

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

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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

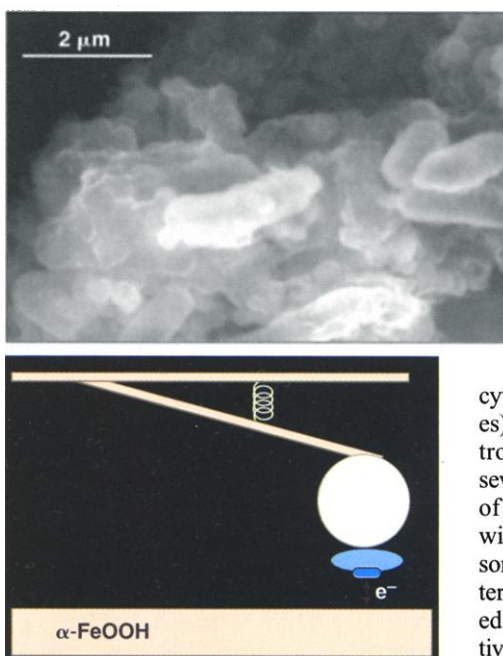
(such as storing chemical energy in the form of adenosine triphosphate) (4).

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 mem-

brane-bound molecules of the respiratory chain. But this route is not open to microbes that use solids like hematite ( $\alpha\text{-Fe}_2\text{O}_3$ ) and goethite ( $\alpha\text{-FeOOH}$ ) 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?

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



**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.

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accomplish this by linking fully functional cells to a small bead at the end of a cantilevered AFM tip and then measuring the forces exerted on the tip in response to its deflection or attraction to an oriented mineral crystal. The cantilever measurement is directly translated into an interactive force measurement by its alteration of the known spring constant. Using this method, Lower *et al.* measure the approach and retraction forces between an individual cell of *S. oneidensis* and goethite ( $\alpha$ -FeOOH) or diaspoire ( $\alpha$ -AlOOH). Although the minerals have the same crystal structure, goethite is used by *S. oneidensis* as a terminal electron acceptor, whereas diaspoire is not. This is because the Fe(III) in goethite can receive an electron but Al(III) in diaspoire cannot.

The affinity of *S. oneidensis* for goethite is strongest under those conditions for which electron transfer from the bacterium to the mineral is expected, that is, in the absence of oxygen. Similar affinities are not observed for diaspoire. On the basis of specific signatures in the force curves, Lower *et al.* argue that a 150-kD protein in the outer membrane of the cell specifically interacts with the goethite surface to facilitate electron transfer. This protein, along with others in the outer membrane of *S. oneidensis*, was previously identified as a putative electron carrier to iron minerals

(16). This result is exciting because it opens up the possibility of using nanomechanical measurements to test biochemical and mineralogical hypotheses about what controls mineral respiration.

By combining nanoscale force measurements with molecular genetics and mineralogy, it should soon be possible to find out which components of the electron transfer pathway in the cell are most important for direct electron transfer to minerals. This could be done by knocking out genes thought to encode outer-membrane proteins involved in electron transfer and comparing the interactive forces between the mutant and a mineral to those between the wild type and the same mineral. If substantial differences were measured, this would be compelling evidence for that particular protein's role in direct electron transfer to the mineral surface. As we learn more about how physical force measurements relate to electron transfer, it may be possible to use this technique to quantitate electron transfer reactions directly.

Once we have identified the components of the electron transfer system, the next challenge will be to determine how the relevant proteins work and how they evolved. Are they similar to other electron transfer proteins that participate in different respiratory metabolisms? Which residues in the proteins are critical to electron transfer? Are the pro-

teins used by one species more efficient than those used by another, and can this be correlated with their environmental niche? What structural properties make minerals good electron acceptors? We are far from knowing the answers to these questions, but Lower *et al.*'s work provides us with an exciting new technique with which to approach them.

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#### PERSPECTIVES: CELL BIOLOGY

## A TIP About Rabs

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Eukaryotic cells are filled with membrane-bound compartments, such as the endoplasmic reticulum (ER) and Golgi apparatus, that form a transport network for newly synthesized proteins. In the exocytic pathway, cargo proteins destined for secretion are inserted into the ER and are transported through the various cisternae of the Golgi. They are then sorted into secretory vesicles in the trans-Golgi network (TGN), which fuse with the plasma membrane, releasing their cargo at the cell surface. In the endocytic pathway, proteins in or at the surface of the plasma membrane are internalized into early endosomes, and then are transported in late endosomes to enzyme-filled sacs called lysosomes, where they are degraded.

Like traffic cops at key intersections, a family of small proteins termed the Rab

guanosine triphosphatases (GTPases) regulate the sorting and transport of cargo proteins. These molecular switches ensure the specific and efficient targeting of vesicles that move cargo between various cellular compartments (1, 2). In addition, Rabs may be required for the formation and movement of transport vesicles, the remodeling of vesicle membranes, the coupling of individual transport steps, and the coordination of protein transport with other cellular processes (3). On page 1373 of this issue, Carroll *et al.* shed light on how Rab GTPases carry out their many tasks with help from the effector proteins that they recruit (4). First, they show that Rabs may recruit effector proteins for loading protein cargo into budding vesicles. Second, they suggest that Rabs enhance interactions between effectors and effector-binding proteins, implying that Rabs are not only involved in effector recruitment. Finally, they propose that Rabs determine compartment specificity, because each Rab appears to be localized to

just one cellular compartment and recruits a unique set of effector proteins only to that compartment.

Transport vesicles—containing both soluble and transmembrane cargo proteins—bud from donor cellular compartments (such as the ER), then fuse with acceptor compartments (such as the early Golgi). After depositing their protein cargo at the plasma membrane, the vesicle membranes are then recycled back to the donor compartment. Sorting of cargo proteins into the correct budding vesicles is important because each donor compartment produces several types of vesicle, and each type has its own specific components. Most compartments are donors for at least two types of vesicle: anterograde vesicles, which carry cargo forward in a pathway, and retrograde vesicles, which return membrane components back to the previous compartment (see the figure). Some compartments, such as the TGN, serve as a donor for more than one type of anterograde vesicle.

Protein cargo is sorted into budding vesicles by cargo receptors that span the compartment membrane (see the figure). These receptors interact with cargo on the inner (luminal) surface of the budding vesicle and with vesicle-forming

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