

Cell Biology Part 2

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Table of Contents

1	Introduction	3
1.1	Important Molecules	3
1.2	Tips + Tricks for Understanding Reactions	4
1.2.1	Naming	4
1.2.2	Important Enzymes	4
2	Cellular Respiration	5
2.1	Glycolysis	5
2.1.1	Energy Investment	6
2.1.2	Energy payoff	7
2.1.3	Result	7
2.2	Intermediate Step	7
2.3	Krebs Cycle	8
2.3.1	Result	10
2.4	Electron Transport Chain (ETC)	10
2.4.1	ATP synthase	11
2.4.2	The Chain	13
2.4.3	When things go wrong	14
2.4.4	Result	15
2.5	Fermentation	16
2.5.1	Alcohol Fermentation	16
2.5.2	Lactic Acid Fermentation	17
2.5.3	Anaerobic Respiration	17
2.6	Gluconeogenesis	18
3	Photosynthesis	19
3.1	Photosystems	19
3.2	Light Reactions	21
3.2.1	Linear Electron Flow	21
3.2.2	Cyclic Electron Flow	22
3.3	Calvin Cycle/Light-Independent Reactions/Dark Reactions	23
3.4	Photorespiration	25
3.4.1	C4	26

3.4.2 CAM	28
4 Conclusion	28

1 Introduction

Cells are always performing activities—moving, eating, communicating, transporting solutes, etc. When performing these activities, cells require energy. To obtain energy, cells must harvest usable energy from their available surroundings effectively. The process of cellular respiration and photosynthesis facilitate the process of obtaining and storing energy to keep a cell alive.

For this handout, obscure info will be in gray.

1.1 Important Molecules

Since the goal of cellular respiration and photosynthesis is to produce energy, we should pay careful attention to any energy-rich molecules. These molecules are color-coded throughout the notes to help you notice when they come into play:

- **ATP (adenosine triphosphate)** is the cell's main unit of energy. Its ability to be quickly broken down and formed allows it to be used on demand wherever it is needed in the cell. Due to its convenience, the goal of cellular respiration is going to be to produce as much ATP as possible.

ATP is made up of a nitrogenous base (adenine), the sugar ribose, and 3 phosphates linked together. To utilize the energy from ATP, the bonds between phosphates are broken, converting ATP into ADP (adenosine diphosphate) and P_i (inorganic phosphate). This can be broken down even further to form AMP (adenosine monophosphate) and P_i .

A single phosphate on its own is called **orthophosphate**, while two phosphates together is called **pyrophosphate**.

- When ATP is made as a result of a reaction catalyzed by an enzyme, it is known as **substrate-level phosphorylation**.
- When ATP is made as a result of the ETC (which uses O_2), it is known as **oxidative phosphorylation**.
- When ATP is made during photosynthesis, it is known as **photophosphorylation**.

- **NAD⁺ (Nicotinamide adenine dinucleotide)** is what is known as an **electron carrier**. This means NAD⁺ stores energy in the form of electrons. It will accept H⁻ (a hydrogen with an extra electron) from molecules, turning it into NADH. The NADH will then release its electrons at the ETC (explained later) to convert that energy into ATP. Each NADH can be cashed in at the ETC for **3 ATP**, although if you account for the energy it takes to transport pyruvate into the mitochondria it's more like 2.5ATP or even 2ATP depending on who you ask.

When NAD⁺ turns into NADH, we say it is **reduced**, since it accepts electrons. NADH is the reduced form, NAD⁺ is the **oxidized** form.

Vitamin **B3 (niacin)** is important for the production of NAD⁺.

- **FAD (Flavin adenine dinucleotide)** is very similar to NAD⁺ except it accepts 2H to become FADH₂. Like NADH, FADH₂ is used in the ETC to generate ATP. Each FADH₂ can be cashed in at the ETC for **2 ATP**, although some sources will say it's more like 1.5ATP.

Vitamin **B2 (riboflavin)** is important for the production of FAD.

- **NADP⁺** is very important in photosynthesis. It serves the same function as NAD⁺, except its energy is used to generate glucose in the Calvin cycle.

1.2 Tips + Tricks for Understanding Reactions

Both cellular respiration and photosynthesis contain many steps in their chemical reactions, all of which are helpful to know. As a result, it can be daunting to try and tackle them all, especially for those with no background in chemistry. Here are some general rules that can help make the process easier.

1.2.1 Naming

If you've dabbled in organic chemistry, then you already know how to name compounds. However, anyone can understand compound names by following these two general rules:

1. If you add something to a compound, then you just add it to its name! For example, hexokinase adds a **phosphate** onto **glucose**, turning it into **glucose-6-phosphate**. See that second part of the name simply shows where we added the phosphate.
2. Enzymes are often named by their substrate. For example, **phosphofructokinase** adds a **phosphate** to **fructose-6-phosphate**.

Keep in mind that many reactions catalyzed by enzymes are reversible. This means that sometimes we'll see reactions happen in *reverse*. For example, the enzyme **pyruvatekinase** takes a **phosphate** from PEP to make **pyruvate**. If we consider the reverse reaction (adding a phosphate to pyruvate), then the name of the enzyme makes a lot more sense.

1.2.2 Important Enzymes

There are a few major types of enzymes you need to know. By being able to identify the key words, it will be a lot easier to understand which steps produce which products:

- **Kinase:** An enzyme that adds a phosphate. Therefore, whenever you see a kinase being used, **ATP** will be either used or produced.
- **Dehydrogenase:** As the name suggests, this enzyme takes away a hydrogen (de-hydrogen) from its substrate. This hydrogen is then given to an electron carrier. Therefore, whenever you see a dehydrogenase being used, **NADH** or **FADH₂** will be produced.
- **Isomerase:** As the name suggests, this enzyme converts a molecule to its isomer. For example, **phosphoglucoisomerase** converts **glucose-6-phosphate** to its isomer **fructose-6-phosphate**.

2 Cellular Respiration

The collection of all chemical reactions that occur within an organism is known as that organism's **metabolism**. Many of these reactions require energy in the form of ATP. Thus, **cellular respiration**, or the harvesting of ATP from glucose, is an important process. Cellular respiration can be summarized in the following reaction:



Here, glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) is broken down using oxygen (O_2), producing carbon dioxide (CO_2) and water (H_2O) as by-products.

This process occurs over several distinct steps, with each step having their own pathways to be memorized:

1. **Glycolysis:** glucose is broken into **2 pyruvate**, yielding a net of **2 ATP** and **2 NADH** in the process. Since 2 pyruvate are produced, each subsequent step happens twice.
2. **Intermediate step:** pyruvate is converted to **acetyl CoA**, producing **1 CO₂** and **1 NADH**.
3. **Krebs Cycle:** acetyl CoA is put into a cycle of reactions to farm as much NADH from it as possible. Each acetyl CoA makes **1 ATP**, **3 NADH** and **1 FADH₂**.
4. **Electron Transport Chain (ETC):** Now we cash in all the NADH and FADH₂ we made. Each NADH is worth **3 ATP**, so $3(2 + 2(1 + 3)) = 3(10) = \text{30 ATP}$. Each FADH₂ is worth **2 ATP**, so $2(2(1)) = 2(2) = \text{4 ATP}$. Putting this all together, we have 30 ATP from NADH, 4 ATP from FADH₂, 2 ATP from glycolysis, and $2(1) = 2$ ATP from the Krebs cycle. $30 + 4 + 2 + 2 = \text{38 ATP!}$

Note that if we plug in our adjusted values, $10(2.5) + 2(1.5) + 2 + 2 = 32$ ATP, or $10(2) + 2(2) + 2 + 2 = 30$ ATP. Thus, even though the cell could *theoretically* generate 38 ATP per glucose, realistically it is more like 30-32 ATP.

In other words, cellular respiration is an incredibly efficient method of extracting energy from glucose. As we will discuss later, sometimes **fermentation** is used as an alternative pathway when O_2 is not available.

For each step, we will go through the pathway together. It's a lot of reactions to remember, so I will put a * next to the most important steps. In addition, I will put a * next to the steps that are **irreversible**, this will become important when we talk about **gluconeogenesis**.

2.1 Glycolysis

Glycolysis occurs in the **cytoplasm** of **all** cells! Refer to the diagram below as we go through all of its steps:

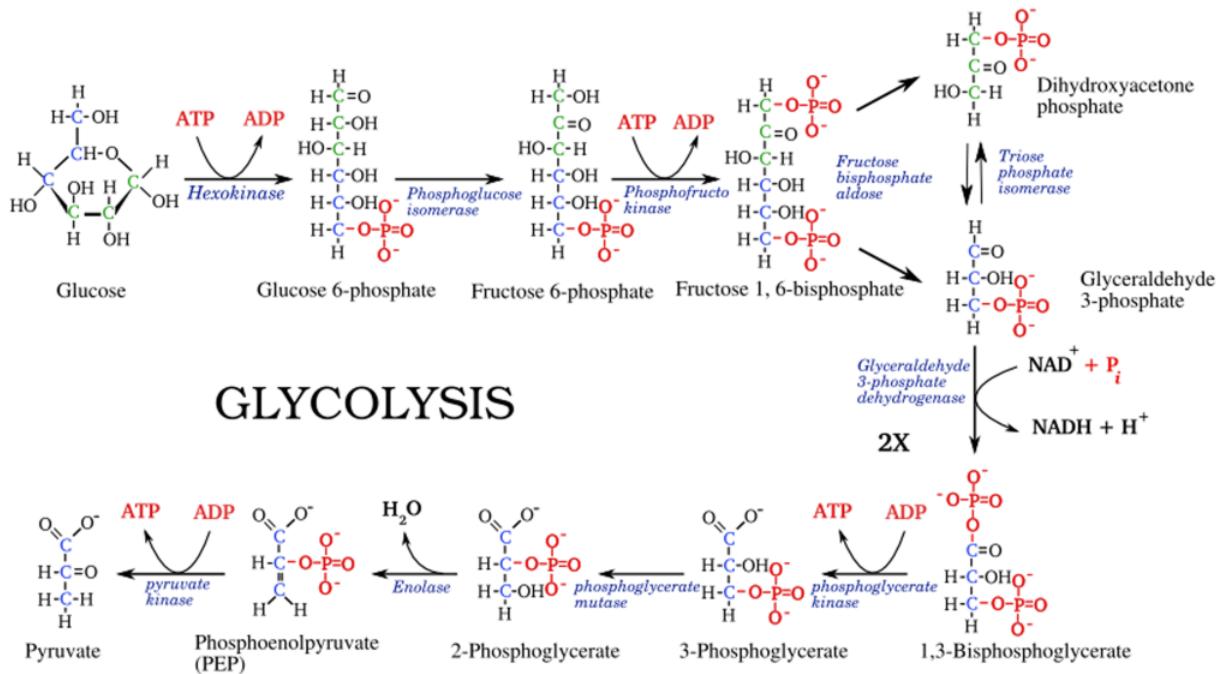


Figure 1: Steps of glycolysis. (Source: Pedaa.com)

2.1.1 Energy Investment

Glycolysis is split into two phases, **energy investment** (top) and **energy payoff** (bottom). The energy investment phase *uses* ATP to convert glucose to 2 G3P (glyceraldehyde 3-phosphate). In the energy payoff phase, G3P will be broken down to *make* ATP.

1. ** Hexokinase uses 1 ATP to add a phosphate to glucose, converting it to glucose-6-phosphate.

Glucose is brought into the cell using **GLUT** transporters. Recall from Cell Bio pt 1, GLUT is an example of *facilitated diffusion*. Free glucose flows from high concentrations outside the cell to low concentrations inside the cell. However, in order to keep a steady flow of glucose and to prevent any glucose from leaving the cell, the cell needs to do something to the glucose coming in. This is why hexokinase is so important: it *traps* glucose inside the cell by adding a charged phosphate to it. This keeps *free* glucose concentrations inside the cell low, maintaining the concentration gradient.

2. Phosphoglucoisomerase converts glucose-6-phosphate to fructose-6-phosphate.
3. ** Phosphofructokinase (PFK-1) uses 1 ATP to add a phosphate to fructose-6-phosphate, converting it to fructose-1,6-bisphosphate. Note that “bis” is used in the name instead of “bi” because the phosphates are not adjacent.

This step is known as the **rate-limiting step** and serves as the main control point for cellular respiration. Compounds that indicate that the cell already has a lot of energy, such as ATP and citrate, *inhibit* PFK-1 while compounds that indicate that the cell needs more energy, such as ADP, *activate* PFK-1.

4. **Fructose bisphosphate adolase** chops the 6-carbon **fructose-1,6-bisphosphate** into two 3-carbon molecules: DHAP and G3P.

- The top half becomes **DHAP** (**dihydroxyacetone phosphate**). DHAP is useful in regulating glycolysis, as it can be used to make triglycerides, but it is often ignored.
- The bottom half becomes **G3P** (**glyceraldehyde 3-phosphate**). In the energy payoff phase, we will break down G3P to get ATP from it.
- **Triose phosphate isomerase** can convert between DHAP and G3P. Most of the time, it turns DHAP into another G3P. This means that we now have 2 G3P. Therefore, all steps that follow will occur **twice**.

2.1.2 Energy payoff

1. **Glyceraldehyde 3-phosphate dehydrogenase** replaces a H^- with a **phosphate**, resulting in **1,3-bisphosphoglycerate**. The H^- reduces a NAD^+ to **NADH**!
2. **Phosphoglycerate kinase** takes a **phosphate** (making **1 ATP**!) from **1,3-bisphosphoglycerate**, turning it into **3-phosphoglycerate**.
3. **Phosphoglycerate isomerase** moves the phosphate on **3-phosphoglycerate**, turning it into **2-phosphoglycerate**.
4. **Enolase** takes 1 H_2O from **2-phosphoglycerate**, turning it into **phosphoenolpyruvate**, better known as **PEP**.
5. **Pyruvate kinase** takes a **phosphate** (making **1 ATP**!) from **PEP**, resulting in **pyruvate**.

2.1.3 Result

As we have seen, glycolysis converted **glucose** into **2 pyruvate**. A net of **2 ATP** was made since **4** ($2 \times$ steps 2,5 of energy payoff) - **2** (steps 1, 3 of energy investment) = **2**.

Furthermore, **2 NADH** was made ($2 \times$ step 1 of energy payoff).

2.2 Intermediate Step

Glycolysis occurred in the cytoplasm of all cells. However, the steps after glycolysis focus on extracting ATP from NADH, which is only possible when O_2 is present. All of these steps occur in the **matrix of the mitochondria**, and thus are only found in eukaryotes. The intermediate step takes pyruvate from the cytoplasm and brings it into the mitochondria, converting it into **acetyl CoA** in preparation for the Krebs Cycle.

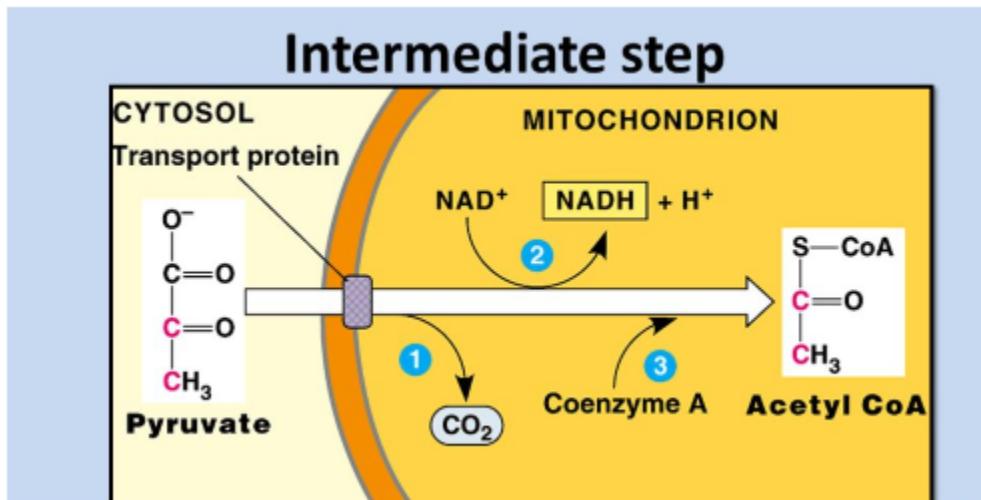


Figure 2: The intermediate step. (Source: SlidePlayer)

***Pyruvate dehydrogenase** is actually a complex of multiple enzymes, allowing it to accomplish three things at once:

1. removes a CO_2 from the top of pyruvate (this is the first time CO_2 is produced!)
2. produces 1 NADH
3. adds a *Coenzyme A* to pyruvate, converting it to **Acetyl CoA**

Vitamin **B5 (pantothenic acid)** is an important component of Coenzyme A.

2.3 Krebs Cycle

Now, the cell takes the acetyl CoA it made and milks as much NADH as it can from it. This is known as the **Krebs Cycle**, or Citric Acid Cycle, or TCA cycle (Tricarboxylic acid cycle)... yeah, it has a lot of names.

The important thing is that it is a *cycle*, which means it will start and end with the 4-carbon **OAA (Oxaloacetate)**. The naming of the intermediates is not as intuitive as it was for glycolysis, but there are plenty of mnemonics out there to help you remember (my [AN] personal favorite is “susy baka is not Obama’s friend”... trust me... it works, like really well).

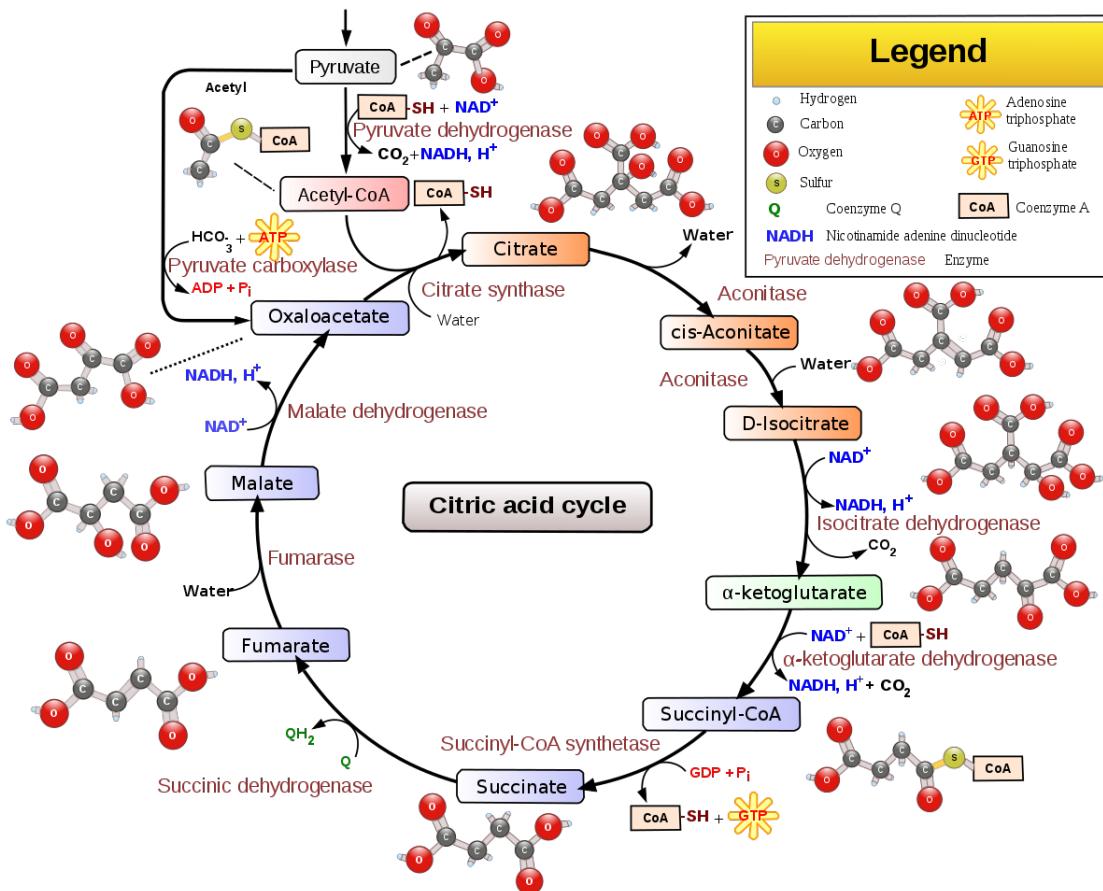


Figure 3: The Krebs Cycle. (Source: Wikipedia)

1. **Citrate synthase** makes 6-carbon **citrate** by adding 4-carbon OAA to 2-carbon acetyl CoA.
2. **Aconitase** converts citrate into **D-isocitrate**, using **cis-Aconitase** as an intermediate.
3. **Isocitrate dehydrogenase** converts **D-isocitrate** to **α -ketoglutarate**. Since it is a dehydrogenase, **1 NADH** is produced in the process. Notice that **1 CO₂** is removed. This means that **α -ketoglutarate** is a 5-carbon molecule.
4. **α -ketoglutarate dehydrogenase** converts **α -ketoglutarate** to **Succinyl-CoA**. Since it is a dehydrogenase, **1 NADH** is produced in the process. Notice that **1 CO₂** is removed. This means that Succinyl-CoA is a 4-carbon molecule.
5. **Succinyl-CoA synthetase** removes a **CoA**, from **Succinyl-CoA** synthetase, turning it into **Succinate**. This also produces **1 GTP** in the process, which is quickly converted into **1 ATP**!
6. **Succinic dehydrogenase** removes **2H** from **succinate** producing **FADH₂** (FAD is Q in the diagram) and **Fumarate**.
7. **Fumarase** turns **fumarate** into **Malate**.
8. **Malic dehydrogenase** turns **malate** into **OAA**, producing **1 NADH** in the process.

And with that final step, we are back to where we began, completing the cycle.

2.3.1 Result

Just to make it clear, let's review what was made during the Krebs cycle.

3 NADH was produced (steps 3,4,8) and since we have 2 acetyl CoA that means we produced $3 \times 2 = 6 \text{ NADH}$. Combining that with the $1 \times 2 = 2 \text{ NADH}$ produced during the intermediate step and the $1 \times 2 = 2 \text{ NADH}$ produced during glycolysis brings us to a total of $6 + 2 + 2 = 10 \text{ NADH}$!

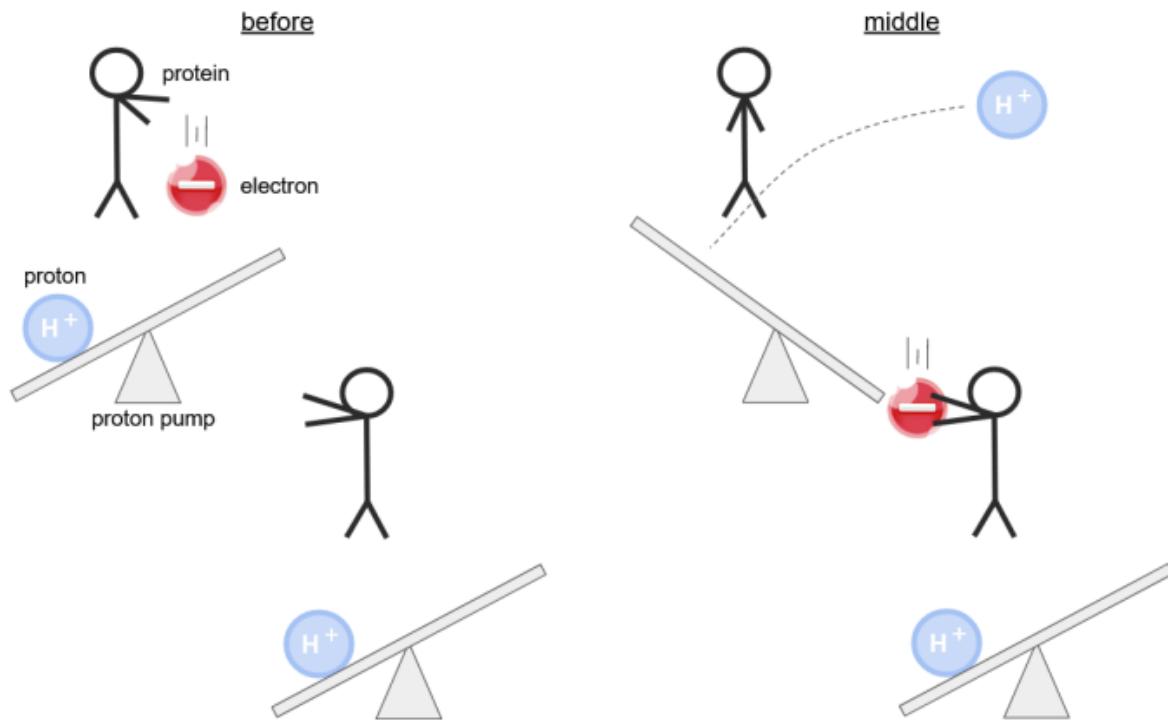
1 FADH₂ was produced (step 6) and since we have 2 acetyl CoA that means we produced $1 \times 2 = 2 \text{ FADH}_2$. Since this is the only step in cellular respiration where FADH₂ is produced, that means our total is **2 FADH₂**!

1 ATP was *indirectly* produced (step 5) and since we have 2 acetyl CoA that means we produced $1 \times 2 = 2 \text{ ATP}$. If we include the net **2 ATP** from glycolysis, that brings us to a total of **4 ATP**!

2 CO₂ was produced (steps 3,4) and since we have 2 acetyl CoA that means we produced $2 \times 2 = 4 \text{ CO}_2$. Combining this with the $1 \times 2 = 2 \text{ CO}_2$ released during the intermediate step, that brings us to a total of **6 CO₂**! This is where the 6 CO₂ in the equation for cellular respiration comes from.

2.4 Electron Transport Chain (ETC)

As the name suggests, the **Electron Transport Chain** is a chain of proteins that transport electrons from one to the next like a game of hot potato. Each of these proteins has their own **electronegativity**, or how strongly they want an electron. As an electron moves down the chain, it is held by proteins with stronger and stronger electronegativity until in the end, the most electronegative is oxygen, the **final electron acceptor**.



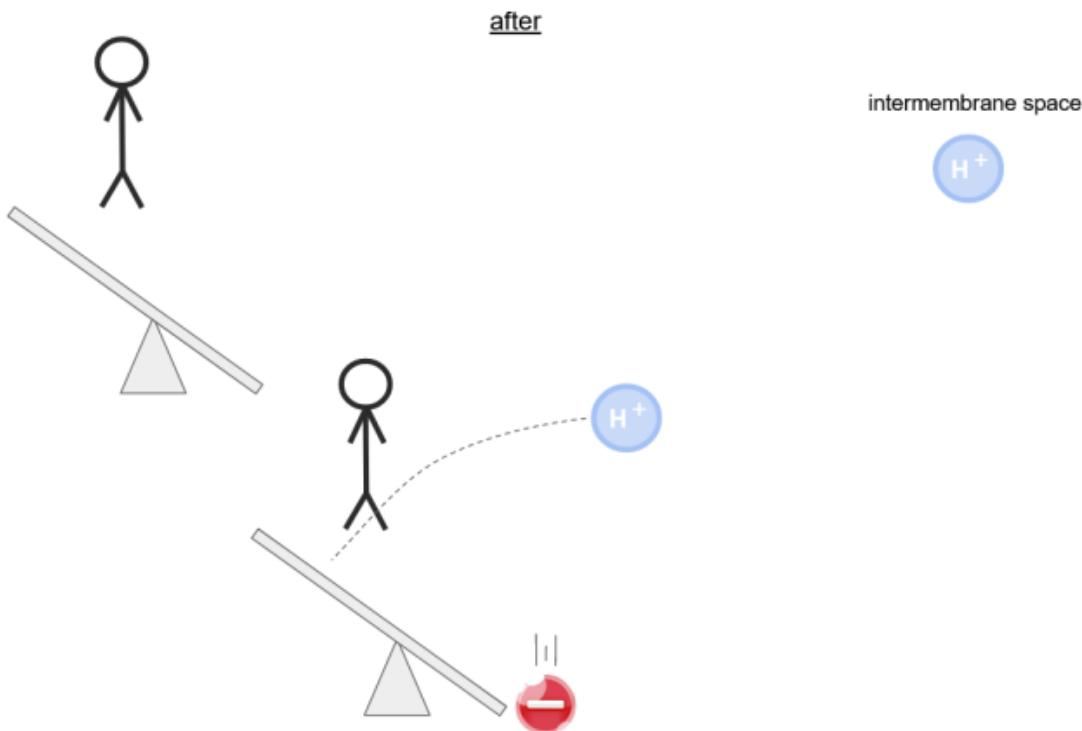


Figure 4: In this three-step figure, an analogy for the electron transport chain is depicted. A protein “drops” an electron through a proton pump, decreasing the electron’s energy level and using that released energy to pump a proton into the intermembrane space. The next protein down the chain does this as well, and the process repeats until the electron reaches the end of the chain. It is important to note that in this figure, height represents potential energy.

(Source: Andrew Ung)

Each time an electron is exchanged, there is a change in energy due to the difference in electronegativity. The ETC uses this energy to pump protons (H^+) from the matrix to the **intermembrane space**. This accumulation of H^+ in the intermembrane space results in an **electrochemical gradient** (both the positive charges and the high concentration will cause protons to diffuse from the intermembrane space to the matrix). Therefore, if there was an open channel in the inner membrane, then protons would freely move across that membrane down their concentration. This movement is called **chemiosmosis**.

The cell utilizes this chemiosmosis by using a very special channel: **ATP synthase**. The movement of H^+ creates a **proton-motive force**, which causes ATP synthase to *rotate*, making ATP from ADP and P_i .

2.4.1 ATP synthase

ATP synthase (sometimes called Complex V) is made of multiple subunits. One of them is **F0** (subunit c), the part that is in the inner membrane and acts a *rotor* (along with γ and ϵ subunits). The other part is in the matrix and is called **F1**. This is the part that actually makes ATP (specifically subunits α and β). In addition, this part is also called the **stator** because it is stationary and holds the rotor in place.

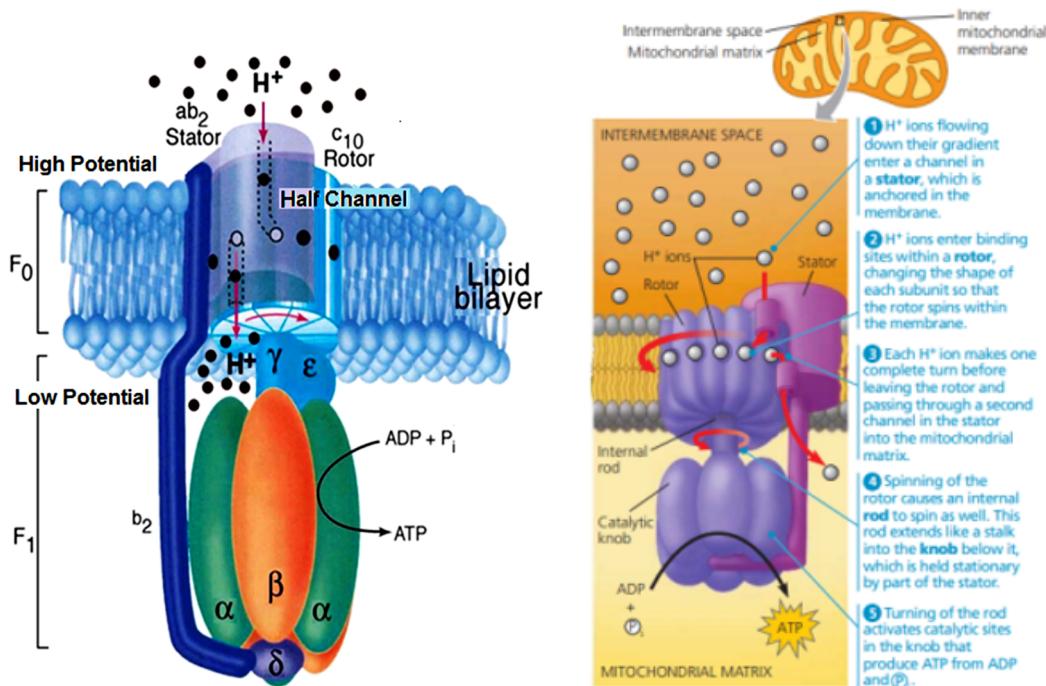


Figure 5: (left) diagram showing components of ATP synthase (Source: Research Gate), (right) diagram showing how ATP synthase uses the proton-motive force to generate ATP (Source: Campbell's 9th Edition)

Example 2.1 (USABO Opens 2018)

4. Select the answer choice that correctly describes which chemical species are oxidized, reduced, and phosphorylated in the process of oxidative phosphorylation.

Choice	Oxidized	Reduced	Phosphorylated
A	H ₂ O only	NAD ⁺ and FAD	ADP
B	NADH and FADH ₂	O ₂	ADP
C	NADH and FADH ₂	CO ₂	ATP
D	H ₂ O only	NAD ⁺ only	ADP
E	NADPH only	FAD only	ATP

Solution: This question is asking about what occurs during the ETC (oxidative phosphorylation). Remember, a molecule is *oxidized* when it *loses* electrons, and a molecule is *reduced* when it *gains* electrons (you can remember this as OIL RIG → oxidation is losing, reduction is gaining). In the ETC, NADH and FADH₂ lose their electrons, which is passed along the chain until it is eventually gained by oxygen. Therefore, **the answer is B.**

2.4.2 The Chain

Now that we understand how the ETC works, let's look at its components (I shortened Complex to "Com", but this is not standard notation):

Com I (ubiquinone oxidoreductase): the largest, most complex, and most important complex in the ETC. Accepts electrons from NADH.

Com II (succinate dehydrogenase): accepts electrons from FADH_2 . This is the only Complex that does NOT pump protons.

- **Q** (Ubiquinol): this is one of the *mobile* electron acceptors in the ETC. As a lipid, Q can move freely within the inner membrane, shuttling electrons from Complex I and II to Complex III.

Com III (ubiquinol-cytochrome c oxidoreductase / cytochrome bc₁ complex)

- **Cytochrome c**: this is the other *mobile* electron acceptor in the ETC. This one is a water-soluble protein and moves around the intermembrane space, shuttling electrons from Complex III to Complex IV

Com IV (cytochrome c oxidase): the electrons from Complex IV are accepted by oxygen, resulting in the **formation of water**.

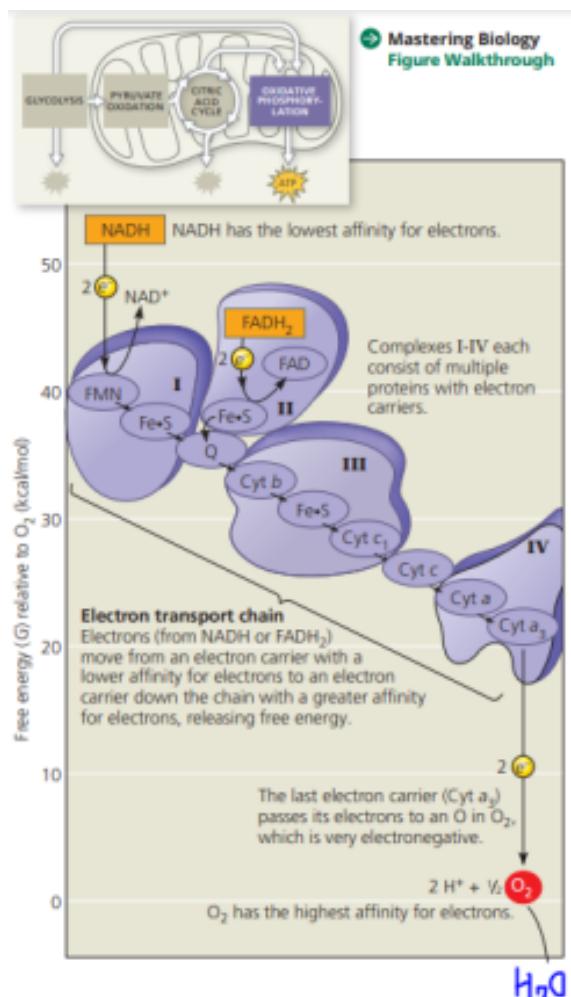


Figure 6: This diagram orders the components of the ETC by free energy.
(Source: Campbell's 9th Edition)

Example 2.2 (USABO Opens 2018)

Questions 7 to 10. Indicate if each of the following statements is **TRUE** or **FALSE** about the electron transport chain in cellular respiration. Use "A" for True and "B" for False.

7. Ubiquinone proteins have a heme group to facilitate electron transport.
8. FADH_2 adds its electrons to complex III while NADH adds its electrons in complex I.
9. Cytochrome a is more electronegative than cytochrome b.
10. Cytochrome c is more electronegative than cytochrome b.

Solution: These questions require a deep understanding of the components of the ETC. Question **7 is False (B)** since ubiquinone (the oxidized form of ubiquinol) is a lipid, not a protein. Therefore, it will not have a heme group. Question **8 is False (B)** since FADH_2 gives its electrons to complex II. Question 9 and 10 are tricky, as you would have had to pay attention to the components of each complex in Figure 6. Cytochrome b is a part of complex III and Cytochrome a is a part of complex IV. We know that the ETC goes complex III → cytochrome c → complex IV. In addition, we know that the parts later in the ETC are more electronegative, with oxygen being the most electronegative. Therefore, **9 and 10 are both True (A)**.

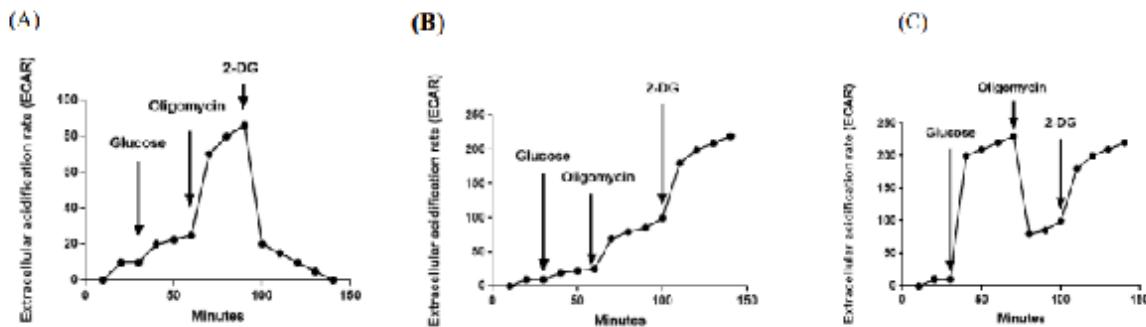
2.4.3 When things go wrong

Many questions like to test your understanding of the ETC by asking you what would happen if something *didn't* go as expected. Let's run through a few scenarios:

- No O_2 : This can happen during strenuous exercise, as your muscle cells use up all the O_2 they have access to. Since O_2 is the final electron acceptor, without it, the electrons in the ETC have no where to go to, halting the chain. Thus, the cell will stop at glycolysis and not proceed any further, since the Krebs cycle generates NADH that is now useless. In the next subsection, we will talk more about the alternative pathways taken in such an event.
This can also happen as a result of drugs, such as **cyanide**, that inhibit components of the ETC.
- A channel for H^+ forms: Some drugs, such as **dinitrophenol (DNP)**, create a new channel in the inner membrane for H^+ to pass through. As a result, H^+ will flow out of the intermembrane space without powering ATP synthase. This increases the pH of the intermembrane space (since there is less H^+ it is less acidic) and the subsequent loss of the electrochemical gradient drastically decreases the rate of ATP production. Since the cell is not producing ATP when it should, the mitochondria may freak out and respond by increasing heat production.
- ATP synthase is blocked: In this case, the ETC functions as normal, pumping H^+ to the intermembrane space. However, with ATP synthase blocked, those H^+ have no where to go to. As a result, the pH of the intermembrane space decreases (since more H^+ makes it more acidic). If the effects are really bad, the H^+ can even leak out of the *outer* membrane, decreasing the pH of the cytoplasm.

Example 2.3 (USABO Semifinals 2018)

83. In macrophages purified from murine spleen, you want to measure extracellular acidification rate (ECAR) in a non-buffered media by adding different chemical treatments in the following order: Glucose, oligomycin (an irreversible inhibitor of ATP synthase), and 2-DG (an irreversible inhibitor of hexokinase). How would ECAR change over different time points in purified macrophages?



also uses H_2O ??? Honestly, I have no clue, which conveys an important message: the cell is full of water so don't start counting where water is used in reactions as that'll just drive you crazy. **All you need to worry about is that the ETC produces water from O_2 .**

2.5 Fermentation

Organisms can be classified based on whether or not they use oxygen. **Aerobes** use O_2 and will die if they lack it. **Obligate Anaerobes** can't use O_2 and will die if they are exposed to it. Meanwhile, **Facultative Anaerobes** get the best of both worlds, using O_2 when it's available and switching to other means when it's not.

If some organisms don't use O_2 , then what do they do after glycolysis? The point of all subsequent steps in cellular respiration were to generate a bunch of NADH that can be then used in the ETC to make ATP. However, if they lack oxygen, then they can't use the ETC.

So do they just stop at glycolysis? NO! Another important role of the ETC that you probably ignored is that it turns NADH back into NAD^+ . By *regenerating* NAD^+ , we can continue to perform glycolysis, which needed NAD^+ to make NADH in the step involving G3P dehydrogenase. Therefore, if the ETC is not an option, anaerobes need to find some other way to regenerate NAD^+ . This is where fermentation comes in handy!

2.5.1 Alcohol Fermentation

Alcohol Fermentation converts the pyruvate from glycolysis into **ethanol** in 2 steps:

1. **PDC** (pyruvate decarboxylase) converts pyruvate to acetaldehyde, releasing **1 CO_2** in the process.
2. **ADH** (alcohol dehydrogenase) converts acetaldehyde to ethanol, converting **1 NADH** to **1 NAD^+** in the process.

Since 2 pyruvate are produced from glycolysis, that means alcohol fermentation releases $1 \times 2 = 2 \text{ CO}_2$ and regenerates $1 \times 2 = 2 \text{ NAD}^+$. Alcohol fermentation most commonly occurs in **yeast**. This is why yeast is used in baking bread (the CO_2 released allows the bread to rise) and brewing alcohol (the ethanol produced is an alcohol).

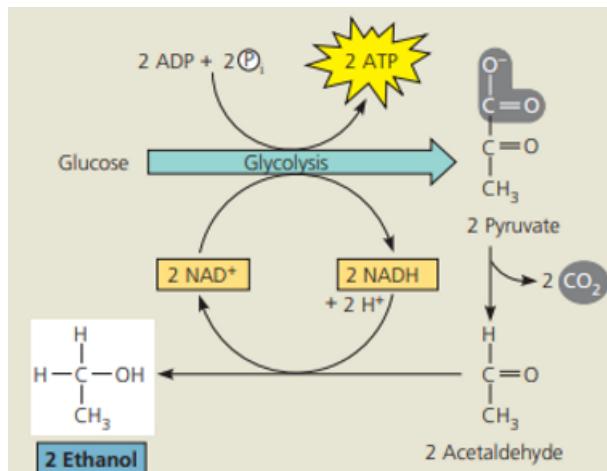


Figure 7: Alcohol fermentation (Source: Campbell's 9th Edition)

Example 2.4 (USABO Opens 2015)

5. A liquid culture of yeast was grown under anaerobic conditions but then had a significant quantity of alcohol dehydrogenase inhibitor added to the medium. Which of the following would best describe the effect on glycolysis due to the presence of the inhibitor?
- Glycolysis would continue with no change in catabolic efficiency.
 - Glycolysis would continue with higher catabolic efficiency.
 - Glycolysis would continue with lower catabolic efficiency and pyruvate would be further broken down through the Krebs cycle.
 - Glycolysis would continue with lower catabolic efficiency and the cells would start to excrete pyruvate instead of alcohol.
 - The rate of glycolysis would slow to a complete stop.

Solution: The whole point of fermentation is to regenerate the NAD^+ so that glycolysis can proceed. In this scenario, NAD^+ can not be regenerated. Therefore, glycolysis can not proceed and our **answer is E**.

2.5.2 Lactic Acid Fermentation

Lactic Acid Fermentation converts the pyruvate from glycolysis into **lactic acid** in 1 step and does *not* release CO_2 . This type of fermentation occurs in human muscle cells after strenuous exercise and in making foods like yogurt.

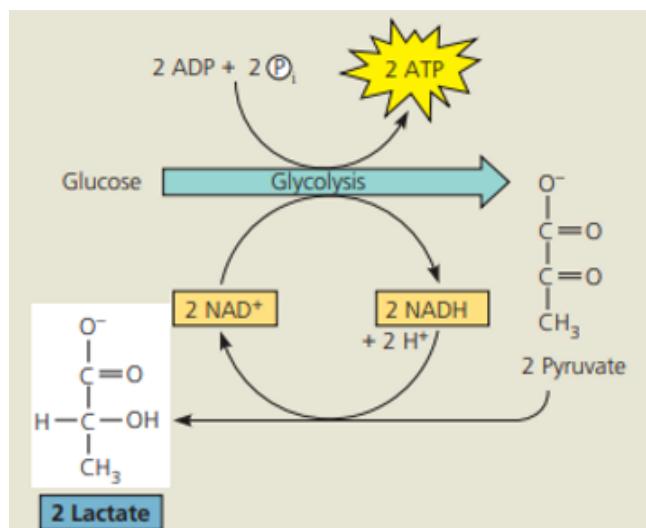


Figure 8: Lactic acid fermentation (Source: Campbell's 9th Edition)

2.5.3 Anaerobic Respiration

Despite what you may have been taught, anaerobic respiration is NOT the same thing as fermentation. Just like how cellular respiration uses the ETC, anaerobic respiration uses a special ETC of its own, except it uses something else in place of O_2 . The most common example is in purple sulfur bacteria, which use sulfate (SO_4^{2-}) from *hydrothermal vents*. As a result, instead of making H_2O , they make H_2S , better known as **hydrogen sulfide**.

2.6 Gluconeogenesis

As we have seen, cellular respiration allows a cell to break down glucose into ATP, the cell's most usable form of energy. However, what happens when a cell runs out of glucose to use? Simple, the cell just needs to make more glucose!

When a cell makes new glucose from other stores of energy, it is known as **gluconeogenesis** (genesis = making, neo = new, gluco = glucose). Essentially, the cell can convert its stores of energy into an intermediate in cellular respiration and since reactions are reversible, it'll go through the steps backwards until it eventually gets turned into glucose. Depending on the source of energy it will get converted into a different intermediate:

- Carbohydrates can just be broken down into glucose
- Fats are made up of a glycerol, which can be converted into G3P, and fatty acid tails, which can be converted to acetyl CoA via **beta oxidation**. Beta oxidation also releases some **NADH** and **FADH₂**, which can be used in the ETC to generate more ATP.
- Proteins are made up of amino acids. Depending on the amino acid, it can be converted into a different intermediate in glycolysis:
 - Most of them are **glucogenic** and can be converted to:
 - * Pyruvate (CT SWAG)
 - * α -ketoglutarate (REP HQ)
 - * Succinyl-CoA (MITV)
 - * Fumarate (PYD)
 - * Oxaloacetate (DN)
 - Some of them are **ketogenic** and are converted into *Acetyl CoA* (TWYP KIL).
- The **Cori cycle** can convert lactate (lactic acid) to glucose, which is then converted to lactate via lactic acid fermentation, which is then converted to glucose... and so on.

Even though I said that gluconeogenesis was like cellular respiration but in reverse, that isn't actually true since some steps were *irreversible*. To get around this issue, gluconeogenesis implements some new enzymes:

- Instead of pyruvate dehydrogenase, **PC** (pyruvate carboxylase) is used.
- Instead of pyruvate kinase, **PEPCK** (PEP carboxykinase) is used.
- Instead of PFK, **FBPase** (fructose 1,6 biphosphatase) is used.
- Instead of hexokinase, **G6Pase** (glucose-6-phosphatase) is used.

3 Photosynthesis

In many ways, **photosynthesis** is very similar to cellular respiration. They both occur in a special organelle, with photosynthesis occurring in the **chloroplast**. They both utilize electron carriers and contain an ETC of sorts, generating ATP. Even their reactions look similar, with photosynthesis simply being the reverse reaction of cellular respiration:



Photosynthesis, seen in plants, phytoplankton, and some protists, captures light energy and converts it into glucose. It is split into the **Light Reactions**, which harvest the energy from light, and the **Calvin cycle**, which uses that energy to form glucose.

3.1 Photosystems

Before we can understand the reactions of photosynthesis, we first need to understand how plants capture sunlight. They do so by utilizing **pigments**, molecules that can absorb light. Each pigment has an **absorption spectrum**, the range of wavelengths of light which it can absorb, and an **action spectrum**, the range of wavelengths of light which the pigment can use to drive a reaction. A pigment is useful when its absorption spectrum matches its action spectrum (that way it can do stuff with the light it is absorbing).

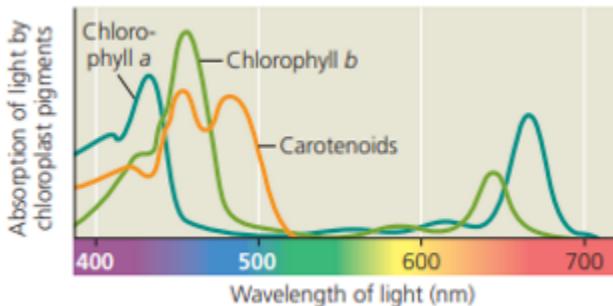


Figure 9: The absorption spectra of the three major pigments in plants. Note that green light is one of the only wavelengths *not* absorbed by pigments. Instead, green light is reflected and hits our eyes, which is why most plants look green! (Source: Campbell's 9th Edition)

Most pigments are lipid-soluble and are thus incorporated into the **thylakoid membrane**. Here are different pigments you may see:

- **Chlorophyll a:** the universal pigment. 3/4 of pigments in green plants is chlorophyll a. Mg forms the center of the chlorophyll molecule.
- **Accessory pigments:** these are additional pigments that typically absorb wavelengths that chlorophyll a misses. Different organisms have unique accessory pigments:
 - **Chlorophyll b:** in plants, green algae, and euglenoids.
 - **Chlorophyll c:** in brown algae and diatoms.
 - **Chlorophyll d and f:** two recently discovered pigments that specialize in longer wavelengths.

- **Bacteriochlorophyll:** in purple sulfur bacteria.
- **Chlorobium chlorophyll:** in green sulfur bacteria.
- **Phycobilin:** in cyanobacteria and red algae. Water soluble.
- **Carotenoids:** the red, orange, and yellow colored pigments that provide *photoprotection* from UV light. Includes **carotenes** and **xanthophylls**.

When a pigment is excited, one of 3 things can happen:

1. **Flourescence:** The pigment radiates light, which is functionally useless for photosynthesis.
2. **Resonance energy transfer:** Energy is transferred to neighboring pigment
3. The electron is transferred to the ETC, leaving an electron hole in the pigment.

To take advantage of resonance energy transfer, hundreds of pigment molecules are arranged into a **Photosystem**. A photosystem consists of an **antenna complex**, where the *antenna pigments* receive the incoming sunlight and transfer that energy via resonance energy transfer to a **reaction center** in the middle of the photosystem, which uses a *special pair* of chlorophyll a molecules to transfer an electron to the ETC. The photosystem can also have additional **light-harvesting complexes**, which are antenna complexes that don't contain a reaction center.

Each photosystem harvests light to excite an electron, sending it along the ETC. However, that leaves behind an electron hole that must be filled. As you will see, electrons from water fill this hole for Photosystem II while electrons from plastocyanin fill this hole for Photosystem I.

Example 3.1 (USABO Opens 2016)

12. The Death Star experiences a power outage. Darth Guha, being the hipster he is, really doesn't want his kale to die. He uses his red lightsaber as an emergency light source for his beloved kale. Anticipating this scenario, Darth Guha engineered his kale to overexpress beta-carotene. What is the engineered kale's photosynthetic rate, compared to wild-type kale, under the red lightsaber?

- A. The engineered kale has a significantly higher photosynthetic rate compared to wild-type kale because beta-carotene absorbs red light better than chlorophyll.
- B. The engineered kale has a significantly lower photosynthetic rate compared to wild-type kale because beta-carotene interferes with the absorption of red light.
- C. The engineered kale has a significantly lower photosynthetic rate compared to wild-type kale because beta-carotene does not significantly absorb red light.
- D. The engineered kale has approximately the same photosynthetic rate compared to wild-type kale because beta-carotene does not significantly absorb red light.
- E. The engineered kale has approximately the same photosynthetic rate compared to wild-type kale because beta-carotene is not an accessory pigment to chlorophyll a or chlorophyll b.

Solution: This question is simply asking about what wavelengths certain pigments absorb best. Beta-carotene is a carotenoid and as we can see in Figure 9, it absorbs blue light best. Therefore, it will have no effect on red-light absorption and our **answer is D**.

3.2 Light Reactions

As the name suggests, the **light reactions** absorb the energy from incoming sunlight and convert it into **ATP** and **NADPH**, which are needed for making glucose. The light reactions come in two types: *linear* electron flow, which produces **ATP** and **NADPH** in a 1:1 ratio, and *cyclic* electron flow, which produces *only* **ATP**. Since the Calvin cycle requires a 3:2 ratio of **ATP** to **NADPH**, both processes are necessary.

3.2.1 Linear Electron Flow

This process involves *both* of the major photosystems involved in photosynthesis. These photosystems go by two names: one name is based on what wavelength of light they absorb best, and the other is based on the order of their discovery. Unfortunately, **Photosystem II (P680)** comes *before* **Photosystem I (P700)** in linear electron flow.

The ultimate goal of linear electron flow is to reduce NADP^+ , forming **NADPH**. Where will the H^- to do so come from? Water! H_2O supplies the electrons that are passed from Photosystem II, along an ETC, to Photosystem I, which then passes it to another ETC that eventually passes it to NADP^+ , forming **NADPH**.

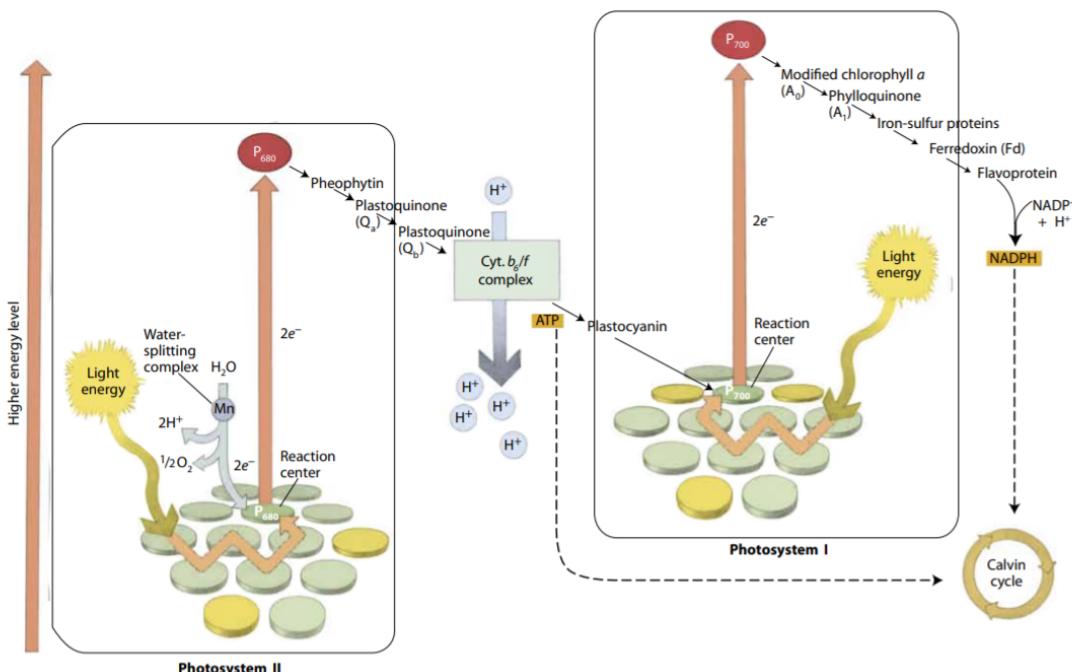


Figure 10: Diagram of linear electron flow. Each photosystem harvests light energy to excite an electron that gets sent along an ETC. The hole left by that electron is filled by the previous step.
(Source: Raven)

Photosystem II, which is located mainly in the **grana thylakoids**, contains a special **oxygen-evolving complex** (which uses Mn^{2+} ions). When Photosystem II takes electrons from water, it splits it into O_2 and 2H^+ . This splitting of water by light is known as **photolysis**. The O_2 becomes O_2 (this is how plants produce oxygen in photosynthesis!) while the H^+ is left inside the thylakoid to contribute to the electrochemical gradient used to make ATP. From there, Photosystem II passes the electron along an ETC:

Pheophytin → Plastoquinone (PQ_a) → Plastoquinone (PQ_b) → **Cytochrome b₆/f complex**

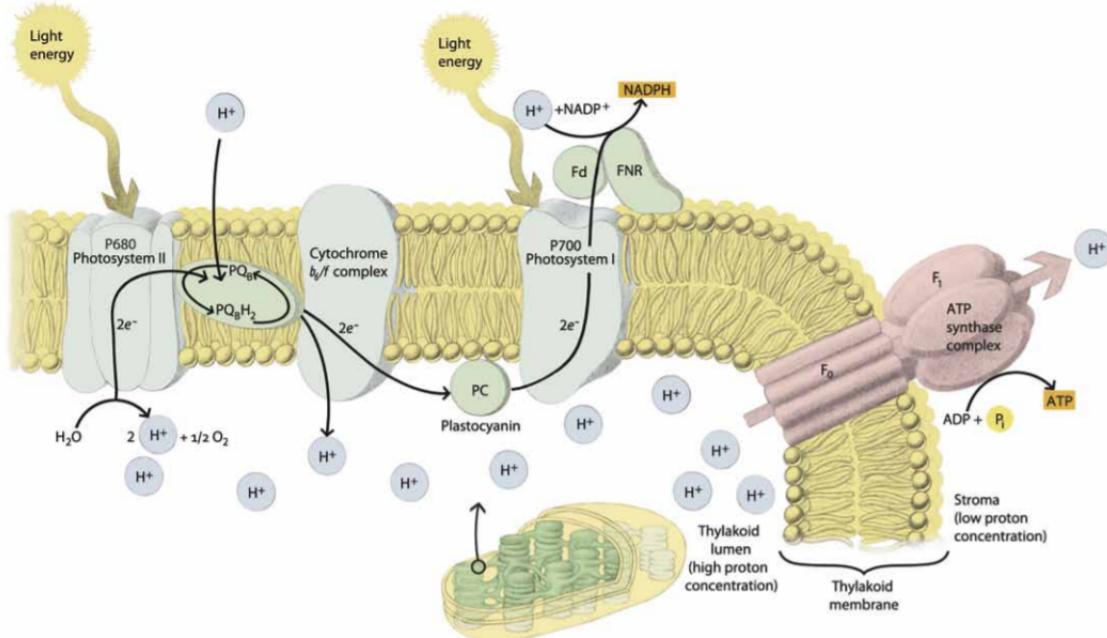


Figure 11: The proton-motive force generated by photolysis and the cytochrome b₆/f complex is used to produce ATP. (Source: Raven)

The **Cytochrome b₆/f complex** mimics the ETC in cellular respiration, using the electron carriers that feed into it to pump protons. However, while in cellular respiration protons were pumped from *inside* (matrix) to *outside* (intermembrane space), here protons are pumped from *outside* (a fluid within chloroplasts called **stroma**) to *inside* (the thylakoid lumen). Then, this electrochemical gradient is used to power ATP synthase as protons flow out of the thylakoids, producing ATP!

Cytochrome b₆/f complex → plastocyanin → Photosystem I

Photosystem I, which is located mainly in the **stroma thylakoids**, uses the electron from plastocyanin to fill its electron hole. This hole was created when Photosystem I absorbed light, sending an electron along the following pathway:

Phylloquinone (A₁) → Iron-Sulfur proteins → Ferredoxin (Fd) → Flavoprotein → NADPH

The final step is catalyzed by the enzyme **FNR (Ferredoxin-NADP⁺ reductase)**. Since our reaction for photosynthesis uses 6 H₂O (which produced 6 O₂), and each water molecule contributes 2 electrons (one from each H), then that means $6 \times 2 = 12 \text{ NADPH}$ are produced!

3.2.2 Cyclic Electron Flow

Cyclic Electron Flow utilizes *only* Photosystem I. Photosystem I feeds an excited electron to the ETC, following the same pathway as in the last part of linear electron flow. However, once the electron reaches ferredoxin, instead of using it to make NADPH, that electron is fed *back* into the Cytochrome b₆/f complex. Electrons passing through this complex power proton pumps, which create an electrochemical gradient for ATP production! In addition, since no electrons are ever fed to FNR, no NADPH is produced!

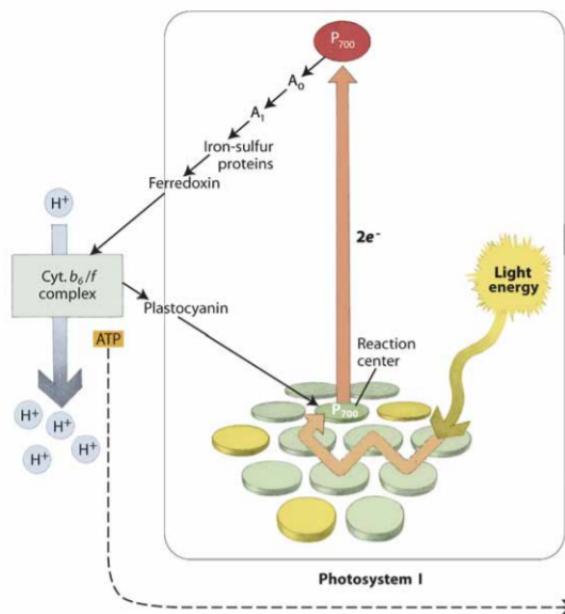


Figure 12: Diagram of cyclic electron flow. (Source: Raven)

3.3 Calvin Cycle/Light-Independent Reactions/Dark Reactions

The **Calvin Cycle** is the stage of photosynthesis where sugar is made. Using the products of the light reactions, carbon dioxide can now be drawn from the air to form sugar through a process called **carbon fixation**. The Calvin Cycle occurs in the *stroma* of the chloroplast. Now we will look at how sugar is ultimately produced.

- 1 CO_2 from the air attaches to the 5-carbon **ribulose bisphosphate (RuBP)**. The enzyme that facilitates this process is called *RuBP carboxylase-oxygenase*, better known as **RuBisCO**. Rubisco is so important that it is the most common enzyme in the world! A short term 6-carbon intermediate molecule is created that is so energetic that it immediately splits in half, forming two 3-carbon compounds.

- Note that the oxygen from CO_2 combines with hydrogen to produce 6 H_2O .

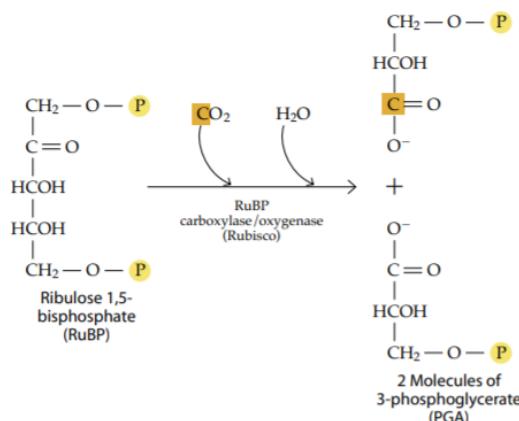


Figure 13: RuBP fixes CO_2 , producing 2 PGA. (Source: Raven)

2. The two compounds that formed are called **3-phosphoglycerate (PGA)**, which you may remember from glycolysis. The next two steps are essentially the reverse of glycolysis, using the **ATP** and then the **NADPH** that we generated during the light reactions to create G3P, or as it's known in the Calvin Cycle, **PGAL**.

- During this process NADPH gets oxidized, *recycling* the NADP^+ that was used up during linear electron flow.

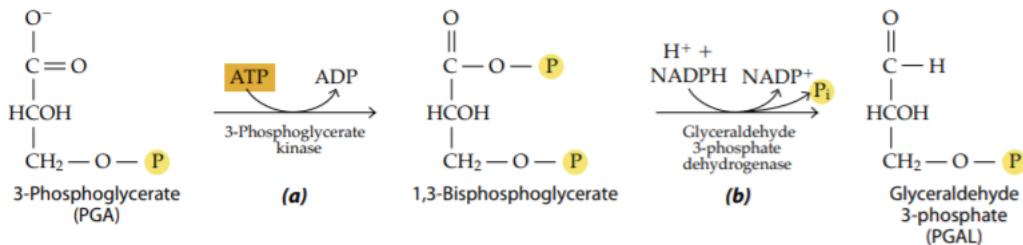


Figure 14: PGA is converted to PGAL, using ATP and NADPH. (Source: Raven)

3. Two molecules of G3P are ultimately used to make 1 molecule of glucose.

- Remember how we needed 6 CO_2 to complete photosynthesis? Each CO_2 molecule produces 2 G3P. However, the Calvin Cycle is a cycle, meaning that it must end with RuBP being regenerated. To do this, 5 molecules of G3P are put back into the cycle to produce 1 RuBP, using up another **3 ATP** in the process. Therefore, to have a net of 1 G3P, 6 G3P must be produced in total since $6 - 5 = 1$. Since each CO_2 produces 2 G3P, it takes *three turns* of the Calvin cycle to produce 1 G3P. Since we need 2G3P to make glucose, there are *six turns* in total.

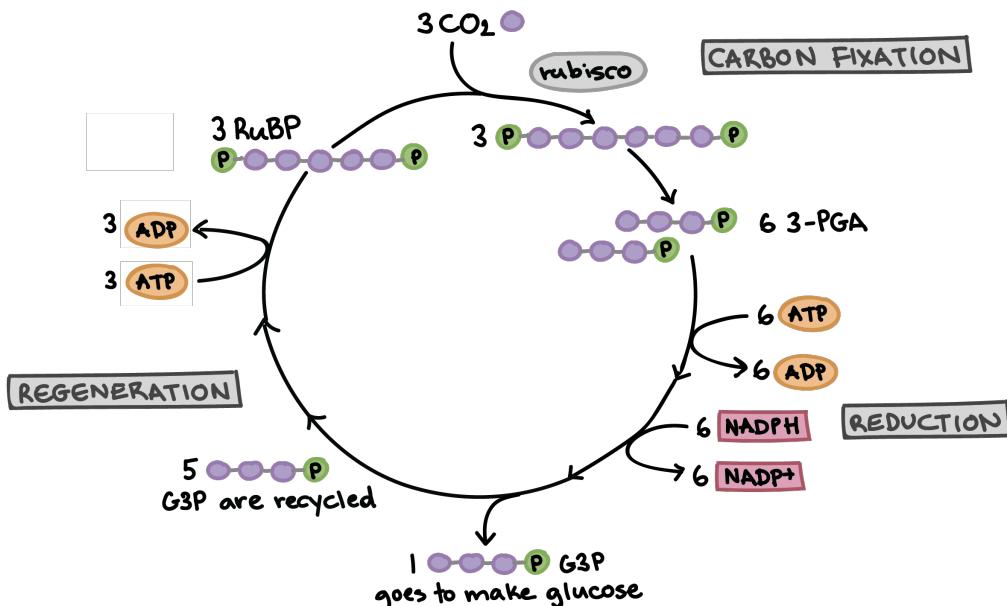


Figure 15: A simplified diagram of the Calvin Cycle, showing carbons and phosphates. Keep in mind that the cycle happens twice to make 1 molecule of glucose. (Source: Khan Academy)

In total, the Calvin Cycle utilized **6 CO₂** to get 2 G3P, or one **glucose** molecule. This process required **18 ATP** and **12 NADPH**, which was provided by the light reactions.

Example 3.2 (USABO Opens 2017)

15. Consider the following experiment similar to those first performed in the 1950's. Individual *Arabidopsis thaliana* plants are grown in a hydroponic solution (without soil). Some plants (Group A) are grown in water labeled (20%) with oxygen-18 and atmospheric air with unlabeled CO₂, while others (Group B) are grown in unlabeled water and atmospheric air with 50% of the CO₂ labeled with oxygen-18. Gases produced by the plant are collected for 20 minutes. After 1 day, the following are analyzed for oxygen-18 content: the collected gas, tissue from the plants, and tissue from the plants baked until dry. The results are as follows, where a + indicates a statistically significant enrichment and a - indicates no enrichment:

	Collected gas	Plant tissue	Dried plant tissue
Group A (H ₂ O labeled)	+	-	-
Group B (CO ₂ labeled)	-	+	+

Assuming that the detailed mechanisms of photosynthesis have not yet been discovered, what would be concluded about photosynthesis from this experiment?

- A. The oxygen atoms in plant sugars (or other organic compounds) are derived from water.
- B. The oxygen atoms in the CO₂ produced by plant respiration originated from water.
- C. The oxygen atoms in the labeled CO₂ were incorporated into plant sugars (or other organic compounds) only.
- D. Oxygen (O₂) is formed from the splitting of water during photosynthesis.
- E. The oxygen in the labeled CO₂ was released as O₂.

Solution: We know that photosynthesis produces O₂ gas and glucose. Looking at the chart, we see that when H₂O is labeled, the gas is also enriched, showing that the oxygen gas in photosynthesis comes from water (remember photolysis). Similarly, when CO₂ is labeled, the plant tissue is also enriched, showing that the oxygen in glucose comes from carbon dioxide (remember Calvin cycle). Therefore, our **answer is C**.

3.4 Photorespiration

The process you just learned only occurs in **C3 plants** (name comes from first intermediate PGA being a 3-carbon molecule), which include most plants. However, it turns out that the enzyme that was so crucial for fixing carbon is actually pretty bad at its job. Rubisco has a lower affinity for CO₂ than it does for oxygen! This means that if there is a lack of CO₂ and O₂ starts building up, as happens when the stomata are closed and there is no gas exchange, then rubisco will start binding to O₂, forming **phosphoglycolate**.

This is a terrible situation and so the plant cell tries to make the best out of a bad position by performing a complicated process known as **photorespiration**, which involves the chloroplasts, mitochondria, and *peroxisomes*, wasting a bunch of ATP in the process!

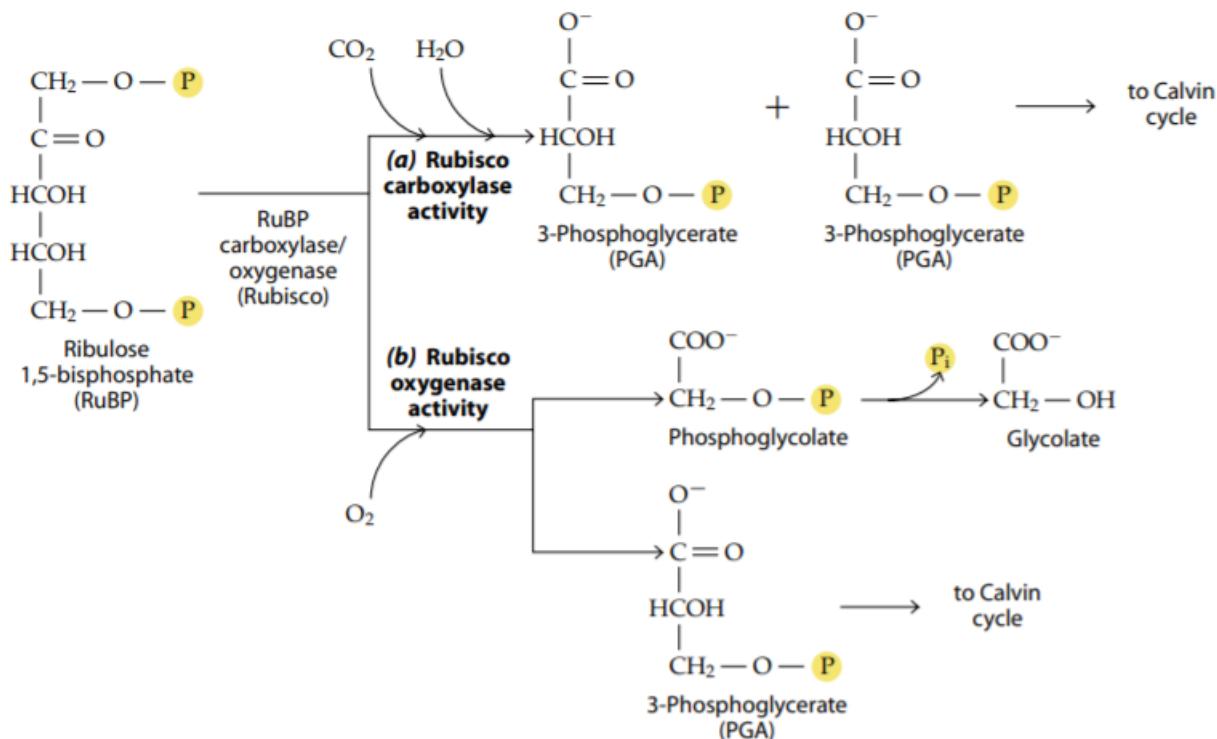


Figure 16: Rubisco binding to O₂ results in phosphoglycolate. (Source: Raven)

Since stomata are usually closed to prevent water loss from transpiration, photorespiration tends to become a big issue in hotter climates. To deal with this, *C4* and *CAM* plants have evolved different pathways, which separate carbon fixation and the Calvin cycle.

3.4.1 C4

C4 plants, which include corn, sorghum, sugarcane, millet, switchgrass, and crabgrass, deal with photorespiration by implementing **spatial separation**, which means that carbon fixation and the Calvin cycle occur *in different cells* at the same time. If the problem is that O₂ builds up, then we simply need to keep the rubisco in special cells that are far away from any O₂.

Also known as the **Hatch-Slack** pathway, C4 plants fix carbon in *mesophyll* cells and use rubisco in **bundle-sheath** cells. To facilitate this, the mesophyll cells are often in a wreath-like arrangement known as **Kranz anatomy** around the bundle sheath cells. Here is how the pathway works:

1. In mesophyll cells, CO₂ is added to the 3-carbon **PEP** using the enzyme **PEP carboxylase**. This creates the 4-carbon sugar **oxaloacetate**.
2. Oxaloacetate is reduced to **malate** and aspartate, which are transported to the bundle sheath cells. There, it is broken back down into CO₂ and pyruvate. The CO₂ goes into the Calvin cycle as normal while the pyruvate goes back to the mesophyll cells to regenerate PEP.

Although this process uses more ATP, the decreased risk of photorespiration makes it worth it.

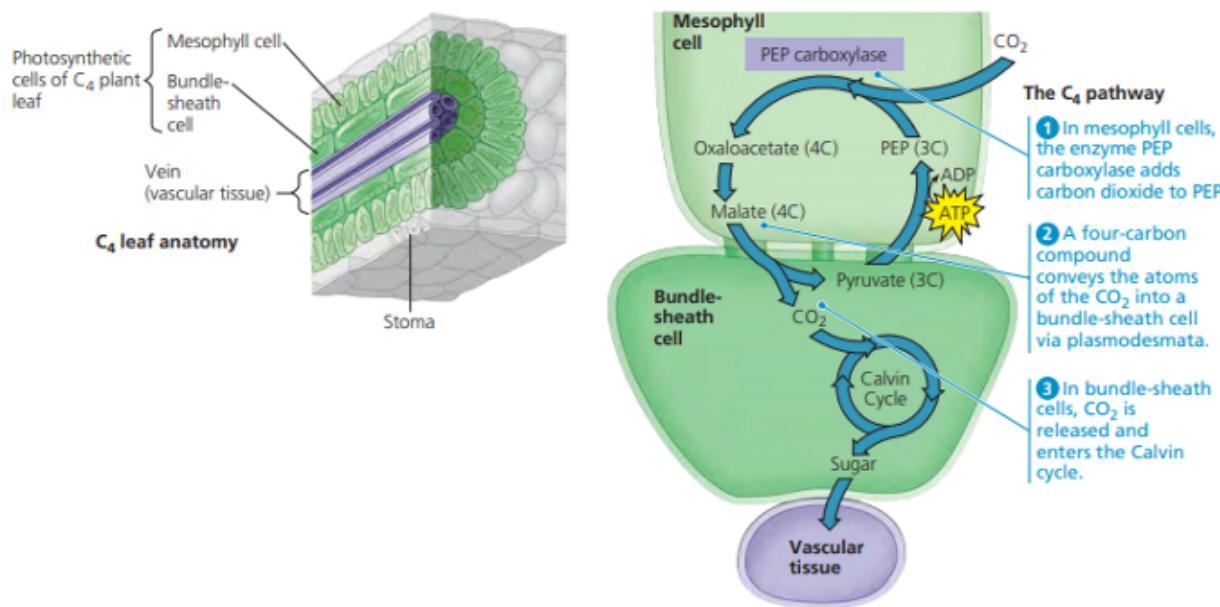


Figure 17: Diagram of the C₄ pathway. (Campbell's 9th Edition)

Example 3.3 (USABO Opens 2016)

11. Botanist Grace is attempting to determine the identity of a plant. She masterfully sections the leaves, and through a light microscope observes that the vessels have a thick layer of concentric mesophyll tissue around them. Which of the following plants could this most likely be?
- A. Chard.
 - B. Crabgrass.
 - C. Kale.
 - D. Rutabaga.
 - E. Squash.

Solution: The “layer of concentric mesophyll tissue” is describing Kranz anatomy, which means we are looking for a C₄ plant. Therefore, our **answer is B**. Many questions will simply ask you to identify whether a plant is C₃, C₄, or CAM, so it’s a good idea to memorize the common examples.

Example 3.4 (USABO Opens 2017)

11. Which of the following incorrectly compares C₃ versus C₄ plants?

	C ₃ plants	C ₄ plants
A.	Rubisco performs initial carbon fixation step	PEPase performs initial carbon fixation step
B.	Loses water and energy through photorespiration	No or very low photorespiration rate
C.	RuBP initially accepts carbon dioxide	PEP initially accepts carbon dioxide
D.	Most plants	Tropical grasses
E.	Spatial separation of carbon fixation and photosynthesis	Temporal separation of carbon fixation and photosynthesis

Solution: The goal of C₄ plants is to minimize the amount of photorespiration in tropical climates by initially fixing CO₂ onto PEP instead of RuBP. For this reason, A through D are all true. E is false, since C₃ plants do not exhibit any form of separation while C₄ plants exhibit spatial separation and, as you will soon see, CAM plants exhibit temporal separation. Therefore, the answer is E.

3.4.2 CAM

CAM (Crassulacean Acid Metabolism) plants, which include orchids, cacti, aloe, pineapple, spanish moss, and agave, deal with photorespiration by implementing **temporal separation**, which means that carbon fixation and the Calvin cycle occur in the same cell *at different times*. In other words, they will fix CO₂ at night when their stomata are open and O₂ levels are low using the same process that C₄ plants use. The difference is that malate is converted to **malic acid**. Then, when it's daytime the malic acid is broken down and the CO₂ is used in the Calvin cycle.

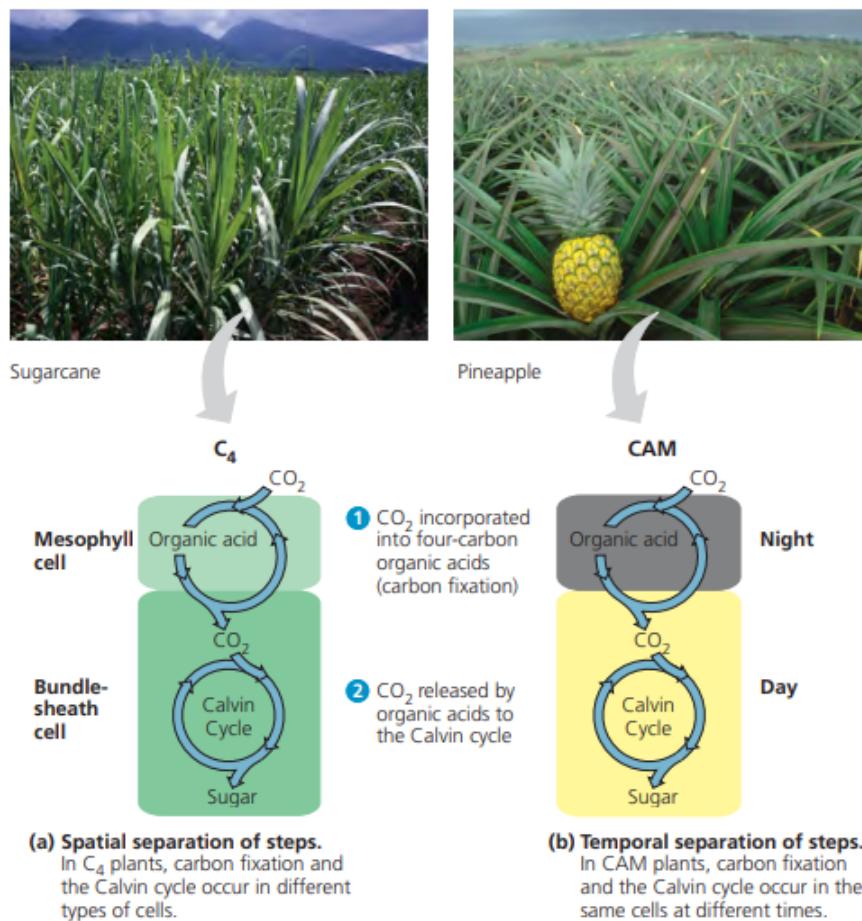


Figure 18: Comparison of C₄ and CAM pathways. (Campbell's 9th Edition)

4 Conclusion

In photosynthesis, we saw how light energy can be transformed into glucose, which in cellular respiration was broken down into ATP. The reactions were complex and involved many steps, but by focusing on the basic ideas and going through each process slowly, we got through it.