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7.014 Introductory Biology, Spring 2005
Transcript – Lecture 9

So today we're going to continue our focus on DNA which I'm personally enthusiastic about at least in terms of being such a fascinating molecule. And I told you the story last time of how we actually came to understand that DNA was the genetic material. And I still see comments that, oh, God, all this stuff is not relevant to the exam. We're trying to construct the exams in ways that test whether you got the concepts and not just whether you memorized every term that you ran into in the textbook.

So I'm hoping that you will see some greater purpose in why I'm trying to talk about some of this. And also I'm sure some of you will forget the details of transformation, of DNA replication we're going to go into as we sort of burrow into it over the next lecture or so, but what I am hoping you may remember ten years from now, even those who don't go in biology, is how experiments are done, how real people do them.

And that was partly what I was trying to tell you. And you guys are pretty good at figuring out the basic principle that someone had to somehow show that a DNA molecule in one organism could change some organism to have a new characteristic. And as I sort of told you with the work from Frederick Griffith. And then his initial stuff wasn't devoted to that at all.

It was trying to solve a very pressing problem which is dealing with pneumonia in a pre-antibiotic era. And then the finding that he got, that this odd result that something in a heat-killed extract could be transferred to a live bacterium sort of set things up for Avery and his colleagues after a number of years of work to make a very powerful argument that DNA was the genetic material.

But, as I said at the end of last lecture, that paper was published in the 1940s. And people didn't immediately say oh, wow, DNA is the genetic material. Often, and we'll see it again with genetics, there's sometimes sort of the body of science the average person thinks about. Science needs to reach to a certain state before an idea can take hold, even if there's evidence supporting it.

Part of the problem was that chemists had isolated DNA. And the way they used to isolate DNA was really rough on it. Crack the cells open. And what happened, it would all get broken down into little pieces of DNA. And people had worked out the basic chemical structure that it was the deoxyribose and how the things were joined together, but nobody had ever seen anything more than just these little pieces of DNA.

And there was a widely held conception that it was just an anonymous tetranucleotide of G, A, T and C. It wasn't clear why the cells made it, but it didn't look like anything that could encode information. Whereas, as I said, something like proteins, those seem to be very different. And so the world wasn't quite ready for it. Another thing, and this came from one of the comments here, was someone said they didn't know bacteria could take up DNA from the environment.

And, in fact, most bacteria can. It happens that streptacoccucci and some other bacteria at certain phases in their lifetime develop this capacity to take up DNA from the outside. Given what I've told you about a membrane and how hard it is to get things across it, you could imagine it's not trivial to get a DNA molecule which is huge from one side to the other. So it doesn't normally happen.

And what happens if you go into a lab and you're cloning something or other, and we'll talk about how to take a couple pieces of DNA and join them together in a test tube and then put them back into a bacterium. If we put it into E. coli that doesn't normally take up DNA you'll find that it's sort of basically black magic. You cook them up with some divalent cations at very high concentrations, you do temperature shifts and various things, or you give them a big jolt of electricity, and the next thing you know you get some DNA inside.

And it's not a very efficient process, but all you need is one molecule to get in one bacterium and then you're in business. So that was another reason that this wasn't accepted right away. Because this was not a phenomenon that could easily be repeated with other bacteria. So it looked like it was something perhaps special to streptococcus. And what did really change people's understanding, or at least bring people to the understanding that DNA could possibly be the genetic material came about from the discovery of the actual structure of -- How the structure of DNA as a long molecule with complimentary strands and the double helix, the little pictures I showed you with the base pairs, which you know about, and how the two strands which now I'm going to start emphasizing run in opposite directions.

We'll come back to that in a little bit, but the 5 prime to 3 prime direction is this way on one end and 5 to 3 on the other. And just remember back here that there's the 5 prime carbon and that's the 3 prime carbon. So this is the 5 to 3 prime direction of the strand. And then it twists up in 3-dimensional space to form this double helix. And you've seen that movie several times.

So once that structure was discovered then people began to see how these could possibly encode information. It was clearly not just a tetranucleotide of G, A, T, Cs. But we didn't move immediately to that understanding. And today, again sort of trying to show you how biological experiments are done and how they're done by real people, I want to just go on and tell you the key things that happened next. So someone who was very struck by the results of Avery when they came out was Erwin Chargaff who was at Columbia.

And, in fact, my colleague Boris Magasanik whose office is next to mine was a post-doc in Chargaff's lab. So I've got a neighbor of mine who worked with Chargaff. And Chargaff was very struck by this result from Avery and his colleagues that you could take DNA and put it in another organism. And here are a couple of quotes from his writing. One that I liked.

I've sort of had a sense of this in my own research career, this kind of thing. "I saw before me in dark contours the beginning of a grammar of biology." He didn't really know quite how it worked but he sort of sensed that somehow here where you could get down to the language that biology was written. So he started some experiments. And I started with the conviction that if different DNA species exhibited different biological activities there should exist chemically demonstrable differences between deoxyribonucleic acids.

So he was able to start just doing some simple chemical experiments to try and look at DNAs from a whole variety of sources and see what he could learn. And this was not at the structural level. This was just at the chemical level. But one thing he learned was that the base content of DNA, that's the A, G, C, T part of it varied widely between organisms. So this was what Chargaff found in his lab, key findings. And that was important because if DNA was just a molecule of GATC, just a tetranucleotide that every organism made then you'd expect to find the same base composition in all organisms.

He didn't, so that finding essentially buried the monotonous tetranucleotide hypothesis. Another thing he found was that DNA was the same in different tissues -- -- from the same organism -- -- but the proteins varied. And that's a characteristic you'd expect of something that was the genetic information from the cell that all cells have to have sort of the major blueprint. And if you had, even though proteins look like an attractive possibility for that because they had so much variation, this kind of finding wasn't consistent with it and it supported the idea that DNA was the genetic material.

Well, the other thing he could do was he could measure the A, T, G and C content of all these different DNAs. And he noticed some similarities then. And he extracted out of that a couple of generalizations. One was that if you looked at the ratio of the purines, those are the ones with the two rings, adenosine and guanidine over the pyrimidines, those are the ones with the single ring which were C and T, it was about one.

Another thing he noticed was that the ratio of A to T was about one and the ratio of G to C was about one. Now, that was an important clue but it didn't lead to any immediate breakthrough, even though maybe now that you know the structure you can see, gee, if I had been there maybe I would have been smart enough to jump on that number. So instead the work that led to the structure of DNA now introduces a couple of other characters who you've heard of a lot, Jim Watson and Francis Crick.

At the time that Avery made his discovery reporting DNA was transformation, and Jim Watson described himself later as a precocious college boy in Chicago who was consumed by ornithology. So he was into bird watching. That's what he was excited about at the time Avery did his experiment. And Francis Crick at that point was a physicist, and he was in the British Navy designing Naval mines.

So that's where those two players were at the time of Avery's results. So then both Francis Crick and Jim Watson ended up in Cambridge, England about 1950. I think Crick got there around 1949 and Jim Watson got there in 1951. Francis Crick was a grad student, 35 years old at the time. I'll show you pictures in a minute. 35 years old at the time and still working on his PhD.

So he was a pretty elderly grad student, if you want to think of it that way. And Jim Watson was a young hot-shot. He had done his PhD working with Salvador Luria who was at Indiana University at the time. Salvador Luria was one of the Nobel Laureates at MIT. He founded the Cancer Center, which is still here right across from the main biology building. And Jim was a very, very bright and brash young guy, and he had done his PhD with Salva and then he went to Cambridge as well.

And the reason they both went to Cambridge was they were attracted by the power of x-ray crystallography. Now, I said a little work about that earlier, that if you take

x-rays and you bounce them off a crystal and then measure the diffraction pattern you can work backwards by Fourier transforms and whatnot to figure out what the underlying crystal structure is. For the purposes of this course the mechanics of how that's done, we don't have to worry about that right now.

You just need to know that you can work backwards from the diffraction pattern to figure out what the underlying structure was. And I told you, when I introduced to proteins, that the first clues that there were these regions of secondary structure, alpha helices and beta sheets came because people saw characteristic reflections in these diffraction patterns of certain proteins.

And I also told you the story of how Linus Pauling had gone to Oxford, had gotten sick and tired of reading detective novels, started to try and explain the refractions in a certain class of proteins and came up with a model for the alpha helix. And so that was the sort of thing that inspired Watson and Crick. They were both interested in how one could get the structure of DNA. Now, Cambridge also had a very good x-ray crystallogram group.

And just in passing it's interesting as to why they didn't come up with the structure of the alpha helix. There were two things. One was just lack of basic knowledge. I told you that the peptide bond, if you remember I emphasized that you cannot rotate it because the electrons are distributed. Pauling was an outstanding chemist. He knew that fact.

And the folks at Cambridge who were doing that didn't learn this until later, so their models were far less constrained because they could have rotation around that bond. And the other one was just an experimental thing that the size of the photographic plates they used in the Cambridge lab were too small in the sense that they missed a key reflection that Pauling knew about and they didn't know about.

So this combination led to them being scooped by the other group. But nevertheless the group at Cambridge was absolutely outstanding and at one of the top places in the world to do. And I showed you a couple of pictures when I was showing you the transition state. Sort of what you get out of working backwards from these diffraction patterns is they can measure regions of electron density, and then you fit atoms or fit molecules to the patterns that you see.

And if it's all working you can explain why there are bumps here. There's an oxygen here and so on. There's another one. This is an ATP that's bound actually in a pocket in a protein. But you can sort of see how beautifully the patterns of electron density deduced from the x-ray crystallography will match the chemical structures that we put on the board. So that was the idea, they were going to work out the structure of DNA.

Now, the thing about Watson and Crick, who at this point looked like this, they didn't look inordinately distinguished. In fact, Jim probably looked like, you've probably seen people who look approximately like that around MIT. He would have fit in right here and no one would have noticed. They were not actually x-ray crystallographers. They were just trying to model other people's data. And the best DNA crystallography data was from a young woman Rosalind Franklin, who was working in London.

A very somewhat uneasy alliance with Maurice Wilkins. And in trying to read the history it's a bit complicated because, at least some of what I've read, I think that when Rosalind Franklin arrived at the lab she was told this DNA structure problem was hers. And Maurice Wilkins in whose lab she was working was told that he was sort of working for her. So there was a bunch of confusion in this.

But, in any case, Rosalind Franklin was collecting crystallographic data. And Watson and Crick located some distance away in Cambridge were trying to come up with models that could explain the structure of DNA. And they learned about Rosalind's data. And it was her data that they used to work out the basis, her crystallographic data that they used when they put together their structure.

So if it hadn't been for her they wouldn't have been able to make their discovery. So part of the reason I'm dwelling on this is I think their discovery of the structure of DNA was arguably one of the great intellectual advances of our time. It just opened doors. The whole field of molecular biology became possible once people suddenly saw that DNA was complimentary strands.

You could almost immediately see how you could copy genetic information. It laid the groundwork for what later turned out to be, you know, recombinant DNA and everything else. So much of this pivots around this one discovery. And I think I wouldn't be doing justice to this finding, which you all have heard about for years and years, if I had let you walk away from here thinking this was too young geniuses who sat down in a room with some crystallographic data and emerged with a structure that sort of changed the course of the study of biology.

And, as you can see, changes our society and everything else. There are a couple of accounts of this, there are numerous accounts. One that I found pretty interesting is called "The Eighth Day of Creation," if you ever want to read an interesting book on science. This was Horace Judson's effort to try and put together a history of this happening.

And with all history he's ultimately -- You know, there are some judgment calls by the historian, but this one certainly he tried to be pretty fair-handed and even-handed and he tried to get at the heart of what was going on. Watson wrote a book called "The Double Helix". Jim Watson's a very colorful character, quite brash particularly when he was younger, and that's reflected in this book.

It's an interesting read. Probably more balanced point of view for sure in "The Eighth Day of Creation". And there are now a lot of other books. But what I did, just to try and do this in about a minute or two, was I took a couple of the key things that happened during their adventure of trying to work out the structure of DNA and just kind of ran some of their missteps together, because even though this was a marvelous discovery it just didn't happen.

So they started out, they were inspired by Linus Pauling's discovery of the alpha helix. And I don't know if you can remember the story, but what Pauling decided to do when he was lying in bed and with a strip of paper trying to work out the structure that was giving these reflections in the crystal structure, he said I'm going to start by ignoring the side chains. So that was a brilliant move in the case of the alpha helix because he was then able to figure out that that hydrogen bond between the carbonyl and the amino group, you could see how if you got helix going it would

repeat at exactly the way that would give the reflections that were observed in the crystallography data.

So that was how Watson and Crick sort of did it. Linus Pauling had shown the way. So they decided they would ignore the side chains of DNA. So they started out by saying we won't consider the As,Ts, the Gs and the Cs. Well, given what you know about the structure of DNA that was not a helpful move in trying to work out the structure of DNA. Another thing, for example, that happened was that Jim Watson has no lack of self-confidence.

And so it turned out when he went to hear scientific talks he didn't take notes. And so he went to hear a talk on x-ray crystallography given by Rosalind Franklin, but he didn't quite remember the numbers right. He got the facts a little jumbled, and he and Francis spent a while trying to design models to data that wasn't the right data. It was just not quite remembered right, so there was kind of an inefficiency there.

And then Jim had a bias almost to the end that the phosphate backbones they knew would somehow be on the inside and the bases would be on the outside of the structure. So if that's your sort of starting place then it's sort of hard. So Watson, excuse me, Francis Crick was beginning to suspect that maybe the bases were important. So he hired a young mathematician. And he said, "Can you see if you could work out whether there would be any chemical attraction between any pairs of bases?" And the young mathematician came back and said that he thought G might go with C and A with T.

And given what happened here you might have thought that a light bulb would have gone off, but it didn't. And, in fact, Chargaff visited them and the light bulb went off for nobody. And, in fact, Chargaff wasn't a terribly big fan of what Watson and Crick were trying to do. So the pieces are piling up but still not there. Then a big experimental advance came from Rosalind Franklin.

And that was she discovered that the DNA that they had been diffracting was actually a mixture of two forms. So there were actually two structures in the mix that were contributing to the diffractions. She was able to separate out the two kinds of DNA, DNA-A and DNA-B she called it. And so now this gave a much clearer diffraction pattern, and that's the diffraction pattern that she saw.

And Watson and Crick managed to get a look at this data. And it's a little complicated how that happened, but Crick realized almost right away that there were two strands running in opposite directions. So he know knew it was 5 to 3 in one direction and 5 to 3 in the other direction like that. So you might have thought they were home-free, but no. Jim Watson immediately built a model that paired like with like, A with A, T with T, G with G.

They wrote it up and they were ready to submit the paper. And they gave a presentation to their colleagues at the lab in Cambridge. And they were shot down. And one of the key things was they learned the chemical fact that most of the textbooks were wrong at that time in the way that they depicted the structure of guanine. If you look in your textbook, excuse me, here. So if you were to look in a textbook today you'd see guanine like this, but there is another way you could draw this.

So this you may remember when we were talking about phosphoenolpyruvate that this is an enol form and this is a keto form. And this is the way most of the textbooks were showing guanine at the time. So they were looking at the structure of guanine in textbooks. And if you were trying to work out schemes for putting bases together you can see what's going on up here would be very different.

And if we have a hydrogen here versus if we have an oxygen, if you're trying to say make hydrogen bonds at that particular position, I think all of you understand hydrogen bonds well enough to see how that would throw you off. So once that insight came, once they learned that then the rest of the structure came pretty fast. And there's a movie about this. One of the nice things in it was sort of trying to recreate the experience where I think it was Watson who was shuffling these base pairs around.

And he suddenly realized that you could set up base pairs with A and T and with G and C, and when you looked at them you could see they were geometrically exactly the same shape. You could just take the shape of the G and C pair and lay it right down on the A and T pair. And then you could see how you could build either a G-C or an A-T pair into the repeating structure of this DNA and it would be compatible.

So they built a model and they thought, we can just hit the lights for a second here maybe. I just want you to see what that first model looked like. It looks like something you could hack together in a chemistry lab. They had the bases cut out of metal. And you can see just, you know, here the retort sort of stands using chemistry and various clamps that you would use for clamping a flask or something if you're doing a chemical lab.

That's the stuff that they were using to put the model together. And they published then a paper in Nature that told about this result. That's the entire paper reporting the structure of DNA. And maybe you can see there's a little hand-drawn double helix right there that captures the elements. That is the paper, and that was in the journal Nature. And it had in it, right near the end, one of the coyest sentences in the scientific literature.

They didn't want to go into all the details that if you had an A paired with G and G paired with a C and you pulled them apart then you could replicate the molecule by redoing it. So all they said was, "It has not escaped our notice that the specific pairings we expostulated immediately suggests a copying mechanism for DNA." So this is a picture of Jim Watson wearing short pants at Cold Spring Harbor in 1953 reporting this structure of DNA.

Cold Spring Harbor is on Long Island. It's been one of the Meccas for molecule biology since the 1940s. They have a famous symposium once a year. The topic changes every year and rarely repeats. And it was at one of those symposia -- This was the year that they discovered the structure of DNA. And there was Watson. So two years ago they had another meeting, a special meeting just exactly this time of year.

It was in February within a couple of days of right now. So I gave this lecture and I showed the student in the class that this year, I said here's a picture of Jim Watson displaying the structure. They're having a meeting 50 years later in 2003. And I'm going down there. I'm asked to give a talk. And I'll come back and I'll tell you what it was like. So I gave my lecture. I dashed out to the airport.

I hoped on the plane. I went down and I registered. They gave me, you know, the stuff to get into my room, a little envelope with the key card and things. And I went up to my room. And I took out the key card. And what did I find myself looking at? The same picture I had shown to the class just a couple of hours earlier. Here's another picture of Jim the way he looked at the time when he made this amazing discovery. That's Salvador Luria who I mentioned.

I tell you about him in a subsequent lecture. I was at another meeting a few years earlier where some of the old-timers were razzing each other, and someone showed this picture. And then they got up and they gave it a title. And that was "Picture of a Man Picking His Own Pockets". So they would tease each other a lot. And I'm hoping maybe you'll get a chance to hear a little bit more about that soon.

This is what Jim Watson looks like now. I asked to get a picture taken just so you could see he's still around and is very active and still very controversial. This doesn't make much of a difference. Here's a picture of Watson and Crick a little bit later just sitting out on a porch in Cold Spring Harbor. It's sort of right on the edge of a bay down there in a very relaxed kind of atmosphere that still permeates molecule biology research to this day.

Francis Crick just died last July at the age of 88, so we've just lost the link to one of the two people who did this amazing experiment. OK. So I want to then set things up for the details of DNA replication. So there was a basic principle that came across from this that you could see how this could work, that DNA was sort of like having a photograph and a negative. And so the information is actually in there twice.

It's just in different forms. And when I tell you about DNA repair in another lecture you can maybe see already how useful that is because if you damage one strand you're not really out of luck because you've still got the information in the other strand. And you could probably, on the basis of that, device a repair strategy if you thought about it. But more importantly for DNA replication finally gave an insight to this thing that had been vexing people forever.

If you had to have all this information for making a cell, and every time a cell divided and you saw how it can happen pretty quickly with something like a bacterium of yeast, how could you accurately copy all that DNA, excuse me, all that genetic information? How is it stored? How could it be done? And once you saw ah, it's just a matter of separating the strands, and if there's an A there put a T there, if there's a C you put a G and so on, was a huge breakthrough.

But that then didn't tell people how DNA replicated or even if this is the mechanism. You can actually come up with all kinds of models for how you could replicate things based on this principle, including crisscrossing between strands and all sorts of things. The predominant model and perhaps the simplest one was called semi-conservative. And it thought of the problem in this kind of way, that if you had two strands of the original DNA molecule and then you pulled them apart that one of the strands here would become one of the strands of the daughter, and then the new one would be here and the same thing would happen on the other side.

And then if you did it again this thing would happen again with a new strand. This time the skinny strand here would be like this, the skinny strand here would be like this, and then this one again. We'd have one that was nearly synthesized plus one of

the originals. So this model was one of the simplest because it kept this strand intact throughout the whole process while some of the other models had them being patched back together, all based on the idea that A pairs with T and G pairs with C.

But proving that this was the correct model was then another important advance. And that was done by Frank Stahl and Matt Messelson. Actually, I think I'll skip this for right now. Matt is a professor up at Harvard, just up at Harvard Square not very far from here, still very active. Frank Stahl is a professor out in Oregon. He's still active.

So one of the differences about this course is a lot of the things I'm telling you about -- And this is pretty old stuff right now, right, molecule biology. The people who did these are still around and very active. This is most of modern biology is a pretty young science, and many of the major characters are still running around and with us today. So, anyway, what Matt and Frank were at Caltech. And they, with a bunch of other students had an apartment.

And they were sitting around trying to work out a way to figure out this model. And they came up with an idea, and that was to see if you could differentially label what we might call "old DNA" and the "new DNA" here. And since it's chemically the same stuff it's a bit of a trick. How do you tell old DNA from new DNA? So their idea was since nitrogen comes in two different isotopes, N14 which is the common one and N15 with is one mass heavier, that maybe you could start out with the DNA, for example, grown in N15.

And then when you started replication switch to N14. And then you'd be able to tell, if you could separate these molecules on the basis of their density since the one with the N15 would be heavier than the one with the N14, then maybe you could work this out. And the story goes, this has been written, they were sitting arguing about this, or talking about this idea at the table.

And it was a good idea but there was a problem. And that was how could you separate the two kinds of DNA based on their density? So they had a piece of fingernail and they were trying to see whether they could get it to float by dissolving more and more sugar. And they figured if they added more and more sugar the water would get denser and denser so the could float the fingernail.

And they weren't able to do it. But all chemists made a periodic, probably some places here at MIT, they had a periodic chart right in their living room. So they went and they looked. And then they looked at sodium. And they went down the periodic table and then they saw cesium. And thought maybe, you know, if you took a solution of cesium chloride and you put it a centrifuge and you spun really hard then you'd get a gradient of varying concentrations, of slightly different concentrations of cesium chloride.

And that they could tune that to a range that would discriminate between the heavy and the lighter forms of DNA. So the experiment they did is known as the Messelson-Stahl experiment. But, as I say, these are names that come from real people. And the idea was pretty simple. They grew the bacteria for many generations -- -- in N15 medium. This is the so-called heavy or H isotope -- -- of nitrogen.

And then at time equals zero in their experiment, when they're ready to start the experiment they switched to medium with N14, which we'll think of as the light or

the L isotope. And then they isolated DNA -- -- after let's say increasing rounds of replication that you could tell simply by measuring how much DNA was in your bacterial culture when the bacteria had doubled their DNA. And this is the data they got which looks something like this.

In fact, in this case the blackboard representation is pretty close. So this is cesium chloride. And it has been centrifuged very hard so that there's a gradient now that's light at the top and a little heavier at the bottom of the gradient. There's a little more cesium chloride per ml here then there is there in the tube. And I'll just give us three little sort of reference marks here.

So what they found when they started was that all of the DNA was at that position down at the heavy end. And then this is after one generation. So the DNA has now doubled. What they found was that all the DNA was now at this intermediate position. And after two generations or two DNA replications they now found that some of the DNA was here, some of the DNA was there.

And if they went to three or more what they saw was they began to pile stuff up there. And I think most of you could probably make the connection between that data and that picture that I've got up there. This is the heavy-heavy DNA. This is the heavy-light. So this would be heavy-heavy, heavy-light, light-heavy. After one round it will all be here. After two we have heavy-light, but this one is light-light, light-light, light-heavy.

And so now we've got light-light, the heavy-light, no heavy-heavy is ever going to show up again. And the longer you do this the more you'll get the light accumulating. A very simple experiment done by real people but enormously powerful because now it showed that this basic idea, you have the photograph and negative, you pull them apart and copy them was right. So at this point you begin to see why data of Avery's that before people had trouble accepting, all of a sudden now it was really you needed a CYD and A was the genetic material.

And this is what sort of ushered in this great burst of molecular biology. So in the next lecture what we're going to start doing now is once you, this is all great, but once we start figuring out how to replicate it we're going to have to get down to enzymes and biochemical steps. And there are some formidable challenges to replicating DNA, and it's also awesome. I'll tell you at the beginning of next lecture how much DNA we have and just how accurate it is.

It always blows me away. I'll see you then. Take care.