Bioenergetics & Metabolism

**Metabolism** is a highly coordinated cellular activity in which many multienzyme systems (pathways) cooperate to:
- Obtain chemical energy by capturing solar energy or degrading energy-rich nutrients
- Convert nutrients into raw materials for the production of macromolecules
- Polymerize monomeric precursors into macromolecules
- Synthesize and degrade biomolecules required for specialized cellular functions

Metabolism includes hundreds of varied pathways, but we will be focusing on the central metabolic pathways that are common to all forms of life.

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**In a metabolic pathway, each step brings about a small chemical change, usually the removal, transfer or addition of an atom or functional group.**
- These intermediates are metabolites

**Catabolism** is the degradative phase in which nutrients are converted into smaller end products
- These pathways release energy as either ATP or reduced electron carriers (NADH, FADH₂, NADPH)

**Anabolism** is the biosynthetic phase where small precursors are built into macromolecules
- These pathways require energy, usually in the form of ATP or the reduced electron carriers

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**ATP is the Universal Energy Currency of Cells**
- High energy phosphoryl compounds are formed during catabolism
- As a means of activating other compounds for further chemical transformations
- ATP is energetically rich, kinetically stable
  - Intermediate group-transfer potential
  - Great versatility in what groups it can transfer
- It converts lower energy compounds into more reactive species
• The carbon oxidation energy in nutrients can be used to create a compound with high phosphoryl transfer potential.
• This energy also can be used to create an ion gradient.
• Either of these result in the end in the formation of ATP.

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**ATP synthesis is driven by energy from nutrients**

- The terminal electron acceptor is oxygen, which has a high reduction potential (high affinity for protons).
- Thus energy is released when electrons are dumped into oxygen (making water).
- This energy is used to pump protons from the mitochondrial matrix into the intermembrane space.
- Thus establishing a pH gradient across the membrane which is used to drive ATP synthesis.

Bioenergetics & Metabolism

**Specialized Functions of Mammalian Tissues**

Bioenergetics & Metabolism

**Fig. 23-12**

Bioenergetics & Metabolism

**Animal cell**

- Mitochondria are protein-synthesizing machines.
- Peroxisomes destroy peroxides.
- Lysosomes degrade intracellular debris.
- Transport vesicles shuttle ligands and proteins between organelles.

**Nuclear envelope:**
- Serves as site of chromosomal DNA replication.
- Nuclear pores are sites of nuclear RNA synthesis.
- Nucleus contains the genes (chromosomes).

**Mitochondria oxidizes fuels to produce ATP.**
Chapter 14
Homework Assignment

- The following problems will be due once we finish the chapter:
  3, 6, 7, 16, 18, 20

- Additional Problem:
  - Write out the ten reaction steps of Glycolysis, using structures to describe the intermediates. Use the correct stoichiometry to show the final products derived from one glucose molecule. Identify the enzyme and any required cofactors for each step. Use arrows to show which reactions are irreversible and which are reversible. Repeat for Gluconeogenesis, highlighting the steps that vary from Glycolysis.

Cellular Respiration

Overview

Stage 1: Acetyl-CoA Production from:
- Glucose
- Fatty Acids
- Amino Acids

Stage 2: Acetyl-CoA Oxidation (The Citric Acid (TCA) cycle)

Stage 3: Electron Transfer & Oxidative Phosphorylation

Glycolysis, Gluconeogenesis and the Pentose Phosphate Pathway

Themes to Follow
Phosphoryl group transfers
Electron transfers
Energy storage and coupling

Things to Know for each Stage:
- Structure of reactant and product for each step
- Enzyme names for each step (not just their acronym)
- “Balance sheet” for ATP, NADH, etc.
- How to use the Mass Action Ratio = $K_{\text{start}}$
Glycolysis (“glucose splitting”)

\[ \Delta G^{\circ} = -146 \text{ kJ/mol} \]

(5% of the total -2840 kJ/mol to CO\(_2\) and H\(_2\)O)

### The Strategy

- Glycolysis has two phases: the “preparatory” phase and the “payoff” phase.
- The “Preparatory” phase splits the 6-carbon glucose into 2 interchangeable 3-carbon units, with a net energy input (2 ATPs).
- The “Payoff” phase rearranges and oxidizes these 3-carbon units, yielding pyruvate, 4 ATPs, and reducing power (2 NADH).
- You should be able to trace
  - The pathway of carbon
  - The pathway of phosphoryl groups
  - The pathway of electrons

### The Overall Reaction

Glucose + 2 NAD\(^+\) + 2 ADP + 2 Pi \rightarrow 2 Pyruvate + 2 NADH + 2 H\(^+\) + 2 ATP + 2 H\(_2\)O

2 Pyruvate + 2 NADH + 2H\(^+\) + 2 ATP + 2 H\(_2\)O

- Note that 4 electrons (as 2 hydride ions) are passed to NAD\(^+\), which, as NADH, is transferred to the respiratory chain in mitochondria.
- These electrons are used for respiration-linked ATP synthesis (of which more, later…)

### The Free Energy Release from the Oxidation of Glucose is Gradual

- The total free energy change from glucose to carbon dioxide and water is \(-2840\) kJ/mol.
- Individual oxidation steps generate \(-60\) kJ/mol.
- Just enough to make one ATP molecule.
- The electrons that are removed in these steps are transferred to coenzymes that are specialized for carrying electrons, such as: NAD\(^+\) and FAD.
Glycolysis
The Preparatory Phase

- The first 5 steps are the preparatory phase
- During this phase:
  - Glucose is activated (twice, by group transfer from ATP)
  - Then split into two 3-carbon units, each of which is phosphorylated, and which can be interconverted

Net Cost: 2 ATPs

Glycolysis
Enzyme Names Tell You What They Do

1. Hexokinase
2. Phosphohexose isomerase
3. Phosphofructokinase
4. Aldolase (aldol condensation)
5. Triose phosphate isomerase

Glycolysis
Do all steps in a pathway proceed equally fast?

- If enzyme is limiting, substrate will accumulate behind it, thus
- To achieve equilibrium, the reaction will have to go forward
- If it is far from equilibrium, its actual ΔG will be very negative
- Making the reverse reaction almost impossible
- Three steps in glycolysis have this characteristic
Free Energy Tells You About the Equilibrium State – Or Does It?

ΔG° kJ/mol
-16.7 1. Hexokinase (HK)
+ 1.7 2. Phosphohexose isomerase
-14.2 3. Phosphofructokinase (PFK1)
+ 23.8 4. Aldolase
- 7.5 5. Triose phosphate isomerase

Glycolysis
The “Payoff” Phase
- The last 5 steps are the payoff phase
- During this phase:
  - The 3-carbon unit is further activated (by inorganic phosphate)
  - This creates one familiar high-energy compound (1,3 BPG), and then another (PEP)
  - Energy is released from PEP as it is converted into pyruvate (4 ATPs + 2 NADH)
  - Catalyzed by the enzyme pyruvate kinase

Net Gain: + 2 ATP, + 2 NADH

Glycolysis
The PFK1 Reaction
F6P + ATP ⇄ F1,6P + ADP
- The equilibrium constant K′eq = 250
- But the mass-action ratio = K′start = 0.04
- Since 250/0.04 = 6250, the prevailing conditions in the cell are ~ 6000x away from equilibrium
- This equates to ~ -20 kJ/mol (between 10³ = 17.1 and 10⁴ = 22.8)
- This is greater than ΔG° = -14.2 kJ/mol so the reaction proceeds

Glycolysis
The “Payoff” Phase
ΔG° kJ/mol
+ 6.3 6. Glyceraldehyde 3-Phosphate dehydrogenase
- 18.8 7. Phosphoglycerate kinase
- 4.4 8. Phosphoglycerate mutase
+ 7.5 9. Enolase
- 31.4 10. Pyruvate kinase
Remember?

Considered high energy if $\Delta G < -25 \text{ kJ/mol}$

Intermediate energy $\Delta G$ is $-5$ to $-25 \text{ kJ/mol}$

Considered high energy

The Big Five: Four + One

Acetyl-(S)CoA

Considered high energy if $\Delta G^* < -25 \text{ kJ/mol}$

Glycolysis

The Actual Energetics of Glycolysis

…based on actual concentrations of reactants and products. Note that 7 are near equilibrium - only 3 of the steps are energetically "irreversible".

How then can glucose be synthesized?

Glycolysis

How is the Pathway Affected by the Energetics of Individual Reactions?

- Three of the reactions have large, negative $\Delta G$’s (you should know which ones by now)
- Which means that they are
  - Far from equilibrium
  - Rate-limiting steps in the pathway
- Seven of the reactions have actual $\Delta G$’s $\sim 1$
- Which means that they are
  - Near equilibrium in the cell
  - Readily reversible
- The former are unique to glycolysis, but the latter are exploited in both glycolysis and gluconeogenesis
**Feeder Pathways for Glycolysis**

**Do We Eat Only Glucose?**

- What happens with other sugars, such as
  - Sucrose?
  - Lactose?
  - Galactose?
  - Fructose?
- What happens with polysaccharides, like
  - Starch (from potatoes, for example…)?
  - Cellulose (…if we ate grass)?
- How do we utilize our own glycogen?
  - The same way as starch?

**Feeder Pathways for Glycolysis**

**More Questions to Ponder**

- How does the breakdown of starch (which we can’t store) differ from the breakdown of glycogen (which we can)?
- What are the advantages of the latter?
- What would happen if we ate glycogen?
- How does amylopectin get broken down, and what is maltose? Dextrin?
- What form do sugars have to be in before they can be absorbed from the intestine? How is this related to lactose intolerance?
- Why is **galactosemia** potentially a bad disease?
Lactic Acid Fermentation

- When there is insufficient oxygen to support aerobic oxidation of the pyruvate and NADH produced by glycolysis, NAD+ is regenerated by the reduction of pyruvate to lactate.
- The reduction of the pyruvate is catalyzed by lactate dehydrogenase.
- Although the reduction of glucose to lactate includes two redox steps, there is no net change in the oxidation state of carbon (C₆H₁₂O₆ versus C₃H₆O₃).
- However, some energy has been extracted, enough to yield 2 ATP.

\[
\text{Glucose} + 2 \text{ADP} + 2 \text{Pi} \rightarrow 2 \text{Lactate} + 2 \text{ATP}
\]

Can you now answer these questions?

- Why is lactate formed in skeletal muscle tissue, and what is its ultimate fate?
- Why is lactate formed in red blood cells, and what is its ultimate fate?
- What is the role of alcohol dehydrogenase in fermentation by yeast?
- Why does bread rise?
- What cofactors are required in the above reactions?

Alcohol Fermentation

- Yeast and other microorganisms ferment glucose to ethanol and CO₂, rather than to lactate.
- Glucose is converted to pyruvate via glycolysis.
- The pyruvate is then converted to ethanol and CO₂ in a two-step process:

\[
\text{Glucose} + 2 \text{ADP} + 2 \text{Pi} \rightarrow 2 \text{Ethanol} + 2 \text{ATP} + 2 \text{CO}_2
\]

Central Role of Gluconeogenesis

- Glucose is the major or sole fuel source for the brain, nervous system, erythrocyte, renal medulla, and embryonic tissue.
- It is made from a limited set of precursors, mainly in the liver.
- Its derivatives fill a variety of structural and functional metabolic roles.
Gluconeogenesis
Building Up versus Breaking Down
• Anabolic pathways use the chemical energy generated during catabolism (ATP, NADH, etc.) to synthesize cellular components
• They are generally reductive, not oxidative
• Though opposing pathways (e.g. glycolysis versus gluconeogenesis) may share many reversible reactions, each has at least one unique reaction, which is also essentially irreversible
• Regulation of the pathway is usually at an early, unique, and irreversible (highly exergonic) step

Making Glucose Differs from Breaking It Down
• Recall that glycolysis has three “irreversible” steps
• Characterized by large negative ΔG’s, and catalyzed by enzymes 1, 2 and 3.
• In gluconeogenesis, each of these steps is “bypassed” by a separate set of unique reactions
• These bypass reactions are exergonic enough to be themselves irreversible

Key Steps in Glycolysis
• Three steps have very large -ΔG’s and are thus essentially irreversible
• One step is an oxidation
• Two steps involve substrate-channeling
• Two steps display substrate-level phosphorylation

Gluconeogenesis Bypass Reaction #1
• Bypassing the last step in glycolysis is the trickiest
  – requiring a large number of steps
  – the cooperation of mitochondrial and cytosolic enzymes
  – Used when the glucogenic precursor is pyruvate or alanine
• An alternative pathway is used from lactate
Pyruvate inside can come from
- Alanine inside
- Pyruvate outside
- Lactate outside

The demand for NADH outside and the availability of lactate governs which pathway is taken by oxaloacetate (inside)

Also, movement of Malate requires a membrane shuttle

Uses malate-aspartate shuttle, see Fig. 19-27

Chapter 14

Bypass Reaction #1

Pyruvate in the matrix is carboxylated by activated bicarbonate (Biotin!) yielding oxaloacetate (OAA)
- Energy comes from ATP

If NADH is not available, OAA is then decarboxylated and phosphorylated (from GTP), yielding Phosphoenolpyruvate (PEP) (+ GDP)

Though the sum $\Delta G^\circ$ is + 0.9 kJ/mol under cellular conditions the actual $\Delta G = -25$ kJ/mol, and is thus essentially irreversible

Overall, 2 high-energy phosphates were used (ATP and GTP); the reverse step in glycolysis gained only 1 (ATP)

Chapter 14

The First Bypass Needs Two Exergonic Reactions

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Chapter 14

The Logic of this Coordinated Scheme

In the matrix, the [NADH]/[NAD+] ratio is about 80; in the cytosol it is 100,000x less (i.e. NADH is scarce!)

But NADH is essential for gluconeogenesis (which step?), so the malate shuttle moves both substrate and cofactor to where they are needed

Alternatively: under anaerobic conditions, where lactate predominates (blood cells, muscle), it is oxidized to pyruvate by lactate dehydrogenase, producing NADH in the cytosol before pyruvate enters the matrix

In the matrix, OAA is still made as before, but then converted directly into PEP, by a mitochondrial isozyme of PEP carboxykinase

And the PEP itself is exported...

Think through the logic...
Chapter 14

**The Role of Biotin**

- Pyruvate carboxylase is a mitochondrial enzyme that requires the coenzyme Biotin.
- Biotin serves as the carrier of activated HCO₃⁻.

**Bypass Reaction #2**

- Catalyzed by Mg²⁺ dependent fructose 1,6-bisphosphatase (FBPase-1).
- Which hydrolyzes (rather than transfers) the C-1 phosphate, gaining substantial free energy.
- The reciprocal effect of fructose 2,6-bisphosphate will be covered later.

**Gluconeogenesis**

- Catalyzed by glucose 6-phosphatase.
- Which hydrolyzes (rather than transfers) the C-6 phosphate, gaining substantial free energy.
- Muscle and brain do not have this enzyme, and must receive their glucose in the bloodstream from diet, or gluconeogenesis in liver or kidney.

**Expensive but worth it!**

Cost of making glucose:

\[
2 \text{Pyruvate} + 2 \text{NADH} + 4 \text{ATP} + 2 \text{GTP} + 2 \text{H}^+ + 4 \text{H}_2\text{O} \rightarrow \text{Glucose} + 2 \text{NAD}^+ + 6 \text{ADP} + 2 \text{GDP} + 2 \text{NADH} + 6 \text{Pi}
\]

**Gluconeogenesis**

- Pyruvate + 2 NADH + 4 ATP + 2 GTP + 2 H⁺ + 4 H₂O → Glucose + 2 NAD⁺ + 6 ADP + 2 GDP + 2 NADH + 6 Pi

**Gluconeogenesis**

- Catalyzed by glucose 6-phosphatase.
- Which hydrolyzes (rather than transfers) the C-6 phosphate, gaining substantial free energy.
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\]
Where Do the Carbons for Glucose Synthesis Come From?

- All the amino acids save leucine and lysine can be converted to pyruvate, or TCA cycle intermediates (we’ll revisit this later)
- Alanine and glutamine are especially important
- Carbons cannot come from β-oxidation (as you’ll see in Chapter 17), though lots of energy does (NADH, ATP, GTP)

Avoid Futile Cycles! (Mostly)

- Since the 3 irreversible steps in glycolysis and the 3 bypass steps in gluconeogenesis all have large negative ΔG’s
- They could be continuously “on”, resulting in no net synthesis of either product, but expending huge amounts of ATP
- For example, if the PFK-1 reaction of glycolysis and the FBPase-1 reaction of gluconeogenesis were to run simultaneously:
  \[
  \begin{align*}
  \text{F-6-P} + \text{ATP} & \rightarrow \text{F-1,6BP} + \text{ADP} \\
  \text{F-1,6BP} + \text{H}_2\text{O} & \rightarrow \text{F-6-P} + \text{P}_i
  \end{align*}
  \]
- This is called a "futile cycle" that is normally prevented by reciprocal regulatory mechanisms

Gluconeogenesis

<table>
<thead>
<tr>
<th>TABLE 14-4 Gluconic Amino Acids, Grouped</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyruvate</strong></td>
</tr>
<tr>
<td><strong>Alanine</strong></td>
</tr>
<tr>
<td><strong>Aspartate</strong></td>
</tr>
<tr>
<td><strong>Glutamine</strong></td>
</tr>
<tr>
<td><strong>β-Ala</strong></td>
</tr>
<tr>
<td><strong>Arginine</strong></td>
</tr>
<tr>
<td><strong>Asparagine</strong></td>
</tr>
<tr>
<td><strong>Histidine</strong></td>
</tr>
<tr>
<td><strong>Histidine</strong></td>
</tr>
<tr>
<td><strong>Alanine</strong></td>
</tr>
</tbody>
</table>

Gluconeogenesis

Could generate heat, though...

Escher Futile Cycles

Pentose Phosphate Pathway

- Present in tissues actively synthesizing fatty acids and steroids such as mammary gland, adrenal cortex, liver, and adipose tissue.
- This oxidative pathway consists of a series of reactions that convert:
  \[
  \text{G6P} + 2 \text{NADP}^+ + \text{H}_2\text{O} \rightarrow \text{R5P} + 2 \text{NADPH} + \text{CO}_2 + 2 \text{H}^+
  \]
- The primary products:
  - Ribose-5-P (R5P), essential for the synthesis of nucleotide derivatives, nucleic acids, and coenzymes (which?)
  - NADPH, used as reducing power in many anabolic pathways, such as fatty acid synthesis

Where else can Glucose Go?

Glycolysis & Gluconeogenesis

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  - NADPH, used as reducing power in many anabolic pathways, such as fatty acid synthesis
**Where else can Glucose Go?**  
**PPP – Oxidative Phase**
- In the Oxidative phase, two oxidation steps occur in one phase of the pathway.
- The generated reducing power is used in many biosynthetic pathways, e.g., fatty acid synthesis, cholesterol and steroid hormone synthesis, etc.
- The R5P is used to synthesize RNA, DNA and coenzymes such as ATP, NADH, FADH$_2$ and Coenzyme A.

**PPP – Non-oxidative Phase**
- In tissues where only the NADPH reducing power is needed: 6 pentoses are converted back into 5 hexoses.
- Reactions are catalyzed by *transketolase* and *transaldolase*.
- The reverse can also take place, the reductive PPP.

**PPP – Regulation**
- Whether G6P enters glycolysis or the PPP depends on the needs of the cell and on the [NADP$^+$].
- When the cell is converting NADPH to NADP$^+$, the level of NADP$^+$ rises, stimulating the first enzyme of the PPP and directing G6P into that pathway.
- When the NADPH level rises, the PPP slows down and the G6P is shunted into glycolysis for fuel production.