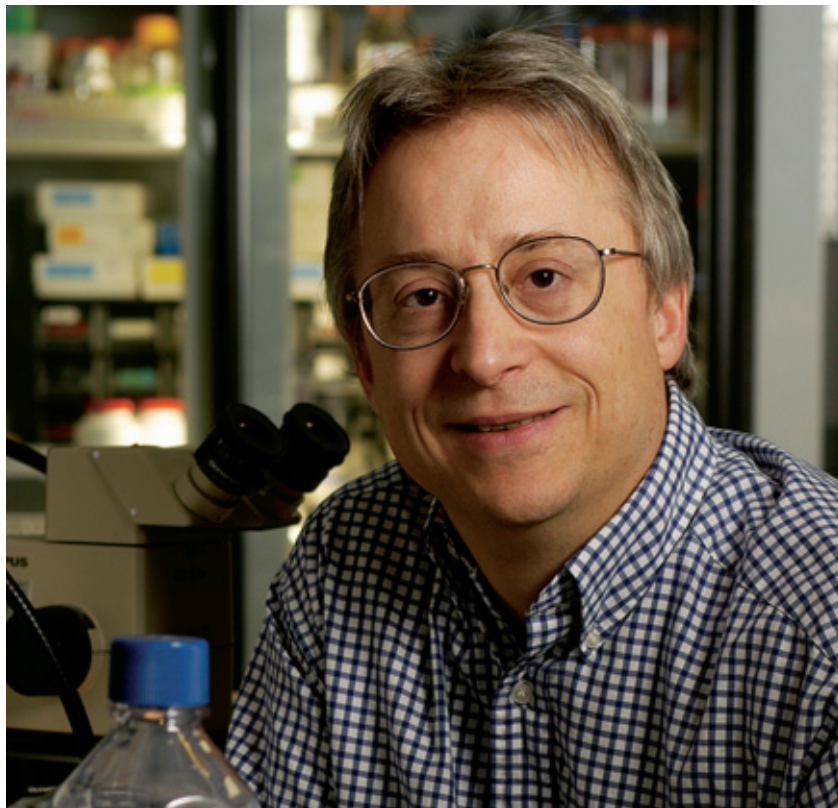


CHOLESTEROL UP CLOSE

Cholesterol is contradictory. On the one hand, the substance is a vital part of cell membranes, and essential for life. At the same time, an overabundance of cholesterol-carrying low-density lipoprotein—the infamous LDL—can lead to atherosclerosis and other diseases.

To help understand how the human body manages this seeming paradox, researchers look at cholesterol up close, at the cellular level. Matthew Scott, an HHMI investigator at Stanford University School of Medicine, says that “the cell has developed elegant control systems to handle cholesterol because it has such a profound impact on the properties of the cell.”

In this series of articles, we take a closer look at some of the cell’s elegant systems for processing cholesterol. In a related account, two Nobel Prize winners summarize the history of cholesterol research.



MYSTERIOUS PROTEIN

Scott, who had been studying the signaling proteins that direct body patterning during embryonic development in the fruit fly *Drosophila* and in mice, found himself drawn into the study of human disease when NPC1, one of two proteins behind a devastating disorder called Type C Niemann-Pick disease (NPC), turned out to resemble Patched, a protein that regulates development.

“When the NPC1-Patched connection was found, a new opportunity arose to learn about development from cholesterol metabolism and vice versa,” says Scott. NPC1 normally helps transport excess cholesterol out of the cell. But if the protein is disrupted, as in NPC, transport processes inside the cell fail, high levels of cholesterol accumulate, and certain cells in the brain and other tissues are damaged or killed. The unfortunate few who are born with the disease rarely live past childhood, because basic bodily functions deteriorate progressively and there is no known treatment.

“The cell has developed elegant control systems to handle cholesterol because it has such a profound impact on the properties of the cell,” says Scott. “But much remains to be learned about the roles of cholesterol in cell properties and signaling mechanisms. The NPC proteins are providing a way to try to understand the components of the cholesterol-processing pathway.”

Two articles published in the March 4, 2003, issue of the *Proceedings of the National Academy of Sciences (PNAS)* describe the structure and cholesterol-binding properties of NPC2, the second protein that can cause NPC, which was discovered three years ago by Peter Lobel, professor of pharmacology at Robert Wood Johnson Medical School–University of Medicine and Dentistry of New Jersey (UMDNJ). NPC2 is responsible for about 5 percent of NPC cases, but apart from the fact that it can bind cholesterol, little else has been reported about it until now. Lobel recruited HHMI investigator Ann M. Stock, also at UMDNJ, to determine the crystal structure of the protein, and together the two labs reported a protein structure with unexpected features.

Other proteins known to bind cholesterol contain a large cavity. The interior of the cavity is hydrophobic, which means that it repels water, but it attracts highly insoluble molecules such as cholesterol. The hydrophobic interior of NPC2 lacks a large cavity, or pocket, that could accommodate a large molecule such as cholesterol, but it contains several small cavities. “What was a surprise to us when we determined the structure was that NPC2 lacked an obvious binding pocket,” says Stock. “The structure of NPC2 is basically a sandwich of two beta sheets with an intriguing pore leading into the protein’s interior. When we inspected the structure, it became obvious that this could be the binding site, but it is too small to accommodate the cholesterol molecule. The NPC2 protein must change its shape when it binds cholesterol.”

Cholesterol plays a very important role in organizing the cell membrane, Philip Beachy says.

SIGNAL TO SIGNAL

Cholesterol molecules shed light on how cells communicate. By Karyn Hede

Most of us are at least a little concerned about cholesterol, but for some scientists, it’s an obsession. Investigators who study cholesterol closely—deep inside the cell—are finding that it has some rather fascinating properties.

“It turns out cholesterol actually has a very important role in organizing the cell membrane,” says Philip A. Beachy, an HHMI investigator at The Johns Hopkins University School of Medicine. “This is a huge area of cell biology right now, and cholesterol is an important player.”

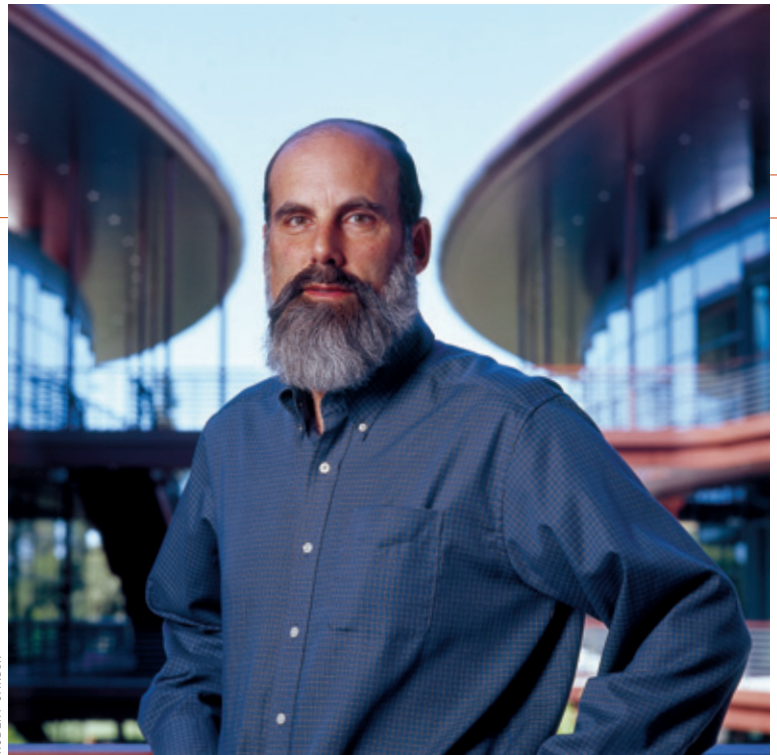
HHMI investigator Matthew P. Scott, a biologist at Stanford University School of Medicine, believes cholesterol can shed light on how cells communicate. In what he calls an “exquisite interplay,” cells send signals to each other as they multiply—transmitting information that determines a cell’s ultimate function and destiny. Thus, “cholesterol is arising as a very interesting molecule for signaling processes, in addition to its known role in affecting membrane properties,” Scott says.

BILL DENISON

Confirmation of their hypothesis came at the annual meeting of the Ara Parseghian Medical Research Foundation in 2002, when Scott and M.D.-Ph.D. student Dennis C. Ko reported results of their genetic studies of NPC2, which are now published in *PNAS* back-to-back with Stock and Lobel's work. (The Parseghian Foundation was established by the legendary Notre Dame football coach, whose three grandchildren were born with NPC. The foundation funds Lobel, Scott, and Stock's work, and its annual meeting brings together dozens of scientists whose work is focused on the disease.)

By studying evolutionarily conserved regions among NPC2 genes in several species, Ko identified and mutated several key amino acids in the protein. He then tested their ability to bind cholesterol and to reverse cholesterol buildup in cells that lack NPC2 function. "What we found were three mutations unable to bind cholesterol and also unable to rescue NPC2-deficient cells," Ko says.

The three amino acids that affect cholesterol binding when mutated are located in the same hydrophobic region of the NPC2 protein that, based on their structural work, Stock and Lobel suggested would bind cholesterol. However, although the convergence of the structural and genetic studies strengthens the case of NPC2 as a cholesterol-binding protein, "exactly what's happening isn't clear," says Stock. "Cholesterol is very insoluble, so it makes some sense that it does not exist in a free state to any significant extent. It may be passed from protein to protein inside the cell. It is logical to hypothesize that cholesterol binding by NPC2 somehow facilitates delivery of cholesterol for export out of the cell. But it is a relatively unexplored field, and there are presently far more questions than answers."



ROBERT CARDIN

Matthew Scott, a biologist at Stanford University School of Medicine, believes cholesterol can shed light on how cells communicate.

PATCHED AND SMOOTHENED

Answers may come from the study of NPC1's evolutionary relative Patched and its binding partners Hedgehog and Smoothened, a trio of proteins that constitute a pathway for regulating basic body patterning during embryonic development. When Hedgehog, a secreted signaling molecule, binds to Patched on the cell surface, it releases Smoothened to transmit signals to the nucleus for eventual activation of a plethora of genes that direct cell specialization. It turns out that cholesterol is a crucial piece of this pathway.

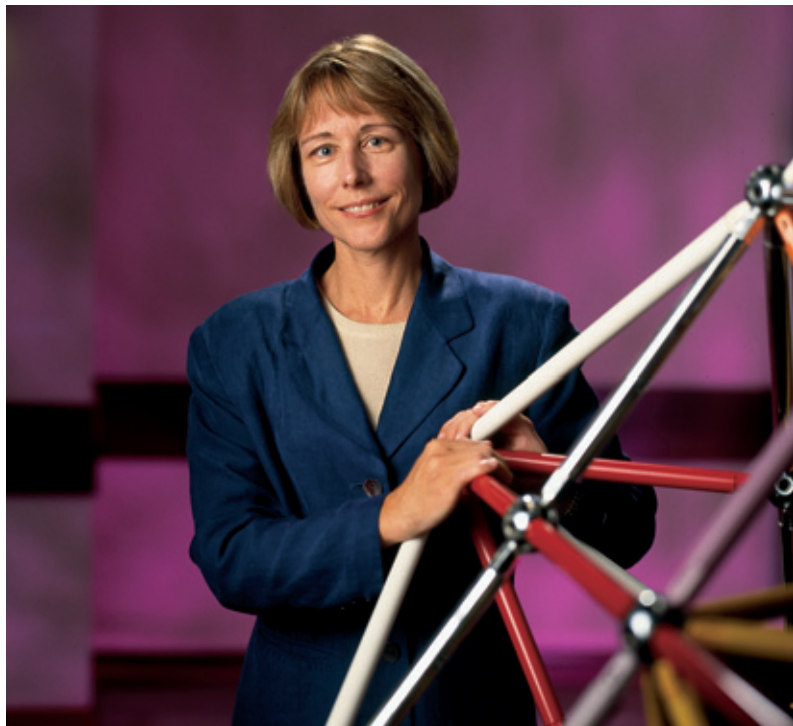
In 1996, a seminal *Science* paper from the laboratory of Philip Beachy at Johns Hopkins showed that the Hedgehog signal is formed through an unusual autocatalytic process that involves cleavage of a precursor protein into two parts and the attachment of a cholesterol molecule to the signaling portion of the molecule.

"It was a bolt from the blue," says Beachy. "The specific modification of a protein by cholesterol is truly unique."

Beachy speculates that cholesterol's insolubility may have been advantageous during evolution to restrict the movement of the nascent differentiation signal. "You can't give an accurate accounting of what evolution was doing, but it may have been advantageous at the dawn of multicellularity to restrict Hedgehog's effects to the cell right next door," says Beachy. "Then you could have three cell types: the one that makes it, the one right next door, and the one just a little farther away. Maybe evolution co-opted this autoprocessing domain to add cholesterol and restrict the range of Hedgehog signaling."

Since his discovery of cholesterol's role in development, Beachy too has been drawn into the study of human genetic disease. He became intrigued by a family of human syndromes, the most well-known being Smith-Lemli-Opitz syndrome (SLOS), caused by a defect in the final step of cholesterol synthesis. Lack of cholesterol in these diseases leads to birth defects—such as brain and facial malformations, reduced branching of the lungs, and defects in the development of the nervous system—that resemble those associated with loss of Hedgehog signaling.

MARC BRYAN-BROWN



Ann Stock worked to determine the crystal structure of the protein behind the devastating disorder Type C Niemann-Pick disease.

“Naturally, we thought what’s probably happening in these patients is that there is not enough cholesterol, Hedgehog isn’t getting made properly, and so there’s a defect in the signaling,” says Beachy. However, after using a mouse model of SLOS in a series of experiments, Beachy’s team ruled out Hedgehog processing as the culprit. Instead, the scientists discovered that reduced cholesterol levels affect the cell’s ability to respond to Hedgehog signals.

In a series of studies using mutant forms of Patched and Smoothened proteins in cholesterol-depleted cells, the scientists narrowed the effect to Smoothened’s inability to undergo conformational change from the inactive to active form. In a paper published in the April 2003 issue of *Nature Genetics*, they showed that the level of cholesterol in the cell membrane in SLOS patients is too low to support the critical transition. “The state of the membrane must be such that Smoothened can make this transition from inactive to active, and cholesterol seems to be a key part of that,” says Beachy.

But a mystery remains. The genetic and biochemical studies, says Beachy, suggest a missing piece of the puzzle—a regulator in between Patched and Smoothened—even though no such player is known to act between the two proteins.

In an article in the August 22, 2002, issue of *Nature*, Beachy and colleagues reported that Patched does not directly suppress Smoothened, as had previously been suggested. Instead, Patched may transport a small molecule, whose identity is not yet known, that affects Smoothened activity. Beachy notes that Patched and NPC1 are both related to a family of bacterial transporters called the resistance-nodulation-division proteins. Another related protein, Dispatched, is responsible for releasing the Hedgehog signal from the cell—perhaps, speculates Beachy, by releasing the cholesterol component that binds Hedgehog to the cell membrane.

“We’ve been studying how Patched regulates Smoothened, and we think that Patched may transport a small molecule that then regulates Smoothened,” says Beachy. “That may be what’s in between Patched and Smoothened. But we don’t know its identity yet.”

What is known is that the Hedgehog signal itself and possibly other pathway components associate with cholesterol-rich domains of the cell membrane, known as rafts, which have been implicated in several cellular processes, including signal transduction and vesicle traffic—the very process that is defective in NPC disease.

“We were all taught 20 years ago that membranes were just this sort of sea of lipids with proteins floating in them,” says Beachy. “Well, it’s not so. Parts of membranes are much more ordered.” In fact, lipid rafts have emerged as a crucial element of many signal-transduction pathways, most prominently in white-blood-cell formation, immune-cell activation, and neuron signaling.

FLOPPY LOOPS AND FLEXIBLE TAILS

Exploring the gateway that processes “bad” cholesterol. By Renee Twombly

On the street, LDL (low-density lipoprotein) is known as bad cholesterol. While cholesterol itself is essential for life—it’s a vital part of cell membranes—an overabundance of LDL in the blood can help lead to atherosclerosis and other diseases.

To better understand how the body normally regulates the buildup of bad cholesterol, researchers at the University of Texas Southwestern Medical Center at Dallas are exploring the gateway created by the LDL receptor, which helps regulate levels of cholesterol in the blood. HHMI investigator Johann Deisenhofer, who shared the Nobel Prize in Chemistry in 1988, leads the team.

Deisenhofer and colleagues wanted to know how the LDL receptor lets go of LDL after snatching it from the bloodstream and dragging it inside a cell, so that the cholesterol transported by the LDL can be used by the cell.

To learn more about this receptor’s structure and functions, the researchers decided to use x-ray crystallography to make a three-dimensional image of the LDL receptor. They therefore had to grow crystals



Johann Deisenhofer, who shared a Nobel Prize in 1988, and colleague Gabrielle Rudenko used x-ray crystallography to make a 3-D image of the LDL receptor.

REID HORN

of the LDL receptor—a task that took Gabrielle Rudenko, an instructor of biochemistry who heads the team in Deisenhofer’s lab, no less than six years. X-ray crystallography requires that proteins with the same shape be neatly stacked together into a crystal. When x-rays are beamed at the crystal, electrons diffract the x-rays, which creates a pattern that is used to reveal the protein’s atomic structure. But this was incredibly difficult, Rudenko says, because the receptor “is inherently very flexible, with lots of floppy loops and flexible tails.”

Rudenko finally found the right chemical conditions to produce a baker’s dozen of identical receptors and then took a “snapshot.” What she and Deisenhofer saw, and published December 20, 2002 in *Science*, is a 3-D picture of what the researchers think the receptor looks like after releasing LDL.

At neutral pH (like the pH in blood), the LDL receptor binds LDL on the cell surface. In this state, the receptor is likely to look “long and floppy and all of the modules are aligned like a long string of beads,” Rudenko says.

However, once internalized in the cell, in a compartment with acidic pH, the LDL receptor seems to snap shut, releasing LDL again. In this state, it acts something like a folding cellphone that can be snapped together to shield its buttons; the receptor similarly doubles over on itself to cover its binding domains. The receptor is then effectively closed, and the LDL is free to be taken apart elsewhere for use by the cell. The receptor recycles back to the cell surface, ready for new duty.

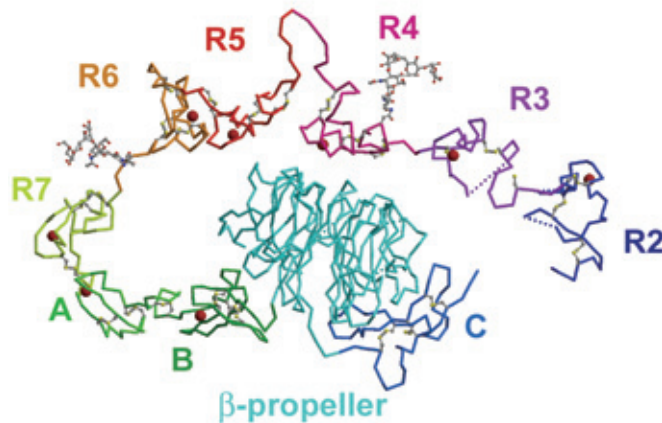
“You want receptors to bind tightly, but then let go of their cargo at the right moment. The system around the LDL receptor does this by decreasing pH and generating an internal competition for the ligand-binding site,” Deisenhofer says. “I would expect to see this kind of action in a lot of receptors that transport molecules into the cell.”

Deisenhofer’s new findings relate to some of his earlier research on how statin drugs work to reduce cholesterol. As reported earlier in *Science* (May 11, 2001), Deisenhofer and colleagues used x-ray crystallography to show how six different statin compounds—such as atorvastatin and simvastatin—inhibit the liver enzyme HMG-CoA reductase, which catalyzes a key step in cholesterol production.

The scientists are now working to understand how alterations in the amino acid sequence in critical regions of the LDL receptor might cause familial hypercholesterolemia, a common inherited disease marked by high cholesterol levels, atherosclerosis, and increased risk of a heart attack early in life. **II**

Like a String of Beads

Schematic of the extracellular domain of the LDL receptor as it is seen at acidic pH in the crystal structure. The ligand binding region (R2–R7) is folded back over the rest of the molecule, and the epitopes R4 and R5, important sites for binding LDL, are buried against the β -propeller, rendering them inaccessible.



A NEW ANGLE ON CHOLESTEROL

In the fight against heart disease, researchers have their eye on a protein called LXR that may be a master switch for cholesterol. By Robert Kuska

Marketed under brand names such as Lipitor and Zocor, the statin drugs help many patients keep their cholesterol in check. But this standard treatment doesn’t work for everyone.

“People are still dying [from heart disease] even though they’re on cholesterol-lowering drugs,” says Helen H. Hobbs, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas and director of the Dallas Heart Disease Prevention Project, a study of heart disease in a population of 6,000 individuals. “Until Americans decide to give up their hamburgers and French fries, we have to figure out how to interrupt this disease in other ways.”

Searching for new approaches, scientists are looking at the problem of cholesterol from a different angle. Rather than focusing on therapies that target production of cholesterol, which is essentially what statins do, researchers are concentrating on the way the body gets rid of surplus cholesterol—and getting promising results.

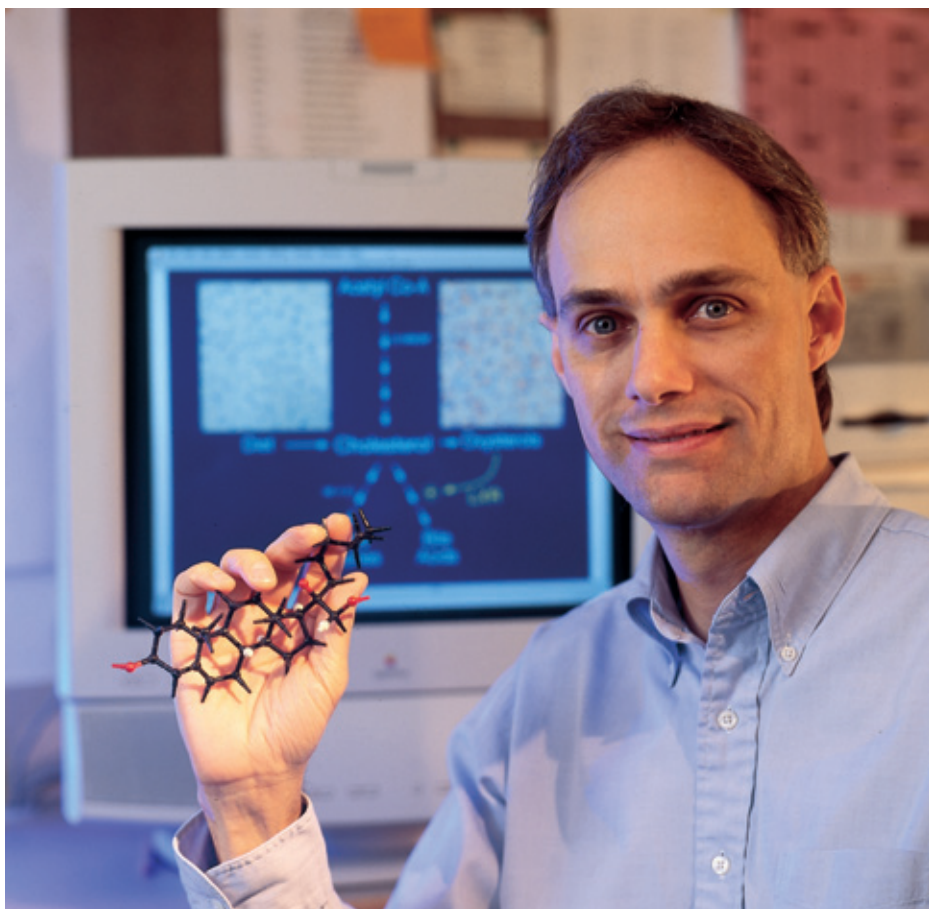
An important breakthrough came not long ago when researchers identified a unique protein, called LXR (for liver X receptor), which appears to serve as a cellular “master switch” for removing excess cholesterol. A logical next step: Try to develop synthetic compounds that might control this intriguing switch—and in turn control the leading culprit in heart disease.

CHOLESTEROL’S BAD RAP

Despite its rotten reputation in the doctor’s office, cholesterol is essential to human health. Cells insert cholesterol into their membranes to help control which substances enter and leave the cells. Cholesterol also is the sole precursor of steroid hormones such as testosterone and estradiol, certain vitamins, and the bile acids in the liver.

How much cholesterol we need varies from person to person. Researchers estimate that a 155-pound male has about 100 grams of cholesterol in his body. Each day, the liver and other tissues produce 600 to 900 milligrams of cholesterol to meet the body’s routine daily demands for the substance. We get into trouble when we have an excess of this tough-to-metabolize substance—which the body cannot entirely get rid of.

The liver, being the only organ equipped to break down cholesterol efficiently, serves as a centralized treatment plant. However, it’s



DOUG HANDEL

David Mangelsdorf studies which genes and proteins are involved in the cholesterol-transport process.

limited by the countervailing effects of two distinct types of lipoprotein, the form in which cholesterol is carried in the blood. High-density lipoprotein (HDL), the so-called “good” cholesterol, picks up globules of cholesterol that cells have pumped to their outer membranes as if they have dropped off a bag of garbage at the curb. The HDLs then transport the cholesterol to the liver, where it is broken down in the bile. But low-density lipoprotein (LDL), or “bad” cholesterol, functions in an opposite fashion, bringing cholesterol from the liver into the body’s cells.

The effects are not surprising. “Studies tell us that high levels of LDL and low levels of HDL are associated with atherosclerosis,” says Hobbs. “Conversely, we know that if you lower plasma levels of LDL you have a profound [preventive] effect on the development of heart disease and a profound effect on reducing the incidence of coronary events in people with established heart disease.”

REVERSE TRANSPORT

Scientists have long hoped to define precisely how cells pump the excess cholesterol to their membranes—a process that’s commonly called “reverse transport”—for pickup and disposal. That knowledge could pave the way for the design of drugs to manipulate the process. (Statins,

the current drugs of choice, largely target an enzyme involved in the body’s own production of cholesterol, a completely different cellular process than reverse transport.) About four years ago, a team of scientists provided an important first clue to the reverse-transport puzzle when they showed that a protein called ABCA1 transfers cholesterol from the cell membrane to HDL. This raised an obvious next question: What activates ABCA1?

The answer came soon thereafter from the laboratory of David J. Mangelsdorf, an HHMI investigator at the University of Texas Southwestern Medical Center at Dallas. As a postdoc, Mangelsdorf had cloned the genes of a dozen previously unknown protein receptors in the cell nucleus. Because the functions of these assumed hormone-binding proteins were unknown, scientists referred to them as “orphan nuclear receptors.” Later, he discovered that one of these orphans—LXR—bound cholesterol metabolites.

Given that most nuclear receptors, once saturated with their activating ligand, signal for the transcription of specific genes, Mangelsdorf followed up with a series of experiments to determine which genes LXR mobilizes; the hope was that this information would lead to genes and proteins directly involved in the cholesterol-transport process. He observed that when mice were given small molecules that stimulated the LXR receptor, their production of ABCA1 was markedly increased. Although the details have yet to be fully worked out, this result suggests that an LXR-stimulating drug would increase cholesterol transport and boost HDL levels, both beneficial effects.

Actually, as Mangelsdorf and colleagues would show, this was just the tip of the LXR iceberg. In these same mice, the scientists noted a prominent decrease in cholesterol absorption. The explanation was that LXR signals ABCA1 to pump out the cholesterol in the intestine, thereby preventing it from being absorbed into the blood. Though this hypothesis has not been confirmed, it suggests that LXR has the potential to limit the absorption of dietary cholesterol, another greatly desired effect.

In the liver, the data are equally striking. Mangelsdorf reported that LXR activates a gene whose protein plays a key role in the synthesis of bile acids, meaning a ramping up of cholesterol degradation—another positive effect. There is also evidence that LXR activates other genes that transport cholesterol into the bile.

Then there is LXR’s beneficial effect on macrophages, a type of white blood cell that ingests foreign material. Peter Tontonoz, an HHMI investigator at the University of California, Los Angeles, and colleagues have published a series of articles showing that LXR aids in the efflux of cholesterol from macrophages, presumably by activating ABCA1 proteins. This discovery has major implications because cholesterol-laden macrophages contribute to the formation of foam cells, a fundamental component of artery-clogging plaques. In addition, the group has



TOM KELLER

Peter Tontonoz thinks LXR-modulating drugs and statins could be combined in the fight against cholesterol.

reported that LXR induces macrophages to produce apolipoprotein E, a plasma protein that has a protective effect against atherosclerosis.

COMPLEX INTERPLAY

These and additional studies show that LXR plays a key role in reverse transport and in ancillary processes as well. They also suggest that the pharmaceutical company that corners the LXR market could stand to benefit handsomely.

There seems to be a big obstacle, however. Mangelsdorf has shown that LXR wears a second metabolic hat: It regulates the metabolism of triglycerides, the body's store of fatty acids that constitute a major energy source. This has raised fears that although LXR-targeted synthetic drugs might flush out the extra cholesterol, they might also cause blood fatty acid levels to spike, which can lead to health problems such as pancreatitis.

"Perhaps the LXR linkage of the two is a way to coordinate the balance between triglycerides and cholesterol," speculates Ronald M. Evans, an HHMI investigator at The Salk Institute for Biological Studies. "They are both packaged and trafficked around in the LDL and VLDL [very low density lipoprotein] particles, which constitute the major delivery systems for lipids throughout the body. So it may not be inappropriate, biologically, for them to be linked. Interestingly, internalization of these particles and their lipids is controlled by another set of nuclear receptors, termed PPARs" (see article on page 17). A mentor to both Mangelsdorf and Tontonoz, Evans is the father of "reverse endocrinology," an approach that led to Mangelsdorf's discovery of LXR.

Many scientists say they are cautiously optimistic that the problem of balance will one day be solved. But first they have to fill in the blanks in the molecular chain of events that activate LXR. "Cholesterol homeostasis does not occur in a vacuum," says Evans. "The physical links between cholesterol and triglycerides in lipoprotein particles reflect a more global coordination in which other nuclear receptors, along with LXR, control the ebb and flow of metabolic energy. Understanding the logic of this molecular circuit is key."

Referring to the many cellular signals, or pathways, activated by LXR, Mangelsdorf notes that "a signaling network resembles the arms of an octopus, all branching out in various directions but all feeding back in some way to one central thing. The question is: What is that central thing? If answered, simplicity will emerge from the complexity."

Meanwhile, researchers have learned the identity of the target genes that LXRs regulate and that are responsible for the undesired increase in fatty acids and triglycerides. According to Mangelsdorf, the ability of LXRs to target both "good" genes (that lower cholesterol) and "bad" genes (that raise fatty acids and triglycerides) may be exploited by the development of new drugs that selectively activate

expression of only the good genes.

He notes, moreover, that the idea of selectively activating a receptor is not without precedent. Cancer researchers have done it for years with tamoxifen, which selectively binds to the estrogen receptor to treat or prevent breast cancer. "The fact that tamoxifen, raloxifene, and estrogen have different activities in different tissues is important," Mangelsdorf says. "It suggests that by giving a selective modulator that's tissue-specific, it might be possible to dial out the bad effects and keep the good effects."

Tontonoz adds that because LXR-modulating drugs and statins target different cellular pathways, they conceivably could be combined to provide a one-two punch to clear out excess cholesterol. He acknowledges, though, that work has only just begun on LXR and the application of its cholesterol-lowering capabilities. "There's a complex interplay between diet, environment, immune responses, and genetic predispositions that will require a lot of investigation," Tontonoz says. "Right now, we're at the point where we can look at individual pathways, but the bigger picture is how all of the pieces fit together—why different people exposed to those same pathways have different responses."

Using the metaphor that the body is a factory, it could be said that the statin drugs fight cholesterol on the assembly line, while LXR helps take it out with the trash. If researchers can ultimately craft therapeutics that adapt LXR's approach, this different angle on controlling cholesterol may be a key to solving the pervasive problem of heart disease. **■**

SEARCHING FOR THE FAT SWITCH

Can we control fat by controlling metabolism?

By Karen F. Schmidt

Like our earliest ancestors, we metabolize food to prepare for both feast and famine. The body stores energy as fat when food is plentiful and burns it later when food is scarce. But we're only starting to learn the details of how that transition occurs.

A key molecular switch appears to regulate the critical balance between fat storage and burning—a finding that could lead to treatments for obesity, cardiovascular disease, and diabetes. Ronald

M. Evans, an HHMI investigator at The Salk Institute for Biological Studies in California, has identified this “fat switch” as PPAR δ , a nuclear receptor in the family known as peroxisome-proliferator-activated receptors. (Nuclear receptors are activated by hormonal fats—fat derivatives that regulate cellular function much as hormones do—which trigger them to turn on specific genes.) Two other PPARs were already known to play a role in fat metabolism. Indeed, drugs activating these two receptors are used to treat hyperlipidemia—a condition characterized by elevated levels of lipids, including cholesterol, in the bloodstream—and type 2 diabetes. But the function of PPAR δ remained a mystery, largely because no ligand had been found to activate it.

Evans's group got around this problem by creating transgenic mice with PPAR δ receptors that were permanently activated in fat cells. In the April 18, 2003, issue of *Cell*, his team describes its experiments and findings on PPAR δ over seven years. Remarkably, young mice with activated PPAR δ weighed 20 percent less than normal counterparts on the same diet, and by 1 year of age, they were 35 percent lighter. Moreover, the trans-

genic mice were protected against weight gain on a high-calorie, high-fat diet, while normal mice became obese. In addition, in 2001, Glaxo researchers discovered a chemical to activate PPAR δ . Evans's group gave the chemical to obese mice that ate all the time because of a defect in leptin, an appetite regulator, and the mice lost weight. “When you activate this fat switch, you increase fat burning,” concludes Evans.

These results raise the possibility of a new approach to obesity treatment—“losing weight by controlling metabolism, rather than behavior,” says Evans—which has pharmaceutical companies such as Lilly and GlaxoSmithKline eagerly searching for drugs to activate PPAR δ . “The exciting thing about nuclear receptors such as PPARs is that we know ligands for them can be drugs and there's never a problem with absorption in the body,” notes Mitchell A. Lazar, director of the Penn Diabetes Center at the University of Pennsylvania School of Medicine.

Lazar, who also studies PPARs, nevertheless cautions that there are many remaining questions about PPAR δ . For example, does tilting the balance toward fat burning raise body temperature? Does PPAR δ , which is ubiquitous throughout the body, play different roles in different tissues? Would a ligand for PPAR δ activate the other PPARs as well?

As he pursues answers to these and related questions, Evans is optimistic about ultimate success—though with possible qualifications. “I am convinced that we can activate fat metabolism in people,” he says. “The issue now is whether it will show a favorable safety profile over a long period of time.” **H**



Investigator Ronald Evans identified a key molecular switch that appears to regulate fat storage and burning.

MISHA GRAVENOR

CHOLESTEROL: A CENTURY OF RESEARCH

Nearly 100 years of research on atherosclerosis has taken us from merely recognizing the disease to understanding its cause and producing an effective therapeutic approach.

By Joseph L. Goldstein and Michael S. Brown

Cholesterol is essential for the functioning of all human organs, but it is nevertheless the cause of coronary heart disease—a condition that is responsible for more than one-third of all deaths in the Western world. It is in fact the number one killer in the United States and in other industrialized nations.

Over the course of nearly a century of investigation, researchers have developed four lines of evidence—experimental, genetic, epidemiologic, and therapeutic—that irrefutably established the causal connection between cholesterol-carrying low-density lipoprotein (LDL) and atherosclerosis. Building on that knowledge, scientists have been successful in developing an effective course of therapy—the statin drugs.

Few other major diseases have been subject to such intensive and ultimately fruitful research. Here, briefly, is how the history of cholesterol research unfolded.

THE EXPERIMENTAL EVIDENCE

The first hint that cholesterol was related to atherosclerosis goes back to 1910, when the German chemist Adolph Windaus reported that atherosclerotic plaques from aortas of human subjects contained 20- to 26-fold higher concentrations of cholesterol than did normal aortas. Three years later, the Russian pathologist Nikolai Anitschov fed pure cholesterol to rabbits, which produced marked hypercholesterolemia and severe atherosclerosis of the aorta. This was the first experimental production of atherosclerosis, and Anitschov's experiment has been repeated many thousands of times ever since in virtually every animal species from pigeons to humans.

Windaus and Anitschov studied aortic plaques rather than the coronary artery plaques that are responsible for heart attacks. Aortic plaques in humans had been noted by 19th-century pathologists, who believed that coronary artery plaques were rare; they also believed that when a thrombotic occlusion of an atherosclerotic plaque did occur in a coronary artery, it was always a fatal event. This view persisted until 1918, when the syndrome of nonfatal myocardial infarction was recognized by James Herrick, a Chicago clinician, who made the first use of the electrocardiograph to diagnose heart attacks in patients who presented with

Joseph L. Goldstein and Michael S. Brown shared the 1985 Nobel Prize in Physiology or Medicine for their research on the mechanism underlying cholesterol metabolism.

crushing chest pain. Herrick provided the first clear demonstration that thrombosis of a coronary artery was not always fatal and that coronary heart disease was responsible for the acute chest pain that had been previously ascribed to all kinds of causes, from indigestion to apoplexy.

THE GENETIC EVIDENCE

The genetic connection between cholesterol and heart attacks was first made in 1938 by Norwegian clinician Carl Müller, who described several large families in which high blood-cholesterol levels and premature heart attacks together were an inherited trait. The genetic understanding of this syndrome, which came to be known as familial hypercholesterolemia (FH), was greatly advanced 25 years later by the astute observations of Lebanese clinician Avedis K. Khachadurian, who delineated two clinically distinct forms of FH in inbred families—the *homozygous* form, in which affected individuals manifest severe hypercholesterolemia at birth (with plasma cholesterol levels of about 800 mg/dl) and heart attacks that occur as early as 5 years of age, and the *heterozygous* form, characterized by levels in the 300- to 400-mg/dl range and premature heart attacks that occur typically between 35 and 60 years of age. The incidence of heart attacks in children with homozygous FH provided strong genetic evidence that hypercholesterolemia alone can produce atherosclerosis.

The mounting clinical interest in cholesterol led to an intense effort in the 1950s to determine the process by which cholesterol was synthesized in the body. Most of the crucial steps in this complex pathway, involving 30 enzymatic reactions, were worked out by four biochemists—Konrad E. Bloch, Feodor Lynen, John Cornforth, and George Popják—in a triumph of technical virtuosity that combined organic



Nobel Prize winners Joseph Goldstein (left) and Michael Brown provided the first molecular link between LDL cholesterol and atherosclerosis.

chemistry, enzymology, and one of the earliest uses of radioisotopes. The major outlines of this pathway were completed by 1960.

THE EPIDEMIOLOGIC EVIDENCE

The epidemiologic side of the cholesterol-coronary connection unfolded in 1955 when John Gofman, a biophysicist at the University of California at Berkeley, used the newly developed ultracentrifuge to separate plasma lipoproteins by flotation. Gofman found not only that heart attacks correlated with elevated levels of cholesterol but also that the cholesterol was contained in one lipoprotein particle, LDL. Gofman also observed that heart attacks were less frequent when the blood contained elevated levels of another cholesterol-carrying lipoprotein, high-density lipoprotein (HDL).

The epidemiologic connection between blood cholesterol and coronary atherosclerosis was firmly established by a physiologist at the University of Minnesota, Ancel Keys, whose classic Seven Countries Study showed that the incidence of heart attacks in 15,000 middle-aged men followed for 10 years was linearly proportional to the blood level of cholesterol. Keys also found that the cholesterol level rose in proportion to the saturated-fat content of the diet. Men living in eastern Finland, where the mean cholesterol level was 260 mg/dl, had eight times more coronary deaths in a 10-year period than men living in a Japanese fishing village where the mean cholesterol level was 165 mg/dl. Men living in Italy, where the mean cholesterol level (200 mg/dl) was intermediate between that of Japan and eastern Finland, had three times fewer coronary deaths than in Finland and three times more than in Japan. Subsequent studies showed that this wide range of cholesterol levels resulted from a correspondingly wide variation of LDL levels in the blood.

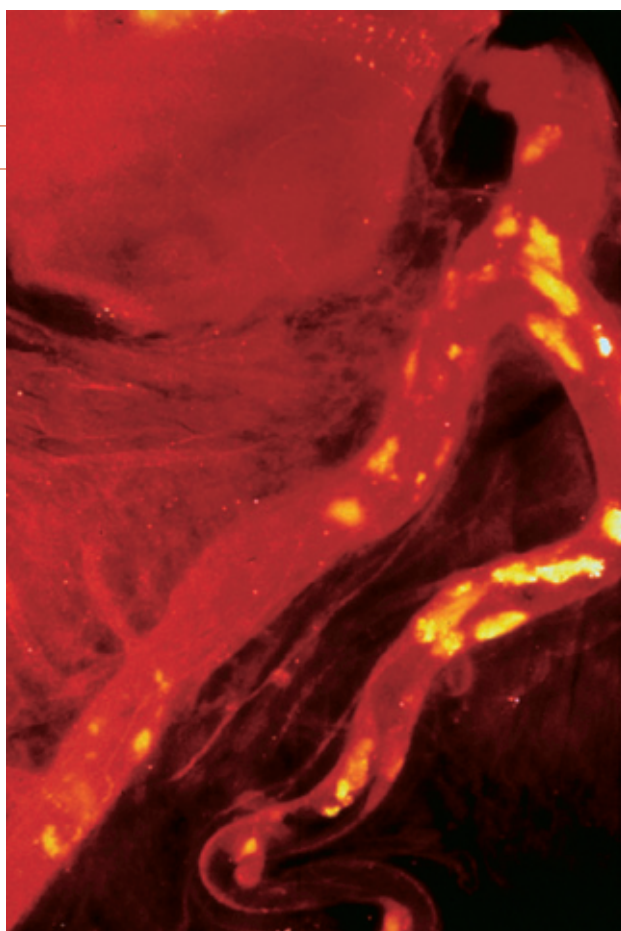
In 1974, the authors of this article discovered that the level of LDL in blood is controlled by the activity of a cell-surface protein we called the LDL receptor, which binds LDL and delivers it to cells where the lipoprotein is degraded; the cholesterol is then used there for metabolic and structural purposes. We also found that FH is caused by genetic defects in this receptor that ultimately block removal of LDL from the blood. These studies provided the first molecular link between LDL cholesterol and atherosclerosis.

THE THERAPEUTIC EVIDENCE

In 1976 Akira Endo, a Japanese scientist at the Sankyo Co. Ltd., discovered a fungal metabolite that could block cholesterol synthesis by inhibiting the enzyme HMG-CoA reductase. This discovery led to the first

Clogged Arteries

Coronary arteries of a 50-year-old man who died of a myocardial infarction. They have been opened lengthwise to reveal the yellow deposits of cholesterol that constitute the hallmark of atherosclerotic plaques. Under ordinary conditions, the cholesterol-carrying particles (called low-density lipoproteins, or LDL) in the bloodstream infiltrate the tissue of the artery wall, where they are partially metabolized and some become oxidized. These oxidized lipids start an inflammatory reaction that leads to cytokine release, tissue damage, and scarring. The result over many decades is the buildup of atherosclerotic plaques that narrow the channel of the coronary artery. The process is accelerated by risk factors such as smoking, hypertension, and diabetes. For reasons apparently related to inflammation, some plaques become unstable. They ultimately rupture, leading to the formation of a blood clot—a thrombosis—that blocks the blood flow in the artery, producing myocardial infarction.



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statin. In collaboration with Endo, we showed 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 Merck & Co. Inc. to develop these drugs for therapeutic use, and in 1986 the FDA approved the first statin for human consumption. In 2003, more than 25 million people worldwide will take statins.

In 1994 the landmark “4S” (Scandinavian Simvastatin Survival Study) was completed. Sponsored by Merck and conducted by physicians in four Scandinavian countries, it showed for the first time that statins, by lowering LDL levels, could not only prevent myocardial infarctions but could generally prolong life. In several large multicenter trials, involving nearly 50,000 people followed for three to five years, treatment with statins lowered LDL levels by 25–35 percent and reduced the frequency of heart attacks by 25–30 percent—even in high-risk people who had “normal” LDL levels at entry. In these individuals, the high risk came from other predisposing conditions such as chronic smoking, hypertension, or diabetes. They benefited from statin therapy presumably because the predisposing conditions render the coronary arteries prone to inflammation at LDL levels considered “normal” in Western societies.

After nearly 100 years of exploration, we now have four lines of persuasive evidence—experimental, genetic, epidemiologic, and therapeutic—that implicate the cholesterol-carrying LDL particle as the primary cause of atherosclerosis. Very few, if any, chronic diseases of adults have ever been subjected to such intensive research, and in very few, if any, chronic diseases of adults has the cause been so convincingly demonstrated in so many ways. **H**