Let's dive in today and look at how geneticists use genetics. I've told you up until now about some of the history of genetics and how it gave rise to our understandings about genetic transmission in traits, about genetic mapping, linkage analysis, how all this helped confirm the Chromosomes Theory.

And we wove in a number of concepts about how scientific theories are developed and data is interpreted and intuitions are made, and then how they're actually proven, what sort of evidence it takes to actually achieve conscientious around theories.

And that often takes sometimes years, many times decades before full contentious is achieved around things. Today I want to turn a little bit to the experimental uses of genetics in a more day-to-day fashion. And you will recall this coat of arms that I put up here.

Function. Gene. Protein. Biochemistry.

Genetics. And I told you about how these were two different ways to study biological function. Today I want to talk a little bit about how we use genetics to study biological function.

And, in particular, I'm going to pick some examples of how we use genetics to study biological function that have to do with the biological functions of biochemistry.

So already we're beginning to look ahead to this connection between gene and protein, which molecular biology will establish for us. So, suppose you want to do genetics.

You've got to study some organism. We talked already about Mendel's choice of organism, the pea. We talked about some of its advantages and disadvantages. Advantages you could get pure breeding strains in the market, you could, when you're done with the experiments, feed it to the other monks. There were a lot of things like that, that were advantageous about the pea, but it had problems of generation time. You would only get, certainly in Europe, a generation or so a year. In Northern Europe maybe you could squeeze a second generation in not so good.

Fruit flies, a very attractive system in many respects because you could grow many, much larger numbers.

The generation time is on the order of two weeks or so to go from a fertilized fly embryo, a fly egg developing into a fly, developing into a mature adult, able itself to have offspring.

So, very attractive. There are other systems that people studied.

And, of course, one of the reasons they study this system is because it's interesting, I'm sorry, because it's tractable.

And the other reason is because it's interesting.

So, tractability is very important to a geneticist, right?

The number of whale geneticists is few, for the most part.

But we also want to choose our system because of what it will tell us about the system we want to study. Like if you want to study distinctive things about the immune system, you might want to study them in mice, or if you could even study them in people, although you can't set up crosses in people. We'll come to that on Monday. If you wanted to study things about basic aspects of development, you might study them in fruit flies.

And if you wanted to study basic biochemistry, the place to study basic biochemistry might best be done in single-celled organisms, which also have to carry out biochemical pathways like glycolysis and synthesis of amino acids and things like that.

They're going to be, by far, the most tractable systems.

And so, people are particularly fond for doing things like studying basic biochemistry and many other aspects of basic molecular biology to studying the organism yeast. Yeast is a friend of human beings.

Certainly, yeast has been an intensely studied organism because of its practical benefits in the making of bread, in the making of beer. So, fermentation processes, dough rising and all that. But yeast also is a tremendously important organism for the geneticist. It is an extremely elegant experimental system. Yeast is a fungus.

It is a single-celled eukaryote. That is true nucleus. It's got chromosomes that pair up. It's cells, through a first order approximation, that are an awful lot like your cells in terms of having all of the basic important eukaryotic organelles in the nucleus, mitochondria, other things like that.

So, yeast is a great model for many purposes. And we're not going to talk much about the cell biology of yeast, but I do want to talk about the husbandry of yeast, how it is that you grow yeast. So, the way a geneticists grows yeast is take growth medium that has lots of rich nutrients.

You could take a broth with lots of amino acids and all sorts of stuff, you know, a little bit of salt, lots of water of course.

And if you take a single yeast cell and it's got lots and lots of rich nutrients in this broth here, you put your yeast cell into the broth, so I will do that. Here's my flask, here's my little rod which has a yeast cell or a couple of yeast cells on the end of it. I put it in there and I grow it at an appropriate temperature.

Let's say 30 degrees, for example, would be a nice temperature.

I could do that if I wanted to. Then a C obviously. I grow it up and I get a culture of yeast in there. And I can tell because this nice clear broth is now all cloudy with yeast that's grown up in it.

Now I want to study these guys, so what I do is I pour them out onto a Petri plate. The Petri plate has on it a medium, a solid medium, an agar medium that again has nutrients.

And if I pour this out, and I pour out a lot of it, what will happen? Well, there will be yeast all over the place and it will be very smootsie. There will be like yeast cells everywhere and it's not very organized. So, what I want to do is I want to take that and I want to dilute it.

I want to take only a little bit of the broth and put a little bit of the broth on my plate. Maybe I'll have diluted it first.

And then I want to spread it around with a little spreader, here's a little glass spreader maybe or something, and push it back and forth, so that really there are just individual single cells scattered randomly, scattered around. And so, then this cell begins to grow and divide and divide and divide and I get a colony.

A little hill of cells all of which descend from a single cell that was put into that position. And the reason I know that they all descend from a single cell is because most of this plate does not have cells on it. Most of the plate is sparse.

I've just got cells, cells, cells, cells scattered about. And because of that I know that these had of been individual events.

These things are called colonies. Now, when yeast grows and divides like that, let's take a moment and talk about its life cycle.

We'll introduce its life cycle here. Yeast proper eukaryote, so it has a diploid stage. It grows as a diploid. And it can undergo mitosis in which all of the chromosomes line up, as we talked about. They've already pre-replicated so that they'll be ready to divide up and give one to each daughter cell, and there you go. N for yeast is 16. Yeast happens to have 16 pairs of chromosomes. Peas had seven. Humans have 23 pairs. Every organism has its own yeast of 16. Now, what we do is we undergo meiosis to make haploid cells, sperm or eggs in the human population. Yeast also undergoes meiosis to make spores.

It sporulates and it produces spores. And it turns out these spores, of course as you would expect, have N chromosomes.

They undergo meiosis just as we drew it on the board.

And these can come in two flavors. They happen to come not in male and females, but A and alpha, there you go. A and alpha cells can mate together to produce, again, a diploid. They fertilize and can produce a diploid. They fuse to do that. And you now get back to a diploid from your haploid. So, this looks just identical to the human genetic cycle here, but there is one difference.

What's the difference? Sorry? Time. Yes, it's true. Yeast can divide much more rapidly. Yeast can have offspring extremely rapidly over a course of a day or so.

And humans take somewhat longer than that. They, for example, have to wait until they get out of college to be able to reproduce mostly. What else? There's one other important thing. It turns out that yeast can also undergo mitosis as a haploid. In other words, the haploid cells of yeast, when it makes individual haploids, they can continue to grow indefinitely. By contrast, your gametes cannot. You do not have an independent human stage in which you are haploid, or your gametes are haploid.

Whereas, yeast can hang out as a haploid for a very long time until it decides it wants to mate. This is very convenient for geneticists. Geneticists like this because it means we can grow the thing as a diploid, we can grow the thing as a haploid.

When we want to mate them, we can mate them together, but we can also study them alone. And, you could imagine, this is going to be really good for studying recessive traits, right? So, that's one of the reasons why geneticists are fond of yeast. There are many reasons geneticists are fond of yeast.

Just growing yeast, it smells very nice in the lab.

For example, try growing E. coli by comparison. So, now, it turns out that yeast is very happy if you grow it on rich medium.

But yeast can grow on minimal media with very few macro molecules.

It needs a carbon source which is some sugar that it can ferment.

It needs a nitrogen. It needs some simple source of nitrogen.

It needs some simple source of nitrogen. It needs a source of phosphorus. It needs some other trace salts and things like that.

And obviously it needs some water. That's it. If you think about what's in a yeast cell, like it's got phospholipid bilayers.

But you're not giving it any phospholipids.

Why is it able to grow? It makes them. What about proteins?

They're made up of 20 amino acids. You're not giving it any amino acids. Why? It makes them. Yeast is extraordinarily self-reliant. You, by contrast, are not as self-reliant.

There are a number of amino acids which, if I don't give you, you can't live because you don't actually have the ability to make those amino acids. But yeast is able to make the vast majority of things. Basically, you almost just needed to give it the elements. As for carbon sources and things like that, it's very happy with a wide variety of fermentable sugars.

You can give it glucose. You can give it sucrose.

You can give it galactose. You can give it fructose and it will deal. So, yeast is very well set up metabolically. So, it's got all of these pathways of the sort Bob has talked about for being able to breakdown the things you give it and being able to synthesize up the things it needs.

Now, yeast, of course, is not stupid. Because if you give it amino acids it will use it. If you give it all sorts of other things it will use it. So, yeast is able to use rich media that have lots of complex nutrients and macromolecules.

So, it has an ability, it has everything it needs to make these things, but it has an ability to regulate that.

So, the processes, the enzymatic pathways that produce complex macromolecules, amino acids, phospholipids, et cetera, will be down regulated, shut off, or at least decreased if you provide it with these macromolecules.

That's an interesting question of how it manages to regulate its biochemistry. Why does it care? Why doesn't it, why not just have those pathways be on all the time? Sorry? Waste of energy.

It needs ATP. It costs money. So, at the beginning probably they were on all the time, but some yeast evolves, or some precursor to yeast evolves that's able to regulate it.

That one is able to be more frugal with its energy.

It outgrows its other ones and then another, dah, dah, dah. Any place you can make a few ATPs here or there, eventually the organism that does it will out compete the organism that doesn't. And so, rather fine control of this, which is a topic we'll come to in a couple of days, gene regulation and other kinds of pathway regulation is very important. OK. So, we want to know how does it do it? What are the enzymes? What are the pathways? How does it actually make, oh, I don't know, arginine? How does it make arginine, amino acid? How would you find out how

yeast makes arginine? How can yeast synthesize arginines?

So, you remember our picture that the biochemist wants to study a problem by grinding up the cell and purifying a component able to do something. So, a biochemist might want to grind up the cell and purify an enzyme that can make arginine.

Form what, of course, is an interesting question?

And then the thing that made the thing that was used to substrate, et cetera, et cetera. What would a geneticist do?

How does a geneticist approach the problem with how does yeast make arginine? Find a yeast that cannot make it, that's what we do. That is. So, what we need is a mutant.

A geneticist wants a yeast that cannot make it.

A geneticist wants mutants. How do you find the mutant? You find the mutant by going on a mutant hunt.

That is what geneticists refer to it as. And it's a very exciting thing.

You go off, load up the guns and go off into the bush on a mutant hunt.

And so, I want to talk about the strategy for a mutant hunt.

How do we look for a yeast that can't make arginine?

Sorry? Cannot. I've got a yeast that can make arginine, because normal wild type yeast can grow on minimal media without arginine supplied. And, when I examine it, it's got arginine in it. Yes? So, who should I find?

Proteins that contain arginine and then it doesn't have the proteins that doesn't have arginine. Interesting. Now, the problem is almost all proteins will have an arginine, or the vast majority of them. And a yeast that lacked all those proteins that didn't have arginine would not be much of a yeast. I think it would be pretty dead. So, it's a good thought if it was a more dispensable function. But that's going to be tough.

Or, maybe I can use the fact that it's dead. Now in a sense, can I find the yeast? Yes? You had a thought on this.

Yes? Kill all yeast that make arginine, excellent.

So, if I had a chemical agent that could kill yeast that can make arginine, I could only get the yeast that make it. How would I do that?

That's a very interesting idea. You're right. You could construct the chemical molecule in the arginine pathway which when it was broken down enzymatically made some toxic product, and only those yeasts that couldn't break it down would be able to grow, et cetera, et cetera, and I could select. That's a very cleaver idea.

But I'd also have to know an awful lot about the pathway in advance.

So, suppose I didn't' know the pathway. Suppose I knew nothing about how arginine gets made. Yes? Excellent. So, I take, I mean geneticists are simpleminded folks and they like simple solutions. Take medium in which you've given the yeast arginine, grow it up, and then pour it out on a plate that doesn't have arginine. Everybody got this idea? So, we're going to take yeast. We're going to grow it up in medium which contains arginine with arginine. So, now yeasts, those mutants that arose by chance that are unable to make their own arginine are still able to grow here. And then we dump it out onto a plate that has minimal media without arginine, no arginine, and those ones that can grow up are the ones that we're not interested in. And the ones that don't appear are the ones we're interested in. But, wait a second, that's the problem, isn't it, because they're not here.

How do we study them if they're not there? What can we do about that?

Yes. You want to see if you can help us. Remove the ones that grew up. So, get in there, scrap them off, now put some arginine on. We're getting to the idea.

Maybe we can set this up more elegantly, though.

Thoughts? How can we, yes? Make a bet? Make a guess?

I can make that guess, but how do I find them?

Here's a simple, simple, simple idea.

Let me try a simple idea. How about I grow up these yeast, and instead of plating them on minimal medium, let's be good to them. Let's plate them on minimal medium.

Good. That's interesting. Let's plate them on minimal medium plus arginine. Or, actually, if we wanted to, we could even plate them on rich medium. We'll be really good to them. Either way. So, now, let's let each one grow up.

And here will be the ones that can grow and the ones that can't grow with arginine. Now let me take a plate that is minimal medium. And now let me take a toothpick, put a little toothpick there and carry over this colony to there.

Let me take a toothpick and carry this guy over to here and a toothpick and carry this guy to here, and a toothpick, and a toothpick, and a toothpick. And all I have to do is keep transferring, one at a time, these colonies.

And now I can see that somewhere there was a colony that grew fine when I gave it, say, rich medium, or minimal plus arginine, and a colony that didn't grow when I put it on minimal medium. That would at least show, so, of course, the issue is I first have to find them by growing them on something where I've given the arginine and then I can see that they can't grow. All right. This is what geneticists basically do. What happens if I grew them on rich medium and I transferred them to minimal medium? Why might something not grow? It might be missing the ability to make tryptophan. It might be missing the ability to make proline. It might be missing the ability to make something else.

So, what I can do is, if I wanted to, make a very broad mutant hunt.

I could just first grow on rich medium and then plate on minimal medium and any yeast that has lost the ability to make some essential nutrient will be evident by its absence on the minimal medium plate.

So, we have for yeasts. Yeasts that are able to grow on minimal media are called prototrophs. They are the wild type that can grow on minimal media. They can make everything themselves.

Yeasts that need help, that cannot grow by themselves, that need help, that need a supplement are called auxotrophs.

Auxo obviously meaning help. So, it's a mutant that has lost the ability to grow on minimal medium and that it needs a supplement of some kind. So, if I wanted to, I could just first collect lots and lots and lots of auxotrophs and then figure out what they need. So, I might collect a large collection of auxotrophs. And then test to see if supplying arginine rescues them. I could also test tryptophan.

So, if I only, only, only cared about finding arginine auxotrophs, I could just grow them on minimal plus arginine and then test them on minimal. And then I would know in advance, these guys all grew with arginine on minimal and didn't grow without arginine, and I'd know it was arginine. Or, if I was in an expansive mood, I could test them on rich medium, collect everybody who's unable to grow on minimal, and then work out what the reason is. Is it arginine?

Is it proline? Is it whatever? And it depends how much work you're interested in doing and how complete the study is you want to do.

Either way, we could end up with a collection of arginine auxotrophs.

Organisms that are mutant for the ability to make their own arginine and require it to be supplied to them in the medium.

All right. I might get, depending on how much work I'm willing to do, dozens of independent colonies unable to grow without arginine. I might get hundreds if I'm willing to do enough work. I can get as many as I want.

Our goal now is to study them and find out why they're unable to do that. I have a quick question? Those yeast cells we plated, where they haploid or diploid? We didn't say, did we? So, should they be haploid or diploid? How many vote diploid?

How many vote haploid? A lot of people vote haploid but aren't willing to express a reason why. Why haploid?

Right. Excellent. Excellent, although genes are not recessive, but OK. A little detail. Phenotypes are recessive. Tell me a little more of what you're thinking about. We'll have it out later on this point, yes. So, suppose we were looking in a haploid.

I take your point, even if on nomenclature I want to push back a bit. So, suppose it's a diploid and suppose we have now two copies of this chromosome here in the diploid. And suppose there's a gene over here that encodes an enzyme that we now is necessary to make arginine, or that somebody knows is necessary to make arginine.

Let's image that that's the case. In order to get haploid yeast that is unable to make arginine due to a mutation in this gene, you need to have some kind of a mutation in this copy.

What about in the diploid yeast? In order to make this yeast unable to grow without arginine, do we need a mutation in both copies?

Well, the answer is probably. The truth is actually a bit more complicated, but let's suppose it was the case that even one copy of the functional gene was sufficient to carry out the enzymatic step, then the answer would be yeah, we'd need a mutation of both copies.

What's the chance of finding a yeast that has a mutation in both copies? It's obviously much less than the chance of finding a yeast that had a mutation of one copy. So, we're much better to go searching in the haploid where the phenotype will be revealed much more easily by virtue of just the single mutation rather than having to, by chance, encounter one that had mutations in both copies.

Now, the reason I'm a little bit cautious here is because notwithstanding the textbooks, it's not always the case that everything like this is a recessive trait. It's possible that auxotrophy for arginine could be a dominant trait.

So, how could that be? Well, auxotrophy could be a recessive trait. Suppose there's some enzymatic pathway, A goes to B goes to C goes to D, and this encodes an enzyme that carries out a particular biochemical step.

Well, if the gene is broken, if the gene is missing, if the gene doesn't make the protein, as you guys all know that

that's what happens, then you don't have the enzyme, you can't do the pathway.

And it is usually the case that having just one copy is sufficient.

Because having a little bit of enzyme the pathway may work slower but it will still work just fine and you'll eventually get arginine made.

But it's occasionally possible, I note since you guys are sophisticated, that sometimes a gene can encode a protein which not only doesn't work but screws up the other working copies of the protein. Suppose the enzyme that did this were a tetramer. It had several subunits that had come together. A mutant copy of an enzyme, when it forms into a tetramer, might somehow disrupt all the other good copies that are around. And that does happen sometimes.

It can happen that you're going to have an inability to make your own arginine be a dominantly inherited trait. So, you actually have to test whether it's recessive or dominant. Often it will be recessive. So, usually most of these simple auxotrophs are recessive traits. Occasionally some are dominant.

So, now, suppose we get a whole collection of Arg auxotrophs, and we'll just give them a name. I don't know. Here's my collection.

We'll call the first one, for lack of anything terribly creative, Arg 1, Arg 2, Arg 3, et cetera, each being an individual strain from growing up originally for a single colony that is unable to produce its own arginine.

We now want to take this collection and characterize it.

How many distinct genes does this affect? Are these mutants perhaps all in the same gene? Are they in a hundred different genes? How could we tell? Now, of course, if you're a biochemist, you already know the protein you can see and dah, dah, dah. But, if you know the answer, well, why are asking then, right? A geneticist goes out to ask this question because he or she wants to know all the possible ways you can disrupt the cell so it cannot make arginine. And we don't know in advance what those ways are, so how are we going to be able to tell whether or not different mutations affect the same gene, the same function in yeast? It's an interesting question.

Geneticists do a variety of tests. The first test that a geneticist does to characterize a mutant is by tests of recessivity or dominance, whichever way you want to put it.

We want to take each mutant and test whether it is recessive or dominant as a phenotype, whether the phenotype, the auxotrophy for arginine is recessive or dominant.

So, here's mutant number one, the mutant cell carrying this mutation here. Conceptually it affects some gene.

I'm going to label it Arg 1. We don't know where it is in the genome.

There are other chromosomes here as well. Here's my mutant cell.

How am I going to find out whether or not the auxotrophy for arginine is recessive or dominant? Yup? With what?

Cross it with a haploid that is a prototroph, or I could just say cross it with wild type, right? Perfect. So, make a cross here, very good, with wild type plus there.

How do I know it's plus there? This is wild type. Wild type is defined as the normal form. And so, because I said this is what we're using as wild type, it's necessarily plus because we're measuring mutations relative to wild type. So, what happens when we get here? We now, when we cross we get a diploid, and Arg 1 plus. Now, how do we know whether or not that phenotype was recessive or dominant? Sorry? It's what shows up when we try to grow it. So, when we cross it, what kind of plate should we grow it on first? Should we grow it on minimal or rich? We better grow it on rich because just in case it doesn't, it can't make its own arginine, we better first let it grow and then test it. So, let's grow it on rich medium. We'll cross these together, grow it on rich medium. So, grow on rich, test on minimal. OK? And we'll be able to check out the phenotype as to whether or not the phenotype is wild type or mutant.

All right. So, we could do that.

And we'll test the first one and the second one and third one and the fourth one. And, for each of these, we'll write down whether it's recessive or a dominant auxotroph.

Now, let me assume that all the ones we're talking about are recessive phenotypes. Because everything I'm about to say is very much harder if it turned out any of them were dominant.

So, we're going to assume. Let's assume now, but it's not always the case, we'll assume that the collection, maybe Arg 100, are all recessive auxotrophies, the phenotype is recessive. Now, how do I tell if they're in the same gene or not? So, now I want to characterize my mutant by some other test that will tell me whether or not Arg 1 and Arg 2 are in the same gene. Suppose Arg 1 and Arg 2 are in different genes. Cross them. What will happen?

Right. So, to repeat that, if I cross together the two mutants and they're in different genes, each will have at least, the each will be contributing a good copy, a functional copy, a wild type copy of one of the genes. So, let's walk this through.

Interesting. Interesting. So, suppose I take a situation where I've got Arg 1, a mutation in a gene over here, on this

chromosome, and on the other chromosome I've got a wild type copy.

My Arg 1 mutant is mutated in a gene here.

I've got this other gene here, which is normal. And I'm going to cross that now by the strain that has a wild type copy here for this first gene, but it has a mutation in the second gene.

When I cross them together, I now get me a diploid cell here, which is Arg 1, a mutation there, plus there, plus copy here, and Arg 2. Will having one copy, one working copy of this gene be enough to make the enzyme?

No? In other words, is the wild type phenotype dominant to this auxotrophy, or is the auxotrophy attributable to this gene recessive? Yes. Why? Because we assumed it.

Why did we assume it? So I would be able to say this, right? OK. If it wasn't we'd be in trouble. But by assuming that we're working with a recessive phenotype, then we know that this will be enough to save the yeast. What about here? Enough to save the yeast so it will grow without arginine.

By contrast, suppose it was the case that this cell here, Arg 1, and suppose our other mutant that we had isolated in our mutant hunt was a mutation Arg 2 in the same gene. Suppose these were the same gene. When I cross them together I now have a cell that is Arg 1, Arg 2. In other words, its genotype is Arg 1 over Arg 2, name of mutation. And can it grow?

No growth without arginine. By contrast, the genotype here is Arg 1 over plus, plus over Arg 2. I could even write Arg 2 over plus, but I just did that to indicate the chromosomes that they came from.

All right. This is called a Test of Complementation because these two genes are able to compliment each other's defect.

If two mutations compliment each other's defect then they are in different genes. OK? Boy, that's a noisy one.

So, we're able to make a Complementation Table.

Suppose I take a bunch of yeasts, wild type, WT, mutant number one, mutant number two, mutant number three, mutant number four. And suppose I cross them with each other in all pair-wise combinations. I've assumed that all of these arginine auxotrophs have a recessive phenotype here.

These are all my Arg mutants, and I'm assuming that this is recessive. What happens when I cross them and I test to see whether they can grow without arginine? If I cross wild type by wild type, can it grow without arginine? Yeah. Normal phenotype. So, plus is going to mean prototrophic. Minus will mean auxotrophic for arginine. What

happens when I cross wild type with mutant number one?

It grows. Why? By assumption, these were all recessive.

I'm only testing recessive ones. Two. Three. Four. When I cross in this direction, wild type by these guys.

This is going to be a symmetric matrix, of course, right? OK. Now, what happens when I cross mutant one by mutant one?

I now have a diploid. Will it be able to grow without arginine? No. Why not? It has no working copies of that gene, so I'm going to put a minus there. What about mutant two with mutant two? Minus. What about mutant three with mutant three? Minus. What about mutant four with mutant four? Minus. Now, what happens when I cross mutant one by mutant two? It depends. It might be plus or might be minus. If they're in the same gene, minus. Different genes, could be plus. So, here's some data.

So, all this is compelled. But the kind of data, ooh, I'll use a color. Isn't that fun? They want me to use colors over there. Here we go. Suppose the data were minus, minus, plus, plus, plus, plus, minus, minus, plus, plus, plus, plus. What would it be?

What conclusion could we draw? Is mutant one and mutant three in the same gene? They compliment each other?

No. But is one in the same gene as two? Yes. In fact, this box and this box here define the genes beautifully.

The groups that failed to compliment define mutations in the same gene.

These are called Complementation Groups because they don't compliment, OK? It's a little complicated but that's all right.

These are called Complementation Groups because all the members of the complementation group, namely Arg 1 and Arg 2, failed to compliment each other. They could be called failure to compliment groups, but it would be too long.

OK? So, there you go. You can take hundreds of mutants and organize them into complementation groups and thereby know which ones go to the same gene. And now, if I want to study the genes, I only have to study the distinct complementation groups.

Last thing, which we'll just have time to do, are what's called tests of epistasis. We'll probably run just a moment or two over on this.

Suppose a biochemist were collaborating with a geneticist and had studied what he or she thought was the pathway for making arginine.

Some precursor alpha goes to precursor beta, goes to precursor gamma, goes to arginine. And suppose specific genes were needed to encode specific proteins.

I'll call them Arg A, Arg B, Arg C to catalyze each step of this biochemical reaction. The geneticist and the biochemist could collaborate with each other to study whether these mutants, these particular genes now that had been identified, affected each step of the pathway. And here's how they might do it.

They might take wild type yeast, mutant, well, they wouldn't know in advance whether or not it was missing the ability to grow on each of, whether it was missing each of these enzymes, but let's think conceptually. Suppose we had a mutant that was, a strain that was wild type, Arg A minus, Arg B, minus, Arg C minus, unable to make this enzyme, this enzyme, this enzyme. And suppose we helped it along. Suppose we gave the mutant arginine. Suppose we supplement and grow it on media with arginine. Which ones will be able to grow with arginine? Can wild type grow if it's given arginine?

What about Arg A minus? B minus? C minus? What if instead we offer it precursor gamma? Will wild type be able to grow if it's given precursor gamma? Sure. What about Arg A minus?

No, because it still is stuck at this step.

It cannot. What about Arg B minus? What about Arg C minus? Really?

It hasn't got this enzyme. What's it going to do with gamma?

It ain't got anything to do with gamma, no enzyme.

Suppose I gave it beta. Wild type, can it grow? What about Arg A minus? No, because it can go from alpha to beta, but it can't go to gamma. It cannot grow.

What about Arg B minus? I've given it beta, but it can't do anything with beta because it hasn't got this gene.

What about Arg C minus? Wait a second. What did I just do?

We're just backward. Sorry. If we gave it gamma, I just got lost here. If we gave it gamma it was able to grow, well, we are completely wrong, guys. It's able to grow here.

Thank you. Let's go back on that. You should have caught me before.

My mistake. If we have it gamma it's able to, if it's a mutant here it can grow because it bypasses this problem. And having gamma is enough.

If I gave it beta, sorry, if I gave it gamma and its mutation was here it can grow. Sorry.

Now, if I gave it here beta, and its mutation was here, it can still grow, right? But if its mutation is here it can't and if its mutation is here it can't. That's better.

I was getting worried there for a while myself. Suppose I gave it alpha. Wild type can grow. If I give this guy alpha, will that help if he's mutant in A? No. Can it help if he's mutant in B? No. Can it help if he's mutant in C?

No. Sorry. There we go. I usually start at the other end of this picture. So, what you can see is these mutants have different phenotypes with respect to being able to supplement them with different chemicals. Now, let me ask in our last two minutes, I'll run two minutes over here. Suppose I gave you a mutant that was a double homozygote. Suppose it was Arg B minus, Arg B minus, sorry, Arg B minus and Arg C minus. Suppose it was a double mutant, it lacked both this and this.

Which line of my table would it resemble? Would it look like the first line, the second line or the third line of my table?

Second line. Why's that? If I'm lacking B, I'm already in trouble here. And also lacking C doesn't matter.

So, I will look, just like a mutant who lacks B.

So, in other words, I'm able, if I know something about the biochemistry of a pathway and I can break my arginine mutants up into different kinds of phenotypes here by their response to different steps in a pathway, I can then look at combinations of mutants.

And I can say if I have a double mutant missing both B and C, does it look like B or does it look C when I put them together?

And it turns out that if it looks like B then B was further upstream in the pathway. So, it turns out that geneticists and biochemists can collaborate based on the phenotype of the organism sometimes to infer aspects of the biochemical pathway.

These are the kinds of things a geneticist does to be able to characterize mutants on a mutant hunt. Next time what I want to do is talk about characterizing mutants in a very different kind of organism, namely the human being.