Mitochondrial Membrane Transport

- **membrane = impermeant** to most everything, esp to H+
- **outer membrane** - porins - molecules 5,000 -10,000d
- **inner membrane** - 70% protein & 30% lipid
  - a. redox proteins of ETC
  - b. ATP synthase
  - c. carrier proteins - phosphate translocases
    - ADP/ATP translocases,
    - pyruvate/H+ symporter 13.16
  - d. glycerol-P & malate shuttles

How Electron Transfer Works

- **REDOX POTENTIAL** (how measured – panel 13.1)
  - empirical measure of tendency to gain e’s
  - strong reducing agent has negative -ΔE₀’
  - strong oxidizing agent has positive +Δ E₀’
  - \( \Delta G_0' = -nF \Delta E_0' \)
  - \( \text{NADH} \rightarrow \text{NAD}^+ + H^+ + 2e^- \) \(-0.32V\)
  - \( \text{H}_2\text{O} \rightarrow \text{O}_2 + 2\text{H}^+ + 2e^- \) \(+0.82V\)
  - \( \Delta G_0' = -(1)(0.023) (1.14) = - 26.2 \text{ Kcal} \)

- **Electron Transfer Chain’s Order**
  - Increasing Redox Potential (from - to +)
  - see fig 12.18 p500
**Components of the ETC**

- **Pyridine nucleotides** NAD⁺ 2.33
  - enzyme bound hydrogen carriers
  - accepts 2e’s and/or protons
  - shows spectral shift @ 340nm

- **Flavoproteins** FMN & FAD 2.33b
  - protein bound hydrogen carriers
  - spectral shift @ 340, 370, & 460 nm

- **Iron sulfur proteins** FeS 12.14b p495
  - non-heme iron electron carriers

- **Ubiquinone** CoQ 12.15 p496
  - semiquinone & hydroquinone
  - mobile membrane bound non-protein hydrogen carriers

- **Cytochromes** (a, a₃, b₅₆₂, b₅₆₆, c₁, c) 12.14 p495 & above
  - “colored proteins” with bound Fe atoms [ferric vs. ferrous]
  - iron porphyrin (heme) bound protein carriers

**How Oxidative Phosphorylation Works – fig 12.18 p500**

**Respiratory Assemblies - Mitochondrial Components**

- **Respiratory Assemblies:**
  - NADH-Q reductase
  - Succinate dehydrogenase
  - Cytochrome-C-Reductase
  - Cytochrome Oxidase

- **Proton Motive Force:**
  - an electrochemical concentration gradient of protons across a membrane coupled to ATP synthase to make ATP... likened to process of osmosis, the diffusion of water across a membrane thus chemiosmosis.

  \[ \Delta pH = 1.0 - 1.4 \text{ pH units} \]

  \[ \Delta \text{charge} = 140\text{mV in}(-) \text{ vs. out}(+) \]

- **ATP Synthase:**
  - creates a hydrophilic channel for H⁺ flow makes 100 ATP per 300 H⁺ per sec [ADP + Pi → ATP]
  - Fo – membrane piece & stalk
  - F1 – soluble piece; 5 proteins rotational models

**Oxidative Phosphorylation - Making of ATP**

- **Synthesis of ATP made via a proton motive force**
  - H⁺ gradient generated by transfer of e’s
  - H’s passed to O₂ to make H₂O through series of redox proteins

- **Mechanism - Chemiosmotic Coupling - Mitchell 1961**
  - fundamental mechanism - arose early in evolution - was retained
  - 3 steps

  - ETC - passage of e through membrane carrier proteins
    - electron flow (hydride ion H⁻ → H⁺ + 2e⁻)
    - generates a proton motive force gradient (pH difference)
    - pH = 1.0 units [8.0 matrix vs. 7.0 peri-mito. space & a membrane potential - charge [140mV in(-) out(+)]
    - ATP Synthase - which links ADP & P... making ATP
      - uncouplers as DNP destroy H⁺ gradient = no ATP
**ATP Synthase Structure...**

'mushroom' shaped complex composed of 2 membrane subunits

**F1 (extrinsic) & F0 (intrinsic)**

Humberto Fernandez (60's) sees lollipops on inner mito membranes

Efraim Racker (1966) isolates lollipop - Coupling Factor 1 - F1

ATP synthase of liver mitochondria 

= about 15,000 present

F1 5 polypeptides (nuclear DNA): 3α, 3β, 1γ, 1ε, and 1c

arranged like sections of grapefruit

3 catalytic sites for ATP synthesis

- one on each β subunit

F0 3 polypeptides in ratio of:

1a, 2b, and 12c (C-ring)

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**Binding Charge Mechanism of ATP Synthesis - A Rotary Motor**

1. H⁺ movement changes binding affinity of synthase's active site, thus when ADP & P bind to active site, they readily condense into ATP (removed from aqueous solution $K_{eq} = 1$ and $\Delta G$ close to zero, thus ATP forms easily)

2. active site (β subunits) changes conformation thru 3 successive shapes:

   L - loose - ADP & P loosely bound to site

   T - tight - ADP & P tightly bound favoring condensation without water

   O - open - site has low affinity to bind ATP - thus releases it

3. conformational changes result in rotation of subunits relative to central stalk (γ)

   α & β subunits of F1 form hexagonal ring that rotates around central axis

   γ stalk extends from Fo & interacts with 3 β's differently as it rotates 360°

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**Pathway of the Protons through Fo**

- rotational model of C-ring & γ stalk

12 C-proteins reside in lipid bilayer (C-ring)

C-ring is attached to γ stalk of F1

H⁺ diffuse through Fo rotating the 12c's of Fo ring

each C protein has a half-channel space with a charged ASP-

C's bind H⁺ (& via shape changes) C-rotates 30° CCW

next C in ring picks up H⁺ & thus the ring cycles thru 360°

release of H⁺ into matrix happens at end of cycle

Karp 5.29°

4 H⁺ moves ring 120° (γ stalk) shifts 120° --> β's change

4 H⁺ result in ATP being made

rotation of C-ring drives γ stalk through 360° & 3 conformations of F1 (L-T-O) to make ATP

Biovisions animation of ATP synthase