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Last time, we discussed standard reduction potential, this E° prime value, that really is a way to quantitate the propensity of a molecule to accept or donate electrons. And this is useful to think about how energy is transduced in biological systems. Because remember, as I have now tried to stress many times, how energy is moved through biology is largely about oxidation and reduction reactions involving carbon and ultimately oxygen.

And so remember that oxidation of carbon is favorable. That is removing electrons from the carbon and giving them to oxygen, exactly what we described for burning wood or burning gasoline. And so that's why coming from the most reduced carbon like fatty acids or other reduced carbon like carbohydrates, not as reduced as fatty acids, that oxidizing that carbon releases energy.

And of course, it means that storing carbon as reduced carbon either as carbohydrates or fatty acids is a way to store energy for later. And so moving from left to right, equilibrium strongly favors moving to the right. That means that ΔG is less than 0 for the oxidation of carbon.

Or we can describe this as moving from a smaller standard reduction potential to a larger standard reduction potential because, remember, the relationship between the change in standard reduction potential is related to the equilibrium constant or ΔG° prime, which is also related to the equilibrium constant by the above formula.

And so we closed last time by saying that with this formula we know that, if we move from a smaller standard reduction potential-- that is donate electrons from a smaller standard reduction potential to a larger standard reduction potential-- that that number, that difference, will then be positive, which is the opposite of ΔG because of that formula. And so a positive change in standard reduction potential, moving from lower to higher, equilibrium is going to favor that direction-- should make sense from everything we discussed before.

And of course, that change tells you the propensity of electron transfer that ultimately is going to be favorable at equilibrium. However, exactly as we discussed for ΔG , whether an exact reaction is favorable is still going to be dependent on ΔG . And so the change in standard reduction potential tells you about the equilibrium constant, and so which direction is going to be net favorable at equilibrium.

However, what actually happens is still going to depend on conditions for all the reasons we talked about previously. Still, this change in standard reduction potential is still a useful concept to think about because it tells us about how energy transfer can occur across redox pairs. And that will be useful in thinking about this process of oxidative phosphorylation and how cells really couple electron transfer to oxygen as a way to do lots of useful things in cells, including making ATP.

Now, one point I want to come back to again, which we discussed at length when we talked about changes in free energy and how that worked for pathways, is that, remember, we discussed that, if we start with wood or oil and all in one step burn it to CO₂, it releases a certain amount of energy. Or we can break that into individual steps, as we did in glycolysis. The same amount of energy is released. But by breaking into individual steps, we can now capture that energy as intermediates such that we can, say, favorably synthesize ATP at a high ATP/ADP ratio in cells.

The exact same thing is true about any of these electron transfers. There is the change in standard reduction potential. Or the change in free energy as one moves from electron transfer from one acceptor to one donor is the same if you go in one step or in many different steps.

And so by doing these stepwise electron transfers in exactly the same way we discussed about for glycolysis, we can capture that energy release in smaller packets. And this is a way to basically better harness this energy release to do useful things for cells. Ultimately, you'll see it's how you make ATP, but it can do other work as well, generate heat, move ions, et cetera.

And so this is really what I want to discuss today. But it's important to remember these concepts when we think through how these systems work. And so we've now spent several lectures discussing the chemical details of how we can oxidize. First, we discussed carbohydrates. Then we discussed fatty acids.

And what we saw is that many of the electrons that are lost from the carbon oxidation are used to generate molecules such as NADH or FADH₂ and effectively charge up a ratio, if you will, of reduce oxidized cofactors. And these molecules then donate those electrons, ultimately, to oxygen. And it's that, how you couple those electron transfers from these carriers to oxygen, ultimately is what oxidative phosphorylation is about as a way to allow cells to convert this energy released into a biologically useful form.

Now, effectively, the way biology does this is by using that favorable electron transfer to oxygen to charge a battery. So really oxidative phosphorylation, if you will, is about a biological battery. And it's really the same concepts that you may have learned in other courses about how chemical batteries work. And so let's just think about that.

What is a battery? Well, a battery is two different solutions of ions usually that is separated into two compartments. It has a unit of volts. That's exactly what the units are for this change in standard reduction potential is volts.

By using that voltage to run a current between the two compartments, you can do work. And if you short circuit a battery, of course, you can get some heat. And so how are these really made?

Well, you basically just need to get that voltage. You need to have something that insulates between those two compartments. And you can then control electron flow from one side of the circuit to the other.

And it's exactly the same issue that happens in biology, except biology needs some insulator between two compartments. How do we create different compartments in cells? Well, we create them with membranes.

And so an important function of membranes in cells, other than things like separating cellular contents from the outside world or breaking up different organelles, is that cells actually require a membrane. They're essential for life because they are what allows an insulation between two aqueous compartments in a way that you can build a biological battery that is really central to how life uses energy as a way to do work.

And so life creates a voltage, which I'm going to call $\Delta\psi$, ΔpH -- we'll come back to that in a little bit-- to basically couple favorable electron transfer from, say, an electron carrier like NAD/NADH to oxygen, so reoxidizing NADH to reduce oxygen to water. That is moving from a lower to a higher standard reduction potential that's favorable. ΔG° is negative. Net transfer is favorable.

This can be coupled to doing some work that otherwise would be unfavorable, in this case pumping protons from one side of the membrane to another. By pumping protons to one side of a membrane or the other, you create a voltage membrane potential, often abbreviated $\Delta\psi$. It's also a pH gradient, so ΔH . The protons have lower pH on one side of the membrane than the other.

And it's this charging of a battery at a membrane that is what is captured by oxidative phosphorylation, what's typically referred to as that process, as a way to make ATP. But of course, this is a useful thing. It's a battery. And so it can also do other work for cells.

Now, I just want to say and really stress that this is really critical, this process. And it's really central to how energy works for cells. You will see that, effectively, this is the key steps in photosynthesis, do exactly the same thing. It's how mitochondria work.

It's also how bacteria work. And really prokaryotes end up doing this at their plasma membranes, whereas mitochondria and chloroplasts end up doing this at intracellular membranes in eukaryotes. But ultimately, it's the same idea of charging this biological battery that really allows cells to convert, do energy conversions in a way that's useful for them to allow otherwise unfavorable things to happen in cells.

Now, this works largely because you have this insulating membrane system, which is, of course, made of lipids. Lipids are what separates the two compartments. Remember, we discussed earlier that these lipid bilayers are useful because they create a hydrophobic interface between two aqueous compartments.

It's also useful because lipids are poor conductors of electrons. And so this really ends up being the insulating system that makes this work. Now, in the previous lecture, we discussed a lot about the polar and non-polar properties of phospholipids really allow them to assemble into these membrane barriers. And I just want to do a slight diversion to revisit this in the context of how this works for bioenergetics.

So remember, you have this lipid bilayer where you separate two aqueous compartments with this hydrophobic membrane. In this membrane are these phospholipids, where you have these polar head groups with two lipid tails.

You can look back to a former lecture where I drew and we discussed what those phospholipids are in great detail. But it's really having that polar, non-polar interface that allows you to form these bilayers that's creating this separation between the aqueous compartments, but also this insulator that allows a battery to be built across the membrane.

Now, there are several consequences, though, to this system that is worth thinking about at least in terms of how lipids and membranes work. Now, a consequence of this is that, if we're going to create this system, now we create a system where we have issues with transport across from one compartment to another.

Now, this, of course, is an opportunity for a layer of regulation because it also allows then these conditions where you could have different chemical conditions, say, different ATP/ADP ratios, NAD/NADH ratios in the different compartments, which, of course, for all the thermodynamic reasons we've already discussed can make things more or less favorable to occur in different compartments in cells. But it also means metabolism then has to have ways to transport stuff across these membranes.

And we've discussed this a couple of times. A few examples-- I talked briefly about the pyruvate carrier. That is, if glycolysis is happening in this compartment in the cytosol and we need to get the pyruvate inside the mitochondria across a membrane for the pyruvate dehydrogenase reaction, now you needed this pyruvate carrier molecule. And it turns out that that transport process is an opportunity for regulation, but it still needs to occur.

We discussed the carnitine shuttle, right? Fatty acid, we generate fatty acyl-CoAs from breakdown of lipids in one side in the cytosolic compartment. We need to get those fatty acyl-CoAs into the mitochondria where they can be oxidized by fatty acid oxidation. And that required that carnitine shuttle system.

Now, ultimately, what mediates this transport is, of course, proteins that can sit within this membrane bilayer. And you can imagine you can have proteins that span the membrane bilayer, such that there's hydrophilic surfaces that are on either face of the membrane and hydrophobic residues in the middle with various ways to make channels and other transport mechanisms to get things from one side of the membrane to the other, all right?

Or you could have complexes that float freely within the lipid bilayer, such that there's a hydrophilic surface that faces one compartment and a hydrophobic surface that faces the inside of the membrane. Now, having these complexes will become very important for ways in which one can regulate processes that occur either within the membrane or by moving things across membranes, like generating moving protons and generating a voltage, or even move large things like proteins across the membrane.

But it has additional consequences because it now means that the properties of the membrane also matter somewhat as well. And this brings us back to the other discussion we had in the last lecture. And that is how the various properties of the fatty acids that exist within this hydrophobic membrane area also, now, can really matter because they're going to affect things about how leaky this is as an insulator between the two compartments, as well as how fluid the membrane is, such that things like these proteins can actually move within this hydrophobic barrier between the two compartments.

In other words, the fatty acid composition, as well as the lipid species themselves, that is which phospholipid, other polar hydrophobic lipid species analogous to phospholipids that form up membranes, can change properties such as how leaky the membrane is or how fluid the membrane is.

You can imagine, if you have a really rigid membrane surface, you're not going to get any flow of materials. It's not going to be very leaky, which might be great if you want to build an insulator between the two compartments. But it's going to be very difficult to regulate transport across that compartment.

And so nature has come up with a bunch of different solutions for how to play with fatty acid and other lipid composition to change the properties of membranes. We don't have time to get into that in great detail in this course, but I want you to appreciate that these things exist. But one thing I want to discuss is that often, when you see phospholipids drawn like this, they're drawn in this way where the polar head group is a circle. And then they'll draw the two lipid tails often with a kink in one of the lipid tails.

That's because many times phospholipids, of the two lipid tails, one is saturated. And one has a monounsaturated in it. And I just want to go back to the models here.

Remember, the monounsaturated, the double bond, ends up being this cis double bond. And so here's two fatty acids. If you put these here in with the head groups on one side-- so this would be esterified to the glycerol, the phospholipid, you end up getting this kink in the membrane, which is much different than two straight chains.

You can imagine that within the membrane itself this ends up basically not letting those lipid tails pack quite so tightly in the membrane, which certainly contributes to an ability to have space to put other stuff in the membrane to allow other processes to happen. Now, recall that there's other ways we can modulate the properties of fatty acids, introduce more double bonds, chain length, et cetera. And different organisms use different strategies to deal with this.

And so plants, remember, have all kinds of polyunsaturated fatty acids, just like we talked about with plant oils being liquid at room temperature. Having more polyunsaturated fatty acids in their lipid tails is one way plants use to keep their membranes more fluid. Animals, remember, have more saturated fatty acids. All right, those saturated fatty acids-- less double bonds, going to be higher melting temperature, more likely to be solid at room temperature.

So that's not going to be very good for membrane fluidity. And it turns out animals use a different strategy to keep membrane fluidity that's not polyunsaturated fatty acids. And that is that they incorporate this molecule shown here on the slide, which is cholesterol, which you're well aware of.

And so animals use you sterols, like cholesterol, as a way to keep their membranes more fluid. And so that cholesterol that is present in animal products is not there to give you heart attacks. It's really there as a way animals use to keep their membranes fluid. And this is really, at heart, why plants are cholesterol free foods and animals have cholesterol. And it's because animals use cholesterol as a way to keep their membranes fluid, whereas plants use a different strategy, polyunsaturated fatty acids.

All right, now, ultimately setting up the system this way is important because it allows for these flexible membranes that can enable ion and other transport between compartments, as well as hold a charge so that you can form this battery which allows oxidative phosphorylation or other processes to occur where you can charge this membrane and ultimately use that voltage to do work, like make ATP, all right?

Now, how this membrane charging occurs, again, is really taking advantage of favorable electron transfer across a series of redox pairs, ultimately, to oxygen or another good electron acceptor. And it's really that favorable electron transfer that's being coupled to this pumping of proton ions to charge a battery. That's the oxidation part.

And then that battery doing work such as driving favorable ADP to ATP conversion, the phosphorylation part of oxidative phosphorylation, is the type of work that you can then do once you generate this chemical battery, although not the only work you can do with that battery. And it's why this is so much more flexible than just making ATP.

All right, so ultimately this process by coupling transfer of electrons from favorable oxidation of carbon to charge up these other electron carriers that ultimately transfer to oxygen is a major way that life uses nutrient oxidation to convert it to biologically useful energy. And this is where we get to this process of what is typically referred to as oxidative phosphorylation, which is a concept or a process that was often abbreviated oxphos. And it was discovered by or first described by a gentleman by the name of Peter Mitchell.

And like many novel or revolutionary ideas, when Peter Mitchell first described or proposed the processes I'm about to tell you about, his ideas were initially dismissed. It was thought at the time, when people were thinking about how do you couple metabolism to, say, charge up your ATP/ADP ratio, that that process would work via the oxidative phosphorylation type reactions that we otherwise described, things like what happens at the GAPDH step or the succinic thiokinase step in the TCA cycle.

Those are two examples where we could directly couple the oxidation of carbon to a phosphorylation event that ultimately allowed us to favorably produce ATP to maintain an ATP/ADP ratio that's high and is present in cells. However, what Peter Mitchell described was really this battery phenomena that we're talking about. And it turned out to be largely right and represents something that's actually quite unique about biological processes even if you think about other biological processes that occur.

And that is what he proposed was the oxidation and phosphorylation events were actually separate processes. That is nature does not have to have these two things touch in order for them to work. That is that you use oxidation to create this battery as one event.

So oxidation generates this potential across a membrane. And then that potential across a membrane is a totally separate event, can drive phosphorylation, ATP, or do other work, ultimately making this a more flexible system for biology.

And so turns out, as I said before, in a prokaryotic cell-- so this here is some kind of prokaryote, OK? Prokaryotes have a membrane that separates them from the outside world. This cell membrane effectively couples that oxidation step to the pumping of protons.

That creates a battery at the cell membrane of the prokaryote. And separate in space, that can then be used to drive ATP synthesis or do other work that's useful to maintain that prokaryotic cell.

Now, in eukaryotes, this process occurs at membranes within the mitochondria. So this here is a mitochondria, OK? So just to remind you of some very basic cell biology-- and so, remember, mitochondria have two membranes.

So there's this outer mitochondrial membrane, outer mitochondrial membrane. And then there's this inner mitochondrial membrane, so two membranes in the mitochondria. Remember, there's this matrix space. The cytosol would be out here.

And then there's this space between the two membranes called the intermembrane space, intermembrane space between the two membranes. If you'll recall, many of these oxidation reactions, the TCA cycle, fatty acid oxidation, pyruvate dehydrogenase step, all of those were happening in the matrix of the mitochondria.

Remember, glycolysis was in the cytosol, but all these other processes were happening in the matrix of the mitochondria. And really what's going on is that this battery is being charged across this inner mitochondrial membrane, OK? So protons are being pumped into the inner mitochondrial membrane into the intermembrane space of the mitochondria and then used to synthesize ATP in the matrix of the mitochondria.

Now, part of this came, many people know-- remember, mitochondria have their own DNA, et cetera. And so really where people thought, think eukaryotic cells came from is basically the mitochondria is an enslaved bacteria or an obligate captured symbiont, if you will, where one cell engulfed a different prokaryotic cell.

And if you track this, think of the inner mitochondrial membrane as a enslaved prokaryotic cell, you can see that you're generating this membrane potential across the inner mitochondrial membrane, which is really equivalent to the bacterial membrane of the enslaved prokaryotic cell.

In both cases, it's creating this battery at a membrane, the inner mitochondrial membrane or the bacterial membrane of a prokaryote, that is then being used to separately in space use that gradient to do work, such as synthesize ATP.

So if I draw this more explicitly as this here being the membrane-- so the inner mitochondrial membrane of a mitochondria or the bacterial membrane of a prokaryotic cell, such that this is outside the bacteria, that's inside the bacteria. This is the intermembrane space often referred to as the cytosol side.

Of course, there's another outer mitochondrial membrane here that, for the sake of this class, you can just think of as being generally permeable. This would be the matrix inside of the inner mitochondrial membrane. And so all this NADH is being generated from nutrient oxidation TCA cycle, fatty acid oxidation, pyruvate dehydrogenase, whatever, within the matrix or within the bacteria.

Those electrons are ultimately being transferred from NADH, reoxidizing it to NAD, to reduce oxygen to water. This is favorable. This is, therefore, coupled to pumping protons.

That creates a membrane potential and gradient across this membrane, which can be used to do work such as driving the synthesis of ATP for other work.

So this all has to happen at the same membrane, but this process does not have to touch that process. It's coupled because you create this battery, this membrane potential and pH gradient, that can be used elsewhere along the membrane to do some kind of work.

Now, I draw this is $\Delta\psi$, ΔpH . You can imagine, if you're moving protons, you're going to create a voltage. You're also going to create a pH gradient.

It turns out I don't have time to discuss this further in the class. A lot is written about how to think about this membrane potential as either a true voltage or as a pH gradient. It turns out that they're useful to think about in different ways for different processes. But if you're interested in this, there's certainly lots written about this on various papers and texts on bioenergetics. And I would encourage you to read more about that. OK.

All right, what I want to do next then is discuss each of these processes in turn, that is how does this work and how does that work, to really understand what's going on in mitochondria and bacteria to carry out this process of oxidative phosphorylation. So first, let's talk here.

First, how does one use favorable electron transfer to make this membrane potential?

Obviously, if we're going to pump protons against a gradient, such that we have different concentration of protons on either side of the membrane, that's going to require energy input. And that energy is going to come from favorable electron transfer. And really this process is taking electrons from, say, a cofactor like NADH, and ultimately transferring those electrons to oxygen to get water.

The standard reduction potential of NAD/NADH is less than the standard reduction potential of oxygen and water. That means that equilibrium is going to favor electron transport in this direction. To say it another way, relative to oxygen-water, NAD/NADH is a better electron donor. Oxygen-water is a better electron acceptor pair.

And so by this electron transfer being favorable, it can be used then to release energy coupled to other processes, such as moving protons and make that otherwise unfavorable process favorable. Now, you'll note that, in doing this, we now regenerate the oxidized cofactor NAD⁺. And so that solves our electron balance problem for glycolysis, solves any electron balance problem that would exist for fatty acid oxidation or the TCA cycle in the mitochondria.

Because ultimately, those electrons are being given to oxygen. And so oxygen, being this final electron acceptor, being stoichiometrically produced to generate water, is effectively water being the alternative waste product to, say, lactate or ethanol that we saw in fermentation, all right?

Now, as we started the lecture with and discussed in glycolysis, if we do this in one step, it's favorable. But it's also favorable if we do it in lots of little steps. By doing it and lots of little steps, just like we did in glycolysis, we can break up that energy release in a way to allow us to do more work, right?

So in glycolysis, we could turn carbohydrate into CO₂-- one step, burning wood, release a lot of light and heat all in one step. That can be used to do work. But it was more efficient to break it up into little steps where we could then capture intermediates that made ATP synthase favorable despite high ATP/ADP ratio.

Same idea here, we could transfer this all in one step. But instead, you break it up into a bunch of individual steps, which can then be coupled individually to pumping protons across the membrane to generate more efficiently this $\Delta \Psi$ Δ pH to charge this battery. And so this occurs across a series of electron carriers, multi-protein complexes, electron carriers, called the electron transport chain.

And you can think of this electron transport chain as being entirely analogous to what we already talked about when we talked about how pyruvate dehydrogenase or alpha-ketoglutarate dehydrogenase works when we talked about the TCA cycle. Remember, these had these different protein complexes, this E1, E2, E3, where electrons were transferred across this chain.

It's exactly the same idea here, a number of multi-polypeptide encoded protein complexes that come together to hold a bunch of electron carriers that break up the energy release, if you will, as we oxidize NADH and reduce oxygen and transfer those electrons along the chain with the complexes themselves coupling the favorable electron transfer to the moving of protons against a gradient across the membrane to work.

And so for this electron transport chain to work, we obviously need a bunch of electron carriers. And why you need more than one electron carrier should now be obvious. Because having different carriers that sit at different standard reduction potentials is going to be useful if we want to build a chain such that we can have favorable oxidation and reduction to move electrons in a favorable direction and couple that to processes that allow us to charge this battery.

And so it turns out that for this electron transport chain to work-- well, we know about some of our electron carriers. So we've discussed NAD, NADH. There's FAD, FADH₂. There was lipoic acid.

Lipoic acid is not part of the electron transport chain. NAD, NADH is a donor. You'll see FAD can be part of the electron transport chain, but there's a different-- or there's additional electron carriers. I want to discuss those now.

So another important one is something called FMN or Flavin Mononucleotide. What FMN, or Flavin Mononucleotide is, is it's effectively FAD. But it's a FAD not in a dinucleotide state, but a mononucleotide state.

So if you take FAD and you remove an AMP group from it, what you're left with is FMN. So just to remind you to be explicit about this-- so.

So you have this flavin group attached to this ribitol group with a phosphate on it. If I put another phosphate and an adenine nucleotide here, an AMP group attached here, that would be FAD. This would be FMN, nucleotide instead of a dinucleotide.

This is FMN in the oxidized form. And just like FAD, it can accept an electron pair. So here's our hydride ion, two electrons if we just draw here the middle part of the molecule.

And so this here would be FMNH₂ or the reduced form of this electron carrier, OK? So flavin mononucleotide-- part of the electron transport chain.

Now, note FMN, FAD, NAD, all of these things are two electron carriers, right? We disguise the mechanism of all of them, just reminded you of it, these hydride ion transfers, two electron carriers. It turns out the electron transport chain also uses metal ions as electron carriers.

And so-- lots of minerals in our diet, things like iron. It turns out elemental iron is a great electron carrier. And it's very important for electron transport chain.

Now, elemental metals are one electron carrier. And so there's a few ways you incorporate iron. One is something called iron sulfur clusters.

And so iron sulfur clusters is, within a protein, you have some cysteine residues. And those cysteine residues can coordinate an iron atom. And so that iron atom can sit in the 3+ versus 2+ state, oxidized, reduced.

So reduction of the iron-- oxidation of the iron, OK? So one electron carrier-- move one electron that way. Also, iron sits within molecules called cytochromes. So what are cytochromes?

This is an iron atom that's basically chelated into a porphyrin very similar to what you saw for hemoglobin. And so you have an iron 2+ or 3+ that's chelated here into this porphyrin ring structure.

OK. Lots of conjugated double bond systems in it, this should look very similar. Porphyrin is the thing on the outside, coordinates iron atom in the middle. That can move between the 2+ and the 3+ state just like we do for iron sulfur clusters, so carry electrons be oxidized or reduced, 3+ or 2+.

And ultimately, this porphyrin can have some R groups added here, can be associated with it or bound to cysteine residues within proteins. Effectively, these molecules varying these R groups and how it's attached to proteins gives you different families of cytochrome is referred to as cytochrome A, the B type cytochromes, and cytochrome C.

These are associated with enzyme complexes, cytochrome C. It's actually covalently bound to the polypeptide itself. Basically, these are named because you have these different conjugated ring structures here. That and how the R groups are will slightly change.

So this will absorb visible light. That will slightly change the absorption spectra. And so classically, based on absorption spectra, these were classified into A, B, and C type cytochromes. And you can imagine, by these properties having slightly different-- affecting the iron in ways that it slightly affects its electron transport properties.

Of course, hemoglobin almost certainly evolved from these molecules as oxygen carrying ways in multicellular life. But of course, the original use was as electron carriers for the processes here that we're going to talk about, to hold that iron as a way to control electron transport across these electron transport chains. All right, so that's iron.

It turns out iron is not the only metal that can be used to do this. And so cells also use copper. And so histidine can coordinate a copper that can move between an oxidized and reduced state, so copper 2+ reduced to copper +, copper + to copper 2+ or copper + oxidized to copper 2+, another one electron carrier.

And so iron and copper can act as one electron carriers. And so you have these two electron carriers. Here's a bunch of metals as one electron carriers. Obviously, you need ways to carry both one and two electrons if you're going to move electrons between these different carriers.

And an important molecule to do that is something called coenzyme Q. Coenzyme Q-- sometimes referred to as ubiquinone ubiquinol, because it's ubiquitous, often abbreviated co-Q. Many of you have probably heard of the supplement CoQ10, very popular supplement out there.

CoQ10 is basically a version of coenzyme Q, ubiquinone, ubiquinol. What this looks like is as follows.

So this here is ubiquinone, the oxidized form. So this middle part is the quinone. And then you have these decorations on the outside, including this R group. This R group is typically a long acyl tail. If it's 10 carbons long, that's CoQ10 supplement.

Having this long acyl chain makes this a very hydrophobic molecule. And it actually lives within the membrane itself as an electron carrier. And so this ubiquinone can pick up an electron as well as a proton. Whoops.

And so by picking up one electron, it can then basically go here to this semiquinone stabilized free radical state and then pick up a second electron to go to the fully reduced form. ubiquinol.

OK. So one electronic carrier and two electron carrier-- so basically, it can carry two electrons from the oxidized, the reduced form, but can transfer them as single electrons by going through this stabilized free radical ubiquinone state. And so, basically, these various electron carriers are associated with these protein complexes within this electron transport chain that ultimately works to couple favorable electron transport down the chain to pumping protons to generate this potential across the membrane.

All right, so this process occurs, again, at the bacterial membrane or the inner mitochondrial membrane and, in animals and most organisms, operates with four large multi-subunit protein complexes, all right?

Each of these protein complexes has multiple polypeptides, as I said. It's coming together to form these complexes just like we described for pH, for instance. Most of the components of this are encoded in the nuclear genome, but a subset of these are encoded in the mitochondrial genome. Now, what exactly is encoded in different mitochondrial genomes varies by organisms.

But across organisms, it's basically mostly complex components of these protein complexes. And so this is why mitochondrial DNA is retained. It's kind of this vestige of this symbiotic relationship. The mitochondria have retained some of the ability to control their own energy transduction, sort of core components of these complexes.

There is one soluble protein. So these complexes are embedded within the membrane. There's also one soluble protein that's associated with the membrane. And then there's coenzyme Q, which also floats within the membrane.

So that's in the membrane. These are embedded in the membrane. And then there's a soluble protein that's associated with the membrane.

OK, now I want to discuss the details of what these protein complexes are and also few comments about how they work. So if this is the inner membrane space and this is the matrix side, that'd be out or in if we're discussing a prokaryote.

The first is we have something called complex I, also referred to as NADH oxidase because it oxidizes NADH back to NAD.

NADH oxidase is a giant complex greater than 900 kilodaltons in total. It has more than 25 individual protein subunits, contains some flavin mononucleotides as well as some iron sulfur clusters as electron carriers.

And it is the least well-understood of all of the protein complexes. In fact, it is still a major field of study to try to understand how NAD oxidase really works. But it effectively is one of the complexes that can pump protons across the membrane as those two electrons are transferred through the complex from NADH ultimately to coenzyme Q that exists within the membrane.

OK, the next complex discuss is complex II. Complex II is succinate dehydrogenase, the exact enzyme that we heard about from the TCA cycle. So what does succinate dehydrogenase do?

Remember, it converts fumarate to succinate. It gave those electrons to FAD to make FADH₂. Well, that occurs within this complex, which can also reoxidize the FADH₂ with those two electrons and give them, also, to coenzyme Q that sits within the membrane.

So I want to point out that this, ultimately, you will see, is why it is that electron transfer from NADH and FADH₂ has different energy yields. And so the electron transfer from NADH to ubiquinone is more of a change in standard reduction potential than from FADH₂ to ubiquinone.

Why use FADH₂? Well, because, remember when we did electron transfers, we used, when we oxidized a carbon, say, in a carbohydrate to a ketone, we tended to use NAD⁺/NADH as the electron transport pair. So this change in standard reduction potential is such that we can make that reaction work and ultimately drive that.

It turns out that putting a double bond in-- this going from a not oxidizing an alcohol to a ketone, but oxidizing the carbon to a double bond-- that basically you need a different change in standard reduction potential to carry out that reaction and make it work. FADH/FADH₂ sits at a higher standard reduction potential than NAD/NADH.

And so that means to further transfer them to something further downstream, like coenzyme Q, there's a bigger change in standard reduction potential here. That can be used to drive protons. There's not enough of a change here. You can't use that to drive protons.

And it's because of these differences in what's actually being oxidized that ultimately leads to this difference in energetics when we think about FADH₂ versus NADH and how much ATP equivalence that they might have for cells. All right, once electrons are in the ubiquinone pool or coenzyme Q pool, they can now be transferred to complex III.

So complex III is also referred to as cytochrome C reductase. You'll see why in a second. It's a 250 kilodalton complex. There are 10 subunits.

It has a bunch of B type and C type cytochromes, as well as some iron sulfur clusters. Shown here on the slide from a textbook is a picture of complex III and what it actually looks like.

Fairly well-understood how it works, this coupling of basically electron transfer through the Q pool can pump protons. And this ultimately allows transfer of electrons to this small soluble protein that lives in the intermembrane space or associated with the outside of the bacteria called cytochrome C-- or outside in the intermembrane space called cytochrome C.

Cytochrome C is a 13 kilodalton protein. It's a very small highly conserved protein with a C type cytochrome commonly used because it's found across many, many different types of organisms. And so it's been sequenced from many, many organisms as a way to identify evolutionary relationships. And then cytochrome C, this is also why it's called cytochrome C reductase because it's reducing cytochrome C to transfer electrons along the chain, ultimately to the final complex, complex IV.

Complex IV-- also referred to as cytochrome C oxidase. And this is ultimately the place where electrons are transferred to reduce oxygen to water. So this is 160 kilodalton protein complex with 13 subunits, uses A type cytochromes and copper as electron carriers and, ultimately, allows you to get electrons from cytochrome C to oxygen.

And so here, again, from the textbook is a picture of complex IV and what it looks like. Also, fairly well-understood-- also couples electron transport to proton pumping. And so this is effectively the electron transport chain as it exists in mitochondria.

And you can draw it a different way as I will do here. So here's complex I, allows oxidation of NADH back to NAD⁺. That oxidation can be coupled to reduction of FMN.

Ultimately, those are passed through some iron sulfur clusters leading to the reduction of ubiquinone. And in the process, this electron transport can pump on the order of four protons, or at least that's what's estimated.

Complex II, which is succinate dehydrogenase from the TCA cycle, really converts succinate to fumarate. That oxidation reaction within the complex leads to the reduction of FAD to FADH₂. That can be reoxidized through iron, ultimately driving the reduction of coenzyme Q from ubiquinone to ubiquinol.

That ubiquinone to you ubiquinol in something called the Q pool within the membrane can then be reoxidized in complex III. That reoxidized in complex III puts electrons through complex III that can be coupled to pumping on the order of two protons.

Those electrons are transferred to cytochrome C. Ultimately, those electrons are transferred to copper in complex IV to cytochrome A and, ultimately, oxygen to water, reducing oxygen to water and pumping on the order of four protons.

And so you can see here that, basically, this is a series of oxidation and reduction reactions that happens across these complexes, such that complex I, the transfer electrons from the Q pool through complex III. And complex IV can pump protons across the membrane and generate this $\Delta\psi$ Δ pH that can be used to do work. And it's really the favorable transfer of electrons either from NAD/NADH or the oxidation of the succinate to fumarate with those electrons being given to oxygen to drive this process.

And so really just to schematize this, in a way, is that, if this is our membrane, NAD⁺ to NADH gives electrons to complex I, which goes to coenzyme Q, which goes to complex III, which goes to cytochrome C, which goes to complex IV and reduces oxygen to water.

That is one way to carry out this set of reactions. The other way is we can convert fumarate-- sorry, succinate to fumarate. That's the FAD. That's complex II, also gives it to the Q pool.

And so you can see that the electron transport chain would be complex I, Q, III, C, IV or II, Q, III, C, IV. It is not I, II, III, IV. It is actually I to Q or II to Q, ultimately going to III.

Now, the way protons are pumped is a process that I don't have time this year to discuss in class. I will post something on Stellar that is just a short section from the textbook that describes at least what is known about how that process works, such as the Q cycle, basically how oxidation and reduction of coenzyme Q, which will take protons on and off the molecule on either side of the membrane, can be used to show how this can be coupled to proton pumping across the membrane. And you can read that if you're interested.

OK. Now, a couple other points about this, this works because you're moving from a lower to a higher standard reduction potential across the chain. And it turns out oxygen is a particularly good electron acceptor. And this is really why oxygen and respiration is really so vital to a lot of the way energy transduction works in metabolism.

Oxygen is now abundant. And being a great electron acceptor is why it's used. But of course, oxygen was not abundant 2 and 1/2 billion years ago.

And it doesn't have to be oxygen for this to work. If you have another electron acceptor that basically makes this process favorable, that can work just as well. And in fact, that happens still in some very extreme environments.

This so-called chemosynthesis basically can use other electronic acceptors to drive the energetics of cells. And you really just need the right ways to build an electron transport chain to make this whole system work, such you can build a battery and use it to do work, OK? So oxygen-- commonly used because it's abundant and good at it, but it's not the only way that this can work. And there are examples in biology where oxygen is not used, that there is other acceptors that are used instead.

Now, I also want to note, as I've alluded to in discussing complex II, this FAD/FADH₂. So when we talk about that in the TCA cycle or in fatty acid oxidation, we discuss it as if it's kind of like NAD/NADH, but it's not. NAD/NADH are basically cofactors that move around between enzymes.

Fad/fadh₂ are not free. They're electron carriers that are part of protein complexes, like what we described up here. And they're part of enzymes like succinate dehydrogenase. So complex II just happens to be an FAD/FADH₂ containing enzyme that's part of the TCA cycle and part of the electron transport chain as a way to transfer electrons in the TCA cycle into the Q pool via this succinate to fumarate conversion.

Acyl-CoA dehydrogenase, the FAD/FADH₂ enzyme we discussed in fatty acid oxidation, could easily be drawn instead in electron transport chain that goes acyl-CoA dehydrogenase, coenzyme Q, III, C, IV, just like we can say succinate to fumarate is the same as complex II, Q, III, C, IV, OK? And so there's really lots of alternative electron transport chains that involve these FAD/FADH₂ containing enzymes that really sit in the membrane and directly transfer electrons to the Q pool.

Ultimately, the goal of all of this is, of course, to create this $\Delta\psi$, which can be used to charge this battery that then can then be done to do work. And so this work includes the phosphorylation of ATP. And so if we charge this battery, we can now use this as a way to couple current in this battery to phosphorylate ADP to make ATP, the phosphorylation part of oxidative phosphorylation.

Now, this occurs also by a large protein complex sometimes referred to as complex V. But remember, complex V, or this ATP synthase machinery, is not really part of the electron transport chain. It is separate in space and is basically something, a way, to utilize this $\Delta\psi$ across the membrane to drive ATP synthesis.

All right, how does this complex V or ATP synthesis work? So this is also a large protein complex, sits within the same membrane, of course, because it has to utilize the membrane potential to function.

And the so-called complex V is also referred to as the ATP synthase or the F₀ F₁ ATPase. And basically, if this is the intermembrane space, this is the inner mitochondrial membrane. This is the matrix.

You have a protein complex that sits within the membrane called F₀. It's actually F₀. The O stands for a Oligomycin because there's a drug called oligomycin that inhibits the F₀, or F₀, part of the molecule.

And then there's this F1 component that is associated with the F0 component that sits on the matrix side of the membrane. And it is effectively the machine that phosphorylates or interconverts ATP and ADP on the matrix side of the membrane.

Now, it's pretty well-understood how this system works. And basically, F1 works by a process called rotational catalysis. How this works was awarded the Nobel Prize in 1997, figured out not all that long ago.

And basically, this is F1 is a large protein complex where you have a gamma subunit that is attached to F0 the couples, basically, proton pumping to this gamma subunit to literally turn the F1 component and change the conformation of attached regions, parts, of this F1 machine.

Because it turns out you can have three different conformations of the rest of F1, the so-called L, or Loose, conformation, the T, or Tight, conformation, or the O, or Open, conformation. And so in the open conformation, ADP and phosphate or ATP can exchange in and out of this complex, all right?

In the loose conformation, you basically have ADP and phosphate bound. And in the tight conformation, this is favored to synthesize ATP. And so you can envision that what actually happens here is that you first have ADP and ATP bind in this open conformation.

Protons are moved. You get rotation around the gamma subunit. That changes the conformation of the O subunit to the loose subunit.

So now, you have ADP plus P_i bound. Another proton moves, changes the conformation of the loose subunit into the tight conformation. Now, ATP synthase is favored.

Another proton moves, comes back to the open conformation. ATP is released from the molecule, and you can run another rotational catalysis cycle again to ultimately drive ATP to ATP synthase using this transfer of protons across the F0 subunit to ultimately rotate this machine to synthesize ATP.

Now, the way F0 works is within the membrane-- and this is always hard to draw-- you basically have a central complex with another set of complexes around it that, within that, have two half-channels that are open to either side of the membrane.

And so if you look at this from the top view, so looking at it straight down, you'd have this central piece with this external piece with the two half-channels that basically can rotate around the central stalk. This is hooked up to the gamma subunit of F1 in which drives rotational catalysis. And how this works is that attached to this inner piece that is open to the half-channels is an aspartate residue.

So this is an aspartic acid residue that's there. And recall that aspartic acid, it's an acid. So it can pick up a proton or release a proton from its acid group depending on the pH on either side of the membrane.

And so, remember, there's a different pH on the two sides of the membrane. And it's really that difference in pH that will allow favorable turning around this center stalk. And so the pH in the intermembrane space is lower.

So that means you're more likely to favor protonation of the aspartate. It can then rotate, such as the channel, that aspartate, is now exposed to the other channel, to the matrix side. There, the pH is higher, so favors deprotonation.

And basically, this will drive motion of this piece around the central stalk. That being coupled to the gamma subunit of F1 will cause the rotation of the F1 subunit changing the attached conformation of the rest of the complex to favor ATP synthesis. And it's really this is how moving of protons back from the intermembrane space to the matrix can drive the synthesis of ATP, the phosphorylation part of this process.

And so now you have a full picture of how carbon oxidation really releases energy in a way that's coupled to do work in biology. So we can, of course, couple this directly, as we did at GAPDH or succinic thiokinase. But mostly, it's captured to charge up these NAD/NADH ratios where favorable electron transport can be used to make this $\Delta\psi$ Δ pH in a membrane that can be used also to synthesize ATP despite a high ATP/ADP ratio because that battery can be used to drive this machine.

Now, I want to point out that the ATP synthase is just like any other enzyme in any pathway. And that is, if conditions are right, there's no reason that this machine has to drive ATP synthesis. It could also consume ATP as a way to create a proton gradient.

That is use ATP hydrolysis to pump protons in the opposite direction into the intermembrane space. Well, why would you ever do that? Well, you would do that because having this battery is useful because ATP is not the only way that cells can use energy to do work.

It turns out that having a membrane potential is useful for other things. So if you're in course nine, you know that action potentials are carried, basically electricity along cells. What is that? That is basically changing membrane potentials across cells.

More generally, membrane potentials can be used to do lots of other types of work. And so ATP is only one way that cells can use biological energy because it can be coupled to otherwise unfavorable reactions. But you can also couple ion gradients to unfavorable reactions and use that to do work-- and so lots of useful ways that $\Delta\psi$ Δ pH can be used by cells.

And so what are some of these? Well, the first one is this can be used to generate heat. So if you're checking your phone right now, your phone is obviously a very useful device to do all kinds of work for you.

You can read whatever you want on your phone, use it to call people. Well, what happens if you short circuit the battery in your phone? Well, it'll get really hot.

And the same thing happens here. Rather than use this $\Delta\psi$ Δ pH to generate ATP, if I just put a hole in the membrane, something referred to as an uncoupling protein, UCP, Uncoupling Protein, this will just allow the leak of protons back across the membrane. Well, that's short circuiting the battery. Energy has to be dissipated somewhere, and it's dissipated as heat.

And this is effectively ways in which biology can create heat. And so uncoupling proteins are expressed in a tissue called brown fat in mammals. Brown fat is a way to generate heat. And it's one way that we maintain our body temperatures.

Now, the system is imperfect. And lots of cells, all metabolism, generates some heat. If you've ever done composting, you know your compost pile gets hot. That's because the organisms there that are metabolized in the material in the compost pile are generating some heat. And that's because membranes aren't perfect. They're somewhat leaky.

Now, as humans, we work hard to maintain a constant body temperature. And there's actually a huge amount of energy that goes into just maintaining our body temperature. But you can imagine how efficiently we couple these processes will determine how many calories we need to maintain heat.

And that's actually a theory of weight control, that those of us out there who have less well-coupled mitochondria can eat more because they waste more energy in generating heat than those of us who are really efficient in doing this. And in fact, there's drugs that allow you to uncouple electron transport generation of $\Delta\psi$ from utilizing that membrane potential. And these are great weight loss drugs.

Dinitrophenol was used middle part of the last century as a weight loss agent. It was very effective. The problem is it's also incredibly dangerous because people will die. Because it turns out, if you uncouple this process and you don't have enough ATP, you die really fast. And so don't use that drug, but it illustrates how you can use this battery to generate heat, another form of energy.

Another big one is membrane transport. So if I want to concentrate something-- and this could be moving proteins. Or it could be moving other ions, like in an action potential. Those are unfavorable processes, right?

I'm causing dissociation of ions across membranes or moving protein from one side of a membrane to another. One can couple those transfer processes to these $\Delta\psi$ as well. And in fact, protein import in the mitochondria depends very much on a membrane potential to get those proteins in.

The mitochondria is an important site of calcium storage for cells. Calcium is concentrated in the mitochondria. And that concentration can be driven by this membrane potential. And of course, this potential can also use to power lots of shuttles, like the carnitine shuttle, and actually create situations where you have different conditions across membranes as a way to favor different chemistries.

And so the last thing I want to mention is, in fact, these shuttles, which is just ways that one can couple membrane potential to drive other things, can also be really important for how metabolism works. And I just want to illustrate one aspect of this. So if I draw here my mitochondria-- let me draw it up here.

If I draw a mitochondria, so here's all these processes are happening here where we have electron transport generating a potential across the intermitochondrial membrane that can be used to synthesize ATP. Well, this synthesis is occurring in the mitochondria.

But ultimately, I need to get that ATP out into the cytosol for it to be useful for the cell to run otherwise unfavorable processes in the cytosol. And so one needs a transporter for this. There's some transporter called the adenine nucleotide transporter.

And this partially takes advantage of the fact that there's a charge difference between ATP and ADP to actually couple membrane potential to exchange of ATP/ADP between the cytosol and the mitochondrial matrix. Now, there's other issues we'll talk about next time as well. And so, remember, some metabolism is happening in the cytosol.

So glycolysis turning glucose into pyruvate is occurring in the cytosol. We've already discussed. That means you got to get pyruvate in the mitochondria for the PDH reaction, but this is also generating NADH in the cytosol.

And if that NADH needs to transfer its electrons to oxygen, we also have to get that NADH into the mitochondrial matrix or, more correctly, transfer those electrons into the mitochondrial matrix. It turns out cells maintain distinct NAD/NADH pools in the mitochondria and the cytosol. That's part of the conditions that make different reactions favorable in each compartment.

And how cells do that, transfer those electrons into the mitochondria, ends up being another important thing that has to be transport that's driven across these membranes where one can use $\Delta\psi$ Δ pH. And we'll talk about that at the start of the next lecture. Thanks.