Bil 255 – Spring Semester

electron transport chain
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

holds

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**

- **mtDNA** - 16,500+ np's... code for 20% mito proteins
  including: **cyto-oxidase subunits I, II, III**
  ATP synthase units 6 & 8
  5 subunits of NADH dehydrogenase
  and 22 tRNA's and 2 rRNA's
mito-DNA... 16,500+ np's...
codes for 20% of mitochondrial proteins (13)

including: cyto-oxidase subunits I, II, III
ATP synthase units 6 & 8
5 subunits of NADH dehydrogenase
and 22 tRNA's and 2 rRNA's

1,000's copies per cell; maternally inherited; frequent point mutations; sequence analysis

mtDNA & Human Evolution
forensic uses of Mito-DNA

genetic variation among peoples
mitochondrial diseases
How Electron Transfer Works

- **REDOX POTENTIAL** (how measured – panel 13.1)
  - empirical measure of tendency to gain e's
  - strong reducing agent has negative -$\Delta E_0'$
  - strong oxidizing agent has positive +$\Delta E_0'$

  $$\Delta G_0' = -nf \Delta E_0'$$

  NADH $\rightleftharpoons$ NAD$^+$ + H$^+$ + 2e$^-$
  -0.32V

  H$_2$O $\rightleftharpoons$ O$_2$ + 2H$^+$ + 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 13.21 p426
Components of the ETC

- **Pyridine nucleotides** \( \text{NAD}^+ \) 3.28
  enzyme bound hydrogen carriers
  accepts 2e's and/or protons
  shows spectral shift @ 340nm

- **Flavoproteins** \( \text{FMN} \& \text{FAD} \) 4.12b
  protein bound hydrogen carriers
  spectral shift @ 340, 370, & 460 nm

- **Iron sulfur proteins** \( \text{FeS} \) 13.22 p426
  non-heme iron electron carriers

- **Ubiquinone** \( \text{CoQ} \) 13.20 p425 semiquinone & hydroquinone
  mobile membrane bound non-protein hydrogen carriers

- **Cytochromes** \( (a, a_3, b562, b566, c1, c) \) 13.23 p427 & above
  “colored proteins” with bound Fe atoms [ferric vs. ferrous]
  iron porphyrin (heme) bound protein carriers

*How Oxidative Phosphorylation Works – fig 13.13 p417*
Respiratory Assemblies

Mitochondrial Components

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

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 gradient generated by transfer of e's 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

1. **ETC** - passage of e thru membrane carrier proteins
electron flow (hydride ion H:\( \text{H}^+ + 2e^- \))

2. 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(+)]

3. **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)**
Humbeto 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, 1δ, & 1ε
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)
Binding Charge Mechanism of ATP Synthesis - A Rotary Motor  

Paul Boyer – 1979

1. H+ movement changes binding affinity of synthases’s active site, thus when ADP & P bind to active site, they readily condense into ATP (removed from aqueous solution Keq = 1 and ΔG close to zero, thus ATP forms easily)

2. active site (β subunits) changes conformation through 3 successive shapes (L-T-O)
   - 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°
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 30o CCW
next C in ring picks up H+ & thus the ring cycles thru 360o
release of H+ into matrix happens at end of cycle Karp 5.29*

4 H+ moves ring 120o (γ stalk) shifts 120o → β's change
4 H+ result in ATP being made

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