

Electron Transport Chain and Oxidative Phosphorylation (ETC/Ox Phos)

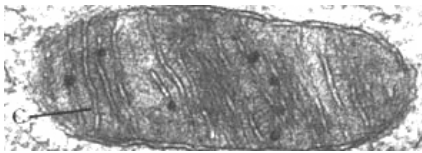
AKA respiratory chain

Importance and Location

- Importance: these are the mechanism by which NADH plus H⁺ and FADH₂ are used to generate ATP
- Location: inner mitochondrial membrane (showing my mammalian bias)

Review of Mitochondrial Structure

- Porous (~5 kDa) OMM
- IMS not just space- filled with enzymes, e.g.
- Tight (and highly convoluted) IMM forming *cristae*
- Matrix



Reducing Potential

- Theoretically ~ 7 ATP could be produced (NADH→O₂ 1.12 V (table of standard reduction potentials))

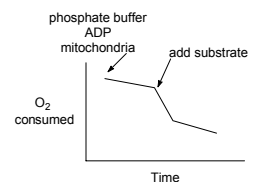
$$\begin{aligned}\Delta G &= -n\Delta E \\ &= -(2)(96.5 \frac{\text{kJ}}{\text{Vmol}})(1.12\text{V}) \\ \Delta G &= -216 \frac{\text{kJ}}{\text{mol}}\end{aligned}$$

- Since ΔG° for ATP *synthesis* is +30.5 kJ/mol, 7.1 ATP (One step, one ATP)
- (3 steps, 3 ATP) Some energy "lost" as heat in order to drive the rxns

Experimental Setup...

...and States

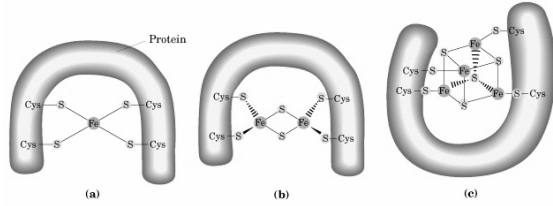
- **State 1** availability of ADP and substrate
- **State 2** availability of substrate
- **State 3** capacity of chain itself
- **State 4** availability of ADP
- **State 5** availability of O₂



Site I Contains Flavin- and Iron-Sulfur-Proteins

Fig. 19-5 Lehninger POB 3rd Ed.

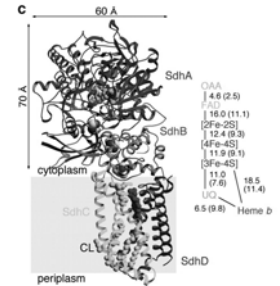
- # proteins > 30
- Important classes
- Enzyme names
- Inhibitors include rotenone



Site 2 Enzymatic Activity is SDH

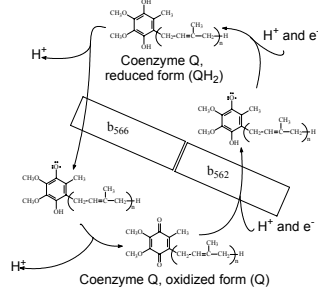
Yankovskaya, et al., Science 299: 700-704, 2003

- # proteins at least 4
- Important classes
- Enzyme names
- Inhibitors include malonate



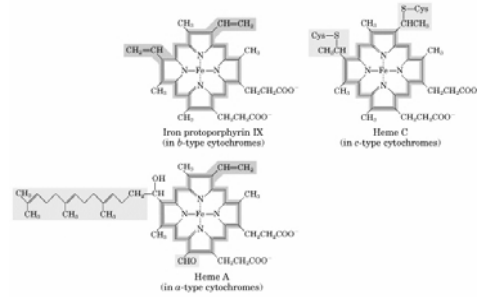
Site 3 is Composed of Cytochromes b

- # proteins \geq 11
- b562 (matrix side) and b566 (IMS side) arguably most important
- Sidedness is important!
- Cytochrome c reductase
- Inhibitors include antimycin



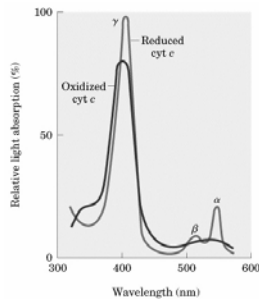
Cytochromes Have Heme as Prosthetic Group

Fig. 19-3 Lehninger POB 3rd Ed.



Studying Cytochromes

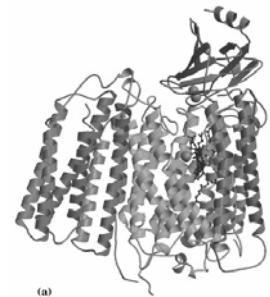
Fig. 19-4 Lehninger POB 3rd Ed.



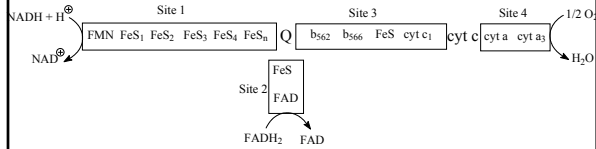
Site 4 Has Copper-containing Cytochromes a and a3

Fig. 19-12(a) Lehninger POB 3rd Ed.

- # proteins \geq 13
- Cyt a and cyt a3 arguably most important
- Cytochrome c oxidase
- Inhibitors include CN⁻, CO, and H₂S



Global ETC



In-depth Summary of the Site Components

Table 19-3

Protein Components of the Mitochondrial Electron-Transfer Chain

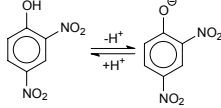
| Enzyme complex | Mass (kDa) | Number of subunits* | Prosthetic group(s) |
|---|------------|---------------------|--|
| I NADH dehydrogenase | 850 | 42 (14) | FMN, Fe-S |
| II Succinate dehydrogenase | 140 | 5 | FAD, Fe-S |
| III Ubiquinone: cytochrome c oxidoreductase | 250 | 11 | Hemes, Fe-S |
| Cytochrome <i>c</i> [†] | 13 | 1 | Heme |
| IV Cytochrome oxidase | 160 | 13 (3-4) | Hemes; Cu _A , Cu _B |

*Numbers 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.

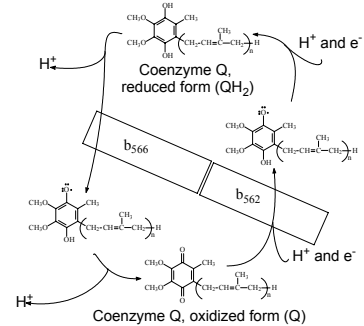
It is Not the Flow of e⁻ per se That Provides Energy for ATP Synthesis: 2,4-DNP as an *Uncoupler*

- Adding a little dilute acid to mitochondria causes ATP synthesis
- A proton gradient



The Q Cycle as an Exemplar

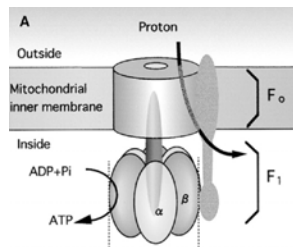
- Protons are ejected at other sites in similar fashion



ATP Synthase

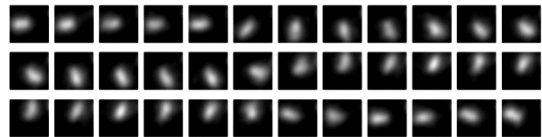
Mehta, et al., *Science* 283: 1689-704, 2003

- Formerly called ATPase (inside out vesicles)
- Cell biologists like the term "lollipop structure"



ATP Synthase Rotates as ATP is Synthesized

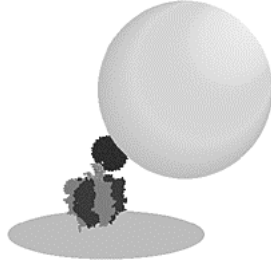
Fig. 19-24(b) Lehninger POB 3rd Ed.



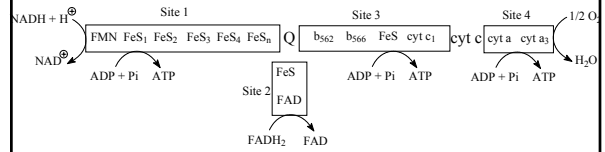
- ~7,000 rpm. This is very fast. My truck operates at about 3,500 rpm...when I get up to about 90 mph
- Rotation deduced by attaching actin filament

Refinement of Technique

- "40-nm gold bead attached through streptavidin (red) linked to biotin, friction drops to one ten-thousandth of that caused by actin, allowing a resolution on the order of 0.1 millisecond."
- The motion of the gold bead, observed through laser dark-field microscopy, reveals that the 120° step is composed of a 90° substep driven by ATP binding and a 30° substep due to product release.

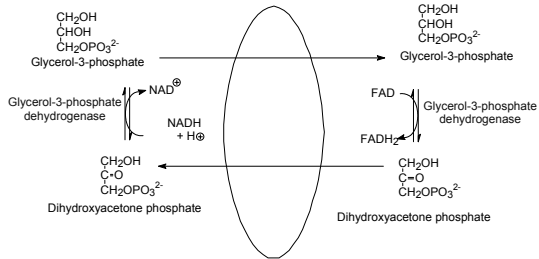


Global ETC With ATP

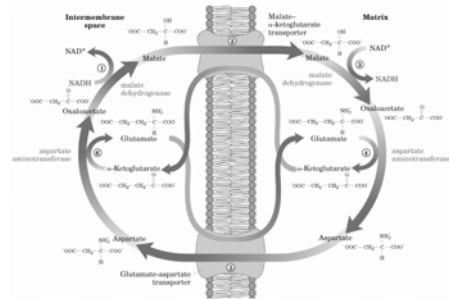


The Glycerol-3-phosphate Shuttle Causes the Loss of 2 ATP per Glucose

- Present in muscle, brain, BAT, ?liver?

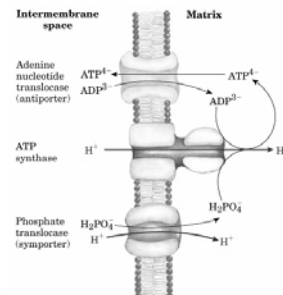


The Malate-aspartate Shuttle is More Complicated, But No Loss of ATP



An ATP/ADP Antiporter is Inhibited by Atractyloside

Phosphate Can Be Obtained by H⁺ Symport or OH⁻ Antiport



ADP Availability Regulates the Pathway

Inhibitors and Uncouplers: The Difference is Important

Table 19-4 Lehninger POB 3rd Ed.

Table 19-4

Some Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation

| Type of interference | Compound* | Target/mode of action |
|--|-----------------|---|
| Inhibition of electron transfer | Cyanide | Inhibit cytochrome oxidase |
| | Carbon monoxide | |
| | Antimycin A | Blocks electron transfer from cytochrome <i>b</i> to cytochrome <i>c</i> ₁ |
| | Myxothiazol | |
| | Rotenone | |
| Prevent electron transfer from Fe-S center to ubiquinone | Amytal | Prevent electron transfer from Fe-S center to ubiquinone |
| | Plasinidin A | |
| | DCMU | |
| | Aurovertin | |
| Inhibition of ATP synthase | Oligomycin | Competes with Q ₀ for binding site in PSII |
| | Verticillidin | Inhibits F ₁ |
| Inhibit F ₁ and CF ₁ | DCCD | Blocks proton flow through F ₀ and CF ₀ |
| | FCCP | |
| | DNP | |
| Uncoupling of phosphorylation from electron transfer | Valinomycin | Hydrophobic proton carriers |
| | Thermogenin | |
| Inhibition of ATP-ADP exchange | Atractyloside | Forms proton-conducting pores in inner membrane of brown fat mitochondria |
| | | |

*DCMU is 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCCD, 8-cyclohexylcarbodiimide; FCCP, cyanide-*p*-trifluoromethoxyphenylhydrazine; DNP, 2,4-dinitrophenol.