5/16/06  The Krebs cycle

MOVING INTO THE MITOCHONDRIA IN EUKARYOTIC CELLS

NOTE: THESE PROCESSES OCCUR IN PROKARYOTIC CELLS TOO
In fact, prokaryotes invented them!
The relationship between mitochondria and microtubules

a. Light micrograph of elongated mitochondria in a living mammalian cell in culture

b. Same cell stained with a fluorescent dye that binds to microtubules. Note how the mitochondria tend to be aligned with the microtubules.

Figure 14–5. Molecular Biology of the Cell, 4th Edition.
Transmission electron micrograph: cross-section of a mitochondria (what other cellular structures can you see?)

EACH MEMBRANE is a lipid bilayer
Matrix. This large internal space contains a highly concentrated mixture of hundreds of enzymes, including those required for the oxidation of pyruvate and fatty acids and for the citric acid cycle. The matrix also contains several identical copies of the mitochondrial DNA genome, special mitochondrial ribosomes, tRNAs, and various enzymes required for expression of the mitochondrial genes.

Inner membrane. The inner membrane (red) is folded into numerous cristae, greatly increasing its total surface area. It contains proteins with three types of functions: (1) those that carry out the oxidation reactions of the electron-transport chain, (2) the ATP synthase that makes ATP in the matrix, and (3) transport proteins that allow the passage of metabolites into and out of the matrix. An electrochemical gradient of $H^+$, which drives the ATP synthase, is established across this membrane, so the membrane must be impermeable to ions and most small charged molecules.

Outer membrane. Because it contains a large channel-forming protein (called porin), the outer membrane is permeable to all molecules of 5000 daltons or less. Other proteins in this membrane include enzymes involved in mitochondrial lipid synthesis and enzymes that convert lipid substrates into forms that are subsequently metabolized in the matrix.

Intermembrane space. This space (white) contains several enzymes that use the ATP passing out of the matrix to phosphorylate other nucleotides.

**Matrix:**
pyruvate dehydrogenase  
Krebs cycle (aka Citric Acid/TCA cycle)
fatty acid oxidation  
urea cycle  
replication, transcription, translation  
HUH?

**Inner membrane:**
electron transport  
oxidative phosphorylation  
transport systems

**Outer membrane:**
fatty acid elongation and desaturation  
phospholipid synthesis

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Figure 5.17  The mitochondrion
Mitochondria consist of a pair of membranes enclosing two fluid compartments: the intermembrane compartment between the outer and inner membranes and the matrix within the inner membrane. The outer membrane is smooth, but the inner membrane loops back and forth to form deep folds called cristae. Mitochondria are the site of aerobic metabolism.
Glucose
\[ \text{H}_2\text{C}=\text{O} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H} \]

Glycolysis
\[ \text{H}_3\text{C}-\text{C}-\text{C}=\text{O} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]
\[ \text{H}_2\text{C}-\text{O}-\text{H} \]

Pyruvate dehydrogenase
\[ \text{pyruvate} \rightarrow \text{pyruvate} \]
\[ \text{Krebs cycle} \]

At each exothermic step, energy is transferred in either ATP or NADH or FADH₂

6 CO₂
Fully oxidized
\[ \text{O} = \text{c} = \text{o} \]
The bridge between glycolysis and the Krebs/citric acid cycle: Pyruvate dehydrogenase

**Reaction:**
pyruvate + CoA + NAD⁺ ----> acetyl-CoA + CO₂ + NADH + H⁺

*Co A = coenzyme A = transfers an acetyl group to a substrate*

*Co A acts as an acetyl courier the same way NADH acts as an electron courier*
Text figure 6.17  Protein, carbohydrates and fats can all furnish substrates for cellular respiration. A variety of carbohydrates can be converted to glucose and processed by glycolysis. If carbohydrates are in short supply, cells can also use fats or proteins as a source of reduced compounds for ATP production.

CIRCLE AT FAR RIGHT INDICATES THE KREBS CYCLE

Krebs cycle = Citric acid cycle = TCA cycle
[TCA = tricarboxylic acid]
Hans Krebs interesting life history
1953 Nobel prize in physiology or medicine
http://www.nobel.se/medicine/laureates/1953/krebs-bio.html

A FOOTNOTE From the CALAMITOUS 20TH CENTURY

Life’s Energy Cycle*

On 14 December 1932, the dean of the medical faculty of the University of Freiburg, Professor E. Rehn, reported to the Ministry of Education,

As an assistant physician Dr. Krebs has shown not only outstanding scientific ability, but also unusual human qualities. His paper on the synthesis of urea in the animal body… will be regarded as one of the classics of medical research.

Four months later, shortly after Hitler seized power, this same dean sent him a “Notification of Immediate Removal from Office,” obediently implementing the Minister’s orders against members of the Jewish race.

Krebs kept several such letters and also various press cuttings from
KREBS CYCLE

GREAT SITE ON THE KREBS CYCLE
http://www.wiley.com/legacy/college/boyer/0470003790/animations/tca/tca.htm

http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/krebs.htm#animat1

Walk through Krebs cycle at this site:
Note that the activity of some of the Krebs cycle enzymes is controlled by allostERIC interactions
http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/krebscyc/krebscyc.htm

Krebs cycle step by step (need MIME plug-in)
http://www.beechtreecommon.org/biochemistry/tcasteps/
What does the citric acid (Kreb’s) cycle accomplish?
What does the citric acid (Kreb’s) cycle accomplish?

Carbons in pyruvate are fully oxidized to $\text{CO}_2$

A couple of ATPs are generated

8 NADH and 2 FADH$_2$ are generated
How does ATP act as a carrier of chemical energy?

ATP has stored potential energy:

\[
\text{ATP} \rightarrow \text{ADP} + P_i + \text{energy} \quad \Delta G = -7.3 \text{ kcal/mole} \quad \text{exergonic reaction (corresponds to an } K_{eq} \text{ of } >10^5
\]

Under cellular conditions, the hydrolysis of ATP creates two molecules of much lower energy and releases a great deal of usable energy

1. The phosphates in ATP can be considered to exist in an activated state: the presence of four negative charges in close proximity destabilizes the molecule -- electrostatic repulsion between negative charges favors hydrolysis
2. Increased hydration ADP and P -- energetically favored
3. Release of a phosphate increases entropy because the PO$_4$ molecule released is capable of resonance forms (delocalized proton and oxygen binding) not possible when phosphate is bound to another molecule

\[
\text{NADH} + \text{C}=\text{O} \quad \rightarrow \quad \text{NAD}^+ + \text{C}-\text{OH} \quad \Delta G = -14 \text{ kcal/mole}
\]

- NADH is also in a high energy state
- convenient source of readily transferable electrons
- NADH is less stable than NAD$^+$ because the nicotinamide ring is no longer stabilized by resonance (delocalized electrons)