Bil 255 – CMB

how cells make ATP
CELLULAR ENERGETICS

How Cells Make ATP

- Autotrophic Metabolism
  - Photosynthesis
  - Photophosphorylation

- Heterotrophic Metabolism
  - Cell Respiration
  - Oxidation of Foods
    - Aerobic & Anaerobic
    - Oxidative Phosphorylation

... primarily by phosphorylation
Primary Mechanisms of Phosphorylation

- **Substrate Level Phosphorylation**

  ![Substrate Level Phosphorylation Diagram]

  mcb 12.3 pg 482
  steps 7 & 10

- **Chemiosmosis (Oxidative Phosphorylation)**
  
  subst-H + NAD \(\rightarrow\) NADH + subst
  NADH \(\rightarrow\) H+ proton motive force \(\rightarrow\) ATP

- **Photosynthetic Phosphorylation**
  
  light + NADP \(\rightarrow\) NADPH \(\rightarrow\) H+

**Key metabolic reaction** = **REDOX Reaction**

\[ AH + BO \rightarrow A + BOH \]
\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + e's \]

Mallery Cellular energetics = ATP
Cellular Respiration

Evolution of aerobic metabolism was a major step in the history of life on planet Earth

Cell Respiration - series cytoplasmic & mitochondrial
- linked enzymatic pathways
- stepwise OXIDATION food molecules- makes ATP
  physiological view: uptake of $O_2$ & release of $CO_2$
  biochemical view: $O_2$ consumption, $CO_2$ production

3 Stages:
1. Digestion - food polymers --> monomers
2. Production of AcoA --> glycolysis & FAoxidation
3. Oxidation of AcoA to $CO_2$ & $H_2O$ --> KC & ETC
4 Cellular Pathways:

- **Glyco-lysis**
  
  \[
  \text{glucose} \rightarrow \text{pyruvate} + \text{NADH} + \text{ATP}
  \]

- **Kreb's Cycle**
  
  \[
  \text{AcoA} \rightarrow \text{CO}_2 + \text{NADH} + \text{GTP} + \text{FADH}_2
  \]

- **Electron Transport Chain (ETC)**
  
  passage of e's from NADH to \( \text{O}_2 \) \( \rightarrow \text{H}_2\text{O} + \text{H}^+ \text{ gradient} \)

- **ATP synthase**
  
  mitochondrial membrane protein which makes ATP as \( \text{H}^+ \) move into mitoplasm
GLYCO - LYSIS

Embden, Meyerhof, Parnas Pathway

Greek (glykos) - "sweet" + "splitting"

- anaerobic = no requirement of oxygen
- cytoplasmic location
- 10 step enzymatic pathway
  hexose --> 2 PYR + 4ATP (2 net) + 2NADH
  • energy investment phase (coupled Rx's)
    phosphorylation of low energy intermediates
  • energy capture phase [mcb12.3 steps 6 & 7 & 10]
  • redox reaction (glyceraldehyde3-PDH)
  • substrate level phosphorylation

Cellular energetics = ATP
GLYCO-LYSIS and Ancillary Pathways

Fates of PYRUVATE

if anaerobic - 1. alcoholic fermentation
   via alcohol dehydrogenase
   2. lactic acid respiration - LDH

if aerobic - Krebs Cycle

- **Shuttles**
  purpose to move e's from cytoplasmic NADH to mitochondrial NADH or FADH$_2$
  - glycerol-P shuttle - skeletal muscle/brain (FADH$_2$)
  - malate shuttle - liver, kidney, heart muscle (NADH)

Cellular energetics = ATP
KEY REACTIONS of GLYCOLYSIS (panel 13.1)

- substrate level phosphorylation (steps 7 & 10)
- redox reaction involving NAD (step 6)

Summary of GLYCOLYSIS

- 2 ATP to initiate pathway
- 2 substrate level phosphorylations makes 2 ATP (net), 2 NADH, and 2 PYRUVATE

Fermentations & Shuttles
Krebs Cycle, Citric Acid Cycle, Tricarboxylic Acid Cycle

a cyclical biochemical pathway resulting in aerobic oxidation of cell fuels, as CH₂O, fatty acids, & amino acids, while making CO₂, H₂O, & ATP.

HISTORY

1910's - enzymatic nature learned - dehydrogenases
1930's - substrates identified = di-COOH's
   experiments on minced flight muscle prep's
1937  - Sir Hans Krebs - citrate synthetase
   acetyl-coA + OAA ----> citrate + CoASH
1948  - cycle localized within the Mitochondria
1961  - Peter Mitchell proposes Chemiosmosis
Overall reaction:
\[
\text{acetyl-CoA} + 3\text{NAD} + \text{E-FAD} + \text{GDP} + \text{P} + 2\text{H}_2\text{O} \rightarrow \text{CoASH} + 3\text{NADH} + \text{E-FADH}_2 + \text{GTP} + 2\text{CO}_2
\]

ENZYMES of KREBS CYCLE
- 5 dehydrogenases: ISDH, \(\alpha\)KGDH, SDH, MDH, & PDH
- 2 hydrolyases: aconitatase & fumarase
- 1 thio kinase: succinyl thiokinase
- 1 synthetase: citrate synthetase
- 2 multi-enzyme complexes (f4.8 p118)
  each with 60 proteins & 5 coenzymes
  1. pyruvate dehydrogenase &
  2. alpha ketoglutarate dehydrogenase

Mallery

Cellular energetics = ATP
Key Metabolic Reactions of KREBS CYCLE

NAD is reduced
substrate level phosphorylation occurs
decarboxylation [-COOH]
acylation via CoASH

Each turn of the cycle

4 protons passed to coe's \(3\text{NADH} \& 1 \text{FADH}_2\)
2 CO2's are released

3 parts of Mitochondrial Oxidation of PYR

1. PYR --> \(CO_2 + H_2O \rightarrow NADH/FADH_2\) Krebs
2. \(e^-\) of NADH/FADH_2 --> \(O_2\) to make \(H_2O\) ETS
3. ADP + P ----> ATP Chemiosmosis

Mallery Cellular energetics = ATP 11
Pyruvate Dehydrogenase Complex

Oxidative decarboxylation of alpha-Keto acid

\[
\text{HOOC-C-CH}_3 \quad \longrightarrow \quad \text{CoA-S-C-CH}_3 \quad + \quad \text{CO}_2
\]

3 enzymes (4.8p118)

a. pyruvate decarboxylase
   12 dimers = 24 identical subunits

b. dihydrolipoyl transacetylase (reductase)
   8 triamers = 24 identical subunits, each 3 lipoates

c. dihydrolipoyl dehydrogenase
   6 dimers 12 subunits with FAD

Mallery

Cellular energetics = ATP
PDH COMPLEX

5 coenzymes

1. CoASH  ecb8.8 p309  pantothenate
2. Lipoate  lipoic acid
3. Thiamine pyrophosphate  thiamin (B1)
4. E-FAD  2.26 p54  riboflavin (B2)
5. NAD+  2.26 p54  niacin
**Mechanism of Action of PDH Complex**

**A:** pyruvate decarboxylase – thiamine pyrophosphate (TPP) removes COOH from pyruvate leaving 2 carbon fragment bound to the TPP.

**B:** lipomide reductase transacetylase – lipoate the 2 carbon group is transferred to one lipoamide arm, and then the other to position for CoASH transfer.

**C:** dihydrolipoyl dehydrogenase-CoASH, FAD, NAD+ acetyl group is transferred to CoASH; the reduced lipoamides transfer 2H's to FAD --> FADH2, and FADH2 passes H's to NAD+ --> NADH
Key Metabolic Reactions of KREBS CYCLE  [+ PDH reaction]

1. NAD is reduced \( \rightarrow \) (NADH)
2. substrate level phosphorylation occurs
   \[ \text{GDP} + \text{P} \rightarrow \text{GTP} \ (\@ \text{ATP}) \]
3. decarboxylation \( \rightarrow (-\text{COOH}) \)
4. acylation via CoASH \( \rightarrow \) (ACoA)

Each turn of the cycle:
- 4 protons passed to coe's  (3 NADH & 1 FADH2)
- 2 CO2's are released
- 1 GDP is phosphorylated to GTP (equivalent to ATP)

fig mcb6e 13.2
The complete citric acid cycle. The two carbons from acetyl CoA that enter this turn of the cycle (shadowed in red) will be converted to CO₂ in subsequent turns of the cycle: it is the two carbons shadowed in blue that are converted to CO₂ in this cycle.
FATTY ACID Metabolism [ beta-oxidation ]

Oxidation Fatty Acids to Acetyl-CoA

3 Steps in Fat Oxidation Cycle

1. oxidation of COOH end of free fatty acid
2. transport of fatty acyl-coA into mitoplasm
3. oxidation of 2 carbon fragments as AcoA
4 enzymes of beta-oxidation

1. **fatty acyl-coA ligase** (on outer mito. membranes)
   \[ \text{FA-COOH} + \text{ATP} + \text{CoASH} \rightarrow \text{FAcoA} + \text{AMP} + \text{PP} \]
   converts cytoplasmic FA to Fatty-acyl-coA

2. **carnitine acyl-transferase 1** (outer mito memb.)
   \[ \text{fattyCoA} + \text{carnitine} \leftrightarrow \text{Fatty acyl-carnitine} + \text{CoASH} \]
   transfers FAcoA to carnitine for transport across mito

3. **carnitine acyl-transferase 2** (in mitoplasm)
   \[ \text{fatty acyl-carnitine} + \text{CoASH} \leftrightarrow \text{FAcoA} + \text{carnitine} \]
   releases FAcoA inside the mitoplasm
4. fatty acyl-coA dehydrogenase \( \text{fig 13.9b} \)

“Beta-Oxidation Cycle”

Four steps for these dehydrogenase enzymes...

\begin{itemize}
  \item [a)] dehydrogenation \( w \text{ FAD} \rightarrow \text{FADH}_2 \)
  \item [b)] hydration - addition of water
  \item [c)] dehydration \( w \text{ NAD} \rightarrow \text{NADH} \)
  \item [d)] thiol clevage \( w \text{ CoASH} \)
    \begin{itemize}
      \item releases a 2c piece = \text{AcoA}
    \end{itemize}
\end{itemize}

**Net result:** each turn of the cycle shortens a long chain fatty acid by 2 carbons generating 1 \text{AcoA}, 1 \text{NADH} and 1 \text{FADH}_2
Balance Sheet Aerobic Oxidation glucose vs 6C FFA

Rule of Thumb... the P to O ratio

1 NADH (via mito ETC) = 3 ATP and 1 FADH$_2$ = 2 ATP

Cell Respiration

to start - GLYCOLYSIS

glyceraldehyde DH + 2 NADH
PGA kinase (via SLP) + 2 ATP
pyruvate kinase (via SLP) + 2 ATP

beta-OXIDATION 6C-FFA (c-c-c-c-c-c)

-1 ATP @ ligase

Krebs Cycle per each PYR

PDH - 2CO$_2$ + 2 NADH + 3 AcoA
per each AcoA

ISDH -2CO$_2$ + 2 NADH + 2 FADH$_2$ = 4 ATP
KGDH -2CO$_2$ + 2 NADH + 2 NADH
thiokinase + 2 GTP + 10 ATP
SDH + 2 FADH$_2$
MDH + 2 NADH

Totals

1 glucose = 2 PYR = 2 AcoA 
-6CO$_2$ + 2ATP + 10NADH + 2FADH2 + 2GTP

B-OX = 10 ATP
Kreb's (3 AcoA) = 72 ATP
+ 82 ATP

total ATP = 36-38
ATP via 2 AcoA alone = 24
82 - 1 = 81 - 38 = + 43 ATP net

MALLERY Cellular energetics = ATP 20
Regulation of Krebs Cycle

controls flow of intermediates [in & out] - 4.18 & 4.19

substrate availability - mass action
allosteric inhibition - PFK-1&2 & feedback inhibition model
covalent modification - reversible phosphorylation...
protein kinases & phosphoprotein phosphatases

4 key enzymes are involved in regulation...

PDH SER-P by kinase - inactive
citrate synthetase  +ADP -ATP/NADH/cit/ScoA
isocitrate dehydrogenase  +ADP/Ca^{+2} -ATP
alpha-keto gluutarate dehydrogenase  +Ca^{+2} -ScoA & NADH