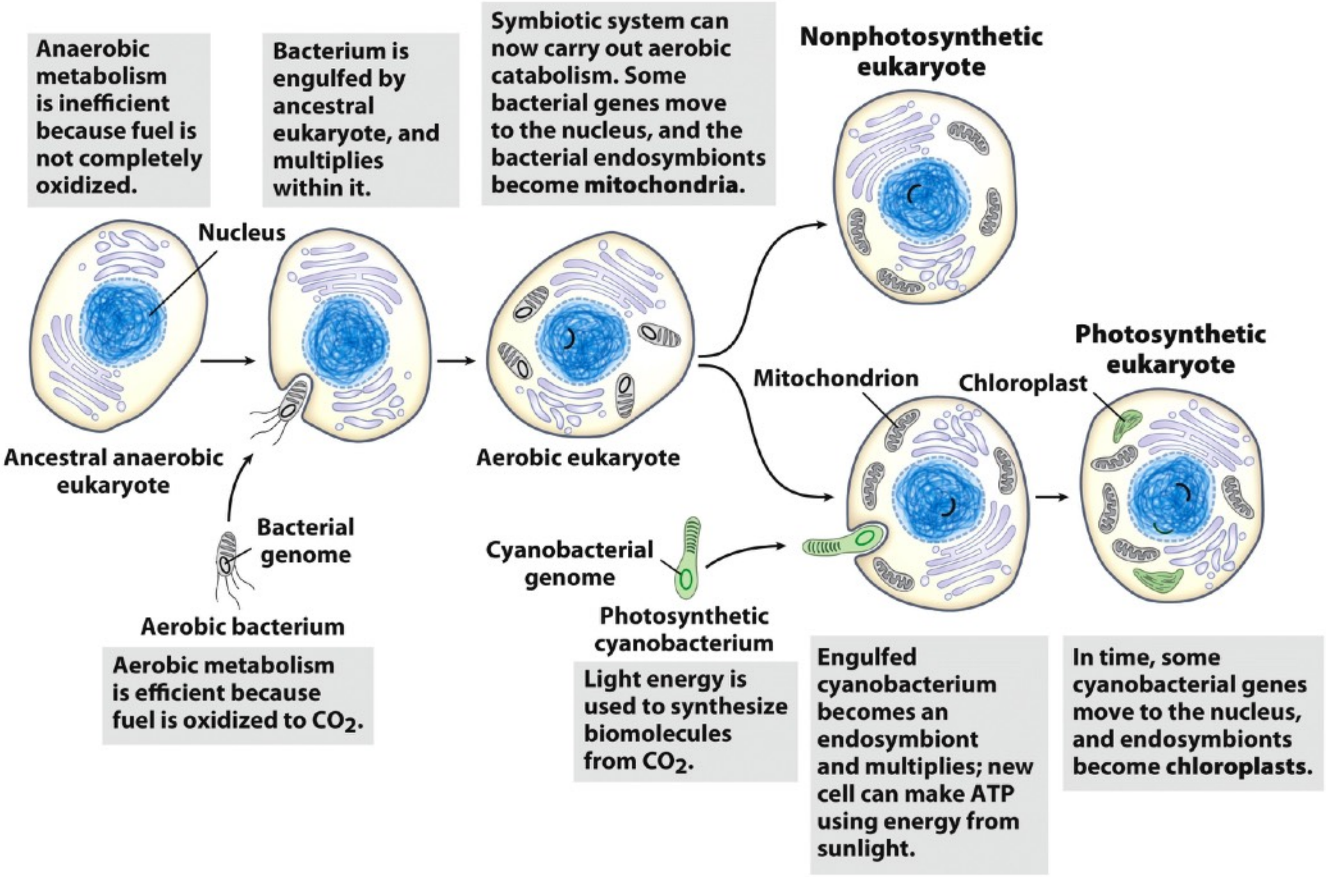
Mitochondria Contain Own Genome

- Mitochondria contain their own genome.
  - Circular double-stranded DNA.
  - Human mitochondrial genome contains 37 genes.
    - Subunits of proteins of respiratory chain.
    - Ribosomal RNA and transfer RNA molecules essential for protein synthesis.
- Great majority of mitochondrial proteins (about 1100) are NOT on own genome.
  - Encoded by nuclear genes.
  - Synthesized in cytosol.
  - Imported and assembled.



Mitochondria Evolved from Bacteria

- Primitive eukaryotes lived anaerobically by fermentation.
  - Establish a symbiotic relationship with bacteria capable of aerobic metabolism.
  - Many bacterial gene moved to host nucleus and bacteria become mitochondria.



Anaerobic metabolism is inefficient because fuel is not completely oxidized.

Bacterium is engulfed by ancestral eukaryote, and multiplies within it.

Symbiotic system can now carry out aerobic catabolism. Some bacterial genes move to the nucleus, and the bacterial endosymbionts become **mitochondria.**

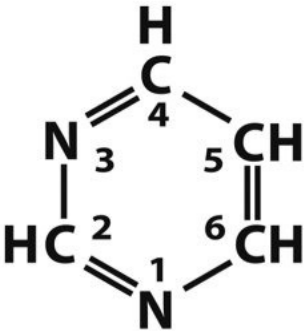
Aerobic metabolism is efficient because fuel is oxidized to CO_2.

Light energy is used to synthesize biomolecules from CO_2.

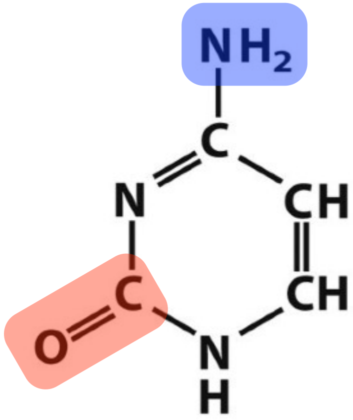
Engulfed cyanobacterium becomes an endosymbiont and multiplies; new cell can make ATP using energy from sunlight.

In time, some cyanobacterial genes move to the nucleus, and endosymbionts become **chloroplasts.**

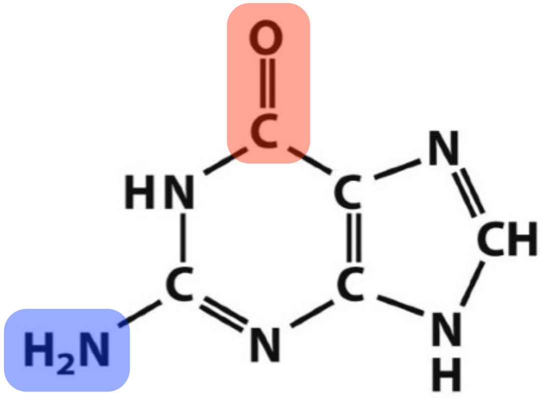
Structures of This Week: Cytosine



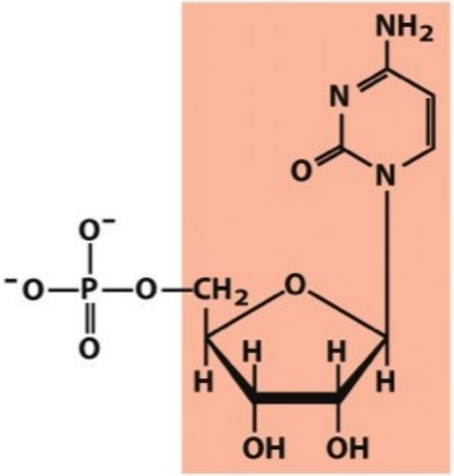
Pyrimidine



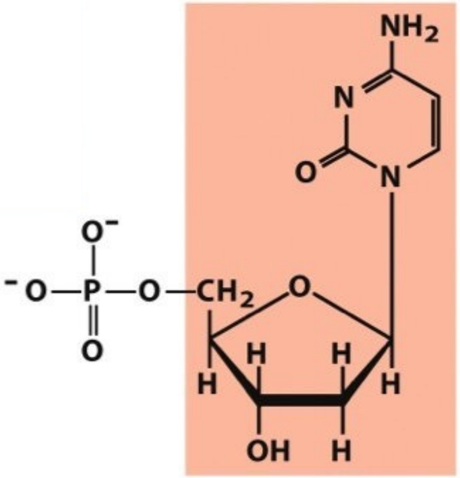
Cytosine



Guanine



Cytidine 5’-monophosphate



Deoxycytidine 5’-monophosphate

Example Question

Which of the following electron carriers is *not* able to transfer one electron at a time?

[✓ A) NADH]
B) FMN
C) FAD
D) Ubiquinone
E) Heme

Example Question

Reduced QH_2 is *not* formed by which of the following?

A) Complex I and NADH
B) Complex II and succinate
[✓ C) Complex III and cytochrome *c*]
D) Fatty acid oxidation
E) Oxidation of glycerol-3-phosphate

Example Question

In Complex III oxidation of 1 mol of QH_2 requires 2 mol of cytochrome *c* because:

[✓ A) cytochrome *c* is a one-electron acceptor, whereas QH_2 is a two-electron donor.]
B) cytochrome *c* is a two-electron acceptor, whereas QH_2 is a one-electron donor.
C) cytochrome *c* is water soluble and operates between the inner and outer mitochondrial membranes
D) molecular weight of QH_2 is twice that of cytochrome *c*.
E) two molecules of cytochrome *c* must first combine physically before they are catalytically active.

Example Question

Which of the following is *not* a feature of complex IV?

A)Cytochrome *c* is a one-electron donor.

B)Oxygen is a substrate.

C)Complex IV is also called cytochrome oxidase.

D)For every electron passed to complex IV, two protons are consumed from the matrix (N) side.

[✓ E)In order to generate two water molecules, complex IV must go through the catalytic cycle two times.]

Example Question

Which of the following is *not* true of the proton motive force (pmf)?

A) One component of the pmf is the chemical gradient of protons.
B) One component of the pmf is the charge gradient of protons.
[✓ C) Generation of the pmf in mitochondria requires succinate.]
D) The pmf is generated by electron transport chain in mitochondria.
E) The pmf drives ATP synthesis in mitochondria.

Example Question

Cyanide, oligomycin, and DNP are inhibitors of oxidative phosphorylation. Which of the following statements correctly describes mode of action of the three inhibitors?

A) Cyanide and DNP inhibit the respiratory chain, and oligomycin inhibits the synthesis of ATP.
[✓ B) Cyanide inhibits the respiratory chain, whereas oligomycin and DNP inhibit the synthesis of ATP.]
C) Cyanide, oligomycin, and DNP compete with O_2 for cytochrome oxidase (complex IV).
D) Oligomycin and cyanide inhibit synthesis of ATP; DNP inhibits the respiratory chain.
E) Oligomycin inhibits the respiratory chain, whereas cyanide and DNP prevent the synthesis of ATP.

Example Question

Upon the addition of 2,4-dinitrophenol (DNP) to a suspension of mitochondria carrying out oxidative phosphorylation, all of the following occur *except*:

[✓ A) oxygen consumption rate decreases.]
B) oxygen consumption rate increases.
C) No additional ATP is produced.
D) the proton gradient dissipates.
E) the rate of transport of electrons from NADH to O_2 becomes maximal.

Example Question

Which of the following statements about energy conservation in the mitochondrion is *false*?

A) Drugs that inhibits the ATP synthase will also inhibit the flow of electrons down the chain of carriers.

B) For oxidative phosphorylation to occur, it is essential to have a closed membranous structure with an inside and an outside.

C) The yield of ATP per mole of oxidizable substrate depends on the substrate.

[✓ D) Uncouplers (such as DNP) have exactly the same effect on electron transfer as inhibitors such as cyanide; both block further electron transfer to oxygen.]

E) Uncouplers dissipate the proton motive force as heat.

Example Question

Which of the following is *correct* concerning the mitochondrial ATP synthase?

A) It can synthesize ATP after it is extracted from broken mitochondria.

B) It catalyzes the formation of ATP even though the reaction has a large positive free-energy change.

C) It consists of F_o and F_1 subunits, which are both transmembrane (integral) polypeptides.

D) It is actually an ATPase and only catalyzes the hydrolysis of ATP.

[✓ E) When it catalyzes the ATP synthesis reaction, the free energy change is actually close to zero.]

Example Question

During oxidative phosphorylation, the proton motive force that is generated by electron transport is used to:

A) create a pore in the inner mitochondrial membrane.
B) generate the substrates (ADP and P_i) for the ATP synthase.
[✓ C) induce a conformational change in the ATP synthase.]
D) oxidize NADH to NAD^+.
E) reduce O_2 to H_2O.

Example Question

Draw the path of electron flow from NADH to the final electron acceptor during electron transport in mitochondria.

For each electron carrier, indicate whether only electrons, or both electrons and protons, are accepted/donated by that carrier.

Example Question

Although molecular oxygen (O_2) does not participate directly in any of the reactions of the citric acid cycle, the cycle operates only when O_2 is present. Explain this observation.

Example Question

When the F_1 portion of the ATP synthetase complex is removed from the mitochondrial membrane and studied in solution, it functions as an ATPase. Why does it not function as an ATP synthetase?