Good morning. Good morning.

So, what I would like to do today is pick up on our basic theme of molecular biology. We've talked about DNA replication.

The transcription of DNA into RNA, and the translation of RNA into protein. We discussed last time some of the variations between different types of organisms: viruses, prokaryotes, eukaryotes, with respect to the details of how they do that in general that bacteria have circular DNA chromosomes typically that eukaryotes have linear chromosomes, etc. What I'd like to talk about today is variation, but variation not between organisms but within an organism from time to time and place to place, namely, how it is that some genes or gene activities are turned on, on some occasions, and turned off on other occasions. This is, obviously, a very important problem to an organism, particularly to somebody like you who's a multi-cellular organism, and has the same DNA instruction set in all of your cells.

It's obviously quite important to make sure that the same basic code is doing different things in different cells.

It's important, also, to a bacterium to make sure that it's doing different things at different times, depending on its environment. So, I'm going to talk about a very particular system today as an illustration of how genes are regulated, but before we do that, let's Ask, where are the different places in this picture?

DNA goes to DNA goes to RNA goes to protein, in which you might, in principle, regulate the activity of a gene. Could you regulate the activity of a gene by actually changing the DNA encoded in the genome? So, why not? Because what? It becomes a different gene. Yeah, that's just a definition.

Why couldn't the cell just decide that I want this gene now to change in some way? Oh, I don't know, I'll alter the DNA sequence in some way. And, that'll make the gene work.

Could that happen? Is that allowed? Yeah, it turns out to happen.

It's not the most common thing, and it's not the thing they'll talk about in the textbooks a lot but you can actually do regulation.

So, the levels of regulation are many, and one is actually at the level of DNA rearrangement. As we'll come to later in the course, for example, your immune system creates new, functional genes by rearranging locally some pieces of DNA, some bacteria, particularly infectious organisms control whether genes are turned on or off by actually going in there, and flipping around a piece of DNA in their chromosome.

And, that's how they turn the gene on or off is they actually go in and change the genome. There's some protein

that actually flips the orientation of a segment of DNA. Now, these are a little funky, and we're not going to talk a lot about them, but you should know, almost anything that can happen does happen and gets exploited in different ways by organisms.

So, DNA rearrangement certainly happens. It's rare, but it's always cool when it happens.

So, it's fun to look at. And, something like the immune system can't be dismissed as simply an oddity. That's an incredibly important thing. The most common form is at the level of transcriptional regulation, where whether or not a transcript gets made is how it's processed can be different. First off, the initiation of transcription that RNA polymerase should happen to sit down at this gene on this occasion and start transcribing it is a potentially regulatable (sic) step that maybe you're only going to turn on the gene for beta-globin and alpha-globin that together make the two components of hemoglobin, and you're only going to turn them on in red blood cells, or red blood cell precursors, and that could be done at the level of whether or not you make the message in the first place. That's one place it can be done.

Another place is the splicing choices that you make.

With respect to your message, you get this thing with a number of different potential exons, and you can regulate how this gene is used by deciding to splice it this way, and skip over that exon perhaps, or not skip over that exon. That alternative spicing is a powerful way to regulate. And then finally, you can also regulate at the level of mRNA stability.

Stability means the persistence of the message, the degradation of the message. It could be that in certain cells, the message is protected so that it hangs around longer.

And, in other cells, perhaps, it's unprotected and it's degraded very rapidly. If it's degraded very rapidly, it doesn't get a chance to make a protein or maybe it doesn't get to make too many copies of the protein. If it's persistent for a long time, it can make a lot of copies of protein.

All of those things can and do occur. Then, of course, there is the regulation at the level of translation.

Translation, if I give you an mRNA, is it automatically going to be translated? Maybe the cell has a way to sequester the RNA to ramp it up in some way so that it doesn't get to the ribosome under some conditions, and under other conditions it does get to the ribosome, or some ways to block in other manners than just sequestering it, but to physically block whether or not this message gets translated, what turns out that there's a tremendous amount of that. It's, again, not the most common, but we're learning, particularly over the last couple of years, that regulation of the translation of an mRNA is important.

There are, although I won't talk about them at length, an exciting new set of genes called micro RNA's, teeny little RNAs that encode 21-22 base pair segments that are able to pair with a messenger RNA and interfere in some ways partially with its translatability. And so, by the number and the kinds of little micro RNAs that are there, organisms can tweak up or down how actively a particular message is being translated.

So, the ability to regulate translation in a number of different ways is important. And then, of course, there's post-translational control. Once a protein is made, there's post-translational regulation that could happen.

It could be that the protein is modified in some way.

The proteins say completely inactive unless you put a phosphate group on it, and some enzyme comes along and puts a phosphate group on it. Or, it's inactive until you take off the phosphate group.

All sorts of post-translational modifications can occur to proteins after the amino acid chain is made that can affect whether or not the protein is active. Every one of these is potentially a step by which an organism can regulate whether or not you have a certain biochemical activity present in a certain amount at a certain time. And, every one of these gets used. This is the thing about coming to a system that has been in the process of evolution for three and a half billion years is that even little differences can be fought over as competitive advantages, and can be fixed by an organism. So, if a tiny little thing began to help the organism slightly, it could reach fixation. And, you're coming along to this system, which has had about three and a half billion years of patches to the software code, and it's just got all sorts of layers and regulation piled on top of it. All of these things happen. But, what we think is the most important out of this whole collection is this guy.

The fundamental place at which you're going to regulate whether or not you have the product of a gene is whether you bother to transcribe its RNA. But I do want to say because, yes? And, which exons you used and which aren't? Yeah, well, there are tissue-specific factors that are gene-specific that can influence that. And, surprisingly little is known about the details. There are a couple of cases where people know, but as you'd imagine, you actually need a regulatory system in that tissue to be able to decide to skip over that exon.

And, the mechanics of that surprisingly are understood in very few cases. And, you might think that evolution wouldn't like to use that as the most common thing because you really do have to make a specialized thing to do that. So, that's what happens on these. That's one in particular where I think a tremendous amount of more work has to happen.

mRNA stability, we understand some of it but not all the factors in this business. I was telling you about translation with these little micro-RNAs is stuff that's really only a few years old that people have come to understand. So, there's a lot to be understood about these things. I'm going to tell you about initiation of mRNAs, because it's the

area where we know the most, and I think it'll give you a good idea of the general paradigm.

But, any of you who want to go into this will find that there's a tremendous amount more to still be discovered about these things.

So, the amount of protein that a cell might make varies wildly.

Your red blood cells, 80% of your red blood cells, protein, is alpha or beta-globin. It's a huge amount. That's not true in any other cell in your body. So, we were talking about pretty significant ranges of difference as to how much protein is made.

How do things like that happen? Well, I'm going to describe the simplest and classic case of gene regulation and bacteria, and in particular, the famous lack operon of E coli.

So, this was the first case in which regulation was ever really worked out, and it stands today as a very good paradigm of how regulation works. E coli, in order to grow, needs a carbon source. In particular, E coli is fond of sugar.

It would like to have a sugar to grow on. Given a choice, what's E coli's favorite sugar? It's glucose, right, because we have the whole cycle of glucose. The whole pathway of glucose goes to pyruvate, which we've talked about, and glucose is the preferred sugar to go into that pathway, OK, of glycolysis.

Glycolysis: the breakdown of glucose. But, suppose there's no glucose available. Is E coli willing to have a different sugar?

Sure, because E coli's not stupid. If it were to refuse another sugar, it wouldn't be able to grow. So, it has a variety of pathways that will shunt other sugars to glucose, which will then allow you to go through glycolysis, etc. Now, given a choice, it would prefer to use the glucose. But if not, suppose you gave it lactose. Lactose is a disaccharide. It's milk sugar, and I'll just briefly sketch, so lactose is a disaccharide where you've got a glucose and a galactose.

Glucose plus galactose equals lactose. So, if E coli is given galactose, it is able to break it down into glucose plus galactose.

And it does that by a particular enzyme called beta galactosidase, which breaks down glactosides. And, it'll give you galactose plus glucose. How much beta-galactosidase does an E coli cell have around? Sorry? None? But how does it do this?

When it needs it, it'll synthesize it. When it needs it, like, there's no glucose and there's a lot of galactose around,

how much of it will there be? A lot. It turns out that in circumstances where E coli is dependent on galactose as its fuel, something like 10% of total protein can be beta-gal under the circumstances when you have galactose but no glucose. Sorry? Sorry, when you have lactose but no glucose. Thank you. So, when you have lactose but no glucose, E coli has 10% of its protein weight as beta-galactosidase. Wow. But when you have glucose around or you don't have lactose around, you have very little.

It could be almost none, trace amounts. So, why do this?

Why not, for example, just have a far more reasonable some compromise?

Like, let's always just have 1% of beta-galactosidase.

Why do we need the 0-10%? 10%'s actually extremely high.

So what. It's a good insurance policy. So, if I only have galactose, I need more. Well, I mean, 1% will still digest it. I'll still do it. What's the problem? Sorry?

So what, I do it at a slower rate. Life's long. Why not? Ah, it has to compete. So, if the cell to the left had a mutation that got it to produce four times as much, then it would soak up the lactose in the environment, grow faster, etc. etc., and we could have competed.

So, these little tuning mutations have a huge effect amongst this competing population of bacteria. And so, if E coli currently thinks that it's really good to have almost non at sometimes and 10% at other times, you can bet that it's worked that out through the product of pretty rigorous competition, that it doesn't want to waste the energy making this when you don't need it, and that when you do need it, you really have to compete hard by growing as fast as you can when you have that lactose around. OK. So, how does it actually get the lactose, sorry, keep me honest on lactose versus galactose, into the cell? It turns out that it also has another gene product, another protein, which is a lactose permease. And, any guesses as to what a lactose permease does? It makes the cell permeable to lactose, right, good. So, the lactose can get into the cell, and then beta-gal can break it down into galactose plus glucose. These two things, in fact, both get regulated, beta-gal and this lactose permease. So, how does it work?

Let's take a look now at the structure of the lack operon.

So, I mentioned briefly last time, what's an operon? Remember we said that in bacteria, you often made a transcript that had multiple proteins that were encoded on it.

A single mRNA could get made, and multiple starts for translation could occur, and you could make multiple proteins.

And, this would be a good thing if you wanted to make a bunch of proteins that were a part of the same biochemical pathway.

Such an object, a regulated piece of DNA that makes a transcript encoding multiple polypeptides is called an operon because they're operated together. So, let's take a look here at the lack operon. I said there was a promoter.

Here is a promoter for the operon, and we'll call it P lack, promoter for the lack operon. Here is the first gene that is encoded. So, the message will start here, actually about here, and start going off. And, the first gene is given the name lack Z.

It happens to encode beta-galactosidase enzyme.

Remember, they did a mutant hunt, and when they did the mutant hunt, they didn't know what each gene was as they isolated mutants.

So, they just gave them names of letters. And so, it's called lack Z. And, everybody in molecular biology knows this is the lack Z gene, although Z has nothing to do with beta-galactosidase. It was just the letter given to it.

But, it's stuck. Next is lack Y.

And, that encodes the permease. And, there is also lack A, which encodes a transacetylase, and as far as I'm concerned you can forget about it. OK, but I just mentioned that it is there, and it actually does make three polypeptides.

We won't worry about it, OK, but it does make a transacetylase, OK? But it won't figure in what we're going to talk about, and actually remarkably little is known about the transacetylase. There's also one other gene I need to talk about, and that's over here, and that's called lack I. And, it too has a promoter, which we can call PI, for the promoter for lack I.

And, this encodes a very interesting protein.

So, we get here one message encoding one polypeptide here.

This mRNA encodes one polypeptide. It is monocystronic. This guy here is a polycystronic message. It has multiple cystrons, which is the dusty old name for these regions that were translated into distinct proteins. And so, that's that mRNA.

So, lack I, this encodes a very interesting protein, which is called the lack repressor. The lack repressor, actually

I'll bring this down a moment, is not an enzyme.

It's not a self-surface channel for putting in galactose.

It is a DNA binding protein. It binds to DNA. But, it's not a nonspecific DNA binding protein that binds to any old DNA.

It has a sequence-specific preference.

It's a protein that has a particular confirmation, a particular shape, a particular set of amino acids sticking out, that it combined into the major groove of DNA in a sequence-specific fashion such that it particularly likes to recognize a certain sequence of nucleotides and binds there. Where is the specific sequence of nucleotides where this guy likes to bind? It so happens that it's there.

And this is called the operator sequence or the operator site.

So, this protein likes to go and bind there. Now, I've drawn this, by the way, so that this operator site is actually right overlapping the promoter site.

Who likes to bind at the promoter site? RNA polymerase.

What's going to happen if the lack repressor protein is sitting there?

RNA polymerase can't bind. It's just physically, blocked from binding. So, let's examine some cases here.

Let's suppose that we look at here at our gene. We've got our promoter, P lack. We've got the operator site here. We've got the lack Z gene here, and we've got the lack repressor, lack I, the repressor sitting there.

Polymerase tries to come along to this, and it's blocked.

So, what will happen in terms of the transcription of the lack operon: no mRNA. So, that's great.

So, we've solved one problem right off the bat.

We want to be sure that sometimes there's going to be no mRNA made.

This way, we're not going to waste any metabolic energy, making beta-galactosidase. Are we done? No? Why not.

We've got to sometimes make beta-galactosidase.

So, we've got to get that repressor off there. Well, how is the repressor going to come off there? When do we

want the repressor off there: when there's lactose present.

So, somehow we need to build some kind of an elaborate sensory mechanism that is able to tell when lactose is present, and send a signal to the repressor protein saying, hey, lactose is around. The signal gets transmitted all the way to the repressor protein, and the repressor protein comes off.

What kind of an elaborate sensory mechanism might be built?

Use lactose as what? So, this is actually pretty simple.

You're saying just take lactose, and you want lactose to be its own signal? So, if lactose were to just bind to the repressor, the repressor might then know that there was lactose around.

Well, what would it do if lactose bound to it? Sorry? Why would it fall off? Yep. More interested in the lactose.

So, if you're suggestion, this is good. I like the design work going on here. The suggestion is that if lactose binds to this here, binds to our repressor, it's going to fall off because it's more interested in lactose than in the DNA. Now, how is the interest actually conveyed into something material? Because the actual level of cognitive like or dislike for DNA on the part of this polypeptide is unclear, you may be anthropomorphizing slightly with regard to this polypeptide chain. So, mechanistically, what's going to happen? Shape. Yes, shape? Change confirmation, the binding act, the act of binding lactose creates some energy, may change the shape of the protein, and that shape of the protein may, in the process of wiggling around to bind lactose may de-wiggle some other part of it that now no longer binds so well to DNA. That is exactly what happens.

Good job. So, you guys have designed, in fact, what really happens. What happens is what's called an allosteric change. It just means other shape.

So, it just changes its shape, that it changes shape on binding of lactose. And it falls off because it's less suitable for binding this particular DNA sequence when it's bound to lactose there. So, in this case, in the presence of lactose, lack I does not bind.

And, the lack operon is transcribed. Yes? Uh-oh. OK, all right designers, here we've got a problem. You have such a cool system, right? You were going to sense lactose.

Lactose was going to bind to the lack repressor, change its confirmation falloff: uh-oh. But, as you point out, how's it going to get any lactose, because there's not a lactose permease because the lactose permease is made by the same operon. So, what if, in fact, instead of getting one of these DOD mill speck kind of things of some repressor that is absolutely so tight that it never falls off under any circumstances, what if we build a slightly sloppy repressor

that occasionally falls off, and occasionally allows transcription of the lack operon? Then, we'll have some trace quantities of permease around. With a little bit of permease around, a little lactose will get in.

And, as long as even a little lactose gets in, it'll now shift the equilibrium so that the repressor is off more, and of course that will make more permease, and shift, and shift, and shift, and shift. So, as long as it's not so perfectly engineered as to have nothing being transcribed, so no mRNA is really very little mRNA. See, this is what's so good, I think, about having MIT students learn this stuff because there are all sorts of wonderful design principles here about how you build systems. And, I think this is just a very good example of how you build a system like this.

Now, all right, so we now have the ability to have lack on and lack off, and that is lack off, mostly off because of your permease problem: very good. Now, let's take a little digression about, how do we know this? This kind of reasoning, I've now told you the answer. But let's actually take a look at understanding the evidence that lets you conclude this.

So, in order to do this, and this is the famous work in molecular biology of Jacobin Manoux in the late '50s for which they won a Nobel Prize, they wanted to collect some mutants.

Remember, this is before the time of DNA sequence or anything like that, and wanted to collect mutants that affected this process.

So, in order to collect mutants that screwed up the regulation, they knew that beta-galactosidase was produced in much higher quantity if lactose was around. The difficulty with that was that wild type E coli, when you had no lactose would produce very little beta-gal, one unit of beta-gal, and in the presence of lactose, would produce a lot, let's call it 1, 00 units of beta-gal. But, the problem with playing around with this is lactose is serving two different roles.

Lactose is both the inducer of the expression of the gene by virtue of binding to the repressor, etc., etc.

But, it's also the substrate for the enzyme because as beta-galactosidase gets made, it breaks down the lactose. So, there's less lactose in binding, and if you wanted to really study the regulatory controls, you have the problem that the thing that's inducing the gene by binding to the repressor is the thing that's getting destroyed by the product of the gene. So, it's going to make the kinetics of studying such a process really messy. It would be very nice if you could make a form of lactose that could induce beta-galactosidase by binding to the repressor, but wasn't itself digested.

Chemically, in fact, you can do that. Chemically, it's possible to make a molecule called IPTG, which is a galactoside analog. And, what it does is this molecule here which I'll just sketch very quickly here, it's a sulfur there, and you can see vaguely similar, this is able to be an inducer.

It'll induce beta-gal, but not a substrate. It won't get digested.

So, it'll stick around as long as you want. It's also very convenient to use a molecule that was developed called ex-gal.

Ex-gal again has a sugar moiety, and then it also has this kind of a funny double ring here, which is a chlorine, and a bromine, and etc. And, this guy here is not an inducer. It's not capable of being induced, of inducing beta-galactosidase expression. But, it is a substrate.

It will be broken down by the enzyme, and rather neatly when it's broken down it turns blue. These two chemicals turned out to be very handy in trying to work out the regulation of the lack operon. So, if I, instead of adding lactose, if I think about adding IPTG, my inducer, when I add IPTG I'm going to get beta-gal produced. When I don't have IPTG, I won't produce beta-gal. But then I don't have a problem of this getting used up. So now, what kind of a mutant might I look for? I might look for a mutant that even in the absence of the inducer, IPTG, still produces a lot of beta-gal. Now, I can also look for mutants that no matter what never produce beta-gal, right? But, what would they likely be? They'd likely be structural mutations affecting the coding sequence of beta-gal, right? Those will happen.

I can collect mutations that cause the E coli never to produce beta-gal. But that's not as interesting as collecting mutations that block the repression that cause beta-gal to be produced all of the time. So, how would I find such a mutant?

I want to find a mutant that's producing a lot of beta-gal even when there's no IPTG. So, let's place some E coli on a plate. Should we put IPTG on a plate? No, so no IPTG.

What do I look for? How do I tell whether or not any of these guys here is producing a lot of beta-gal? Yep?

So, no IPTG, but put on ex-gal, and if anybody's producing a lot of beta-gal, what happens? They turn blue: very easy to go through lots of E coli like that looking for something blue.

And so, lots of mutants were collected that were blue.

And, these chemicals are still used today. They're routinely used in labs, ex-gal and stuff like that, making bugs turn blue because this has turned out to be such a well-studied system that we use it for a lot of things. So, mutants were found that were constituative. So, mutants were found that were constituative mutants. Constituative mutants: meaning expressing all the time, no longer regulated, so, characterizing these constituative mutants.

It turns out that they fell into two different classes of constituative mutants. If we had enough time, and you could

read the papers and all, what I would do is give you the descriptions that Jacobin Maneaux had of these funny mutants which they'd isolated and were trying to characterize, and how to puzzle out what was going on.

But, it's complicated and hard, and makes your head hurt if you don't know what the answer is. So, I'm going to first tell you the answer of what's going on, and then sort of see how you would know that this was the case. But, imagine that you didn't know this answer, and had to puzzle this out from the data.

So, suppose we had, so if there were going to be two kinds of mutants: mutant number one are operator constituents.

They have a defective operator sequence. Mutations have occurred at the operator site. Mutant number two have a defective repressor protein, the gene for the repressor protein.

How can I tell the difference?

So, I could have a problem in my operator site.

What would be the problem with the operator site?

Some mutation to the sequence causes the repressor not to bind there anymore, OK? So, a defective operator site doesn't bind repressors. Defective repressor, the operator site is just fine, but I don't have a repressor to bind at it. So how do I tell the difference? One way to tell the difference is to begin crossing the mutants together to wild type, and asking, are they dominant or recessive, or things like that?

Now, here's a little problem. E Coli is not a diploid, so you can't cross together two E colis and make a diploid E coli, right? It's a prokaryote. It only has one genome. But, it turns out that you can make temporary diploids, partial diploids out of E coli because it turns out you can mate bacteria. Bacteria, which have a bacterial chromosome here also engage in sex and in the course of bacterial sex, plasmids can be transferred called, for example, an F factor, is able to be transferred from another bacteria. And, through the wonders of partial merodiploid, you can temporarily get E colis, or you can permanently get E colis, that are partially diploid. So, you can do what I'm about to say. But, in case you were worried about my writing diploid genotypes for E coli, you can actually do this.

You can make partial diploids. So, let's try out a genotype here.

Suppose the repressor is a wild type, the operator is wild type, and the lack Z gene is wild type. And, suppose I have no IPTG, I'm un-induced. I have one unit of beta-gal. When I add my inducer, what happens? I get 1,000 units of beta-gal.

Now, suppose I would have an operator constituative mutation.

Then, the operator site is defective. It doesn't bind the repressor. Beta-gal is going to be expressed all the time, even in the absence. All right, well that was, of course, what we selected for. Now, suppose I made the following diploid.

I plus, O plus, Z plus, over I plus, O constituative, Z plus. So, here's my diploid. What would be the phenotype? So, in other words, one of the chromosomes has an operator problem.

Well, that means that this chromosome here is always going to be constituatively expressing beta-gal.

But, what about this chromosome here? It won't. So, this would be about 1, 01, give or take, because it's got one chromosome doing that and one chromosome doing this, and this one would be about 2, 00. Now, that quantitative difference doesn't matter a lot. What you really saw when you did the molecular biology was that when you had one copy of the operator constituative mutation, you still got a lot of beta-gal here even in the absence of IPTG. So, that operator constituative site looked like it was dominant to this plus site here.

But now, let's try this one here. I plus, O plus, Z plus, over I plus, operator constituative, Z minus. What happens then?

This operator constituative site allows constant transcription of this particular copy. But, can this particular copy make a working, functional beta-gal? No. So, this looks, when you do your genetic crosses, you find that the operator constituative, now, if I reverse these here, suppose I reverse these, I plus, O plus, Z minus, I plus, O constituative, Z plus, same genotypes, right, except that I flipped which chromosome these are on.

Now, what happens? This chromosome here: always making beta-gal and it works. This chromosome here: not making beta-gal.

Even though it's regulated, it's a mutant. So, in other words, from this very experiment, you can tell that the operator site is only affecting the chromosome that it's physically on, that it doesn't make a protein that floats around.

What it does is it's said to work in cys. In cys means on the same chromosome. It physically works on the same chromosome.

Now, let's take a look, by contrast, of the properties of the lack repressor mutants. If I give you a lack repressor mutant, I plus, O plus, Z plus is the wild type.

I constituative, O plus, Z plus: what happens here?

This wild type is one in 1, 00. This guy here: 1,000 and 1, 00, and then here let's look at a diploid: I plus, O plus, Z plus, I constituative, O plus, Z plus. What's the effect? The I constituative doesn't make a functioning repressor. But, I plus makes a functioning repressor. So, will this show regulation?

Yeah, this will be regulated just fine. This works out just fine, and in fact it'll make 2,000, and it'll make two copies there.

But again, the units don't matter too much. And, by contrast, if I give you I plus, O plus, Z minus, and I constituative, O plus, Z plus, what will happen?

Here, I have my mutation on this chromosome. But, it doesn't matter because I've got my mutation on this chromosome in the repressor. I've got a mutation on lack Z here, but as long as I have a functional copy, one functional copy of the lack repressor, it works on both chromosomes.

It will work on both chromosomes, and so in other words this lack repressor, one copy works on both chromosomes. In other words, it makes a product that diffuses around, and can work on either chromosome, and it's said to work in trans, that is, across.

So, the operator is working in cys. It's operating on its own chromosome only. A mutation in the operator only affects the chromosome it lives on, whereas a functional copy of the lack repressor will float around because it's a protein, and that's how Jacobin Maneaux knew the difference.

They proved their model by showing that these two kinds of mutations had very different properties. Operator mutations affected only the physical chromosome on which they occurred, which of course they had to infer from the genetics they did, whereas repressor, a functional copy repressor, could act on any chromosome in the cell.

So, OK, we've got that. Now, last point, what about glucose?

I haven't said a word about glucose. See, this was a big deal to people.

This model, the repressor model, we have this repressor. What about glucose? What's glucose doing in this picture?

So, glucose control: so here's my gene. Here's my promoter, P lack. Here's my operator, beta-gal.

It's encoded by lack Z. You've got all that. When this guy is present, sorry, when lactose is present, the repressor comes off. Polymerase sits down. Wait a second, polymerase isn't supposed to sit down unless there's no glucose.

We need another sensor to tell if there's glucose, or if there's low glucose. So, we're going to need us a sensor that tells that. Any ideas? Yep?

Yeah, if you work that one through, I don't think it quite works. But, you've got the basic idea. You're going to want another something, and it turns out there's another site over here, OK? There's a second site on which a completely different protein binds. And, this protein is the cyclic AMP regulatory protein, and it so happens that in the cell, when there's low amounts of glucose, let me make sure I've got this right, when there's low amounts of glucose, what we have is high amounts of cyclic AMP. Cyclic AMP turns out, whereas lactose is used directly as the signal, cyclic AMP is used as the signal here. When the cell has low amounts of glucose, it has high amounts of cyclic AMP. Now, what do you want your cyclic AMP to do? How are we going to design this?

It's going to bind to a protein, cyclic AMP regulatory protein, it's going to sit down, and now what's it going to do?

Is it going to block RNA polymerase?

What do we want to do? If there's low glucose, high cyclic AMP, we sit down at the site, we want to turn on transcription now, right? So, what it's got to do is not block RNA polymerase, but help RNA polymerase. So, what it actually does is instead of being a repressor, it's an activator. And what it does is it makes it more attractive for RNA polymerase to bind, and it actually does that by, actually it does it slightly by bending the DNA.

But, what it does is it makes it easier for RNA polymerase to bind.

It turns out that the promoter is kind of a crummy promoter.

It's actually just like, remember the repressor wasn't perfect; the promoter's not perfect either. The promoter's kind of crummy.

And, unless RNA polymerase gets a little help from this other regulatory protein, it doesn't work.

We have two controls: a negative regulator responding to an environmental cue, a positive activator responding to an environmental cue, helping polymerase decide whether to transcribe or not, and basically that's how a human egg goes to a complete adult and lives its entire life, minus a few other details. There are some details left out, but that's a sketch of how you turn genes on and off.