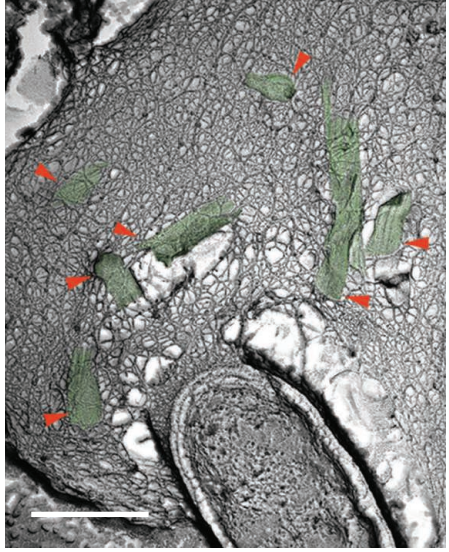
lize microtubules, thus allowing bacterial movement in the cytoplasm (*8*). The *Shigella* VirA virulence factor is secreted into the cytoplasm by a "syringe and needle" mechanism called a type III secretion system, designed to translocate virulence factors from the bacterial cytoplasm to the host cell cytoplasm. VirA creates a tunnel inside the host cell cytoplasm by breaking down the microtubule infrastructure (see the figure). This not only facilitates a bacterium's movement through the cytoplasm but also helps other bacteria move faster because they are able to follow the same path. Yoshida *et al.* show that VirA is a protease that specifically cleaves alpha-tubulin, a major component of microtubules. *Shigella* mutants lacking VirA not only are unable to move inside host cell cytoplasm but also are deficient in causing bacillary dysentery in a mouse model of infection. In addition, mutants that express an inactive form of VirA protease are also attenuated, demonstrating that specific enyzmatic activity acting on microtubules is absolutely required for *Shigella* virulence. A main question that remains to be answered is the half-life of the *Shigella*-induced tunnels, because microtubules are dynamic structures that regenerate quickly (as fast as 0.18 μm/min).

In other pathogenic microorganisms, proteases play an important role in virulence by acting on the host cell's actin filament rearrangements. Pathogenic *Yersinia* species evade the innate cellular immune response by injecting Yops (*Yersinia* outer proteins) into host cells through a type III secretion system. Among Yops, YopT inactivates RhoA, a host cell guanosine triphosphatase. By cleaving RhoA, it prevents the protein's function in regulating the formation of actin stress fibers (*9*).



Felling the infrastructure. Freeze-fracture electron micrograph image of a mammalian cell infected with *Shigella*. The bacterium breaks down microtubules (green, red arrowheads) during the course of infection (*1*). Scale bar, 0.2 μm.

Although little is known about early stages of the replication cycle of retroviruses, viral proteases appear to be critical. After entry into a cell's cytoplasm, wild-type foamy viruses as well as mutant forms that are defective in an aspartic protease travel along microtubules toward the microtubule-organizing center, the structure from which microtubules radiate. However, whereas the subsequent import of the wild-type retroviral genome and the nucleocapsid protein Gag into the host cell nucleus is observed, incoming nucleocapsids and genome from mutant viruses remain at the microtubule organizing center. This correlates with the detection, only for the wild-type virus, of a specific viral protease–dependent Gag cleavage product early after infection, demonstrating that cleavage of Gag protein by a viral protease, leading to viral core disassembly, is absolutely required for release from microtubules and productive infection (*10*).

Because we are now facing a lack of new antimicrobial molecules, especially of antibiotics, we need further insight into how a microorganism's effector molecules interact with host molecules to usurp host cell function. High-throughput screening chemical libraries has identified small, easy-to-make reagents that can alter or enhance biochemical properties of microbial enzymes, such as inhibitors of the CagA adenosine triphosphatase from *Helicobacter pylori* (*11*) or searching for virulence inhibitors against *Chlamydia pneumoniae* (*12*). Perhaps VirA is such a target.

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ECOLOGY

A Starving Majority Deep Beneath the Seafloor

The rocks and sediments beneath the seafloor may harbor most of Earth's microorganisms. Molecular approaches are beginning to provide clues regarding the energy sources fueling their metabolic activity.

Bo Barker Jørgensen and Steven D'Hondt

Over the past 20 years, scientific drilling into sediments and basaltic crust all over the world ocean has revealed the omnipresence of microscopic life deep beneath the seafloor. Diverse communities of prokaryotic cells have been discovered in sediments and rock reaching a subsurface depth of 1 km. Most of these microorganisms have no cultured or known relatives in the surface world and are still only characterized by the genetic code of their DNA. Recent studies (*1*–*4*) have shed light on the ways in which they differ from microorganisms in the surface world and on the energy sources that support life in this buried ecosystem.

About 20 years ago, R. John Parkes and Barry Cragg started to systematically enumerate microorganisms in deep cores (*5*). Much later, rigorous contamination tests performed on the drill ship (*6*) showed that the cells detected were indeed indigenous to the deep subsurface. The cell counts were used for a bold extrapolation to the global ocean floor. The astonishing conclusion was that this "unseen majority" of microorganisms accounts for 55 to 85% of Earth's prokaryotic biomass and about 30% of the total living biomass (*7*).

The first drilling expedition focused entirely on deep biosphere exploration was launched in 2002 by the Ocean Drilling Program (ODP, Leg 201) (*1*). The target was the eastern tropical Pacific, with sites ranging from the continental shelf to ocean depths of 5000 m. By drilling through the seafloor and—at open-ocean sites—down to

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the basaltic crust, sediments with ages up to 35 million years old could be sampled (*8*).

At all sites, prokaryotic cells (bacteria and archaea) were detected below the seafloor. Their numbers dropped from more than 10^8 cm^{-3} at the sediment surface to less than 10^6 cm^{-3} just above the ocean crust, with an average density much greater than in the ocean above. Occasional high cell numbers (up to 10^{10} cm^{-3}) coincided with sediment horizons in which more energy was available from counterdiffusing methane and sulfate (*9*).

These large population sizes remain the greatest mystery of the deep biosphere. Although marine sediments harbor Earth's largest reactive carbon pool, the organic matter becomes increasingly unreactive with depth and age and would seem to be practically inaccessible for microorganisms several million years after its burial. How, then, can there be sufficient energy for all these organisms to metabolize and grow?

The metabolic activity of the subsurface populations can be calculated by transport-reaction modeling of pore water solutes that are consumed or excreted by the microorganisms. For example, the mean metabolic activity per cell can be estimated by comparing the bacterial numbers and the predominant bacterial energy metabolism, such as sulfate respiration. For the eastern Pacific seabed, the mean sulfate respiration is 10^{-18} mol per cell per year (*8*, *10*). Because microbial cells must metabolize a certain minimum amount of substrate before they can double their cell size and divide into two daughter cells, their minimum doubling time can also be calculated. On the basis of this calculation, the mean generation time of deep subseafloor microorganisms is more than 1000 years.

This extremely slow growth cannot be reconciled with our understanding of the minimum energy requirements for life. All actively growing organisms must keep their enzymatic machinery going above a critical level to maintain vital cell functions such as replacement of degraded enzymes, repair of DNA damaged by high-energy radiation from natural radionuclides, and, presumably, the maintenance of an electrochemical gradient across the cell membrane (*11*).

A possible explanation for the low apparent rates of deep subsurface metabolism could be that most subseafloor cells are not active but dormant or even dead. However, when a highly sensitive fluorescence technique (catalyzed reporter deposition–fluorescence in situ hybridization or CARD-FISH) was used to detect the presence of ribosomes—a component of all living and active cells that is rapidly degraded upon their death—the results showed that many of the subsurface cells were alive (*12*).

The identity and physiological state of the inhabitants of the deep subsurface are now being elucidated with the powerful toolbox of DNA-, RNA-, and biomarker-based techniques. DNA encoding for 16*S* ribosomal RNA (a key gene for the phylogenetic identification of prokaryotic organisms) extracted from sediments provides thousands of genetic codes that reveal novel lineages of microbial life. Most of the genetic types belong to groups that have no cultured relatives; they are currently classified under provisional names such as "Japan Sea 1 Candidate Group" (bacteria) or "Marine Crenarchaeotic Group I" (archaea) or, even more exotic, "South African Gold Mine Euryarchaeotic Group" (*2*).

The physiology and potential function of these groups in the deep biosphere remain totally obscure, however, and their environment provides little clue as to their physiology. Future genomic research will reveal how 16*S* genes are coupled with key functional genes in the same genome, thereby relating identity and function. Quantitative analyses of intact polar lipids from cell membranes can also be used to identify the active populations of microorganisms (*3*). To date, however, approaches based on DNA, RNA, and biomarkers have provided contradictory conclusions about even the basic question of whether bacteria or archaea dominate the deep biosphere (*3*, *12*, *13*).

A crucial problem is the extremely low energy flux per cell in the deep subsurface. The search for additional energy sources has focused on molecular hydrogen (H_2), which is generated by chemical alterations in young basaltic crust along the mid-oceanic ridges (*14*). However, most of the seabed lies on old, crack-permeable crust, in which the potential oxidants for H_2 (such as oxygen or nitrate) seem to persist long enough to preclude a substantial H_2 supply (*1*). Another possible source of H_2 may come from the decay of natural radionuclides of potassium, thorium, or uranium in the sediments; energy released by this decay dissociates water molecules into free radicals and molecules such as H_2. Hence, this nuclear energy is not only destructive to microbial cells but may also support their metabolic activity.

Lin *et al*. (*15*) have estimated the radiolytic H_2 production rates for a sedimentary basin to be on the order of 10^{-8} nM H_2 s^{-1}. For comparison, sulfate reduction rates fueled by buried organic carbon in subsurface sediments of the eastern tropical Pacific Ocean correspond to H_2 consumption rates of 3 to 60 × 10^{-8} nM H_2 s^{-1} (*1*, *8*). These numbers suggest that water radiolysis

Fresh material from the deep subsurface. A fresh sediment core has just been retrieved from the deep subseafloor in the eastern tropical Pacific Ocean at a water depth of 5000 m during Leg 201 of the Ocean Drilling Program. The 10-m-long core is carried down from the drill deck for microbiological and geochemical sampling on board the drilling vessel JOIDES Resolution. The staff wear gas masks to avoid inhaling toxic hydrogen sulfide and explosive gas hydrate; both are metabolic products of the deep biosphere.

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could be the principal source of microbial energy in deep-sea sediments that are much more depleted in organic matter than the eastern tropical Pacific sites discussed here. Such sediments with extremely low organic carbon flux cover large regions of the ocean floor, for example, in the central North and South Pacific Ocean.

This potential energy source is particularly interesting in that it is independent of biomass production by photosynthesis. It does not even require an external oxidant. Water radiolysis produces not only H_2 but also oxidants such as H_2O_2 or O_2, which may be directly used for the energy-generating reoxidation of H_2. Although the rich communities at deep-sea hydrothermal vents also live on inorganic chemical energy, for example, from H_2 or H_2S, they depend on O_2 produced from photosynthesis. An extreme low-energy subsurface biosphere driven by radioactivity would be different from all other ecosystems on Earth: It could proceed on a planet without surface life and solar energy.

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GEOCHEMISTRY

How Melted Rock Migrates

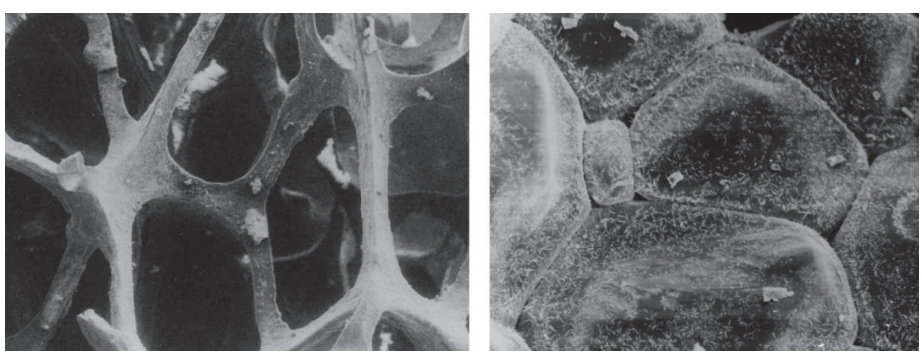
Magma flows through rock by different mechanisms than previously thought, which may cause a reevaluation of how data from Earth's mantle is interpreted.

Marian Holness

For most nongeologists, the idea of liquids moving through solid rock is a strange one. But liquids of one sort or another are thought to be ubiquitous in the Earth. There are the familiar hydrothermal fluids, dominated by water, which occur in the very shallow crust (the Old Faithful geyser in Yellowstone National Park in the United States is a dramatic example). But in the deeper parts of the Earth there are hydrous and carbon dioxide (CO_2) liquids formed by the heating of rocks as the minerals containing these molecules break down. At still higher temperatures, the rocks start to melt, generating a silicate liquid. The how and why of liquid flow through rocks is a very important problem in geology. This is because movement of liquid within the Earth is one of the primary ways that mass moves around and results in so-called geochemical differentiation. It was the movement of iron-rich liquids down to the center of the Earth that formed the core, for example. On page 970 of this issue, Schiano *et al.* (*1*) report new insights into flow mechanisms and the effects of fluid flow on the rock record .

Our understanding of what happens in the deep Earth is limited by our inability to get down there for a direct look. We are therefore reliant on three different sources of information: remote probing by geophysical methods such as seismic imaging; examining rock fragments that have been ripped off conduit walls and brought up to the surface by erupting lava; and laboratory experiments. All have their limitations. Geophysics can give hints as to what might be happening on a long length-scale, but can say very little about what may be happening on the grain scale. The fragmentary samples of the deep Earth that emerge with erupting lava flows have been separated from their original surroundings, and so the original spatial context is lost. And experiments are hampered by the difficulties of replicating the slow time scales typical of Earth processes within the time scale of a research grant. A further, perhaps not obvious, problem is that sometimes we do not carry out the right experiments. Researchers do not always know what to look for. We design experiments to investigate what we think might be there but sometimes, by chance or a fine instinct, we do something completely different and unexpectedly, serendipitously, happen upon a new and deeper understanding. The problem of silicate melt moving through its source rock provides an excellent example of this (*2*).

Driven by metallurgical insights, we thought for a decade or so that the distribution



Crystalline yin and yang. Porosity of texturally equilibrated polycrystals revealed in electron microscope images, showing the interplay of liquid and solid. This interconnected geometry of the melt phase was thought to dominate liquid flow in the mantle before Schiano *et al*. demonstrated that transcrystalline melt migration may also be important. (**Left**) A view of the pore structure in aluminum once the solid grains have been removed [reprinted from (*7*) with permission]. The elongated channels that form at three-grain junctions are evident (width of the image is 5 mm). (**Right**) Electron microscope image of quartz grains (with dimensions of about 100 μm) equilibrated with water at 6 kbar and 800°C, showing triangular ends of pores on three-grain junctions.

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