Metabolism

- Sum total of all chemical reactions that occur within an organism
- Also refers to specific chemical reactions at the cellular level
Chemical reactions

- 2 factors govern fate of a chemical reaction
  - Direction
    - Many cells use ATP to drive reactions in 1 direction
  - Rate
    - Catalysts called enzyme can speed the reaction rate

\[ aA + bB \leftrightarrow cC + dD \]
Energy

- Ability to promote change
- 2 forms
  - Kinetic - associated with movement
  - Potential - due to structure or location
    - Chemical energy - energy in molecular bonds
Covalent bonds in glucose store energy.

(a) Kinetic energy

(b) Potential energy

a: © Bob Daemmrich/The Image Works
2 Laws of thermodynamics

1. First law
   - Law of conservation of energy
   - Energy cannot be created or destroyed

2. Second law
   - Transfer or transformation of energy from one form to another increases entropy or degree of disorder of a system
Increase in entropy

Highly ordered → More disordered
Change in free energy determines direction

- Energy transformations involve an increase in entropy
- Entropy - a measure of the disorder that cannot be harnessed to do work
\[ H = G + TS \]

- **H**: enthalpy or total energy
- **G**: free energy or amount of energy for work
- **S**: entropy or unusable energy
- **T**: absolute temperature in Kelvin (K)
Spontaneous reactions?

- Occur without input of additional energy
- Not necessarily fast
- Key factor is the free energy change
$$\Delta G = \Delta H - T \Delta S$$

- **Exergonic**
  - $\Delta G < 0$ or negative free energy change
  - Spontaneous

- **Endergonic**
  - $\Delta G > 0$ or positive free energy change
  - Requires addition of free energy
  - Not spontaneous
- Hydrolysis of ATP
- $\Delta G = -7.3$ kcal/mole
- Reaction favors formation of products
- Energy liberated can drive a variety of cellular processes
Cells use ATP hydrolysis

- An endergonic reaction can be coupled to an exergonic reaction
- Endergonic reaction will be spontaneous if net free energy change for both processes is negative
Glucose + phosphate $\rightarrow$ glucose-phosphate + H$_2$O
$\Delta G = +3.3$ Kcal/mole
endergonic

ATP + H$_2$O $\rightarrow$ ADP + P$_i$
$\Delta G = -7.3$ Kcal/mole
exergonic

**Coupled reaction:**
Glucose + ATP $\rightarrow$ glucose-phosphate + ADP
$\Delta G = -4.0$ Kcal/mole
exergonic
Enzymes

- A spontaneous reaction is not necessarily a fast reaction
- Catalyst - agent that speeds up the rate of a chemical reaction without being consumed during the reaction
- Enzymes - protein catalysts in living cells
Activation energy

- Initial input of energy to start reaction
- Allows molecules to get close enough to cause bond rearrangement
- Can now achieve transition state where bonds are stretched
Enzyme

Reactant molecules

Transition state

Activation energy ($E_A$) without enzyme

Activation energy ($E_A$) with enzyme

Change in free energy ($\Delta G$)

Free energy ($G$)

Progress of a reaction
Overcoming activation energy

- 2 common ways
  - Large amounts of heat
  - Using enzymes to lower activation energy
    - Small amount of heat can now push reactants to transition state
Lowering activation energy

- Straining bonds in reactants to make it easier to achieve transition state
- Positioning reactants together to facilitate bonding
- Changing local environment
  - Direct participation through very temporary bonding
Other enzyme features

- **Active site** - location where reaction takes place
- **Substrate** - reactants that bind to active site
- **Enzyme-substrate complex** formed when enzyme and substrate bind
Substrate binding

- Enzymes have a high affinity or high degree of specificity for a substrate
- Used the example of a lock and key for substrate and enzyme binding
- Induced fit - interaction also involves conformational changes
Other requirements for enzymes

- Prosthetic groups - small molecules permanently attached to the enzyme
- Cofactor - usually inorganic ion that temporarily binds to enzyme
- Coenzyme - organic molecule that participates in reaction but left unchanged afterward
Enzymes are affected by environment

- Most enzymes function maximally in a narrow range of temperature and pH
- Outside of this narrow range, enzyme function decreases
Overview of metabolism

- Chemical reactions occur in metabolic pathways
- Each step is coordinated by a specific enzyme
- Catabolic pathways
  - Result in breakdown and are exergonic
- Anabolic pathways
  - Promote synthesis and are endergonic
  - Must be coupled to exergonic reaction
Enzyme 1  Enzyme 2  Enzyme 3

Initial substrate  Intermediate 1  Intermediate 2  Final product

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Catabolic reactions

- Breakdown of reactants
- Used for recycling
- Used to obtain energy for endergonic reactions
  - Energy stored in energy intermediates
    - ATP, NADH
2 ways to make ATP

1. Substrate-level phosphorylation
   - Enzyme directly transfers phosphate from one molecule to another molecule

2. Chemiosmosis
   - Energy stored in an electrochemical gradient is used to make ATP from ADP and $P_i$
Redox

- Oxidation
  - Removal of electrons
- Reduction
  - Addition of electrons
- Redox
  - Electron removed from one molecule is added to another
\[
Ae^- + B \rightarrow A + Be^-
\]

- **A**
  - Has been oxidized
  - Electron removed
- **B**
  - Has been reduced
  - Electron added
Energy intermediates

- Electrons removed by oxidation are used to create energy intermediates like NADH
- NAD⁺ Nicotinamide adenine dinucleotide
- NADH…
  - Oxidized to make ATP
  - Can donate electrons during synthesis reactions
Two electrons are released during the oxidation of organic molecules.

The 2 electrons and H⁺ are then added to this ring, which now has 2 double bonds instead of 3.

Nicotinamide

Nicotinamide adenine dinucleotide (NAD⁺)

NADH (an electron carrier)
Anabolic reactions

- Biosynthetic reactions
- Make large macromolecules or smaller molecules not available from food
Many proteins use ATP as a source of energy

- Each ATP undergoes 10,000 cycles of hydrolysis and resynthesis every day
- Particular amino acid sequences in proteins function as ATP-binding sites

- On average, 20% of all proteins bind ATP
The energy to make ATP comes from catabolic reactions that are exergonic.

ATP hydrolysis provides the energy for cellular processes that are endergonic.
Regulation of metabolic pathways

1. Gene regulation
   - Turn on or off genes
2. Cellular regulation
   - Cell-signaling pathways like hormones
Regulation of metabolic pathways

3. Biochemical regulation

☐ Competitive inhibitors- compete for access to active site

☐ Noncompetitive inhibitors- bind outside the active site
  - Allosteric site- binding causes conformational change in enzyme active site inhibiting enzyme function
  - Feedback inhibition- product of pathway inhibits early steps to prevent overaccumulation of product
Cellular respiration

- Process by which living cells obtain energy from organic molecules
- Primary aim to make ATP and NADH
- Aerobic respiration uses oxygen
  - $O_2$ consumed and $CO_2$ released

**Organic molecules + $O_2 \rightarrow CO_2 + H_2O + Energy$**
Glucose metabolism

- 4 metabolic pathways
  1. Glycolysis
  2. Breakdown of pyruvate to an acetyl group
  3. Citric acid cycle
  4. Oxidative phosphorylation
Stage 1: Glycolysis

- Glycolysis can occur with or without oxygen
- Steps in glycolysis nearly identical in all living species
- 10 steps in 3 phases
  1. Energy investment
  2. Cleavage
  3. Energy liberation
3 phases of glycolysis

1. **Energy investment**
   - Steps 1-3
   - 2 ATP hydrolyzed to create fructose-1,6 bisphosphate

2. **Cleavage**
   - Steps 4-5
   - 6 carbon molecule broken into two 3 carbon molecules of glyceraldehyde-3-phosphate

3. **Energy liberation**
   - Steps 6-10
   - Two glyceraldehyde-3-phosphate molecules broken down into two pyruvate molecules producing 2 NADH and 4 ATP

Net yield in ATP of 2
**Energy investment phase**

Glucose

```
CH2OH
H
OH
H
HO
```

Step 1: ATP

Step 2: ATP

Step 3: ATP

**Cleavage phase**

Fructose-1,6-bisphosphate

```
\begin{align*}
\text{CHO} & \quad \text{CHOH} \\
\text{HO} & \quad \text{HO} \\
\text{H} & \quad \text{H} \\
\text{OH} & \quad \text{OH} \\
\end{align*}
```

Step 4: ATP

Step 5: ATP

**Energy liberation phase**

```
\begin{align*}
\text{CH}_3 \text{COO}^- & \quad \text{CH}_3 \\
\end{align*}
```

Step 6: NADH

Step 7: ATP

Step 8: NADH

Step 9: ATP

Step 10: ATP

Two molecules of glyceraldehyde-3-phosphate

Two molecules of pyruvate
Glucose is phosphorylated by ATP. Glucose-6-phosphate is more easily trapped in the cell compared to glucose.

The structure of glucose-6-phosphate is rearranged to fructose-6-phosphate.

Fructose-6-phosphate is phosphorylated to make fructose-1,6-bisphosphate.

Fructose-1,6-bisphosphate is cleaved into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.
6. Glyceraldehyde-3-phosphate is oxidized to 1,3-bisphosphoglycerate. NADH is produced. In 1,3-bisphosphoglycerate, the phosphate group in the upper left is destabilized, meaning that the bond will break in a highly exergonic reaction.

7. A phosphate is removed from 1,3-bisphosphoglycerate to form 3-phosphoglycerate. The removed phosphate is transferred to ADP to make ATP.

8. The phosphate group in 3-phosphoglycerate is moved to a new location, creating 2-phosphoglycerate.

9. A water molecule is removed from 2-phosphoglycerate to form phosphoenolpyruvate. In phosphoenolpyruvate, the phosphate group is destabilized, meaning that the bond will break in a highly exergonic reaction.

10. A phosphate is removed from phosphoenolpyruvate to form pyruvate. The removed phosphate is transferred to ADP to make ATP.
Stage 2: Breakdown of pyruvate to an acetyl group

- In eukaryotes, pyruvate is transported to the mitochondrial matrix
- Broken down by pyruvate dehydrogenase
- Molecule of CO$_2$ removed from each pyruvate
- Remaining acetyl group attached to CoA to make acetyl CoA
- 1 NADH is made for each pyruvate
Pyruvate is made in the cytosol by glycolysis. It travels through a channel in the outer membrane and an $H^+/pyruvate$ symporter in the inner membrane to reach the mitochondrial matrix.

The acetyl group is transferred to coenzyme A via pyruvate dehydrogenase and will later be removed and enter the citric acid cycle.
Stage 3: Citric acid cycle

- Metabolic cycle
  - Particular molecules enter while others leave, involving a series of organic molecules regenerated with each cycle
- Acetyl is removed from Acetyl CoA and attached to oxaloacetate to form citrate or citric acid
- Series of steps releases $2\text{CO}_2$, 1 ATP, 3 NADH, and 1 FADH$_2$
- Oxaloacetate is regenerated to start the cycle again
The cycle begins when the acetyl group from acetyl-CoA is oxidized to form citrate.

3. Oxaloacetate is converted to α-ketoglutarate.

4. α-Ketoglutarate is oxidized with CoA to form succinyl-CoA. This releases CO₂ and NADH is formed.

5. Succinyl-CoA is broken down to drive the synthesis of GTP from ADP and F₆P, thereby forming ATP.

6. Succinate is oxidized to make water.

7. Tautomerase converts oxaloacetate to make malate.

8. Malate is oxidized to oxaloacetate.
Stage 4: Oxidative phosphorylation

- High energy electrons removed from NADH and FADH$_2$ to make ATP
- Typically requires oxygen
- Oxidative process involves electron transport chain
- Phosphorylation occurs by ATP synthase
Electron transport chain

- Group of protein complexes and small organic molecules embedded in the inner mitochondrial membrane
- Can accept and donate electrons in a linear manner in a series of redox reactions
- Movement of electrons generates H⁺ electrochemical gradient/ proton-motive force
  - Excess of positive charge outside of matrix
Free energy change

- Movement from NADH to O\textsubscript{2} is a very negative free energy change
  - Spontaneous in forward direction
- Highly exergonic
- Some energy used to pump H\textsuperscript{+} across inner mitochondrial membrane and create H\textsuperscript{+} electrochemical gradient
ATP synthase

- Enzyme harnesses free energy as H\(^+\) flow through membrane embedded region
- Energy conversion- H\(^+\) electrochemical gradient or proton motive force converted to chemical bond energy in ATP
- Racker and Stoeckenius confirmed ATP uses an H\(^+\) electrochemical gradient
- Rotary machine that makes ATP as it spins
1. The ATP synthase and bacteriorhodopsin were incorporated into membrane vesicles.

2. ADP and P_i were added on the outside of the vesicles.

3a. One sample was kept in the dark. No ATP was made.

3b. One sample was exposed to light. ATP was made.
Yoshida and Kinosita demonstrate that the $\gamma$ subunit of the ATP synthase spins

- Masasuke Yoshida, Kazuhiko Kinosita, and colleagues set out to experimentally visualize the rotary nature of the ATP synthase
- Released membrane embedded portion and adhered it to a slide
- Visualize $\gamma$ subunit using fluorescence
- Added ATP to make reaction run backward
- Rotated counterclockwise to hydrolyze ATP
  - Rotate clockwise to synthesize ATP
**HYPOTHESIS** The ATP synthase is a rotary machine.

**STARTING MATERIALS** Purified complex containing 1 γ, 3 α, and 3 β subunits.

1. Adhere the purified γα₃β₃ complex to a glass slide so that the base of the γ subunit is protruding upwards.

2. Add linker proteins and fluorescently labeled actin filaments. The linker protein recognizes sites on both the γ subunit and the actin filament.
3. Add ATP. As a control, do not add ATP.

4. Observe under a fluorescence microscope. The method of fluorescence microscopy is described in Chapter 4.
This figure shows a series of micrographs from Noji et al. (1997) Nature 386, 296-303.
Other organic molecules

- Focus on glucose but other carbohydrates, proteins and fats also used for energy
- Enter into glycolysis or citric acid cycle at different points
- Utilizing the same pathways for breakdown increases efficiency
- Metabolism can also be used to make other molecules (anabolism)
Proteins → Amino acids

Carbohydrates → Sugars

Fats → Glycerol Fatty acids

Glycolysis:
- Glucose
- Glyceraldehyde-3-phosphate
- Pyruvate

Acetyl CoA

Citric acid cycle

Oxidative phosphorylation
Anaerobic metabolism

- For environments that lack oxygen or during oxygen deficits
- 2 strategies
  - Use substance other than O$_2$ as final electron acceptor in electron transport chain
  - If confined to using O$_2$, carry out glycolysis only
    - Pyruvate converted to lactate or lactic acid in muscles or ethanol is yeast
    - Fermentation – produces far less ATP
(a) Production of lactic acid

Pyruvate + NADH + H^+ → Lactic acid + NAD^+

(b) Production of ethanol

Pyruvate → Acetaldehyde + NADH + H^+ + CO_2 → Ethanol + NAD^+