MACQUARIE University

Making ATP: Oxidative Phosphorylation

Abidali Mohamedali

School of Natural Sciences

T: 02 9850 8292; E: Abidali.mohamedali@mq.edu.au

Objectives

- Oxidative Phosphorylation
- Respiratory States and Respiratory Control
- Mitochondrial Transport Systems
- Energy Yields from Oxidative Metabolism
- The Mitochondrial Genome, Evolution, and Disease
- Oxygen as a Substrate for Other Metabolic Reactions

- Textbook Chap. 14

Aerobic respiration

- We make **ATP** at a rate of nearly **10^{21} molecules per second**, equivalent to producing our own weight in ATP every day.
- How is this massive amount of energy extracted from nutrients? **Glycolysis and the citric acid cycle** by themselves generate relatively **little ATP directly**.
- Six substrate oxidation steps: one in glycolysis, another in the pyruvate dehydrogenase reaction, and four more in the citric acid cycle, collectively reduce **10 moles of NAD^+ to NADH and 2 moles of FAD to $FADH_2$ per mole of glucose**.
- Reoxidation of these reduced electron carriers in cellular respiration generates most of the energy that is then used for **ATP synthesis from ADP**.
- In eukaryotic cells, NADH and $FADH_2$ are reoxidized by electron transport proteins bound to the inner mitochondrial membrane.
  - ❖ Reduced cofactors give up their electrons in the ETC
  - ❖ Electrons drive proton pumping in the mitochondria
  - ❖ The final reduction occurs when molecular oxygen accepts electrons to become water (aerobic respiration)
- So how do these protons make ATP?
  - ➢ **Enter ATP synthase!**

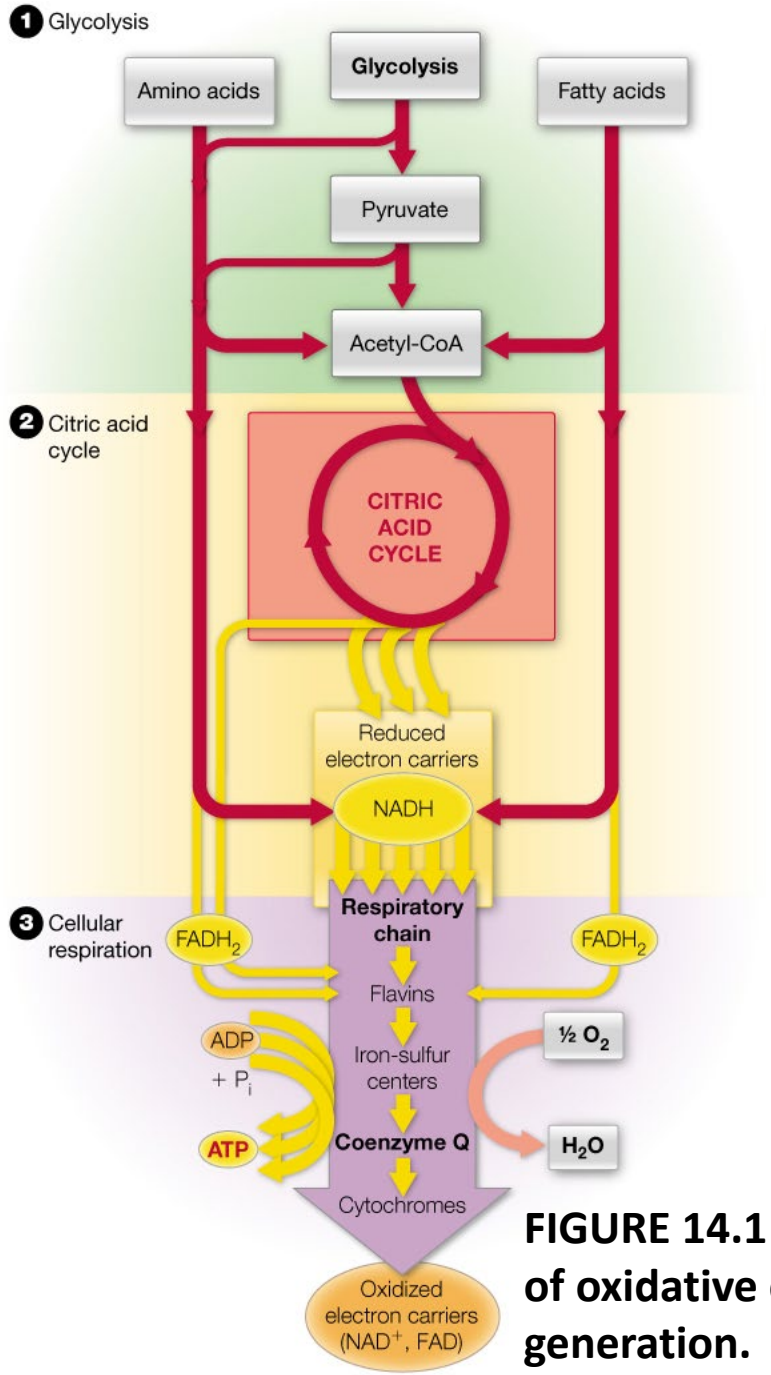


FIGURE 14.1 Overview of oxidative energy generation.

Electron Transport Chain and Oxidation Phosphorylation are located in the inner mitochondrial membrane

ETC:

- 4 integral membrane complexes (I to IV) and two mobile electron carriers
- 3 chemical reactions.
- 1-electron and 2-electron transfers depending on the cofactor.

OxPhos

- 1 integral membrane complex (V) coupled to ETC

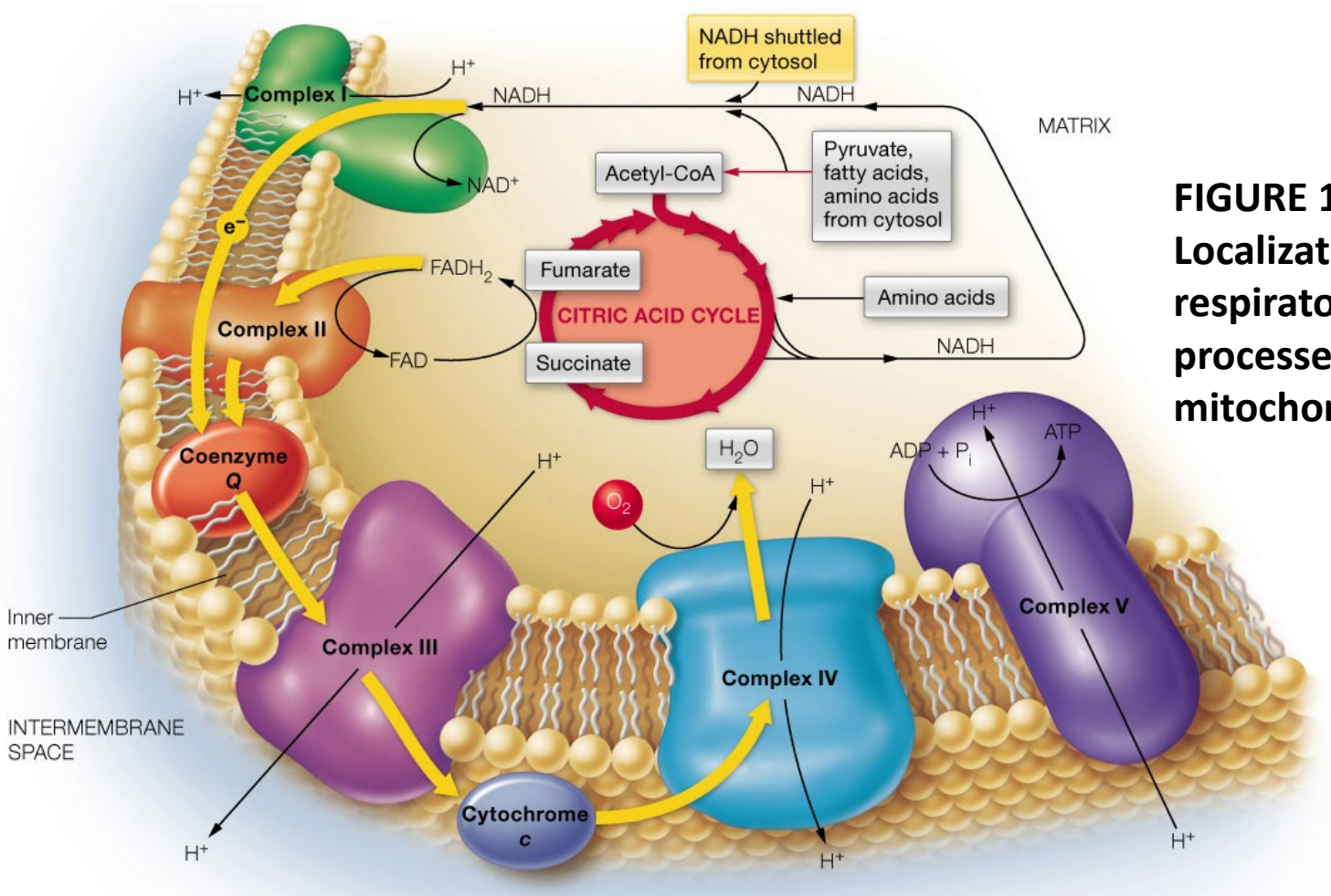


FIGURE 14.2 Localization of respiratory processes in the mitochondrion.

(b) Overview of oxidative phosphorylation. Reduced electron carriers, produced by cytosolic dehydrogenases and mitochondrial oxidative pathways, become reoxidized by enzyme complexes bound in the inner mitochondrial membrane. These complexes actively pump protons outward from the matrix into the intermembrane space, creating an energy gradient whose discharge through complex V drives ATP synthesis.

Key experimental observations leading to our understanding of ATP synthesis

- Oxidative phosphorylation (OxPhos) requires **an intact mitochondrial membrane**
- The inner mitochondrial membrane is impermeable to ions that will discharge an electrochemical gradient: H^+, OH^-, K^+, Cl^-
- Electron transport chain pumps H^+ into the intermembrane space, creating an electrochemical gradient across it
- Compounds that make holes in the membrane (e.g. 2,4-dinitrophenol) destroy this gradient – stop ATP synthesis.

Phosphate-to-Oxygen (P/O) Ratio

- The P/O ratio is the number of ATP molecules synthesized per pair of electrons carried though electron transport
- Consider the transfer of electrons from NADH to O_2:

$$\mathrm{NADH} + \mathrm{H^+} + \tfrac{1}{2}\mathrm{O_2} \rightleftharpoons \mathrm{NAD^+} + \mathrm{H_2O}$$

$$\Delta G^{\circ\prime} = -nF\,\Delta E^{\circ\prime} = -2(96485)(0.82 - (-0.32)) =$$

$$-220\ \mathrm{kJ/mol}$$

- The P/O ratio for the transfer of electrons from NADH is about 2.5; from succinate (through complex II) about 1.5
- P/O ratios may be non-integers because the oxidation of NADH and succinate is not directly coupled to ATP synthesis

Identification of "Coupling Sites" in Electron Transport

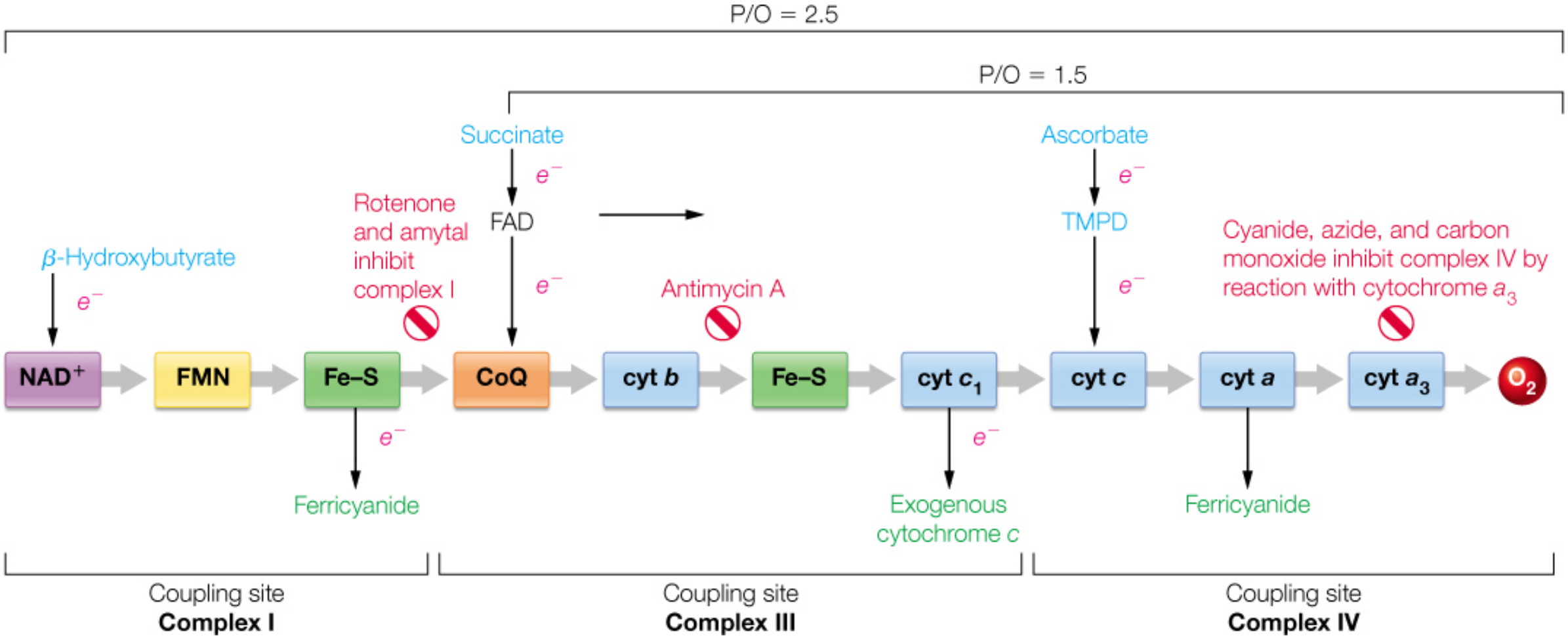
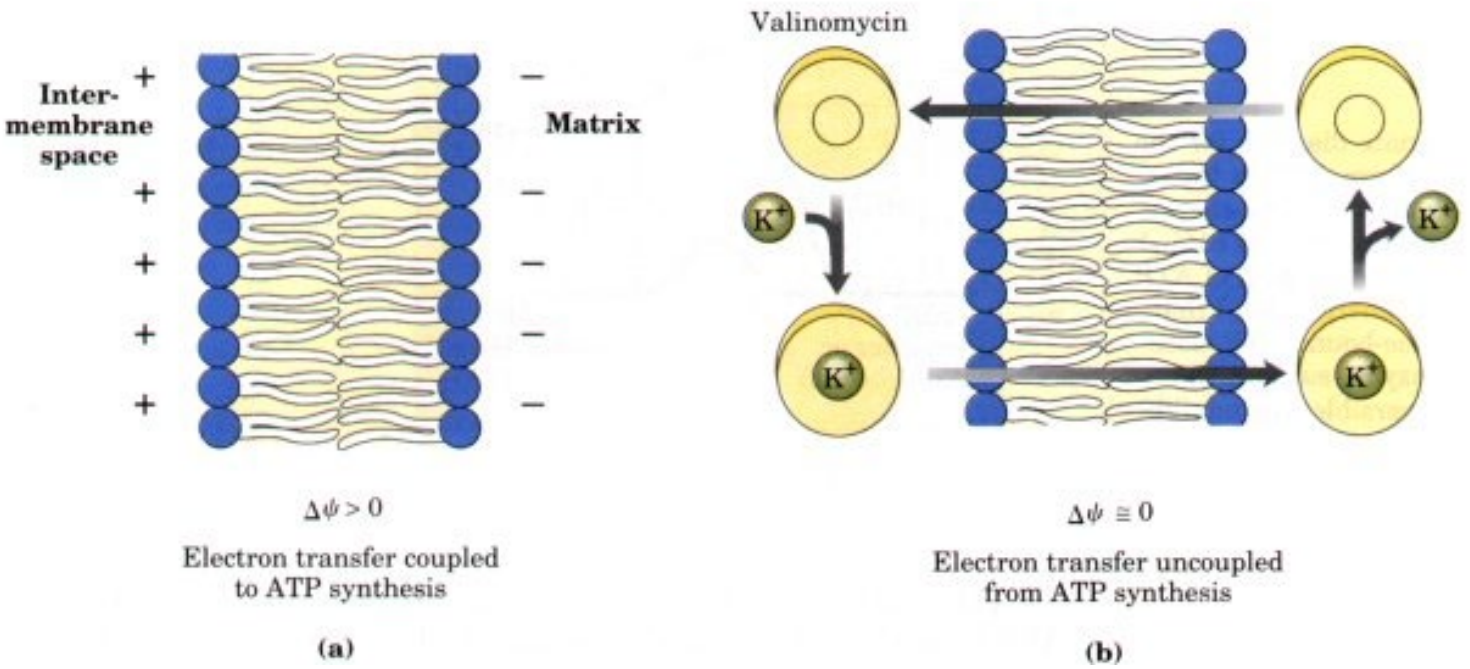


FIGURE 14.16 Experimental identification of "coupling sites."

Experiments using inhibitors and artificial electron donors (e.g., ascorbate – Vit. C) demonstrated that complexes I, III, and IV were capable of driving ATP synthesis, but not complex II

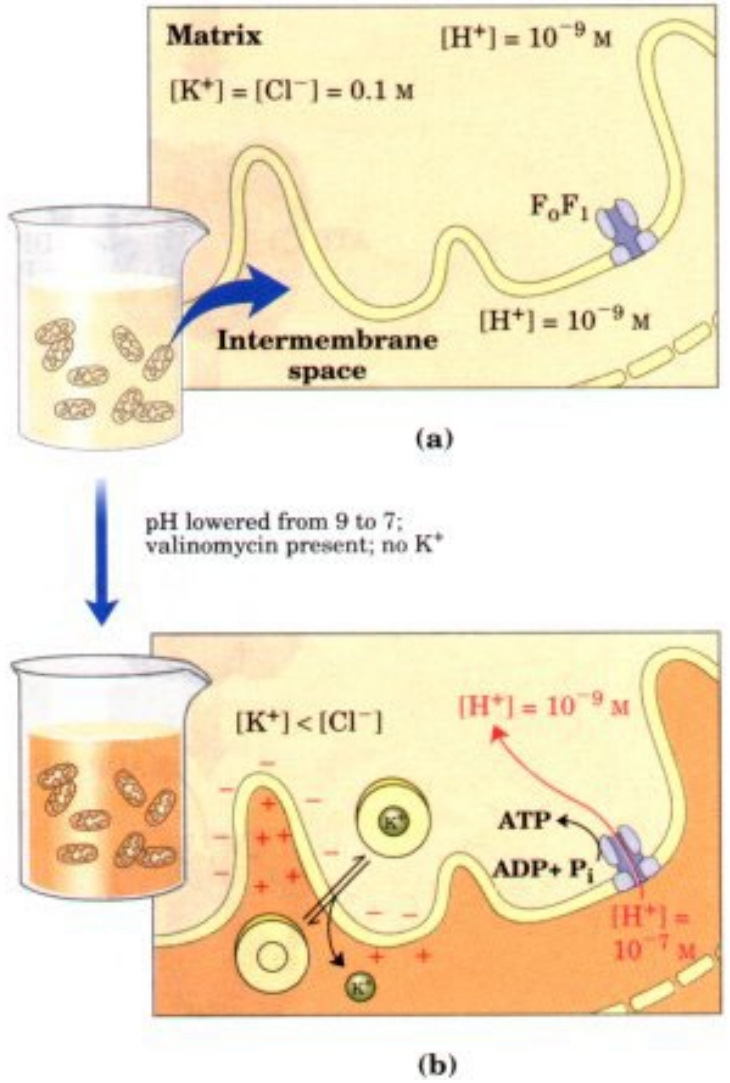
Addition of an uncoupler slows down ATP synthesis

- The toxic ionophore, valinomycin forms a lipid-soluble complex with K^+ (an independent 1974 experiment)
- This reduces the chemiosmotic gradient, which slows down ATP synthesis:



Artificial pH and charge gradient drives ATP synthesis even in the absence of electron donors

- Incubate isolated mitochondria in pH 9 buffer with 0.1 M KCl. No oxidisable substrates present
- Resuspend intact mitochondria in pH 7 buffer with valinomycin but no KCl
- pH gradient of 2 units and valinomycin-transported K^+ generates a charge gradient
- ATP synthesis occurs!



Chemiosmotic theory: Peter Mitchell: Nobel Prize in Chemistry 1978

- Links the proton gradient from electron transport to ATP synthesis.
- Mitchell's theory (1961) proposed:

  "the free energy of electron transport is conserved by pumping H^+ from the mitochondrial matrix to the intermembrane space to create an electrochemical H^+ gradient across the inner mitochondrial membrane. The electrochemical potential of this gradient is harnessed to synthesize ATP"

Chemiosmotic Coupling and ATP Synthesis

Chemiosmotic coupling model (Mitchell, 1961): electron transport drives the active transport of protons from the mitochondrial matrix to the intermembrane space; creating an electrochemical gradient.

The dissipation of the electrochemical gradient through complex V provides the free energy for ATP synthesis

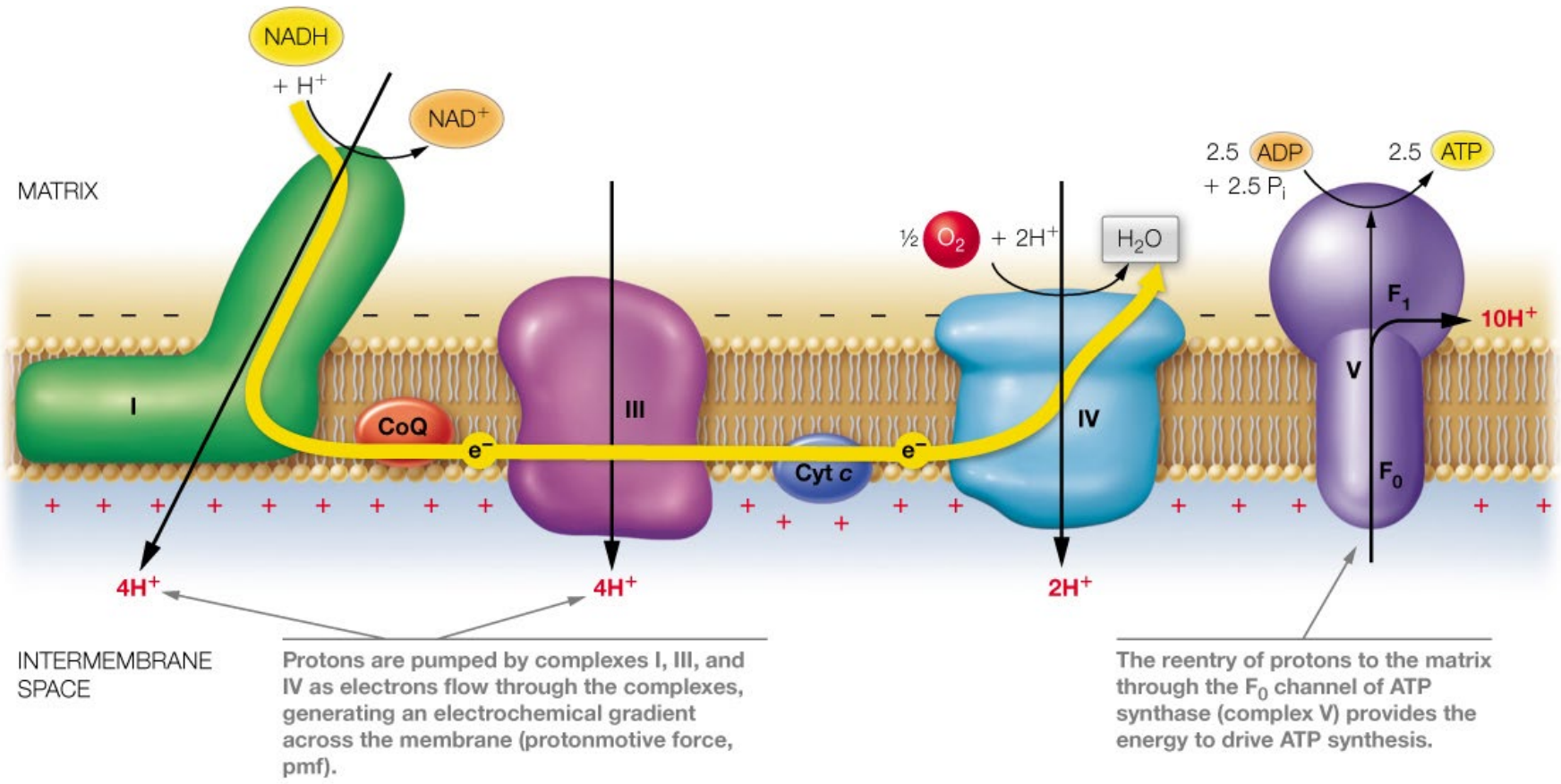
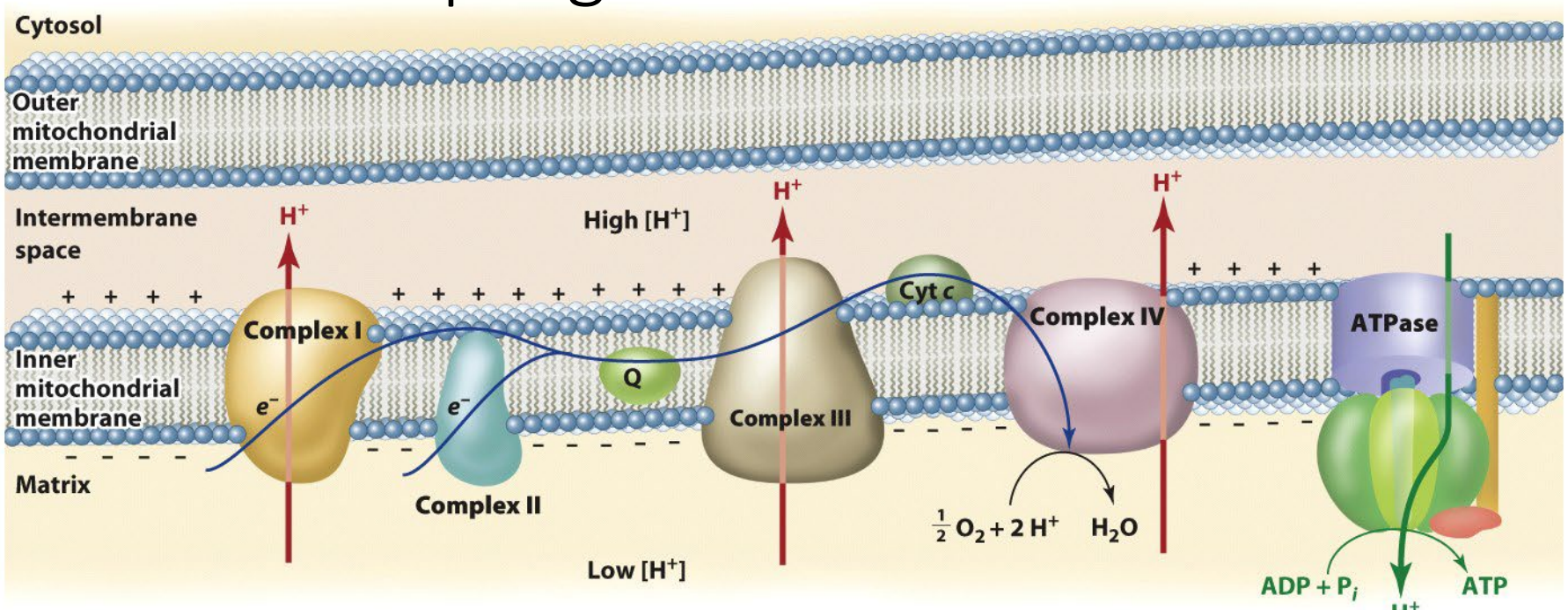


FIGURE 14.17 Chemiosmotic coupling of electron transport and ATP synthesis.

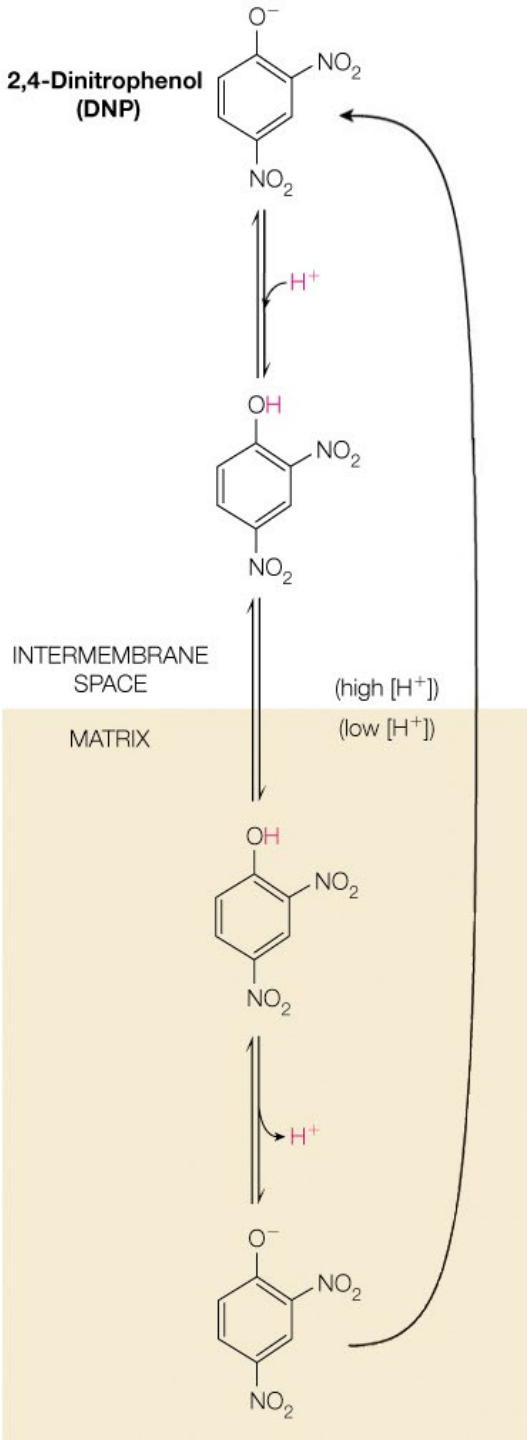
Coupling of ETC and OxPhos



- Complexes I, III and IV pump protons into the intermembrane space – increasing [H^+] and positive charge
  - Complex II provides e^-'s to Complexes III and IV for [H^+] pumping
- The matrix loses [H^+] and accumulates negative charge
- This charge difference across the inner mitochondrial membrane is the electrochemical gradient
- This gradient drives Complex V: ATP synthase, by pumping out H^+

Experimental Evidence for Chemiosmotic Coupling

- pH gradient and membrane potential both contribute to an electrochemical gradient (protonmotive force, pmf)
- Key electron transport proteins span the inner membrane
- Intact membranes are necessary for oxidative phosphorylation
- Uncoupling compounds, such as DNP:
  - allow electron transport to occur without generating ATP
  - act as ionophores and dissipate the proton gradient



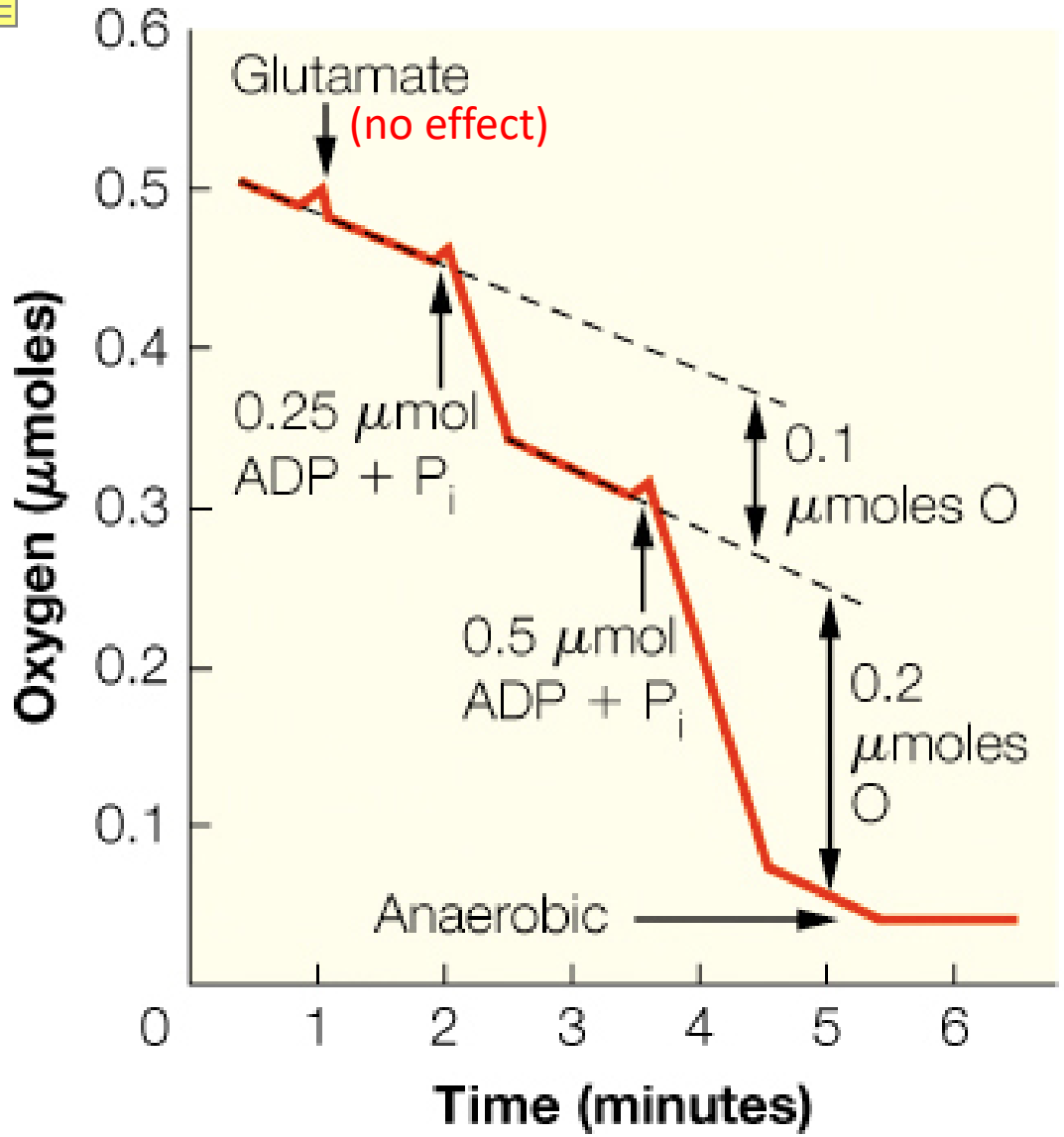


FIGURE 14.24 Experimental demonstration of respiratory control.

Respiratory Control by ADP and P_i

- Isolated mitochondria are placed in a chamber with 0.5 µmol of O_2
- Mitochondria take up oxygen during respiration
- Electrons are quantitated by oxygen uptake as measured in µmol of O (not O_2); ATP synthesis is quantitated in terms of phosphate incorporation into ATP
- Initially only slow oxygen update, until **ADP and P_i are added**.
- **Twice the amount** leads to **twice the oxygen consumption**!

Effects of an Inhibitor and Uncoupler on O_2 Consumption and ATP Synthesis

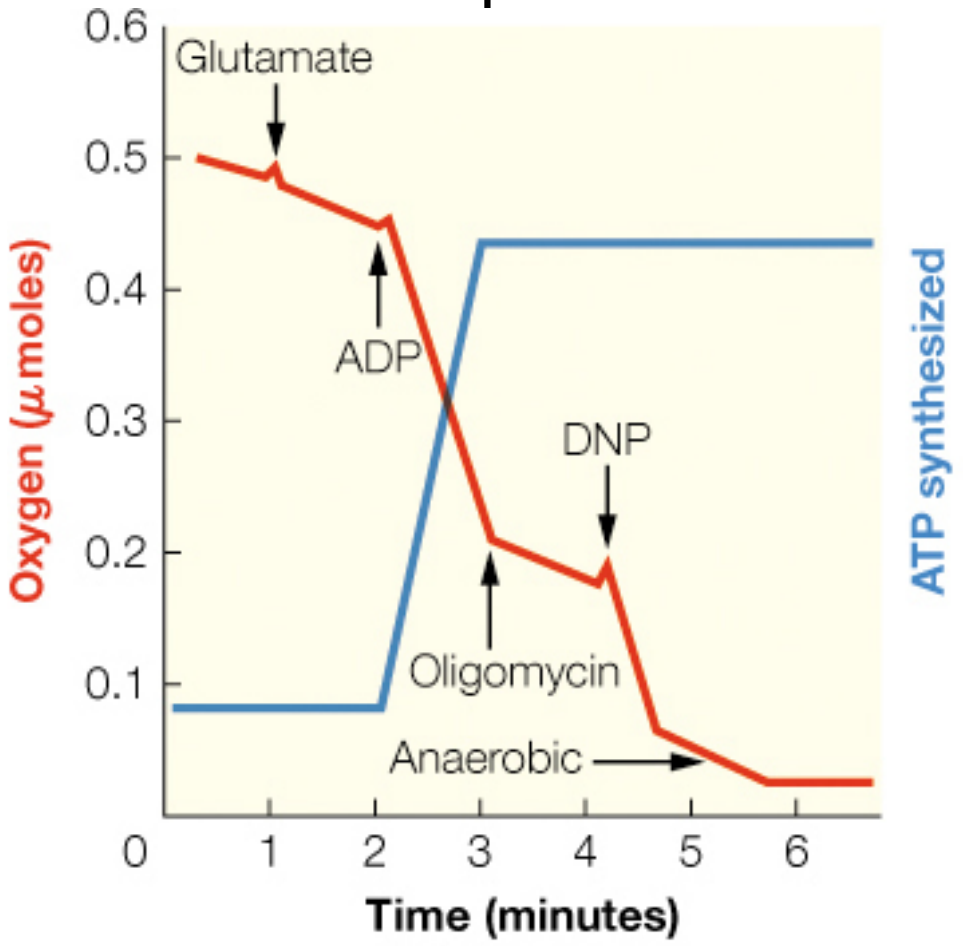
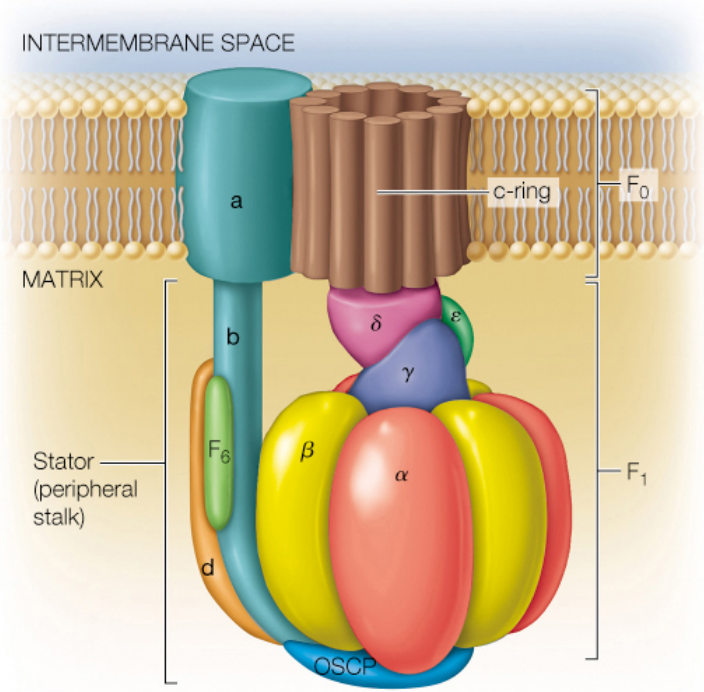


FIGURE 14.25 Effects of an inhibitor and an uncoupler on oxygen uptake and ATP synthesis.

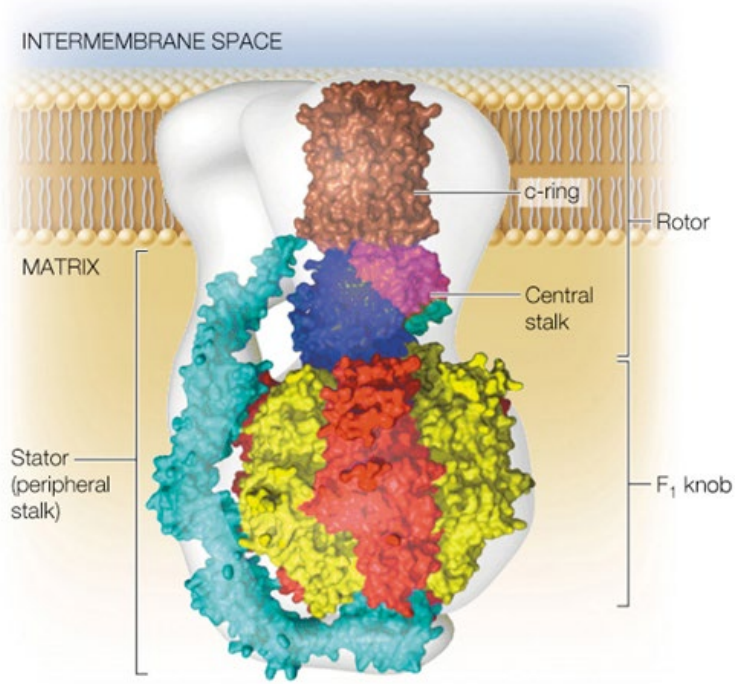
- In a similar experiment, the addition of ADP results in the production of ATP and an increase in O_2 consumption
- Addition of the ATP synthase inhibitor **oligomycin** slows the O_2 consumption rate and prevents ATP synthesis
- The addition of **dinitrophenol (DNP)** results in an increase in O_2 consumption (flow through the electron transport chain increases), but there remains no synthesis of ATP (**because the uncoupler destroys the proton gradient**)

Complex V: ATP Synthase

- Complex V is also called the F_0F_1 complex
- The F_0 c-ring (10 subunits in yeast, 8 in higher eukaryotes) and the a subunit are embedded in the mitochondrial membrane, while the F_1 "knob" protrudes into the mitochondrial matrix
- The F_0 subunit also consists of b, d, F_6, and OSCP subunits which, along with a, comprise the stator (which prevents the rotation of the three αβ dimers of F_1)
- The F_1 knob is composed of three αβ dimers arranged around a central stalk (the "rotor") consisting of the γ, δ, and ε subunits



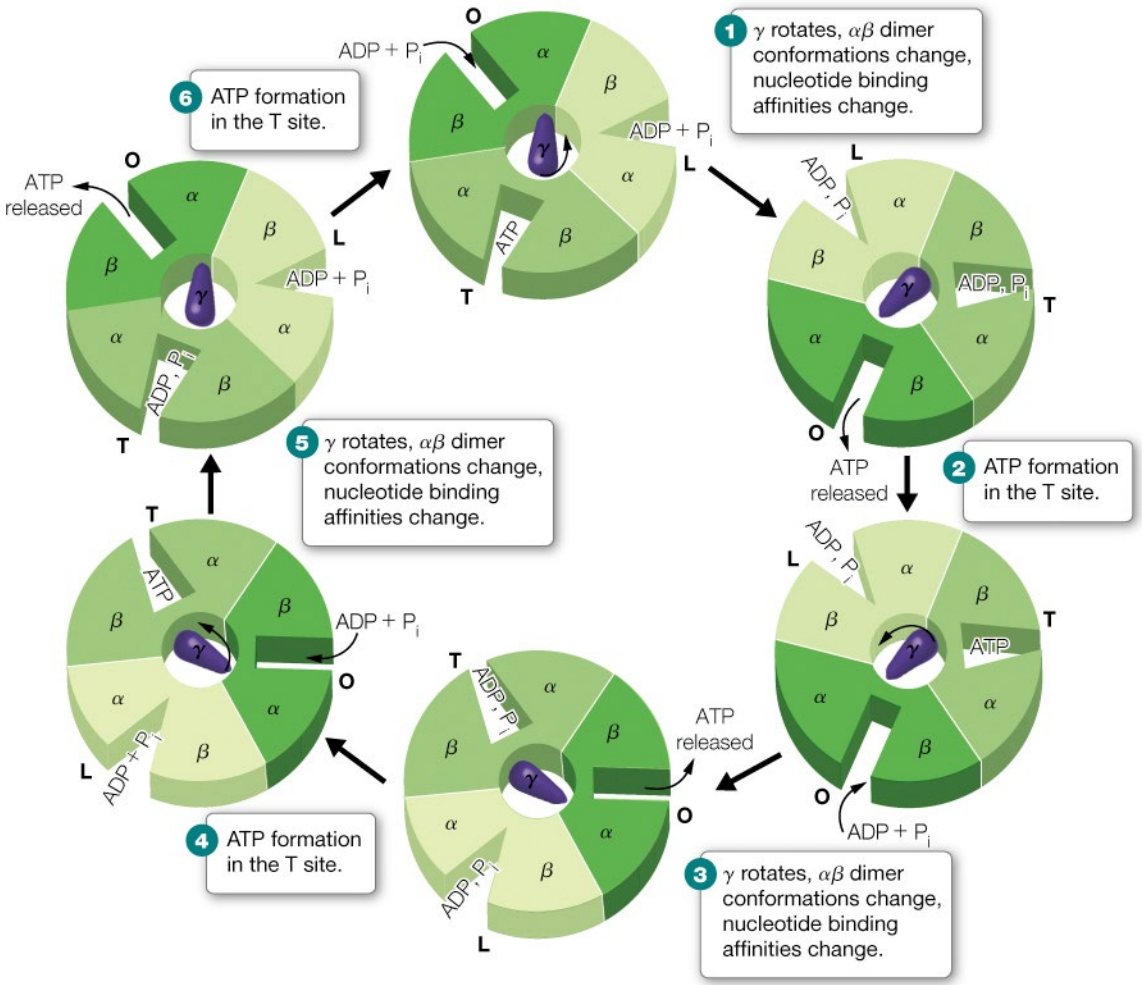
(a) The F_0F_1 complex, also called ATP synthase or complex V, contains an F_1 knob that projects into the mitochondrial matrix and is connected by a central stalk to the F_0 base. The globular F_1 knob contains three $\alpha\beta$ dimers, arranged about the central stalk composed of the γ, δ, and ε subunits, also part of the F_1 complex. The central stalk and the c-ring of the F_0 complex compose the "rotor" of ATP synthase. The remainder of the F_0 subunits (a, b, d, F_6 and OSCP) make up the "stator," a structure that prevents the rotation of the three $\alpha\beta$ dimers of F_1. This model is based on the X-ray crystal structures of the yeast and bovine mitochondrial F_0F_1 complex.



(b) The yeast mitochondrial F_0F_1 X-ray structure has been superimposed on cyroelectron microscopy reconstructions of the bovine complex. The subunits are colored as in panel (a).

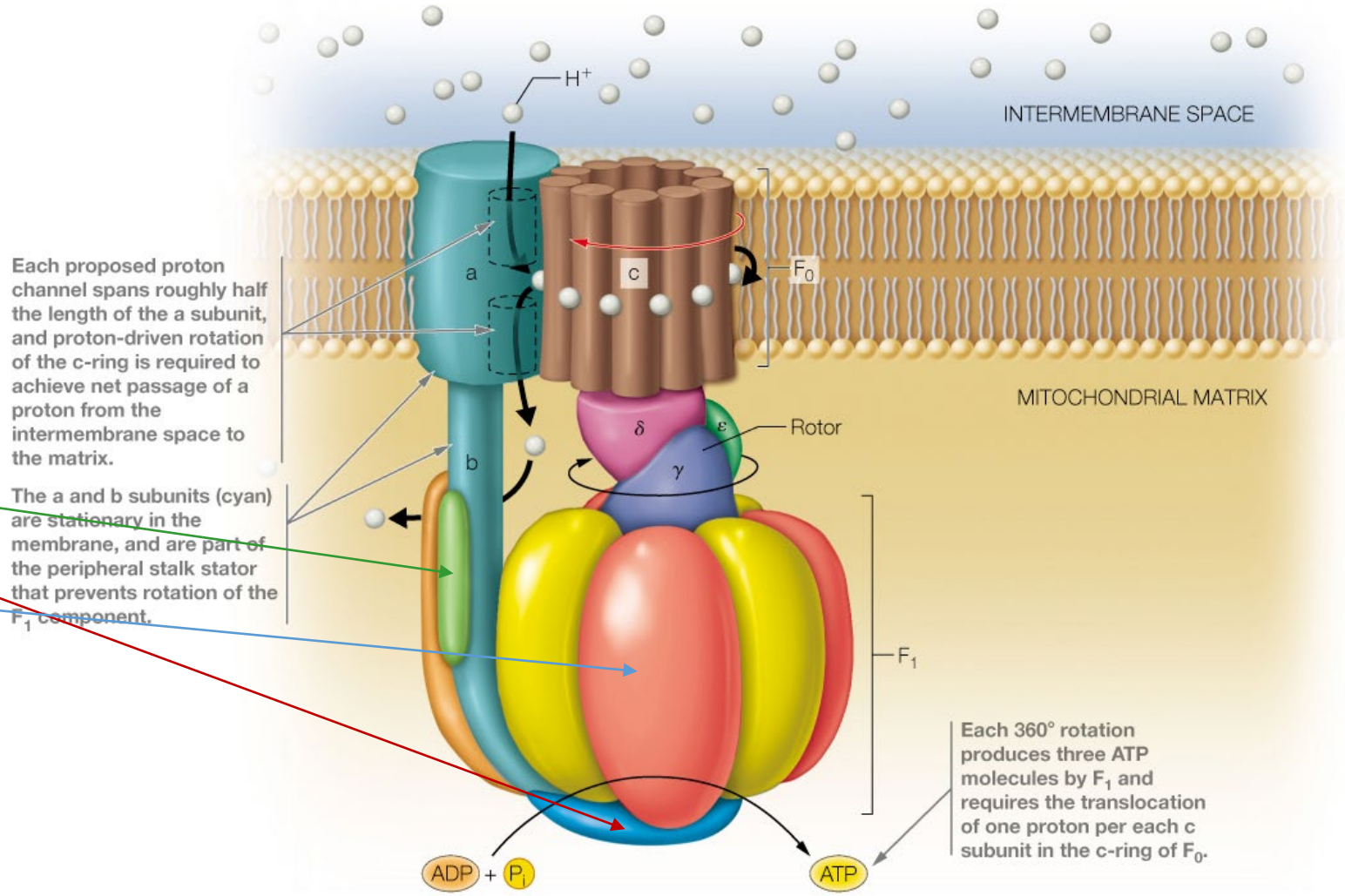
Binding-Change Model for ATP Synthase

- The three αβ dimers of F_1 exist in three different conformations, L (loose), T (tight), and O (open)
- The γ subunit rotates counterclockwise (driven by the passage of protons through F_0) while the F_1 components are held in a fixed position by the stator
- As the γ subunit rotates, it interacts differently with each subunit and simultaneously causes conformational changes in all three dimers
- These conformational changes allow for the binding of ADP and P_i, the synthesis of ATP, and the release of ATP



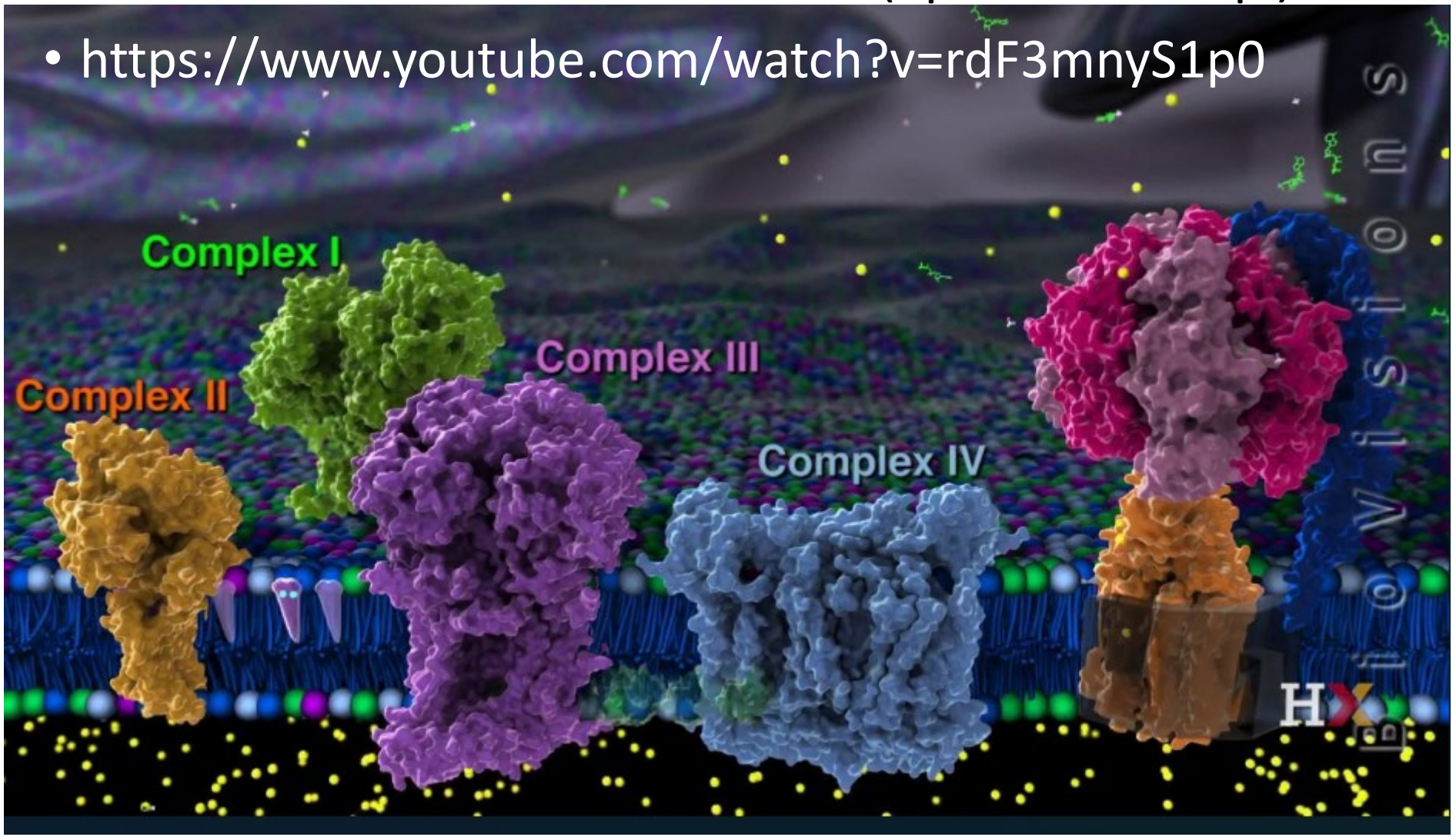
Proton Gradient and ATP Synthesis

- ATP synthase is different from a standard motor: the rotating part is in the membrane
- The central stalk and the c-ring of the F_0 complex compose the "rotor" of ATP synthase. This rotates as protons enter from the intermembrane space to the matrix.
- The remainder of the F_0 subunits (a, b, d, F_6 and OSCP: oligomycin sensitivity conferral protein) make up the "stator," a structure that prevents the rotation of the three $\alpha\beta$ dimers of F_1.
- This model is based on the X-ray crystal structures of the yeast and bovine mitochondrial F_0F_1 complex.



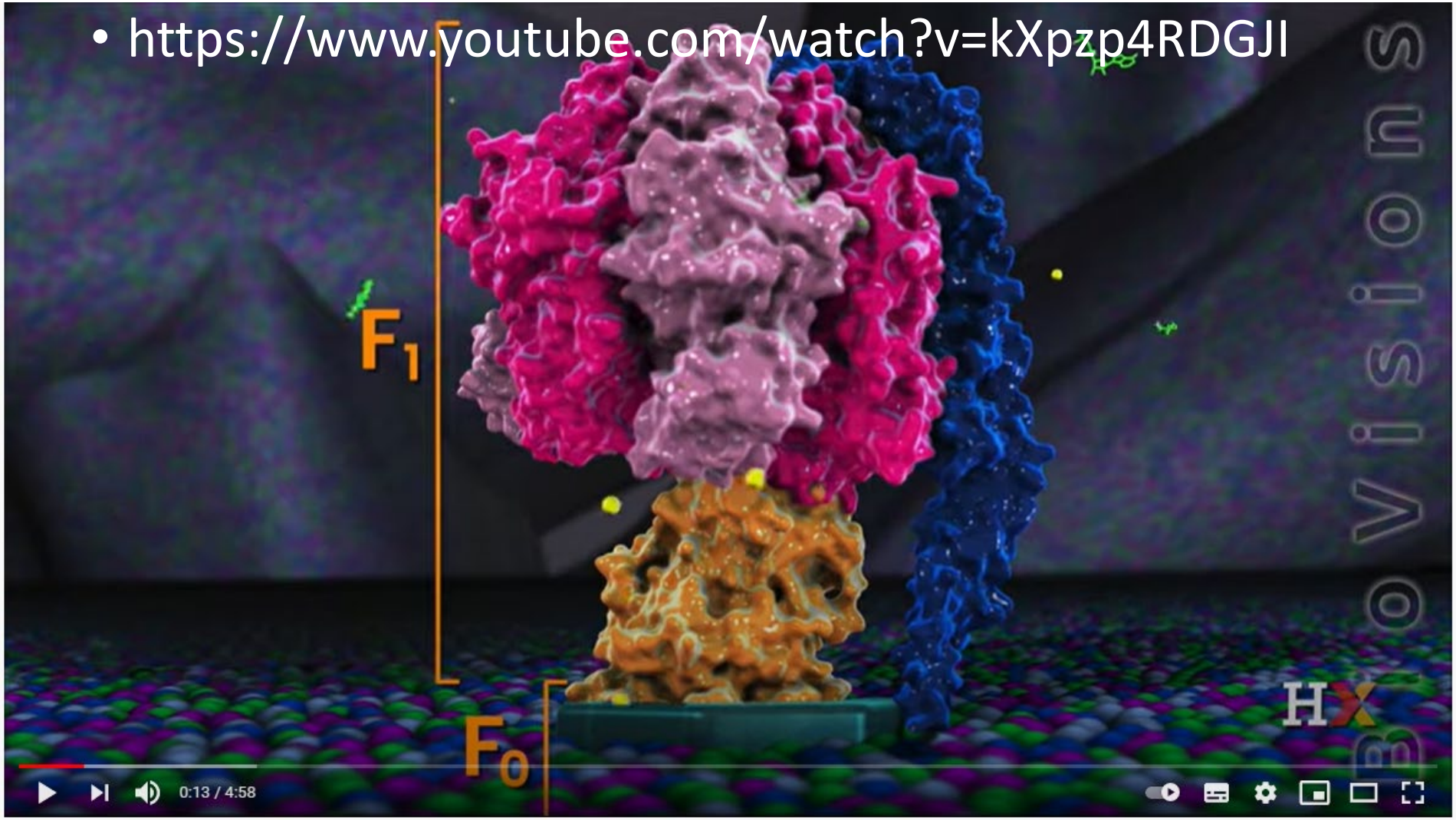
Youtube video on ETC! (quick recap)

- https://www.youtube.com/watch?v=rdF3mnyS1p0



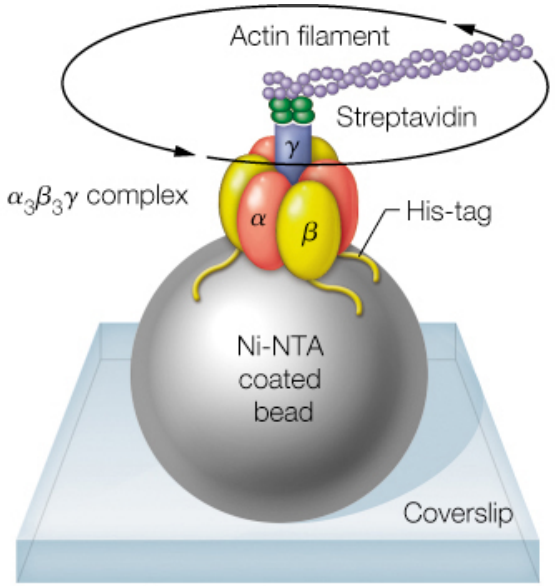
Youtube video on OxPhos!

- https://www.youtube.com/watch?v=kXpzp4RDGJI



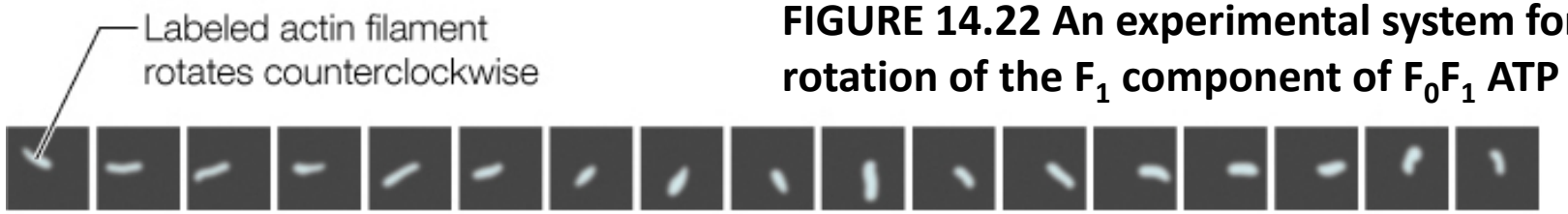
Evidence for the Rotation of the γ Subunit

- The cloned gene encoding $F_1\beta$ subunit was modified with a His-tag to allow binding to a nickel-coated bead immobilized on a glass coverslip
- A fluorescent-labeled actin filament was attached to the γ subunit
- The addition of ATP to this complex caused the hydrolysis of ATP, and the rotation of the γ subunit within the complex was visualized by fluorescence microscopy (below)



(a) The cloned gene encoding the F_1 β subunit was modified by adding a sequence coding for a polyhistidine tag (His-tag) that allows binding to a nickel-coated bead (Ni-NTA coated bead). After in vitro assembly with α and γ subunits, the F_1 complex was immobilized on the bead and attached to a glass coverslip. Streptavidin is a protein used to couple fluorescent-tagged actin to the γ subunit.

FIGURE 14.22 An experimental system for observing rotation of the F_1 component of F_0F_1 ATP synthase.

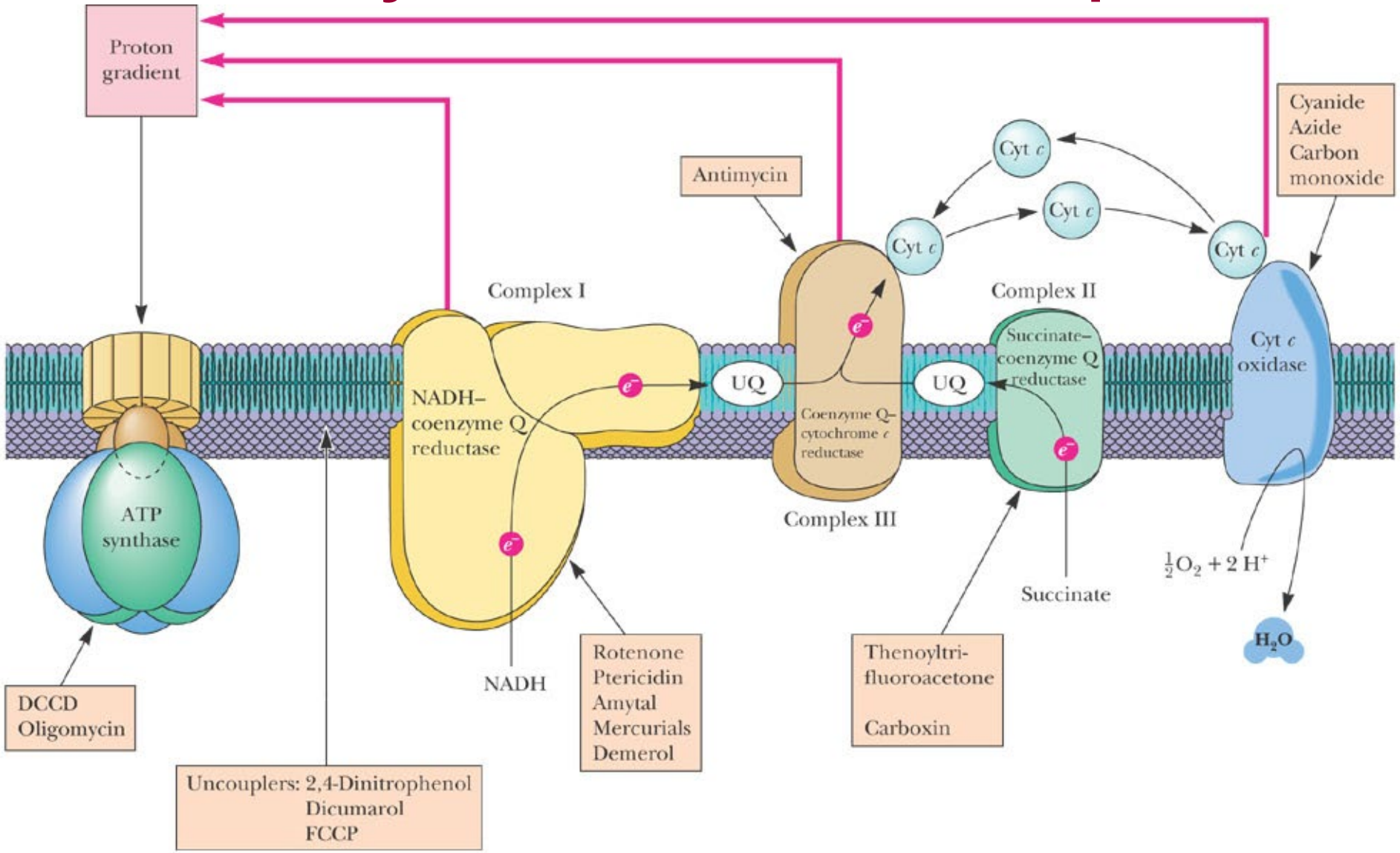


(b) Fluorescence microscopic examination showed that, following addition of ATP and its hydrolysis by the $\alpha\beta$ catalytic subunits, the actin molecule rotated, which proved that the γ subunit itself was rotating.

P/O ratio measures ATP synthesized per mole of oxygen reduced

- P/O (or the ADP/O) ratio is the number of ATP molecules made for each pair of electrons transferred down the electron transport chain to O_2
- Experiments that give P/O show how much ATP we get from each substrate:
  - **NADH: about 2.5 (10 protons required)**
  - **Succinate/FAD: about 1.5 (6 protons required)**
  - **Ascorbate: 1.0 (4 protons required)**
  - **glucose: 32 ATPs (counting glycolysis, CAC, ETC and OxPhos)**

Summary: Inhibitors and Uncouplers



Summary of electron and proton flow in ETC/OxPhos

Electron flow

- Complex 1 to CoQ
- Complex II to CoQ
- CoQ to Complex III
- Complex III to Cyt c
- Cyt c to Complex IV

Proton flow

- Matrix to inner membrane space
    1. Complex I
    2. Complex III
    3. Complex IV
- Inner membrane space to matrix
    - Complex V

Summary of ETC/OxPhos chemical reactions:

4 redox reactions

- Complex I: NADH oxidised and FMN (flavin mononucleotide) reduced"

  $2\ NADH + FMN \rightarrow 2NAD^{+} + FMNH_2$

- Complex II: succinate oxidised and FAD (flavin adenine dionucleotide) reduced

  $succinate + FAD \rightarrow fumarate + FADH_2$

- *Complex III: no chemical reaction*
- Complex IV: cytochrome c oxidised and oxygen reduced

  $red.\ CytC + ½\ O_2 \rightarrow CytC + H_2O$

- Complex V: ADP oxidised and phosphate (P_i) reduced

  $ADP + P_i + 4H^{+} \rightarrow ATP + 2\ H_2O$

Control of oxidative mechanism

- An adult human requires between 200-250 ATP molecules a day for metabolism
- Yet, the total amount of ATP present is <0.1 mol. Thus ATP is constantly used up as it is generated.
- When more ATP is required suddenly, it needs to be generated rapidly – this leads to control of OxPhos.

Substrate and Product level control

Low cell charge

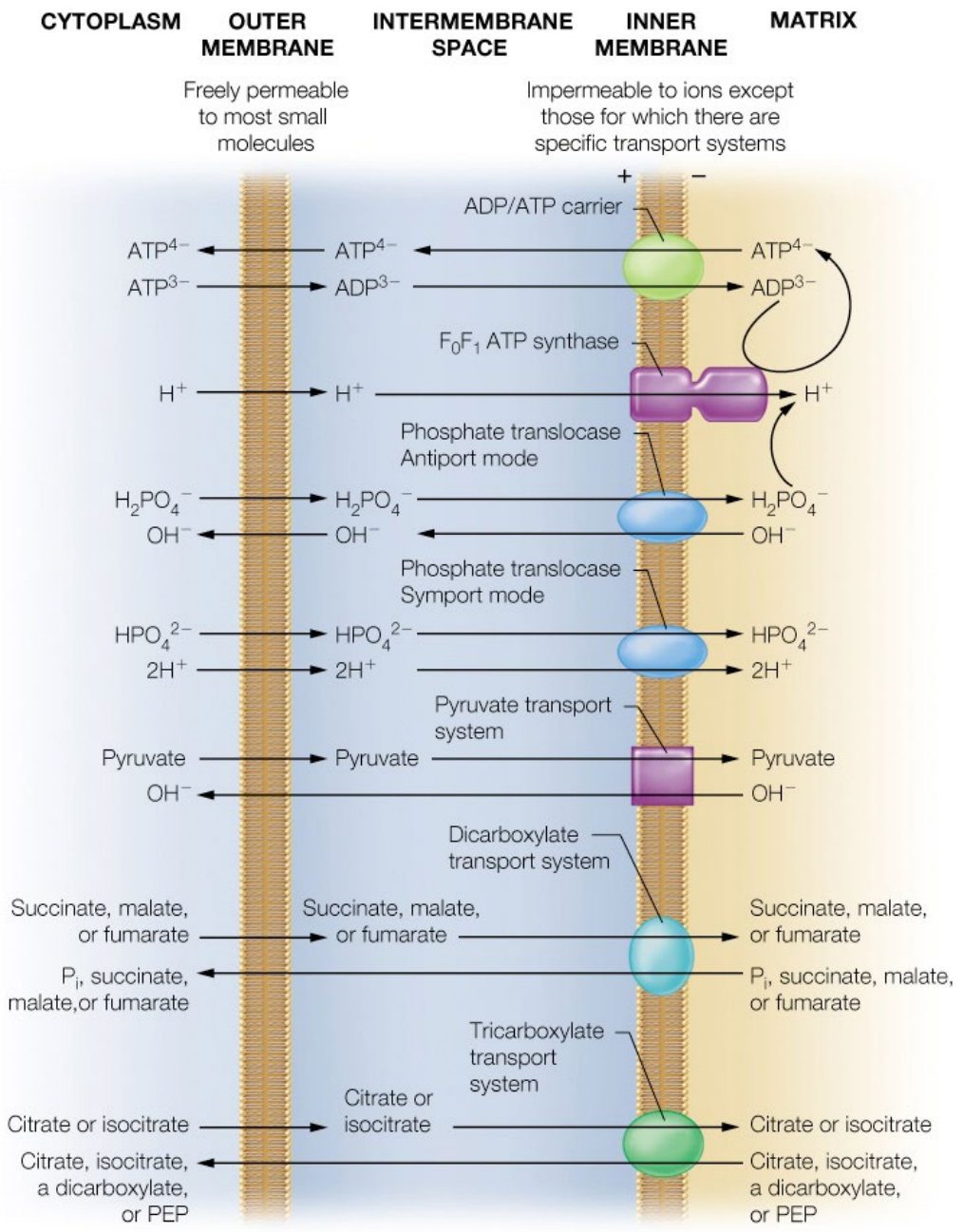
- High [NADH]/[NAD^+] leads to greater the Cyt *c* oxidase activity: more respiration
- Lower the [ATP]/[ADP][P_i]; greater the Cyt *c* oxidase activity: more respiration

Substrate concentration

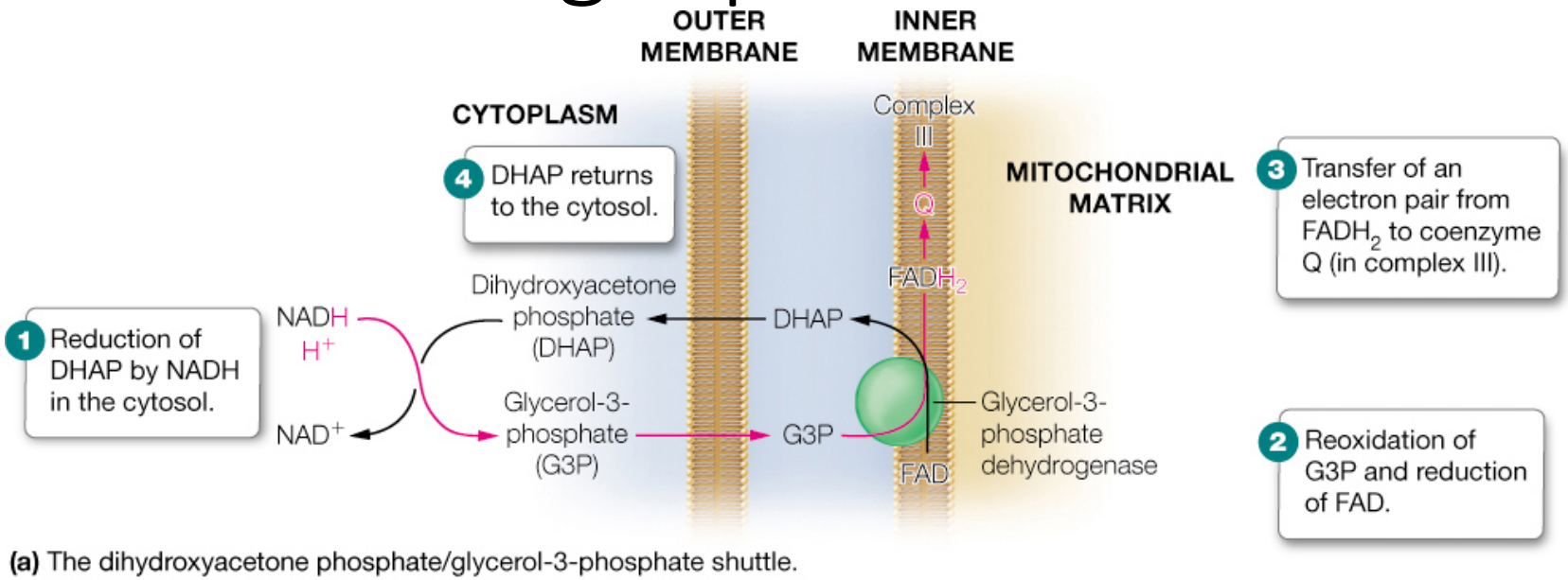
- Regulation of glycolysis, gluconeogenesis and citric acid cycle control [NADH] and [ATP] available to ETC/OxPhos
- Fatty acid degradation (later lectures) also provides CAC metabolites and thus regulate ETC/OxPhos indirectly.

Transport Through Mitochondrial Membranes

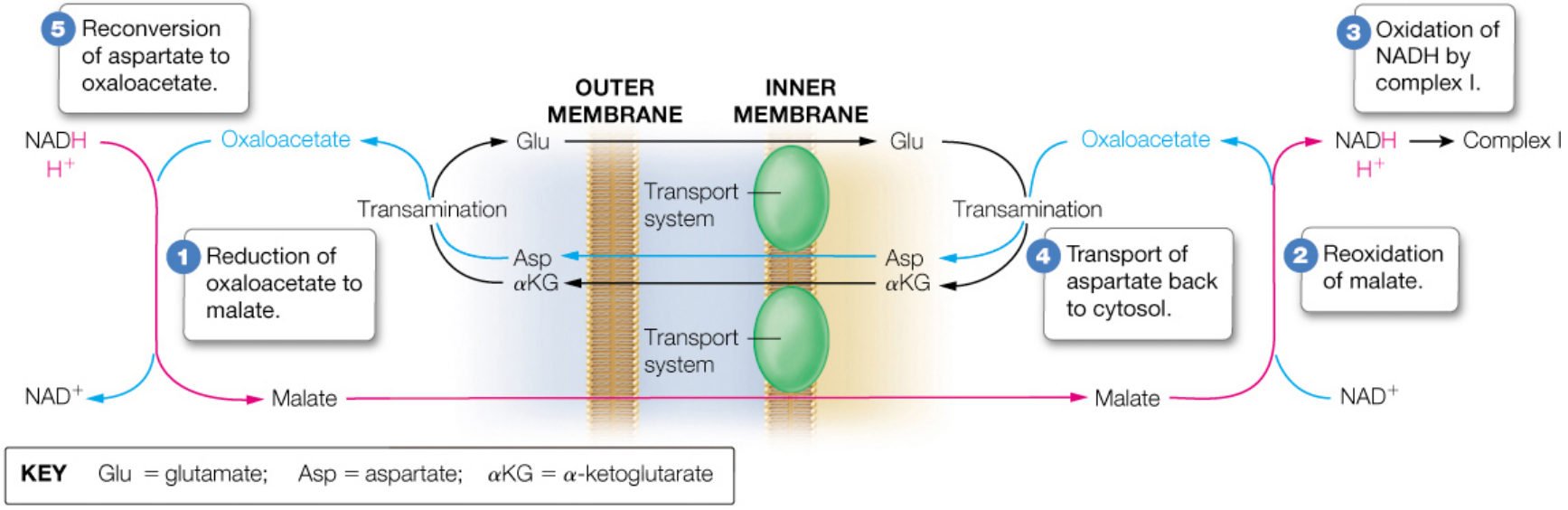
- The outer mitochondrial membrane is freely permeable to most small molecules up to about 5000 Da, but inner membrane permeability is severely limited
- Thus, transport across the inner membrane requires a transport system
- As the matrix is negatively charged relative to the cytoplasm, transporters utilize cotransport with protons or exchange with hydroxide ion to import metabolites



Transport of Reducing Equivalents

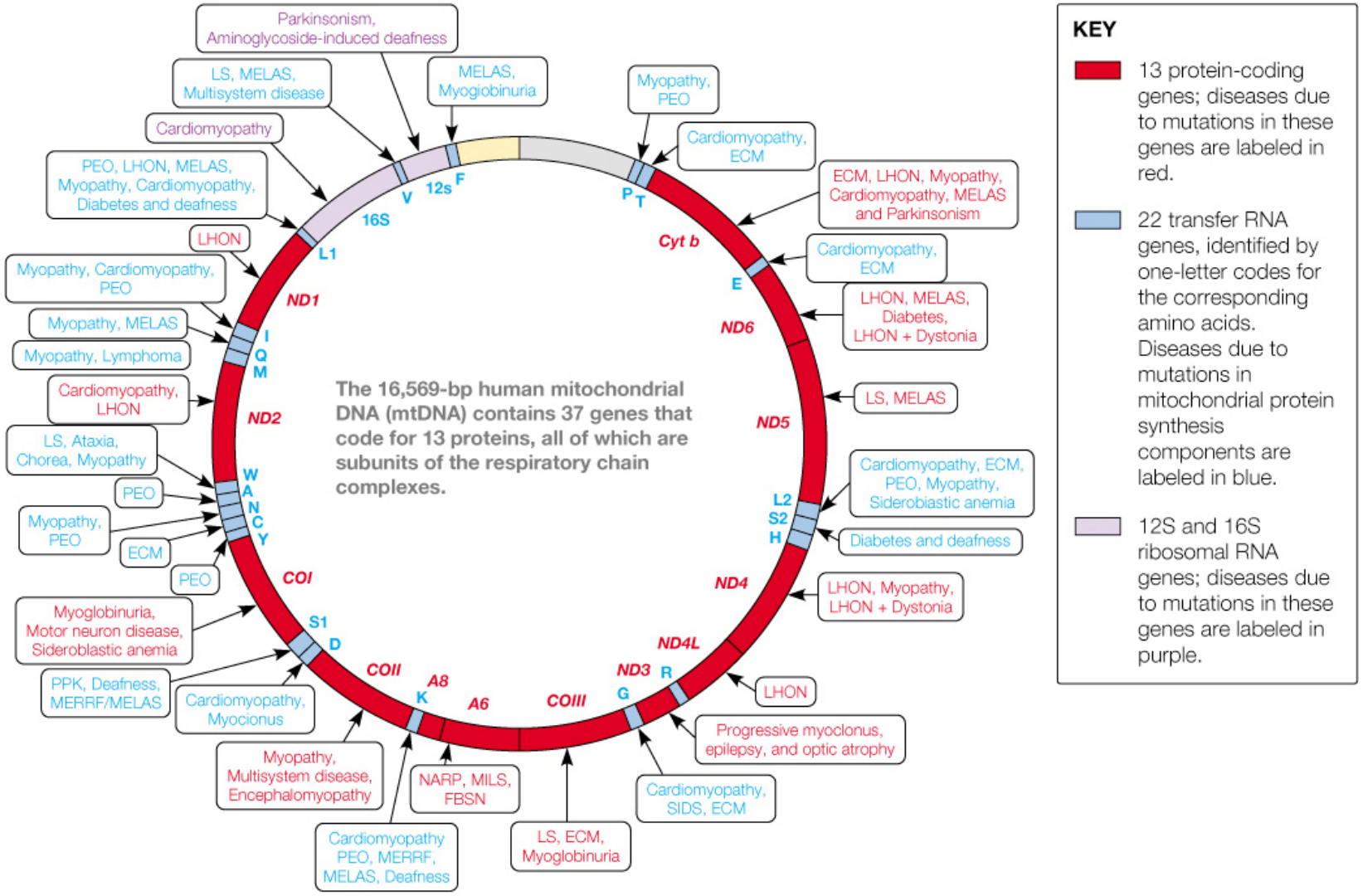


(a) The dihydroxyacetone phosphate/glycerol-3-phosphate shuttle.



(b) The malate/aspartate shuttle.

Morbidity Map of Human Mitochondrial DNA

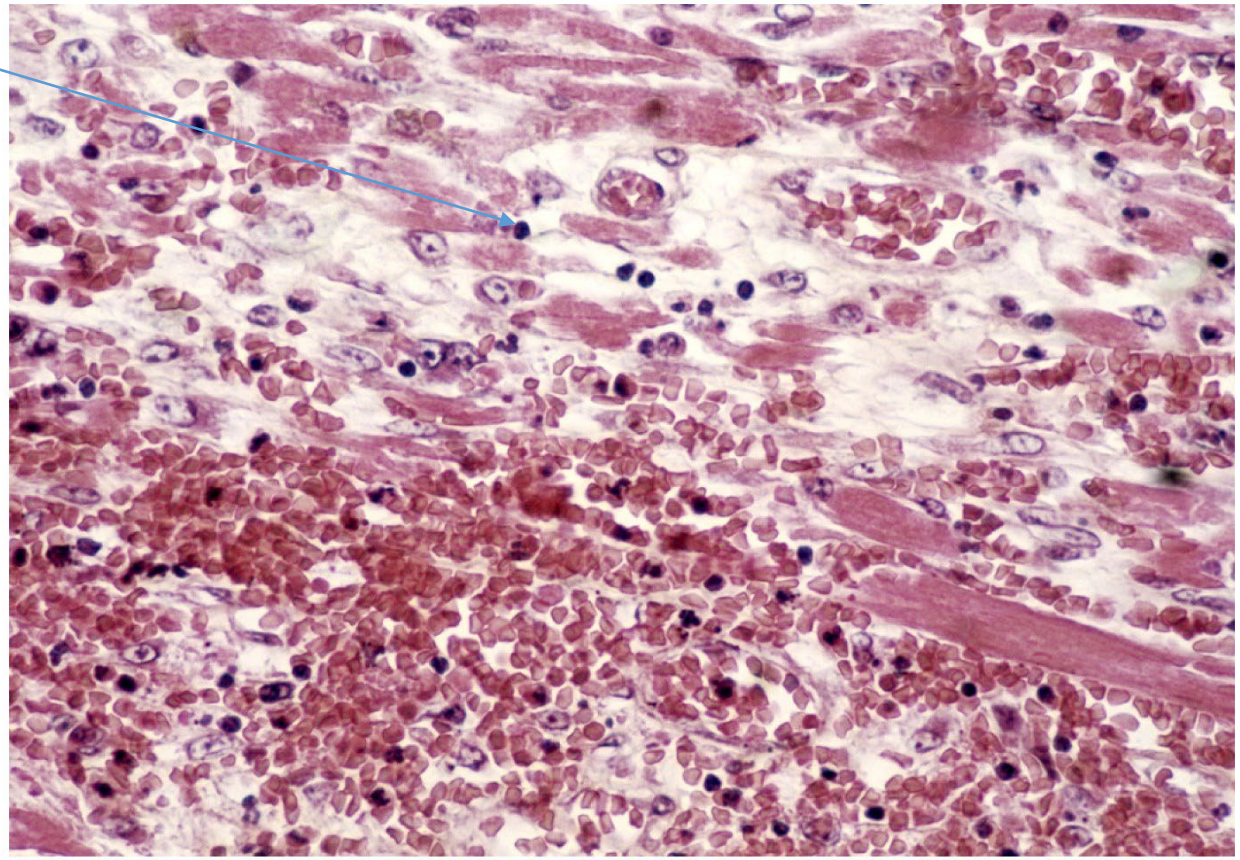


(b) A morbidity map of the human mitochondrial genome.

MACQUARIE University

Disadvantages of aerobic metabolism

- Although the aerobic metabolism of glucose is 16 times more efficient than anaerobic glycolysis, oxygen deprivation renders cells irreversibly damaged: heart attack/stroke leads to necrosis



Box 18-5

Production of reactive oxygen species

- Occasionally oxygen is partially reduced, making the reactive oxygen species (ROS), containing the superoxide radical:

$$O_2 + e^- \rightarrow O_2^-\bullet$$

- This leads to the formation of other radical species – all of them can extract electrons from other molecules, leading to chain reactions.
- **Several degenerative diseases:** Parkinson's, Alzheimer's and Huntington's diseases, are associated with oxidative damage to mitochondria.
- Oxidative damage is also associated with the aging process.

Cellular antioxidant is the enzyme, Superoxide dismutase

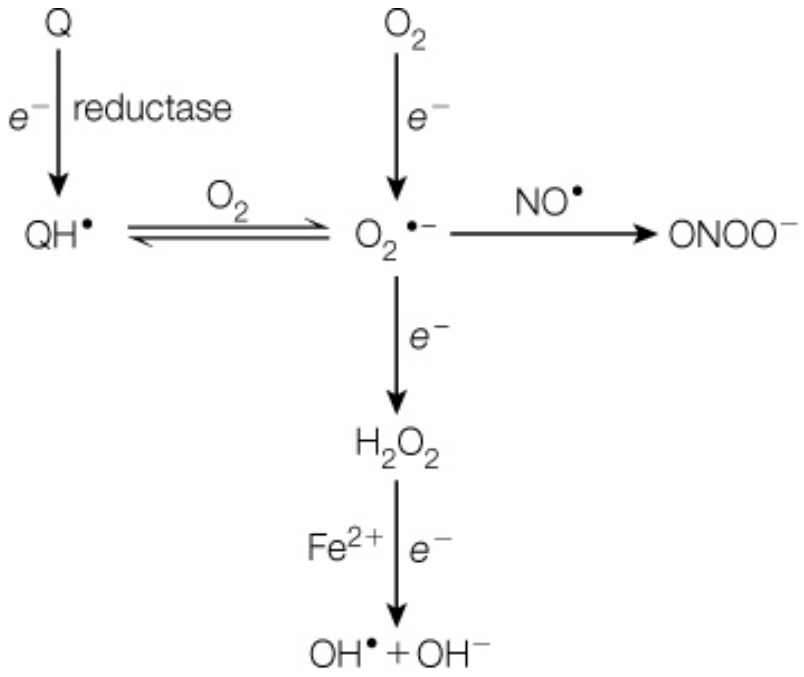
- The enzyme superoxide dismutase (SOD), present in nearly all cells, stops reactive oxygen species and make them harmless:

$$2O_2^{-}\cdot + 2H^{+} \rightarrow H_2O_2 + O_2$$

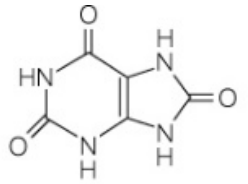
- Mitochondrial SOD is an Mn-containing tetramer, while eukaryotic cytosolic SOD is a dimer containing copper and zinc ions.
- SOD is our first line of defence against ROS.

Oxidative Stress and Reactive Oxygen Species

Oxidases often generate reactive oxygen species (ROS), which can damage biological molecules

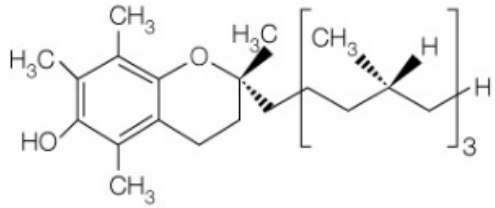


Organisms may prevent biological damage through antioxidant compounds (nonenzymatic means) or enzymatic means such as superoxide dismutase (SOD)



Uric acid

From nucleic acid breakdown



α-Tocopherol (vitamin E)

The antioxidant vitamin E

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2$$

The SOD reaction

ETC + OxPhos summary

- Oxidative phosphorylation converts most of the energy captured from oxidative reactions to ATP
  - this process takes place in the mitochondria
- ATP synthesis during oxidative phosphorylation is coupled to the electron transfer from NADH and $FADH_2$ to O_2
- This electron transfer is performed by the respiratory chain, comprising of four multisubunit protein complexes called complexes I–IV as well as ubiquinone/ubiquinol and cytochrome *c*

ETC + OxPhos summary - 2

- Complex I, III, and IV pump protons from the mitochondrial matrix across the inner membrane, generating an electrochemical gradient termed proton motive force
- This proton motive force drives the synthesis of ATP from ADP and P_i by ATP synthase (complex V)
  - about 1.5 molecules of ATP are produced per molecule of $FADH_2$ oxidized, while about 2.5 molecules of ATP can be obtained per molecule NADH oxidized
- Overall 32 ATPs are generated from 1 molecule of glucose via glycolysis, citric acid cycle and ETC/Oxphos.