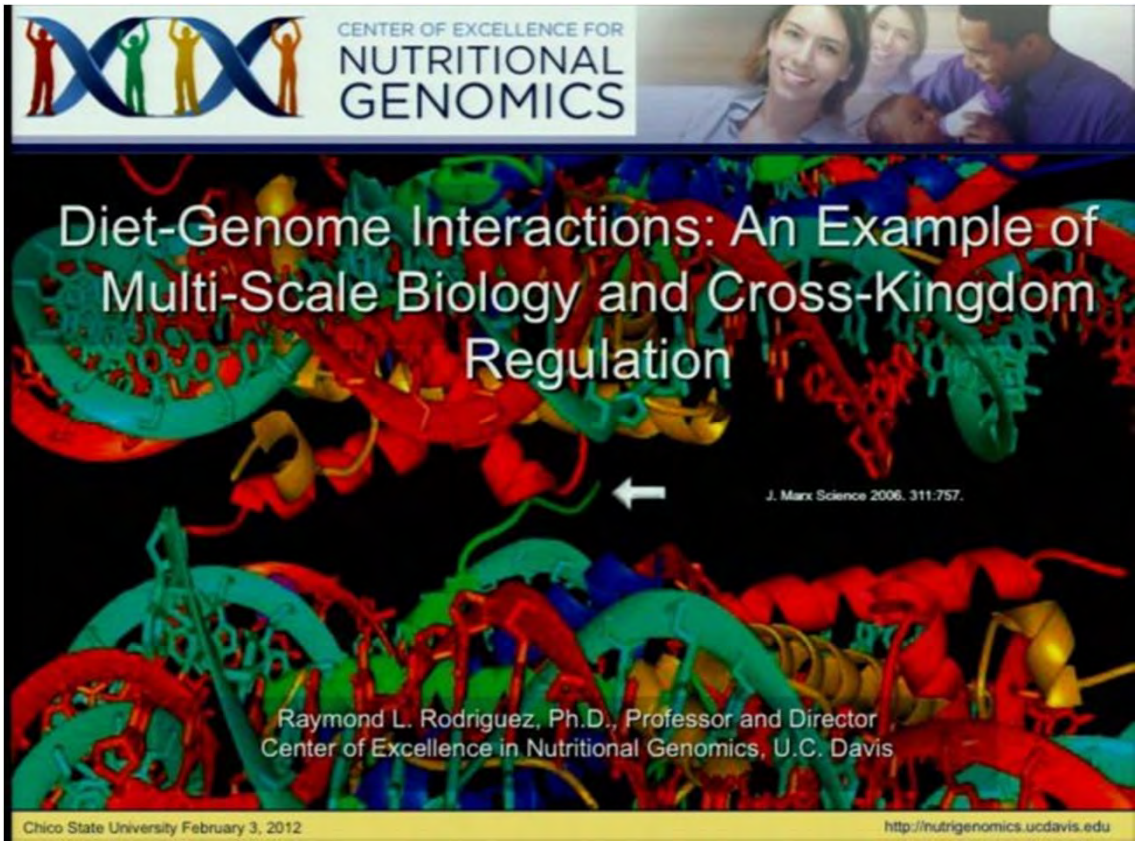
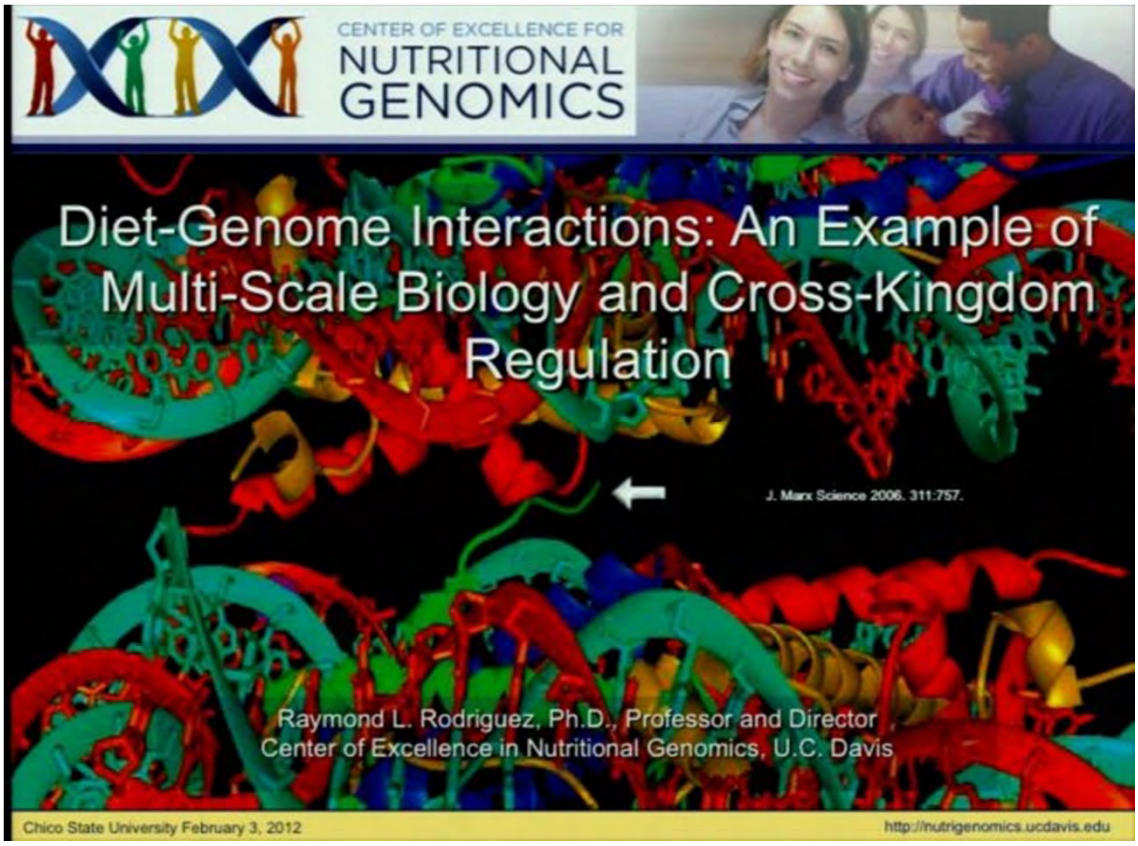


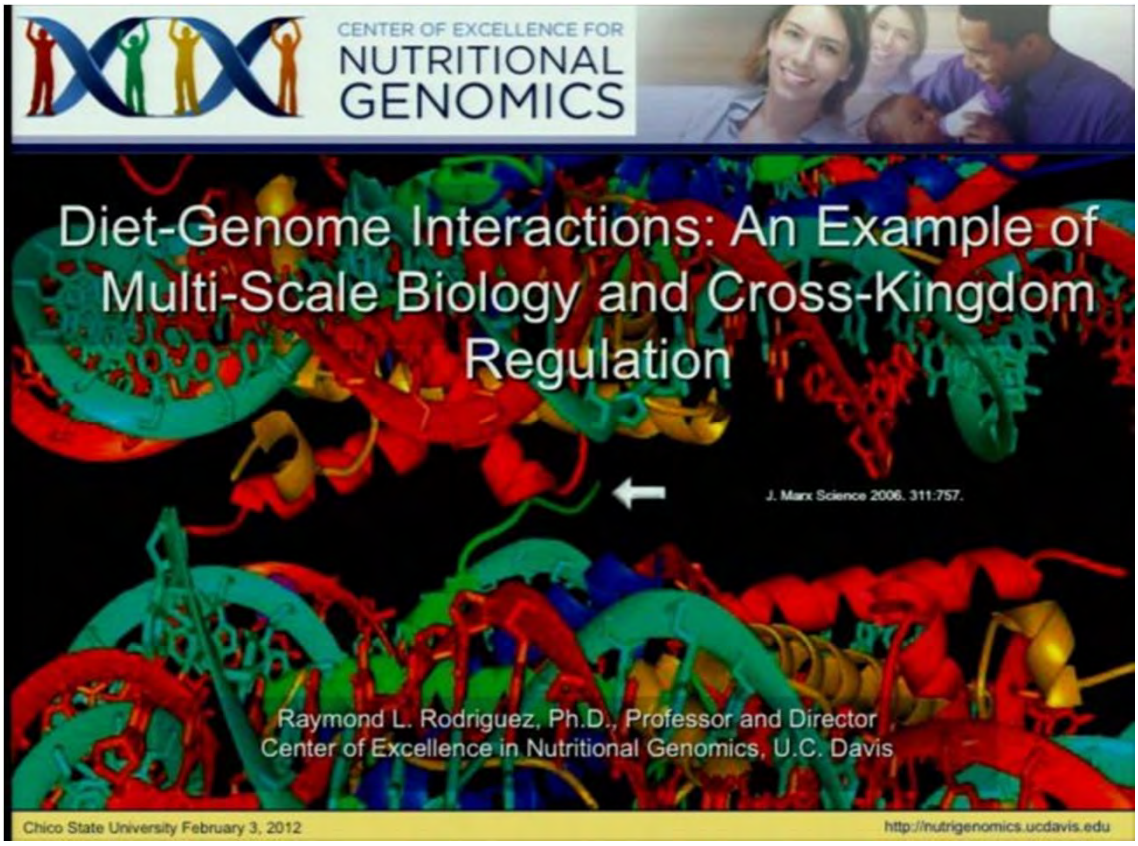
Prof. David Keller, CSU, Chico, Introducing: We're starting off with a really fabulous speaker, Ray Rodriguez of UC Davis. Ray has been at Davis since 1977. Basically, he was instrumental in starting biotechnology in California, [Inaudible] at UCSF. One of the first vectors that's commonly used in molecular biology, he helped create, and has not stopped since then [laughter]. Basing in the cutting-edge of research still…At U.C. Davis [inaudible] he's a director, Center of Excellence for Nutritional Genomics, and he'll talk today about [inaudible] genetics, the area that I'm really interested about, and unfortunately we didn't discuss it in genetics class [laughter], so--

Dr. Rodriguez: Well thank you David for that introduction and it's nice to be a back at Chico again. And while I was talking to some of the faculty next door, none of us could remember exactly when I was here last time but--I think I'm talking on a different subject so can you be assured it's a different seminar. And I was talking to one of the faculty, our emeritus faculty, before and I was telling her how my career has sort of gone in a circuitous route and it’s come back exactly where I really wanted to be 40 years ago. And that is--I'm just trying to understand that really wonderful interaction between human genes as they are represented in human genome. And our dietary--the dietary environment which is mostly plants. I know meats and dairy play an important role, but our bodies really sort of evolved working with our plant environment. And one of the first classes I took from Jean Langenheim at UC Santa Cruz was plants and human affairs and we--all we talk about was that that interaction, that interdependence, that connectedness between humans and plants and I'm still interested in that topic.

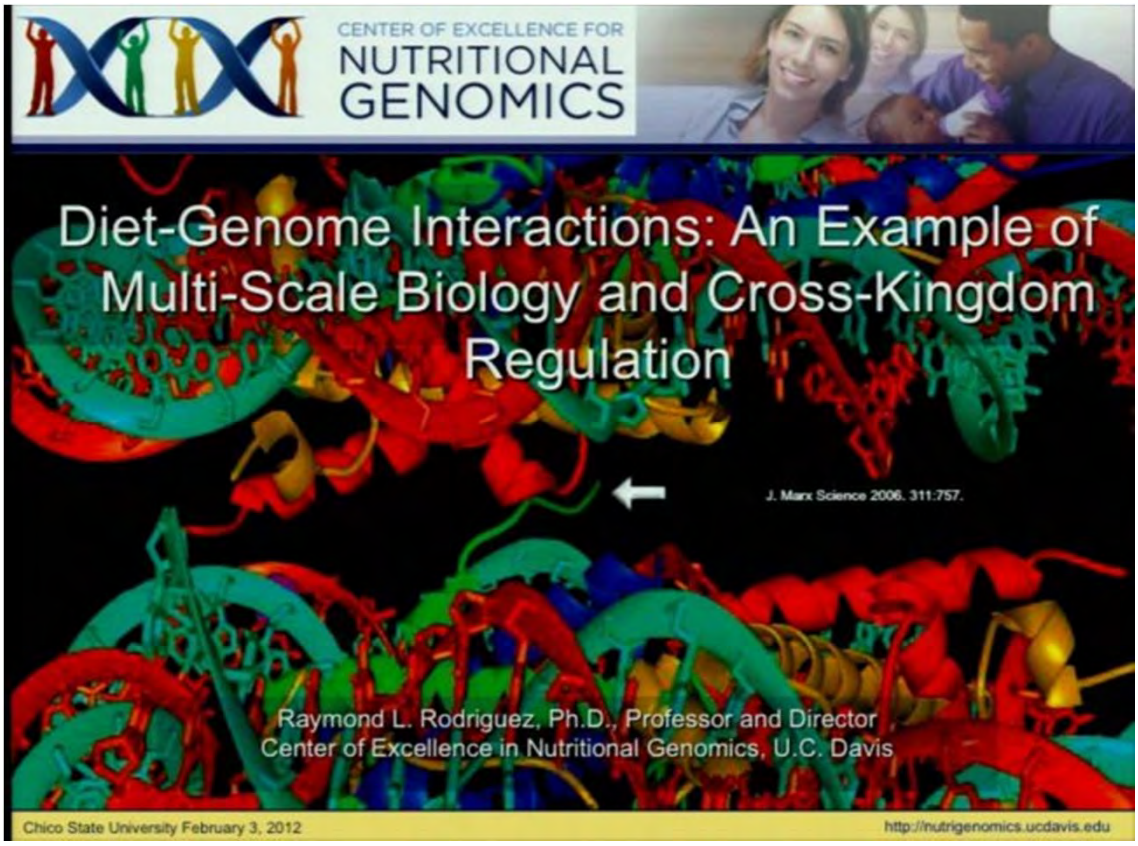


I should start by also telling you that I got three or four questions before I even got to the podium here about the microbiome and that's the microbial flora that you carry around that you inherited when you first started suckling as an infant after your birth. You inherited a microbiome and that there's tremendous exciting things coming out of the microbiome and I'm not going to talk about any of them. And I was telling people I don't have enough time left on my career to get into the complex world of microbiome, but I'll tell you some of the things that I've just heard recently. Jeffrey Gordon, of course at Washington University in Saint Louis is leading the charge in microbiome and he did a classic experiment which some people think he may get the Nobel Prize for. And that's why he was working with gnotobiotic mice and these are mice that as soon as they pop out of the womb, they put them right in isolation and they never have a microbiome of their own, they're always sterile. Except he took half the siblings, and half the siblings he raised in a regular environment, the other half were raised in these in little sterile bubbles. And he did an interesting thing, he gave the siblings, the non-gnotobiotics siblings, a high caloric diet and fattened them up. Took a little bit of their fecal material and then infected their sterile siblings and the mice got fat. First example of a transferring of a Human Phenotype from one sibling to the next--not through human genes, but through microbial genes. And now we're working with our collaborators and--at the International Centre for Diarrheal Disease Research in Dhaka, Bangladesh, one of the world leading centers in studying childhood malnutrition, and guess what they're doing. They have their gnotobiotic mice all arranged and they have these sick little babies, probably 6 to 12 months old who now already exhibiting stunting and wasting and malnutrition and they're going to infect those mice with their fecal material to see what kind of effect it has, and everybody's got their fingers

crossed.

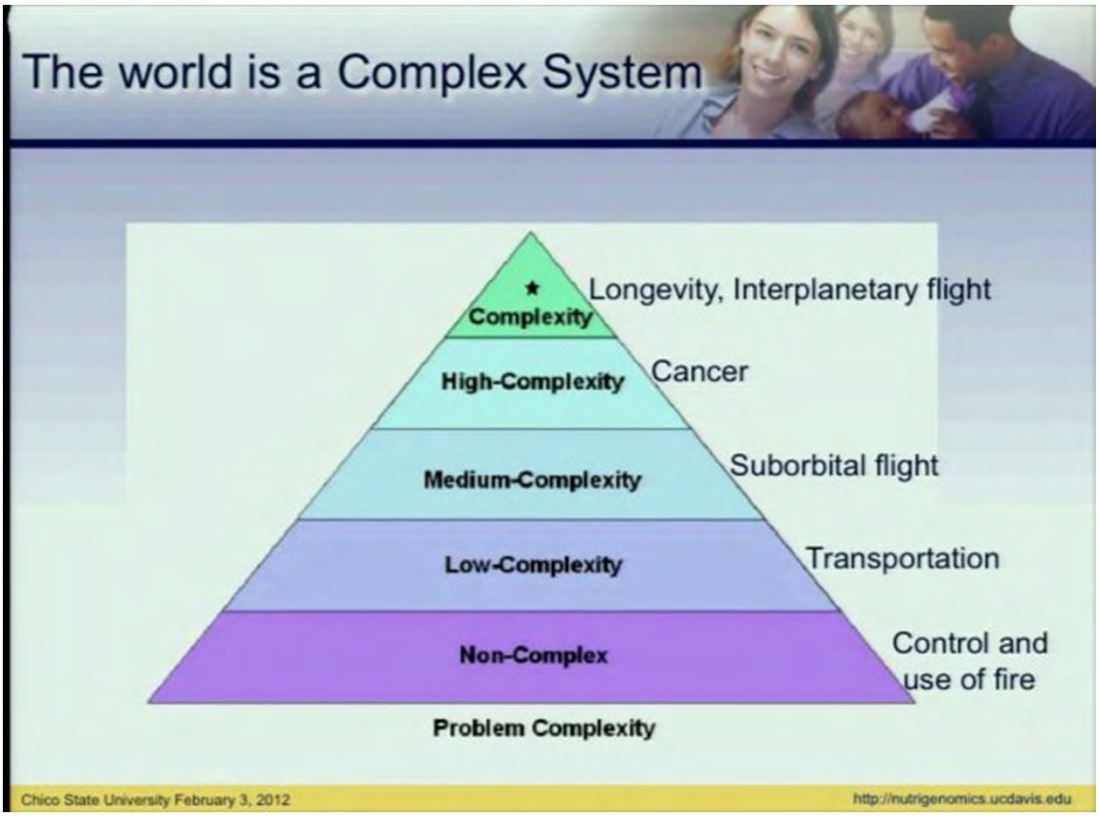


I get--assume they'll get--they'll see stunting in their little sterile mice, I don't know. But that's an area that is extremely exciting and I wish I had enough time to participate in it…But I'm just going to talk about this complicated problem here, diet-gene interactions, as an example of multi-scale biology and cross-kingdom regulation. And that just goes back to the fact that we are really in--we're interacting with our environment more intimately than we even think. You know, I grab an apple out of the refrigerator and I eat and I have a basic idea of what it's doing, but do I really have an idea of what it's doing? And I'll talk about some papers which have really sort of opened everybody's eyes on how intimate that relationship between diet and genes really is. This was a paper that we took out on science and it's actually two nucleosomes that are actually talking to each other. And they're talking to each other by their histone H4 tails, and on their H4 tails are a series of arginines and lysines. And at position 16, at the amino terminal that tailors the lysine, as you know, lysines are positively-charged amino acids and it's talking to a negatively-charged amino acid on one of the H4 tails adjacently. And that delicate little touching, I emphasize this because I'm not talking about sledgehammer effects, I'm not talking about drugs, I'm talking about delicate gentle little actions--interactions between dietary factors and genetic material that make big things happen. We call it "Dynamical Systems." But just this little touching and interaction here will cause the chromatin to either condense up and shut gene expression off because if once chromatin condenses, the transcriptional apparatus can't access DNA anymore, or it'll cause the opposite. It'll cause the chromatin and the nucleosomes to decondense and unwind, exposing a large stretches of DNA where transcriptional complexes can bind and activate genes.

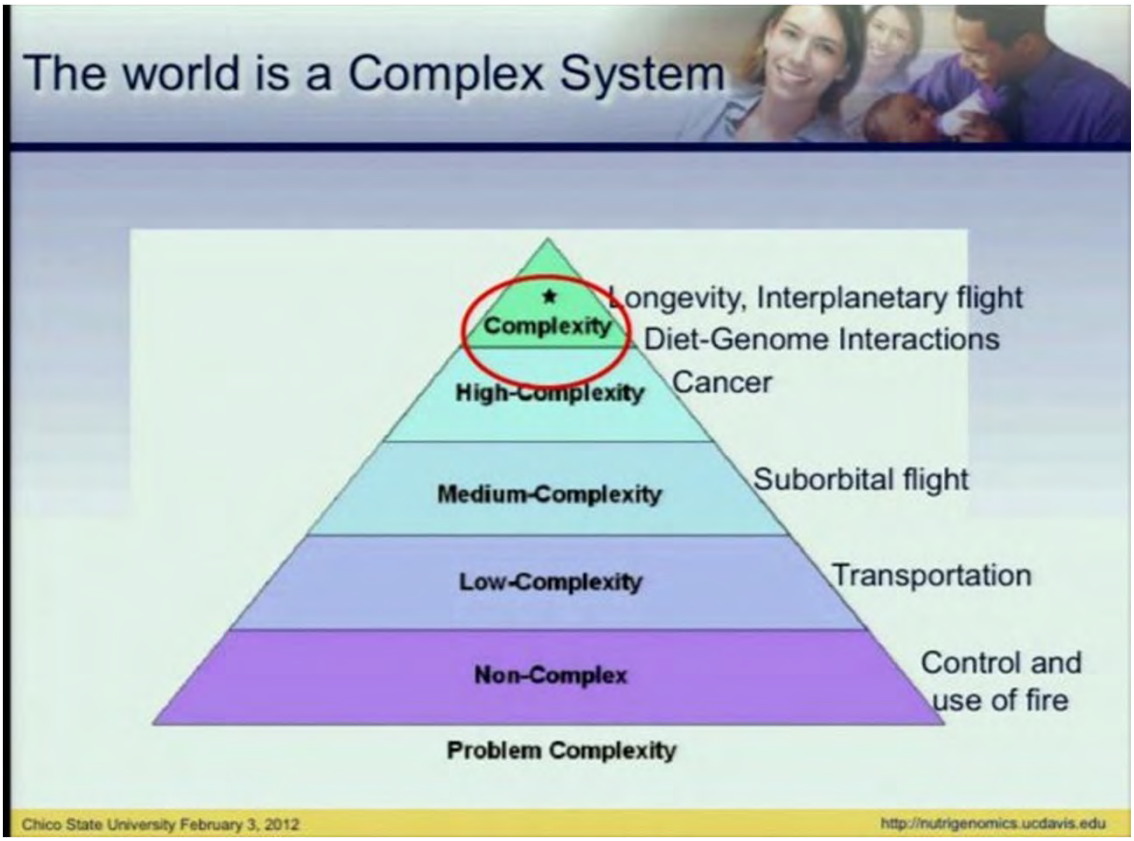


Now, one of the pro--dietary factors that we're working on is a small peptide from the soy that has the ability to promote this interaction and--all right, actually to inhibit this interaction and cause a decondensation in chromatin, allowing certain genes to be turned on. Now, I wouldn't be too excited about this if some of the genes they turned on were good genes or--and some of the genes they turned on were bad genes, but the results that we've found is that all the genes that this little peptide seems to turn on, all seemed to be good genes. They seem to be the genes that you would want to be up-regulated slightly to monitor against damage to DNA, to inappropriate cell division and growth. Yeah, those are the genes we want, those are called chemo-preventive genes or genes that prevent cancer. So I'll--two parts, one is the epigenetic part, the diet interaction across geno--across kingdom interactions. And the other part is where I see biology is in the 21st century, and that involves complex systems.

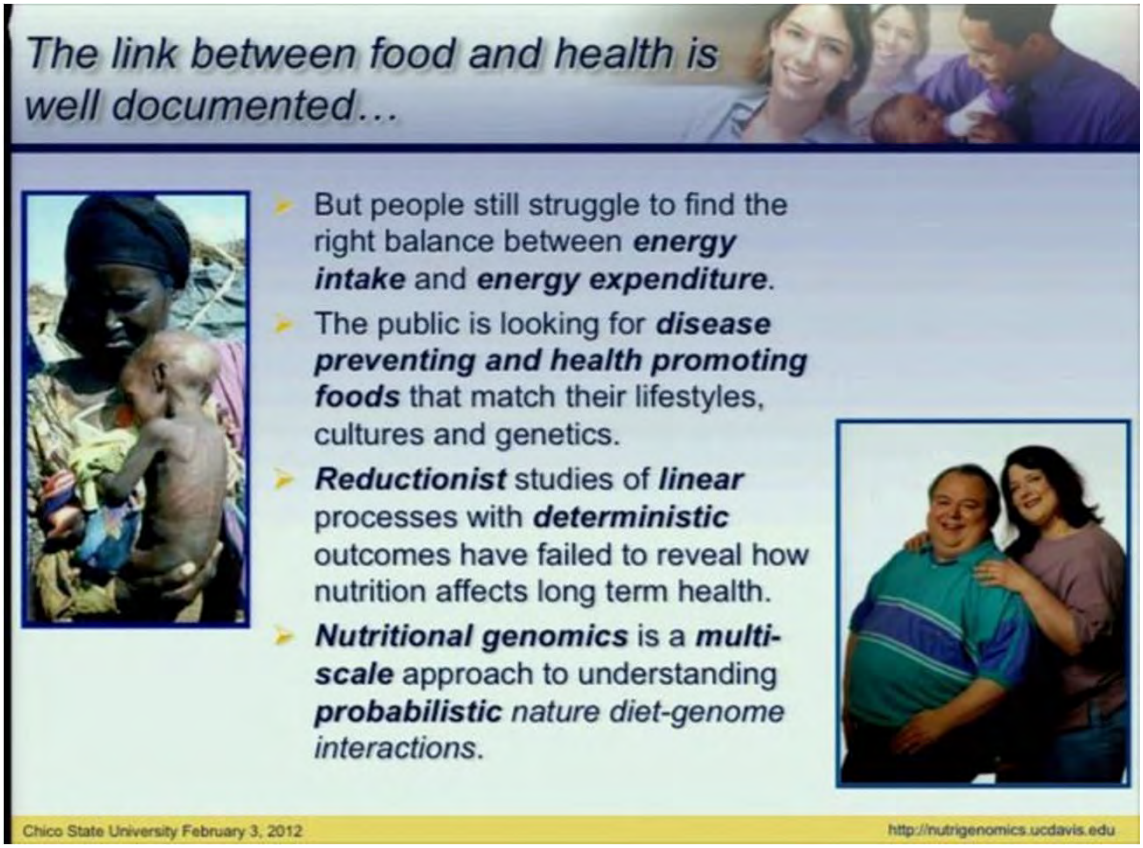
And so, these are the problems that we're left with here in the 21st century. So graduate students, this is what you've got to work on, okay? Whether it's climate change and energy sufficiency, pandemics, cancer, food sufficiency, and chronic disease, they're all tough problems. And they fall in that category, what we call, complex problems, part of complex systems. And I want to make sure that I'm not talking about complicated problems in complicated systems, but mathematicians, bio-mathematicians, use this term “complex system” to mean certain things.



And here, I've drawn the complexity pyramid where we're--we're really looking at complexity increasing, increasing from non-complex systems. If I need a fire, I know how to start a fire, if I have a--if I cut my finger, I know what to do about that. Now--you know, it isn't complex system anymore. Then, low complexity systems like transportation, getting from point A to point B, driving from Davis to Chico, yeah I had to key--I had to be awake, I had to be thinking, but I sort of knew how to get here. As opposed to--and then there's suborbital flight, getting from here to New York City or Washington, DC. Now, it's getting a little bit more complicated, but the good news is that all the principles of engineering, material science, it's all there and we know how to do that. Cancer, oh, that's getting complicated. Longevity and interplanetary flight, they fall in that category of totally complex or a complete complex system, because what this means is we don't have even the information to solve that problem. We would have to discover the knowledge to solve that problem before we can actually solve the problem.



Cancer is actually sort of falling in this category here, it's extremely complicated. We know parts of the puzzle and then there's some things we don't even know about. And maybe the microbiome is playing a role here. And I was talking earlier about this conversation that goes on between our microbiome and our genome and we'll talk about conversation between our dietary environment and our genome. We don't even know what language that is, we haven't decoded that language yet and that will be necessary to solve these complex systems.



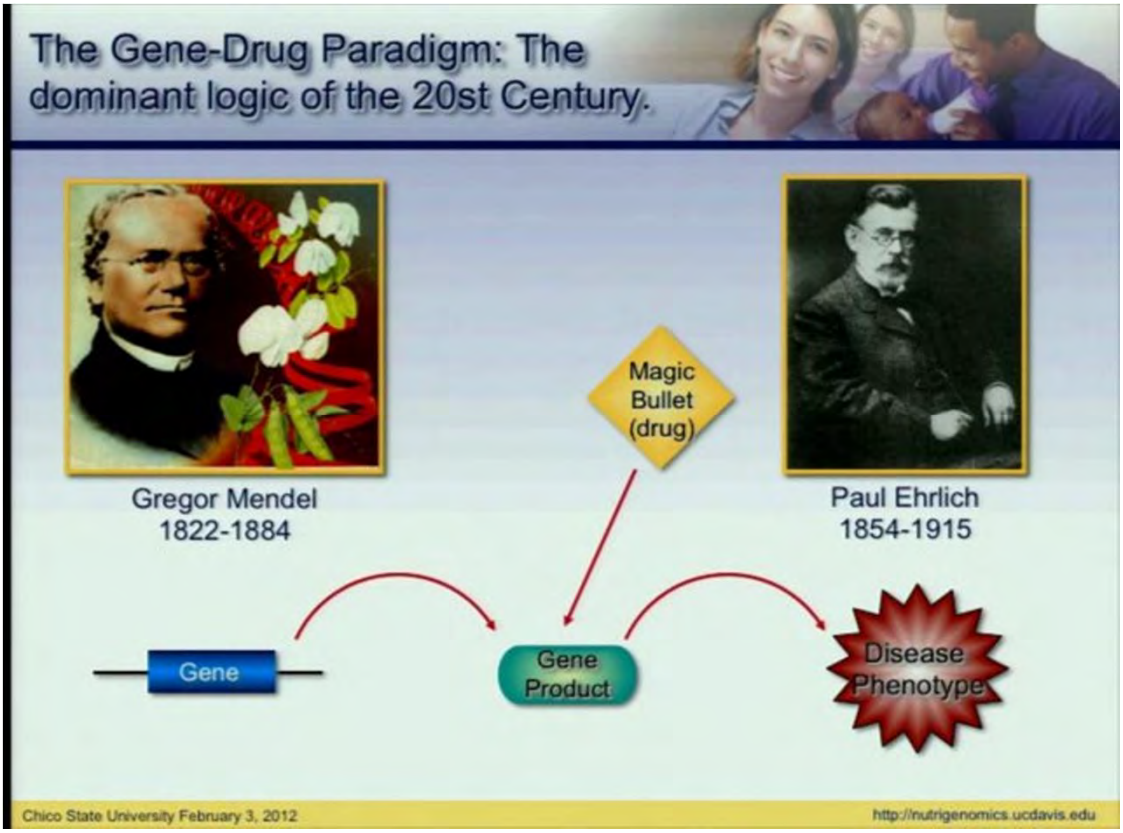
So this is--I just always start off with this slide because it's an obvious one that the link between food and health is well documented.

And in spite of that, people still struggle with getting the right balance of energy intake and energy expenditure.

Here in 2012, the public are looking for disease preventing and health promoting foods that match their lifestyles, their culture and their genetics…

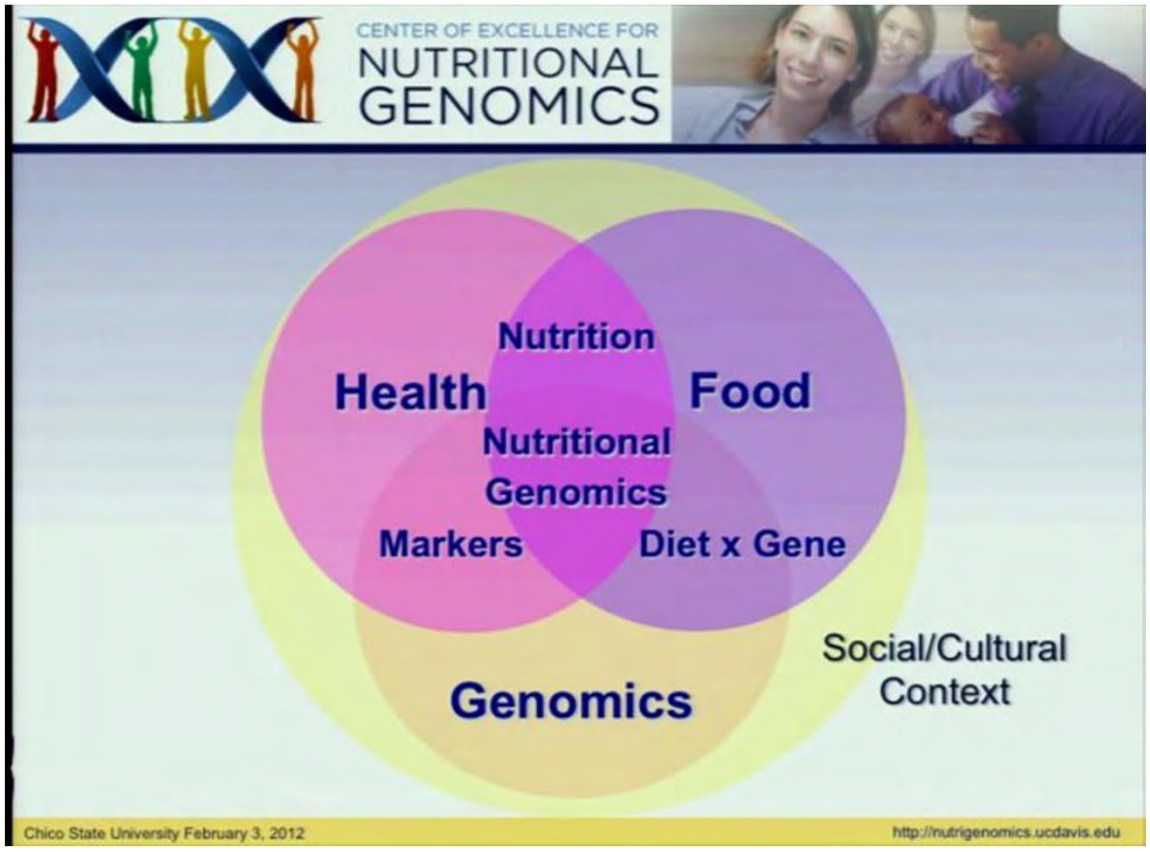
reduction studies of linear processes with deterministic outcomes, all of that--that's what you learn in your biochemistry lab--are reductionist processes of--reductionist studies of linear processes with deterministic outcomes have failed to reveal how nutrition affects long-term health outcomes. So we need to have--we need to look at this a little differently.

And nutritional genomics is that multi-scale approach to understanding the probabilistic nature of diet-gene interactions. And now we live in a world of probabilities. We live in a world where we're assessing risk in terms of percents, P-values. But you know if people didn't believe in probability, they wouldn't be going to Las Vegas and spending billions of dollars a year, you know, at the Black Jack table.



So this is the problem that we've been struggling with. These are my 2 heroes, Paul Ehrlich and his magic bullet, antitoxin and Gregor Mendel and you know, discovery of the Mendelian Principles first, second laws. The problem is and this is what we've been studying, I've been studying this for many years

A single gene makes a product that causes disease, all I need is that magic bullet to stop that disease. This is the paradigm that we are still laboring under in 2012, this is the paradigm that the pharmaceutical industry still embraces even though experts tell them that diseases are actually the result of a vast constellation of genes interacting with each other over time sensing input from the environment--that's what causes the disease. And so, to really make an effective drug, you'll need to have four or five molecules working at four or five places in these vast networks to subdue that disease process. They don't like to hear that, they want a drug target and a drug lead of one-to-one interaction.



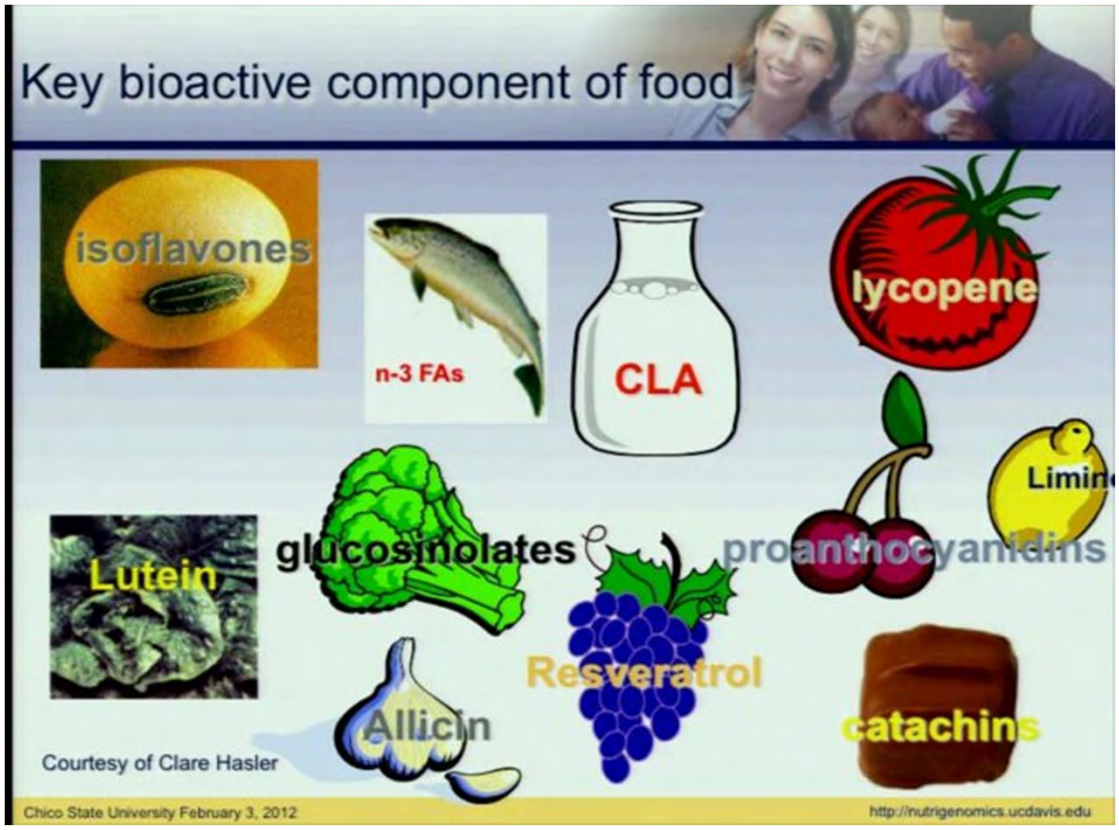
So here we are in 2012, and nutritious--Center of Excellence for Nutritional Genomics is working in this highly interdisciplinary space between food and health, genomics. We're looking at nutrition, we're looking at biomarkers, we're looking at diet-gene interactions. And the interesting thing now, it becomes even more complicated.

All of that takes place in a sociocultural context. Every one of you eats differently, you eat differently for a reason, whether you're a male or female, or whether you're an Asian, you're a Caucasian, or whatever. There's cultural things, some people don't eat meat, some people do, some people eat pork, some people don't, some people drink milk, some people don't. So we have to approach this in this sociocultural context.

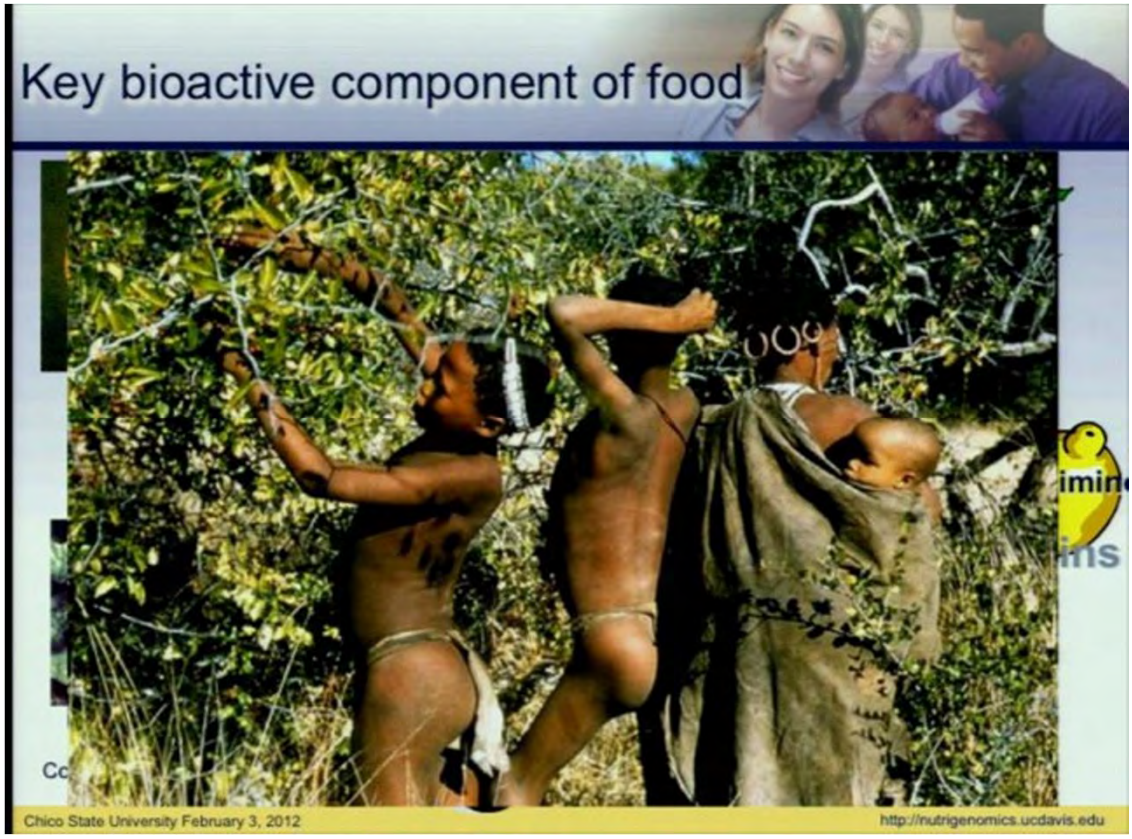
So this is what we believe. I think most of you will believe that there are things in the diet. We'll call them "Dietary signals." They are good things like macronutrients, micronutrients, essential vitamins, and minerals as well as some bad things like xenobiotics, well, I don't want to forget our friends here, phytochemicals, and that they cause this kind of health outcomes. Health, longevity, I'm all for that. Pathophysiologies, I'm not--that doesn't sound good, death and disease. Yeah so it depends on what the input is and that'll determine the outcome and we've struggled with many years how this occurs.

What's the mechanism? And we have all heard of antioxidants, antioxidants are good, I encourage you to take them. They don't answer the whole question or all the questions. And we know that some of these compounds here are co-factors for enzymes, some affect gene expression, some affect it directly, some affect it indirectly. And as the indirect actions of diet and genome which people have ignored for many years, we're now starting to look at those.

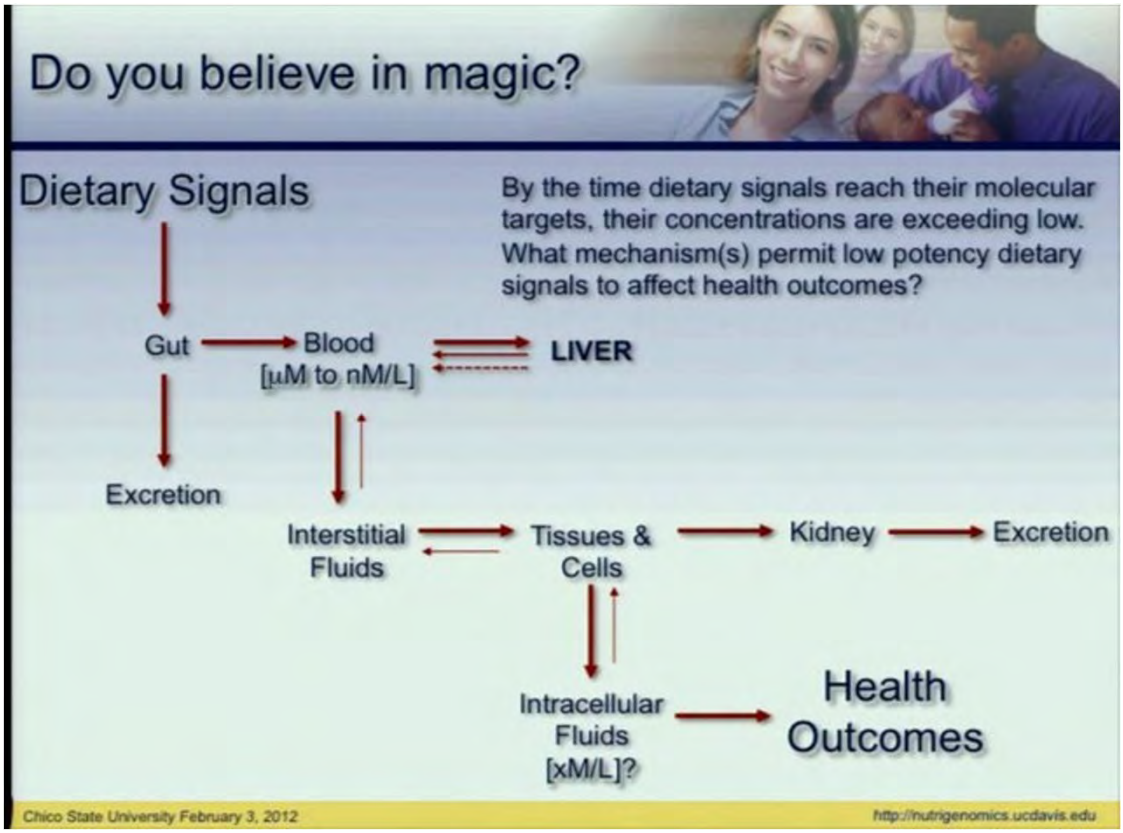
Yeah, so what's going on there?



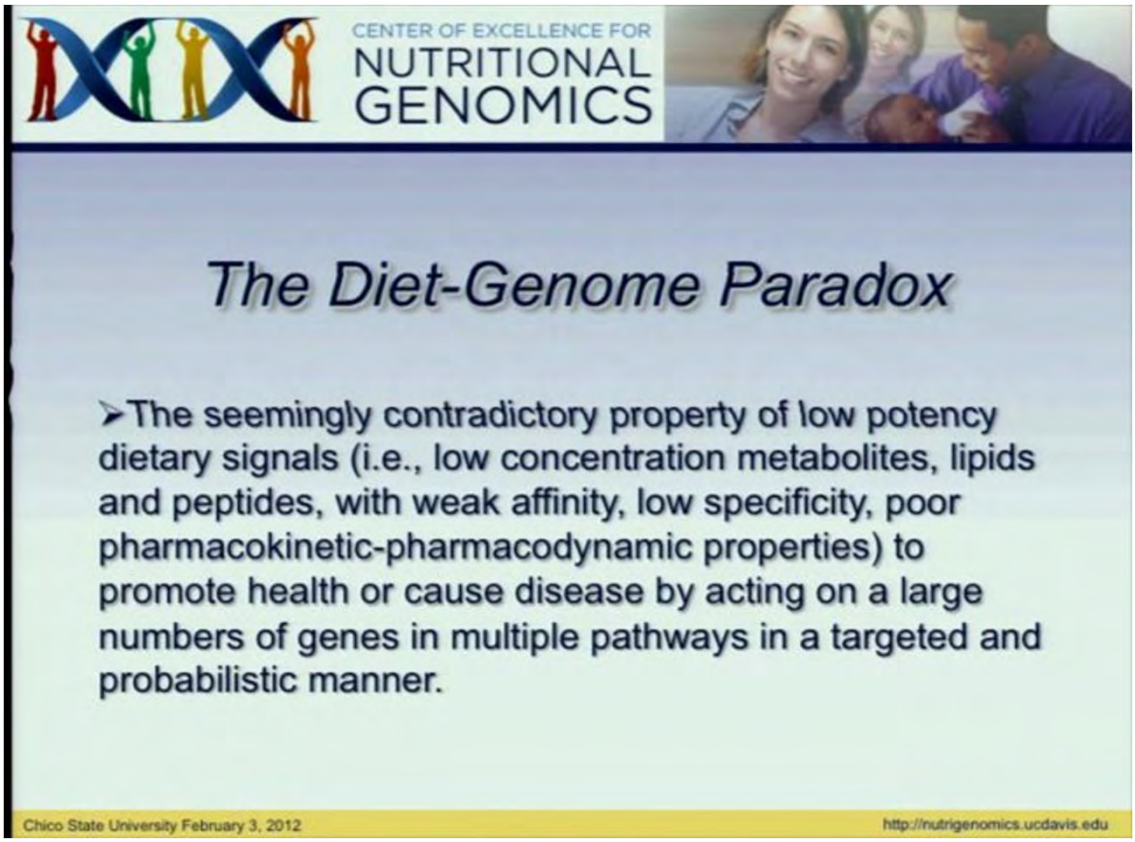
So these are all the things that you've read about in the news, in the magazines, and you probably even incorporated many of these in your diet, catachins from dark chocolate, lycopene in tomato, glucosinolates from broccoli, lutein. I take my centrum silver, I don't know if you got--some of you are too young to take centrum silver, but if you look on the back, there's a little--says a floor glow, a little emblem, a little orange flower. And so what they're doing is they're taking the petals of marigolds that they grow in the Midwest and they're grinding them up, extracting the lutein out of them which is a carotenoid and putting it in your multivitamin. And that's really important because it's that lutein that works at the back of the eye in the macula that protects the macula from oxidation and degradation and prevents you as you get older from getting age-related macular degeneration. So that's why people are interested in lutein, that's why they're putting it in multivitamins, a lot about omega 3 fatty acids and isoflavone. I'm going to spend a little bit time talking about resveratrol, one of the polyphenolics in red wine and I guess I'll confess, now, I'm drinking six ounces of red wine, primarily, Pinot Noir, every night. Because Pinot Noir has got the maximum amount of resveratrol on it. I had a--I've got a former student in Japan, he's now a professor there, and he says, "Well, you drink six ounces every night? I drink a whole bottle on Friday night." I said, "Why do you do that?" He says, "Well, because in Japan, you can't drive and drink so I just get it all out of the way and--." He wanted to know whether that was good or bad. You know, I said I like the--a little bit every day, yeah.



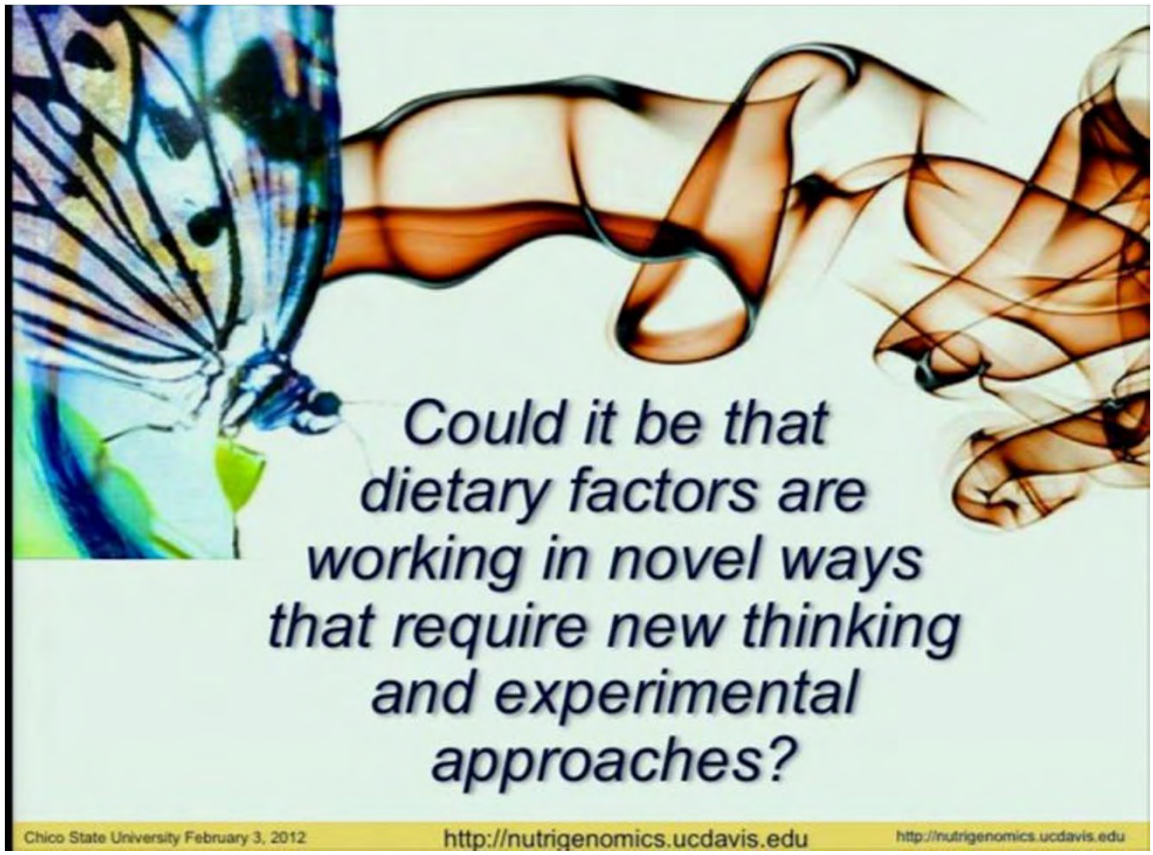
Just a reminder, you know, everything we're talking about in terms of our physiology, our micro--our biochemistry, our metabolism, started here. Our--all of those systems including the signal transduction systems that help regulate everything started with humans nibbling and chewing on seeds, on leaves, on spouts, on tubers, on roots. That's how the process of signal transduction, that's how signals--external signals work their all--their way all the way to the nucleus to turn on genes that we need under various conditions. So we have forgotten that. Now we have a--we created very large plump, polypoid tomatoes and large plump grains of weed and rice. giving us lots of starches and carbohydrates of various types, but we've maybe forgot about those phytochemicals that I had on that previous slide.



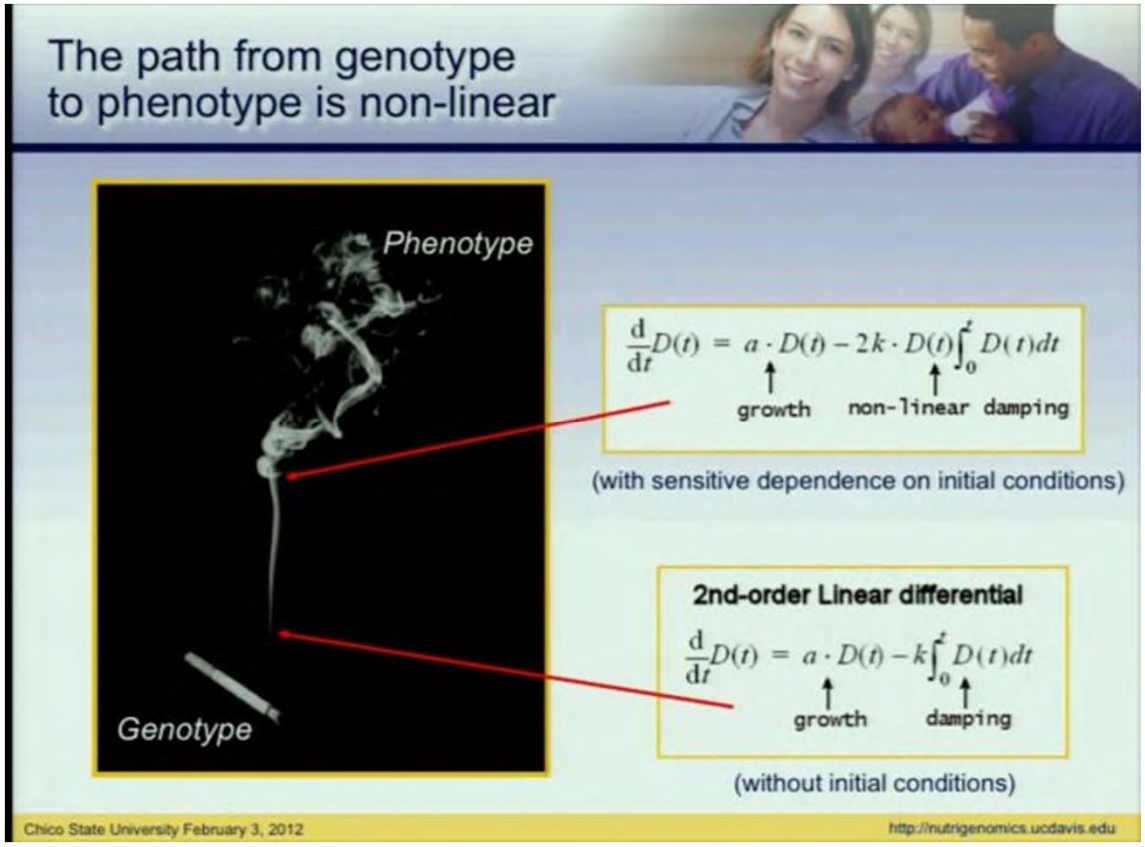
So, do we believe in magic? We believe--I think most of us agreed in the previous slide talking about dietary input and health outcomes. But if you really believe in that slide, how do you believe in this slide? And that is we take our dietary signal whether it's a cup of green tea or one of those Lipton green tea drinks and immediately it goes into the gut, a lot of it is destroyed by the acid in the gut and much of it is excreted. But still, there's enough in the gut to get into the blood, but it doesn't go directly to the organs, it goes to one organ, it goes to the liver and you can see that a lot of it goes into the liver and then some of it comes out. And some of it comes out in a different form so it's biotransformed in the liver. And then it gets into interstitial fluids, it gets into tissues and cells, and then eventually, it gets into intracellular fluids and eventually gets into the nucleus where it does something. And by this time, concentrations are so low that metabolomists with their very expensive and powerful instruments can't even find it. But I thought from those previous slides that if we ate this and we ate that, that we will be healthy. So there's a disconnect from what we know, what we feel and what we believe, how our bodies evolve, and what we can find in the laboratory. And so, what's--again, what people are labeling--laboring under are those linear deterministic processes. I heard--read a paper the other day that talked about the law of mass action. If you don't have enough of these molecules in the cell, they won't bump into each other frequently enough that you can't see the effect. I'll give you an example of that. Others--I threw in another paper, well, the levels were in the nanomole amount and that was just too level--too little to have any effect.



So on one hand, we've got a paradox here and that's a--I guess here by diet-genome paradox. The seemingly contradictory properties of low potency dietary signals, low concentration metabolites, lipids, peptides and others with weak affinity, low specificity, poor pharmacokinetics, and pharmacodynamic properties to promote health and cause disease by acting in a large number of genes--on a large number of genes in multiple pathways in a targeted and probabilistic manner. That's how it works. I know that in my gut, that's how it works. I can't prove it yet, but I know that we are--we cannot expect to see at the cell level that those dependent log-linear response that you expect to see in the biochemistry lab. There's something else at play.



Could it be that dietary factors are working in novel ways that require new thinking and experimental processes and approaches and you probably can't figure what this is. Here's a butterfly and that's turbulence. This is the butterfly effect, a small effect working way out here causing a big effect over there. And if that can happen in terms of global climate, why can't it happen inside your cell? I believe it does.

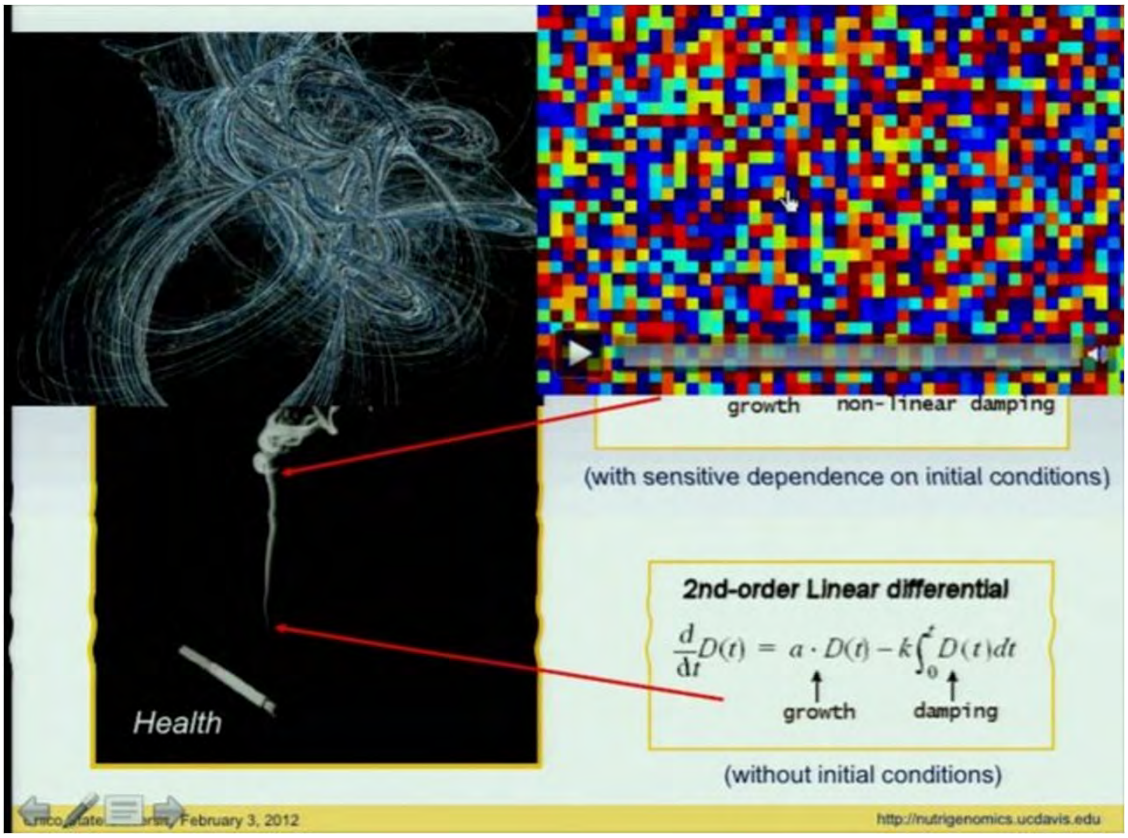


So I like to use this metaphor, Man Ray's photograph of a cigarette. I love it. It's beautiful, black and white, done in the 30s. I don't smoke. I don't like it for that reason.

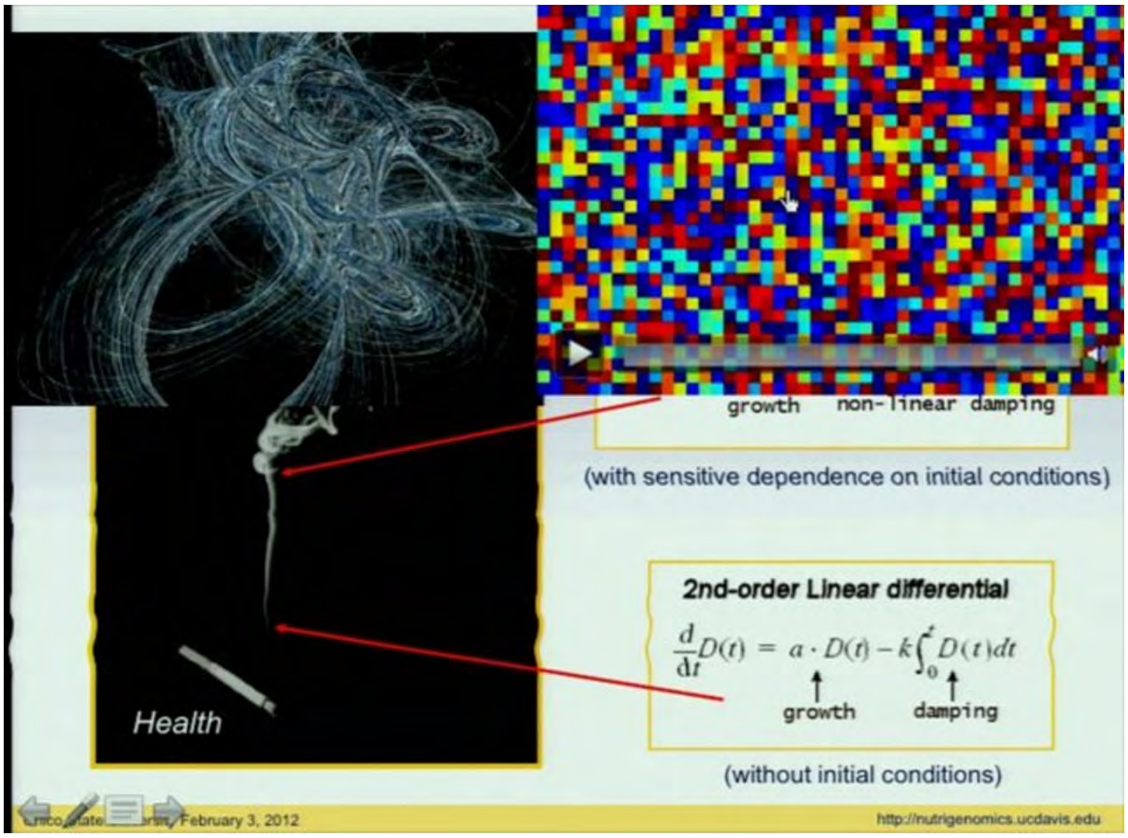
I like it because it's a wonderful metaphor for genotype-phenotype. We're not dealing with that previous slide I showed you, Mendel and his single gene producing a protein creating a disease phenotype. That's a linear path from gene to disease and that is not what we're finding anymore. What we're finding is, if this is a genome, we're finding some linear processes that get a little turbulent, then they get wildly turbulent, and then they get unpredictable. And it's--this is what we're seeing. So if you look at the phenotype, if I look at you from the outside, I see this and the challenge is to de-convolute ourselves back through this chaotic area here down into this turbulent area then back to the gene. That's a real challenge. That's what we call a highly, a computationally intensive process. And there're only a few biologists, mathematicians, in the world that can do that.

So here, this is what we're trained to do in the lab, look at the second-order linear differential equations, we can predict this.

But by the time we get to here, you can see we've entered in a nonlinear function, with inter DNA nonlinear function. Things are getting a little crazy, but they look crazy but they're not really crazy. This is that--a dynamical system, it's that ordered--those ordered processes that appear on what looks chaotic.

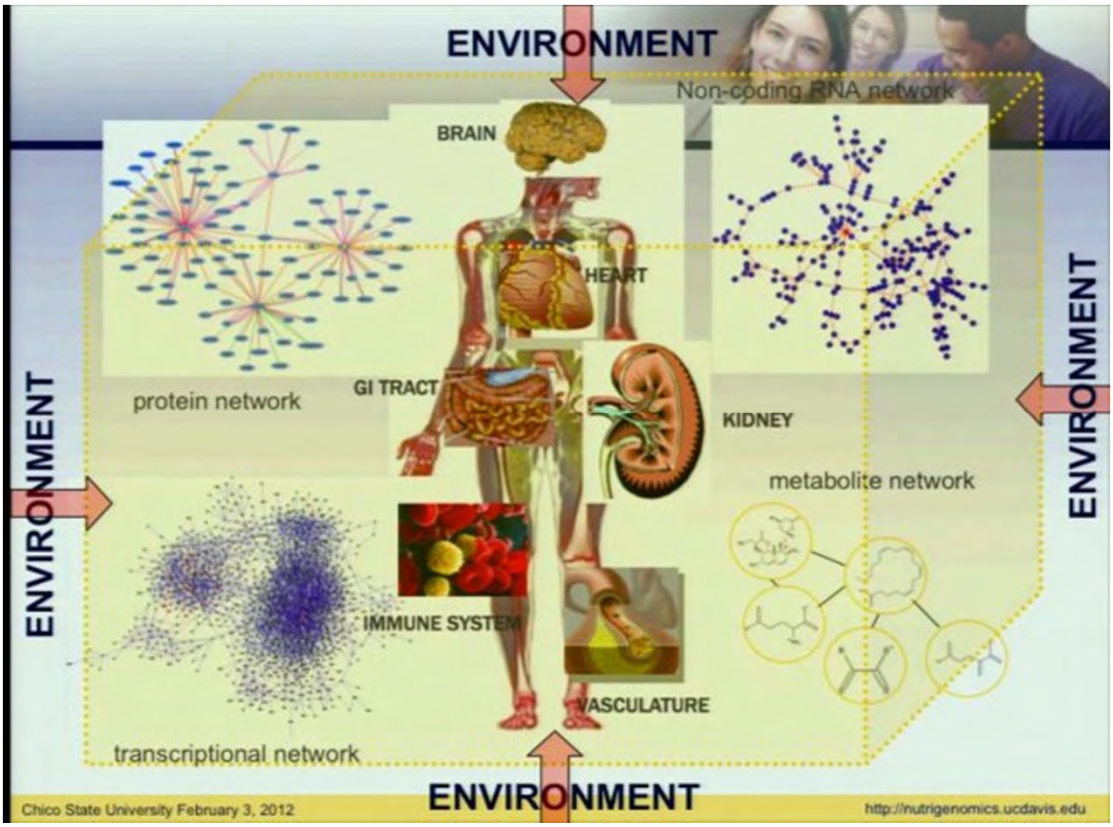


And if you could--if you could blow that up, you might see something that looks like that. You can see, it looks chaotic, it looks a little turbulent, but really what we see are cycles, repeating cycles and patterns. And I believe that that's how our metabolism and our physiology works in response to nutritional input or the lack of nutritional input, or too much nutritional input or too little or bad in nutritional input. We will see these patterns form, they will form and then dissolve. They'll respond to nutritional input and then they will return. We call it homeostasis. So what the people in complexity theory will say now, now that you form this dynamical system, and that's what we see here, then decisions have to be made in the cell. How do we get back to homeostasis? So they come up with this idea of emergent adaptive behaviors. So complex systems have four components: One, they have many parts; secondly, those parts are connected; thirdly, those parts are interdependent--partly interdependent, not completely under interdependent; and thirdly, due to that connectedness and interdependence, you can have the emergence of adaptive behaviors. That cells and enzymes will start to do things that they have to do to get you back into that homeostasis. Can that happen in a--in nature? And the answer is absolutely. I have a little video that--on fireflies in Kentucky, I can't get it to play at my computer anymore but these are synchronized fireflies. You do that in the night, you're walking around, you see a firefly blinking here, one blinking there, one blinking there, just blinking randomly and all of the sudden, others kick in and they start blinking randomly. No, they start blinking together and in about a minute, these fireflies are blinking in a synchronous fashion, flash, flash, flash, right there in the air. No conductor, no one said how to do this. Each one is an independent event so the probability of those synchronized flashing is the product of all those independent events coming together.



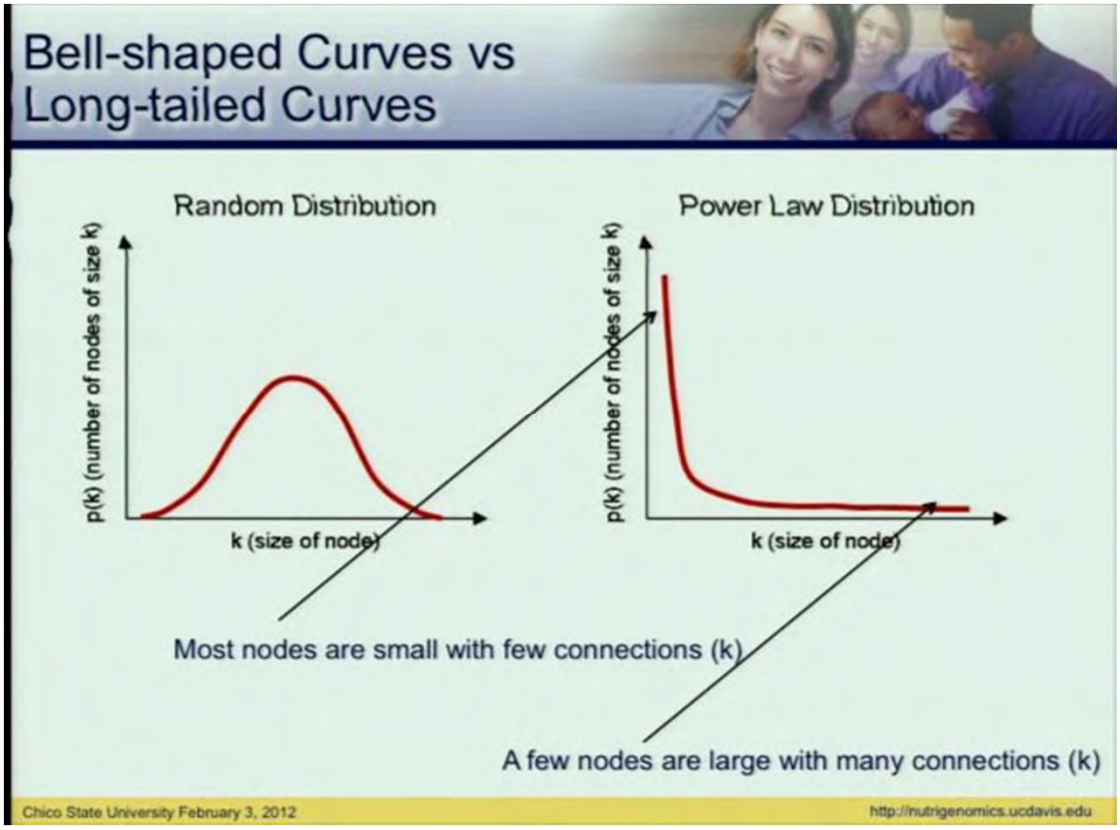
It shows you that emergent adaptive behaviors can occur in nature. So what these smart computer guys done, they generated a little example of this. Components that are connected interdependent where I--an emergent adaptive behavior can appear, and they just apply the few rules. If red is on then blue is off, if blue is on then green is on, and a blue and green are on then yellow is off, yeah, let's just try that, those kind of rules.

And they come up with this little process here--yeah, let's see if we get it going. There it goes. There's random interaction--they only flickered for a few seconds and already they're starting to develop a pattern and this pattern would go on for a couple of minute. Many, it looks--for something that's supposed to be random, this looks really non-random. This looks like that kind of turbulent area over here. And if we let it go, then what we form here is a traveling wave. And a traveling wave is exactly what hormones do in your body. During embryogenesis, every organism has hormones that involve--using this traveling wave concept, spreading throughout the body to trigger the development of the organism. So they can do that on a computer, I'm sure we can do it in the body.

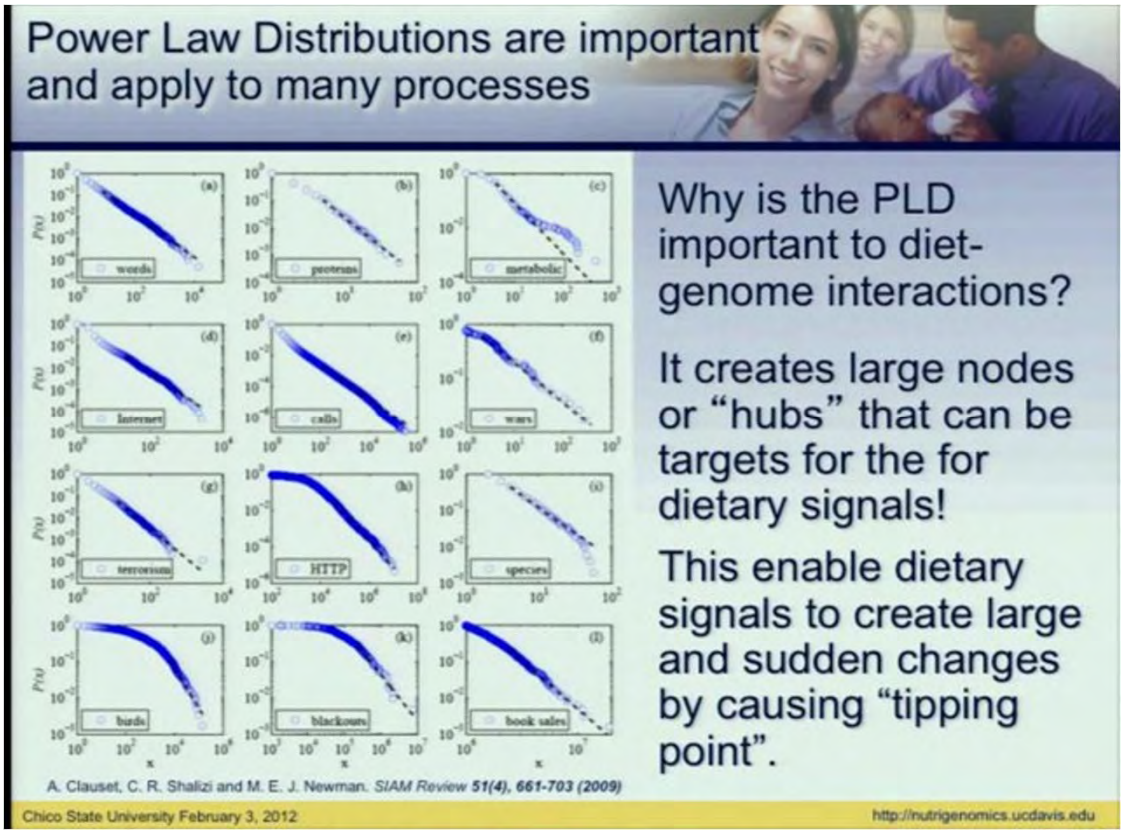


So, the human body is a series of different systems, you know that, and each system has a protein network, a micro RNA network--I'll come back to micro RNAs in a second, a transcriptional network called the transcriptome, and the metabolome, metabolite network. They're all interacting with each other, touching each other, some bind together to do some work, the interesting thing is that they also touch other networks, these networks are all touching each other in the cell. And it's interesting, it's all the possible decisions that can be made, that's what you need in a complex system. I need to be--I need to be able to make trillions of decisions, but the nutritional input and the tools that I have to work with will define the number of decisions that I can do and I will choose to do the right one or the wrong one. The right one of course, leads to longevity and health and longevity. And the wrong one leads to early death.

So the human body is what we call a complex system and everybody that you talk to now is--and it's highly influenced by the environment. The most important thing that we get from our environment besides air is nutrition and we get that input two or three, four, five, times a day. The quality that nutrition over time will determine how well these networks work.

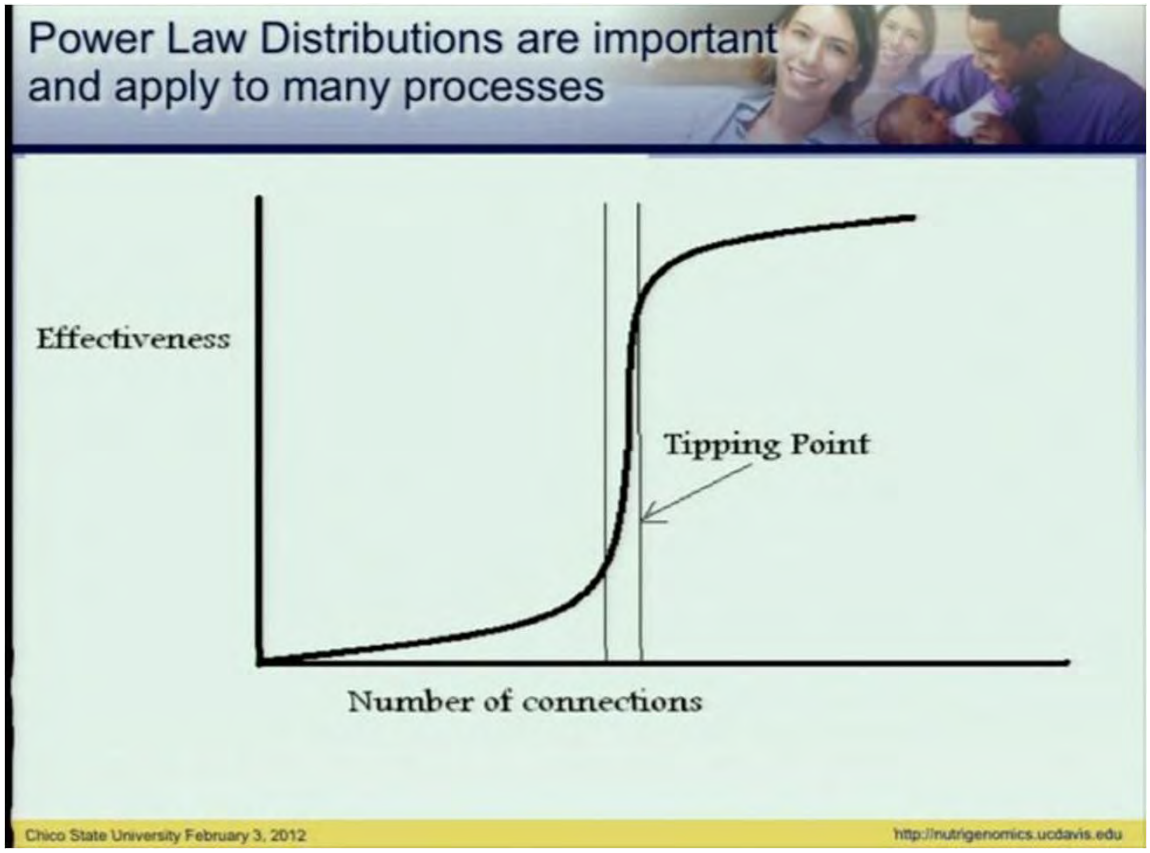


I want to talk about these networks. There's an interesting thing about networks, they're not--they don't have obey Gaussian distribution, they're not bell-shaped curves, they're long tail curves or power-law distributed. So this is a new concept I picked up a couple of years ago, I thought that it was just bell-shaped curves. No, they're long tail power-law distributed networks. And they're everywhere, they're everywhere in society and now we know that they exist actually inside the cell. They had actually exist in the cosmos. And what it says is that there are few thing, a few things. I have a--whatever this things are most nodes have a few connections and a few nodes have lot of connections. Sort of like me and my Facebook friends. I got a few connections, but some of my friends have got 500 connections, my son-in-law has got a thousand connections. And if I--and they'd calculated mathematically, if I choose the most popular friend on my Facebook, they'll have ten times as many connections as I will have. And if that person picks the most popular person they know, they'll have ten times as many friends as he has or she has. This creates some very interesting possibilities and things that I didn't know about, I now appreciate them more, you find that all throughout nature here.



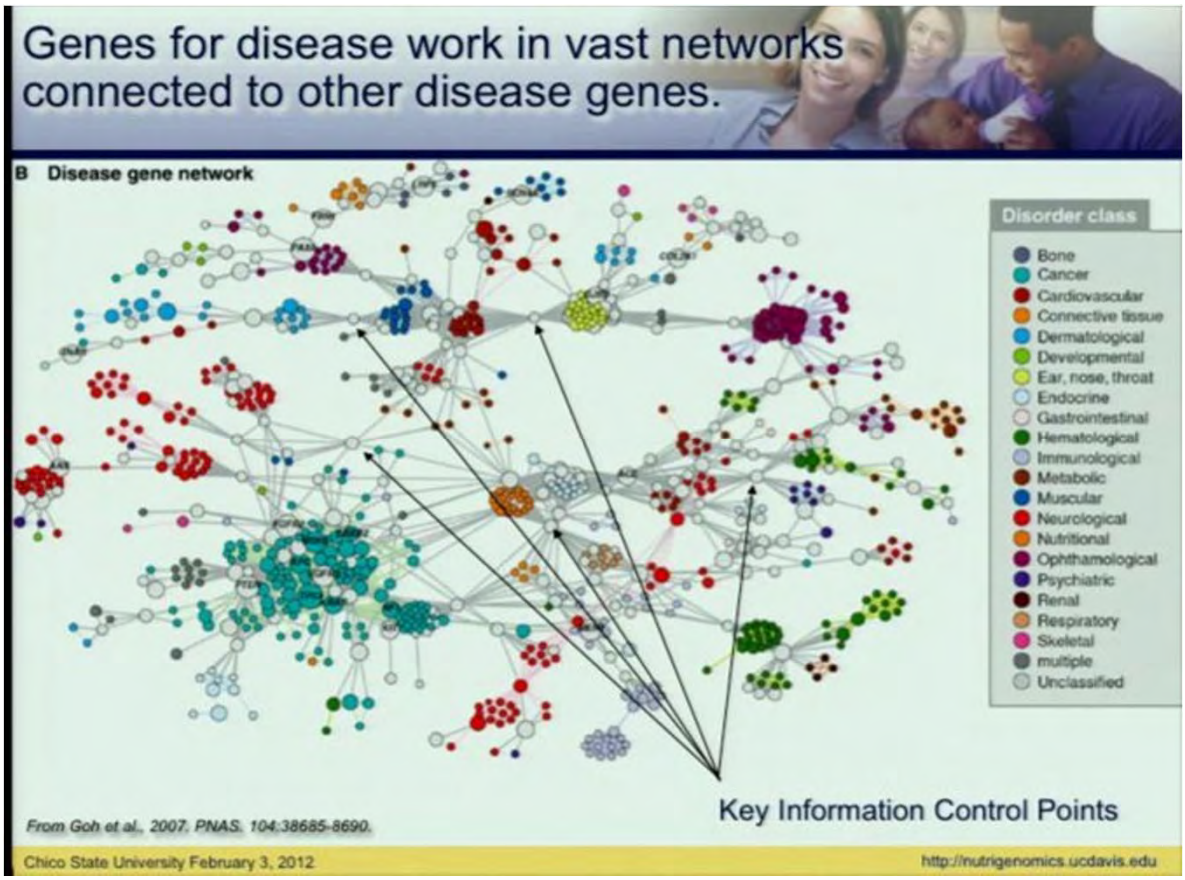
Words: a few words or--most words are used a few times, a few words are used a lot of times. Proteins: most proteins have very few interactions; a few proteins have a lot of interactions. Metabolites: a few metabolites have a--a most metabolites, that's most up hear, have a few interactions; a few metabolites have a lot of interactions. Wars: most wars kill a few people; a few wars kill a lot of people. Booksellers: a few booksellers sell a lot of books, but most booksellers sell a few books. You see it everywhere in nature, in society. Any others here that I know? Blackouts: some blackouts--most blackouts only last for a few minutes; a few blackouts last for a long time. So this is a new concept that's come out of book like "Connected," come out of books like "Linked," come out of book like "Tipping Point," that are now telling us that what we see in society, we can actually extrapolate that back down to biological systems and get it into the cell. And I'll show you how that works.

But just before I do that, I would say that, why are power-law distributed networks important to diet-genome interactions? There's a reason for this. When it creates large nodes or hubs that can be targets for dietary signals. So if I'm a weak dietary signal, remember that my diet-genome paradox said, low concentration, low affinity, poor pharmacokinetics, poor pharmacodynamics; so if I'm this weak little molecule, I don't have time to go around and touch and probe every possible gene and protein. No, I'll go to the hub and that's the one I'll interact with. I'll touch that one and hopefully, I'll cause it to do something and whatever it does because it's highly connected, it's going to pull all those other genes with it. That's called a tipping point. So this enables dietary signals to create large and sudden changes by causing tipping point or, in mathematics, we call it a dynamical system. Yeah. And there's a tipping point.



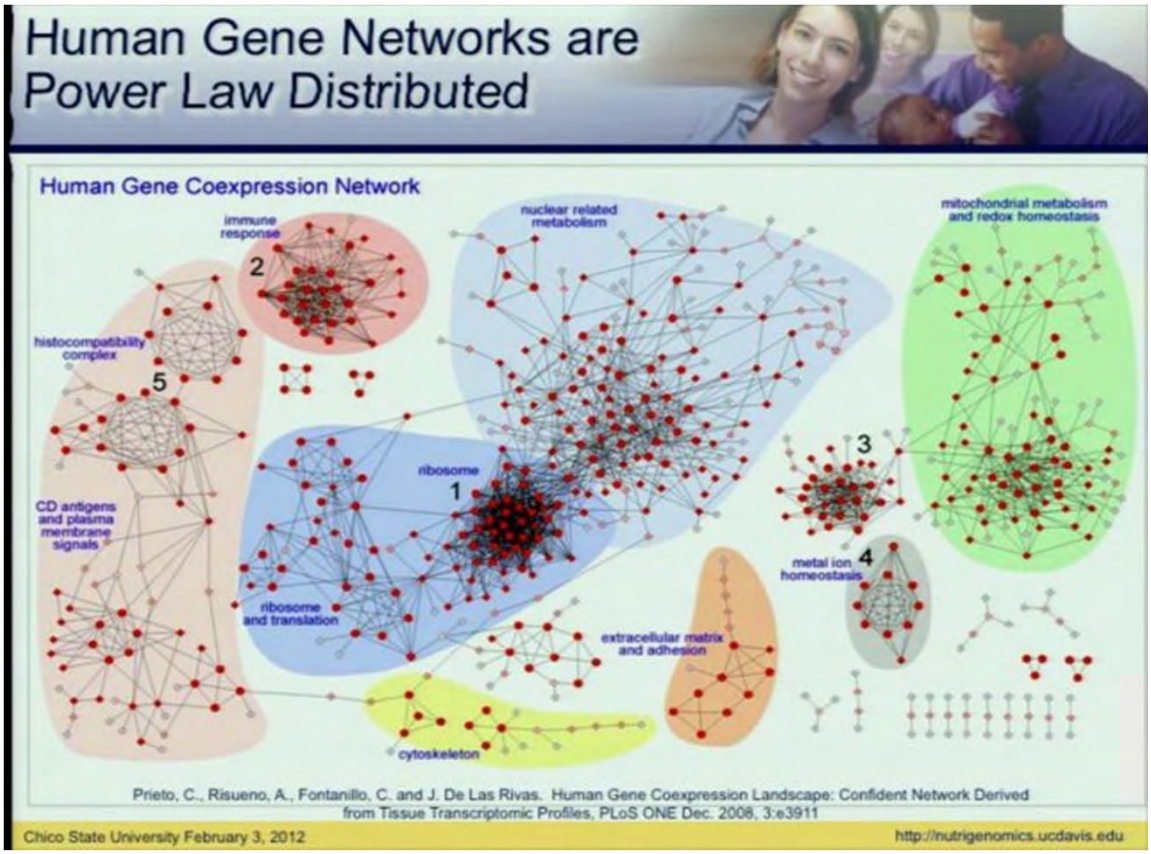
And this is talking about social networking, the number of connections a person has, the more effectiveness you can have in getting something done. This is the Arab Spring. This is a--what do they call it, a flash mobs? Yeah

At some point, you can see as the numbers of connections start to increase in your network, as nodes start to form, at some point, you're going to reach a critical stage where you're going to get this huge jump, nonlinear, no dose dependency, you get this huge jump and that goes back to my first slide. We've got this little dietary peptide that's coming in and it's gently touching the 5--the amino terminal end of histone 4 affecting lysine 16, just gently touching it and putting in an acetyl group on the end of that amino acid to neutralize that charge, can have a huge tipping point. So maybe that's how dietary signals are working to create big effects.

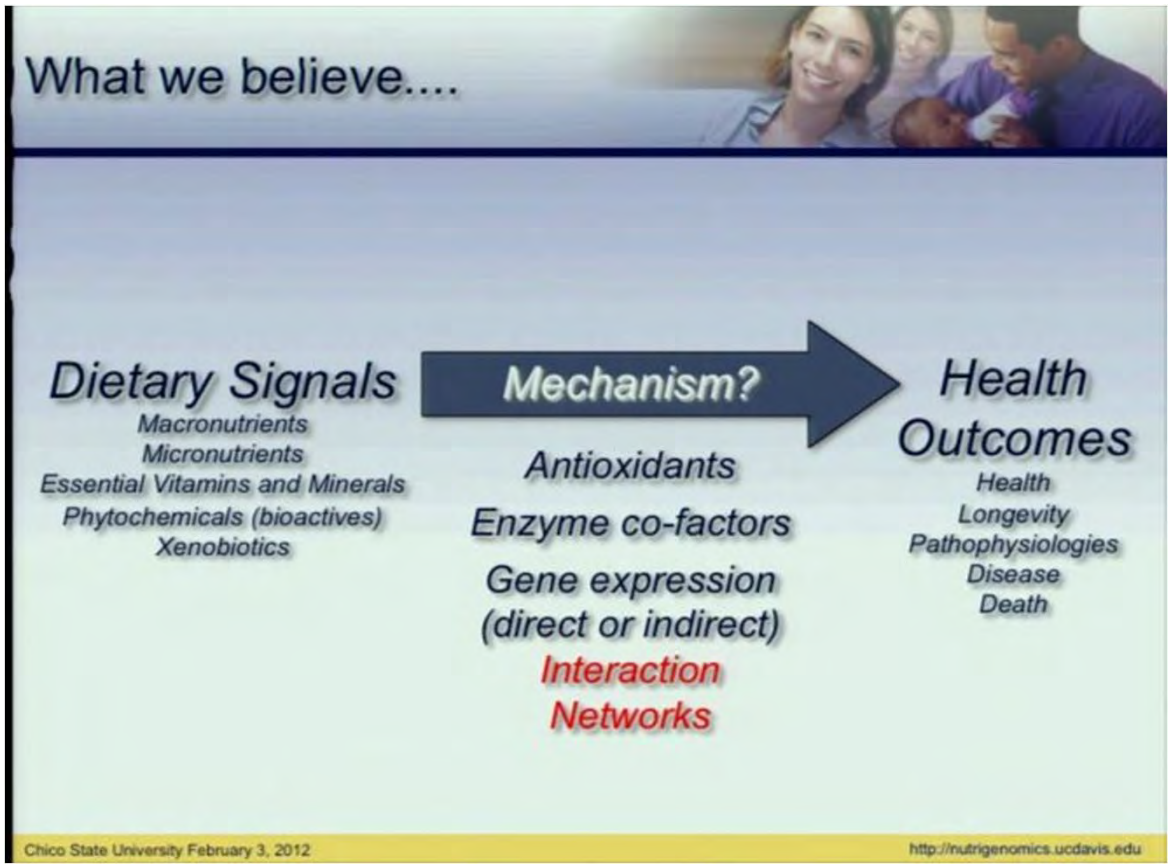


Do networks occur in human cells? And the answer is yes. This was a landmark paper done by Laszlo Barabasi, the founder of network theory, and Marc Vidal, a Harvard Biologist. And Mark asked Laszlo, "Does this really work in human cells?" They published a paper in 2007 called the "The Human Diseasome." And here, you can see all of the various genes that are involved in diseases and look how they're connected. So these are all cancer genes here, and I'm not sure, I can't read some of the--I think this is--what is this red, a cardiovascular, that there're some nutritional and respiratory things here. But the key thing is look down here, this gray circle here, unclassified. All these unclassified are these nodes right here. We spend so much time characterizing individual genes involved in this huge processes like cancer, we forgot to look at those nodes that connect this cluster here to that cluster, this cluster to this cluster.

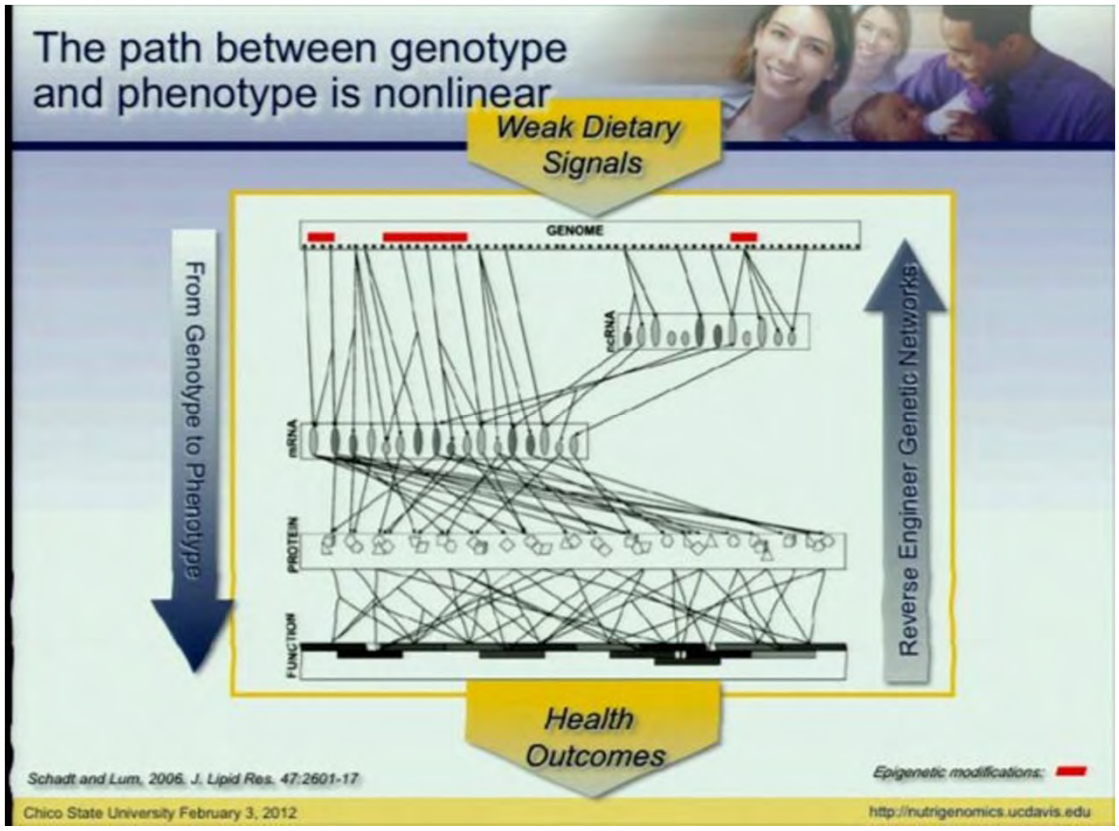
So there a lot--oh, these are all of my poor net--my poor Facebook friends that don't have a lot of connections. The vast majority of genes don't have a lot of connections as they have few connections here, and these are the ones, these nodes right here, whatever they are, they've got a lot of connection. We call them party hubs or party nodes, and if I only have some resveratrol or a little bit of isoflavone, or a little bit of glucosinolate, a tiny amount, I'll try to affect those hubs. As opposed to going out here and trying to cause a major change in physiology by interacting with some of these molecules out here. Yeah, so those are the key control points everybody's interested in.



Here's another example, this is a co--a human gene, coexpression that were genes that seem to go on and off together. There is the immune response, overlaps a little bit with histocompatibility complex. Here, we go down--oh, look at this, this--whatever the genes are in here, it jumps over here to cytoskeleton, cytoskeleton jumps back up here to ribosome and translation, goes through here, gets up into nuclear-related metabolism. Over here, we have extracellular matrix and adhesion, those are important ones. Those are the things really lead to metastatic cancers. Here is iron--metal iron homeostasis, mitochondria, and you could see how the mitochondria, metabolism, and redox homeostasis interacts out here with this network. So yes, networks exist in the cell, they exist between the products of genes, the RNA's of genes, the metabolites that are produced by those products. And if that's true and if we believe in Facebook, then we should be able to promote or produce these large effects by creating tipping points by working at some of the key nodes, maybe this node here.



So it's these interaction networks which I believe is helping to explain this idea here.

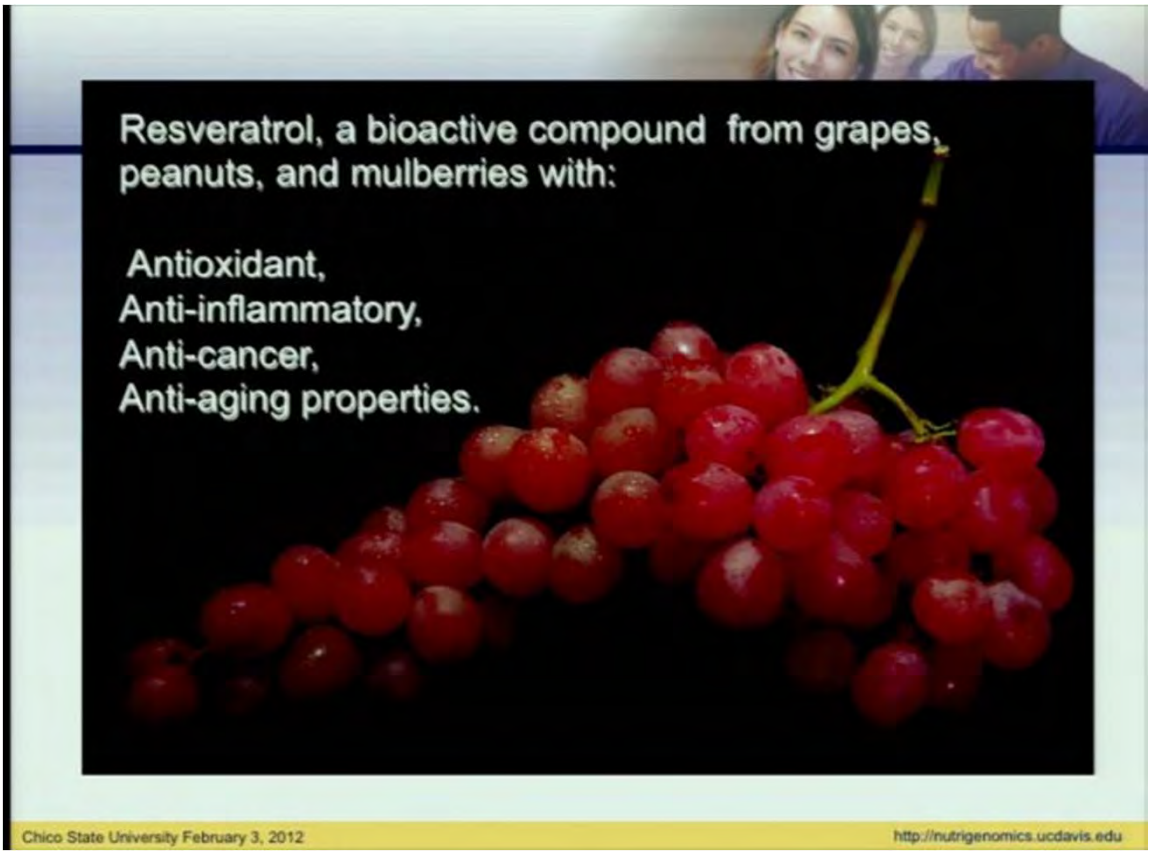


This is a friend of mine's, he's a member of our center, Doctor Eric Schott I guess that there are a couple of people in the world who can deconvolute the path from phenotype back to genotype and he's one of them. And borrowed these from his 2006 paper.

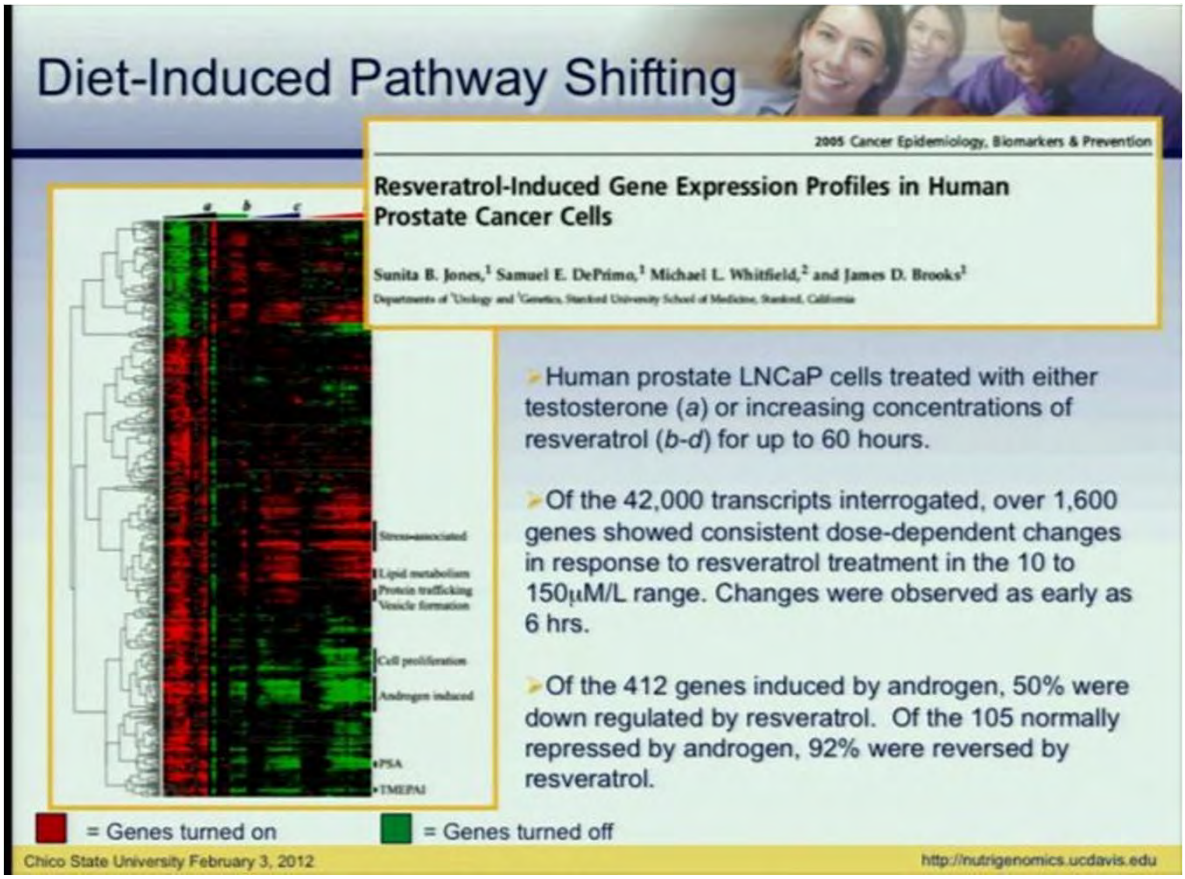
And he's just typically looking at the genome here, the genome making RNA's, RNA's are making proteins. Proteins like to interact with each other and when they do, they produce functions and you can see what's happening here, we've a got lot of functions, there are--a lot of them are more overlapping, and a lot of them are due to this interaction between different proteins which come from these RNA's, and look, we've got a little wild card over here, microRNA's or noncoding RNA's which can come in and affect the way these RNA's are expressed and then let's not forget epigenetics.

We have these genes up here, but some of them could be epigenetically silenced. Cytosine methylation, CpG islands, turn genes off. So just because you have a gene, doesn't mean you're using it. So starting with weak dietary signals, we have these health output, this is a path of genotype to phenotype.

The challenge is to reverse engineer our way back to those genetic and epigenetic effects that cause this whole process in the first place.



Let's talk a little bit about resveratrol. Yeah, everybody's heard about it, you don't have to drink wine to get it, you can get it out of grape skins--antioxidant, anti-inflammatory, anticancer, antiaging, anti everything. So this has been a real popular compound for many years and there are a lot of products that are based on this, there are companies that are based on this. The scientists who just--who determined the mechanism of how resveratrol works, they started a company and they've made millions. So what's going on here with these little poly ethanolic that comes out of the skin of red grapes and primarily, the Pino Noir grape.

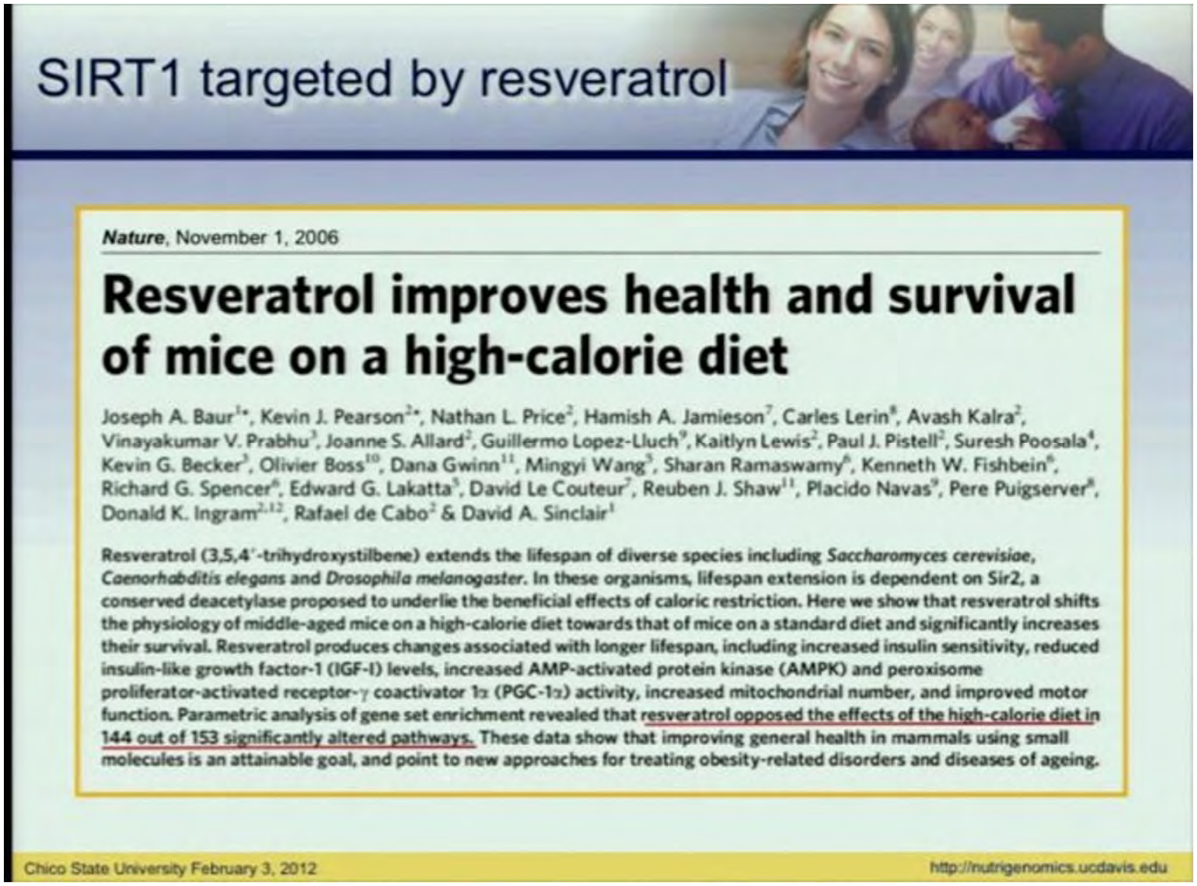


This was a study that was done at Stanford looking at prostate cells. These are special prostate cells that are primed to turn cancerous with the addition of testosterone. These are LNCaP cells. And what we're looking at here is about 1600 genes here, and this is what the gene expression looks like when you add a little bit of the testosterone. The green is genes are turned off, and the red are genes are turned on. So you can see how powerful the guys out there, guys my age are worried about your prostate. This testosterone can have a huge effect on gene expression and all these genes are turned on and some of the genes that are turned on, I'm not happy with. The first thing you see is increased PSA level, yes, that PSA is turned on. This is an RNA down here which is a hallmark of prostate cancer. Self proliferation, don't want that, and so on. So what's shown here is increasing amounts of resveratrol being added to these cells, and you don't have to be a genomicist, you don't have to know anything about microarrays to see that the more resveratrol you add in this direction, this columns, I've got the amount here, thought to elaborate, the LNCaP cells.

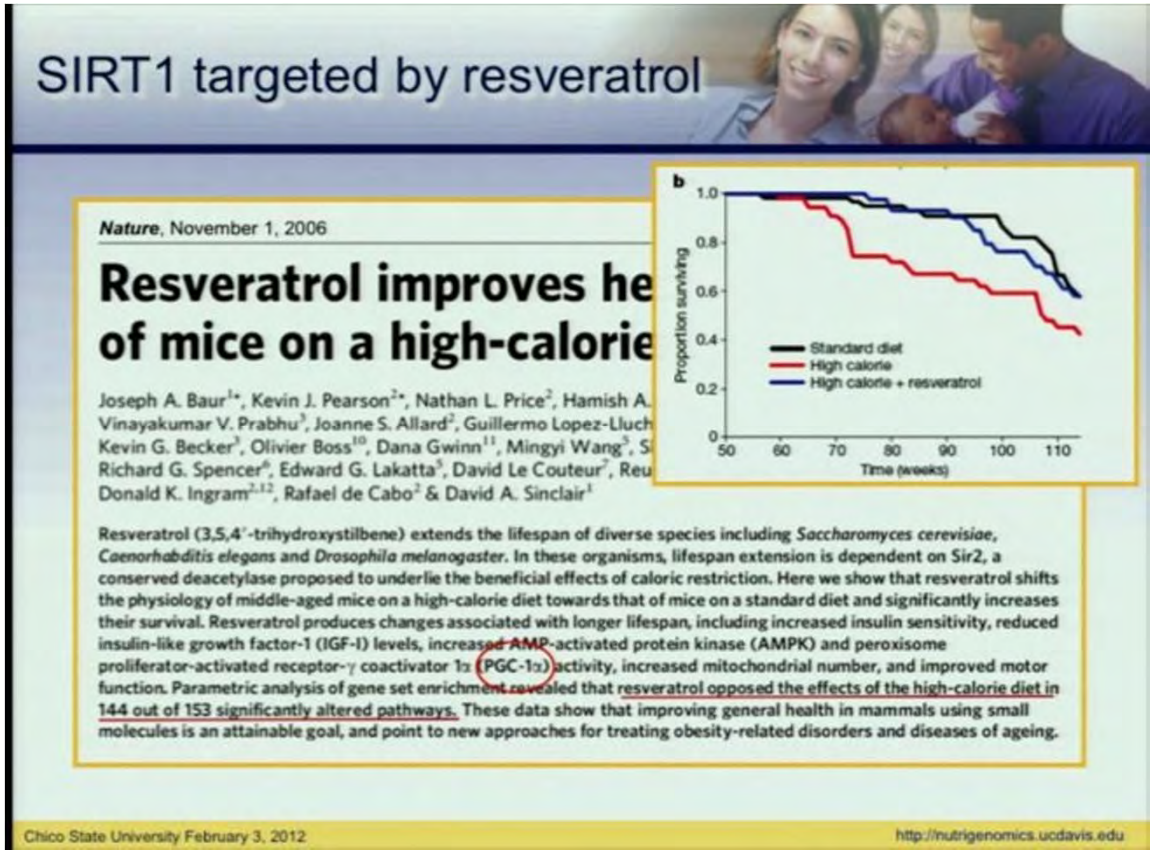
42,000 genes were interrogated here; 1,600 of them showed this consistent dose dependent which changes in response to resveratrol from a 10 to a 150 micro mole per liter, which is not very much, and you can see these genes being turned off. It isn't random, it wasn't all on or all off. It seems to exhibit that targeted effect, some of the genes that you want off, were turned off by resveratrol.

Of the 412 genes induced by androgen, 50 percent of them were down, regulated by resveratrol. Of the 105, that are normally repressed by androgen, 92 were versed by resveratrol. So it looks like this polyphenolic from grape skins is coming in and reversing the

effects of androgen which wants to push these cells to become cancerous, resveratrol's pushing it back. That wasn't the only observation.



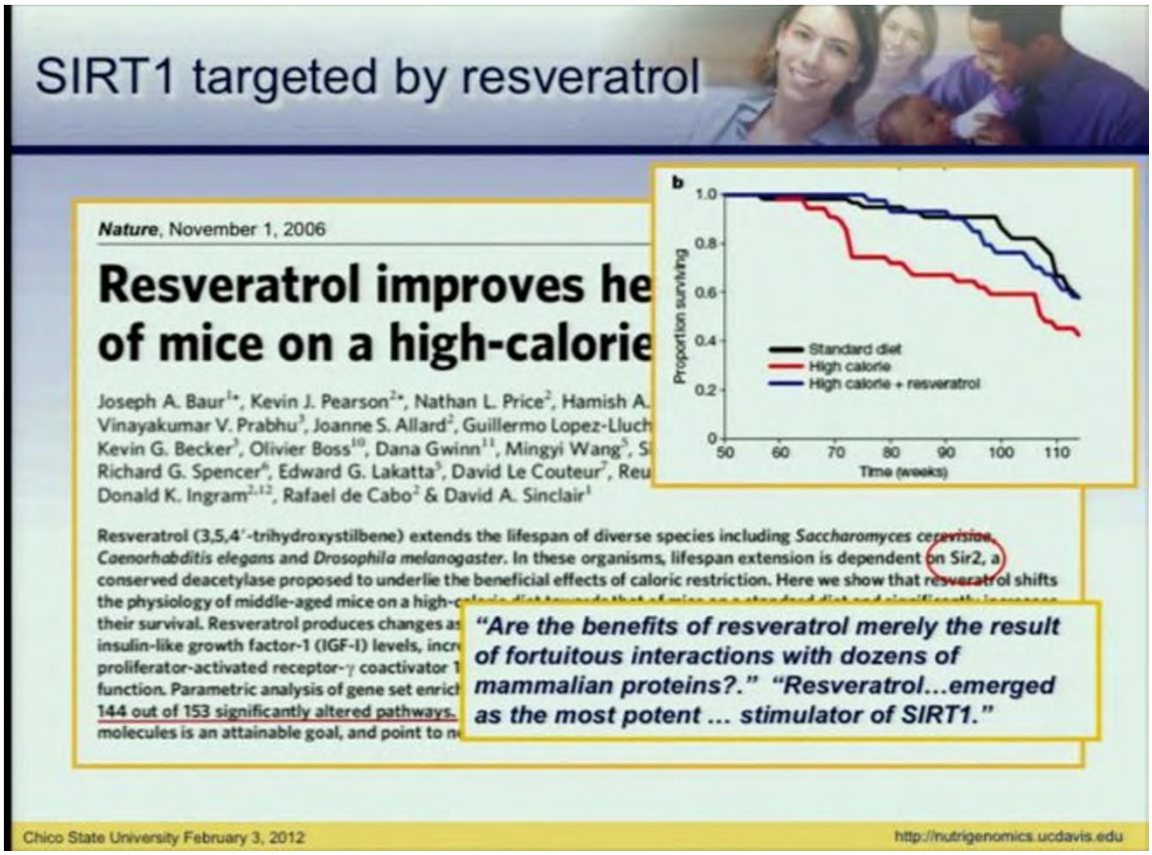
The next one here was by David Sinclair who discovered that, how is it pushing these genes back, it's turning genes off and how was it doing that? This was a landmark paper, 2006, resveratrol improves the health and survival of mice on high caloric diet. So this was the McDonald diet study that everybody talked about in the news in 2006, 2007. Taking this poor mice and then fattening them up with a high calorie diet, and what did you find? They died earlier, their lifespan decreased.



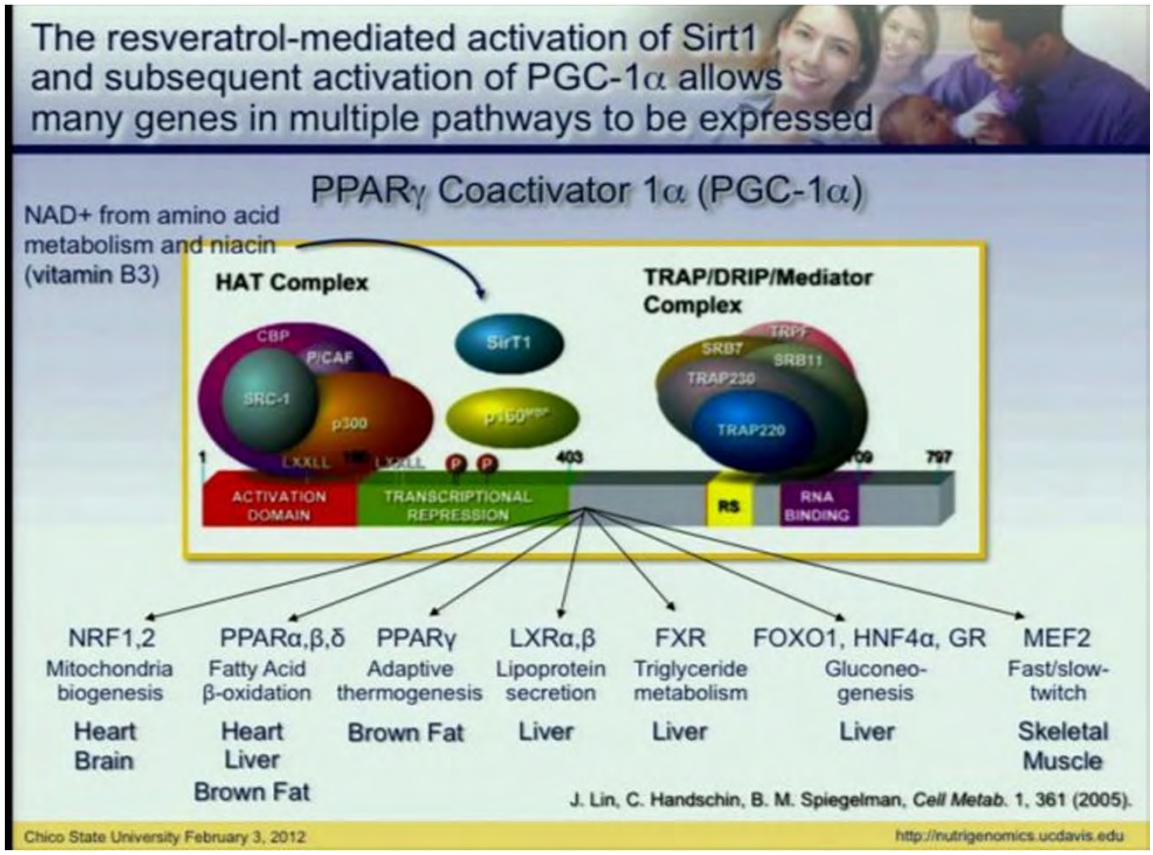
And it's shown--I think right over here, yeah, I think the black line is the normal fed mouse on normal mouse chow, the red line are the mice on a high caloric diet, and you can see in terms of survival, we're looking here proportions surviving and it start to drop off faster than the normal mouse. But look at that blue line--those are the high caloric mice that were fed high concentrations of resveratrol. And that I read--when I read this paper, I practically jumped out of my chair because I thought I was misreading it. Here he says that resveratrol opposes--opposed the effect of the high calorie diet in 144 out of 153--I thought he was going to say, genes. No, he said, significantly altered pathways. He's talking about 144 pathways that the caloric diet had turned on, resveratrol's turning them back. So that must mean that resveratrol is working on thousands of genes.

(slide 53) And he gives us a hint here, he's saying that the way he thinks its doing it he's working on his very powerful coactivator, PPAR-gamma coactivator--coactivator 1A, PGC-1alpha, which is a hub. Yeah it's a hub, that's what, and what--you'll see what PGC-1alpha does.

(slide 54) And he throws another hint in here, yeah, and it's being accomplished, it affects this SIRT2 dependent activation of the SIR2lants.



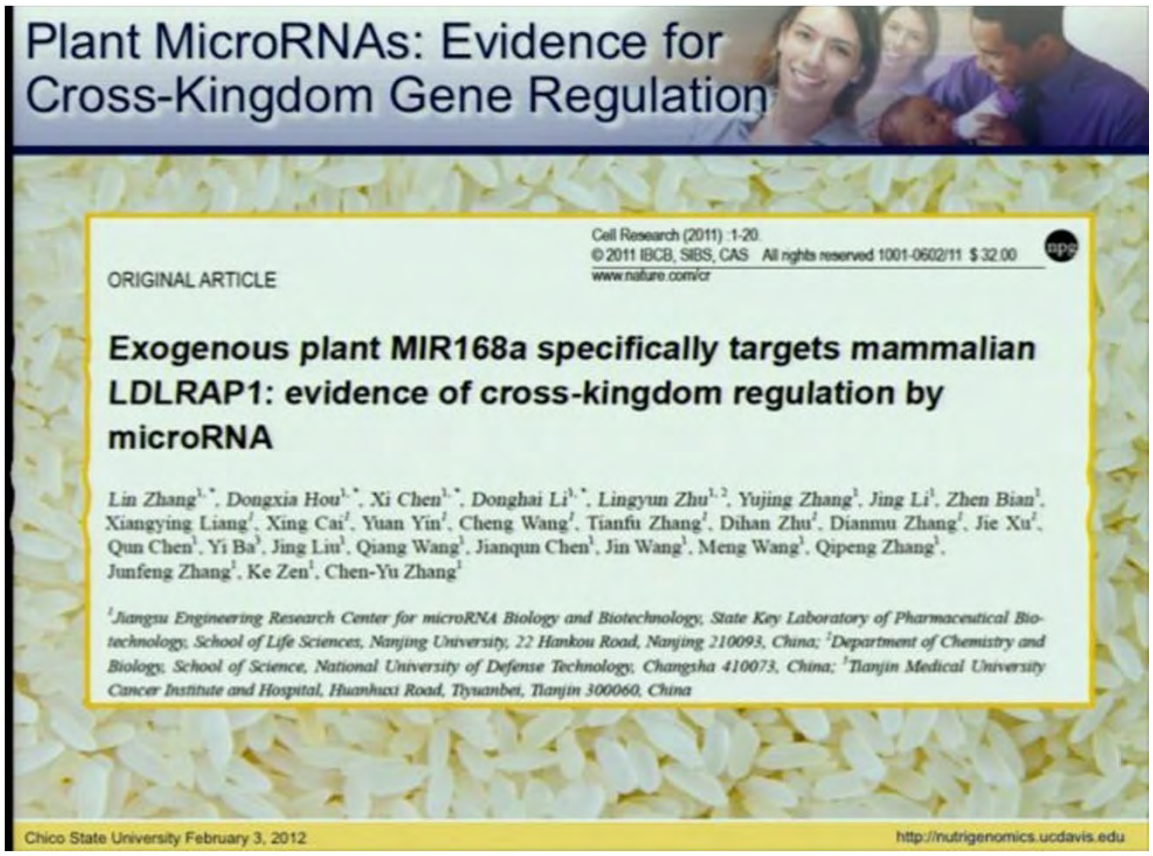
Finally, I just pulled this out of paper. I thought this was an interesting philosophical question. Are the benefits of resveratrol merely the results of fortuitous interactions with dozens of the million proteins? He knows it isn't, it's not random interaction, just somehow targeted interactions. And he says that resveratrol emerged as the most potent stimulator of SIR2 (in humans we call it SIRT) and that's a histone deacetylase, an enzyme that plucks the acetyl groups off of histones, silencing genes.



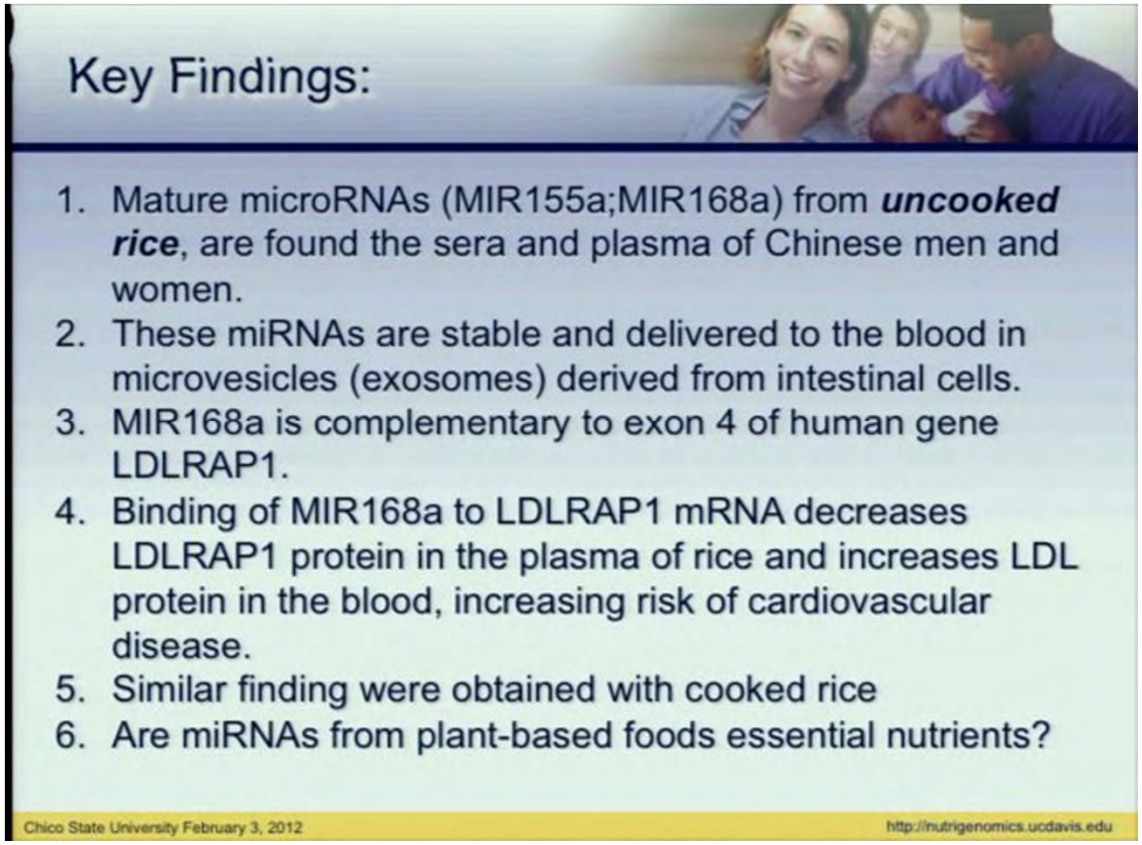
So here is a PGC-1alpha, this is PGC-1alpha and these are all of its friends. You can see it's a large, multi-coactivator complex and one of its friends is a SIRT1.

And it has the ability to alter or affect the expression of all of these transcription factors which in turn affect the metabolism all of these types of metabolism in all of these different tissues. So you can see, this small dietary signal hit the right target, it got the party node, it goes in there and it altered through this interaction here. And I should finish off, yeah…

NAD plus from amino acid metabolism in niacin, I can't get away from nutrition here. And resveratrol stimulate the activation of SIRT1 which helps PGC-1alpha do all of its jobs. Again, going back to networks, hubs, tipping points, all being accomplished not by high concentrations, but by very small amounts that get in to the cell. I'll tell you, in this particular study--talking about high concentrations--he was highly criticized because the levels of resveratrol he was giving the individual mouse was equivalent to I think a 100 bottles of wine a day for a few human, okay? So he was proving a point, but I think that even lesser levels, what would happen if he would see small effects? He uses high concentrations to see a big effect, but I think over a long period of time, you'd see the same positive effect with smaller amounts.



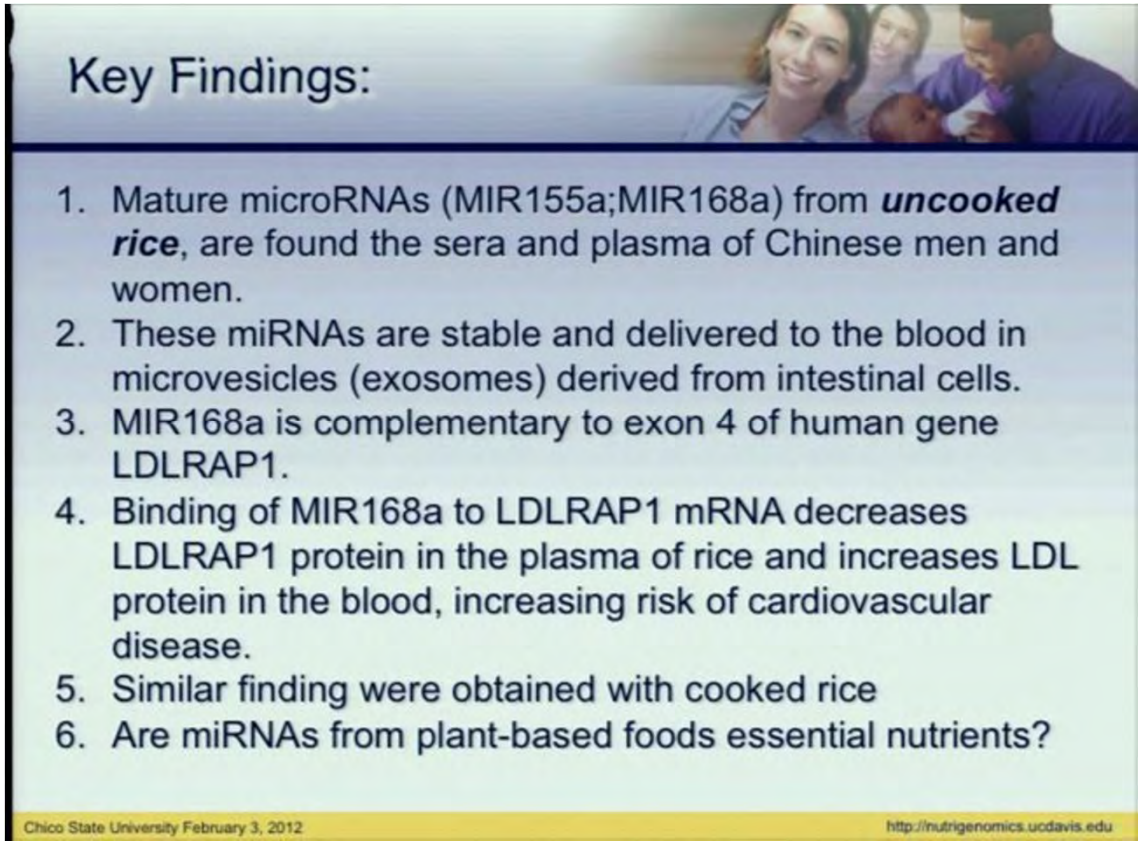
This was another landmark paper that just came out I think last year? Exogenous plant microRNA's specifically target mammalian LDL receptor, adaptor protein 1, evidence of cross kingdom regulation in microRNA's. What is all this about? Remember, there's--those noncoding RNA's include some microRNA's which are made in various parts of the genome. They don't make proteins, they have the ability to come over and either activate or deactivate other RNA's from being expressed. So they're form of regulators and they don't--they're not near the genes that are regulated, they're sprinkled around on the genome. And what they're saying here is that these can get into our body and regulate genes in our cells.



And I have a little bit of cooked rice here, I just summarized quickly the findings of that experiment just to let you know why this was such a important paper, it was--and I read this paper and it was 20 pages long and most of it was controls. And I looked at the acknowledgment, they acknowledge people at NIH, there were two guys at NIH and one at Mount Sinai in New York, and I could tell they didn't want this paper published. They didn't believe it; they said you have to prove it. So after 20 pages and tons of graphs and controls, they got this published. And what they found was mature microRNA's, and there's two of them here, from uncooked rice are found in the serum and plasma of Chinese men and women, okay? So it got from the rice, into the stomach, all--through all of that, that stomach acid into the gut and into the blood.

These microRNA's are stable and delivered to the blood in micro vesicles, we call them exosmoses, that are derived from our--from the intestinal mucosa, those villi in the intestinal mucosa, they take things in and they bud things out. And when they bud them out, the membranes of the encapsulated RNA's and proteins that now go around the body get into the bloodstream. They think that maybe that's how HIV is spread around the body as well.

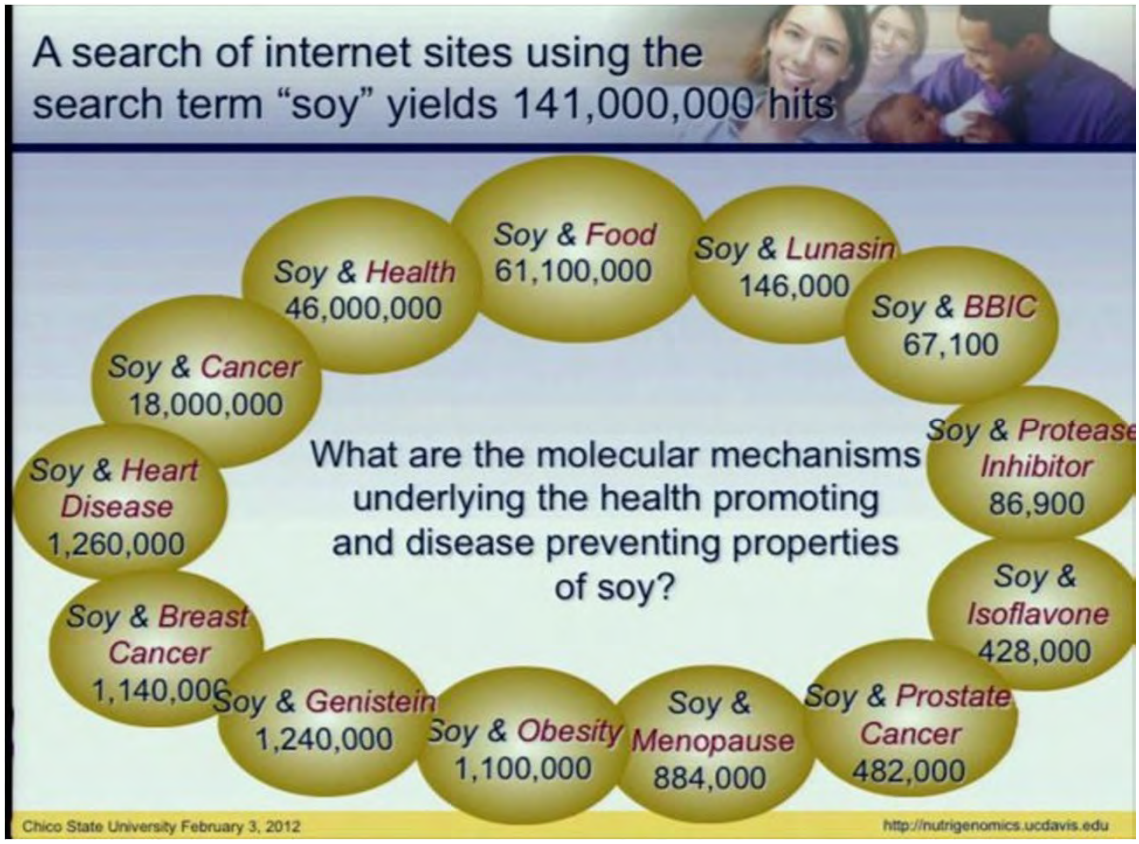
This one particular microRNA 168a is complementary to exon 4 of the human gene, this is the LDLR 8P1, that means it's complementary, that means if it binds that region of the exon 4 in this gene. The next thing that's going to happen is the endonuclease is cut, it's going to cut that RNA and that RNA is now silenced and inactivated.



Binding of these RNA to this particular LDLRAP1, messenger RNA decreases this amount--this protein in the plasma of rice and increases LDL proteins in the blood, increasing the risk of cardiovascular disease. That's not good. But interesting that the things that you're eating are coming in, going into the stomach, into the intestine, getting into the blood, hitching a ride on his little exosomes, and in this case because there's complementary between the rice microRNA and exon 4, it inactivates that protein and your cholesterol level goes up.

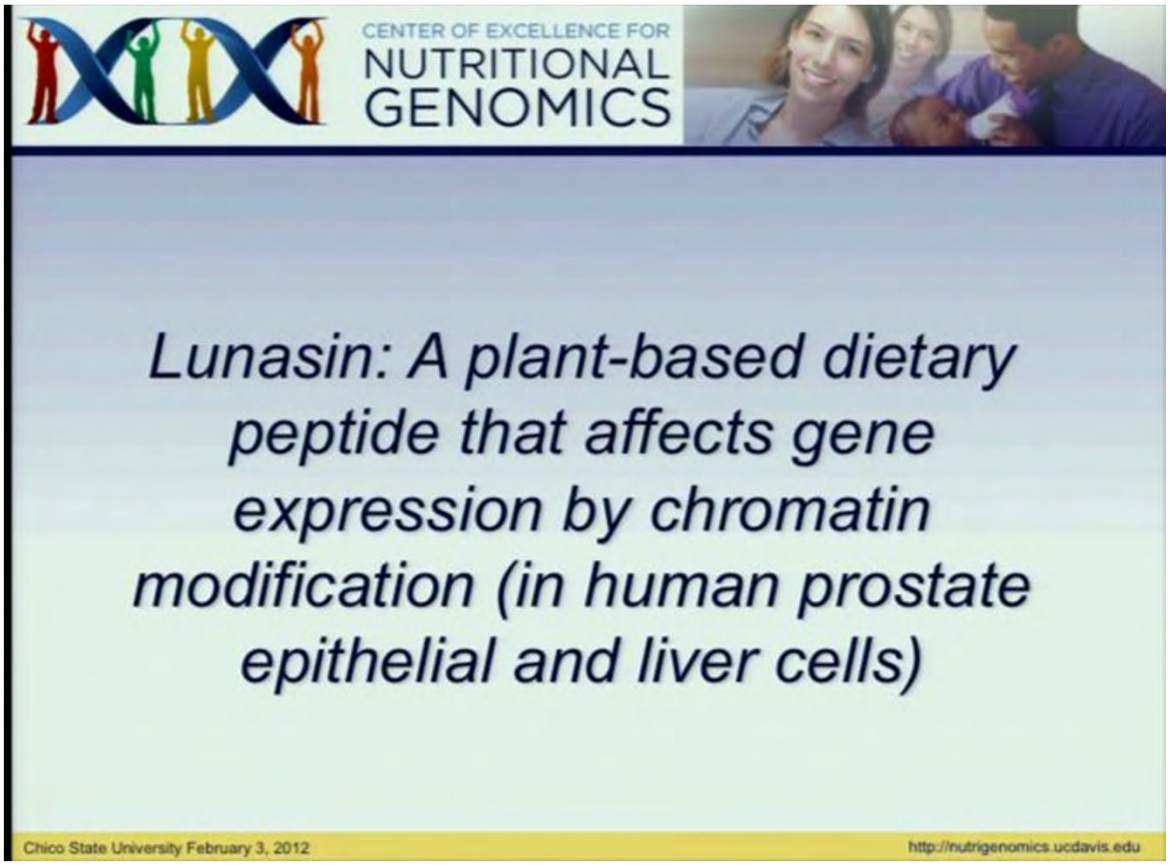
Similar findings were obtained with cooked rice, is that it?

Are microRNA's from plant-based foods essential nutrients? That's what people are asking now. So this is an example of an interaction we're not really happy about, but it's something that we're seeing all--it's emerging out of Asia right now, we're seeing reports out of China, out of Bangladesh, out of India, that these rice based-diets are causing, as they're more and more calories per day, you're starting to see cardiovascular disease, hypertension, type 2 diabetes.

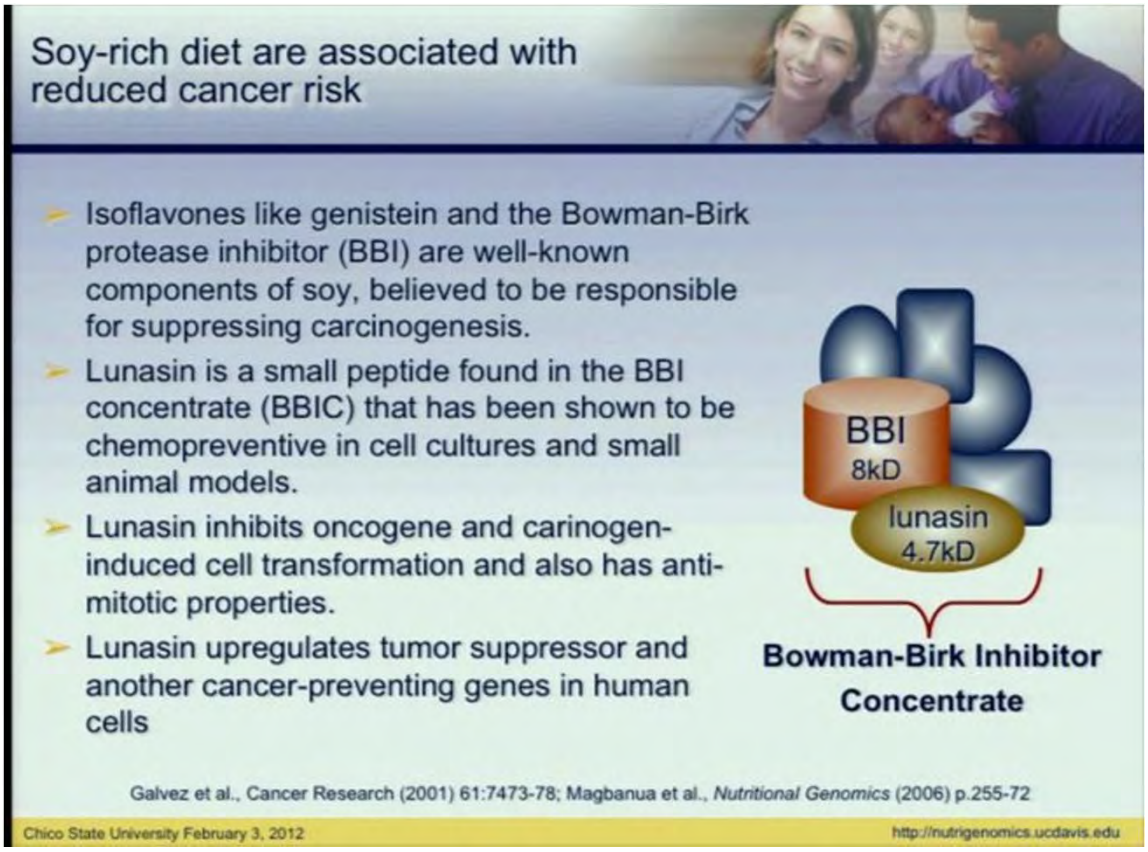


I'm just going to speed around real quick here. There's a lot to talk to about that I won't get to, but I'll get to the punch line on my isoflavone. So the protein that we're working with, this little isoflavone, excuse me, this little peptide from soy, lunasin, if you go online and start Googling soy and food, you start--finding a lot of hits. I did this a couple of years ago at 61 million hits, isoflavone in health cancer, heart disease, breast cancer, genistein, obesity, menopause, prostate cancer of isoflavones, protease inhibitor, Bowman-Birk inhibitor complex and Lunasin.

So what are the molecular mechanisms underlying the health promoting and disease preventing properties of soy? We want to know that. And it seems to be in soy, there's two components--there's the isoflavone component and there's the peptide component. That's why this Bowman-Birk inhibitor over here, that's a peptide.

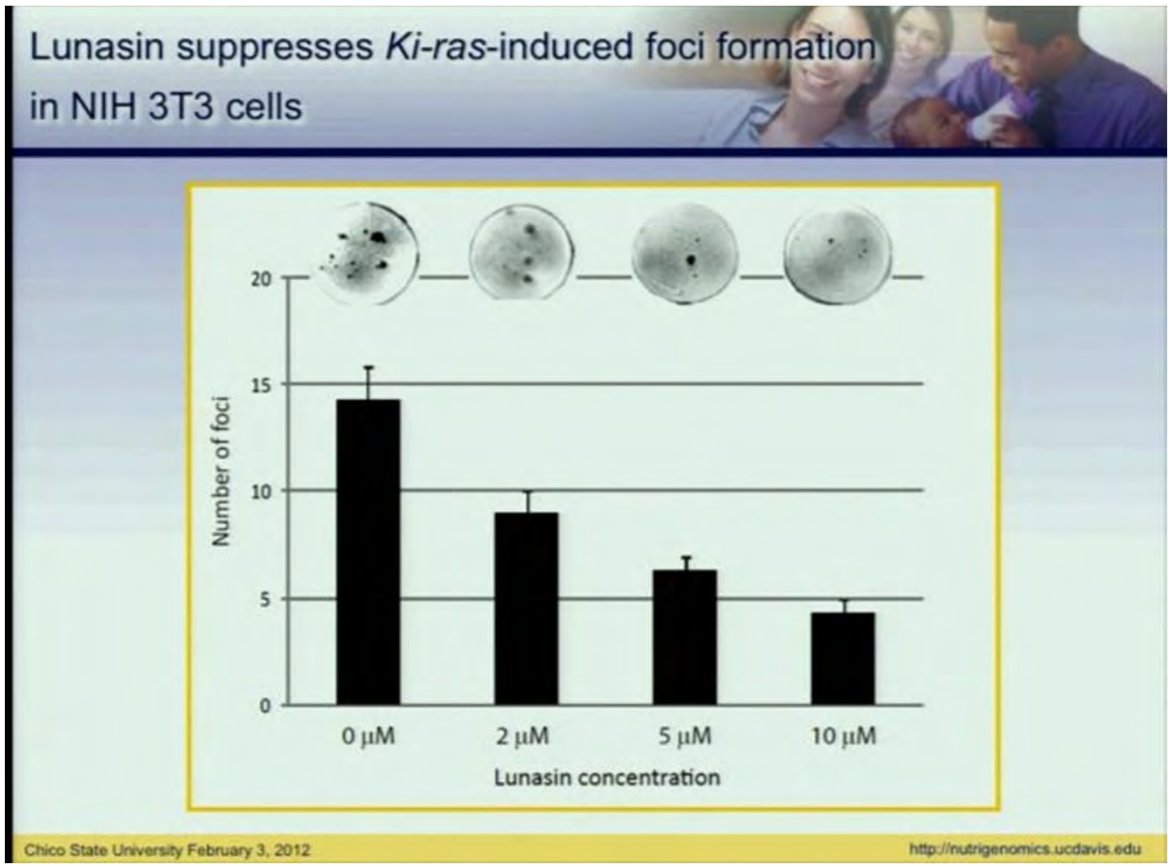


And we'll see that, I think, Lunasin, a plant based dietary peptide that affects gene expression by chromatin modification in human prostate epithelial cells and liver cells.

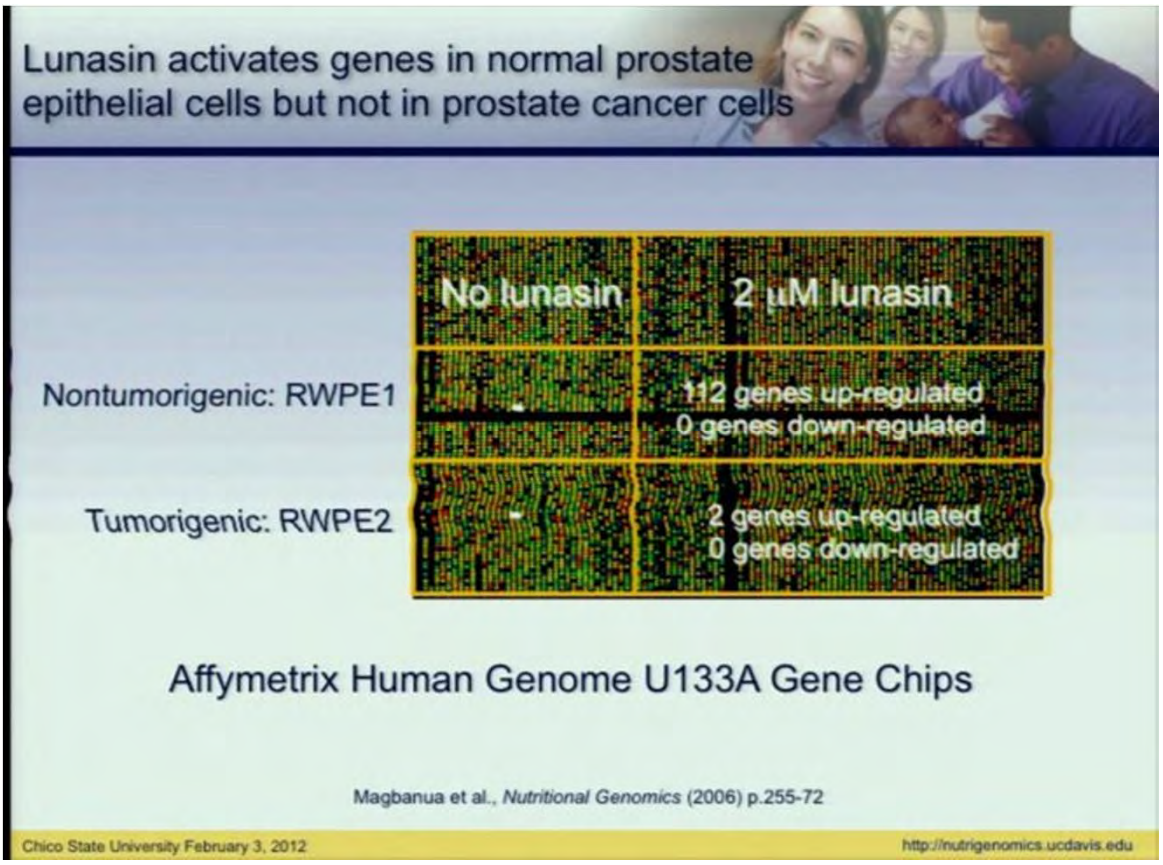


And you can see here is the Bowman-Birk inhibitor concentrate or complex and Lunasin is part of it. Lunasin inhibits oncogene and carcinogen-induced cell transformation and also has antimitotic properties.

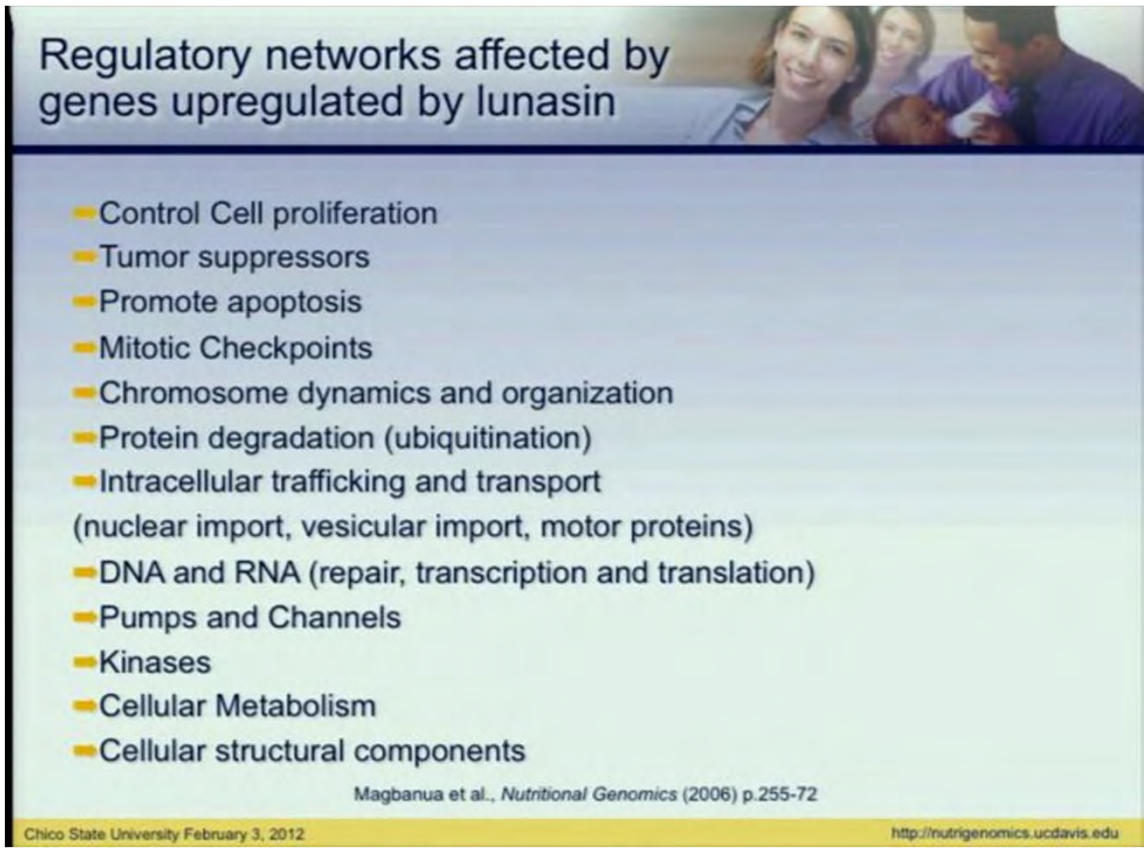
Lunasin upregulates tumor suppressors and other cancer-preventing genes in humans. We were surprised about this. We had some prostate cells, some healthy ones, we had the cancer-controll--RWPE1 is the normal, RWPE2 is the cancer control--we treated with a small amount of Lunasin to find out that Lunasin up-regulated about 120 genes.



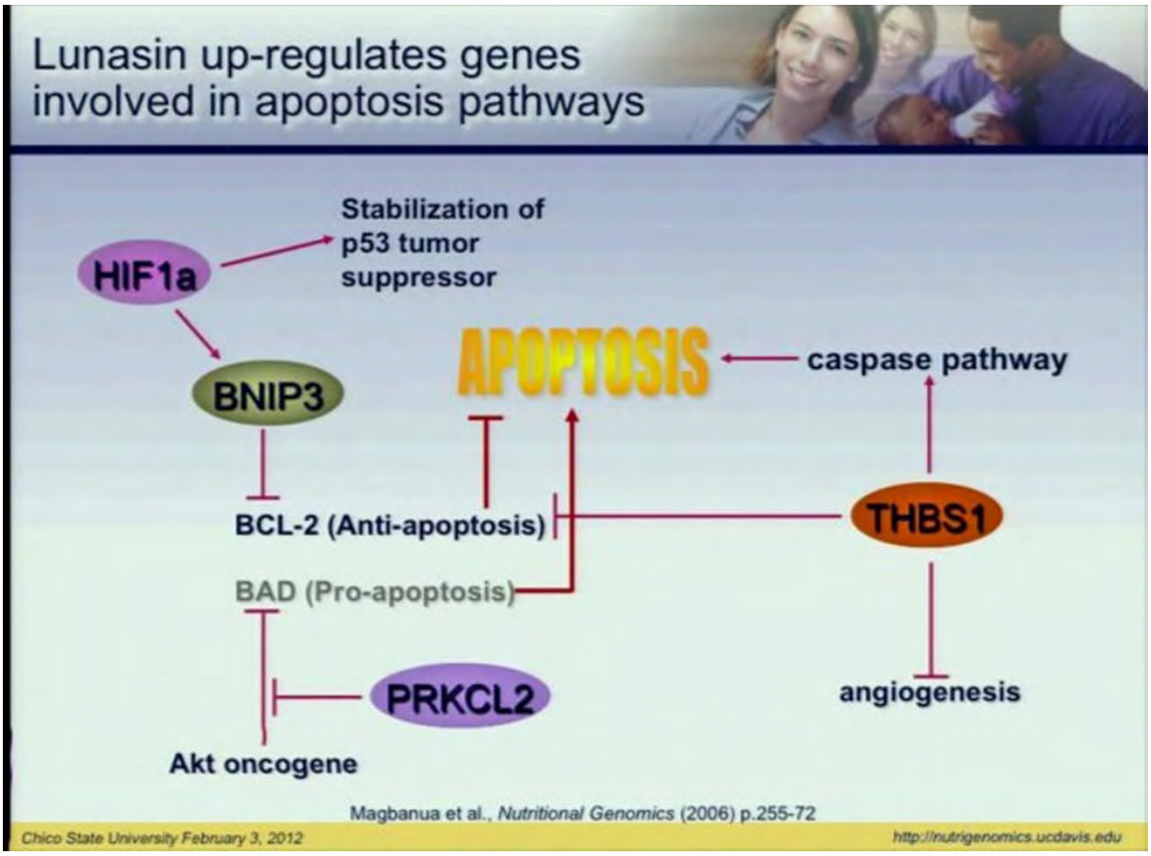
And most of the genes that up-regulated were those that were involved in….


Lunasin activates genes in normal prostate epithelial cells but not in prostate cancer cells
No lunasin
2 μM lunasin
Nontumorigenic: RWPE1
112 genes up-regulated
0 genes down-regulated
Tumorigenic: RWPE2
2 genes up-regulated
0 genes down-regulated
Affymetrix Human Genome U133A Gene Chips
Magbanua et al., Nutritional Genomics (2006) p.255-72
Chico State University February 3, 2012
http://nutrigenomics.ucdavis.edu

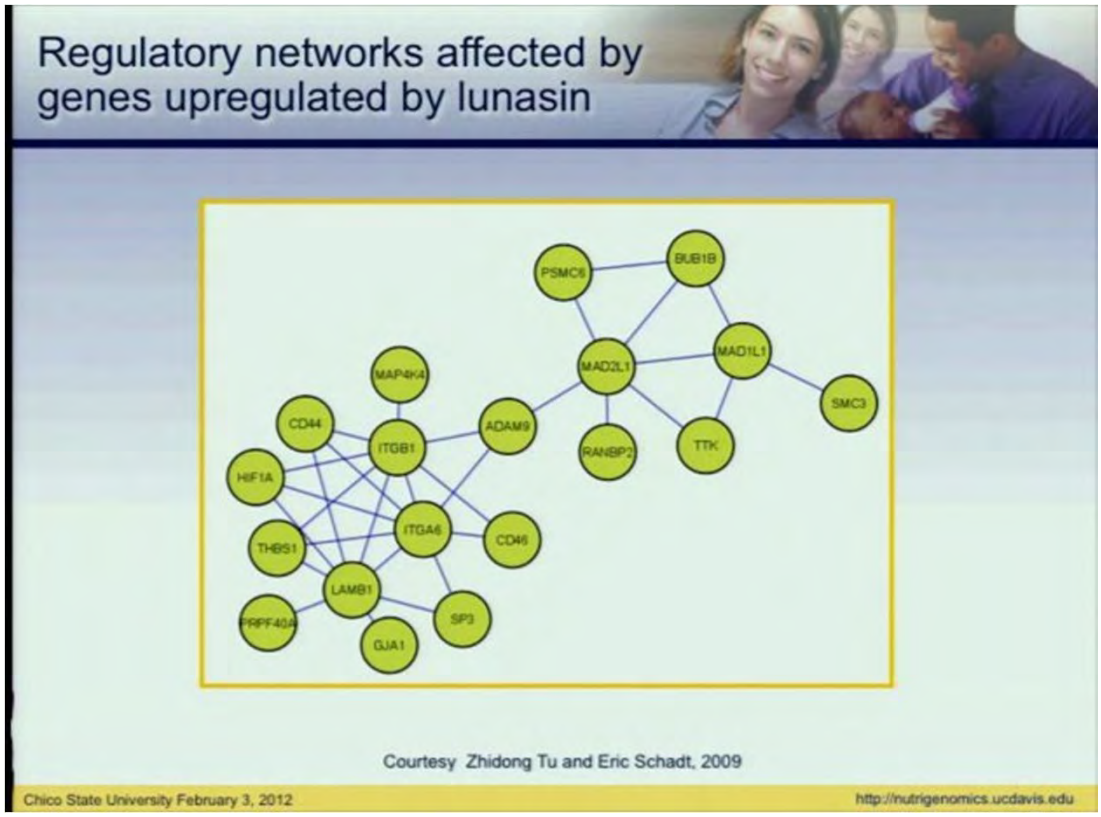
112 genes….



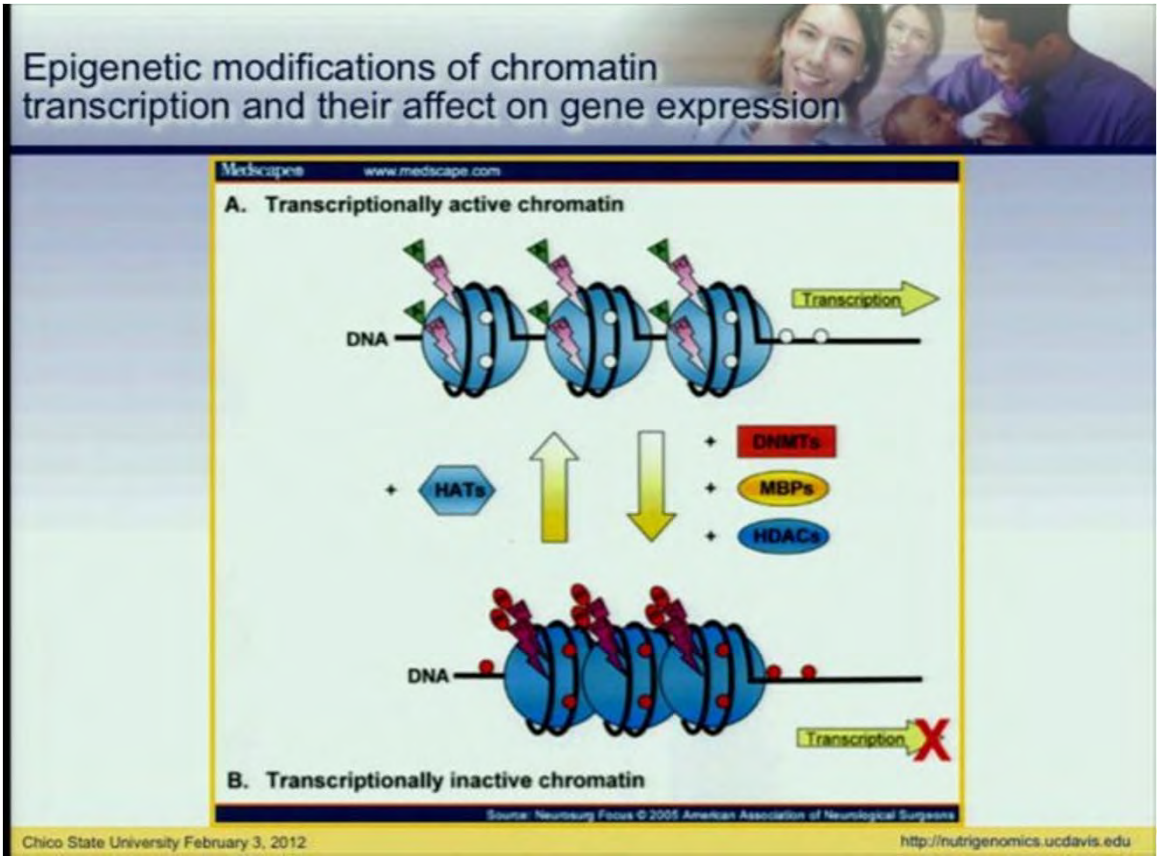
That were involved in control of cell proliferation, tumor suppression, promote epitosis, that's good, mitotic check points, common to all of those genes that you would want to be expressed to prevent cancer were being turned on by this gene. And I'll tell you, not by a lot, maybe one and a half fold, two fold, two and half fold were being up-regulated. And I'm glad to see that because Lunasin is not a drug, it's a dietary factor, so it's going to work very gently on these genes. And so what we saw was a slight up-regulation for all of these important functions here. This is just an example of some of the genes that were up-regulated.



HIF1A stabilizes this tumor suppressor p53, it activates PF3 which is a transcription factor that blocks BCL-2, BCL-2 in turn blocks epitosis, that's bad. So now, we found an inhibitor of an inhibitor of epitosis. It does a number of other things which I won't talk about right now.



This is our little network. I didn't have time to talk to you about the most up-regulated genes that were--that we found--were HIF1A and Thrombospondin 1, where is Thrombospondin 1? Where the most upregulated genes in that longest of 112. But when we do network analysis, where we look at the--who genes are talking to, who they're hanging out with, who they're touching and running with, are a highly upregulated where genes were not there, its LAMB1 which was a key node here. And LAMB1 is a extracellular matrix protein that's critical. Cell adhesion, metastasis, all of those things that are involved in producing cancer, it's interacting with lot of the genes that we found. This little network is connected with this little network, this little network over here is involved in all mitotic check points. That many of these genes like, for example like MAD2L1, prevents chromosomes from segregating into cells, daughter cells after mitosis. If they are damaged or they're not completely separated, you don't want to create any abnormal problems in the genome, so that gene has to be on, so you can look at it in terms of the most up-regulated, you get one picture, if you look at the most interconnected, you get a different picture, and this actually turns out to be a very important protein that we're looking at right now.



So this is what the Lunasin has the ability to do and here we have an open complex and here we have a closed complex. You can see transcriptions turned off on the closed complex, it's turned on in the open complex, and the open complex is characterized by these little A's which are acetyl groups, where do acetyl groups come from? Acetylcholine, where does acetylcholine come from? Metabolism of proteins, starches, and lipids. So again, dietary input promote--producing the precursors of these compounds, acetylation relaxes the chromosome so you can get a transcription. So what we've found is Lunasin has the ability to promote these acetylations, relaxing the chromosin and the nucleosome so its transcription can occur.

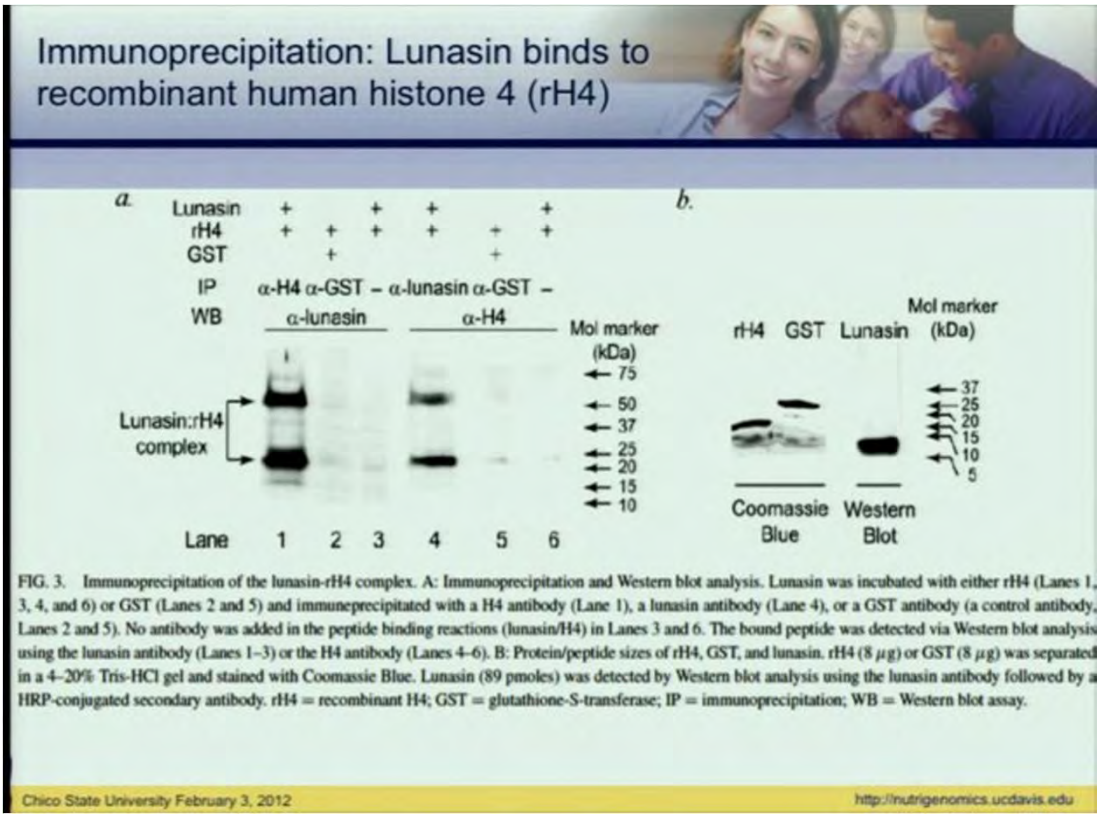
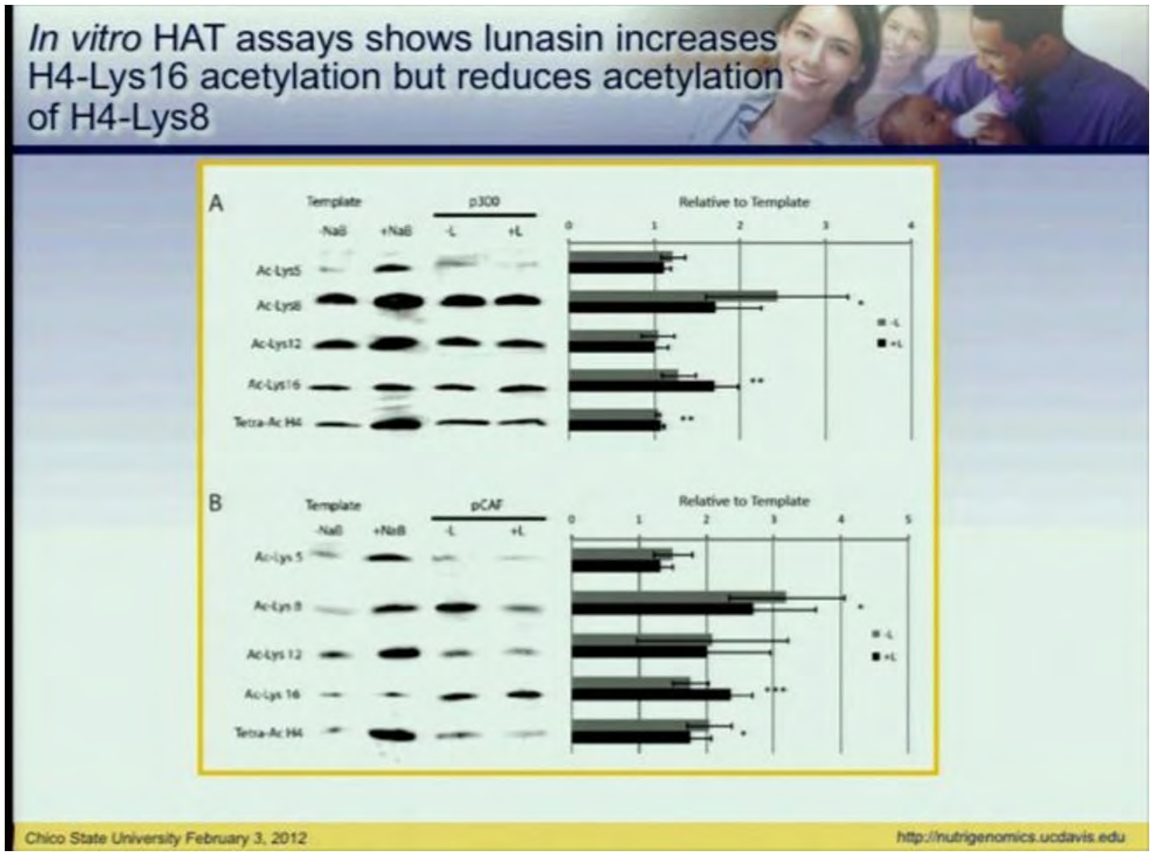
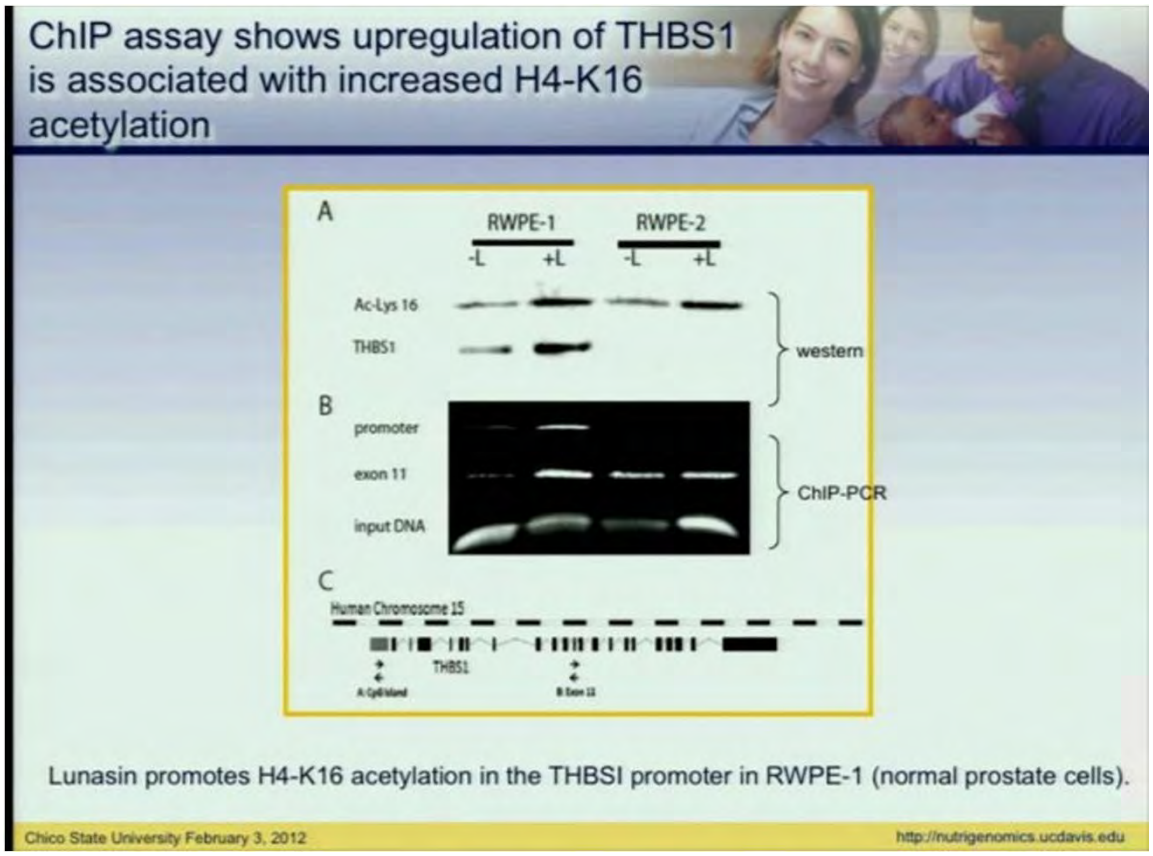


FIG. 3. Immunoprecipitation of the lunasin-rH4 complex. A: Immunoprecipitation and Western blot analysis. Lunasin was incubated with either rH4 (Lanes 1, 3, 4, and 6) or GST (Lanes 2 and 5) and immunoprecipitated with a H4 antibody (Lane 1), a lunasin antibody (Lane 4), or a GST antibody (a control antibody, Lanes 2 and 5). No antibody was added in the peptide binding reactions (lunasin/H4) in Lanes 3 and 6. The bound peptide was detected via Western blot analysis using the lunasin antibody (Lanes 1–3) or the H4 antibody (Lanes 4–6). B: Protein/peptide sizes of rH4, GST, and lunasin. rH4 (8 μg) or GST (8 μg) was separated in a 4–20% Tris-HCl gel and stained with Coomassie Blue. Lunasin (89 pmoles) was detected by Western blot analysis using the lunasin antibody followed by a HRP-conjugated secondary antibody. rH4 = recombinant H4; GST = glutathione-S-transferase; IP = immunoprecipitation; WB = Western blot assay.

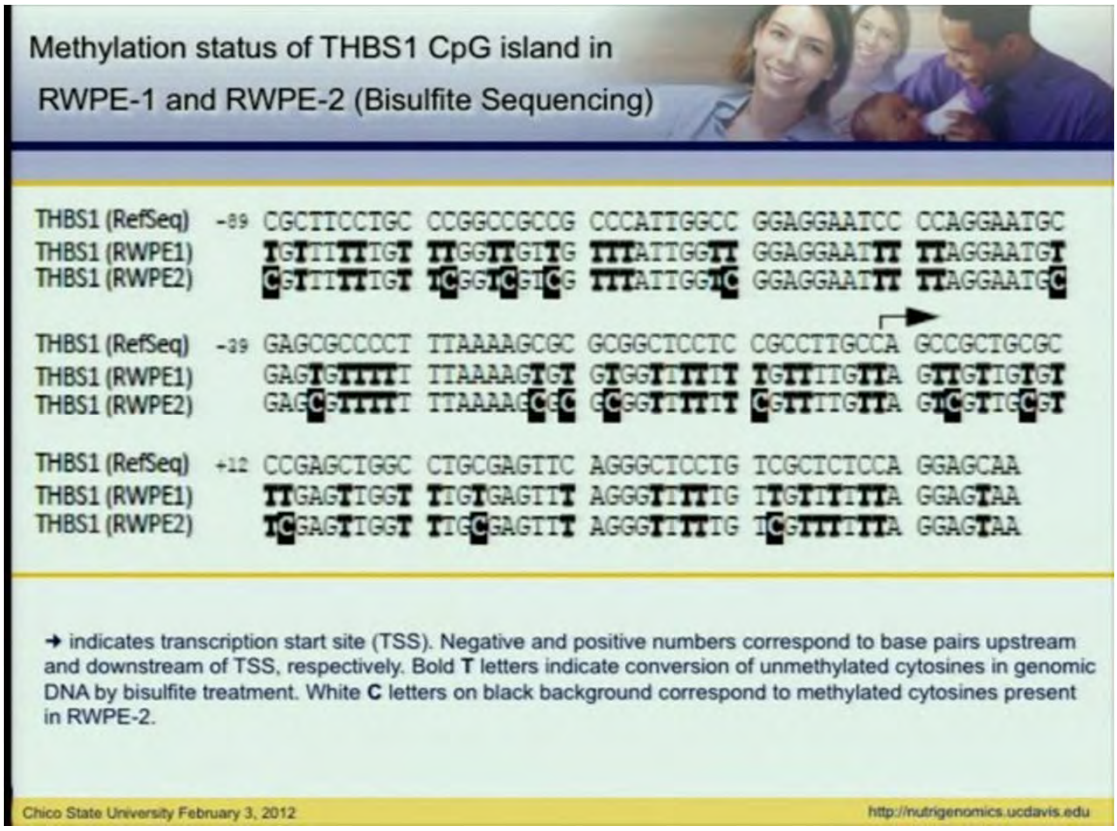
Those are all the experiments…



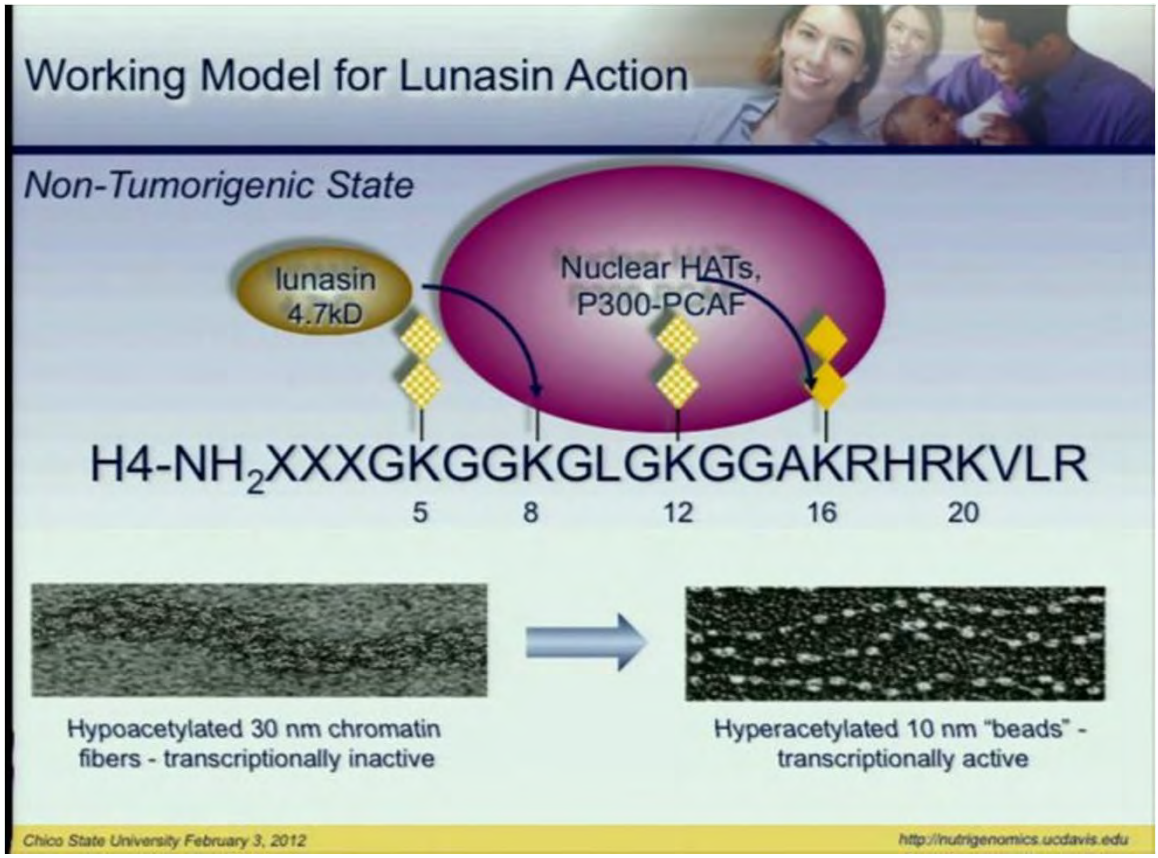
that show you that…



Lunasin actually binds histone H4.



I just want to go the last slide here, one of the last slides. Lunasin did all these good things and the normal prostate cell, it had really no effect on the cancer cell. That tells me two things. One, that Lunasin is not a drug, it will not cure cancer, it prevents the onset of processes that can cause cancer. So that's a preventative and that's why you would want to include it in your diet. The other thing that tells me that there's something in those cancer cells that have been changed in response to that--all those neoplastic processes, something is changed and is profoundly different from the DNA in the non-transformed prostate cell. And we did--we looked at that, we looked at DNA methylation of CpG islands around the promoter of one of the up-regulated gene thrombospondin, and sure enough we found that in the RWPE-2 transform prostate cells, that it was full. These T's represent--T's and C's represent methylated C's, methylated C nucleotides. We know that because we knew this bisulfide sequencing, bisulfide sequencing means you take DNA, and if the DNA--if the C's are unmethylated, then the bisulfide will turn them into a T. If they are methylated they're resistant. And so you can see all of these T's on RWPE-1, they used to be unmethylated C's, but in RWPE-2 we see this C was--retained that C, that C--here is a transcription point, look at all the C's. So the reason we believe that Lunasin does not have any effect on this cancer cell is that there are so many processes that occurred on that DNA. That DNA is now hyper methylated, genes are silenced, and Lunasin cannot pluck off the methyl growth of the cytosines. It can affect the acetyl groups on histones, but it can't affect that methylation.

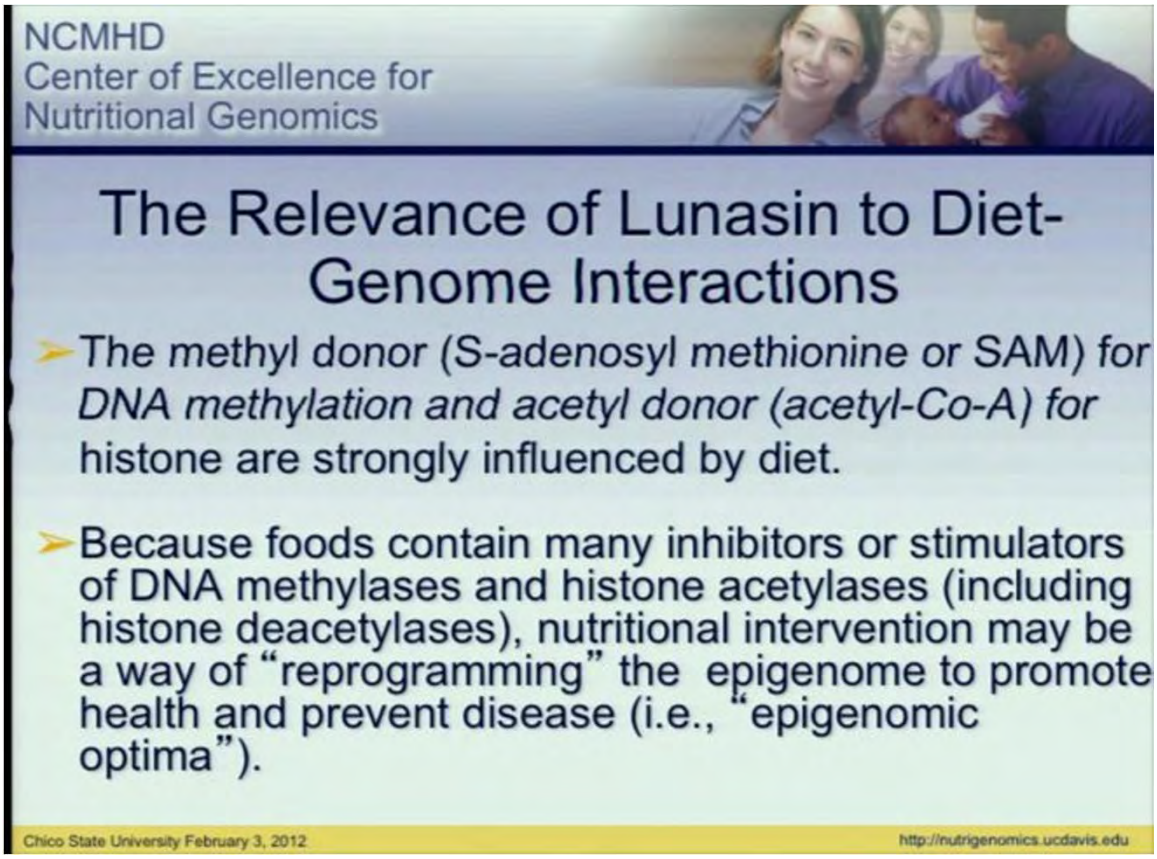


So I just wanted to give you a summary of what we think is going on. Here is the H4 tail of histone 4, here is that critical lysine residue over here, lysine 16.

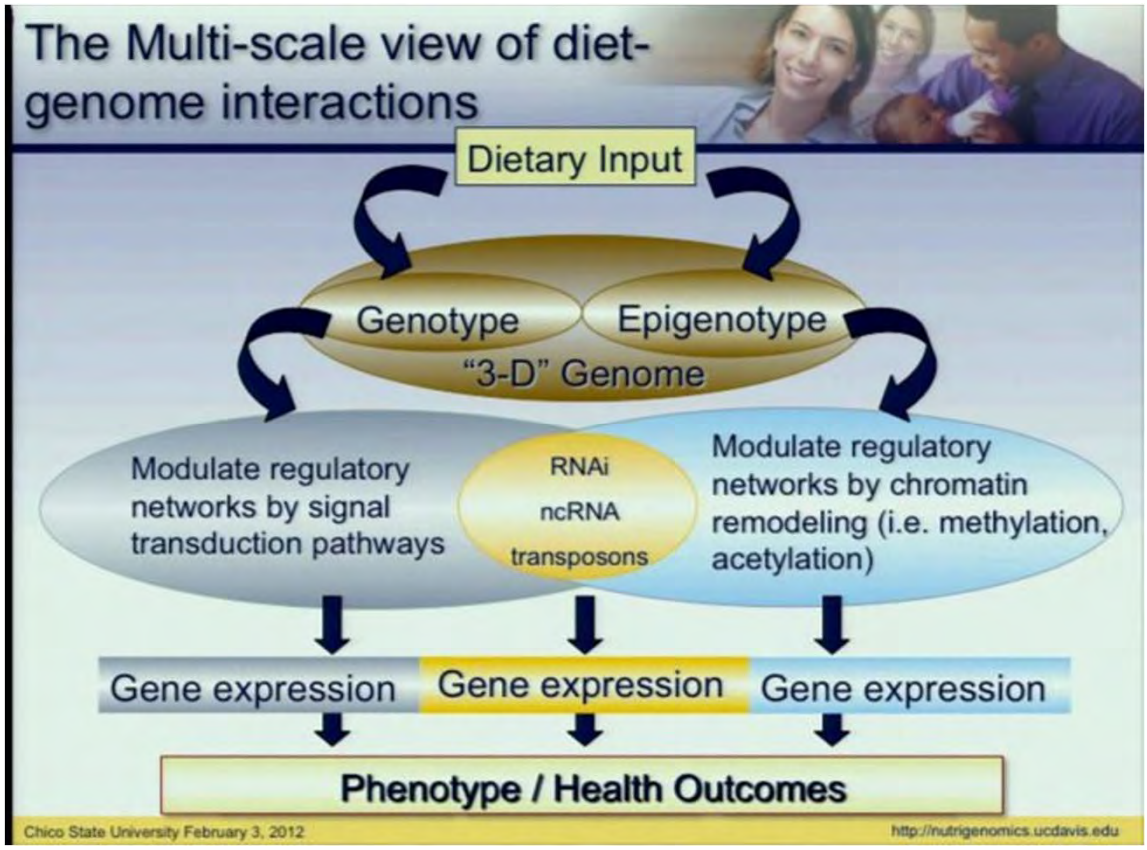
And what we think is--Lunasin is being--is coming in and binding to lysine 5 and 8.

And that binding brings in these histone acetylation proteins that put on acetyl group here on lysine 16.

The consequences is that highly condensed chromatin, this 30 nanometer structure, it gets decondensed into these 10 nanometer beads where transcription can't occur. That's the hypothesis that we're working on.



So the relevance of Lunasin to diet-genome interactions, the metal groups that were on those DNA, the acetyl groups that are on the histone, all come from diet, they're strongly influenced by diet. And second, because foods contain many inhibitors and stimulators of DNA methylation, histone acetylation including histone deacetylases, nutrition interventions maybe a way of reprograming the epigenome to promote health and prevent disease, we'd call that our epigenetic optima, which is a really--going to be a difficult thing to achieve.



So we're left now with this multi-scaled view of the genome where dietary input comes in and affects the genotype, the genotype working through regulatory process, like signal transduction would produce a gene-expression profile, which will produce a phenotype. That was five minutes ago, actually more--that was more 20 years ago. Now we know that there is an epigenotype.

And that together, the genotype and the epigenotype gives us what we call our 3D genome.

We're now starting to see all aspects of the genome more clearly. The epigenotype can produce, again, changes in gene expression by methylation and acetylation of histones in DNA that will produce a pattern of gene expression that produces a phenotype.

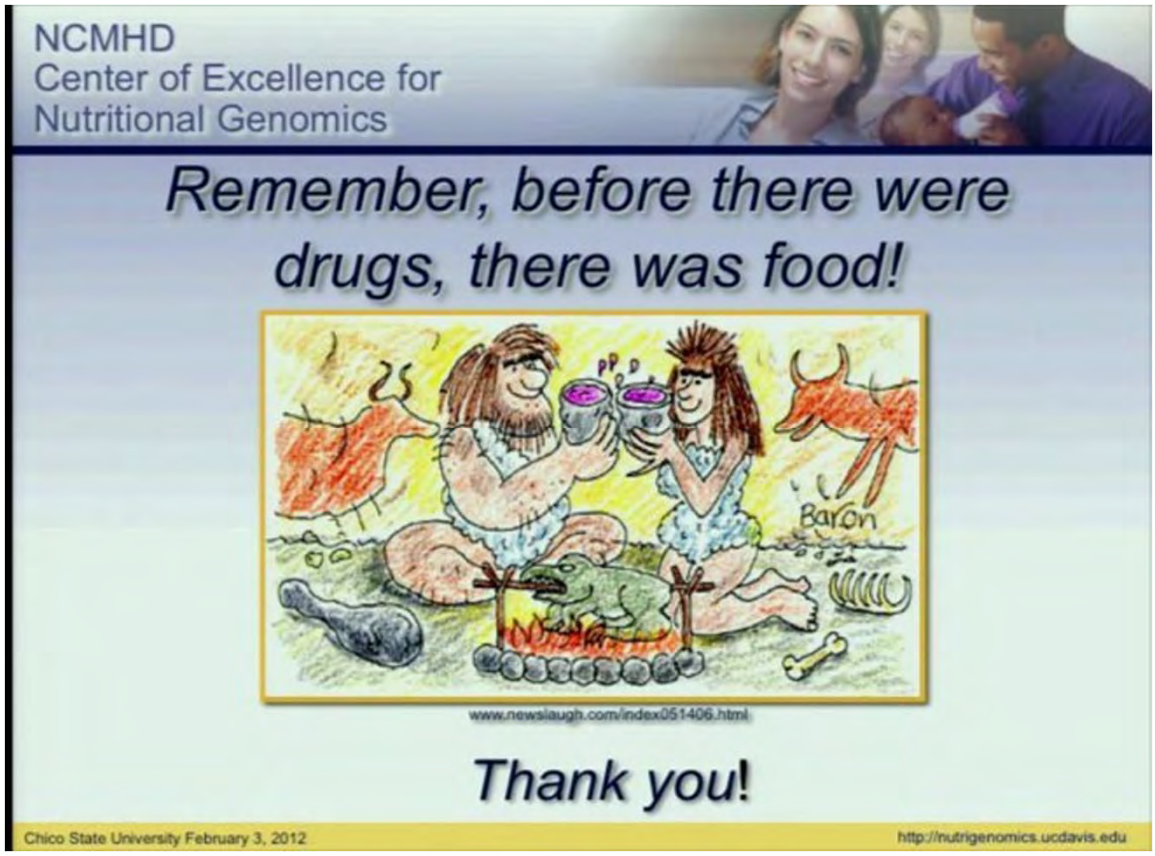
And it gets more complicated.

We got those little microRNA's that we'd now…

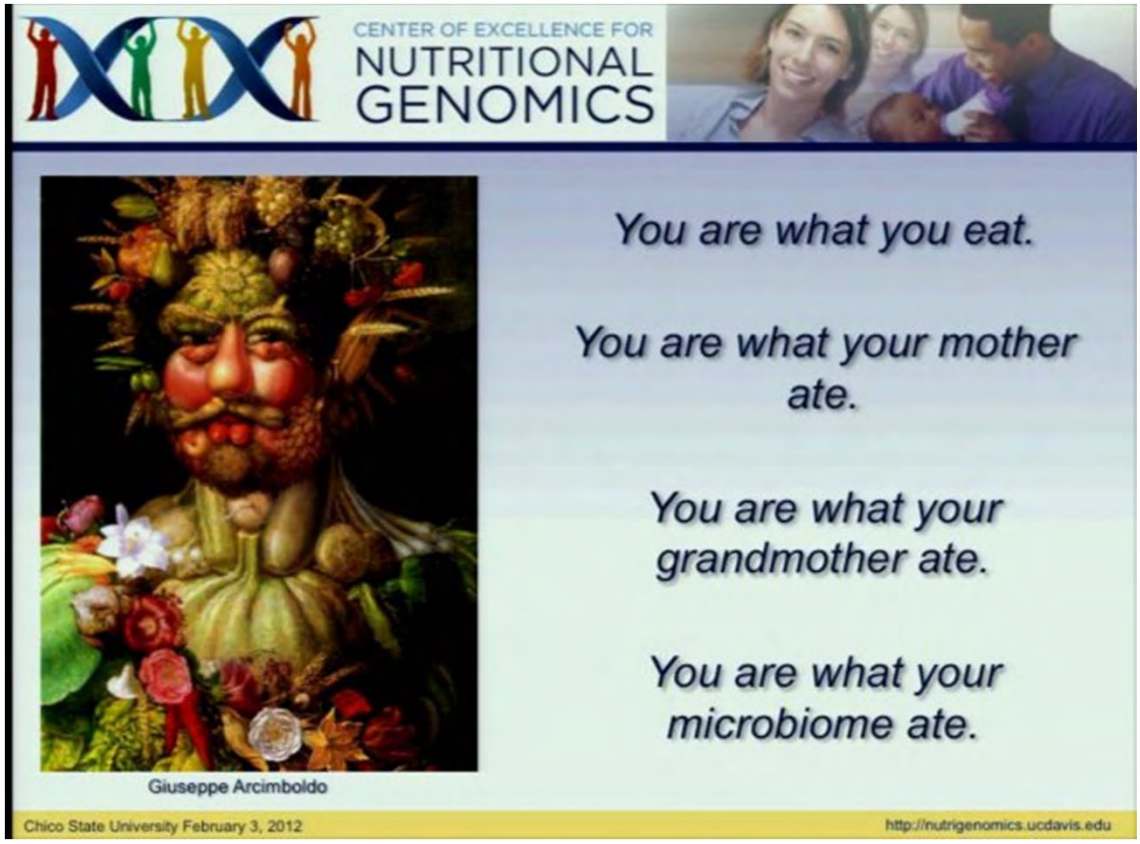
I showed you, you can ingest in rice that would produce it's own gene expression pattern.

Which comes together to produce the complete phenotype

That's why it's so hard to deconvolute from phenotype back to genotype because all of this processes are involved.



So I think I just have two slides here just to end up and remind you that before there were drugs, there was food. And I tell you, I think we live in a society where there's too much dependence on drugs to fix a complex issue. Using one drug to fix a complex system may work, it may have lots of side effects, and it may not work.

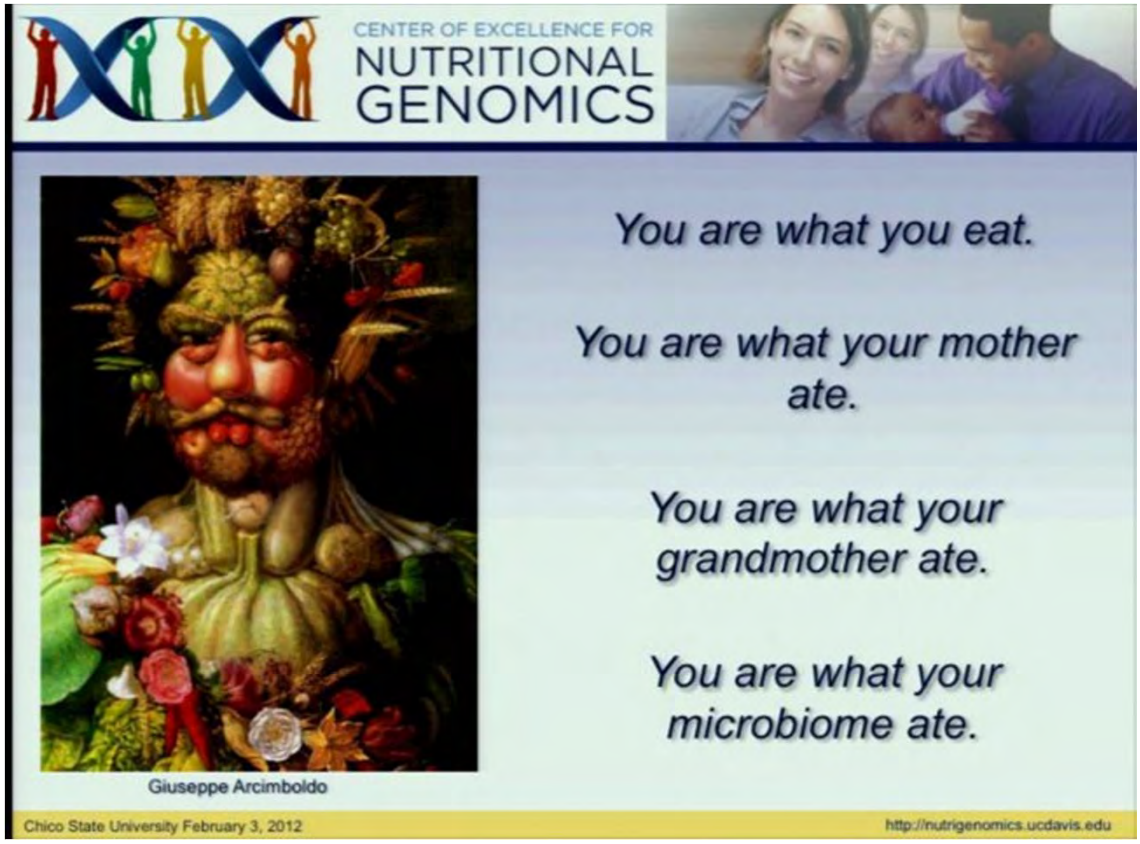


Yeah, you are what you eat, you are what your mother ate.

You are what your grandmother ate

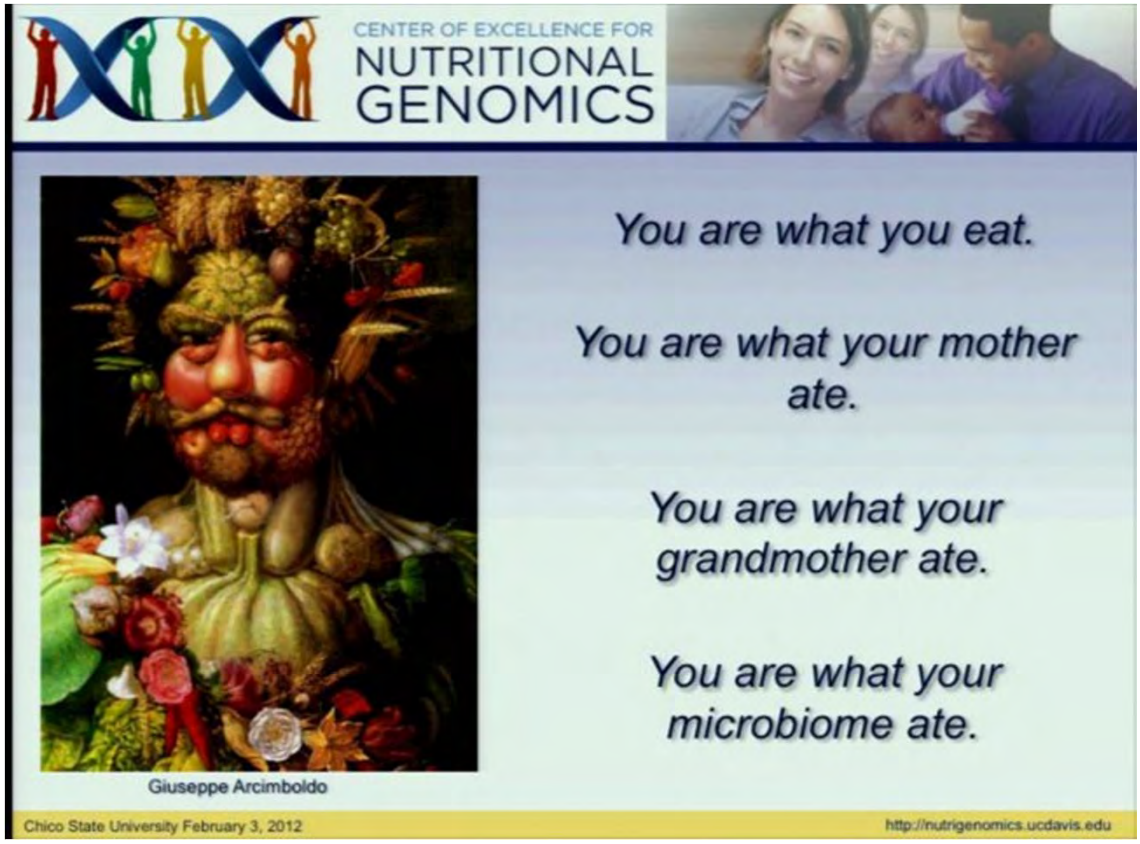
And you are what your microbiome ate. Unfortunately, we don't have time to talk about that. So I just want to thank you guys for your patience and letting me sort of rumble through a lot of different things, but I think--the conclusion that I have for myself is I love being a biologist in the 21st century. I can start to see how biological process might work, I couldn't see that 10 years ago, I think I can see it, how it works now and I think there could be a lot of exciting outcomes in the future.

Remember, we're talking about a complex system. Networks interacting with each, you plucked one of them out and you expect that that's going to do all the job, no it--you know, nature has done all the work for us, they've provided a nutritional environment that we could partake of with the right balance. One of the things we're working on is what we're calling next-generation therapeutic nutrition and that is the correct formulation of foods, not necessarily ingredients although somethings will have to be fortified, that will give you the full complex of nutrients you need to achieve a health and wellness, and to prevent onset of disease early--early onset of disease. This disease are going to happen, we're not talking about longevity. But I really don't believe in too much in over doing it on supplements. Although I do take supplements, one of what I take is vitamin D. Why do I take vitamin D? Because vit--the source of vitamin D is cholesterol and we've all tried to reduce our cholesterol levels.

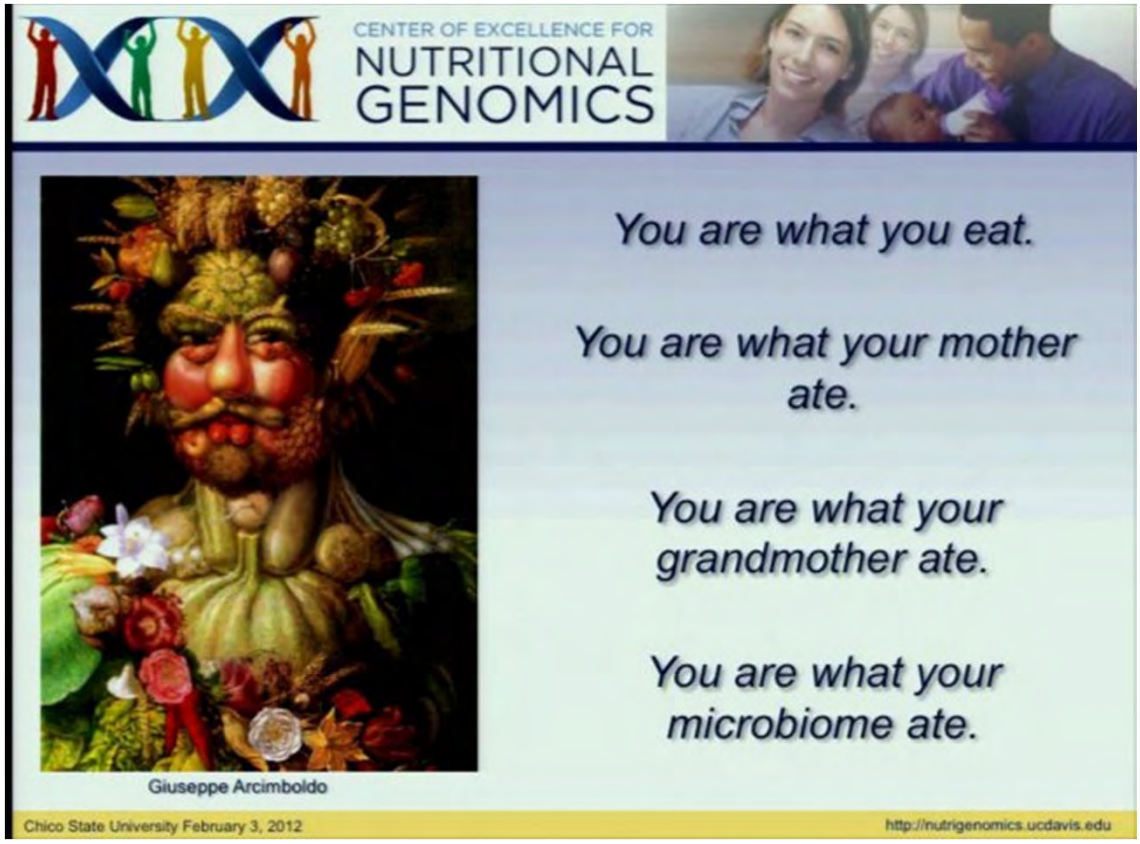


The other source is sun, I'm wearing too much clothes today that would not get any vitamin D through my skin and plus, you're getting up at this latitude, we're getting very little vitamin D anyway. So that's one where I think it makes sense to supplement and I do that. I believe in nutritional diversity, having a lot of different things on your plate and a lot of different colors on your plate. They already do this in India, they do this Japan. Japan has a philosophy for health, eat 30 different things a day. Now, that's a lot though. I don't--normally most Americans don't eat 30 different things a day. But that just tells you how there's--that diversity and complexity is probably the key moderation, mixing things together, a little bit of meat, omega 3's, plant-based foods, I think that's the best way to do it. Yes? On the corner over there?

You know, dietary restriction activates SIRT1. So dietary restriction is the painful--a way to accomplish what resveratrol does with--through SIRT1. So dietary restriction, it activates SIRT1, it activates PGC-1 alpha and goes off and does all those things. But that--but you see that benefit of about 30--excuse me, about 20 percent caloric restriction. That means you're going to go to bed hungry every night. If you can--you can learn to live that way but it's--that's a tough way to do it. So that's why they were so much interested in resveratrol because they thought, basically, you got caloric restriction in a pill. Just take that if you don't want to have to do caloric restriction. So, people are still looking for quick answers to complex problems, but yeah, there is that connection between that resveratrol, PGC-1 alpha, and caloric restriction. How about that question next to you? Yeah, up there?



There's an interesting paper out of--by David Sinclair and other Harvard professor called the Xenohormesis Hypothesis. Really, a couple of beautiful words, Xenohormesis Hypothesis. It means a little bit of stress in your life is good. And what they're talking about is that plants are antenna for environmental stress. They can't run inside, they can't put on the jacket, they got to stay exactly where they are. So they've learned to pick up stressful conditions in the environment and then they take those stressful conditions and turn them into phytochemicals of various types in the leaves. Then the insects come in and they eat those compounds, they're eaten by mice and other rodents and mammals. And the signals get transferred around the environment starting with the plant, insects, mammals. So what he--what they're saying is that those compounds served a purpose, some of them are actually insecticide. They had--plants will die if they eat them, others repel the plant, others are eaten by the vector and they go off and they communicate that stressful message. And what is the stressful message? Get healthy enough to reproduce because times are bad and that's how nature was--has been talking for a long time. So those compounds are there for a reason and that's why I said and now that we live in a highly managed agricultural ecosystem, those plants are not needed to, you know, they're not being attacked so much by insects. Yes there's climate change, but I'm worried that have we lost those phytochemicals that were normally there in the leaves, we normally picked up. And because we're now--we have this highly very mechanized form of agriculture.



And I tell you, I asked one of the superstars in this area, Doctor Gurdev Khush who is the winner of the Japan price, who was the winner of the world food price for making IR8 which IR8 was a rice variety led to IR64 which prevented massive world famine in the 70's which they had predicted. And I asked him, I said "Gurdev, is there any chance that in a rush to plump up those rice grains, to fill and full of starch and proteins, that you could have left behind some nutrients that are not present there?" And he says, "You know, 10 years ago, I had said no. Today, I'd say is a possibility." So that's what happens when you're plumping things up, and I tell you, I'm a fan of modern agriculture. I come from UC Davis so--but I think that in that rush to increase caloric content energy, we may have lost some of those other little phytochemicals, you know, that's why I go to My Farmer's Market, I don't know if you got one. And during the summer, they're selling these what they call non-irrigated figs, these little shriveled little things that they picked up off the sides of the hills and they've never seen any irrigation.