[SQUEAKING]

[RUSTLING]

[CLICKING]

PROFESSOR: OK. So today we're going to continue our discussion about photosynthesis. And last time, I introduced the reactions of photosynthesis and pointed out that really what we're talking about is oxidation reduction reactions. And so the net reaction of photosynthesis-- water plus carbon dioxide goes to carbohydrate and oxygen-- is really the opposite of burning wood. So if burning wood, carbon oxidation, is favorable, reversing this, obviously, needs energy input. And that energy input is light that's coming from the sun.

And we started to discuss a bit about how this reaction works. And I mentioned that photosynthesis is broken down into light reactions and dark reactions. And so the light reactions are really along the same lines of the oxidative phosphorylation electron transport chain that we described. And that is basically using energy from the sun to use water as an electron donor to generate NADPH, as well as oxygen.

In doing so, light from the sun makes this process favorable. That favorable electron transport can then be used to generate delta psi, delta pH, which can create ATP, do other work just like oxidative phosphorylation. Of course, photosynthesis comes first, oxidative phosphorylation came second, even though we taught it in the reverse order. And these reactions also produce NADPH, which can be used in the so-called dark reactions to synthesize carbohydrates.

Now, the dark reactions, again, I mentioned, can be done in the light or the dark. They don't need light. They use the NADPH from the light reactions, as well as the ATP produced from the light reactions to take CO2 and turn it into carbohydrate. And that allows plants to store carbon, reduced carbon energy, for later, such that when the sun is not shining they can then use that carbohydrate to oxidize it in all of the reactions we've already learned about and make sure they're able to generate ATP, keep their ATP-ADP ratio high at night, and then switch back to using photosynthesis when the sun is out during the day.

Now, today what we're going to discuss is both the light and the dark reactions in great detail to fill in how those processes happen. So first, we're going to start with the light reactions. And so the light reactions, if it's like oxidative phosphorylation, that is we're going to generate a membrane potential, build a battery, that is to occur at a membrane.

And so those membranes are the cell membrane of a bacteria, or prokaryote, or an intracellular membrane of a eukaryotic photosynthetic organism. And that intracellular membrane is, of course, the chloroplast that you learned about in grade school. And so the chloroplast is the photosynthetic equivalent of the mitochondria. Oxidative phosphorylation in eukaryotes happen at a mitochondrial membrane. And photosynthesis in eukaryotes happens at a chloroplast membrane.

And so, just to be explicit about this, so if this is our photosynthetic bacteria, some prokaryotic cell, it will use photosynthesis to make delta psi, delta pH across the cell membrane. And then it can utilize that to provide energy for the organism, very similar to what we said about oxidative phosphorylation in a bacteria.

And you can imagine that then there was some early eukaryotic cell that ended up engulfing this photosynthetic prokaryote. And in the process, the ATP generated could be exchanged for ADP across those membranes. And in the end, this is how one ends up with this double-membraned organelle within the engulfed prokaryotic species, with having the membrane potential built across this-- oops-- having the membrane potential built across this inner mitochondrial membrane-- or inner membrane of, in this case, the chloroplast.

And so, if you remember, we drew the mitochondria as having this double membrane structure, the inner mitochondrial membrane, where oxidative phosphorylation happen. Protons were pumped into this intermembrane space. And chloroplasts have basically the same structure, but it's a little bit more complicated, at least on the surface. And that's because chloroplasts have evolved this further to have what, essentially, are extreme cristae, those folds of the mitochondria called cristae. These extreme folds is basically what happens in a chloroplast.

So if we draw here a giant chloroplast-- so this here would be the outer membrane of the chloroplast. So the chloroplast also has an inner membrane. So this here is then the inner membrane of the chloroplast.

You can imagine that if you had this extreme folding in of the cristae, but actually pinched off this little piece of membrane here, you would now end up with stacks of membrane within the chloroplast. You still here have this inner membrane here that goes all the way around, but now you have these little membrane stacks that now exist within the chloroplast. These here is called grana. And this inside of these stacks, what is called the lumen, is really the equivalent of the intermembrane space of the mitochondria.

So the inside of this is basically equivalent to the intermembrane space of the mitochondria, such that if I draw one of these pinched off pieces of membrane in gigantic form, you're basically pumping protons into the lumen, into this intermembrane space equivalent. And so you can imagine that you have this electron transport chain set up, where electrons are transferred from water to NADP+, making oxygen an NADPH. That is, electron transport is made favorable by light that generates a membrane potential across this membrane, which is, by the way, this membrane-- which is the same there-- this is called the thylakoid membrane. Thylakoid-- T-H-Y-L-A-K-O-I-D-- membrane. So you build a potential across this thylakoid membrane, and then that potential can be used to synthesize ATP within this other space, this wider space here, which is called the stroma, which is the equivalent of the matrix of the mitochondria.

And so basically, the chloroplast is set up in a very similar way to the mitochondria, but because it makes these membrane stacks, it looks like it's a little bit different. But it's really pumping protons into the intermembrane space, which would happen in the mitochondria an equivalent space, which is the lumen of these stacks, is what happens within the chloroplast. That charge on the membrane, that delta psi, delta pH, can then be used to do work like synthesizing ATP. That happens in-- rather than-- and that happens in the stroma of the matrix equivalent, the stroma of the chloroplast.

And so effectively, you have NADPH, an ATP produced in the stroma. And so it turns out the dark reactions, when we talk about them later, will occur here in the stroma of the chloroplast. And the light reactions, of course, are happening over here at the thylakoid membrane.

Now, this is actually a cool innovation that nature has come up with for this. And because by making these extreme cristae, you can imagine that there's a very small space here that you're pumping protons into. That means that fewer protons pump will allow you to generate a higher potential, a higher gradient of protons, delta pH. And so that means that that then can be used to drive energy in a more efficient way then you might get using this much larger intermembrane space. And so light reactions here at the thylakoid membrane within the chloroplast, dark reactions here in the stroma of the chloroplast.

All right. So that's the anatomy. Now, let's get back to what's actually happening here in these light reactions.

So of course, the trick of the light reactions is to come up with some way to make water a good electron donor, such that you can have favorable electron transfer to NADPH, which is, of course, what's necessary to have energy released to generate delta psi, delta pH. And so, of course, to do this, we still have to follow all the same thermodynamic rules that existed before, that we talked about for oxidative phosphorylation. So you should remember from those lectures that electron transfer is going to be favorable if we move from a lower to a higher standard reduction potential.

So if we just draw this out. So here's a physiological range of standard reduction potentials from negative 1.6 to positive 0.8 volts. So this here would be a range of standard reduction potentials.

So remember, a change in standard reduction potential that's positive, that means moving this direction down, as I've drawn this axis from smaller to larger. I realize I drew it upside down in the way you probably are normally used to looking at this. But basically, if you go from a more negative to a positive number, or a more negative to a less negative, or a positive to a more positive, basically, a change in this direction, which is going to have a positive change in standard reduction potential, is going to end up having a negative change in delta g-- not prime. And so that means equilibrium is going to favor electron transport moving down in this direction.

So now, let's just give this some real numbers. So the standard reduction potential of oxygen water sits down here. It's 0.86. You don't have to worry about exact numbers. The standard reduction potential of NADH, or NADPH, they're the same. The nicotinamide group is here. It's about negative 0.32.

And this is why oxidative phosphorylation works is because there is a net positive change in standard reduction potential. That is, you go from a lower to a higher standard reduction potential if we do electron transport in the oxidative phosphorylation. That means energy is released as we transfer electrons from NADH to oxygen. So that is favorable. That favorable energy release is what can be coupled to proton pumping to generate delta psi, delta pH, and allow the cell to do other work.

Now, you cannot change the properties of water just because you add light to it. So it still sits at this standard reduction potential. But what happens in photosynthesis is basically you use light to excite an electron. That effectively moves water up here to a more negative electron up here, to generate an electron that's up here at a more negative standard reduction potential than NADPH, which, of course, now moving down, becomes favorable.

And so here, now you're going from more negative to here. And then from there to there, each of those is favorable. This means you can use this favorable electron transfer, the energy release from the favorable movement of electrons from this excited electron, to NADPH, generate NADPH. And then you can also use-- donate those to the electron transport chain and OXPHOS with oxygen-- with water-- with oxygen as the final electron acceptor to net generate delta psi, delta pH in OXPHOS.

And so this works to generate it in photosynthesis, and this works to generate it in oxidative phosphorylation. And we've not violated any laws of thermodynamics. And we're able to, in both cases, create this membrane potential, build this battery, that now allows you to do work such as synthesize ATP, keep the ATP-ADP ratio high, allow the cell to do all the unfavorable reactions that it needs to do.

So now, let's talk a bit about this process. So of course, this happens with visible light, but I want to start with asking a fundamental question. Why do we use visible light? Or, to put it another way, what is visible light, anyway? Well, visible light is, of course, just electromagnetic radiation, a photon on the electromagnetic radiation spectrum. And visible light is defined by what we see.

So if we draw this out-- this is hopefully something that you've covered in a physics class somewhere along the way. So if this here is the electromagnetic magnetic spectrum as drawn out-- so down here you have your short wavelength electromagnetic radiation, down here you have your long wavelength things-- so higher energy, lower energy-- and on this end, X-ray, UV light. And you get to the visible spectrum and we go from the purple light down to the red light. And eventually, we get into the infrared light, and ultimately, down to microwaves, and basically shorter wavelength and longer length wavelengths, more energy, less energy.

And so just to give you some numbers, so this here is on the order of 300 kilojoules, is on the order of 170 kilojoules. And so ADP to ATP conversion-- so we before talked about in kcals per mole, but it's on the order of 30 kilojoules for that. And so this part of the electromagnetic spectrum is the right order of magnitude, it turns out, to transduce energy in a realm that matches the energy required, or release, from the ATP to ADP conversion reaction.

You also know from just popular use, you go out in the sun when it's a nice day, you put sunscreen on. Why do you do that? That's because the UV light that's filtered through our atmosphere from the sun can cause sunburns. That is, it can cause damage to our skin, to other biological molecules. Obviously, X-rays, you go get an X-ray done, you get covered with lead in all the places they don't want to have the X-ray done. Why? Because X-rays can cause damage. We use those to treat cancer.

And so things down on this end, too much energy damages biological molecules. This is the sweet spot where you don't get a lot of damage to the biological molecules. And the energy is right to synthesize-- to transduce energy on a magnitude that works for biological systems.

Come down to this end, well, now you get to be too little energy. You get down to the infrared and the microwaves. This is like your TV remote control. This is the cell phones that if you were in class as opposed to listening to this online, you would be checking instead of listening to me. So this direction, too little energy is transduced to actually matter for the biological systems. And so that's really why this spectrum of light is what's involved in photosynthesis.

However, I also want to remind you that visible light is a human construct. It's what we as humans can see. And photosynthesis evolved long before humans. We needed photosynthesis first before any animal life can evolve. And so this visible light designation is completely arbitrary.

And in fact, we evolved from photosynthetic organisms. And so it's really photosynthetic pigments that evolved first to capture light in this range that we then repurposed in order to see. So in essence, photosynthesis defines the visual spectrum, because what we see is defined by the pigments that came-- because it evolved from photosynthesis.

The carotenoids, the carrot, the why do eat carrots that are good for our eyes? They have the pigments in carrots, carotenoids-- which we'll draw in a little bit-- that are ultimately related to our visual pigments. And we didn't invent those. Photosynthetic organisms invented those to work in this spectrum to harvest light from the sun for energy. And we repurposed those molecules as a way for us to see.

So how does this work? Well, again, I got to remind you of a little bit more physics, a little quantum physics. This is not-- obviously, we're not going to go into this at the level you get in a physics class, and hopefully you've seen this before.

But you remember, if you think back to physics and physical chemistry, that electrons sit in discrete orbitals within molecules. So we can draw those orbitals like this. And so you'd have some electron pair sitting there in some orbital in a molecule. And if you deliver light with the right amount of energy, the right quanta of energy, you can now excite one of those electrons up here into a orbital with a higher energy. And of course, that's not stable, so it will decay.

And when that electron decays back to the ground state, this then re-releases another photon of light at a longer wavelength. So longer wavelength light is emitted, and that's effectively fluorescence. So you excite something, electron bounces up into a higher orbital, it decays, releases some of the energy that it absorbed as it transitions back. That is fluorescence. This happens very, very fast.

And basically what photosynthesis does is it takes advantage of this excited state. And it effectively transfers that electron away, creating a charged separation prior to that electron decaying back into the ground state orbital. This occurs very fast. And so photosynthesis has to occur very, very fast in order to make this work. And in essence, this is the way that photosynthesis is able to capture energy and connect it to a biological system that allows us to build an electron transport chain.

And so in essence, photosynthesis, the way the energy is absorbed, is it's what you learned in physics. The right quanta of light causes an electron to excite into a higher energy orbital. And then that electron is transferred away, creating a charged separation before that energy of that excited electron can decay as fluorescence. That creates a charged separation. And that charged separation now has an electron such that that electron can now be favorably transferred to NADPH. That energy released in that favorable transfer can be coupled to make delta psi, delta pH. And then that translates as energy in exactly the same way we learned about for oxidative phosphorylation.

So to do this, what do we need? Well, we need a pigment that can absorb a photon of the right wavelength-- or, put it another way, of the right energy-- and then quickly transfer that electron away. And so you need two things. You need light acceptors that can absorb energy of the right energy, the right wavelength of light, and redox carriers to transfer those electrons away.

So what are the light acceptors? Well, these are basically pigments that can absorb visible light. And redox carriers are electron carriers, which we've been talking about now for several lectures.

So let's start with the light acceptor. So this is something that's tuned to the right energy, the right wavelength. And so this is chlorophyll.

So we all know plants contain chlorophyll. That's important for photosynthesis. Well, what is chlorophyll? Well, it's a conjugated pyrrole, very similar to heme, except remember, heme had iron in it. Saw that for hemoglobin, saw it again in the electron transport chain. And I'm going to show you chlorophyll. Chlorophyll is going to look a lot like that, except it has a magnesium conjugated to the tetrapyrrole rather than iron.

And so here you have tetrapyrrole with a magnesium in the middle, conjugated double bond system all the way around. Makes sense, that should absorb visible light, all those conjugated double bonds. There's some R group decorations hanging off over here. Chlorophyll also has this additional decoration over here, including a long lipid tail here to make this-- it will sit within a membrane. Oops. Apologies. I misdrew that. Lipid tail hangs off down there.

But this here is effectively what the structure of chlorophyll looks like, with this R and R prime being specific groups. You can look up if you're interested. But basically, these are the decorations that define what I refer to as A type and B type chlorophylls. Remember, we had A, B, and C type cytochromes that were basically similar molecules with slightly different decorations on them, the types defined slightly by the absorbance properties of these pigments. But effectively, what's key about chlorophyll is that it's this conjugated, double bond system in the tetrapyrrole that ultimately allows it to absorb visible light.

Now, chlorophyll is, of course, the most famous pigment, but it's not the only pigment that photosynthetic organisms used. So they also use other pigments to absorb visible light. And so there is a class of pigments that look very similar to chlorophyll. You can look up the structures if you want. They're called phytobilins. They're also tetrapyrroles, very similar to chlorophyll, except, for instance, pheophytin does not contain any magnesium or other thing conjugated in the center, so no magnesium. Otherwise, it looks similar to chlorophyll.

And then there's other ones. There's so-called phycoerythrin, which you might guess is red. So that's a red pigment. There's phycocyanin, which is, you might guess, is a blue pigment.

And then maybe the most famous one that about that's not chlorophyll are the so-called carotenoids. This includes things like beta-carotene. I think we all know that beta-carotene is orange. What does beta-carotene look like? I'll draw it out for you. There's beta-carotene, another long conjugated, double bond system.

Obviously, you don't need to memorize the structures of any of these. You can always look up structures of these pigments. The point is that nature has a variety of these structures, all with conjugated double bond systems, all well-tuned to absorb visible light. And this, of course, also then becomes a major pigment for our visual system, repurposed from the plant using it for chlorosynthesis-- for photosynthesis.

And why have all these pigments? Well, the idea is because if we say here, what's the absorbance then across the electromagnetic spectrum, that the purple, the short wavelength, being down here, and the red, the long wavelength, being on this end, well now, effectively, you have chlorophylls that cover that spectrum. And so you'll have some peak here for say A type chlorophylls. And then you'll have some other peak for B type chlorophylls. These are obviously approximate.

And so that's basically the A and B type chlorophylls. Why are plants green? Because they absorb in two peaks on either end, and so that reflects the green light in the middle.

But now you have these other pigments that will fill in the middle. And so the beta-carotene, the orange beta-carotene, will allow you to-- plants, they'll have an absorption spectrum that will peak somewhere in that range. And then you'll have the phycoerythrins with an absorption spectrum that will peak somewhere in that range. And then the phycocyanins with an absorption spectrum that will peak somewhere in that range.

And basically, by covering all of these different pigments, plants and other photosynthetic organisms can now adapt to different light niches within the natural world, really capturing the full spectrum of visible light. And the nice thing is this also leads to all the beautiful colors and whatnot that exists across photosynthetic organisms, different plants, algaes, and bacteria, all different colors. And basically will use these different pigments to ultimately cover this visual spectrum, this right spectrum with the right amount of energy to carry out the processes that's necessary to run the light reactions of photosynthesis.

Now, even though there's this variety of pigments, effectively, all photosynthetic organisms, as far as we know, work in a very similar way, in that they basically arrange these pigments always at a membrane, always here at this thylakoid membrane in the chloroplast-- or, I guess it would be the cell membrane of a prokaryote. And these are organized into a structure called the light harvesting complex.

And basically, what a light harvesting complex is, is it's pigments arranged in a structure that basically can channel energy from the photons captured down into a place called the reaction center. So you can imagine that you'd have all kinds of pigments arranged in a way that basically will absorb photons, release photons as fluorescence, ultimately going down to this reaction center. And it's in the reaction center that really the magic of photosynthesis happens.

So what happens at the reaction center is pretty well understood from the structure of the photosynthetic reaction center that was solved about 30 years ago in the purple photosynthetic bacteria. And this is very illustrative for how it works. And so if you look here at the slide, this here is basically a graphical representation of what that reaction center looks like.

And so effectively, you have a bunch of, in this particular case, molecules up here. But really, the reaction center is what exists down here. And in there, there is a pair of chlorophylls-- we'll talk about in a second. Those are they're shown graphically over here. And they will effectively transfer electrons around in this direction to these quinones further away from the special pair of chlorophyll. So electrons will be transferred from the chlorophyll after it's excited down to these quinones, creating a charged separation that ultimately is going to allow you to then have a high-energy electron that can be built into an electron transport chain.

And so let's draw that graphically out over here. And so here if we have our special pair of chlorophylls-- so chlorophyll, if you look at it here, is relatively a pyrrole. Like heme, it's a flat molecule, and so these will stack on top of each other. So if they're stacked on top of each other and this is looking at them head on, you have this special pair of chlorophylls. You'll eventually-- you'll absorb a photon of light, and that will create a charge separation a cross between that special pair of chlorophylls.

That electron, before it can decay as fluorescence, as would normally happen, it's very rapidly transferred out to a pheophytin molecule. And then it's very rapidly transferred again to a quinone called QA.

All right, so just show that again here on here. So here's our special pair. You absorb a photon of light, charge separation at the special pair, electron transfer to the pheophytin, and then down to this quinone. And that's a very fast reaction. And you can see that it's actually moving within space that's roughly the distance from one side of the membrane to another. So on an atomic level, quite a long distance.

And so from this quinone, it then will be transferred again to a second quinone. That's a slower step. I want to remind you how electrons are transferred on quinones. And so we talked about this already when we talked about electron transport.

So here is a-- so this here would be an oxidized quinone just like we saw for coenzyme Q, ubiquinone. So this would be oxidized quinone and photosynthesis. Electron, you can pick up electrons one at a time, so that will generate this semiquinone. Remember, with the stabilized free radical, now you can pick up a second electron. And that ultimately generates the reduced, the fully reduced quinone, the quinol. We talked about this with coenzyme Q and oxidative phosphorylation. Remember, ubiquinone to ubiquinol, similar thing happening in photosynthesis. Also, a quinone can pick up electrons, go from the oxidized to the reduced state.

And if you remember, in oxidative phosphorylation, you could then, as you transfer the electrons across from the oxidized and reduced state of the quinones, you'll pick up a proton. If you pick up those protons and release those protons as you transfer electrons on different sides of the membrane, that can be coupled to pump protons across the membrane as you transfer electrons across the system, exactly what happens here in photosynthesis. Except now that electron is basically coming from transfer of an excited electron from the special pair to the pheophytins, and ultimately to the quinones. And then from the quinones, can now be transferred down on electron transport chain.

To show this in a quantum way, if you will-- so if this here is the special pair of chlorophyll and we draw here the pair of electrons in the special pair in the ground state, if you get a photon of light of the right energy, you excite one of those electrons here into a higher orbital. Before that can decay, if we transfer that electron out, basically take that electron and transfer it out, and instead here give it to the pheophytin-- so here's some orbital in the pheophytin-- it comes, it picks up this electron that's transferred away from the special pair.

Well, now, what you have is you basically have a negative charge here and a positive charge up on the special pair. So you've created this charge separation. That electron can then be further transferred down the electron transport chain to the quinones, that then transfer them across the quinones, who are pumping proton. And ultimately, down an electron transport chain, ultimately with the final electron acceptor being in NADP+ to make NADPH.

And you're still left with this positive charge on the special pair. Obviously, that's not a very stable state, so we've got to resolve that. How can that be resolved? Well, lots of things will-- that's a pretty good electron acceptor. And so now, even something like water, which is highly abundant, can be an electron donor, and ultimately fixes that positive charge on the special pair by taking electron from water, which, of course, generates oxygen.

This part of the reaction is carried out by a complex called the water splitting complex- We: don't have time to get into this-- and some manganese-containing enzyme. And it basically controls the transfer of an electron from water to oxygen, which, hopefully, is at least intuitively you'll see why that's favorable, because you really have this true positive charge on the special pair. And almost anything is a good electron donor to that, including water.

And so it's really this charge separation by fast electron transfer through the reaction center from the special chlorophyll pair ultimately down to quinones that creates this charged separation that is ultimately where you can pull an electron from water to generate oxygen. And if we come back over here, it's how we made this whole process work, how we made water on a good electron donor.

That is, we didn't make the standard-- didn't change the standard reduction potential of the oxygen-water pair. Instead, what we did is we basically used light to excite an electron in chlorophyll such that that excited electron now can be favorably-- sits at a low enough standard reduction potential such that it's favorable to transfer it to NADPH. And then we fix that charge hole, if you will, by pulling an electron from water, and that generates oxygen.

Of course, once we're able to get this favorable electron transfer, now we can couple that to the pumping of protons. That allows us to make delta psi, delta pH. We charge a battery, and that does work, just like we saw before. So why these concepts were so important to understanding how energy transduction and biological systems work, and why I spent so much time talking about standard reduction potentials, delta psi, delta pH, et cetera.

Now, I want to describe these concepts and build them into the context of a chloroplast electron transport chain. I'm going to show you what happens in chloroplasts in higher plants. Conceptually, even though the details may not be 100% the same across all photosynthetic organisms, conceptually what I'm going to show you is exactly the same, regardless of whether it's a photosynthetic bacteria or a chloroplast from a plant.

So if we draw here-- this here being the thylakoid membrane-- so I'm going to draw it such that this here is the stroma side of the membrane. This is the lumen side of the membrane. Remember, the stroma side would be equivalent to the matrix of the mitochondria, where the lumen side would be equivalent to the intermembrane space of the mitochondria. So thylakoid membrane, stroma side, lumen side.

We have a complex here. This complex is called photosystem II, abbreviate PSII. Photosystem II is going to be linked up to the water splitting complex, which will basically pick up an electron, of course, injecting light that will transfer electrons through effectively what is a Q cycle, what we alluded to happens in oxidative phosphorylation-- I didn't have time to describe it in great detail-- that can pump protons across the membrane, as electrons are transferred into the next complex, which is called cytochrome bf.

The next complex, cytochrome bf, also can pump protons across the membrane. Those electrons then get transferred to a soluble protein in the lumen called plastocyanin. From there, those electrons now get transferred to another complex called photosystem I, or PSI.

Electrons in photosystem I can end up in another complex called ferredoxin, ultimately transferring those electrons to NADP+ to generate NADPH. And of course, that will happen here on the stromal side of the membrane, because that's where the dark reactions are going to take place.

This has the effective generating a delta psi, delta pH across the membrane. And of course, that can be used then, that delta psi, delta pH can be used by a chloroplast F0, chloroplast F1 to generate ATP, just like we described from oxidative phosphorylation, with the ATP also being generated on the stromal side of the membrane.

So this should look very similar to what we described for mitochondrial electron transport. So you see we have multiple complexes, including a cytochrome C-like protein that sits on, in this case, the lumen side of the thylakoid membrane, plastocyanin. Cytochrome C sits in the intermembrane space of the mitochondria. You transfer electrons, including via Q, quinone-like, cycle that pumps protons, have other complexes that pump protons, ultimately transfer electrons to generate NADPH.

There's two complexes here that absorb light, photosystem II and photosystem I. Electron transfer, I guess, classically, would go photosystem II, cytochrome bf, plastocyanin, photosystem I, ferredoxin. Water is the donor, NADPH as the acceptor, generates delta psi, delta pH. That delta psi, delta pH can then be used to do work, including synthesis of ATP, as done by an F0F1-ATPase, just like exists in the mitochondria.

Of course, this evolved first. And so oxidative phosphorylation looks like photosynthesis, even though we talked about it, oxidative phosphorylation first and then photosynthesis. So you will, of course, looks like oxidative phosphorylation, but, of course, remember, it's really not that photosynthesis looks like oxidative phosphorylation, it's that oxidative phosphorylation looks like photosynthesis.

Now ultimately, the reason this works is because photosystem II and photosystem I can absorb photons, which ultimately, by the processes we discussed, is what makes electron transfer favorable from water to NADP+ to generate oxygen and NADPH.

Now, I want to take you through just to show you how this works, because it turns out it's also possible to short circuit this in a way that can favor more delta psi, delta pH production relative to NADPH production, which is important, because you can imagine that a photosynthetic organism has to balance its needs for NADPH and ATP-- both useful molecules, but you want to balance your needs for both of them. And it turns out that this middle part, photosystem I, works with cytochrome bf in a way that allows it to balance that.

So if I draw this out in a little bit more detail-- so here's negative 1.6 volts and here's positive 0.8 volts. So that's, again, our standard reduction potential. So remember, water and oxygen is going to sit down here. You're going to have in photosystem I a special pair called P680. That's the wavelength of light.

At the special pair in-- so this here would be photosystem-- sorry, photosystem II. That's a special pair within photosystem II, absorbs photon. That photon now becomes an excited electron that can be transferred away. That ultimately makes water a good electron donor to resolve that charge separation at this special pair.

That electron is ultimately transferred away, coming down here to cytochrome bf. So this moves from a higher to a lower standard reduction potential that's favorable. So this should go here through the pheophytin, QA, QB, et cetera, through a Q-like cycle to get to cytochrome bf and pump a proton. That would then transfer to plastocyanin. That would then transfer, ultimately, to photosystem I.

So there's photosystem I, which also can absorb a photon, excite that electron, which can be rapidly transferred away. But this time, rather than water being the electron donor, it's the electron transfer from plastocyanin that resolves the charged separation on the special pair as you excite and transfer the electrons out, ultimately coming here to ferredoxin, which is at the right standard reduction potential to reduce NADP+ to NADPH.

And so this here also would come through quinones. A0, A1 is what those quinones are called in photosystem I. That's not important. It's exactly the same thing as what we described before. And ultimately, this is drawing out, basically, what happens in the chloroplasts, how photosystem II and photosystem I fit in with electron transfer in terms of moving across standard reduction potentials.

Now, what's cool is that photosystem I, rather than transferring the electrons to make NADPH, also can instead transfer electrons as an alternative back to cytochrome bf. Well, if it comes back into cytochrome bf, you can see that basically this now creates a loop where you have favorable electron transfer here, excite, favorable electron transfer, excite, favorable electron transfer. In essence, this here will then pump a proton by basically transferring the electron back, allowing you to run this middle part between photosystem I, cytochrome bf, and plastocyanin, create delta psi, delta pH at cytochrome bf. And that allows you to make ATP without generating NADPH, or make a battery, delta psi, delta pH, do whatever work without generating NADPH.

That's also shown here on this slide, drawn out in a way that may be slightly neater than what I drew out over there, but effectively illustrates the same thing. And it's cool that there's this flexibility to run photosynthesis in a way that you can net generate NADPH, but you can also generate just delta psi, delta pH, allowing the plant to meet its energetic needs, because delta psi, delta pH can keep ATP-ADP ratio high when the lights are on. And then that way, the plant has all the energy it needs to fight entropy and be alive as a cell. Whereas, it can also tune it, then, to generate the NADPH it needs to make reduced carbon that it can then store for later.

Now, to make the reduced carbon that can be stored for later, ultimately, now we have to basically take CO2 and turn it into carbohydrate, turn it into glucose. This CO2 into carbohydrate, turning it into glucose, that hexo-sugar that the plant can then pack away with alpha-1,4 linkages as starch to burn later when the lights are off, requires reduction. That reduction electrons have to come from somewhere. Well, ultimately, they can come from water via NADPH. And those are the dark reactions of photosynthesis. And so let's discuss those next.

And so the dark reactions, really, are CO2 plus NADPH going to carbohydrate plus NADP+. These are reactions that can happen either in the dark or the light. They're unique to photosynthetic organisms. That's who can net carry out this reaction, fixing carbon as glucose, as reduced carbon and glucose.

These reactions, these dark reactions, are going to occur here in the stroma of the chloroplast. And why is that useful? Because this is where the NADPH and the ATP that's going to be required for these dark reactions to take place. It should be clear to you, NADPH is the electron donor, but you also need additional energy input because this is effectively reversing glucose oxidation. That releases energy, need energy input, and so you're going to see you also need ATP to ADP conversion. By happening in the stroma, you have the place where the NADPH and ATP is being produced as products of the light reaction.

So these dark reactions of photosynthesis were discovered in the 1950s by a gentleman by the name of Melvin Calvin. As a result, sometimes the dark reactions are referred to as the Calvin cycle to honor him as the discoverer.

And basically, the experiment that Calvin did is he took carbon dioxide that was labeled with radioactive carbon-- so C14 carbon dioxide-- he basically fed it to a photosynthetic algae and found that the first compound that incorporated the radioactive CO2 was a 3-carbon compound. And that 3-carbon compound was 3-phosphogylcerate. Good old 3-PG from glycolysis. And so he found that you could get these reactions of CO2 going into 3-phosphoglycerate is the first thing to incorporate the radioactive carbon. That worked in the light or the dark, and that's where the term dark reactions came from.

Now, it should be, hopefully, clear to you that if I can generate 3-phosphoglycerate, you now know how to make glucose. Because now you can go back and look at your notes from the gluconeogenesis lecture, and if you had a source of 3-phosphoglycerate, you can just run it through the reactions of gluconeogenesis, and ultimately make glucose.

Turns out that the way this happens in total is the so-called Calvin cycle, really happens in three phases. So the first phase is called fixation. And so what is fixation? That's basically Calvin's experiment. It's using CO2 to generate 3-phosphoglycerate.

The second phase is referred to as reduction. So what's reduction? Well, that's basically gluconeogenesis. So it's using 3-phosphoglycerate and turning it into glucose. Gluconeogenesis, we're reducing carbon. This is the step, of course, so you need electrons from somewhere. They're going to come from NADPH. We need NADPH for that. You'll also need ATP.

And then the last step is called regeneration. And what you will see is that the Calvin cycle is a cycle. That is, you're going to have to have an entry point and an exit point. And just like we talked about for the TCA cycle, if we feed two carbon acetyl CoA units into the TCA cycle, we have to have an acceptor, oxaloacetate, in order to run the cycle. And if we pull anything out of the cycle, we need another way to add carbon back to the cycle. And so regeneration is really anaplerosis for the cycle. That is, you need to generate the acceptor that you can keep running this as a cycle.

As a general overview, it works as follows. And so it turns out that the cycle works with this molecule as, I guess, if we're going to draw an analogy to the TCA cycle, where oxaloacetate was the initial acceptor, this is the analogous molecule to that. This is a molecule, it's a 5-carbon sugar. So it's pentose phosphorylated-- it's a pentose and a ketose phosphorylated on the one and the five position.

So this is ribulose 1,5-bisphosphate, which I will abbreviate R15P. So ribulose 1,5-bisphosphate will pick up a CO2 molecule, and it will make two 3-phosphoglycerate molecules. So five carbons plus one carbon equals six carbons. Break them in half, two 3-phosphoglycerate molecules.

Once you have that 3-phosphoglycerate, I can now use ATP to phosphorylate that 3-phosphoglycerate. That'll give me 1,3-bisphosphoglycerate. I can now run the photosynthetic version of the GAPDH reaction, which is exactly the reaction that you saw in glycolysis, except now we're going to use NADPH rather than NADH, as we described for glycolysis and gluconeogenesis.

And so that's going to give me glyceraldehyde 3-phosphate, glyceraldehyde 3-phosphate, use triose-phosphate isomerase to make dihydroxyacetone phosphate. And I can run aldolase reaction to make fructose 1,6-bisphosphate, can release a phosphate from fructose 1,6-bisphosphate. Now I have fructose 6-phosphate. I can isomerize fructose 6-phosphate to glucose 6-phosphate. And of course, if I take the phosphate off, that's glucose.

Or, more importantly, what would the plant do? It would then do a mutase reaction to make glucose 1-phosphate and pack that away in starch, much like we discussed for putting glucose into glycogen. And then get that glucose back out as glucose 1-phosphate, glucose 6-phosphate, and send it to glycolysis to be oxidized to get energy.

This is great, but you can see that this is not going to work unless I can regenerate a source of ribulose 1,5-bisphosphate. Turns out, you can do that from fructose 6-phosphate. Obviously, ATP is going to be required here, too, because there's only one phosphate here. You need two phosphates for the ribulose 1,5-bisphosphate. So more ATP is needed. And ultimately, that, in a non-stoichiometric way, is how the cycle works.

And so if we break this up then into the different phases, this phase up here would be fixation, this phase down here is reduction, and this phase over here is regeneration. And that's basically the Calvin cycle.

Let's go through these now one at a time. Let's first talk about fixation. Fixation is catalyzed by an enzyme called RuBisCO, which stands for ribulose-1,5-bisphosphate carboxylase-oxygenase. So RuBisCO, if you want a bit of trivia, is the most abundant enzyme on earth. It's about 50% of the protein in chloroplasts. It's an incredibly inefficient enzyme. It has a turnover number about three per second. And that's why it's so abundant, because it's a crappy enzyme. And so therefore, it needs to have a lot of it around in order to work.

It evolved in a pre-oxygen atmosphere, and now, of course, there's lots of oxygen in the atmosphere. And CO2 and oxygen will both compete to react with the enzyme. And it turns out that that's a issue for photosynthesis, and it's a big deal for agriculture.

Now, the way RuBisCO works is that there is a lysine in the active site of the enzyme. And that lysine is covalently bound to a CO2 molecule. And that CO2 molecule covalently bound to the lysine is not involved in the reaction. But basically, if you don't have enough CO2 around to carboxylate the lysine, now you can't carry out the reaction. It's one way to ensure that there's enough CO2 that this will work.

But effectively, what that CO2 does is coordinates a magnesium atom that ultimately coordinates and positions the ribulose 1,5-bisphosphate within the active site of the enzyme. So here is ribulose 1,5-bisphosphate bound into the active site of RuBisCO. I'll show you quickly how the RuBisCO reaction works.

And so if we take the enol-- or, the keto form of ribulose 1,5-bisphosphate and redraw it in the active site as the keto form so it's-- that will react with CO2. You add water. And then ultimately, what we are left with from the top half of the molecule, we get this 1,3-phosphoglycerate molecule, and on the bottom part also get a 3-phosphoglycerate molecule. So two 3-phosphoglycerates generated. So ultimately, what's happening is we're adding a CO2 basically between the two and three carbon of ribulose 1,5-bisphophate, breaking the molecule in half to get two 3-phosphoglycerate molecules.

Just to quickly show you what happens if at this step if you instead replace this with oxygen, so instead have oxygen add there instead. Well, now you end up with this situation. So now you have that instead. Now, when water gets added, you end up generating from the bottom half of the molecule, of course, a 3-phosphoglycerate. So that's not a problem. But from the top half of the molecule, rather than generating a 3-phosphoglycerate, now you have this 2-carbon unit, which is called phosphoglycolate. And it turns out that phosphoglycolate is not a good thing for the plant to have.

You basically started with 5-carbon ribulose 1,5-bisphosphate, and you end up with 3-phosphoglycerate and a 2-carbon phosphoglycolate. No carbon is added there, and now the plant has to deal with the phosphoglycolate. So it has to regenerate the ribulose 1,5-bisphophate. So naturally, if you're going to take-- rebuild that 5-carbon molecule, that's going to require energy input. And you haven't fixed a CO2.

So in the end, what's required is actually more ATP and NADPH to solve the phosphoglycolate problem with no net gain for the plant. And so as a result, oxygen competing with carbon dioxide is a big deal for plants. By the way, dealing with phosphoglycolate-- which, of course, plants have a way to do-- is a process called photorespiration. Photorespiration requires all this ATP and NADPH, so it requires all this extra light energy from the plant that's really not being used for any good purpose for the plant itself.

Now, the process I described is the standard photosynthesis process, and it's what happens in so-called C3 plants, called C3 because a 3-carbon intermediate is made. It turns out that there is also a class of plants called C4 plants that basically use a 4-carbon intermediate. They do the same thing 3 plants do, but they basically have a system that basically uses some extra ATP and NADPH in order to generate a shuttle to concentrate CO2 to run the RuBisCO reaction that I just showed.

And so that is shown here on this slide. And so this is what happens in C4 plants. And so effectively, you fix a CO2 using-- basically, making oxaloacetate. So that's the 4-carbon unit. You run an NADPH-driven version of the malate dehydrogenase reaction to ultimately generate malate. And then turn that malate back into pyruvate, releasing CO2 back over here into the different parts.

So use a PEPCK-like reaction, send it to the chloroplast, where you then regenerate CO2 for the RuBisCO enzyme. And you send that pyruvate back out as a way to run a cycle that basically concentrates the CO2. So this requires extra ATP, requires extra NADPH. But in the end, it saves the plant the trouble of having to deal with this phosphoglycolate.

Not surprisingly, this has evolved in places with high light. So tropical plants are more likely to be C4 plants because they have more light around, more ATP, NADPH any way, that allows them to compete with each other and concentrate CO2 in a way that allows them to run the RuBisCO reaction.

All right, reduction, the next phase up here. There's actually not a lot to say. This is basically just gluconeogenesis using NADPH. And of course, you can use it to generate glucose, but plants would rather generate glucose 1-phosphate and store it as starch or something else, some storage sugar for the plant itself.

Now, what should be clear to you is that, well, we started with a 5-carbon unit and we added a 1-carbon unit. And in the end, if we're going to net generate a glucose, we're going to have to run that cycle six times, because we need a 5-carbon unit to come out the end, as well as build a 6-carbon unit just from CO2. And so regeneration is really how do we go from net six CO2 to one glucose molecule. That is, how can we combine that reduction step while also regenerating ribulose 1,5-bisphosphate to keep running this as a cycle?

And so, in essence, what we need is we need to take six 5-carbon units-- so that's a total of 30 carbons. And we're going to have 6x CO2 molecules. There's another six carbons. So that's a total of 36 carbons in. And then we're going to have to allocate those carbons to regenerate six 5-carbon units. So the 30 carbons, we need it for the cycle, as well as one glucose, the remaining six carbons, that hexose.

How this happens is confusing. I'm sorry, I didn't invent this. Nature invented this. But it's essentially accomplished via series of 2-carbon and 3-carbon swaps. And why it's 2-carbon and 3-carbon swaps and not-- I don't know, why didn't nature just come up with something more straightforward is well grounded in the chemistry of how these reactions happen. We'll describe the chemistry of the swapping reactions in great detail next time.

You'll see that the swaps occur between aldosis and ketosis. And so there's actually good evolutionary reasons. You'll see that those reactions will be analogous to things that we've seen before. And effectively, nature, rather than making it easy for you to memorize things, came up with a way that actually fit what happens that enables carbon rearrangement in a way that will make this pathway work.

And so before I go into the details of exactly how this happens, I just want to lay out for you at a very, very high level how those swaps can work to accomplish this goal. So basically, If we're going to start with five carbons and we're going to add CO2, what's that going to generate? Well, we're going to generate, by the RuBisCO reaction, 3-carbon units.

So if I start with 6 of these and 6 of these, I can generate 12 3-carbon units. I can take of those 12 3-carbon units and obviously combine them via the reduction reactions, gluconeogenesis, ways to generate 6-carbon units. And that ultimately is going to be the glucose that I get out.

So it turns out if I do this reaction five times-- so that's 10 3-carbon units-- but if I take some of-- so that means I'm going to get in the end five 6-carbon units. Well, if I use two of those units and I do a swap with the remaining two of these 3-carbon units-- that is, I transfer two carbons from here to there-- what am I going to get? Well, if I-- that means two carbons. 6 minus 2 is I get a 4-carbon unit. 3 plus 2 is a 5-carbon unit. That's good. That's what I'm trying to get. So I took two there, two here.

So that stoichiometry adds up here. I had 12 of these made. 5 plus 5 plus 2 equals 10, 5 of them down here to these guys, 2 of them with this swap with the 3-carbon units. Now I end up getting two of the six 5-carbon units that I need to make.

Well, now I'm left here with two 4-carbon units. I steal here. I took one away here, two away there. 5 minus 2 minus 1 is 2 left, so I still have two 2-carbon units here to go. So I can use my remaining 2-carbon units to react with those 4-carbon units.

If I now move three carbons from here to there, 6 minus 3 is 3, 4 plus 3 is 7. Now I generate a 7-carbon unit, two of these, two of these. Now, if I take two carbons from my 7-carbon unit, give it to the 3-carbon unit, what do I have? Well, now I have-- I've made two different 5-carbon units. So 2 plus 2 plus 2 equals 6 5-carbon units back. So it can work.

I will end today by drawing out the details of how it works. And then I will redraw them next time, and we'll go through in great detail how it works.

So let's start here with our ribulose 1,5-bisphosphate, carry out the RuBisCO reaction. I get two 3PG molecules. I can run those 3PG molecules using ATP and NADPH, ultimately to generate 3-phosphoglycerate molecules. I can isomerize-- I'm sorry, glyceraldehyde 3-phosphate molecules-- I can isomerize those to also generate dihydroxyacetone phosphate molecules. And, of course, this is what I need to generate FBP, and ultimately generate fructose 6-phosphate. And that fructose 6-phosphate, of course, can be used to generate glucose. And I'm not going to draw the steps for it.

OK. All stuff you know. RuBisCO reaction and then gluconeogenesis, ultimately, to get glucose.

OK. Now, here's where it gets interesting. So now let's use our fructose 6-phosphate and our glyceraldehyde 3-phosphate. So I can generate a 5-carbon sugar called xylulose 5-phosphate. That's a five-carbon sugar. If I use an epimerase and phosphorylate it with ATP, now I can regenerate a ribulose 1,5-bisphosphate.

I've also now will generate this 4-carbon sugar called erythrose 4-phosphate. And I can take this erythrose 4-phosphate, swap three carbons, which will give me the 7-carbon sugar called sedoheptulose 7-phosphate, as well as a 3-carbon glyceraldehyde 3-phosphate. Now I can swap two carbons again, and that gives me a ribose 5-phosphate and another-- so this is ribose 5-phosphate-- and another xylulose 5-phosphate. So X5P, xylulose 5-phosphate.

I can now do exactly the same thing I did before with the xylulose 5-phosphate where I do in an epimerase reaction and phosphorylate it, so epimerase from the ribose 5-phosphate. I can do and isomerase reaction and phosphorylate it. And in the end, I now generate 5-carbon ribulose 1,5-bisphosphate.

So if I start with six ribulose 1,5-bisphosphates and six CO_2 molecules, what do I end up with? Well, now I end up with 12 glyceraldehyde 3-phosphates. If I allocate two of them here, five of them here, and five of them here, that allows me to generate five fructose 6-phosphate molecules. If I allocate two of them there, pull one of them out as glucose, and the remaining two here, I get two of these and two of these, can carry this through. Two of these and two of those, is two of these and two of those, two of these and two of those, so I end up with two here, two here. That's two, four, six 5-carbon units ending up as ribulose 1,5-bisphosphate.

And so I can feed six CO_2's in, get a glucose out, and net regenerate six ribulose 1,5-bisphosphates, all at the cost of six ATPs there, another twelve ATPs here to do the gluconeogenesis reactions, and another six-- I'm sorry, twelve NADPH's to generate all of these 3-carbon units. And in the end, pull out one glucose molecule.

We'll start with this. I know it's very confusing. I will redraw that out. I'll go through it again at the start of the next lecture. Thank you.