Genes and Genomes: Impact on Medicine and Society

Genes, Genomes, and Medicine
October 16, 2003

Michael Brown, M.D., and Joseph L. Goldstein, M.D., University of Texas Southwestern Medical Center, Dallas, TX
Fatricide: When Genes and Diets Collide

Introduction by Andrew R. Marks

Andrew R. Marks: Our next two speakers are Joe Goldstein and Michael Brown. Joe is the Paul J. Thomas Professor of Medicine and Genetics and chair of the Department of Molecular Genetics at UT Southwestern and the regental professor at University of Texas. Mike Brown is the Paul J. Thomas Professor of Molecular Genetics and director of the Johnson Center for Molecular Genetics at UT Southwestern. Together they were awarded the Nobel Prize in Medicine or Physiology in 1985. Their work has touched the lives, I would venture to say, of everybody in this room, because it seems that everybody today is either on the cholesterol-lowering drug statin or knows somebody who is.

Indeed the work of Brown and Goldstein, as they're affectionately referred to, stands as one of the most powerful models for the application of basic studies, in this case the discovery of the LDL receptor that provides the molecular link between cholesterol and heart disease. These basic studies forming the basis of developing novel therapy, in this case cholesterol-lowering agents, have treated millions of patients.

Today they will tell us about recent work identifying genes that control metabolic pathways that have become maladaptive in our modern lifestyle, despite millions of years of evolution. Welcome to Columbia.

Cholesterol and Coronary Atherosclerosis

Joseph L. Goldstein: Thank you for the nice introduction, and also Tom Jessell, thank you for organizing such a great program, and Mike and I are delighted to be here to help Columbia celebrate their 250th anniversary. And so speaking of
celebration, all year we've heard about the most celebrated molecule in biology, DNA. And now Mike Brown and I would like to tell you about another celebrated molecule, cholesterol. And although it lacks the panache of DNA, cholesterol is important to medicine because it is the root cause of coronary atherosclerosis, which is the most frequent cause of death in the U.S. and other industrialized countries. It's surprising that coronary heart disease was recognized as a major clinical problem only in the early years of the twentieth century. But by 1950 the disease had reached epidemic proportions, and today coronary heart disease causes more than one-third of all deaths in the Western world, and that's the bad news.

But the good news is that in the twentieth century, scientists have discovered that the disease is caused by cholesterol, and most specifically by lipoprotein carriers that transport cholesterol in the blood. And by the end of the twentieth century, scientists had developed powerful drugs that lower these toxic lipoprotein particles and prevent heart attacks.

So in part one of our joint lecture I will tell you how the link between cholesterol and coronary atherosclerosis was established, and then I'll tell you about the disease familial hypercholesterolemia and how it led to the discovery of the LDL receptor which is a molecule that controls the blood-cholesterol levels in humans. And in part two Mike will tell you about some of recent research on the transcriptional regulation of cholesterol on metabolism and its link to garden variety forms of high blood cholesterol.

Now here's a cross-section of a coronary artery of a 50-year-old man who died suddenly of a heart attack. And this atherosclerotic plaque began to form forty years ago when this man was a teenager, and it began when the cholesterol-carrying lipoproteins infiltrated the bloodstream and entered the artery wall where they underwent oxidation. And the oxidized lipoproteins initiated an inflammatory reaction that over forty years led to this damage that you see and scarring and eventually the buildup of a plaque that narrowed the channel of the blood vessel. And this insidious process of plaque formation was augmented by aggravating factors, such as smoking, high blood pressure, and a high-fat diet. And when a plaque becomes unstable it eventually ruptures, leading to the formation of a blood clot, a thrombosis, that suddenly blocks the blood flow of an artery, and then the heart muscle supplied by the artery dies from lack of oxygen and it produces what's called an acute myocardial infarction, or a heart attack.

Now the lipoprotein that initiates this atherosclerotic plaque is called low-density lipoprotein, or LDL. And LDL is made in the liver, and it's secreted into the blood, and in humans LDL is the major cholesterol-carrying lipoprotein in the blood plasma. And now look. The cholesterol that LDL carries, as shown right here, is a hydrocarbon molecule composed of four rings and a side chain, and it's totally insoluble in water. And this insolubility allows cholesterol to perform its most vital function in the body, which is to act as a component of the lipid-rich
plasma membrane that forms a water-resistant barrier around all cells in the body. But the insolubility of cholesterol creates a transport problem; in order to reach its sites of metabolism outside the liver, the cholesterol must be solubilized so that it can be transported in the bloodstream, and this is the function of LDL.

These LDL particles are spherical particles that are around 22 nanometers in diameter, about the size of a small virus. And the core of each LDL particle consists of 1,500 molecules of cholesterol ester, which is a cholesterol to which a long-chain fatty acid is attached. And this hydrophobic core is surrounded by a polar coat composed primarily of phospholipids and a protein called apoprotein B. And this polar coat solubilizes the particle in water and allows it to be transported in the plasma. And the link between LDL cholesterol and coronary atherosclerosis is based on four lines of evidences—epidemiological, genetic, experimental, and therapeutic. And by itself any one of these lines of evidence might not be completely convincing, and in fact for many years there was a cholesterol controversy. But when one considers all four lines of evidence in concert, the argument becomes irrefutable.

The History of Cholesterol Research

The first link between cholesterol and atherosclerosis was established in 1913 when a Russian pathologist, Nicolai Anitschkow, fed pure cholesterol to rabbits and produced atherosclerotic plaques. And this was the first experimental production of atherosclerosis, and Anitschkow's classic experiment has now been repeated many thousands of times in virtually every species, from pigeons to humans.

At the time of these experiments, pathologists believed that a thrombotic occlusion of an atherosclerotic plaque in a coronary vessel was always a fatal event, and the clinical syndrome of nonclinical myocardial infarction was not recognized until 1918 when the American clinician James Herrick made the first use of the electrocardiograph, the EKG, to diagnose heart attacks in patients who presented with crushing chest pain. And Herrick provided the first demonstration that thrombosis of a coronary artery was not always a fatal event, and that coronary heart disease was responsible for the syndrome that had been previously diagnosed as indigestion or apoplexy.

In 1938 the connection between cholesterol and heart attacks to humans was firmly established on genetic grounds when the clinician Carl Müller in Norway described families in which high blood cholesterol was present from birth, and in which early heart attacks occurred in these hypocholesterolemic relatives. The disease came to be known as familiar hypocholesterolemia, and I'll have more to say about that in a moment. The mounting clinical interest in cholesterol led to an intense effort to understand the pathway by which cholesterol was synthesized in the body, and this pathway was worked out in the 1950s by four biochemists, Konrad Bloch, Feodor Lynen, Cornforth, and Popjak. And actually Bloch's work
began here at Columbia P & S when he was a postdoctoral fellow with Rudolf Schoenheimer in the department of biochemistry.

This slide shows you the cholesterol biosynthetic pathway as worked out by the four biochemists. And at the time it was a tour de force in biochemistry; no pathway of this complexity and magnitude had ever been worked out, and the challenge was to figure out how the two carbon acetyl-CoA could be converted to the 27 carbon cholesterol which has these four rings and a side chain. And to make a long story short, it involved a series of 25 different enzymatic steps, and a key enzyme that you'll hear more about in the talk from Mike and me is, which formed malonic acid. One of the really brilliant insights in working on this pathway was the realization that squalene, a 30-carbon straight hydrocarbon, could be folded in such a way that with the proper enzymatic conversions it would then lead to the classic sterol side chain.

So remember, as I mentioned before, cholesterol is totally insoluble in water, and in order to be transported in the blood it must first be incorporated into LDL particles. And LDL was first identified as a risk factor for coronary disease in 1958, when John Gofman, a medically trained biophysicist at the University of California in Berkeley, used the newly developed ultracentrifuge to separate plasma lipoproteins by floatation. Gofman found that heart attacks correlated with elevated levels of plasma LDL, and Gofman was also the first scientist to discover that heart attacks were less frequent when the blood contained elevated levels of another lipoprotein, HDL. So Gofman's discovery of LDL as a risk factor for heart attacks was followed over the years by an avalanche of confirmatory epidemiological studies, such as the Seven-Country Study of Ansel Keys, and the Framingham Study that was supported by the NIH.

Now according to Richard Peto, an eminent epidemiologist at Oxford, the total body of epidemiological data over the last 45 years implicating LDL as the cause of atherosclerosis is more convincing than the total body of data implicating smoking as a cause of lung cancer. It was at this point in the century of cholesterol and coronaries that Mike Brown and I entered the picture. In 1973 we discovered that the level of LDL in blood is controlled by a cell surface receptor that we named the LDL receptor. And we also found that Müller's familiar hypercholesterolemia was caused by mutations in the LDL receptor gene. And this was the first molecular link between cholesterol and atherosclerosis.

In 1976 a Japanese scientist Akira Endo discovered a fungal metabolite that could block cholesterol synthesis by inhibiting the enzyme HMG-CoA reductase. And this was the first statin drug. And Mike and I collaborated with Endo to show that the inhibition of cholesterol synthesis led to an up regulation of LDL receptors, which explained how these drugs could selectively lower LDL, the bad cholesterol, without lowering HDL, the good cholesterol. We encouraged the Merck company to develop these drugs, and in 1986 the first statin was approved.
for human use. This year the statins will be consumed by more than 30 million people worldwide.

In 1994 a landmark epidemiological study called the 4S Study was completed, and conducted by scientists in four Scandinavian countries. The 4S Study was the first of six large prospective trials to show that statins by lowering LDL could not only prevent myocardial infarctions but they could actually prolong life. And this therapeutic triumph provided the final link in the cholesterol-coronary chain, complementing the experimental link, the genetic link, and the epidemiological link.

**Familial Hypercholesterolemia**

So now let me tell you how Mike Brown and I got interested in cholesterol, and a little bit about our work on LDL receptors, which has contributed to the genetic link in this story.

So our interest in cholesterol was stimulated by taking care of patients like the little girl shown here. This little girl has a homozygous form of familial hypercholesterolemia which I'll refer to homozygous FH. Her plasma LDL level was elevated tenfold above normal since the time of birth, and her total plasma cholesterol level was 1,000 milligrams per deciliter. Some of the excess LDL particles deposited in her skin, as you can see here, forming these bumps which are called xanthomas. Whenever this little girl bruises her skin, the capillary blood vessels break, allowing the excess LDL to escape from the bloodstream and enter the skin tissue, where the LDL particles undergo oxidation. And the oxidized LDL deposits in macrophages and initiates an inflammatory process that produces these xanthomas.

And this same process that you see here on the slide, the formation of these xanthomas in skin, occurs in her coronary arteries, and this little girl suffered multiple myocardial infarctions before age seven. In FH homozygotes LDL is really the only risk factor for atherosclerosis. This little girl does not smoke, she has no hypertension and she doesn't have a type A personality.

Well, Mike Brown and I first saw two children with this disease when we were research associates at the NIH 35 years ago. We were awestruck by their striking clinical picture, and we decided to work together to try to figure out how a genetic defect in a single gene could produce these high levels of LDL which ultimately led to the severe atherosclerosis.

Although homozygous FHs is a rare disease, the heterozygous form of FH is the most common genetic disease in humans throughout the world in all populations that have been studied. And this next slide summarizes the clinical features of the heterozygous and homozygous form of FH; the heterozygous occur in one in five hundred individuals in every population that's ever been studied. These
individuals have a two-and-a-half-fold elevation on average in their plasma LDL from the time of birth, and because of the sustained increase in their LDL, they begin to have myocardial infarctions at around 35 to 45 years of age, if they are not treated. Now, because of treatment, this number fortunately is being delayed to later years in life.

The rare homozygotes who have to inherit a gene from both of their heterozygous parents to have this disease in a severe form occurs at a frequency of one in a million; this is like the little girl I just showed you the picture of. They have a plasma LDL level that’s greater than six-fold from the time of birth and because of the magnitude and very high increase in LDL from the time of birth, these individuals typically have heart attacks between the age of 5 and 15 years, and we’ve actually seen one FL homozygote in Toronto who had a first heart attack at 6 months of age.

Now, 5 percent of all individuals that have a heart attack under age 60 have the heterozygous form of familial hypercholesterolemia which, as I mentioned, occurs in one in 500 people, so this is an important public-health problem as a single-gene disease.

**Identifying the LDL Receptor**

Now our approach to working out the molecular defect in FH was to study the skin fibroblast in tissue culture, comparing LDL metabolism in cells from normal subjects from those with patients with the homozygous FH, and we began our studies in 1972 in UT Southwestern in Dallas. And to make a ten-year story short, we found that fibroblasts require cholesterol for growth in tissue culture, and that cells could obtain this cholesterol from two sources: they could either synthesize cholesterol from the cholesterol synthetic pathway that I showed you earlier; or they could take up cholesterol by the uptake of LDL that was derived from the fetal-calf serum in the tissue-culture medium. In order to take up this LDL, the cells produced a specific cell-surface receptor that we call the LDL receptor, and cells from patients with homozygous FH turned out to lack LDL receptors, and since these mutant cells could not take up LDL from the serum, they obtained all their cholesterol from growth from the endogenous synthetic pathway.

The availability of mutant FH cell lines that lacked LDL receptors allowed us to work out the biochemical steps in this LDL receptor pathway, which is a prototype for the general process of receptor-mediated endocytosis. LDL receptors are concentrated in specialized regions of the plasma membrane called coated pits. These coated pits pinch off to form coded vesicles, the coded vesicles shed their coats and ultimately fuse with other vesicles to form an endosome, which has an acidic pH compared to the coated vesicle, and once the receptor-bound LDL is in the endosome the acidic pH causes the LDL to dissociate from the receptor, and the receptor is recycled to a recycling vesicle,
going back to the cell surface where it then concentrates again in coated pits to
pick up another particle of LDL.

Each LDL particle, as I mentioned, contained 1,500 molecules of cholesterol
ester, and each receptor makes about 1,200 trips in and out of the cell during its
30-hour life span. Each trip is about 15 minutes or so, and so one has an
enormously efficient mechanism for transporting a molecule like cholesterol into
a cell; it's probably one of the most efficient transport mechanisms ever designed
in nature.

Back into the endosome where the LDL dissociates from the receptor in the acid
environment, and that LDL particle, this part of the endosome then fuses with
other vesicles to enter a lysosome where the hydrolytic enzymes of the lysosome
degrade the LDL particle, releasing the cholesterol which is used for various
structural purposes and regulatory purposes that Mike will talk about later in his
talk.

**LDL Receptor Structure**

So by 1982 we were able to purify the LDL receptor and then with the help of all
the exciting technology that had come around with recombinant DNA, we were
able to clone the cDNA in gene for the receptor in 1984, and the deduced
sequence of the receptor was quite revealing. It showed a molecule of 839 amino
acids that had five distinct domains. The first domain consisted of multiple
repeats that served as a ligand binding domain the apoprotein B of LDL. And
then the second domain, this A-B-C, is a region of the receptor that senses the
acidic pH in the endosome and somehow leads to a conformational change in
the receptor in such a way that the LDL is released from the receptor, so that the
receptor can recycle back to the cell surface. And then there is a region that has
sugar molecules, and then there's a region that anchors in a receptor in the
membrane, and then there's the targeting signal in the cytoplasmic domain that
tells the receptor to go to coated pits.

Now this is a very complicated structure for structural biologists to solve because
it turns out to be there are cysteine residues in this receptor, there are thirty
disulfide bonds, and so it took many years for Hans Deisenhofer in Dallas to
solve this structure, and just in the last year he and his postdoctoral fellow Gabby
Rudenko, after 15 years of work, have been able to solve the structure of the
extra cellular domain in the LDL receptor, and it revealed a very interesting point
to illustrate this recycling mechanism that I mentioned to you.

So here we are looking at—this is the structure by Rudenko and Deisenhofer—
this is these repeats, these seven repeats, that bind the apoB of LDL, so a
neutral pH LDL would presumably be bound up here, and this part of the
molecule would not be where it is right now. But in acidic pH what happens is
that this A-B-C part of the molecule which exists as a β-propeller in the three-
dimensional structure now binds to several of these repeats, these repeats 3, 4 and 5, in such a way that it ejects the LDL particle that's bound up here, and this now allows the receptor to recycle to the cell surface to pick up a new particle of LDL, and then releases the LDL to now go into lysosome. At least that's the hypothesis. The structure's only been done in one confirmation and now has to be done in various different confirmations, in different pHs, to prove whether this is in fact right or wrong, but it's consistent with the biological information that Mike and I and our students and postdocs have derived from mutagenesis studies. But in any event, it's gratifying to have a sequence to begin to think about after all of these years.

Now, as I've already mentioned to you, the FH had mutations in their LDL receptor gene that disrupt receptor structure and function. This just happens to be a map of the LDL receptor gene on the short arm of chromosome 19. There are 18 exons, and this just shows the position and the types of the first 100 mutations that we and our colleagues in Dallas worked out from 1984 to 1990. And then after the first 100 we decided to move onto other things. And now other people have gotten involved in a big way and there are more than 1,200 different mutations in this very relatively short LDL receptor gene that have been identified in patients with FH. With few exceptions, virtually every unrelated family that has this disease, familial hypercholesterolemia, has a different mutation in the LDL receptor that affects the structure and ultimately the function of the receptor.

Now I'd like to turn to the question of how does these LDL receptor mutations produce a disease, how does the deficiency of receptors raise the plasma LDL levels in patients with FH? And so our studies showed that in normal humans, the majority of LDL receptors in the body are expressed in the liver where they act to remove LDL from plasma and by receptor-mediated endocytosis. The FH heterozygotes, as I mentioned, have half the normal number of functional LDL receptor genes, and they thus have one normal LDL receptor allele and one mutant LDL receptor allele, and they remove LDL from plasma at half the normal rate. And as a result they have twice the level of LDL in plasma compared to a normal individual. And then the FH homozygotes, the ones shown here, have absolutely no receptors, have a very huge level of LDL in their plasma that's only removed at a very low rate by nonspecific, non-receptor-mediated process.

So a physiological approach to therapy in the FH heterozygote would be to increase the activity of the receptors encoded by the normal allele. If one could get them to work twice as hard, then one would be able to create a state that would look more like the normal, and then these increased number receptors would remove the LDL from plasma at an enhanced rate. So this would be a pharmacological form of gene therapy. And this brings me to the next topic, designing a rational therapy for FH heterozygotes.
Treating for Familial Hypercholesterolemia

And now the mechanistic insight into how drugs could be used to lower LDL levels came from knowing that cells can obtain cholesterol from two pathways, both of which are under the same feedback control. So when cholesterol synthesis in the liver is decreased, the cell responds by increasing the synthesis of LDL receptors, which in turn leads to an increased removal of LDL from the plasma. And this is precisely how the statin drugs appear to work. Now the statins are competitive inhibitors of the HMG-CoA reductase enzyme, and when you ingest the statin drug, it goes directly to the liver where the drug inhibits HMG-CoA reductase, which in turn reduced cholesterol synthesis and raises LDL receptors in the liver because of the existence of this feedback system, which Mike will actually tell you about in more detail.

But the overall effect of administering such a statin drug is to decrease the plasma LDL level, and when the plasma LDL now enters the liver and its cholesterol is now available in the liver, one has a restoration of the normal cholesterol content of the liver. So one has a drug that actually works by lowering the plasma LDL without essentially affecting the level of cholesterol in the liver; it's almost a perfect system. You could not ask for a better drug or a better system for a drug to operate on.

Now, there are 6 statins that are now approved for human use and are taken by 30 million people worldwide, and the 3 most commonly prescribed statins are Lipitor, Zocor, and Pravachol, and I'm sure that these drugs are very, very familiar to many of the older professors in the audience, as Andy pointed out earlier.

This slide shows you how different individuals respond to the statins. The FH homozygotes who have no receptors show a very poor response, as would be predicted from the model that I showed you. The FH heterozygotes who have one normal LDL receptor allele and one mutant LDL receptor allele when maximally treated with statins have a very good response, a 40-percent drop in their cholesterol level. And normal subjects actually who have two genetically normal LDL receptor alleles show an even greater response, when maximally treated, and that's because their receptors are metabolically suppressed for reasons that Mike will tell you about in a moment.

So these statins clearly lower plasma LDL levels in FH heterozygotes and in so-called normal hypercholesterolemic individuals. Do they decrease heart disease? And this is a summary, actually a meta-analysis of the first five clinical trials that were done between 1994 and 1998 on 30,000 subjects followed for years, half receiving statins, half receiving placebos. And the plasma LDL in these clinical trials dropped by 28 percent and the heart attacks over the next 5 years dropped by 31 percent. These—you didn't see the larger response I showed you on the
last slide because these patients were not really maximally treated; they were taking many different drugs and they were given one standardized dose.

The most recent study done by Collins and Peto at Oxford was just published last year. The Heart Protection Study involved a single study of 20,000 subjects followed for years. Plasma LDL was reduced by 33 percent, heart attacks and strokes were decreased by 33 percent over this five-year period.

Now these individuals, these 60,000, 50,000 people were only followed for five years with a 30-percent reduction in coronary events. And the question is what happens if they had been taking this drug for 10 years, or 20 years, or 30 years, would one ultimately prevent heart attacks had they started 10 or 20 years earlier? And that's a question that will eventually be answered over many years, but many people believe that one will see a dramatic effect if these drugs are started at a much earlier age.

Now the vast majority of these 50,000 people in this study are not heterozygous FH, but they're people with genetically-normal LDL receptor genes, the people who are so-called polygenic hypercholesterolemia, a garden-variety hypercholesterolemia. And that brings me to the question, why do people with normal LDL receptor genes have LDL levels high enough to cause heart attacks? And I just can't answer that question; I have to ask Mike to come up and answer that question for you.

"Normal" and "High" Cholesterol

Michael S. Brown: Okay, if you could just leave that on, it's the same talk, go back to where it was before.

Well, let me just start by saying that I am not the descendent of Alexander Hamilton's wife's first husband. Nor did I attend any sessions at Columbia when I was in high school. But I have a very high regard for Columbia and it's for that reason that, I think, Joe and I were very honored by the invitation to—we're going through somebody else's talk, we just have to go back to that, that's it now—what we want to do is go down to this thing, the slide show, just cancel it. Now how do we go back onto—okay. All right, so, all right.

Joe has told you about LDL receptors, and how their normal job seems to be in part to protect—not only to deliver cholesterol to cells but also remove LDL from plasma, and thereby to protect us against high levels of LDL which lead to heart attacks. And the question then is if 499 out of 500 of us have normal LDL receptor genes, why do one-third die of heart attacks? And the answer comes down to the definition of normal.

Here you see the plasma cholesterol level in industrialized countries like the United States, Western Europe, and the rest of North America, and what you can
see is for a 40-year-old man, the mean cholesterol level is about 210 milligrams per deciliter. And we know that the incidence of heart attacks rises as the plasma cholesterol level rises, but it's already high in people with normal, at least statistically normal, plasma cholesterol levels. And that is very hard for some people to accept. Why should we have heart attacks with normal LDL cholesterol levels; LDL can't be the cause of this because we have normal levels.

Well, this is only normal when you look at these industrialized countries. If you were able to measure the cholesterol level of everybody in the world, this is the curve you would get. Now what you would find is that the median value for most of the world is a plasma cholesterol level of about 150. Now there's a simple reason for that; there are a lot of Chinese people in this world, but the fact is that for the majority of human beings who live outside of industrialized countries that's what the cholesterol level is, that's what the cholesterol level is in primates, that's what the cholesterol level is when we're first born before we've done anything to change the cholesterol level. And so one can look at our whole society and see that we're all above the ninetieth percentile. So it's not surprising that we're seeing heart attacks in this whole group.

Now what's the reason why we're faced with these high cholesterol levels in North America and Europe? The answer is not a mystery. This is from *Time* magazine. I don't know if any of you can see the—to show you how old this is, this is Walter Mondale and Gary Hart up there. So we've known about the diet and cholesterol for a long time, and in fact there's a huge amount of data that says the reason we see these high cholesterol levels in Western countries is because of the diets that we eat that are rich in cholesterol and saturated animal fats.

And the question is why should these diets raise the plasma LDL cholesterol level, why shouldn't the LDL receptor just keep removing the cholesterol from the blood? Well the answer is contained in something that Joe referred to, and that's the regulation, the feedback regulation of the LDL receptor. So if one considers the liver, so then the liver of somebody living in China has lots of LDL receptors and LDL levels are kept low; if somebody has familial hypercholesterolemia in the heterozygous state there's a 50-percent reduction and plasma LDL builds up to twofold above normal, but in people living in—who eat high-fat diets, the cholesterol is absorbed in the intestine, and it's delivered directly to the liver. So all of the cholesterol that you eat does not hang around in the bloodstream very long; it's cleared in the liver almost in one pass through that organ. And the cholesterol is taken into the liver by a totally separate receptor called the chylomicron-remnant receptor, and that receptor basically concentrates—all of the cholesterol that you eat gets concentrated in your liver cells. And in fact, it's contained within the liver membranes, because all of the cholesterol in cells is contained within membranes in the cell, since cholesterol is insoluble.
And then what happens at that point is something that Joe illustrated, and that is the feedback mechanism that controls the production of LDL receptors comes into play. And a signal is sent into the nucleus of the cell, and the gene encoding the LDL receptor is partially suppressed. It's not suppressed by 50 percent, or we would all look like FH heterozygotes, but it's suppressed by about, oh, say, 10 or 20 percent, which is probably enough to raise the plasma cholesterol level from its normal 150 up to the 210 to 300 values that we customarily see in this country. And so it's the down-regulation of LDL receptors by dietary cholesterol and saturated fatty acids that leads to the elevation of LDL in plasma.

**How LDL Receptor Is Regulated**

And the question we'd set out to answer several years ago is, how is this information transmitted to the nucleus? After all, cholesterol is insoluble, it's only in the membranes of cells, and how does the nucleus of the cell know that the membrane of the cell is saturated with cholesterol? And so several years ago Xiaodong Wang and Mike Briggs in our laboratory purified transcription factor from nuclear extracts that controls the production of LDL receptors, and we call it the sterol regulatory-element binding protein or SREBP. We pronounce it S-R-E-B-P, some people pronounce it srebp, but it's an unpronounceable name basically, like an Al Capp cartoon character.

But anyway, what we purified from the nucleus of cells was a classic transcription factor; it has a DNA binding and dimerization domain called the bHLH-Zip domain, and this part of the LDL receptor binds to an enhancer in the five-prime flanking region—this part of SREBP binds to an enhancer in the five-prime flanking region of the LDL receptor gene and also other genes involved in cholesterol metabolism, and turns on their transcription. So this part of the—which we purified from the nucleus is a classic factor that stimulates gene transcription.

But the SREBP differed from all of the other transcription factors that were known at that time, because when we cloned the cDNA we found that this transcription factor was actually just the first third of a very large protein of 1,200 amino acids, and following this transcription factor domain there's a membrane-attachment domain that has two transmembrane helices that is inserted into the membranes of the endoplasmic reticulum of the cell. So SREBP is not a nuclear transcription factor, it's a membrane-bound protein. And then at its carboxy-terminus, it has another six hundred amino acids that act in a regulatory fashion.

Now in order to see whether this SREBP could ever get into the nucleus, we made an antibody against this amino-terminal Zip domain and used it to stain cultured human cells. And what we found was that if we grew cells in tissue culture in the presence of sterols, so they were basically overloaded with sterols, they don't want to transcribe the LDL receptor or the cholesterol biosynthetic genes, and as a result the SREBP is not in the nucleus, it's outside of the
nucleus, and it turns out that this staining is in the endoplasmic reticulum. But if we simply deprive the cells of sterols so they become hungry for sterols, then there is a proteolytic reaction that occurs that sends this Zip domain directly into the nucleus of the cells. So the transcription of the LDL receptor gene is controlled by the controlled proteolysis of this membrane-bound transcription factor, SREBP. And over the last seven or eight years we’ve been able to work out the mechanism by which this occurs. And let me summarize it here.

When SREBP is made, it forms a complex with another protein in the endoplasmic reticulum, and that protein is called SCAP (SREBP Cleavage Activating Protein). And that protein is the key to the whole regulatory system.

This SCAP-SREBP complex, after it is formed, if cells have been deprived of sterols, this complex now is incorporated into vesicles that leave the endoplasmic reticulum and move to the Golgi complex of the cell. And within the Golgi complex the SREBP encounters two proteases, first a serine protease called site-1 protease that clips the SREBP here in the lumen of the Golgi, and it separates the two halves. But this bHLH domain is still bound to the membrane because it has one transmembrane helix. And at that point it's cleaved by a second protease called site-2 protease, which is a zinc metalloprotease, a very unusual membrane-embedded zinc metalloprotease, that actually clips the SREBP within the transmembrane helix and releases the HLH domain so that it can go the nucleus and activate transcription.

When sterols accumulate within this membrane of the endoplasmic reticulum, the sterols bind to SCAP and cause a conformational change in SCAP. And when that happens, the SCAP-SREBP complex no longer leaves the endoplasmic reticulum, it no longer moves to the Golgi. The SREBP that's in the nucleus is rapidly degraded, and no new SREBP is made because the SREBP is trapped in the Golgi, and as a result of that the gene transcription declines. So cholesterol turns off SREBP by blocking the movement, by building up in the membrane of the ER, endoplasmic reticulum, and blocking the movement of SCAP to the Golgi.

Now, how is it that this cholesterol buildup in the ER causes the SCAP-SREBP complex to be retained? Well, this is the most recent part of the story. We’ve discovered a protein in the ER membranes called Insig, and Insig acts as the anchor for the SCAP-SREBP complex. So when the sterol content of this membrane is low, the SCAP-SREBP complex gets incorporated into these budding vesicles and goes to the Golgi. But when the cholesterol content of this membrane is elevated, SCAP undergoes this conformational change which we can detect biochemically, and that causes it to bind to Insig, and Insig holds it back in the ER.
Studying the SREBP Pathway in Mice

So we've been able to identify the transport protein SCAP, the holdback protein Insig, and the two proteases that process SREBP; all of that has been done through the use of somatic cell genetics in tissue-culture cells. But the question is then does this whole system apply to the liver? And is this system, this SREBP feedback system, really the reason why the LDL receptor gene is suppressed in people who eat high-fat diets? Well, to answer that question in experimental animals we've returned to the SREBP pathway, and we want to study the role of this SREBP SCAP complex in the liver of mice. And to do that we took advantage of a mutational form of SCAP that we had discovered in tissue-culture cells, in tissue-culture cells that were mutated and became resistant to the feedback actions of cholesterol. And the mutation that occurred, that made cells resistant to the feedback regulation by cholesterol was a point mutation within the SCAP gene that substituted an asparagine for aspartic acid at position 443.

When SCAP has this one-point mutation, then it still binds SREBP and it still carried SREBP to the Golgi, but it's no longer turned off by cholesterol. This mutation prevents SCAP from binding to Insig. So SCAP can't bind to Insig, and therefore the SREBP continued to be processed. And in tissue-culture cells that have this mutation, they continue to take up LDL and can't turn off the receptor, and therefore they become with cholesterol. And to test whether this system really works in the liver, we made a transgene encoding—well, first of all before I get to that, let me just show you the biochemical phenotype in tissue-culture cells.

So now this is an immunoblot using an antibody against the amino-terminal domain of SREBP. And it's performed on nuclear extracts from CHO cells in tissue culture. If we take wild-type cells and grow them in the absence of sterols, the SREBP has been processed and we find the—the nuclear form in the nucleus. But if we just add a small amount of sterols, the SCAP no longer moves to the Golgi, and SREBP is no longer processed. But if the SCAP in cells that contain this mutant form of SCAP, they're markedly resistant to feedback regulation by cholesterol.

So we made a transgene encoding of that mutant SCAP, and together with Bob Hammer in Dallas we made a transgenic mouse that expresses, simply expresses, this transgene. Of course this transgene creates a dominant defect because when it binds SREBP, it carries it to the Golgi, so that's a basically—and it can't be turned off by cholesterol—so that's basically a gain of function. And so what we see here is that we get the same phenotype in the livers of these animals that we do in tissue-culture cells; that is, when wild-type mice are placed on a chow diet, we find SREBP in the nucleus; when the animal is fed a diet containing cholesterol, the SREBP is no longer in the nucleus, the LDL receptor gene, and all the enzymes of cholesterol biosynthesis are repressed. But if the animal has this single copy of this mutant SCAP gene, then the amount of...
SREBP in the nucleus is elevated, and there's a marked resistance to feedback suppression.

And what that does is when the animal is on a—not even fed cholesterol, just because of the overproduction of cholesterol that occurs with this SREBP in the nucleus, the livers of these animals are quite enlarged and they're white here because they're absolutely filled with fat, not only cholesterol but also triglycerides and fatty acids. We call this *foie gras de mouse*.

So this one experiment says that the liver has SREBPs, that the SREBPs are responsible for feedback regulation, and that if you defeat the feedback regulation by putting in a non-suppressible form of SCAP, then you get this overload of the liver with cholesterol and the suppression of LDL receptors. So we believe that this SREBP mechanism is what controls LDL receptor activity. And of course the converse to this thing happens when animals are treated with one of the statin drugs that blocks HMG-CoA reductase. Here you see immunofluorescence of a normal hamster liver, and the SREBP is not in the nucleus, it's outside of the nucleus, because this animal is making cholesterol and keeping its own SREBP partially suppressed. But now if you block cholesterol synthesis by giving this statin drug, you see all these nuclei of the liver lighting up. So we believe that this is the mechanism by which the statins lower cholesterol levels in humans.

**Searching for Other Polymorphisms**

Well now to summarize my talk and Joe's talk, let me just say that—let's go back to this bell-shaped curve, and this is the bell-shaped curve when looked at through the eyes of a LDL receptorologist. So here's the bell-shaped curve of cholesterol again in Western countries, and the people down here at the low end are people who manage to have very active LDL receptors that keep their plasma LDL level low. Now not all of these people are eating a low-cholesterol diet, some of these people down here are eating quite a high-cholesterol diet. But they obviously have other mechanisms, something's different about them, that prevents their LDL receptors from being suppressed even though they're eating a high-cholesterol diet. On the other hand, we have the homozygous FH patients who are not even on the curve; they have two mutant LDL receptor genes so they can't make any LDL receptors, and they're way off the curve. The FH heterozygotes are stuck up at the top here because they only have one normal gene and one mutant gene. And we believe that all the people in here have been moved out of the normal range because they've eaten this high-cholesterol, high-saturated-fat diet and thereby metabolically suppressed their receptors through the SREBP feedback system.

Now, so as I say, this—the problem from a medical standpoint—point of view—the interesting thing is the splay in this bell-shaped curve. I mean even in our society this, you know, we see people with cholesterol levels of 160, like me,
despite the fact that I don't eat a very healthy diet. And on the other hand, there are some people who just look at an egg and their cholesterol goes sky high. So there are clearly a lot of genetic influences that influence our response to this environmental challenge, and some of those genetic influences are very strong, and I think the answer to the whole problem is shown on the next slide.

As far as we could tell, at least what they told us publicly was, that Bill Clinton had, you know, very low cholesterol levels and was the picture of health, despite his pizza diet. So if we can understand Bill Clinton's genes then maybe we'll understand what it is. And maybe by some of the approaches that Professor Lander described to us, we may be able to understand all of the other modulatory genes. One can imagine, for example, that there might be polymorphisms in genes involving the absorption of cholesterol, or in the conversion of cholesterol to bile acids, or in other metabolic pathways that cholesterol can have. There also could be polymorphisms in these regulatory genes; we're looking for polymorphisms in SCAP and in Insig and in the SREBPs themselves that could underlie some of this variability. Our feeling is that whatever it is, it's going to be complex. We know there are other single-gene mutations that can elevate LDL, but most of the garden-variety LDL elevations are going to be due to this high-fat diet, playing on the background of multiple variable genes that make us all different one from the other. And that's the work that we're continuing to do in our laboratory. Thank you very much.