[SQUEAKING][RUSTLING][CLICKING]

MATTHEW VANDER HEIDEN: Last time, we discussed oxidative phosphorylation, which is how to couple the NADH generated from sugar, fatty acids, et cetera; the oxidation of carbon as a way to carry out favorable electron transfer to oxygen and use that energy release to charge a battery-- that is, create this delta psi/delta pH that can subsequently be used by the battery to do work, including the synthesis of ADP.

And of course, this can occur at a physiological ATP/ADP ratio and, therefore, allow the cell to couple ATP to ADP conversion to other unfavorable processes in the cell. Now, all of these reactions occur at the mitochondria. And I drew, here, a schematic mitochondria for us just to start. Remember, the mitochondria has its own genome.

This is a vestige of the symbiotic relationship that developed long, long ago, where two prokaryotes came together, leading to this symbiotic relationship-- but of course, the mitochondrial DNA encoding many of the key components of this electron transport chain and ATP synthesis machinery that remains in the mitochondria today.

Now, ultimately, this process involves the reoxidation of NADH back to NAD that occurs, that complex I of the electron transport chain. Those electrons are transferred into the Q pool, which then go into complex III to cytochrome c to complex IV, ultimately being transferred to oxygen as the final electron acceptor. And it's this process of favorable electron transport-- that is, electrons moving from a lower to a higher standard reduction potential-- that is coupled to the pumping of protons and the generation of this delta psi/delta pH.

Now, we also discussed complex II of the electron transport chain, which is identical to succinate dehydrogenase in the TCA cycle. This is involving that FAD/FADH2, which can, itself, also transfer electrons to the Q pool, ultimately passing those to complex III to cytochrome c to complex IV, again, with oxygen as electron acceptor, ultimately forming what is essentially two different variations on the electron transport chain as a way to generate delta psi/delta pH.

Now I show here, on the slide, that there's really many versions of this electron transport chain, and another one we discussed is shown down here. So up here is the typical one to regenerate NAD+ using complex I to the Q pool to III to c to IV. Here's the one involving complex II, so dehydrogenase reaction via complex II to the Q pool, complex III, c, IV.

And then there's yet a third one here from fatty acid oxidation. So this is Acyl-CoA dehydrogenase. Again, like complex II, involves FAD/FADH2. This occurs at the membrane, transfers electrons to the Q pool to III to c to IV, ultimately reducing oxygen to water. And so the net effect of these various electron transport chains were shown up here is generating this delta psi/delta pH, which can then be used to do work.

Now, the cool thing is is that this is occurring, in this case, at the inner mitochondrial membrane. And so you can do work in lots of different ways. It doesn't have to be next to the complex. You have a charge across the membrane that can be used different places in space, such as so-called complex V or the F1F0-ATPase, which can use dissipation of that proton gradient to drive that rotational catalysis machine we described last time and, ultimately, take ADP plus phosphate and synthesize ATP.

But that's not the only work it can do. You can uncouple the whole thing. We discussed these uncoupling proteins, which, effectively, just let protons leak back across into the membranes, that short-circuiting this potential. And that's a way to generate heat.

Other type of work we can do is we can use this proton gradient to power the transport of other ions. We discussed this happening with ADP/ATP exchange because, of course, ADP-to-ATP conversion needed in the cytosol to favor unfavorable processes there. Need to exchange those in and out of the mitochondria, so you can regenerate ATP with the ATP synthase machinery.

And yet another thing we talked about is you can use this as a way to concentrate ions, such as calcium. And the mitochondria ends up being a major calcium store for cells.

Now, we discussed last time that this is great having all of these oxidation processes-- the TCA cycle, fatty acid oxidation-- all occurring in the matrix because it gives a location for NAD/NADH to happen where it has easy access to this electron transport machinery and the ability to regenerate the NAD necessary to keep these processes going.

But of course, the very first process we talked about happens in the cytosol, and that's glycolysis. And we discussed how you can bring pyruvate into the mitochondria through this mitochondrial pyruvate carrier. Then it has access to the pyruvate dehydrogenase reaction, which, of course, generates acetyl-CoA and NADH in the right place. However, if you go way back to when we discuss fermentation, remember, the reason we had to send pyruvate to fermentation was to regenerate that NAD in the cytosol that's necessary to keep glycolysis going.

Now, of course, if we're going to fully oxidize pyruvate carbon in the mitochondria, we still have to solve this electron balance problem for glycolysis. And as we discussed, we can solve that by giving those electrons to oxygen. But now you see that there's this additional complication-- is that these electrons in the form of NADH need to get inside the mitochondrial matrix or get to the electron transport chain as a way to use oxygen to ultimately dispose of those electrons. And so, effectively, that NADH needs to be gotten to complex I, which would be the matrix in the way we've drawn it here.

Now, really, the way that this happens is not direct. So you don't transport NADH from the cytosol into the mitochondria. And that's partially because one of the benefits, remember, of having compartmentalized metabolism is your ability to have different conditions; different ATP/ADP ratios, NAD/NADH ratios in different compartments because that can favor different chemistry.

And so the way that you get electrons into the matrix or get electrons to the electron transport chains doesn't occur directly. You do not transport NAD or NADH directly. Instead, you use so-called shuttles, which are analogous to some of the other shuttles we've talked about, in order to get materials across membranes. In this case, this is a way to get electrons generated from oxidation reactions in the cytosol-- production of NADH in the cytosol, things like glycolysis-- to the electron transport chain so that they can ultimately be transferred to oxygen.

And so these shuttles, or redox shuttles, actually come in several different ways that cells can deal with cytosolic electrons. And so these shuttles also, in essence, help cells also establish these different conditions, these different NAD/NADH ratios in different compartments. And so you can imagine that the shuttles themselves could be regulated as a way to do this.

Now, there's two major redox shuttles that allow you to move electrons from the cytosol into the mitochondria, and we'll discuss those two here next. These are the two that probably are being looked for if you get this question on an MCAT exam or something like that. They're the ones that are described in the text as the way to get electrons to the electron transport chain.

But however, as we go through them, you will see that there's a couple different ways one can do this. They basically involve oxidation and reduction of a pair of molecules on either side of the membrane. And there's lots of ways you can do that in addition to these other shuttles. It'll become more clear what I mean by this, but I guess I just want you to know that these are not the only ways one could shuttle electrons into the mitochondria. These are just the major ways that have been discussed and are usually discussed in textbooks.

So the first shuttle system is something called the glycerol phosphate shuttle. And so the glycerol phosphate shuttle involves a reaction that we've already discussed. So here's our old friend, dihyroxyacetone phosphate from glycolysis. Remember when we talked about the generation of and metabolism of glycerol, in order to make triacylglycerides, phospholipids-- remember we described that you can take dihyroxyacetone phosphate.

If you use NADH to reduce that ketone to the alcohol, that generates this molecule, glycerol phosphate. And of course, because you're reducing the ketone to the alcohol, something else has to be oxidized. You reoxidized NADH back to NAD+, effectively disposing of those electrons from, say, glycolysis that generated NADH to regenerate NAD+.

Now, rather than use this glycerol phosphate and lipid synthesis, turns out there's a complex that can directly transfer those electrons to FAD to generate FADH2. And this complex sits within the inner mitochondrial membrane, facing the cytosolic side of the membrane. Here's the inner mitochondrial membrane. This would be the matrix.

And so transferring those electrons to FAD to reduce to FADH2 reoxidizes the glycerol phosphate back to dihydroxyacetone phosphate. And of course, in this membrane, you can, in this complex, transfer those electrons by oxidizing the FAD and sending them to the coenzyme Q pool. That can take the ubiquinone, makes ubinquinol. Those electrons can go to complex III. That can go to cytochrome C. They can go to complex IV, ultimately ending up reducing oxygen to water using oxygen as a final electron acceptor.

Again, this is effectively another alternative to the electron transport chain. And so I show that here on the slide. And so we've discussed-- the complex II-- this is the FAD/FADH2 containing enzyme is complex II.

Succinate dehydrogenase sits in the inner mitochondrial membrane, converts succinate to fumarate. Those electrons end up on up FADH2, can get transferred to the Q pool, reoxidizing FAD to complex III to cytochrome C to complex IV to oxygen to water. We already discussed acyl-CoA dehydrogenase-- same thing. FADH2 containing reaction in there is also in the membrane-- ultimately allows another version electron transport chain. And here's yet a third one involving an FDA using enzyme.

The difference is is that these have the oxidation reduction reaction occurring on the matrix side-- succinate to fumarate on the matrix side-- the introduction of a double bond for fatty acid oxidation and the matrix side. This one here instead faces the cytosolic side. So glycerol 3 phosphate can be reoxidized to dihydroxyacetone phosphate-- looks like I have those arrows drawn backwards, sorry-- ultimately sending electrons into this glycerol phosphate shuttle, reducing FADH2, which can be reoxidized by giving those electrons to the Q pool to complex III, to cytochrome C, to complex IV, ultimately with water serving as the final electron acceptor, as I've drawn here.

Now, if you notice, if we use this system or any of these systems, these alternative FAD/FADH2 using electron transport chains, you don't use complex I. Remember, complex I will pump a proton, complex II will not. And that means that if you use any of these FADH2 using alternative electron transport chains, you pump fewer protons into the cytosol.

And so if you donate the electrons to complex I from the matrix, you pump a proton as you go through complex I to the Q pool if you go through the glycerol phosphate shuttle, you avoid complex I, because you give those electrons directly to the glycerol phosphate shuttle. And FAD/FADH2 enzyme-- those end up in the Q pool. Less protons are pumped.

And this is why sometimes in textbooks, you'll see that you get less ATP per NADH produced in the cytosol than you get for NADH produced in the mitochondria. And this is one of the ways that you end up with these accounting yields for glucose oxidation-- something we'll talk about in a minute. But the deeper reason for this, which I want you to appreciate, is because FADH2 has a higher standard reduction potential.

So the standard reduction potential of FAD/FADH2 is greater than it is for NAD/NADH. In essence, that means that there's less change in standard reduction potential for transferring FADH2 electrons to oxygen as there is for transferring electrons to oxygen. And so less energy released, less protons pumped, and so that's why you get less ATP, if you will, produced going through the glycerol phosphate shuttle. All right.

Next, I want to discuss another alternative shuttle to get electrons into the mitochondria. And this involves much more what I was alluding to earlier. And that's oxidation and reduction of a pair of metabolites that are transported across the membrane on either side of the membrane, effectively moving electrons from one compartment in the cell to another. Now, to understand how this shuttle works, I have to-- because it's a little more complicated, I have to first describe that there's a relationship between amino acids and alpha-keto acids.

And so, what do I mean by that? Well, here's a alpha-keto acid that we've talked about a lot. So this is oxaloacetate. Remember, it's an alpha keto-acid because this ketone is alpha to this carboxylic acid-- so an alpha-keto acid. Turns out that alpha-keto acids are related to amino acids in the following way. And so if I take that alpha-keto group and instead make it an amino group, this here is the amino acid aspartame.

Now, we'll discuss later in the course the chemistry that allows you to interconvert this alpha-keto group with this amino group. But that's effectively where your amino acids come from-- how your amino acids are related to other aspects of carbon metabolism-- Alpha-keto acids that are generated in metabolism. And so there's one example-- oxaloacetate and aspartate, an alpha-keto acid, an amino acid. Here's another one.

So this is alpha-ketoglutarate-- an alpha-keto acid. And if we change that alpha group to an amino group, now we get the amino acid glutamate. All right. To remind you, the three letter abbreviation for aspartate is a-s-p. The one letter is D. The three letter abbreviation for glutamate is g-l-u. And the one letter abbreviation is E. And I just remind you of that in case I use those abbreviations.

But effectively, you can note that if you couple these swaps to each other-- that is, if I take oxaloacetate and turn it into aspartate while at the same time taking glutamate and turning it into alpha-ketoglutarate, you can see that that is a reaction where all atoms are conserved. That is, if I turn this amino acid into this alpha-keto acid while at the same time turning that alpha-keto acid into this amino acid, I have not gained or lost any atoms or molecules in the process. And we will see later in the course that these types of interconversions is exactly how you run these reactions to do these interconversions between alpha-keto acids and amino acids.

OK. Why am I telling you this now? Because it turns out this is central to understand the other major shuttle, which is referred to as the malate-aspartate shuttle as another way to get electrons and NADH from the cytosol into the mitochondria. All right. So how does this happen?

So here if you have-- here's oxaloacetate. This is occurring up here in the cytosol. OK. And if we take oxaloacetate in the cytosol and we utilize the malate dehydrogenase reaction that we heard about from the TCA cycle-- so basically this is the reverse of the malate dehydrogenase reaction we discussed in the TCA cycle. We're going to reduce this ketone to the alcohol.

NADH is reoxidized to NAD+. That will generate malate. And now we've regenerated NAD+ in the cytosol by putting those electrons onto oxaloacetate to make malate. All right.

Now, we can take that malate, send it across the mitochondrial membranes via transporter to generate, on the matrix side of the membrane, malate, whereas from the TCA cycle, we also have malate dehydrogenase that can now reoxidize that carbon from the alcohol to the ketone and regenerate oxaloacetate effectively by carrying out the same reaction through a redox pair on two sides of the membrane. Effectively what I've done is I've moved the electrons from NADH in the cytosol to now being NADH in the mitochondrial matrix, where this can now donate the electrons to complex I, to the Q pool, to complex III, to cytochrome C, to complex IV, and ultimately to oxygen, just like it would for the TCA cycle. All right.

Now, the simplest thing, of course, would just be to send the oxaloacetate back out to the cytosol. But that's not what happens. Instead, what happens is you turn that oxaloacetate into aspartate. This is an alpha-keto acid. Turn it into the amino acid. If you do that, you basically at the same time turn glutamate into alpha-ketoglutarate all right.

Turns out it's that alpha-ketoglutarate that is exchanged when you bring malate into the cell. So malate is exchanged for alpha-ketoglutarate. And at the same time, you exchange the aspartate for a glutamate.

All right. So now you can take that aspartame, turn it back into oxaloacetate. To do so you need to balance this. So you return your alpha-ketoglutarate back into glutamate. And effectively, this is the malate aspartate shuttle. OK. So electrons from oxaloacetate to malate, and then malate back to oxaloacetate moves the electrons on NADH from the cytosol into the mitochondria. To get that malate across the membrane, you exchange it for alpha-ketoglutarate and to maintain carbon balance across the-- and nitrogen balance-- across the whole thing.

At the same time, you also exchange an aspartate for a glutamate, which allows you to basically interconvert the oxaloacetate and the aspartate on either side of the membrane via interconverting glutamate and alpha-ketoglutarate. So somewhat confusing systems, but recognize that the net effect is moving NADH from one side of the membrane to the other. And that occurs because you're interconverting to redox pairs in opposite directions-- in this case oxaloacetate and malate-- on either side of the membrane.

And that's why what I alluded to earlier, you can imagine that you could easily come up with other shuttle systems where you have a redox reaction on one side of the membrane and the reverse of that redox reaction on the other side of the membrane. And as long as you can maintain carbon balance, the net effect is moving electrons across the membranes from one compartment to another. And that is a way to allow you to generate NADH that's now in the matrix, which has access to complex I of the electron transport chain as we discussed now several time.

All right. Now we're ready to go through and discuss the accounting that you see-- the accounting that comes up all the time that is in textbooks and perhaps you memorized from previous classes about how the ATP yield works for different pathways. So hopefully it's clear to you by now that these numbers that you get are estimates. And that's because there is no direct relationship between NADH or FADH to NATP. These interconversions occur via oxidative phosphorylation, these electron transport chains, delta psi/delta pH.

And so all of these estimates take into account various assumptions about how efficient the electron transport chain is, oxidative phosphorylation works, bounded, of course, by the change in standard reduction potential, what's the free energy released from that? What's the free energy to synthesize ADP to ATP. Of course, those require assumptions. And it's, in the end, why if you read different textbooks and different sources you will find numbers that are actually a range. There is no fixed number for what the equivalent is of an ATP for an NADH. And that's because it really depends on several variables.

So let's go through what those variables are, because if we can understand that, you really understand how this system works. So the first variable-- at least if we're talking about something like glucose, where we generate NADH in the cytosol, we have to consider the redox shuttles. And hopefully you appreciate now that you have this need to regenerate NAD in the cytosol to keep glycolysis going. You got to get those electrons somehow to the electron transport chain.

And I just described for you two different ways you can do that. Now, if I use the glycerol phosphate shuttle, I'm skipping complex I altogether. And so that's not pumping any protons.

If I'm using the malate aspartate shuttle, well, I have all the energetics of doing this gymnastics. But in the end, now I can use complex I and pump protons. And so the yield of ATP, if you will-- how many protons I can pump to generate delta psi/delta pH will be different if I use the glycerol phosphate shuttle or a shuttle like the malate aspartate shuttle.

The second variable in consideration is, what is the efficiency of proton pumping by the various electron transport chain ETC complexes? That's not so straightforward and, of course, is going to depend on a couple of things. Remember, we spent a long time talking about ATP/ADP, and how it's the ratio of ATP/ADP that was really the energy there, because, remember, thermodynamics is dependent on that log of the reactants over the product's term in the delta G equation.

Well, that's true also for the NAD/NADH ratio. And it's also true for how high the membrane potential is. And so how efficient this pumping is going to be is going to be depending on what is the delta psi to begin with, and what is the NAD/NADH ratio or the FAD/FADH2 ratio, because all of those things will affect how much free energy there is to be able to move protons from one side of the membrane to the other.

Once I get those protons across the membrane, then there's the question of how-- and for lack of a better word, I'm going to say tight the membrane is to proton leak. That is, you can imagine that to make ATP, I have to send those protons back through the ATP synthase, complex V, F1F0-ATPase, whatever you want to call it. And if some of those protons just leak back across the membrane, well, that's how we generate heat.

And so how tight that membrane is, that is how coupled delta psi is, delta pH is to ATP synthesis is another variable. And of course, the last one is, is the efficiency of H+ turning the F0F1-ATPase, which is the same considerations that we already talked about for number two. That is, the higher delta psi/delta pH, the more free energy that's stored there and the higher or lower the ATP/ADP ratio is, the easier or harder it is to synthesize ADP.

And so basically, these different things, two through four, in general takes into account a term called coupled respiration. By coupled respiration is really, how tightly is oxidation-- moving electrons on the electron transport chain to oxygen-- to phosphorylation-- synthesizing ATP from ADP? And so, how tight or how well-coupled is respiration to phosphorylation really is a variable that is going to very much depend on conditions.

And so ultimately, to give numbers for what is the ATP produced from various oxidation processes, one has to make assumptions about all of these things, how coupled respiration is and which redox shuttles are used? If it's something like glycolysis, it involves the cytosol. And this is why these numbers are made up. And it makes no sense in my view to memorize these numbers.

But I think it is important to understand where those numbers come from. Because if you understand that, that means you understand all the assumptions and why the numbers are variable, you really understand the bioenergetics of what's going on in metabolism. All right.

So now let's go through one of these calculations just to fully illustrate what I'm talking about. So let's go through the calculation for glycolysis. So if I run glycolysis and turn glucose into two pyruvate. So what do we get from that? Of course, we get 2 ATPs produced and we get two NADHs produced in the cytosol.

OK. So this is the cytosol. This is the mitochondria. All right?

We're going to completely oxidize that pyruvate. We send that pyruvate into the mitochondria. We run the PDH reaction. That gives us two acetyl-CoA, which is a yield of two more NADHs. All right?

If we now take those acetyl-CoAs and we burn them completely, two cycles around the TCA cycle. OK, now we get two more FADH2s and six NADHs-- one FADH2, three NADHs from each turn, as well as a GDP. And so that's a total of two FADH2, six NADHs, and two GTPs. And so now if I come together, what is our yield?

Well, from the mitochondria I've generated a total of two FADH2s and six, seven, eight NADHs. And then up here, there's an additional two NADHs that are made in the cytosol, and so have to access the electron transport chain via a shuttle. A typical number that's given is you get 1.58 ATP for something that has to access via shuttle, or on FADH2, that would make sense, because FADH2 is really the same as a glycerol phosphate shuttle.

And so if I do that, that would be three ATP from the two glycolysis NADHs and three ATP from the two TCA cycle FADH2s, because the NADHs produced in the matrix can generate POM protons via complex I. Oftentimes, that's said to be, oh, that's about 2 and 1/2 ATP. So 8 times 2.5 is 20 ATP. And if we had all these numbers together, 20 plus 3 plus 3 plus 2 plus 2, that is a total of 30 ATP per glucose that's completely oxidized to CO2. OK?

So 26 ATP over here plus another 2 from GTP plus another 2 from glycolysis is a total of 20 ATP per glucose. That is a number. It made certain assumptions. Obviously, if I assume that this is not the glycerol phosphate shuttle but instead the malate aspartate shuttle, maybe I bump this number up by multiplying that by 2.5. Maybe I use different conversion factors. And if you read in textbooks, you get numbers that range from 30 to 36. I think the highest I've ever seen is 38 ATP per glucose.

And the fact that you actually see this range really reflects the different authors of textbooks, or whoever writes these things, makes different assumptions about all of these things over here to understand how to make these conversions. But the important thing is realizing that these are not real numbers. All right.

Let's quickly go through and just do the similar exercise one last time just to come back to. I'm not going to go through as much detail. But we talked about that 6:0 fatty acid that we did accounting for earlier. If you look back in your notes, what we wrote down is that from completely oxidizing that fully saturated six carbon fatty acid, we got one ATP, 11 and ADHDs, and five FADH2s. Remember, all these are being generated in the-- all these NADHs and FADH2s are already in the mitochondria. And so if we just use the same conversion factors that we used for over there in our calculation for glycolysis, what we get is 27.5 ATP for the NADHs and 7.5 ATP for the FADH2s, which is a total of 35 ATP for-- 35 plus the one, so a total of 36 ATP from complete oxidation of our six carbon fatty acid, which is certainly greater than the 30 ATP we got from complete oxidation of glucose.

That makes sense. Fatty acids are more reduced than glucose-- more calories, should be able to get more from it. And this calculation illustrates that we get that.

The typical calculation in textbook usually describes complete oxidation of a 16:0-- a fully saturated 16 carbon fatty acid. That's palmitate. That's one of the most abundant fatty acids in the cell. This, if you completely oxidize it, the number and a lot of textbooks is 106 ATP per palmitate completely oxidized to CO2.

If you're so inclined, you can go through and do the calculations yourself and see if you agree with that. But the key point of all of this accounting is that none of this are real numbers. And I hope you now better appreciate what energy really means-- that is, how oxidation of carbon by being favorable can be coupled either to direct charging of an ATP/ADT ratio as occurs in the GAPDH step of glycolysis, as well as the succinic thiokinase step of the TCA cycle. But most energy transduction involves this charging up NAD/NADH ratios, or reactions that are coupled to FAD/FADH2 via these membrane complexes that ultimately lead to this favorable electron transport from lower to higher standard reduction potentials.

That favorable electron transport can be used to create a battery, a delta psi/delta pH, which can then be coupled to do other work, including synthesizing ATP at the high ATP/ADP ratio that can then be used by other reactions in the cell that would otherwise be unfavorable and allow cells to basically extract energy from its environment in order to fuel the processes that are unfavorable and are necessary to sustain life. All right. Great.

So thus far, we have focused almost entirely on how you oxidized carbon as a way to release energy. And that's great. But of course, that reduced carbon has to come from somewhere to begin with. And every one of us learned in grade school that the energy for all life ultimately comes from the sun. And that's, of course, photosynthesis.

And now I want to turn to beginning to discuss photosynthesis and how it works. In other words, how does life harvest solar energy in a usable form that ultimately involves using atmospheric CO_2, which gets reduced to generate reduced carbon that, of course, originally occurred because plants-- or, any photosynthetic organism has the same issues as us. That is, to fight thermodynamics, maintain, and survive. It constantly has to be charging an ATP/ADP ratio in order to fuel the otherwise unfavorable processes that are required to maintain order in the cell.

This has to occur during the day when the sun is out and there's light energy photosynthesis. But also has to occur at night, or at times when there is no sun. And so thus, plants basically stored energy as reduced carbon so that they had something to eat at night-- that is, to keep their ATP/ADP ratios high-- at night so they can do all these catabolic processes at night.

Now, fortunately for us, the fact that photosynthetic organisms stored all this reduced carbon allowed other life, like us, to evolve that is entirely dependent on photosynthetic organisms either directly or indirectly for food. And so photosynthesis also is the process that ultimately oxygenated our atmosphere. Aerobic life, also something that was necessary for us to survive because oxygen is such a key electron acceptor, really key to our energetics-- well, the fact that photosynthetic organisms put oxygen in our atmosphere also was really essential for aerobic life, us, to ultimately evolve.

And so photosynthesis is often, in biochemistry courses, almost a sidelight and forgotten process. But of course, it is really, really central to how life works, because no life could exist without photosynthesis and the conditions that allowed aerobic-- most multicellular life that we know of that's not photosynthetic, including ourselves, could not have evolved without photosynthesis oxygenating the atmosphere and generating all this reduced carbon. That, really, is where we get our energy from as food.

Now, you also learned in grade school, when you learned that life depends on energy of the sun, that the process that life uses is this mysterious photosynthesis. Well, as we go through this over the rest of today and in the next lecture, I want you to appreciate what photosynthesis is. And that is how life really takes solar energy and harvests that energy in a biologically useful form, bearing in mind all of the concepts that we've already talked about, because that will ultimately help you understand how that energy from the sun is used to make all this reduced carbon, this carbohydrate and fat that is energy that we can use for later, and also, of course, allows how photosynthetic organisms can use light from the sun to just power things directly from that solar battery, and ultimately, of course, you should appreciate that these things all came first.

Photosynthesis obviously existed first. And so most of what we already talked about-- well, we'll draw parallels when we talk about photosynthesis to the stuff we already know. Of course, photosynthesis came first. And the stuff you've already learned about evolved from photosynthesis.

All right. Now, like all pathways, photosynthesis also has to follow the same rules and laws of thermodynamics that were true for all pathways. So plants and animals have to follow the same rules.

And so what is photosynthesis? Well, it's ultimately CO2 in the atmosphere that gets reduced to carbohydrate. That's classically photosynthesis. But, of course, plants also make fatty acids. OK?

This is carbon reduction. Remember, carbon oxidation generally is favorable. Carbon reduction is not. Delta G naught prime for this is greater than 0. This direction is favored.

That direction is not, which means you need energy input to reduce that CO2 to carbohydrate or fatty acid. And of course, it's the sun's energy, the solar energy, that ultimately must be done to make this favorable. Now we're reducing carbon. That means we need to add electrons.

Those electrons have to come from somewhere. And so we're reducing the carbon. That means something else has to be oxidized. Just like when we oxidized carbon, those electrons had to go somewhere, something else had to be reduced. That was pyruvate to lactate and fermentation, or oxygen to water via all of the oxidative phosphorylation things we just talked about.

And so in this case, those electrons have to come from somewhere. And those electrons ultimately, for photosynthesis-- this reduction, the electrons come from oxidation, ultimately, of water, which will generate oxygen. And this is effectively then the reverse of what you learned about for the mitochondrial electron transport.

Why this? Well, water is very abundant, has been for all of life on Earth. Water is-- obviously, life depends on water. And so you can oxidize the water to generate oxygen. This will obviously generate electrons that can be used to reduce carbon.

And it's effectively the photosynthetic oxidation of water to make oxygen that was the source of oxygen in the atmosphere that, really, today makes aerobic life possible. Now, of course, the fact that oxygen is a good electron acceptor-- we've been talking about this forever. This means that, of course, you might guess that delta G naught prime for this process is not going to be favorable. To put it another way, oxygen is a great electron acceptor and water is a poor electron donor.

And so, really, the way photosynthesis-- the magic of photosynthesis, if you will, is making water a good electron donor, because, ultimately, that is what is going to make this whole thing work energetically. And so you probably could guess that if these processes are really about these electron transfers, we need electron carriers. This is true for photosynthesis, just as it was true for oxidative phosphorylation.

But remember, we'll describe a lot of the same electron carriers that are used. But as we described them, remember that the photosynthesis came first. And so the other processes, like OXPHOS, that use these electron carriers, use them because they were first used in photosynthesis. And then they were repurposed for oxidative phosphorylation.

Now, to discuss this, I want to first start by introducing an electron carrier that's important for this that's really a variation on NADH. And it's an electron carrier that's found in-- excuse me-- all plants, all animals, all life, and is very important in anabolic pathways like photosynthesis. Remember that anabolism, building stuff, has to be distinct from catabolism, breaking stuff down, for a number of reasons.

We saw this in glycolysis and gluconeogenesis, glycogen synthesis, glycogen breakdown. Remember, delta G naught prime can only favor one direction. And so in the direction that's favorable, it works. In the other direction to actually make it happen, we have to make delta G less than zero. That has to happen by energy addition. And so now we had to have these separate pathways.

You also need separate pathways because that allows separate regulation, avoids futile cycle, and enables cells to match needs with processes. And as we already discussed, one way to keep these anabolic and catabolic processes distinct is by building separate compartments, right? It's useful to have separate compartments, different conditions in different compartments that can favor different things happening.

However, not all cells have separate compartments. Prokaryotes don't. And you might think of situations where you clearly want to have two different types of reactions happening in the same compartment. And so what if you want to both do oxidation and reduction in different reactions in the same compartment?

Well, clearly you need some way that will favor some reactions being oxidation and other reactions being reductions. And if you simply rely on NADH/NAD+ as your oxidant or your reductant, this now becomes a problem. All right? So a solution is, well, let's make a distinct electron carrier for the oxidation reactions in the cells. So that's what this is.

Turns out, you keep your NAD/NADH to largely favor the oxidation reactions you want to do. And let's create a different currency for reduction. And this currency ends up being a different molecule, NADPH/NADP+. And you use this as a cofactor pair for reduction.

Well, what is NADPH? Well, it's effectively the same thing as NADH. But now you mark it as a separate pool with a two prime phosphate on the ADP portion of the molecule. Let me draw it out for you.

OK. So this here is NA-- so if I took this phosphate off, this would be NADH, put a two prime phosphate on, now I mark this as a separate molecule pool, NADPH. A is an adenine. Remember, it's a nicotinamide adenine dinucleoside. OK?

So here's a adenine, phosphate, phosphate, other nucleoside with the nicotinamide group. This here is in the reduced form. Seen this now a million times, but we can oxidize it as possible. That generates this two electron hydride ion. So remove two electrons and now this nicotinamide form is here in the NADP+ or oxidized form of the molecule.

And so effectively by marking this as a separate pool with that two prime phosphate, you can have a different NADH/NAD+ ratio in cells. Then you have NADH/NADP+ plus ratio and cells. Use one co-factor fair pair to favor oxidation and the other co-factor pair to favor reduction. And so I want to give a quick aside on NADPH because it's really also NADPH that can be very important in keeping the cytosol of cells reduced.

So what do I mean by that? Well, you remember you learned from Professor Yaffe. We talked about proteins that you can have these inside the side cytosol. You'd have your cystine residues on proteins be in this reduced form. And outside the cell, you'd form these disulfide bonds, which is basically oxidized cystine.

And so in the cytosol, you want your cystine residues reduced. And outside of the cell, you end up with these disulfide bonds on proteins. Why does cystine tend to get oxidized outside the cell? Well, there's lots of oxygen in our atmosphere. Oxygen's a good electron acceptor.

It's very good at oxidation. In fact, that's what gives it this property that makes oxidative phosphorylation work. And so it turns out that these cystines, these sulfhydryl groups, like to give their electrons to oxygen. And that leads to these more oxidized things outside the cell.

In fact, oxygen is very good at accepting electrons from lots of donors. So if you have iron 2+ sitting around outside the cell, oxygen is very good at oxidizing that iron 2+ to iron 3+. That's rust. And this process, in the end, ends up transferring electrons to oxygen and can generate lots of oxygen species, including oxygen radicals.

And so you can get things like this O2- superoxide, ultimately hydrogen peroxide, also comes from oxygen picking up electrons. And as you know, very reactive molecules like this can damage membranes, damage proteins, kill cells. What do we use hydrogen peroxide for in daily life? Use it to clean wounds, kills a bunch of bacteria.

And ultimately, production of these so-called ROS, or reactive oxygen species, in excess can be very bad for biological systems. And this is one of the arguments for why antioxidants are good for us. They protect us and ourselves from these reactive oxygen species.

OK. Now, cells, of course, have endogenous ways to deal with this, to keep cystines in our cytosol reduced, to keep our cytosol in this reducing environment, prevent this ROS damage. And really, if you're going to have things to deal with these reactive molecules, what do you want? Well, you want something that's going to buffer the damage that these things can cause.

That is, if these things are going to react with lipids and proteins and cause damage and you want to keep the cytosol reduced, you really want to have a series of molecules that will actually, instead, undergo this process before it can damage critical structures in the cell. And a key system cells use to do this is something called glutathione. So what is glutathione?

Well, glutathione is a small molecule tripeptide. All right? And it's among the most abundant small molecules found in cells. And effectively, what glutathione does is it serves this purpose of reacting with nasty stuff before it can damage other biomolecules and hurt the cell.

And so by tripeptide, what it is is it's a tripeptide of glutamate with a gamma peptide bond to glycine and then cystine-- so glutamate glycine cystine tripeptide that looks like this.

OK. So this here is the amino acid glutamate. Here's the alpha carbon with the carboxylic acid and amine groups. So you would typically think of the peptide bond of glutamate was in a chain as being from this nitrogen to the next amino acid and that carbon to the next amino acid.

But in this case, it uses the nitrogen on the side chain, the so-called gamma linkage here, to form a peptide bond with-- oops, sorry. This would be glutamic acid. It uses this gamma carboxylic acid on the side chain of glutamate to form a peptide bond with glycine.

OK. So this is glutamate. This is glycine. And then this has a peptide bond to cystine, which contributes that sulfhydryl group that can either exist in this reduced form. Or, if there's two of them together, you can go into this oxidized form.

And so, often, this is drawn as glutathione GSH in the reduced form. And if you have two glutathione molecules that then basically form a disulfide bond to give you this GSSG oxidized form of the molecule. And NADPH plays a key role in the cell, because that's an oxidation reduction reaction in keeping glutathione in the reduced form.

So if you have two GSH molecules that can cycle to a oxidized GSSG-- so this is reduced, this is oxidized. On the other side you can cycle those electrons. If something gets oxidized, something else has to be reduced. So you can have NADP+ and NADPH. This is reduced. This is oxidized.

And so by maintaining a high ratio of NADH to NADP+, you can keep a high ratio of reduced to oxidize glutathione in the cell and keep the cell in a protected reducing environment where you have these glutathione molecules to react with other things before it causes damage to the cell. All right. A little bit of a diversion, but a very important system that cells use related to oxidation reduction and NADPH, and needed a place to talk about it somewhere in the course.

All right. Now back to photosynthesis. Now, for photosynthesis, this reduced NADPH molecule's of course useful, because it can serve as a source of electrons to donate carbons to reduce CO2. So if you're going to reduce CO2 to make carbohydrate or fat, you need this source of electrons. Those can come from NADPH, which can be oxidized to NADP+. And so perhaps at this point, you've already guessed or maybe already know that photosynthesis uses the favorable electron transfer from water to generate NADPH.

And to do that, you use the energy of the sun. Now, of course, if you're going to do a favorable electron transfer, that can be coupled, just like we talked about for oxidative phosphorylation, to make delta psi/delta pH. And if we can use that favorable electron transfer to make delta psi/delta pH, we can also use it to do other work, including making ATP exactly as we described for oxidative phosphorylation. And at the highest level, this is exactly how photosynthesis traps solar energy and uses that energy to both remake reduced carbon and generates a battery for the photosynthetic organism that can be used to make ATP or do other work, provided that sun is available.

And so classically, photosynthesis can really be separated into, I guess, two distinct processes. All right? So you have this process of taking solar energy, using that solar energy to charge up a battery, delta psi/delta pH, which of course can be used to generate ATP or do other work, and at the same time gives you an electron donor, NADPH, that is useful to generate reduced carbon.

Once you generate, high ATP/ADP ratio, high NADPH/NADP ratio. Now we can use that to be couple to do something else unfavorable. And that would be something like reduced CO2 to generate a carbohydrate.

And typically, when we talk about photosynthesis, the way it's often described is how you reduced CO2 to carbohydrate. And we'll do that too. But of course, we'll then see once you have carbohydrates, obviously, it's just further reduction of carbon to make fatty acids and lipids.

We'll discuss later how we can use those to make amino acids, which would allow you to make proteins. And, of course, we can also store those carbohydrates as disaccharides or polysaccharides, storage sugars, which is what plants do. They make starch, they make sucrose, et cetera, as a way to store that reduce carbon so that they can have a source of energy, do the oxidation reactions, to deal with their energetic needs when the sun is not shining. All right.

So let's break down these two processes. And so to just be very explicit, the first part is basically trapping energy to make a battery. All right?

And so this means that we need to generate delta psi/delta pH via favorable electron transport, exactly what we describe for OXPHOS. But, of course, photosynthesis was first. This would then allow us to use ATP to fill our energy needs. And we get NADPH if we set it up in the right way, which is important for carbon reduction.

Then we need to use NADPH and ATP via pathways to describe how we can use that to reduce CO2, and ultimately produce glucose, which allows us to store energy for later. All right. So that is, at a very high level, what photosynthesis is. And, of course, this occurs in plants. All right?

But perhaps more importantly, it also occurs in algae and other unicellular eukaryotes as well as in prokaryotes-- photosynthetic prokaryotes, bacteria. And, of course, photosynthetic bacteria, unicellular eukaryotes, algae-- these really are the heroes of life. These are the things that life started from, and generated the oxygenated atmosphere, and ultimately led to the evolution of higher plants, but also enabled the formation of animal life, and really anything that lives off of eating other organisms.

Just a few facts about photosynthesis because it's really quite an amazing process-- and so about 10 to the 17th kilojoules, at least by some estimate, of energy is harvested on our planet from photosynthesis every year. Just to give you a perspective of what that number means, that is 10 times the worldwide energy use by all people on Earth.

So if you add up all the energy that we as humans use in the world, it is tenfold less than the amount of energy that is harvested by photosynthesis, by photosynthetic organisms around the world. Photosynthetic organisms, when they do this, consume CO2. All right? So that is removing CO2, the greenhouse gas from our atmosphere.

And this occurs on a massive scale. So this is better than any man-made engineering solution that we have come up with by far. And I think this really illustrates the awesome power of biology to solve some of the biggest problems that face our society today. So world energy problems-- photosynthesis harnesses a ton of energy. Global warming, greenhouse gases-- photosynthesis, better than almost anything at dealing with these CO2 that is produced.

However, this also illustrates what some of the problems are and what some of the issues are with fossil fuels, because what are fossil fuels. Well, they're effectively reduced carbon that is trapped in the ground somewhere, that is effectively old photosynthesis. So these are organisms in many, many, many, many years ago that did photosynthesis, made reduced carbon that was effectively trapped in the ground. And so the fact that photosynthesis transducers 10 to 17th kilojoules of energy per year and we're using 1/10 of that amount of energy, if that's all coming from fossil fuels, that means we're using 1/10 of a year's worth of photosynthesis-- and, of course, a year is not entirely stored in the ground-- per year.

So that's a lot of energy. And it really comes down to why people say fossil fuels can't last forever, and also why the net release of these things can change our atmosphere, because, of course, once upon a time, there was much more CO2 in the atmosphere. And photosynthesis, over millennia, trapping these things as fossil fuels really has lowered CO2 in our atmosphere to the levels that they exist today, and how, basically, burning fossil fuels on the scale that we are is able to change our atmosphere. So you can view this both as glass half full and glass half empty.

It really says understanding photosynthesis could be a key to solving some of these problems. But it also points out why some of these things might be problems to begin with. All right. Now we want to discuss first how photosynthesis traps energy.

And you'll see that it is similar to oxidative phosphorylation in many, many ways. So it's going to occur at a membrane. All right? It's going to involve a series of protein complexes where we're going to favor, we're going to couple favorable electron transport along a electron transport chain to favor proton pumping. That is going to generate a membrane potential delta psi/delta pH.

In order to do that, of course, we need favorable electron transport from something that is lower standard reduction potential to something that is higher standard reduction potential. That is what is going to make it favorable, allow proton pumping. That, when it generates delta psi/delta pH, we can now harness that as a way to synthesize ATP or do other work, as we've now talked about a lot. Looks a lot like oxidative phosphorylation and it makes sense. Oxidative phosphorylation came from photosynthesis.

However, there are some really big differences between the two. And that is in photosynthesis, as I've already alluded to, the electron donor is going to be water, which will generate oxygen. And the electron acceptor is NADP+, which will generate NADPH. And you might now be asking, well, how on Earth can this be possible? Because, remember, the standard reduction potential of NADH is less than the standard reduction potential of our oxygen water pair.

And it's really this that makes the change in standard reduction potential positive, which means that delta G is going to be negative, which is why electron transport and oxidative phosphorylation is favorable and can be coupled to make delta psi/delta pH. Now, adding a phosphate to NAD/NADH does not change its standard reduction potential in a way that is at all meaningful to impact what goes on. And so this is really where energy input from the sun becomes critical. And that is-- it is this light energy from a photon that in the end makes water a good electron donor, such that all of these other things are satisfied that electron transport is favorable going from lower to higher standard reduction potential with NADP+ ultimately being the final electron acceptor, such that the change in standard production potential is positive. And that really is the magic of photosynthesis.

And, of course, once you do that, now you have delta psi/delta pH. You can use that to make ATP, do other work for the photosynthetic organism. And you also get NADPH, which is an electron donor for carbon reduction. And the net result is that you get this familiar formula that you learned in grade school for photosynthesis-- CO_2 plus water with light goes to oxygen plus carbohydrate, which is, of course, the exact opposite of combustion.

We talked a lot about how burning wood combustion is the opposite reaction that's favorable-- lots of energy released. And so the energy input here is obviously the opposite of that to do this opposite of combustion and stores lots of energy. All right, so again, I want to say why use water as an electron donor? Well, because it's abundant. There's nothing particularly special about water other than its abundance, just like we talked about in oxygen is not really being special other than that it's a good electron acceptor and abundant.

You could obviously build systems that do something other than oxygen and water. And so there are extremophiles out there that instead do hydrogen sulfide to make reduced sulfur as another-- sorry, oxidize sulfur as a way of doing photosynthesis. Exact same concepts would apply is what we're going to talk about here. All right.

Now, the parts of photosynthesis, as you might guess, are spatially distinct. That is, we're going to generate delta psi/delta pH and NADPH. Those are basically this electron transport chain reactions, charge the battery, et cetera. And then imagine if we're going to fix CO2 as carbohydrate. Well, effectively that's more like what we talked about with gluconeogenesis.

That's the chemical reactions that we just have to build a pathway for, like gluconeogenesis. Now these two things are often referred to as the light and dark reactions of photosynthesis. And that's because the first set of reactions generating the membrane potential and making NADPH requires solar energy inputs or requires light from the sun. This fixing of CO2, which will just use electrons from NADPH to reduce CO2, can occur-- of course, it can occur in the light. But it doesn't need light. So it can occur in the light or the dark, hence called the dark reactions.

And so these light reactions can really be broken up into water plus NADP+ goes to NADPH plus oxygen. And the dark reactions are NADPH plus CO2 goes to carbohydrate plus NADP+, which of course gives us our net-- water plus CO2 goes to carbohydrate plus oxygen. And in the next lecture, what we'll talk about is we'll go through the details of how these light and dark reactions work. But when we do so, bear in mind that we're really going to follow all of the concepts and rules that we talked about for past thermodynamic considerations of pathways as well as what we've been discussing about how oxidative phosphorylation works. Thanks.