Haste makes waste: spliffs 'n' strips

If you've ever burnt newspaper, or watched a cloud of flour explode (that's more of a science fair kind of thing, and exposes my nerdy past), you know that glucose (which is linked together $\beta (1 \rightarrow 4)$ in cellulose, right?), and other simple carbohydrates have plenty of energy that can be released by oxidation. Of course, it is hard to capture much of the energy from a burning newspaper in any useful way. In contrast to the burnin' spliff situation, the cell is amazingly good at capturing the energy that the oxidation of glucose yields. The actual percentages are in the range of 40% of the total energy captured as newly made ATP, and the other 60% lost as heat. When compared to the commonly used internal combustion engine, which captures about 10% of the energy released by burning fuel as mechanical work, you can see that the cell has the energy game down to a...science...or something.

There are many variations of energy harvesting reactions in the biosphere. However, one method that is widely used in all three domains (archaea, eubacteria, and eukarya) is the oxidation of glucose. This is the main way that all the organisms that we commonly encounter, including ourselves, renew their vital stores of ATP which are used for nearly all the mechanical, chemical, osmotic, and electrochemical work that organisms need to do. Furthermore, some of the chemical processes involved in the complete oxidation of glucose can be harnessed to extract chemical energy from other biomolecules such as fats, and can even be used to provide molecules for anabolism (the production of larger things from smaller ones).

So you can see that an understanding of the oxidation of glucose and the resulting production of ATP is a gateway to thinking about a lot of cellular metabolism. I want to describe the key features of this process, which can be divided into three main sections of glucose metabolism: **glycolysis**, the **Krebs cycle**, and the **electron transport chain**. I am most interested in laying out the underlying concepts. So we will try to avoid getting bogged down with massive numbers of structures, or enzymes, or allosteric tricks, or covalent controls. All of these things add up to the molecular symphony of cellular respiration, but if your interest lies in plumbing these depths further, there are courses, books, papers and experts floating around who can tell you about this stuff until you yourself would need a non fat latte. For now, I just want you to understand the flow of things (dude...) that allows the impressively efficient capture of energy from glucose that we call "the slow burn" (warning: before you try to impress your BILD2 teacher with this term, you might want to know that I made it up, and we will have to wait till the rest of the world catches up with our argot). The basic principle is that the oxidation of glucose is done very gradually, sort of like burning little strips of newspaper to keep your hands warm for a long time (maybe not the best analogy for San Diego...)

Redox: what's in a name?

Before we learn how glucose oxidation is done bit by bit, we want to delve a little into just what we mean by glucose oxidation. Why is it called that anyway? Can we get a chemical handle on the term? Again and again you hear that glucose gets oxidized to yield energy. The total oxidation of glucose is written as follows:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 12\text{CO}_2 + 6\text{H}_2\text{O}
\]

What we want to understand a little better is why this is called oxidation. Intuitively we would say that the carbon of the glucose is getting oxidized, since each atom is now only bound to oxygen! As you may recall from chemistry (but it’s ok if you don’t, or haven’t heard this yet) the most accurate and useful definition of oxidation is the loss of electrons. That is what oxidation is in the formal sense. So let's try to understand where electrons are going and coming, because it will help us understand the logic of cellular energy catabolism. In normal chemical reactions, oxidation of something (loss of electrons) is always accompanied by something else getting reduced, that is, gaining electrons. This makes sense because you can't create or destroy electrons in the course of normal reactions (to do that you'll have to go down to the physics department). And that is why you often hear the term "redox", which refers to the tightly coupled pair of things, reduction and oxidation, going on in a balanced fashion during normal chemical changes. But the conservation of electrons also makes the decision of who is getting Ox'd or Red'ed a little tricky. We think that the glucose is being oxidized in the above reaction. Well, if redox is
the word of the day, then what is getting reduced? Look at the O atoms that are in the O2 on the left, and end up in the water on the right. Each initially had an oxidation state of 0 (that is zero) as part of the O2 molecules and each ends up as part of the water, with an oxidation state of -2. So the O atoms in the water have each have gained two electrons in going from molecular oxygen to water. Where do these electrons come from? They come from the glucose, which has C's bound to a mix of H and OH groups. So is it the transition from this fancy arrangement of bonds in the carbohydrate to the simple CO2 that gives up the electrons? Yes, but how to be convinced of this? The change in the oxidation number of the C's in the glucose is a bit trickier to arrive at (no elemental form to provide easy reference, and all those different bonds along the sugar structure, an electron accounting nightmare!). But there is an easy way to picture how the glucose provides the electrons that finally end up in water. The easiest way to do it is to just follow the hydrogens and electrons step by step. Carbohydrates have the unit formula CnH2nOn, so lets consider the oxidation of a simple one carbon version of a carbohydrate. This "sugar" doesn't exist as such (well, ok, its formaldehyde, not to be used in baking). This case will involve the exact same flow of electrons, but we will only think about one carbon instead of 6. The redox chemistry of total glucose oxidation (and O2 reduction) is exactly the same, but happens at 6 carbons (bit by bit) as opposed to one. In fact, you can generalize the one carbon example to glucose yourself! ("College is the process of grabbing the handle of the knowledge spoon"; that is what this ancient professor of mine used to say when I was an undergraduate. I thought he was a jerk, but now I see he had a point!)

So the complete redox reaction that includes the oxidation of our "one carbon" sugar would look like this

\[
\text{CH}_2\text{O} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O}
\]

Now we will separately consider the RED and the OX (sounds like a farm tale) to show the movements of the electrons.

**a) "Ox" of the carbohydrate.** That is, only the electron removal or loss part. We go all the way to the Lewis electron dot structures, because it actually makes it very easy to see the changes that are happening. Note the simple carbohydrate has 12 outer shell electrons total.

\[
\begin{align*}
H & \quad \text{C}\vdash\cdot\text{O} \\
& \rightarrow \cdot\text{C}\cdot + \cdot\text{O}\cdot + 2\text{H}^+ + 2\text{e}^-
\end{align*}
\]

So now we have 1 elemental carbon (4 shell electrons), 1 elemental oxygen (6 shell electrons), two H+ ions and the two electrons! All perfectly allowed chemistry. So where do these electrons go?

**b) "Red" of the elemental oxygen to make water.** Like we figured out before to the O2, but first we break the O molecule in two, which somehow has to happen in the course of making water. The uninvolved O will be used in a moment...

\[
\text{O}_2 \rightarrow \cdot\text{O}\cdot + \cdot\text{O}\cdot
\]

Next we dump the now found electrons to their final resting place. Notice that the neutral O atom is just hanging out, for the moment. Not red, but maybe kinda blue...you know, can't find a partner, that lone O's got those atomic blues...

\[
\cdot\text{O}\cdot + \cdot\text{O}\cdot + 2\text{H}^+ + 2\text{e}^- \rightarrow \cdot\text{O}\cdot + \text{H}_2\text{O}
\]

**c) Cooking with leftovers : C and O find each other at long last.** The last step now involves combining the atoms that are left over from the Red and the Ox parts. So that is, one neutral C, one neutral O from the Red part, and one neutral O from the Ox part. What is nice is that no electrons are involved. By simply using the elemental electrons present in the Lewis structures, we get the very stable
and responsible CO₂. The key to this section is that these particular ways of doing things do not occur in
the cell as we have pictured them above. That is, the mechanism by which these atoms, bonds, molecules
and electrons interconvert is different, but the starting and final molecular players are the same.

\[ \cdot \hat{C} \cdot + \cdot \hat{O} : + \cdot \hat{O} : \rightarrow O::C::O \text{ or, } CO₂ \]

The important point is the no matter what the route, or mechanism, whether it be some on-paper-only
splitting of the reactions into individual Reds and Oxs, or the cellular oxidation of sugars inside the cell,
the exact same starting and finishing molecules are involved, and so the exact same changes in redox
chemistry are occurring in either case. So now we know that when the carbons of glucose are being
turned into CO₂, electrons are being spit off, and eventually recombined with elemental oxygen to give
water, and energy.

In a funny way, the separating of the reactions into distinct phases of oxidation and reduction is
biologically accurate in one sense. Although the cell does not split up things the way we did above, it is
true that the biological oxidation of glucose carbons to CO₂ and the reduction of elemental oxygen to
water do indeed occur at different times and places in the cell. This is done by using electron carriers,
called NAD⁺ and FAD, that can accept and temporarily hold the electrons the come from the oxidation,
and then drop them off where the reduction of oxygen is going on. So the way the cell separates the
oxidation and reduction reactions is a lot fancier (but no harder to understand) then our illustrative tricks
used above, but the oxidation of C and the reduction of O are separate processes, and that is a key to
getting the most bang for our glucose buck!

It’s alive...!!! On to Biology

Moving electrons in real life- In the reactions that I show above, that is, the half reactions
which show us where the electrons are coming and going, the electrons are depicted as e⁻, and there is
always enough H⁺ ions to provide charge balance.

\[ CH₂O \rightarrow \cdot \hat{C} \cdot + \cdot \hat{O} : + 2H⁺ + 2e⁻ \]

It turns out that a better way to view the movement of electrons in biological redox reactions is still with
accompanying H's, but instead of there being free electrons and H⁺ ions, each two electrons "ride" an H⁺
in the form of an anionic hydride ion, H⁻. Although you may not have heard of such a thing as a
hydride ion before, they are actually pretty common in chemistry, and are used to reduce things in organic
chemistry laboratories all the time. So the hydride way to view the same reaction is like this

\[ CH₂O \rightarrow \cdot \hat{C} \cdot + \cdot \hat{O} : + H⁺ H⁻ \]

The hydride ion is a more useful way to think about the electrons that result from a biological oxidation.
Its not that H⁻ ions are ever floating around free in the cell. Rather, the hydride is a better way to think
about electrons because the molecular carriers that temporarily store the electrons from the oxidized
atoms do so by picking up a hydride from the oxidation reactions, and later delivering the hydride to the
right place.

So what are these carriers? There are two principle ones, called NAD⁺ and FAD. These two
molecules are beautifully designed to pick up electrons from things getting oxidized. They have very
different structures but each has just the right chemistry to grab those electrons (in the form of H⁻) and
form a reduced version of the carrier. Here, look. Once again, for convenience, we will consider a "one
carbon" sugar so as not to get bogged down in many carbons.

\[ CH₂O + NAD⁺ \rightarrow \cdot \hat{C} \cdot + \cdot \hat{O} : + H⁺ + NADH \]

\[ CH₂O + FAD \rightarrow \cdot \hat{C} \cdot + \cdot \hat{O} : + FADH₂ \]
Most of the electron carrying the we will look at involves the NAD+/NADH pair. But one FAD is used in the Krebs cycle, and so I include it. The actual chemistry that occurs in these two reactions is somewhat different, but the underlying concept, that each can accept an H- and an H+ (which comes along for the ride in the NADH reaction or is part of the covalent structure in the FADH2 reaction) and later give it back up, is the same.

Now we can do the biology, and it is quite amazing how the cell has figured out how to make all this happen in such a subtle and elegant manner. The harvest of energy from glucose occurs in three steps. First the glucose is broken in two, in a process called glycolysis. Then the product from this process is fed into the Krebs cycle, that step by step removes electrons from the participants, resulting in CO2 (ah ha!) and all the liberated electrons stored in carrier molecules. Finally these electrons are fed into a third process, called the electron transport chain, in which elemental oxygen is converted into water by acquisition of the electrons from the carriers, resulting in enough energy to make an impressive number of ATP molecules.

**Glucose has a breakdown: glycolysis**

This set of reactions alone has a little bit of oxidation that is part of the chemistry, and yields a few ATP. In the context of this writing, glycolysis might appear to be just a prelude to the other processes that yield lots of ATP. But don't think it is trivial or unimportant in biology. Any organism that lives in environments with no oxygen uses glycolysis in a major way to get the energy it needs. Also, even in organisms that can and do use the oxygen-requiring later steps to get the most energy out of glucose, glycolysis is fundamentally important. Along with providing the starting molecules for the later steps, in many cases glycolysis serves as the main way to get a burst of ATP when it is needed quickly. A familiar example is in the sudden use of muscles, such as needed in sprinting, powerlifting, or getting out of bed for Bio 1. As for the actual process of glycolysis, I will mention only the main features that help us to understand what is going on. Purves has beautiful depictions of the structures that arise in the course of this process, on pg 148-149. Feel free to take a look!

**Glycolysis starts off on the wrong foot.** The first thing that happens is that two ATP are used to phosphorylate the sugar on the 1 and the 6 carbons. Using up 2 ATP. Hmmm, not exactly what we had in mind... Next, the resulting molecule (actually called fructose 1,6 bis phosphate) is broken in half (glyco LYSIS - get it?). The easy thing is that although there are two different products that result from this fragmentation, one (dihydroxyacetone phosphate) is interconvertible into the other (glyceraldehyde 3-phosphate), and only glyceraldehyde 3-phosphate, called G3P, is further processed for the extraction of energy. (Although the dihydroxyacetone phosphate has many important uses, it isn't quite so important in the production of ATP). So eventually, you make 2 G3P, and do the same thing to each of them. So far, not so good. Used up 2 ATP, broke our glucose in half. No energy yet. Now what? The next steps are where the energy comes from. We will only talk about what happens to a single G3P, but remember that since two are made per glucose, it all happens twice per glucose. The next reaction is a fancy one, and adds another phosphate onto the G3P, and at the same time oxidizes it by moving and H+ to NAD+ with the products being 1,3 DPG and one of those NADH. Ready?

\[
\text{G3P} + \text{PO}_4^{2-} + \text{NAD}^+ \rightarrow 1,3 \text{DPG} + \text{NADH} + \text{H}^+ 
\]

"So where's the energy?" You say. Well now we're in a position to make an ATP. woooo-hoo (Homer Simpson, Ph. D.) The phosphate that was added on in the production of 1,3 DPG reaction, due to the exact final structure, is a very high energy phosphate. Meaning that it can be transferred to ADP to give

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ATP very efficiently. The way to think about it is that the phosphorylation of ADP requires a fair amount of energy. But the loss of the phosphate from the 1 position of 1,3 DPG gives off a TON of energy. So when you transfer the phosphate from 1,3 DPG to ADP, the net process is still one where net energy is given off, and the amount is \((\text{TON} - \text{fair amount})\) to be exact.

So that is one way that the energy of glucose can be trapped: by making molecules that have very high energy phosphates that can be transferred to ADP, making ATP with a net loss of energy. Remember that the capture of glucose's oxidation energy in ATP production is about 40% efficient, and you can now see why that might be so. We have to lose more energy than it takes for the ATP to be made in order for the process to occur to a large extent. But we will leave these considerations for the thermodynamic analyses that are in the realm of more advanced courses.

This process of directly transferring a "chemically correct" phosphate group from a starting molecule to ADP to make ATP is called substrate level phosphorylation. I guess because the starting molecules are substrates, and it can happen in the test tube with only the molecules involved and the appropriate enzyme (in the case above it is called phosphoglycerokinase). For a long time people thought that all production of ATP occurred by some variation of this simple idea. But surprises abound in biology.

Well, at least now we've broken even in our process: we have spent 2 ATP to get glucose into the right state of mind, and then by splitting it in two, and doing the above chemistry on the two available G3Ps, gotten 2ATPs back, and 2NADH. Now if we could only get a little more ATP from the remaining reactions, we would be making an energy profit. And that is what happens. The molecule left over from the above reaction is called 3-phosphoglycerate (3PG), and is a simple 3-carbon molecule with a phosphate group attached to one end. Another set of enzymatic reactions are now harnessed to once again make ATP by substrate level phosphorylation. The details are not so important here, but what happens is that first, the phosphate is moved to the 2 position, then a water molecule is removed to make, once again, a molecule with a very high energy phosphate. This molecule is called phosphoenoylpyruvate, or PEP.

Once again, we end up with a molecule that has a phosphate so cranked up and rarin' to go that it can be added to ADP to make ATP with energy to spare. One more substrate level phosphorylation, as shown below, and of course, this particular reaction happens twice per glucose, because we get two carbon PEPs for each 6-carbon glucose that we want to harvest energy from.

\[
P\text{EP} + \text{ADP} \rightarrow \text{pyruvate} + \text{ATP}
\]
A little bookkeeping- before we decide what to do with the two pyruvates that have come out of the bottom of the glycolytic pathway, let's stop for a second and assess what we got out of the process. We have only focused on a few of the steps of glycolysis, looking mainly at how molecules are generated that can turn ADP into ATP by virtue of the high energy phosphates that those molecules end up having as part of their structure. The total set of inputs and outputs are

\[
\text{Glucose} + 2\text{ADP} + 2\text{NAD}^+ \rightarrow 2\text{pyruvate} + 2\text{ATP} + 2\text{NADH} + 2\text{H}^+
\]

Of course, now you know that a lot more is going on than is shown in this simple picture, but this is the net outcome. We have netted two ATPs. And there has been a bit of oxidation and reduction. Two NAD$^+$ have been reduced to NADH, and the glucose has been partially oxidized. In the case of glycolysis, the oxidation of the carbons occurred in the step in which 1,3 DPG was made, and that helped create a molecule that could successfully convert ADP into ATP. But from now on its a whole new world. The remaining oxidation of glucose to its final state will happen again by carriers (mostly NAD$^+$) picking up electrons in the form of H- (see above), but the way that this chemistry gives rise to ATP will be very very different.

What goes around: The Krebs cycle

Now we're left with pyruvic acid, which has the formula CH$_3$COCOOH. You might think that I, like some mean-o organic chemistry teacher (actually I love organic chemistry) have just gratuitously written that structure for no good reason. But no! From what we already know about biological redox reactions, you can see something very clearly in this 3-carbon molecule. One end (the right one) has a carbon that is about as oxidized as it can get (bonded to two oxygens...). The other end has a carbon that is still a rich source of electrons (in the form of covalently bonded H's) and so can still be oxidized to get energy. The middle carbon is intermediate in it's oxidation state. So what does the cell do? It systematically oxidizes these remaining two carbon atoms (the left and middle one) step by step. To do this it first separates these two carbons by covalently attaching them to a very versatile carrier molecule called coenzyme A, or CoA. CoA is also referred to as CoASH, or SHCoA, because an -SH group is used to covalently link most of the molecules that are being carried to the rest of the CoA carrier. These two carbons are so commonly found together that they have a functional group name, called an acetyl group, abbreviated Ac. So in this case, the acetyl group in pyruvate (those two left carbons) get attached to CoA as shown.

\[
\text{CH}_3\text{COCOOH} + \text{CoASH} + \text{NAD}^+ \rightarrow \text{CH}_3\text{CO}-\text{SCoA} + \text{NADH} + \text{H}^+ + \text{CO}_2
\]

pyruvate \hspace{2cm} \text{AcCoA}

You will notice that below the fancy product is the word "AcCoA". This is pronounced "acetyl co a" and it is an incredibly important molecule in biology. We are just going to see it used as a molecular shovel, to load the furnace of the Krebs cycle with fresh CH-CO- groups, like lumps of coal. But suffice it to say here that AcCoA is used in all sorts of anabolic reactions too, as a way to provide 2-carbon building blocks in the synthesis of many molecules.

Although we are not going to discuss it much here, the enzyme that converts pyruvate into AcCoA, called pyruvate dehydrogenase (PDH) is a central control point in metabolism. It is regulated by many different molecules that indicate greater or lesser need for the operation of the enzyme. It has a very complex and wonderful quaternary structure that is the subject of many people's research efforts. But for now, it is just supplying "oxidizable" 2 carbon units to the Krebs cycle.
The cycle in low focus: carbon number only - We are going to think about the Krebs cycle in the simplest possible way. There are many opportunities to learn the details, and it seems like most biology and biochemistry students learn the details over and over again, but are never taught the simple underlying features. So we will break the mold! We will learn the simplest features, and leave the structural and molecular details up to your interest, later courses, or both. So what is this simple way? It turns out that the Krebs cycle is most easily viewed by considering the number of carbons that each molecule in the cycle has. The 2-carbon fragments of glucose left over from the conversion of pyruvate to AcCoA get fed into the Krebs cycle by direct covalent attachment to the last molecule of the cycle to make the first molecule of the cycle (that's why it's a cycle). Or in terms of carbon number, you go from the four carbon oxaloacetate to the six carbon citrate.

oxaloacetate

\[ \text{OAA} + \text{AcCoA} \rightarrow \text{citrate} + \text{CoA} \]

(4 carbon) (6 carbon)

Or:

\[\begin{array}{c}
\text{O} \\
\text{O}_2\text{C} - \text{C} - \text{CH}_2 - \text{CO}_2^- + \text{AcCoA} \\
\end{array} \rightarrow \begin{array}{c}
\text{OH} \\
\text{O}_2\text{C} - \text{C} - \text{CH}_2 - \text{CO}_2^- + \text{CoA} \\
\text{CH}_2 - \text{CO}_2^- \\
\end{array}\]

Then the citrate undergoes 7 enzymatic reactions that gradually oxidize the six carbon molecule back to the 4 carbon oxaloacetate, ready to accept another acetyl group from AcCoA, and thus start the cycle all over again. Does this make sense that we would lose carbons from the starting molecule? Sure, because our final product (like way above on this sheet) is CO$_2$, and that is where the carbons end up. As you now know, or maybe sense, oxidizing the 6-carbon citrate by production of CO$_2$ must mean that something is getting reduced (remember redox?) in order for those electrons to be removed. Sure enough, the turning of the cycle results in the reduction of three molecules of NAD$^+$ to NADH (at different parts of the cycle), and one molecule of FAD to FADH$_2$. So that is where the redox is going on. Cs from the carbon skeleton of the starting molecule are being turned into CO$_2$, and carrier molecules NAD$^+$ and FAD are getting reduced to NADH and FADH$_2$. It you look closely at the Krebs cycle in Purves (pg 153) or somewhere, you will notice also that an ATP is produced, somewhere around "6:15" if you consider it a clock face. Where does this come from? During one of the cycle reactions, enough energy is produced to convert GDP into GTP. GTP is structurally very similar to ATP (it has a different base, guanine instead of adenine) and the GTP can transfer its high energy phosphate to ADP. So the Krebs cycle has a little bit of the substrate level phosphorylation that we saw in glycolysis, but a whole lot of oxidation (of C) and reduction (of NAD$^+$ and FAD).

A little more bookkeeping - So now where are we in the slow burn? Well, we have taken a molecule of pyruvate, removed the juiciest two carbons, put them aboard a 4-carbon molecule to give a 6-carbon molecule, and then oxidized that back to the same 4-carbon molecule. We have gotten CO$_2$, and electrons bound to carriers, and even an ATP in the deal. The total ledger sheet looks like this:

\[\text{pyruvate} + \text{ADP} + 4\text{NAD}^+ + \text{FAD} \rightarrow 3\text{CO}_2 + 4\text{NADH} + \text{FADH}_2 + \text{ATP}\]
To keep things a bit simpler, I have left out the \( H^+ \) and \( PO \) and such. One of the NADHs comes from the reaction between pyruvate and \( \text{AcCoA} \), shown earlier.

There are two important general points. One is that nowhere in any of the reactions of the Krebs cycle does molecular oxygen, that is, \( O_2 \), play a role. The other point is that although we only consider the Krebs cycle as a way to oxidize the two "left" carbons of pyruvate, and this is an important reaction, the intermediates of the Krebs cycle are used for all sorts of biosynthetic reactions including many anabolic reactions where these molecules are diverted to be used as building blocks for many things. For this reason, the Krebs cycle plays a central role in the integrated biochemistry of the cell, including the catabolism that we talk about here, and numerous other reactions including anabolic ones where things get made.

**Goin' down that final trail: the electron transport chain**

You may notice a striking dichotomy between the way we described oxidation of glucose in the first section when we were simply figuring out oxidation and the actual way that glucose gets oxidized by glycolysis and the Krebs cycle. Specifically, the question that comes to mind is "Where the oxygen?". Indeed, even though the glucose carbons have been completely converted into \( CO_2 \) (one was spit off when we made \( \text{AcCoA} \) from pyruvate, and two more were made from a single turn of the Krebs cycle) \( O_2 \), the oxygen that is so prominent in the net reaction, has still not made an appearance. It is the electron transport chain, also called the respiratory chain, that gets \( O_2 \) into the picture. In the simplest terms, the electron transport chain takes the electrons stored in NADH and FADH\(_2\), and delivers them to \( O_2 \) in order to make \( H_2O \). Here is an example

\[
\text{NADH} + H^+ + 1/2O_2 \rightarrow \text{NAD}^+ + H_2O
\]

\( O_2 \) gets reduced, NADH gets oxidized. This reaction (and the analogous one with FADH\(_2\)) yield a lot of usable energy (remember our burning newspaper?). So how is this process, the reduction of \( O_2 \) into water, accomplished in such a way as to convert ADP into ATP? If you only knew about the way that ATP is made in glycolysis, you might guess that some molecule somewhere in the process that has phosphate on it is converted into something that can transfer that phosphate to ADP and so produce ATP. That is, some kind of substrate level phosphorylation trick that gives you the ATP. For years some of the greatest biochemists of days gone by assumed that this had to be what was going on, and they looked diligently for the unknown intermediate molecule that would serve as the source of a phosphate group that could be pumped up enough to be added to ADP. But NO! That ain't how it goes. Finally, it was realized that something very different must be going on the harvest the energy from the conversion of oxygen into water.

**Location location location**- It turns out that the energy of electrons flowing from NADH (or FADH\(_2\)) is converted not into some chemical form, but rather the energy is stored as a gradient of charge and ions across a membrane (remember the containment problem). Specifically, as electrons move from, for example, NADH to \( O_2 \), the energy that is given off is used to force \( H^+ \) ions across the inner membrane of the mitochondrion to create a charge and ion imbalance that can do chemical work.

Generation of this gradient is accomplished by the electrons from these carriers being added to and then dumped from a set of different molecules, some of them membrane proteins and some of them simpler molecules (left side of picture below). What is incredibly important is that this can only happen because this collection of molecules and reactions is highly and specifically organized in space and time, in relation to each other and to the direction of the mitochondrial inner membrane (the double line in the picture). So as the electrons flow through all of these intermediate carriers on their way to \( O_2 \), \( H^+ \) ions are pushed across the inner mitochondrial membrane to create a gradient that can do work. The idea and experimental evidence that a transmembrane proton gradient was how the mitochondrion makes ATP from reduction of \( O_2 \) to water was a ground-breaking divergence from the idea of substrate level phosphorylation. This view of ATP production, called the chemiosmotic hypothesis, was put forth by Peter Mitchell in the 1960's, and resulted in his winning the Nobel prize.
A rotary engine? Since the acceptance of the chemiosmotic hypothesis, there has been an intense effort to understand the mechanistic details of this process. That is, what molecular events allow the misplaced protons do the work of making ATP from ADP? Well, even a tiny imbalance in the number of charges across a membrane will result in a massive amount of stored energy of repulsion as the ions strive to avoid each other. The force that results from this imbalance is called a proton-motive force (force from the movement of protons). The exact way that the energy stored in these restless protons is captured by the cell is through an amazing protein complex called ATP synthase (pictured as the greenish bullet-thing in the picture…).

This complex of proteins crosses the inner mitochondrial membrane and has a channel that will allow $H^+$ to re-cross the membrane, back down the gradient. As the protons cross the membrane through the ATP synthase channel, it appears that energy is converted into mechanical rotational energy, that is, it appears that parts of the ATP synthase molecule actually turn and generate rotary force that can be used to make an ADP from an ATP (right side of picture). Sort of like a water wheel, or a turnstile when people are pushing hard against it to get outside. This rotary model of ATP synthase action is a hypothesis with a great deal of experimental evidence behind it, but it is just the most current model. The development of such a detailed picture of ATP synthesis is the result of the work of many people. Three leaders in this tour de force of biochemistry are Paul D. Boyer John E. Walker, and Jens C. Skou, who were jointly awarded the 1997 Nobel prize in chemistry for their long work on the ATP synthase (Boyer, Walker) and related proteins (Skou) that create or use ion gradients to do chemical work. But the story is far from over...