All cells must do work to stay alive and maintain their cellular environment. The energy needed for cell work comes from the bonds of ATP. Cells obtain their ATP by oxidizing organic molecules, a process called cellular respiration. Although many organic molecules can be oxidized, glucose, a main product of photosynthesis, is the primary fuel molecule for the cells of living organisms.

Every living organism, autotroph and heterotroph, must do cell respiration. In fact, the metabolic pathways used in the process of cellular respiration are the same in virtually all eukaryotic organisms as well as most prokaryotic organisms. Recall that organisms that do photosynthesis (or properly, manufacture their own fuel molecules) are called autotrophs. Heterotrophs obtain their fuel molecules "pre-formed" by other organisms. Animals, fungi and many protists are heterotrophs, as are most bacteria. Plants and some protists are autotrophs, as are some bacteria.

Most eukaryotic organisms are aerobic (oxygen requiring). Aerobic cell respiration is required in order to obtain enough energy (ATP) from the oxidations of fuel molecules for these organisms to survive. In aerobic respiration glucose is oxidized to water and carbon dioxide. Oxygen is required as the final electron acceptor for the oxidations. Most organisms are obligate aerobic organisms.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2 + 686 \text{ kcal energy (ATP + Heat)}$$

Not all cell respiration is aerobic. All organisms do some type of anaerobic respiration during times of oxygen deficit, although it may not be sufficient to sustain the organism's ATP needs for many species. Fuel molecules oxidized without oxygen yield smaller amounts of ATP.

The fermentations involve the partial breakdown of glucose without using oxygen. Many prokaryotes have a variety of fermentation pathways, using a number of different fuel molecules. By definition, the end product for the fermentations is an organic molecule. In aerobic cellular respiration, the final electron acceptor is oxygen, hence, the emphasis on oxygen in aerobic cell respiration. In addition, some prokaryotes use anaerobic electron transfer respiration pathways in which their final electron acceptor is an inorganic molecule such as sulfate, iron, or nitrogen compounds.

Some organisms are obligate anaerobes. They cannot survive in the presence of oxygen. The Clostridium bacteria that cause botulism poisoning, tetanus and gangrene are obligate anaerobes. Other anaerobes are metabolic anaerobes; they lack the enzymes needed to do aerobic cell respiration. Many of our intestinal bacteria, such as the Lactobacillus bacteria, are metabolic anaerobes. Some organisms will survive nicely in the absence of oxygen but will do aerobic respiration when oxygen is available. Yeast organisms and E. coli are two such facultative organisms.
Aerobic and Anaerobic Respiration Pathways

Oxidation-Reduction Reactions in Cell Respiration
The oxidations of fuel molecules in aerobic cell respiration use specialized electron carrier molecules, most of which in eukaryotic organisms are located in the membranes of the mitochondria. These electron transport molecules gain and lose electrons at specific energy levels. This is very similar to the electron transport molecules used in photosynthesis.

One of the most important of the electron transport molecules in cell respiration is NAD+. Electrons are passed through an electron transport chain to form ATP by chemiosmosis, a process sometimes called oxidative phosphorylation or electron transport phosphorylation.

Not all of the ATP produced during cell respiration is by chemiosmosis. Some ATP is also synthesized by a direct transfer of phosphate from a substrate molecule to ADP. This process is called substrate-level phosphorylation. We will discuss this more when we do the details of the cell respiration pathways.
Cell Respiration - An Overview of the Processes
As with many metabolic processes, cell respiration has a number of stages.

Glycolysis
The initial stage of glucose metabolism, or cell respiration, is a process called glycolysis, which splits a glucose molecule into two molecules of pyruvate, a 3-carbon compound. Glycolysis occurs in the cytosol of the cell. What follows glycolysis depends on the presence or absence of oxygen and/or the enzymes needed.

If oxygen is available and the organism has the enzymes to do aerobic respiration, the pyruvate molecules will be oxidized in the next stages of aerobic respiration. The reactions of aerobic respiration after glycolysis occur in the mitochondria and include the Krebs cycle and the electron transport chain.

If oxygen is not available, or if the organism lacks enzymes needed for aerobic respiration, the pyruvate molecules will proceed with fermentations.

In order to obtain sufficient ATP for survival, most organisms must do aerobic cell respiration.

Cellular Respiration - The Pathways
Glycolysis - Overview
• Glucose is “activated” for the oxidations by two ATP-consuming reactions. Glucose must be "primed", or destabilized, in order to become reactive.
• Glucose is then broken into two molecules of the 3-carbon compound, Pyruvate.
• In addition:
  • Two molecules of NADH are produced.
  • A net of two molecules of ATP are produced. (Four molecules of ATP are produced during glycolysis, but 2 molecules are consumed in activating the glucose.)
• Glycolysis always occurs in the cytosol (cytoplasm) of the cell.
• Glycolysis is the most widespread metabolic pathway in living organisms, today and evolutionarily. The earliest prokaryotes probably had a glycolysis pathway.
**Glycolysis Specifics**

Glucose + 2ATP + 2NAD$^+$ + 2ADP + 2P $\rightarrow$ 2 Pyruvate + 2NADH + 4ATP*

*Net gain of 2ATP*

**Summary of Glycolysis**

Inputs
- Glucose
- 2 ATP*
- (and 2NAD$^+$ + 2 ADP + 2 P)

Outputs
- 2 Pyruvate
- 2 NADH
- 4 ATP*

* Therefore the net energy yield is 2 ATP

- The ATP generated is by **substrate-level phosphorylation**
- All steps are enzyme mediated
- Glycolysis occurs in the cytoplasm of the cell
- Glycolysis is the initial cell respiratory pathway of all **eukaryotic organisms**.
Following glycolysis, the presence of absence of oxygen, and/or the organism's ability to use oxygen in respiration, determines whether pyruvate will be oxidized to yield more energy in **aerobic respiration** or reduced to a stable molecule in the **fermentations**. We will discuss first what happens after Glycolysis when no oxygen is available – The **Fermentations**. Then we will discuss the processes involved in **Aerobic Cellular Respiration**

**The Fermentations – Cell Respiration in the Absence of Oxygen**
The overwhelming majority of living organisms must do aerobic cellular respiration to stay alive. Fermentations and other anaerobic pathways provide insufficient ATP to sustain life for most organisms. Yet, when no oxygen is available for aerobic cell respiration, eukaryotic organisms, and some prokaryotes, will complete glucose metabolism with the fermentation reactions. Fermentations are an alternative pathway for pyruvate after glycolysis occurs.

For some microorganisms, fermentation is a way of life. Some lack the enzymes to do the Krebs cycle; for others, oxygen is toxic. These are the strict (or obligate) **anaerobes**. Others, such as yeasts and *E. coli* are facultative organisms. When oxygen is available, they do aerobic respiration. When oxygen is not, they perform a fermentation.

NADH carries very high energy electrons, but those electrons can be used to make ATP only in the presence of oxygen. In the fermentations the NADH electrons produced in glycolysis will be used to reduce pyruvate to some other organic molecule, which becomes the final electron acceptor of cell respiration. This is needed to recover NAD⁺ for more glycolysis; **no additional ATP energy** is obtained in the fermentation processes beyond the two ATP produced during glycolysis.

**Fermentation Details**
- Although a number of different fermentation pathways are found among the bacteria, only two fermentation pathways, which are genetically determined, are found in Eukaryotic organisms.
  - **Alcoholic Fermentation**
  - **Lactic Acid Fermentation**
- Pyruvate from glycolysis functions as the electron acceptor for the NADH produced in glycolysis.
- NADH is used to reduce pyruvate to some stable organic molecule, freeing the NAD⁺ (or regenerating NAD⁺) for more glycolysis.
- The organic molecule is the final electron acceptor. No additional ATP is produced.
- No additional ATP is produced after the initial ATP production from glycolysis.
Lactic Acid Fermentation

\[
\text{NADH} \rightarrow \text{NAD}^+ \\
\text{Pyruvate} \rightarrow \text{Lactic Acid}
\]

\[
\begin{align*}
\text{GLYCOLYSIS} & \quad \text{C}_6\text{H}_12\text{O}_6 \\
2 \text{ ATP} & \quad \text{energy input} \\
4 \text{ ATP} & \quad \text{energy output} \\
2 \text{ ADP} & \\
2 \text{ pyruvate} & \\
2 \text{ NAD}^+ & \\
2 \text{ NADH} & \\
2 \text{ ATP net} & \\
\text{LACTATE FORMATION} & \\
2 \text{ lactate} & \\
\text{electrons, hydrogen from NADH}
\end{align*}
\]

Alcoholic Fermentation

\[
\text{NADH} \rightarrow \text{NAD}^+ \\
\text{Pyruvate} \rightarrow \text{Acetaldehyde} \rightarrow \text{Ethanol (Ethyl Alcohol)} \\
+ \text{CO}_2
\]

\[
\begin{align*}
\text{GLYCOLYSIS} & \quad \text{C}_6\text{H}_12\text{O}_6 \\
2 \text{ ATP} & \quad \text{energy input} \\
4 \text{ ATP} & \quad \text{energy output} \\
2 \text{ ADP} & \\
2 \text{ pyruvate} & \\
2 \text{ NAD}^+ & \\
2 \text{ NADH} & \\
2 \text{ ATP net} & \\
\text{ETHANOL FORMATION} & \\
2 \text{ acetaldehyde} & \\
2 \text{ ethanol} & \\
2 \text{ CO}_2 & \\
\text{electrons, hydrogen from NADH}
\end{align*}
\]

Anaerobic Electron Transport  (Those versatile Prokaryotes)
Some anaerobic bacteria have an electron transport system and oxidize a variety of molecules. Some inorganic substance, such as sulfur or nitrogen molecules, becomes the final electron acceptor, rather than oxygen. ATP production is small, but sufficient for the anaerobic bacteria. Aromatic H$_2$S, hydrogen sulfide, is a common end product.
Aerobic Cellular Respiration
Aerobic Cellular Respiration is comprised of two three stages following glycolysis, that occur in the mitochondria of the cell: the oxidation of pyruvate, the Krebs cycle reactions in the mitochondrial matrix (the inner compartment of the mitochondrion) and the electron transport chain reactions that occur in the inner mitochondrial membrane.

The Mitochondrial Matrix (Inner Compartment) Stages
The second and third stages of aerobic respiration comprise the oxidation of pyruvate and the Krebs cycle. Both occur in the mitochondrial matrix.
- Pyruvate molecules are oxidized and lose a CO₂ forming acetyl. NAD⁺ picks up the electrons and H⁺ from the oxidation forming NADH.
- The two-carbon acetyl unites with and is carried to the Krebs cycle by coenzyme A (CoA). More oxidations occur in the Krebs cycle, releasing two more CO₂ for each pyruvate molecule and yielding many more NADHs as well as 1 FADH₂ and 1 ATP.

The Inner Mitochondrial Membrane Stage
The final stage of aerobic respiration is the electron transport chain and the chemiosmotic synthesis of ATP. Since the energy to synthesize ATP is from the oxidation-reduction reactions, the ATP formation is also called oxidative phosphorylation.
- Oxygen is the final electron acceptor for the oxidation-reductions that start with NADH in the electron transport chain.
- The electron transport chain reactions take place in the inner membrane of the mitochondria.

When oxygen is available, as much as 36 - 38 ATP can be generated from one glucose molecule.
Oxidation of Pyruvate to Acetyl
- The two Pyruvate molecules from the original glucose are transported into the inner matrix of the mitochondria by facilitated diffusion
- Each pyruvate is oxidized releasing $\text{H}^+$ to reduce $\text{NAD}^+$ to NADH
- CO$_2$ is removed producing Acetyl (A 2-carbon compound)
- Acetyl combines with Coenzyme A to form Acetyl-CoA, which can enter the Krebs cycle.

For one glucose molecule (two pyruvate molecules), we obtain:
- 2 CO$_2$
- 2 NADH
- 2 Acetyl C0-A

Note: When the level of ATP is high in a cell, the cell can convert acetyl-CoA into lipid molecules that can be stored for later energy use. This is one way that excess calories, no matter the nutrient source, are converted to fat.

The Krebs Cycle
The Krebs cycle is a means to remove energy rich H$^+$ (with its electrons) (originally part of the glucose molecule) that can subsequently be used to generate ATP in electron transport via chemiosmosis. The leftover carbon is given off as CO$_2$.

Essentially, the acids of the Krebs cycle are substances that under the right conditions (i.e., The Krebs Cycle) can be oxidized (That is donate H$^+$ with its electrons).

For each glucose molecule, two ATP are produced in the Krebs cycle by substrate-level phosphorylation, one for each acetyl Co-A molecule that enters the Krebs cycle. (Recall that the glucose molecule has already gone through glycolysis and has been converted to two molecules of Pyruvate in the cytoplasm prior to starting the Krebs cycle.)
A closer Look at the Krebs Cycle

Any cycle requires a substance to start the cycle (which will also be the end of the cycle). For the Krebs cycle the starter is oxaloacetic acid (oxaloacetate), a 4-carbon acid that is regenerated at the end of the cycle. The enzymes needed to do the Krebs cycle are located in the mitochondrial matrix.

Acetyl-CoA combines with oxaloacetic acid to begin the cycle forming the 6-carbon citric acid (citrate). Co-A is released to pick up more acetyl.

For each turn of the Krebs cycle we get:
- 2 CO₂ given off (plus 1 from pyruvate oxidation to acetyl) = 3 CO₂
- 1 ATP produced (by substrate phosphorylation)
- 1 FADH₂
- 3 NADH (plus 1 from pyruvate oxidation to acetyl) = 4 NADH

The Krebs Cycle – Specifics

The Krebs cycle will turn two times for each glucose molecule. Two turns of the Krebs cycle (including the preparation step of pyruvate → acetyl will produce:
- 6 CO₂
- 2 ATP produced (by substrate phosphorylation)
- 2 FADH₂
- 8 NADH
**Electron Transport Chain**
The enzymes, proteins and electron carriers needed to do electron transport are found in the inner membranes of the mitochondria. ATP is produced by using a $H^+$ concentration gradient to run the ATP synthesis pumps as electrons are passed along the electron transport molecules in a series of oxidations and reductions.

As the electrons are passed from one carrier to the next, the energy released is used to move $H^+$ ions from the mitochondrial matrix through the inner membrane into the intermembrane space of the mitochondrion. As the $H^+$ concentration builds, it provides a $H^+$ gradient that passes through a protein channel pore in the membrane that works with the enzyme, ATP synthase, to generate ATP in the mitochondrial matrix. Peter Mitchell won the 1978 Nobel prize in chemistry "for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory". ATP is synthesized in the thylakoid membranes of the chloroplast by a similar mechanism.

The Electron Transport Chain

The Electron carriers, FADH$_2$ and NADH, produced in the Krebs cycle (and in glycolysis), provide the electrons and hydrogen needed for ATP synthesis.

**Oxygen** is required as the final electron (and hydrogen) acceptor, producing **water** as the end product of aerobic cellular respiration. The $H^+$ and $e^-$ passed through the carriers combine with oxygen in the final step. (Recall that CO$_2$ is also a product of aerobic cellular respiration.)
How much ATP do we get from oxidizing glucose in aerobic cellular respiration?

- The electrons and $H^+$ from each NADH produced in the Krebs cycle and the oxidation of pyruvate to acetyl Co-A provides sufficient energy to produce about 3 ATP by chemiosmosis.
- The electrons and $H^+$ from each FADH$_2$ produced in the Krebs cycle provides sufficient energy to produce about 2 ATP by chemiosmosis (FAD is a lower energy electron transfer molecule and enters the transport chain in mid-chain, rather than at the start)
- The electrons and hydrogen from each NADH from Glycolysis provides sufficient energy to produce about 2 ATP (The hydrogens and electrons have to be transferred from NADH in the cytoplasm to the mitochondria)

**Summary of ATP Production from the complete aerobic metabolism of one glucose molecule**

**From Electron Transport phosphorylation:**
- 6 NADH from Krebs X 3 ATP each = 18 ATP
- 2 NADH from Pyruvate to Acetyl X 3 ATP each = 6 ATP
- 2 NADH from Glycolysis X 2 (3) ATP each = 4(6) ATP
- 2 FADH$_2$ from Krebs X 2 ATP each = 4 ATP

**From Direct ATP synthesis (Substrate phosphorylation)**
- 2 ATP directly from Krebs = 2 ATP
- 2 ATP directly from glycolysis = 2 ATP

**Maximum Total ATP from 1 glucose = 36(38) ATP**
**Aerobic (Cellular) Respiration Summary**
The complete aerobic respiration of glucose requires the following:
- Glycolysis
- Pyruvate Oxidation and the Krebs cycle
- Electron transport phosphorylation
- **Oxygen**, the final electron acceptor in the electron transport system, combines with Hydrogen to form water.
- Carbon Dioxide (CO₂) is released during aerobic respiration.
- 36 ATP can be produced for each glucose molecule.
- The Krebs cycle and electron transport occur in the **mitochondria**.
  - **Glycolysis** occurs in the cytosol.
- All steps are catalyzed by enzymes

**Generating Heat From Cell Respiration - Thermogenesis**
There are times when generating heat rather than ATP is desired. For example, organisms, such as bats, need to increase body temperature rapidly when they wake. There are special proteins, called **uncoupling proteins**, that separate the H⁺ proton flow in electron transport from the ATP synthase pump, so that heat is produced instead of ATP.

Plants also have uncoupling proteins for heat generation. Arum lilies attract carrion beetles and flies for pollination by exuding odors that smell like carrion. Skunk cabbage elevates its body temperature as much as 10 - 12° C above ambient air temperature for flowering. Plants emerge through snow in the spring use thermogenesis, too.

![Arum Thermogenesis](image-url)
**Versatility of Metabolic Pathways**

We present aerobic respiration from its typical start with glycolysis using glucose fuel. We know, however that fats and proteins can also be used to provide energy for cells. How do these molecules fit into cellular respiration?

Using other fuel molecules in the energy releasing pathways.

- Other carbohydrates -----> Glucose -----> Glycolysis
- Proteins -----> Amino Acids -----> Pyruvate -----> Krebs Cycle or
- Proteins -----> Amino Acids -----> Krebs Cycle or
- Proteins -----> Amino Acids** -----> Glucose -----> Glycolysis

** Amino acids in this group are converted to pyruvate and metabolized "back" to glucose to provide glucose to brain and nervous system cells and developing red blood cells.

Note: All amino acids must be deaminated prior to being used for fuel.
- Lipids: Glycerol -----> Glycolysis (Glyceraldehyde 3 Phosphate)
- Lipids: Fatty Acids -----> Acetyl -----> Krebs Cycle
- Alcohol -----> Acetyl -----> Krebs Cycle
Some Notes

• Some of the steps in nutrient inter-conversion can work in both directions. Acids from the Krebs cycle can be used to synthesize some amino acids, and acetyl can be used to synthesize fatty acids. (About half the amino acids are non-essential in this sense; they can be made from other amino acids or from other acids in the cells.)

• Fats are more energy rich than carbohydrates. A gram of fat potentially can produce two times as much ATP as a gram of carbohydrate. Most moderate muscle activity, such as breathing and heartbeat, routinely uses a mixture of fats and carbohydrates. However, use of fatty acids for fuel is a strictly aerobic process. All anaerobic respiration must have glucose. Also, fatty acid fragments cannot normally cross the brain membrane barriers so that the brain does not use fats for fuel.

• During starvation or fasting, or when there is insufficient carbohydrate for energy needs, the body uses its protein from body tissues to produce glucose fuel molecules for the brain and red blood cells. (Some amino acids can be converted to pyruvate and by reversing the steps of glycolysis to glucose.)

• When fat reserves are mobilized in response to insufficient calories or insufficient carbohydrate in the diet, some of the fatty acid fragments combine to form ketone bodies rather than acetyl. These ketone bodies enter into circulation. Muscle and some other tissues can use ketone bodies for fuel, and ketone bodies can provide energy to some brain cells. However, some ketone bodies contain carboxyl groups forming keto acids that can cause ketosis, a condition that lowers the pH of the blood and impairs health.