PERSPECTIVES: MOLECULAR MEDICINE

The Cholesterol Quartet

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Low density lipoprotein (LDL), the major cholesterol-carrying lipoprotein in human plasma, is the offending agent in coronary heart disease, which causes one-third of all deaths in the United States. Originally, LDL was implicated in heart disease through epidemiologic and genetic observations in humans and animal models (*1*). Its involvement has been confirmed by the recent and repeated demonstration that LDL-lowering drugs, called statins, reduce heart attacks and prolong life (*2*).

What determines the amount of LDL in plasma, and why do so many people have enough LDL to cause heart attacks? In medicine, common problems are often solved by studies of uncommon genetic diseases. In the case of LDL, answers have emerged from unraveling the aberrant genes underlying four disorders that elevate plasma LDL and cause premature heart attacks. The final two molecular defects of this quartet have been described within the last 6 months (*3*–*5*), and one of them is reported on page 1394 of this week's *Science* (*5*). Remarkably, all four defects raise the amount of plasma LDL by impairing the activity of hepatic LDL receptors (LDLRs), which normally clear LDL from plasma.

LDLs are composed of a collection of spherical particles with an average diameter of 22 nanometers. The average LDL particle contains a hydrophobic core of 1500 molecules of cholesteryl ester surrounded by a polar coat composed primarily of phospholipids and a 513-kilodalton protein called apolipoprotein B-100 (apoB-100) (*6*). LDLs are secreted from the liver as larger precursor particles (average diameter, 55 nanometers) called very low-density lipoproteins (VLDLs), whose cores contain triglycerides as well as cholesteryl esters (see the figure). VLDL-triglycerides are removed in the capillaries of muscle and adipose tissue, and the particles then undergo exchange reactions with other lipoproteins. The net effect is to reduce the size of VLDLs, restricting the core lipids to cholesteryl esters, and removing all proteins except apoB-100, thereby producing LDLs (*6*).

LDLs circulate in human plasma with a mean life-span of 2.5 days. The particles are removed from plasma when apoB-100 binds to LDLRs on the surface of liver cells (*7*). The bound LDL is internalized by receptor-mediated endocytosis in coated pits, the internalized LDLs are degraded in lysosomes, and the liberated cholesterol enters the cellular cholesterol pool (see the figure). The number of LDLRs expressed by liver cells is controlled by negative-feedback regulation (*7*). When the concentration of cholesterol in hepatocytes rises, transcription of the *LDLR* gene is suppressed, and LDL is retained in plasma. In contrast, when hepatic cholesterol levels fall, *LDLR* gene transcription is induced, LDL is taken up more rapidly, and the amount of LDL in plasma falls. This mechanism explains most of the LDL-lowering action of statins, which deplete hepatic cholesterol by blocking an enzyme in the cholesterol synthetic pathway (*7*).

Hepatic pools of cholesterol are influenced by many variables, including absorption of dietary cholesterol from the intestine and excretion of hepatic cholesterol into bile. Recent genetic studies (*3*, *4*) have shown that both absorption and excretion of cholesterol are controlled by a pair of adenosine triphosphate–binding cassette (ABC) transporters, called ABCG5 and ABCG8, that are believed to act in concert to pump cholesterol out of cells (see the figure). In the intestine, ABCG5 and ABCG8 re-excrete cholesterol that has entered gut epithelial cells from the gut lumen, thereby limiting cholesterol absorption. In hepatocytes, ABCG5 and ABCG8 secrete cholesterol into bile (*3*, *4*).

The quartet of monogenic disorders that cause LDL to accumulate in plasma include familial hypercholesterolemia (FH), first described in 1938 (see the table) (*8*). The primary defect in this disorder, a deficit of LDLRs, was discovered in 1973 (*7*). Recently, more than 600 mutations in the *LDLR* gene have been identified in patients with FH (*7*). FH patients who are heterozygous for the LDLR defect produce one-half the normal number of LDLRs, and on average they have a 2.5-fold elevation in the number of LDL particles in plasma. The incidence of heterozygous FH is at least 1 in 500 people in all populations so far studied, making this disorder one of the most common monogenic diseases. It is the most frequent cause of premature coronary heart disease resulting from a single gene defect, and accounts for 5% of heart attacks in patients 60 years of age or less. The rare FH homozygotes (1 in 1 million) have LDL levels that are 6- to 10-fold above normal. These individuals have virulent coronary atherosclerosis, often dying from heart attacks in childhood (*7*).

The second of these genetic disorders, familial ligand-defective apoB-100 (FDB), was distinguished from FH in 1986 (*9*). This disease is caused by mutations in the gene encoding apoB-100, which reduce the protein's ability to bind to LDLRs, thereby retarding plasma clearance of LDLs (*6*). Heterozygous FDB is common in Europeans (1 per 1000). The syndrome is similar to heterozygous FH, although not as severe. The rare FDB homozygotes have higher levels of LDL than the FDB heterozygotes (see the table).

FOUR MONOGENIC DISEASES THAT ELEVATE PLASMA LDL AND CAUSE HEART ATTACKS

Human disease	Prevalence in population	Typical plasma LDL-cholesterol level*	Mutant gene product	Mechanism for decreased LDL receptor function
		mg/dl		
Familial hypercholesterolemia			LDL receptor	Nonfunctional receptors
Heterozygous	1 per 500†	300		
Homozygous	1 per million†	650		
Familial ligand defective apoB-100			apoB-100	Decreased binding of LDL to receptors
Heterozygous	1 per 1000‡	270		
Homozygous	<1 per million‡	320		
Autosomal recessive hypercholesterolemia	<1 per 10 million§	470	ARH	? Altered location of receptors in liver
Sitosterolemia	<1 per 10 million	100 to 600 depending on diet	ABCG5 and/or ABCG8	Suppression of receptor gene transcription

*Typical adult plasma LDL-cholesterol is 120 mg/dl in the United States (*6*). †All populations. ‡Primarily in individuals of European descent. §Primarily in individuals of Italian and Middle Eastern descent.

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The third member of the Cholesterol Quartet, the rare autosomal recessive disorder sitosterolemia, was delineated in 1973 (*10*). Affected individuals accumulate a unique form of LDL that contains abundant plant sterols (sitosterol, campesterol, and stigmasterol), in addition to cholesterol. Sterol accumulation results from two abnormalities: the increased absorption of dietary cholesterol and plant sterols, and the reduced excretion of these sterols into bile (*11*). Together, these defects lead to a buildup of cholesterol in the liver, which suppresses transcription of the *LDLR* gene, causing LDL to accumulate in plasma. Recently, two groups led by Hobbs (*3*) and Patel (*4*) have used positional cloning to trace the sitosterolemia defect to loss-of-function mutations in genes encoding the two ABC transporters, ABCG5 and ABCG8. The authors postulate that these two transporters may pair up as heterodimers and that a deficiency of either partner may abolish ABC transporter activity.

The Hobbs group (*5*) now reports the molecular defect in the fourth member of the Cholesterol Quartet, autosomal recessive hypercholesterolemia (ARH). This syndrome, whose severity approaches that of homozygous FH, is distinguished from FH on genetic grounds: Obligate heterozygous parents of ARH patients have normal plasma LDL, unlike the heterozygous parents of FH homozygotes, who have 2.5-fold elevations in plasma LDL (*12*).

Young adults and children with ARH exhibit severe hypercholesterolemia, premature coronary heart disease, and massive deposits of LDL-derived cholesterol in the skin. Isotopic tracer studies show that these individuals, like FH homozygotes, have a severe defect in the removal of LDL from plasma (*13*). Yet, LDLR activity in cultured fibroblasts from ARH patients was almost normal, and no mutations were found in the *LDLR* gene of these patients.

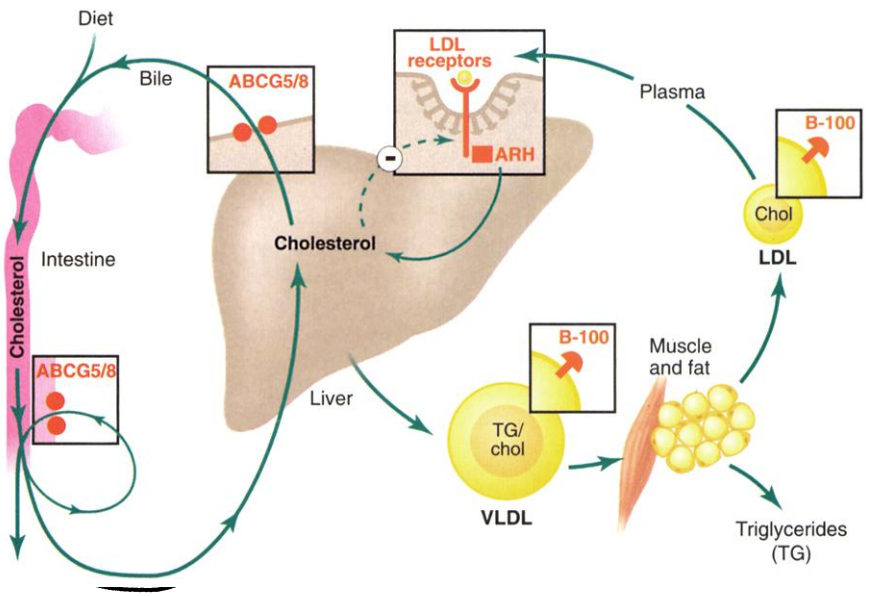
Hobbs and colleagues have now traced the molecular defect to the gene encoding a previously undescribed cytosolic protein, ARH, which contains a phosphotyrosine-binding (PTB) domain (*5*). Other PTB domains bind to the cytoplasmic tails of cell surface receptors that contain an NPXY motif (*14*). This tetrapeptide was originally identified in the LDLR, where it is essential for incorporation of the receptor into clathrin-coated pits during endocytosis (*15*). Although firm biochemical data are not yet available, it seems reasonable to speculate that ARH binds to the NPXY motif of LDLRs. This binding might facilitate the entry of receptors into coated pits, or it could participate in receptor cycling from the cell surface to endosomes and back.

In patients with ARH, why is LDLR activity disrupted in the liver in vivo but not in fibroblasts in vitro? There are two possible answers. First, in fibroblasts ARH activity might be replaced by another PTB-containing protein. Second, ARH may be required only in polarized epithelial cells, such as hepatocytes, in which the receptor must cycle selectively between the cytoplasm and only one surface of the cell.

The defect in each member of the Cholesterol Quartet elevates LDL by decreasing LDLR activity in the liver, directly or indirectly. This is consistent with data in experimental animals showing that it is impossible to raise plasma LDL levels markedly unless LDLR activity has been disrupted by genetic defects (*16*) or by down-regulation through ingestion of a high-cholesterol diet (*17*). All four diseases lead to premature heart attacks at an age that is roughly inversely proportional to the amount of LDL in plasma. This correlation establishes a direct causal link between plasma LDL and coronary atherosclerosis.

How does elevated plasma LDL produce the complex lesions of atherosclerosis (*18*), with their hallmark features of inflammation, necrosis, cellular proliferation, and lipid deposition? The answer may lie in the unsaturated fatty acids of the cholesteryl esters and phospholipids of which LDL is composed. One of these, arachidonic acid, is the precursor of inflammatory prostaglandins. Within the artery wall other unsaturated fatty acids of LDL can undergo oxidation to generate toxic aldehydes and epoxides that induce the production of inflammatory cytokines (*19*). Thus, although the principal job of LDL is to transport cholesterol, and although its metabolism is regulated in response to cellular demands for cholesterol, the pathological consequence of its accumulation may be traced to the localized deposition of its fatty acids at sites of damage in artery walls.

Most of the hypercholesterolemia in the population is attributable not to single-gene defects, but rather to high-fat diets compounded by poorly defined susceptibility genes. Findings from the quartet of monogenic cholesterol diseases emphasize the importance of LDLRs and suggest that certain susceptibility genes may dictate the degree of suppression of LDLRs in response to different amounts of dietary cholesterol. Two types of transcription factors have been shown to mediate this suppression. One is a family of membrane-bound transcription factors, sterol regulatory element binding proteins (SREBPs), that are liberated from cell membranes in response to cholesterol depletion. After their release, they enter the nucleus and activate genes involved in the synthesis of cholesterol and fatty acids and in the uptake of cholesterol by LDLRs (*20*). The second transcription factor is the liver X receptor (LXR), a nuclear receptor that is activated by certain oxygenated derivatives of cholesterol (*21*). Upon activation LXR increases production of ABCG5 and ABCG8 (*3*), and also of one of the SREBPs (SREBP-1c) (*22*, *23*).



Not all in the diet. The quartet of hypercholesterolemias. In these four monogenic diseases, the inability of defective LDLRs to remove cholesterol-carrying LDLs from plasma causes an increase in plasma LDL and the deposition of atherosclerotic fatty plaques in arteries, leading to heart disease. The mutant gene products of the cholesterol quartet are shown in red; also depicted are the points where their normal counterparts act in the cholesterol pathway. [Not shown are the intermediate density lipoproteins (IDL), which are highly atherogenic intermediates in the conversion of VLDL to LDL (*6*, *7*).]

Recent progress in understanding the Cholesterol Quartet of monogenic diseases and exposure of the mechanisms underlying cholesterol regulation create a sense of optimism that new and more powerful ways may soon be found to raise LDLR activity and to lower plasma LDL, with the goal of preventing atherosclerosis and coronary artery disease.

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PERSPECTIVES: MICROBIOLOGY

How Bacteria Respire Minerals

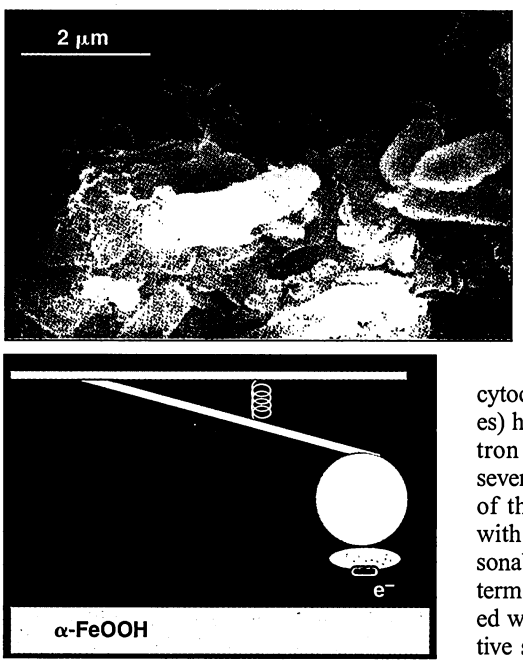
Dianne K. Newman

Ever since the late 1600s, when Anthony van Leeuwenhoek observed samples from the scurf of his teeth under the microscope, microbiologists have known that solid surfaces are a welcome home for bacteria (*1*). Leave a sterile glass slide in any water body, and within a few days it will become entirely covered by microorganisms. By attaching to and transforming minerals, microbes play an important role in the weathering of rocks near the surface and perhaps even at depth (*2*). Yet the mechanisms underlying these transformations are not well understood.

One of the most intriguing examples of microbial interactions with rocks is the use of minerals for respiration. How bacteria do this has remained a mystery, in part because we have not been able to observe what goes on at the molecular level. High-resolution studies of the microbe-mineral interface have been done with techniques such as transmission electron microscopy, but the activity of the organisms is destroyed during sample preparation. On page 1360 of this issue, Lower *et al.* (*3*) use a modified atomic force microscope (AFM) that allows us to observe bacteria while they respire minerals.

The thought of respiring a mineral may seem suffocating, but bacteria have been doing it for billions of years. Respiration is the process of harvesting energy by transferring electrons from an electron donor to an electron acceptor. Typically, this transfer occurs down a respiratory chain embedded in the cell membrane: Specific molecules hand electrons from one end to the other, thereby generating a potential across the membrane that can be harnessed to do work (such as storing chemical energy in the form of adenosine triphosphate) (*4*).

For respiration to succeed, a terminal electron acceptor, such as oxygen, must exist to receive the electrons. Before the evolution of oxygen in the atmosphere, microorganisms had to respire with alternative electron acceptors. Most terminal electron acceptors that bacteria use for respiration, such as oxygen, nitrate, and sulfate, are soluble. They can thus make their way to the cell to receive electrons from the membrane-bound molecules of the respiratory chain. But this route is not open to microbes that use solids like hematite (α-Fe_2O_3) and goethite (α-FeOOH) as electron acceptors because these minerals are effectively insoluble under environmentally relevant conditions (*5*). Simple dissolution and diffusion of ferric iron to the cell therefore cannot be the answer (ferric iron is the constituent of the mineral that receives electrons). The bacteria must have other strategies to transfer electrons to minerals during respiration. The question is, what are they?



Making contact. (**Top**) Environmental scanning electron micrograph (ESEM) of the bacterium *Shewanella oneidensis* on the surface of an iron mineral. (**Bottom**) The outer-membrane proteins that this organism uses to contact the mineral during respiration may now be identified with AFM.

Several mechanisms have been proposed. First, bacteria may solubilize the minerals by producing chelating molecules. The addition of synthetic chelators has been shown to stimulate microbial electron transfer to iron minerals, but to date, no evidence has been found that bacteria use this mechanism in respiration (*6*, *7*). Second, they may use soluble shuttles, such as organic compounds with quinone moieties, to transfer electrons from the cell to the mineral (*8*). These shuttles may be exogenous substances or may be produced by the organisms themselves (*9*). The third, and possibly dominant, mechanism is that they directly transfer electrons from the cell surface to the mineral. A variety of biomolecules (including cytochromes, quinones, and dehydrogenases) have been identified as part of this electron transfer pathway (*10*–*12*). Of these, several are located on the outer membrane of the cell and presumably make contact with the mineral directly. This seems reasonable, given that the initial rate and long-term extent of electron transfer are correlated with the mineral's surface area and reactive site concentration (*13*, *14*). Yet the nature of the electron transfer event has remained obscure.

Lower *et al.* (*3*) present the first quantitative measurements of the nanoscale interactions between *Shewanella oneidensis*, a well-studied mineral-respiring microorganism (*15*), and two different minerals. They

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