

So today we are going to continue where we left off last time talking more specifically about variations on the theme of life.

And last year I tried to do this lecture using PowerPoint and it was a total disaster so I'm going back to the board. You will have the PowerPoint slides. They'll be on the Web to download to summarize basically what I'm drawing on the board.

But it will be slightly different on the board. But I found that for this material it really doesn't work to exclusively use the PowerPoint.

So last time we talked about, remember, my life on earth abridged where -- -- we had photosynthesis making glucose or organic carbon plus oxygen? And then the reverse of this was respiration.

And then we had elements cycling in the middle. And I said this is very, very abbreviated of how all life on earth works. And so today what I'm going to do is tell you that that's not right. That's grossly oversimplified.

And there are some really interesting variations on the theme of how to extract energy and carbon and reducing power and electrons from the earth's system to create life.

And it's mostly microbes that have these diverse possibilities.

And, again, even what I'm going to talk to you about today is oversimplified. If you go to a microbiology textbook you'll find just about every possible combination of energy sources, carbon sources and electron sources in some microorganisms somewhere to get through life. So I'm giving you, again, the simplified version because otherwise it gets way too complicated. So all of life needs carbon and energy, and a lot of other elements, too, but these are the main axis upon which we're going to order our universe today.

So for carbon the choices are inorganic or organic.

So this would be CO<sub>2</sub> and this might be glucose or sugars, any sugars. And then on the energy axis they can use solar energy, as in photosynthesis, or they can use chemical energy.

And within the chemical energy sources they can be inorganic or organic like sugars, etc. And often here you have reduced compounds such as hydrogen sulfide, ammonia, and we'll talk about these.

So these are the ways we divide up the possibilities for carbon and energy sources to be alive. All organisms also need to have an energy currency in the cell. And you've talked about this a lot already in the biochemistry lectures so I'm, again, just giving you the impressionist view of this. You know the details.

This is just to get you organized. And so all life uses redox reactions. And in your handouts for today there's a primer on redox reactions just in case you want to review that.

And one of the key reactions we'll talk about today is the conversion of NADP. If you put energy in you can reduce it to NADPH.

So that's a reduction. And the reverse you get energy out when it's oxidized. Now, we're going to be talking about oxidation and reduction today. And then they all use ATP which you've talked a lot about here. And the couple here is ADP. Put energy in.

You make ATP which is a high energy intermediate. And in converting it back to ADP that energy can be released. And this is used in the biochemistry of the cell. So all cells have these two energy conversion processes in common. OK, so let's look at just summarizing what we're going to go over today. This is a summary of options for life. See also Freeman, Chapter 25. There is some discussion of this.

And we can divide life here between what we call autotrophs.

These are organisms that can make their own organic carbon.

In other words, they can convert carbon dioxide to organic carbon.

Heterotrophs are organisms that can only use organic carbon.

They rely on the guts of other organisms in order to get through life. And so now we're going to systematically go through these processes that fall under each one of these. Oxygenic photosynthesis is the one we've been talking about last time and in my abbreviated version of life on earth.

And this is carried out by eukaryotic organisms, plants, trees, etc., and also by prokaryotic organisms.

Those are the cyanobacteria, microscopic photosynthetic plants.

They use CO<sub>2</sub> and sunlight. So our first variant on this theme we'll get into is a group of bacteria that do anoxygenic photosynthesis. Oxygenic means they evolve oxygen.

These guys use solar energy but they don't evolve oxygen.

And we'll get into how that works. And then there's a group of organisms that still use CO<sub>2</sub>. And in the very similar pathway the Calvin Cycle is photosynthesis. But they use chemical energy in order to make these intermediates to fix CO<sub>2</sub>. OK, so let's talk about those first. And so we're going to talk about the autotrophs.

And all of them share this pathway, CO<sub>2</sub> to C<sub>6</sub>H<sub>12</sub>. This would be glucose.

And it takes ATP to run this reaction and it also takes reduced NADPH -- -- to run this reaction. It also takes this enzyme ribisco which you've talked about I'm sure, ribulose biphosphate carboxylase.

And this is the enzyme that initially takes the CO<sub>2</sub> from the atmosphere and binds it to an organic carbon.

Now, in a detailed version of this is what's called the Calvin Cycle or the Calvin/Benson Cycle. I don't know which one your book calls it. Calvin got the Nobel Prize but Benson was the graduate student that did all the work, so you should recognize that.

Anyway, you studied this in great deal. But an interesting factoid is that ribisco is the most abundant protein on earth.

That tells you how important this reaction is for sustaining life on earth. So notice that in order to drive this reaction, which is the Calvin Cycle, it requires energy and reducing power. So where do they get it?

Well, there are three ways that autotrophs can get energy and reducing power to drive this reaction. And the first is oxygenic photosynthesis. And the second is anoxygenic. And the third is chemosynthesis.

OK, those first three there. So now we're going to go through each of these and look at how they work remembering that all of them are generating ATP and NADPH in order to drive that. So all of the autotrophs have that in common. Well, oxygenic photosynthesis is the one that you know well already. You've studied it in great detail in biochemistry. So we're going to, again, give you the abbreviated version here just so you have a template to map these other ones onto.

These are what are known as the light reactions of photosynthesis, the Z scheme taking solar energy, splitting water, evolving oxygen and synthesizing ATP and NADPH. This is all familiar, right? Very familiar. I'm just writing it in a cartoon version. OK, so this is the NADPH and ADP that goes to fuel that process.

OK, so now, well, at least I can do it on that board.

Let me do it on this board. Anoxygenic -- -- is almost exactly like this process, but instead of splitting water these guys oxidize hydrogen sulfide. So here's our ATP and NADPH.

And they use sunlight to do this.

So these are called photosynthetic bacteria. And they were around very early on the earth. Long before the earth's atmosphere was oxygenated these were the guys that were able to use solar energy and make organic

carbon but without evolving oxygen.

Then somewhere along the line some cell evolved, had some mutations and somehow figured out that water, this abundant source of water was a much better electron donor than hydrogen sulfide.

And once the biochemistry figured this out, you can see the simple substitution here, the whole earth started going in a different direction. So this is an interesting example of how a small biochemical innovation can dramatically change the whole nature of the planet. Now, these guys are still around on earth. In fact, I'm going to show you some.

I'll explain this at the end, but I have some captured in here.

See that little purple band? Those are those guys.

I've got other little tricks in here but I'll save those.

Well, you cannot really see the purple band. But you can come up later and look at it. Those are photosynthetic bacteria.

So they're still around on the earth but they're stuck in places where there's no oxygen. So they have a rather restricted niche on the planet now, but they're still extremely important. What did I do? Oh, here it is.

So one of the places that they can be found, and if you're interested in them a great place to go find some is out at the Mystic Lakes in Arlington which is a permanently stratified lake so the bottom of the lake is always anaerobic. There's never oxygen there.

In a typical lake like that you have a lot of mud on the bottom and you have a lot of hydrogen sulfide coming out of the mud from bacterial processes that we'll talk about. And you have light here.

And so you have a gradient here of this is oxygen and this is  $H_2S$ .

And these photosynthetic bacteria have to live somewhere where there's enough light to photosynthesize and enough hydrogen sulfide to use in this part of the reaction. But they're very sensitive to oxygen so they cannot be in the oxygenated part of the lake.

So you find them in a layer. It's called the squeeze. They have to have light so they have to be up, but they cannot have oxygen so they have to be down. And they need hydrogen sulfide so they have to be down. So they're layered in lakes.

OK. So what about these guys, chemosynthesis?

They don't rely on solar energy. Again, they're still driving the Calvin Cycle reducing CO<sub>2</sub> from the air into organic carbon, but they're not using sunlight. So what do they do? They get their energy -- -- from redox reactions. And let's just show you an example.

Redox reactions couple to the conversion of oxygen to H<sub>2</sub>O. So oxygen is involved in these reactions. And one organism, for example, can take ammonia and convert it to nitrite. Another type of organism can take nitrite and convert it to nitrate. And there are other organisms that can take hydrogen sulfide and convert it to sulfate.

And some can take hydrogen sulfide, oh, no, take iron, ferrous iron, Fe<sup>2+</sup> and convert it to Fe<sup>3+</sup>. So in all of these cases what is happening to these compounds? Are they being oxidized or reduced?

I heard an oxidized. Yes, they're being oxidized.

So these reduced compounds, relatively reduced compounds can be utilized by oxidizing them. The organism can release the energy that's needed. ATP is generated here.

And NADPH is generated by any of these redox couples. So using this energy then the cell takes the reduced NADPH and the ATP and it runs the Calvin Cycle, chemosynthesis. OK. Now, you may think that these are kind of strange, weird bacteria that live in strange pockets of the earth where there's no oxygen. And who cares anyway? They're outdated.

They dominated the earth way back in the early stages of the earth but they're not so important now. Well, that's not true. They're incredibly important. In some ecosystems they're the total base of the entire ecosystem. But also on a global scale, as you'll learn, you should have a feeling for this by the end of this lecture, but also when we talk about global biogeochemical cycles you will learn that these microbes are really messengers for electrons in the environment. Without them the redox balance of the earth would not be maintained, OK? You cannot have nothing but oxidizing reactions or nothing but reduction reactions and have a system sustain itself. So it's these microbes that are playing a really important role in maintaining the redox balance of the earth. OK. Now, one system that I'm going to show you in that DVD, that will do much better justice to it than my drawings here, that's a deep-sea volcano in case you didn't recognize it. And this is 2500 meters at the bottom of the ocean, very, very deep. And there is intense heat. I mean just think of a volcano on the surface of the earth. Intense heat and reduced compounds are found in the earth's mantle that are ready to erupt through this deep-sea volcano. And you have sulfate in the sea water that percolates through here. And as it percolates in and gets drawn into the volcanic stuff that's coming out of here it's reduced to hydrogen sulfide coming out of the volcano.

But you have oxygen in the water in the deep-sea. And we'll be talking about this when we talk about ocean

circulation. But the oceans have a global ocean circulation where the surface water that's in equilibrium with the atmosphere actually sinks and travels along the bottom of the ocean. So there is oxygen in the bottom of the ocean, unlike many lakes where you don't have oxygen.

And we'll talk about that difference. And in the hot vents the water coming out of here can be very, very hot, but there's a gradient right as it comes out meeting the colder sea water.

And so what you have here is a perfect incubator for chemosynthetic bacteria -- -- that use the hydrogen sulfide in chemosynthesis to fix carbon dioxide using the oxygen here. And that forms the base of the entire food web in the deep ocean because there's no light down there.

There's no photosynthesis. There's only chemosynthesis.

And just a little story that goes back to when I first came to MIT as an assistant professor in 1976. You weren't even born. But when I was young we used to go the Muddy Charles Pub periodically after work and have beers. And there was a professor, in this department actually, John Edmond, who passed away several years ago but who used to be there. It was sort of like our Cheers.

And I'll never forget the day he came back from a cruise.

He came to the pub. He was a chemist and I'm a biologist.

And he said you will not believe what we found on the bottom of the ocean. He had gone down in Alvin, this two-person submersible vehicle.

And he started talking about these giant clams and these giant tube worms and all of these things, and I thought he had had one too many beers. I found it hard to believe. Well, it turned out that that was the first discovery of these deep-sea vents and he was on that expedition. And through that collegial relationship I actually ended up with one of the clam shells from the clams there, which is one of the giant clams.

Their meat is blood red because they have a special kind of hemoglobin that they use to keep the oxygen tension perfect for these chemosynthetic bacteria. If the oxygen is too high they cannot do this because it will spontaneously oxidize the H<sub>2</sub>S.

So the oxygen tension is very critical.

And they have a special kind of hemoglobin that does that.

So these clams had symbiotic chemosynthetic bacteria.

Well, since then these vents have been discovered everywhere and ecosystems similar have been discovered on the surface.

And there are all kinds of different vents.

You're going to learn about not only hydrothermal vents, hot vents in this video, but also cold seeps they're called where you have methane bacteria that are really important. OK.

So these are the main ways in which organisms can get energy to convert CO<sub>2</sub> to organic carbon. Then you have all these heterotrophs, the ones that use the organic carbon, and they have various ways of doing that. You've learned in biochemistry the primary way, which is very powerful, and that is using aerobic respiration to do that.

And so we are just going to abbreviate that here.

That's our reverse of photosynthesis. So heterotrophs.

So we have first aerobic.

And let me jump ahead with the slides.

OK, there you are. So this is a cartoon version of aerobic respiration. So we'll just put glucose, we'll come down to the Krebs' Cycle. And we are going to let electrons flow here and have oxygen be the final electron acceptor creating water. So we've really just accomplished the absolute reverse of photosynthesis and we've made NADH in doing this and we've made ATP. So these guys are getting the energy out of the glucose that all of the other organisms made.

And oxygen is the terminal electron acceptor when there's oxygen around. But there are lots of environments, as we've talked about on earth, where there isn't oxygen.

And there are bacteria that can take advantage of those environments.

And instead of having oxygen be the terminal electron acceptor there are a number of other elements that they can use, compounds that they can use.

For example, there are some that use nitrate and they reduce it to nitrous oxide. N<sub>2</sub>. Ammonia. All the relatively reduced forms of nitrogen. And so this called anaerobic.

And this process is called nitrification. And if it weren't for these bacteria, these anaerobic bacteria that can reduce nitrate, nitrogen would never return to the atmosphere. Remember last time we talked about nitrogen fixation, how specific types of microbes can take N<sub>2</sub> from the atmosphere and pull it into the ecosystem?

Well, if you didn't have these bacteria doing this process that nitrogen would never get back to the atmosphere.

They're central to closing the nitrogen cycle.

Then there are some that can use sulfate and reduce it to hydrogen sulfide. As you can imagine, these are critical to creating the hydrogen sulfide that's used in these other processes.

There are some that use CO<sub>2</sub> and convert to methane.

These are methanogenic bacteria, and they're incredibly important in the global carbon cycle and in the methane cycle.

Methane is a really powerful greenhouse gas, and we're going to talk about that later. And then there are some that can take Fe<sup>3+</sup> and reduce it to Fe<sup>2+</sup>. And the same for manganese.

So you should be starting to sense a sort of symmetry here, right, that these anaerobic bacteria are fulfilling functions on the earth. Let me write these down.

These are sulfate reducers, these are methanogens, and these are iron reducers and manganese reducers.

So these will all become extremely important when we talk about the global biogeochemical cycles of all of these elements.

It's these microbes that make sure that the cycles can continue and don't run into a dead end of oxidation or reduction.

OK. Before we go to the movie, I just want to say if you look at Table 25.2 in your textbook, I think it's that one.

I'm assuming I'm using the most recent version.

You'll see a variation of this theme in which there will be some entries of organisms that don't fall into these categories that I've just shown you. And that is to say that there are organisms that use light energy and organic carbon energy at the same time. For every variation that's possible there's an organism that's evolved to take advantage of it. I've just oversimplified it here, but you should know that. And the bottom line is if it's thermodynamically possible. And, again, this whole lecture could have been done in a thermodynamic mode. We could have looked at which redox couples were energetically possible and then assigned those to particular microbes. But for now I just want you to get the overview. But for anything that's thermodynamically feasible there's a microbe out there that's doing it.

And, in fact, microbiologists actually comb through redox tables and put together different redox couples and hypothesize.

I ought to be able to find an organism that does this in that environment. And then they go out. And they can almost always actually find it. So they're incredibly versatile. And it gives you a really good strong feeling for the power of thermodynamics in driving the evolution of these biochemical processes.

Finally, before we show you the movie I want to show you what this thing is all about. There was a Russian microbiologist back in the previous century named Winogradsky -- -- who wanted to isolate some of these photosynthetic bacteria. And knowing what their characteristics were he went out and got himself some mud and some pond water. And he set up what we've come to call a Winogradsky column.

This is a Winogradsky juice bottle, but it works the same. And what you do is you put mud in the bottom and you put pond water here.

And the pond water has basically an inoculum. It has representatives of all different types of bacteria. They might be spores. If they don't like the environment they're in they sporulate and then they just don't germinate. But presumably in pond water you have everything that could possibly grow in here. And in the mud you add a source of sulfate. And so you might add calcium sulfate and you might add a little organic matter, you know, plant parts or something just to jumpstart it.

And eventually you set up a gradient here of hydrogen sulfide and oxygen.

And over time the organisms grow along that gradient.

So you'll end up down here with the anaerobic respiration.

In fact, the organisms generate this gradient. When you start out the whole thing is oxygenated.

And what you should think about in this context is what happens.

How do these gradients get generated when you start out with a completely mixed system, everything in there, everything oxygenated? Eventually you have anaerobic -- First you'll just have anaerobic respiration, right?

Anything that can use organic carbon and oxygen is going to go like mad, and that's what's going to draw the oxygen down.

Then you'll have anaerobic respiration here.

You'll have photosynthesis up here, evolving oxygen. You'll have chemosynthetic bacteria here because they

need a little bit of oxygen but they also need some of this hydrogen sulfide and photosynthetic bacteria here.

Well, they're like down here. Because they need light but cannot have oxygen. And so you can set these up. And this purple band here tells you that you've got your photosynthetic bacteria.