from defects in the production of progenitor red blood cells, which are made after hematopoiesis shifts from the yolk sac to the liver at stage E12 of embryonic development. Expression of ADAR1 increases in the liver at this stage, suggesting that there is a critical transcript in liver tissue that requires efficient editing at this time. Apparently, the activities of ADAR1 and ADAR2 do not overlap sufficiently to enable one enzyme to take over the responsibilities of the other (defective) enzyme.

There are probably other transcripts that are edited by ADAR1, but chimeric mouse embryos do not survive long enough to allow other phenotypes to emerge. In their next set of experiments, Wang *et al.* induced teratomas (embryonic tumors) in nude mice by injecting them with either wild-type or ADAR1 heterozygous ES cells. The teratomas were composed principally of neural tissues in which the amount of editing of known transcripts could be measured. In stark contrast to heterozygous ADAR2 newborn mice that had no decrease in RNA editing (*6*), the teratomas formed in nude mice from heterozygous ADAR1 ES cells showed decreased editing of glutamate receptor (GluR-B R/G and GluR5 Q/R) and serotonin receptor transcripts (but no decrease in editing of the GluR-B Q/R site). Thus, ADAR1 editing is very dependent on whether two copies or only one copy of the gene are expressed. This sensitivity to gene dosage could arise if, for example, lower levels of ADAR1 result in splicing of the transcript (that is, removal of noncoding introns) before editing, which would remove the essential ECS element.

In the heterozygous ES cells, a truncated protein (that has only the dsRNA binding domains) derived from the mutated copy of the *ADAR1* gene cannot be detected by Western blot analysis. Even if this truncated protein interfered with editing, this would not negate the possibility that ADAR1 is sensitive to gene dosage. However, clarification of the possible interference in the editing process by the truncated protein will have to await generation of ADAR1-deficient mice in which the entire gene has been deleted.

Fruit flies that completely lack any ADAR activity have been generated—this is much simpler to achieve in flies than in mice because flies have only one *ADAR* gene that is expressed exclusively in the CNS (*7*). The phenotype of the mutant flies shows parallels with that of the ADAR2 heterozygous mice. The fruit flies are viable and have a normal life-span, but they walk poorly, are unable to fly, and suffer progressive brain neurodegeneration. Editing is completely eliminated at known target sites in RNA transcripts that encode ion channels. The fly phenotype is consistent with the notion that RNA editing is important primarily in the fly CNS.

The generation of mice deficient in either of the two RNA-editing genes establishes beyond a doubt the importance of RNA editing. But what is the purpose of this nuclear editing process? Does it "correct mistakes" in the genome, as appears to be the case for the Q/R site in GluR-B transcripts, or does it produce a diversity of protein products that do slightly different jobs? Only with the discovery of additional edited transcripts and testing of the functions of the edited and unedited forms will this question be answered. The evidence that editing is important and more widespread than previously thought seems particularly appropriate this year as we busily interpret the human genome sequence. The lesson that was learned from the mitochondrial and chloroplast genomes almost 20 years ago—that there is more to predicting the coding sequences of genes than simply identifying exons—should be remembered as we behold the final genome sequence of human nuclear DNA.

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An Absorbing Study of Cholesterol

Hooman Allayee, Bryan A. Laffitte, Aldons J. Lusis

Sterols are essential constituents of the membranes of animal and plant cells. Although structurally very similar, the sterols synthesized by animals and plants differ in the nature of their side chains; for example, the plant sterol sitosterol has the same ring structure as cholesterol (an important animal sterol) but differs in the side chain by an additional ethyl group (see the figure). Plant sterols taken in by animals in their food cannot be used by mammalian cells and are not normally absorbed. The cellular machinery that allows selective absorption of animal sterols but not those of plants is defective in a rare, recessive disorder called sitosterolemia. Patients with this disease accumulate large amounts of plant sterols in most tissues, have elevated plasma cholesterol, and develop coronary heart disease at an early age (*1*, *2*).

On page 1771 of this issue, Berge *et al.* (*3*) report the identification of mutations in two genes in sitosterolemia patients. The genes are new members of the ATP-binding cassette (ABC) family of transporters. Last year, another member of the gene family, *ABCA1*, was found to be mutated in Tangier disease. This disorder is characterized by defective efflux of cholesterol from cells, which results in an inability to make high density lipoproteins (HDLs) (*4*). That finding proved to be a treasure trove for the field of lipid metabolism because it identified the transporter responsible for removing excess cholesterol from cells. The Berge *et al.* study seems likely to yield similar riches. In addition to clarifying how plant sterols are excluded from animal cells, their results address a longstanding mystery in the lipid metabolism field: How is absorption of cholesterol regulated given that cholesterol appears to be passively taken up by intestinal cells?

The intestine is a major barrier to the uptake of plant sterols: Less than 5% of dietary plant sterols are normally absorbed compared to 40% of the available cholesterol. Plant sterols also appear to be preferentially removed from the body by excretion into bile. Berge *et al.* provide strong evidence that ABC transporter proteins pump plant sterols out of intestinal cells into the gut lumen, and out of liver cells into the bile duct. Although the transporters preferentially pump out plant

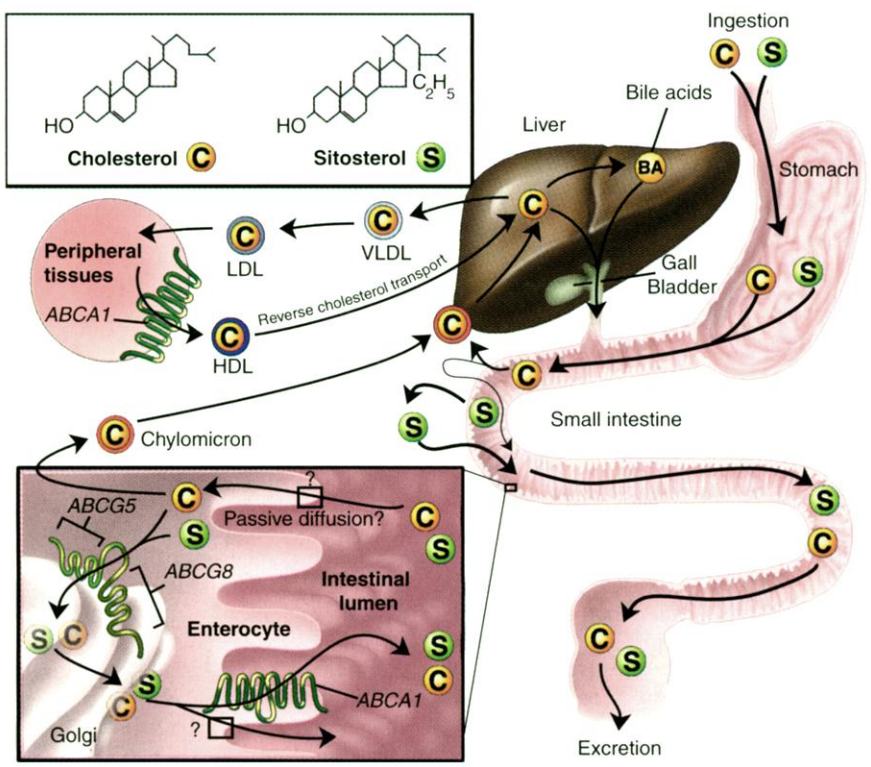
H. Allayee and A. J. Lusis are in the Department of Medicine and the Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles (UCLA) School of Medicine, Los Angeles, CA 90095, USA. B. A. Laffitte is at the Howard Hughes Medical Institute, UCLA School of Medicine, Los Angeles, CA 90095, USA. E-mail: hallayee@ucla.edu; jlusis@mednet.ucla.edu

sterols, they also appear to transport cholesterol out of cells because the absorption of cholesterol is dramatically increased in sitosterolemia. Together with other recent studies, the following picture of sterol absorption emerges: Dietary sterols passively enter intestinal cells and a proportion of them are actively pumped back into the gut lumen by the ABC transporter proteins (see the figure). The control of cholesterol absorption is important for maintaining the correct levels of cholesterol in the blood and tissues and is achieved by modulating the expression of the ABC transporters.

In a positional candidate gene approach, the authors used a combination of mapping information and functional data to identify the genes that are defective in sitosterolemia. Importantly, the sitosterolemia disease locus had been mapped to human chromosome 2p21 by Patel and colleagues 2 years ago (*5*). The authors also reasoned that the sitosterolemia genes might be regulated by the liver X receptor (LXR) as this nuclear hormone receptor has been implicated in cholesterol homeostasis. Thus, the investigators used DNA microarrays to search for messenger RNA transcripts whose expression increased in response to a synthetic ligand for LXR. One such transcript encoded an ABC transporter (ABCG5), the gene for which mapped to chromosome 2p21. This gene happened to be adjacent to a second ABC transporter gene (*ABCG8*) that was switched on and off at the same time as *ABCG5*. Both genes were expressed in cells of the liver and intestine, and both were induced by a high-cholesterol diet fed to mice. Analysis of mutations provided strong evidence for the involvement of *ABCG8* in four out of nine sitosterolemia families. The evidence that *ABCG5* may be mutated in sitosterolemia patients is suggestive but not conclusive. The coordinated regulation of the two genes implies that the proteins they encode may unite to form an active transporter; thus, mutations in either gene could give rise to sitosterolemia. Had *ABCG5* and *ABCG8* not been located so close together in the genome, their genetic mapping and subsequent identification could have been much more difficult.

Members of the ABC superfamily of transporters are integral membrane proteins that couple the energy derived from the hydrolysis of adenosine triphosphate to the transport of various substrates across cellular membranes. Active transporters consist of either a single polypeptide with two ABC domains and 12 transmembrane spanning helices (full transporter) or two polypeptides each with one ABC domain and six transmembrane spanning helices (half-transporters). Most half-transporters are located within intracellular membranes, whereas full transporters are usually found in the plasma membrane. Defective ABC transporters have been implicated in a variety of diseases including cystic fibrosis (ABCC7 transports chloride ions), adrenoleukodystrophy (ABCD1 transports very long chain fatty acids), and Tangier disease (ABCA1). Although ABCA1 is required for reverse cholesterol transport from peripheral tissues back to the liver for excretion, it may also be important in the regulation of intestinal cholesterol absorption (*6*, *7*).

Nuclear receptors such as LXR are transcription factors that remain inactive in the absence of ligand. The binding of ligand to the receptor induces a conformational change that turns on the transcriptional activity of the receptor. The natural ligands for LXR are oxysterols, hydroxylated forms of cholesterol (*8*). The biological role of LXR is to detect high concentrations of cholesterol and to respond by increasing the expression of genes that limit its accumulation. In response to cholesterol, LXR (i) increases the synthesis of bile acids in the liver by inducing expression of cholesterol 7α-hydroxylase (*9*), the rate-limiting enzyme of bile acid synthesis; (ii) increases cholesterol efflux from peripheral tissues (stimulating reverse cholesterol transport) by inducing *ABCA1* and *ABCG1* expression (*7*, *10*); and (iii) inhibits cholesterol absorption in the intestine by activating ABCA1 and perhaps ABCG5 and ABCG8 (see the figure). The Berge *et al.* study suggests that ABCG5 and ABCG8 are regulated by LXR as their expression increased in response to an LXR ligand. However, final proof awaits the identification of the transcriptional regulatory sequences of these genes and examination of gene expression in mice deficient in LXR. The ability of LXR ligands to block cholesterol absorption should be examined in animals lacking either *ABCG5* or *ABCG8* to determine the relative importance of



Sterol metabolism in the body. The human diet includes sterols from both animal and plant sources, such as cholesterol (C) and sitosterol (S), respectively. (**Bottom box**) Ingested sterols enter gut epithelial cells (enterocytes) through an unknown route, perhaps by passive diffusion. The ABCG5 and ABCG8 proteins unite to form heterodimeric transporters in the plasma membrane and intracellular membranes (such as those of the Golgi) of enterocytes. These active transporters preferentially transport plant sterols (but also some cholesterol) out of enterocytes into the gut lumen, thereby decreasing sterol absorption. ABCA1 may participate in this process. Consequently, only a small percentage of the plant sterols that enter enterocytes are absorbed by the body. (**Main figure**) Absorbed sterols are packaged into chylomicrons for transport to the liver. In the liver, cholesterol and plant sterols may be (i) transported to peripheral tissues by lipoproteins (very low density lipoproteins, VLDLs; and low density lipoproteins, LDLs); (ii) converted to bile acids (BA); or (iii) transported out of the liver into the bile for excretion. In peripheral tissues, the ABCA1 transporter delivers cholesterol to high density lipoproteins (HDLs) for transport back to the liver.

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these transporters in this process. The possible regulation of *ABCG5* and *ABCG8* (and other known target genes) by LXR suggests that LXR may be an excellent target for developing drugs to decrease serum cholesterol.

Although a full understanding of the regulation of cholesterol absorption in the intestine will require more work, the identification of genes mutated in sitosterolemia provides important insights into this process. Four ABC proteins have now been implicated in the regulation of cholesterol homeostasis. Future studies will need to determine the structural and functional properties of these proteins and whether they act in concert or in separate pathways of cholesterol metabolism.

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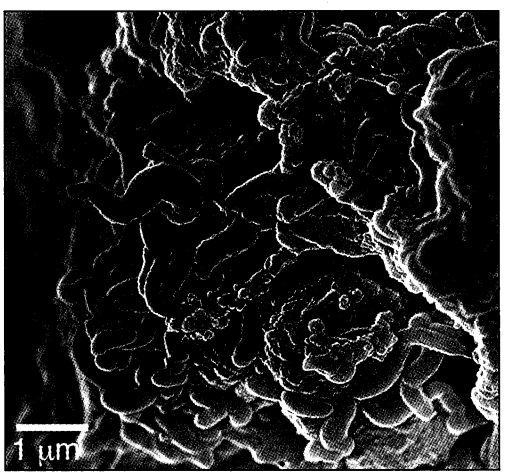
PERSPECTIVES: BIOGEOCHEMISTRY

Sulfate Reducers—Dominant Players in a Low-Oxygen World?

Crisogono Vasconcelos and Judith A. McKenzie

Sulfate-reducing bacteria may be one of the oldest forms of life on Earth. They can be traced back billions of years in the geologic rock record to the Early Archean (3900 to 2900 million years ago), when oxygen concentrations in Earth's atmosphere were low. Ancient sulfate-reducing bacteria left their first mark on their environment in pyrite minerals (FeS_2) as old as 3400 million years (*1*). Today, these microorganisms are widespread in marine and terrestrial aquatic environments. Their ability to adapt to extreme physical and chemical conditions enables them to play an important role in global geochemical cycles (*2*), but their role in the formation of ore deposits has remained controversial. Strong support for such a role is now provided by Labrenz *et al.* on page 1744 of this issue (*3*), who have discovered sulfate-reducing bacteria that can tolerate low levels of oxygen and can precipitate zinc sulfide minerals.



The role of microbes in ore deposit formation. Scanning electron microscopy image of vibrio-shaped sulfate-reducing bacteria that are intimately associated with dolomite crystals produced in a culture experiment conducted at room temperature (*9*). The bacteria are 3 to 5 μm in length. The sample was prepared by chemical fixation and critical-point drying.

Throughout geologic history, the sulfur cycle was strongly correlated with the carbon cycle because the two cycles are intrinsically connected through microbial metabolism. The sulfur cycle thus constitutes one of the best examples of the impact exerted by living organisms on geochemical cycles (*4*). Dissimilatory sulfate-reducing bacteria use sulfate mainly as an electron acceptor (without assimilating sulfur) in the anaerobic oxidation of inorganic or organic substrates such as $H_2 + CO_2$, lactate, acetate, and propionate. As a consequence of their metabolism, large amounts of reduced sulfide ions are produced and accumulated in their natural habitats. The sulfide ions combine with available metal ions to form insoluble products, most commonly FeS_2, leading to the production and transformation of natural mineral deposits (*5*).

The importance of this major biogeochemical process is evident in the fluctuations in the sulfur isotope content of marine sulfate during the Phanerozoic, that is, during the past 570 million years. Bacterial sulfate reduction controls the isotopic composition of marine sulfate, driving the $^{34}S/^{32}S$ isotopic ratio, expressed as $\delta^{34}S$, to more positive values during periods with increased deposition of carbonaceous sediments. For example, in the early Phanerozoic, the $\delta^{34}S$ value of marine sulfate (as recorded in marine deposits) increases by about 15 per mil, indicating a period with increased microbial sulfate reduction within anoxic (oxygen-deficient) sediments called black shales (*6*). Intervals with increased activity of sulfate-reducing bacteria, and associated increased impact on geochemical cycles, can thus be deciphered from the geologic record.

The role played by sulfate-reducing bacteria in natural processes is undoubtedly very important under anoxic or oxygen-free conditions. Anyone who has ever stepped into black stinky mud and smelled the H_2S released has experienced firsthand the activity of sulfate-reducing bacteria. These microbes undoubtedly play an important role in the early diagenetic alteration of sediments rich in organic matter. Their importance, however, for other geologic phenomena, such as the formation of sulfide ore deposits, remains controversial, not least because of their air intolerance. But this is not the case for the bacteria discovered by Labrenz *et al.* (*3*), which can tolerate low levels of oxygen (they are aerotolerant). These bacteria may be important players in geochemical cycling and in the concentration of metals into sediment-hosted sulfide ore deposits.

Using scuba divers to gain access to a flooded mine tunneled into a Pb-Zn ore deposit, Labrenz *et al.* were able to retrieve samples containing microbial biofilms. Applying microscale techniques, they demonstrate that the collected aerotolerant sulfate-reducing bacteria assemblage has the ability to form a pure precipitate of sphalerite (ZnS). The bacteria can scavenge zinc from waters with very low zinc concentrations (less than 1 part per million), essentially stripping the water of the metal. This observation has interesting implications for understanding how economic ZnS deposits may have formed. And it has even more exciting implications for possible biotechnological applications. Imagine if these aerotolerant sulfate-reducing bacteria could be used to remove trace metals, such as Zn, As, or Se, from contaminated drinking water! Because

The authors are at the Geological Institute, Eidgenössische Technische Hochschule-Zentrum, 8092 Zürich, Switzerland. E-mail: chris.vasconcelos@erdw.ethz.ch

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