Oxidative phosphorylation

Overview of oxidative phosphorylation. The electron transport chain forms a proton gradient across the inner mitochondrial membrane, which drives the synthesis of ATP via chemiosmosis.

Why do we need oxygen?

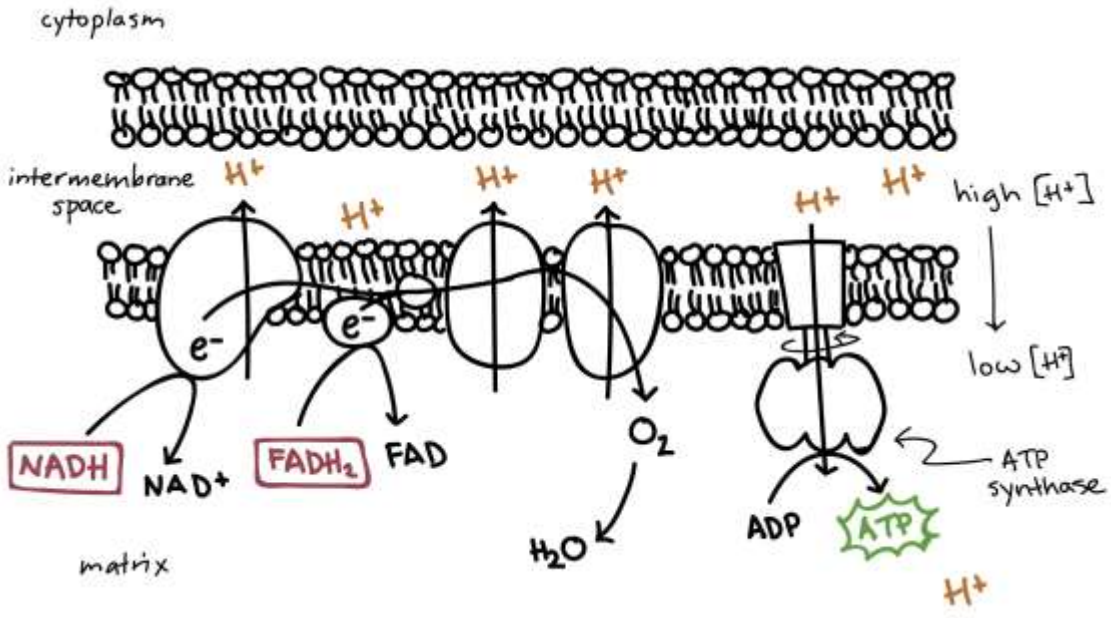
You, like many other organisms, need oxygen to live. As you know if you've ever tried to hold your breath for too long, lack of oxygen can make you feel dizzy or even black out, and prolonged lack of oxygen can even cause death. But have you ever wondered why that's the case, or what exactly your body does with all that oxygen?

As it turns out, the reason you need oxygen is so your cells can use this molecule during oxidative phosphorylation, the final stage of cellular respiration. Oxidative phosphorylation is made up of two closely connected components: the electron transport chain and chemiosmosis. In the electron transport chain, electrons are passed from one molecule to another, and energy released in these electron transfers is used to form an electrochemical gradient. In chemiosmosis, the energy stored in the gradient is used to make ATP.

So, where does oxygen fit into this picture? Oxygen sits at the end of the electron transport chain, where it accepts electrons and picks up protons to form water. If oxygen isn't there to accept electrons (for instance, because a person is not breathing in enough oxygen), the electron transport chain will stop running, and ATP will no longer be produced by chemiosmosis. Without enough ATP, cells can't carry out the reactions they need to function, and, after a long enough period of time, may even die.

In this article, we'll examine oxidative phosphorylation in depth, seeing how it provides most of the ready chemical energy (ATP) used by the cells in your body.

Overview: oxidative phosphorylation



Simple diagram of the electron transport chain. The electron transport chain is a series of proteins embedded in the inner mitochondrial membrane.

In the matrix, NADH and $FADH_2$ deposit their electrons in the chain (at the first and second complexes of the chain, respectively).

The energetically "downhill" movement of electrons through the chain causes pumping of protons into the inter-membrane space by the first, third, and fourth complexes.

Finally, the electrons are passed to oxygen, which accepts them along with protons to form water.

The proton gradient produced by proton pumping during the electron transport chain is used to synthesize ATP. Protons flow down their concentration gradient into the matrix through the membrane protein ATP synthase, causing it to spin (like a water wheel) and catalyse conversion of ADP to ATP.

The **electron transport chain** is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called **chemiosmosis**. Together, the electron transport chain and chemiosmosis make up **oxidative phosphorylation**. The key steps of this process, shown in simplified form in the diagram above, include:

- **Delivery of electrons by NADH and $FADH_2$.** Reduced electron carriers (NADH and $FADH_2$) from other steps of cellular respiration transfer their electrons to molecules near the beginning of the transport chain. In the process, they turn back into NAD^+ and FAD, which can be reused in other steps of cellular respiration.
- **Electron transfer and proton pumping.** As electrons are passed down the chain, they move from a higher to a lower energy level, releasing energy. Some of the energy is used to pump H^+ ions, moving them out of the matrix and into the inter-membrane space. This pumping establishes an electrochemical gradient.
- **Splitting of oxygen to form water.** At the end of the electron transport chain, electrons are transferred to molecular oxygen, which splits in half and takes up H^+ to form water.
- **Gradient-driven synthesis of ATP.** As H^+ ions flow down their gradient and back into the matrix, they pass through an enzyme called ATP synthase, which harnesses the flow of protons to synthesize ATP.

We'll look more closely at both the electron transport chain and chemiosmosis in the sections below.

The electron transport chain

The **electron transport chain** is a collection of membrane-embedded proteins and organic molecules, most of them organized into four large complexes labelled I to IV. In eukaryotes, many copies of these molecules are found in the inner mitochondrial membrane. In prokaryotes, the electron transport chain components are found in the plasma membrane.

As the electrons travel through the chain, they go from a higher to a lower energy level, moving from less electron-hungry to more electron-hungry molecules. Energy is released in these “downhill” electron transfers, and several of the protein complexes use the released energy to pump protons from the mitochondrial matrix to the inter-membrane space, forming a proton gradient.

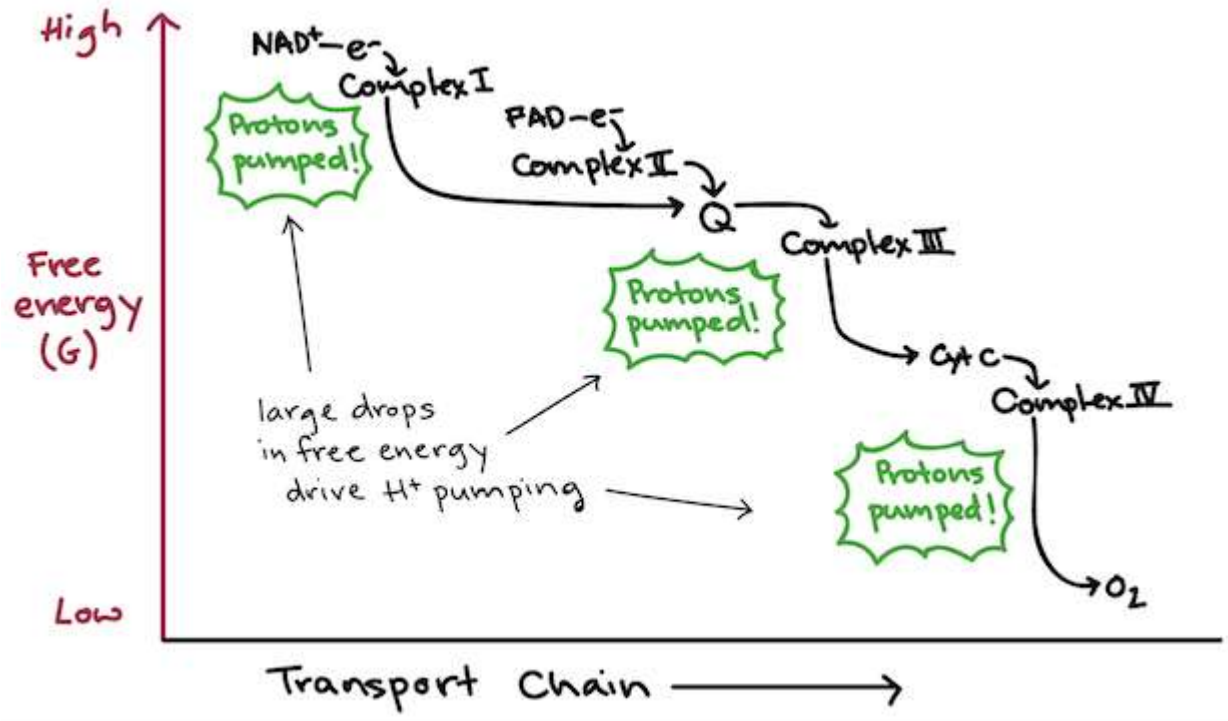


Image based on (and partially traced from) original diagram by Ryan Gutierrez.

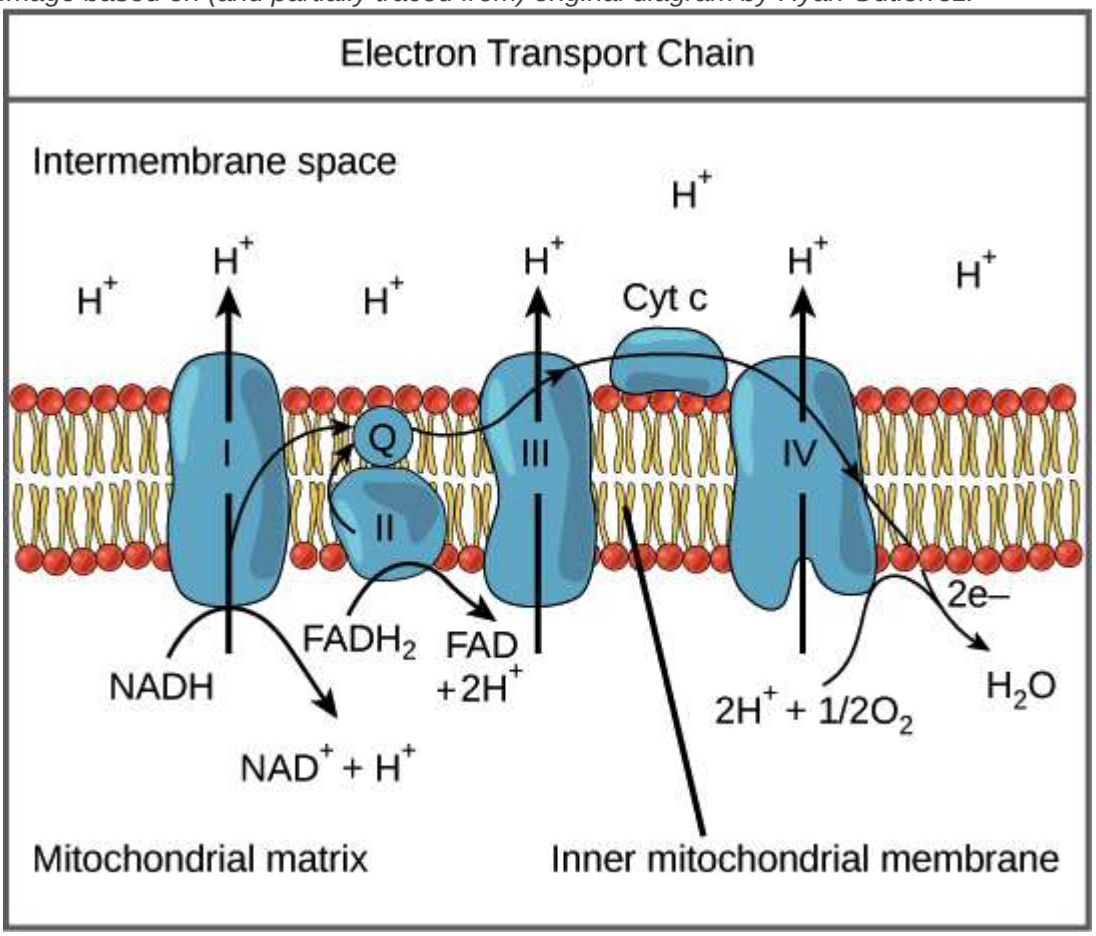


Image of the electron transport chain. All the components of the chain are embedded in or attached to the inner mitochondrial membrane. In the matrix, NADH deposits electrons at Complex I, turning into NAD^+ and releasing a proton into the matrix. $FADH_2$ in the matrix deposits electrons at Complex II, turning into FAD and releasing 2 H^+. The electrons from Complexes I

and II are passed to the small mobile carrier Q. Q transports the electrons to Complex III, which then passes them to Cytochrome C. Cytochrome C passes the electrons to Complex IV, which then passes them to oxygen in the matrix, forming water. It takes two electrons, 1/2 O_2, and 2 H^+ to form one water molecule. Complexes I, III, and IV use energy released as electrons move from a higher to a lower energy level to pump protons out of the matrix and into the inter-membrane space, generating a proton gradient.

All of the electrons that enter the transport chain come from NADH and $FADH_2$ molecules produced during earlier stages of cellular respiration: glycolysis, pyruvate oxidation, and the citric acid cycle.

- **NADH** is very good at donating electrons in redox reactions (that is, its electrons are at a high energy level), so it can transfer its electrons directly to complex I, turning back into NAD^+. As electrons move through complex I in a series of redox reactions, energy is released, and the complex uses this energy to pump protons from the matrix into the inter-membrane space.
- **$FADH_2$** is not as good at donating electrons as NADH (that is, its electrons are at a lower energy level), so it cannot transfer its electrons to complex I. Instead, it feeds them into the transport chain through complex II, which does not pump protons across the membrane.

Because of this "bypass," each $FADH_2$ molecule causes fewer protons to be pumped (and contributes less to the proton gradient) than an NADH.

Complex I. NADH transfers its electrons to complex I. Complex I is quite large, and the part of it that receives the electrons is a flavoprotein, meaning a protein with an attached organic molecule called flavin mononucleotide (FMN). FMN is a **prosthetic group**, a non-protein molecule tightly bound to a protein and required for its activity, and it's FMN that actually accepts electrons from NADH. FMN passes the electrons to another protein inside complex I, one that has iron and sulphur bound to it (called an Fe-S protein), which in turns transfers the electrons to a small, mobile carrier called ubiquinone (Q in the diagram above).

Complex II. Like NADH, $FADH_2$ deposits its electrons in the electron transport chain, but it does so via complex II, bypassing complex I entirely. As a matter of fact, $FADH_2$ is a part of complex II, as is the enzyme that reduces it during the citric acid cycle; unlike the other enzymes of the cycle, it's embedded in the inner mitochondrial membrane. $FADH_2$ transfers its electrons to iron-sulphur proteins within complex II, which then pass the electrons to ubiquinone (Q), the same mobile carrier that collects electrons from complex I.

Beyond the first two complexes, electrons from NADH and $FADH_2$ travel exactly the same route. Both complex I and complex II pass their electrons to a small, mobile electron carrier called **ubiquinone** (**Q**), which is reduced to form $QH2$ and travels through the membrane, delivering the electrons to complex III. As electrons move through complex III, more $H+$ ions are pumped across the membrane, and the electrons are ultimately delivered to another mobile carrier called **cytochrome C** (**cyt C**). Cyt C carries the electrons to complex IV, where a final batch of H^+ ions is pumped across the membrane. Complex IV passes the electrons to O_2 which splits into two oxygen atoms and accepts protons from the matrix to form water. Four electrons are required to reduce each molecule of O_2 and two water molecules are formed in the process.

Complex III. Like complex I, complex III includes an iron-sulphur (Fe-S) protein, but it also contains two proteins of another type, known as cytochromes. **Cytochromes** are a family of related proteins that have heme prosthetic groups containing iron ions. (Have you heard of the protein haemoglobin, which transports oxygen in the blood? Haemoglobin also has heme groups, but they bind oxygen rather than electrons.) In complex III, electrons are passed from one

cytochrome to an iron-sulphur protein to a second cytochrome, then finally transferred out of the complex to a mobile electron carrier (cytochrome C). Like complex I, complex III pumps protons from the matrix into the inter-membrane space, contributing to the H^+ concentration gradient.

Complex IV. From complex III, cytochrome C delivers electrons to the last complex of the electron transport chain, complex IV. There, the electrons are passed through two more cytochromes, the second of which has a very interesting job: with the help of a nearby copper ion, it transfers electrons to O_2 splitting oxygen to form two molecules of water. The mechanism of the transfer is pretty cool and worth looking up, but basically, the heme group and the copper ion bind tightly to the oxygen molecule and hold it in place until it is fully reduced (has gained electrons and protons to form water). The protons used to form water come from the matrix, contributing to the H^+ gradient, and complex IV also pumps protons from the matrix to the inter-membrane space.

Overall, what does the electron transport chain do for the cell? It has two important functions:

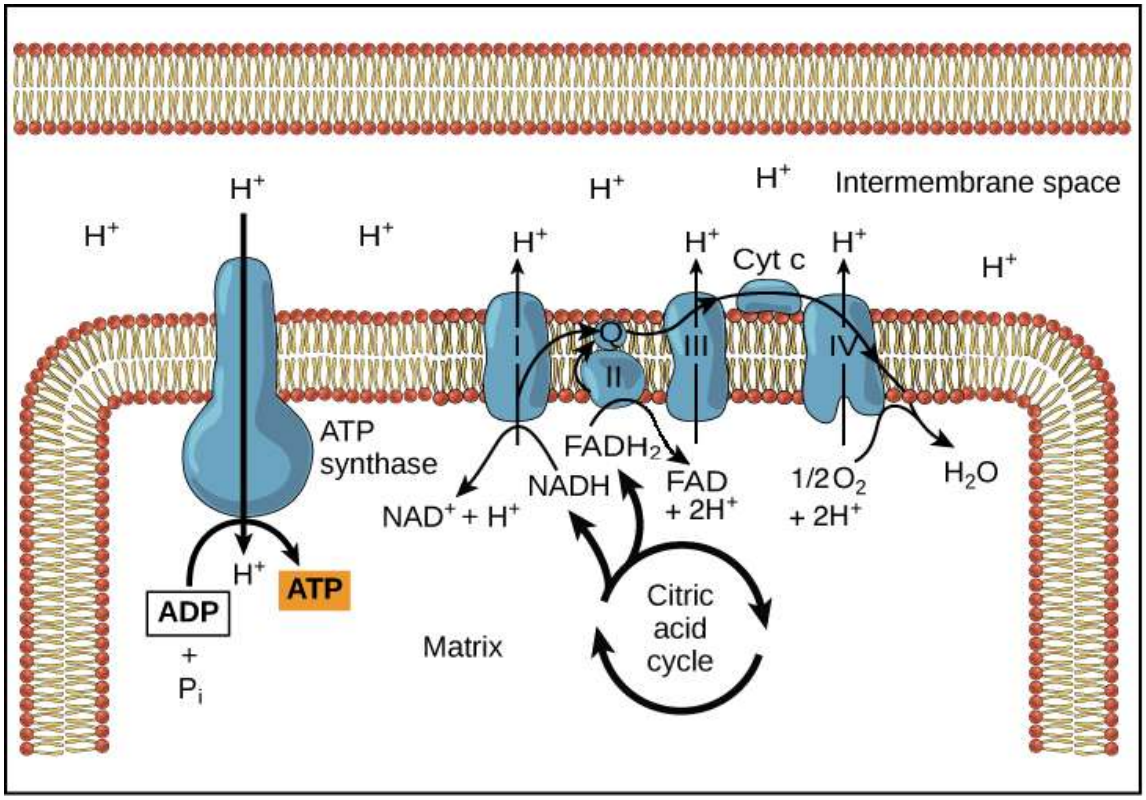
- **Regenerates electron carriers.** NADH and $FADH_2$ pass their electrons to the electron transport chain, turning back into NAD^+ and FAD. This is important because the oxidized forms of these electron carriers are used in glycolysis and the citric acid cycle and must be available to keep these processes running.
- **Makes a proton gradient.** The transport chain builds a proton gradient across the inner mitochondrial membrane, with a higher concentration of H^+ in the inter-membrane space and a lower concentration in the matrix. This gradient represents a stored form of energy, and, as we'll see, it can be used to make ATP.

Chemiosmosis

Complexes I, III, and IV of the electron transport chain are proton pumps. As electrons move energetically downhill, the complexes capture the released energy and use it to pump H+ ions from the matrix to the inter-membrane space. This pumping forms an electrochemical gradient across the inner mitochondrial membrane. The gradient is sometimes called the **proton-motive force**, and you can think of it as a form of stored energy, kind of like a battery.

Like many other ions, protons can't pass directly through the phospholipid bilayer of the membrane because its core is too hydrophobic. Instead, H^+ ions can move down their concentration gradient only with the help of channel proteins that form hydrophilic tunnels across the membrane.

In the inner mitochondrial membrane, H^+ ions have just one channel available: a membrane-spanning protein known as **ATP synthase**. Conceptually, ATP synthase is a lot like a turbine in a hydroelectric power plant. Instead of being turned by water, it's turned by the flow of H+ ions moving down their electrochemical gradient. As ATP synthase turns, it catalyses the addition of a phosphate to ADP, capturing energy from the proton gradient as ATP.



Overview diagram of oxidative phosphorylation. The electron transport chain and ATP synthase are embedded in the inner mitochondrial membrane. NADH and $FADH_2$ made in the citric acid cycle (in the mitochondrial matrix) deposit their electrons into the electron transport chain at complexes I and II, respectively. This step regenerates NAD+ and FAD (the oxidized carriers) for use in the citric acid cycle. The electrons flow through the electron transport chain, causing protons to be pumped from the matrix to the inter-membrane space. Eventually, the electrons are passed to oxygen, which combines with protons to form water. The proton gradient generated by proton pumping during the electron transport chain is a stored form of energy. When protons flow back down their concentration gradient (from the inter-membrane space to the matrix), their only route is through ATP synthase, an enzyme embedded in the inner mitochondrial membrane. When protons flow through ATP synthase, they cause it to turn (much as water turns a water wheel), and its motion catalyses the conversion of ADP and Pi to ATP.

This process, in which energy from a proton gradient is used to make ATP, is called **chemiosmosis**. More broadly, chemiosmosis can refer to any process in which energy stored in a proton gradient is used to do work. Although chemiosmosis accounts for over 80% of ATP made during glucose breakdown in cellular respiration, it's not unique to cellular respiration. For instance, chemiosmosis is also involved in the light reactions of photosynthesis.

What would happen to the energy stored in the proton gradient if it weren't used to synthesize ATP or do other cellular work? It would be released as heat, and interestingly enough, some types of cells deliberately use the proton gradient for heat generation rather than ATP synthesis. This might seem wasteful, but it's an important strategy for animals that need to keep warm. For instance, hibernating mammals (such as bears) have specialized cells known as brown fat cells. In the brown fat cells, **uncoupling proteins** are produced and inserted into the inner mitochondrial membrane. These proteins are simply channels that allow protons to pass from the inter-membrane space to the matrix without traveling through ATP synthase. By providing an alternate route for protons to flow back into the matrix, the uncoupling proteins allow the energy of the gradient to be dissipated as heat.

ATP yield

How many ATP do we get per glucose in cellular respiration? If you look in different books, or ask different professors, you'll probably get slightly different answers. However, most current sources estimate that the maximum ATP yield for a molecule of glucose is around 30-32 ATP[2,3,4]. This range is lower than previous estimates because it accounts for the necessary transport of ADP into, and ATP out of, the mitochondrion.

When authors and teachers quote different numbers of ATP molecules per glucose, it's often because they make different choices about what processes to account for in the total ATP yield. Neither the 38 ATP figure nor the 30-32 ATP figure is incorrect. They are just measuring slightly different things.

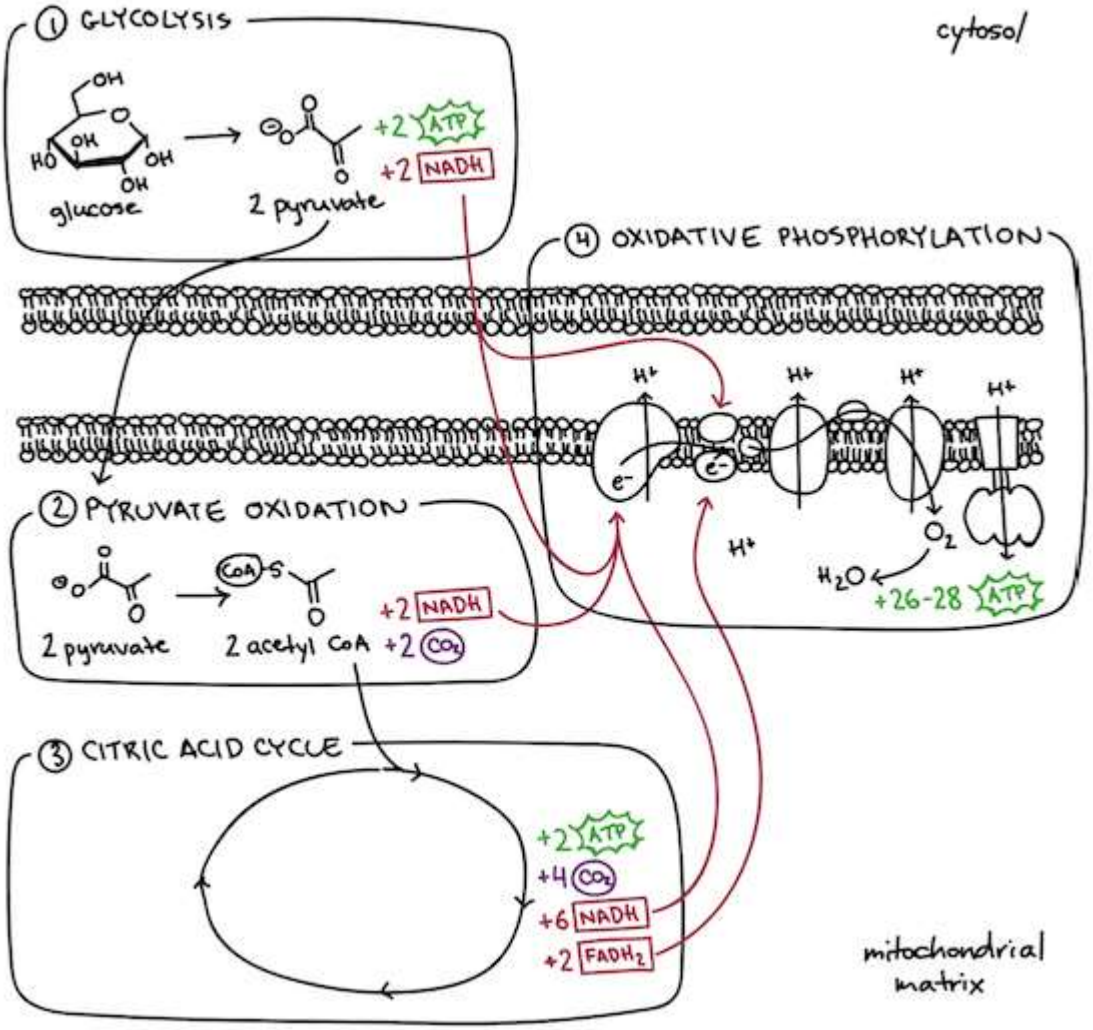
- The 38 ATP figure assumes that every single proton pumped in the electron transport chain as a result of electrons harvested from glucose goes towards synthesizing ATP. In other words, none of the energy of the proton gradient is used to power other transport processes. (The 38 ATP figure also assumes that an efficient shuttle mechanism is used to carry electrons from NADH made in glycolysis to the electron transport chain. With an inefficient shuttle, the maximum figure in this scenario would be 36 ATP.)
- The 30-32 ATP figure accounts for the fact that, in a real cell, not all of the energy of the proton gradient can go towards making ATP. Instead, some of it must be used to transport molecules into and out of the mitochondrial matrix. For instance, ADP must be transported into the matrix so it can be made into ATP, and ATP must be transported out so it can be used by the cell. The 30-32 ATP figure accounts for transport of ATP and ADP, which uses up energy from the proton gradient (meaning that there is less energy left to drive ATP synthesis, yielding fewer ATP)[5,6].

In real cells, the yield of ATP per glucose molecule would likely be even lower than 30-32 ATP. Cells often use the proton gradient to transport other molecules (in addition to ATP and ADP), and some of the proton gradient's energy may also be lost if the inner mitochondrial membrane is "leaky" to protons.

Where does the figure of 30-32 ATP come from? Two net ATP are made in glycolysis, and another two ATP (or energetically equivalent GTP) are made in the citric acid cycle. Beyond those four, the remaining ATP all come from oxidative phosphorylation. Based on a lot of experimental work, it appears that four H^+ ions must flow back into the matrix through ATP synthase to power the synthesis of one ATP molecule. When electrons from NADH move through the transport chain, about 10 H^+ ions are pumped from the matrix to the inter-membrane space, so each NADH yields about 2.5 ATP. Electrons from $FADH_2$, which enter the chain at a later stage, drive pumping of only 6 H^+ leading to production of about 1.5 ATP.

With this information, we can do a little inventory for the breakdown of one molecule of glucose:

Stage	Direct products (net)	Ultimate ATP yield (net)
Glycolysis	2 ATP	2 ATP
	2 NADH	3-5 ATP
Pyruvate oxidation	2 NADH	5 ATP
Citric acid cycle	2 ATP/GTP	2 ATP
	6 NADH	15 ATP
	2 $FADH_2$	3 ATP
Total		**30-32 ATP**



One number in this table is still not precise: the ATP yield from NADH made in glycolysis. This is because glycolysis happens in the cytosol, and NADH can't cross the inner mitochondrial membrane to deliver its electrons to complex I. Instead, it must hand its electrons off to a molecular “shuttle system” that delivers them, through a series of steps, to the electron transport chain.

- Some cells of your body have a shuttle system that delivers electrons to the transport chain via $FADH_2$. In this case, only 3 ATP are produced for the two NADH of glycolysis.
- Other cells of your body have a shuttle system that delivers the electrons via NADH, resulting in the production of 5 ATP.

In bacteria, both glycolysis and the citric acid cycle happen in the cytosol, so no shuttle is needed and 5 ATP are produced.

30-32 ATP from the breakdown of one glucose molecule is a high-end estimate, and the real yield may be lower. For instance, some intermediates from cellular respiration may be siphoned off by the cell and used in other biosynthetic pathways, reducing the number of ATP produced. Cellular respiration is a nexus for many different metabolic pathways in the cell, forming a network that’s larger than the glucose breakdown pathways alone.