

SALLY BENSUSEN

Bacterial Gene Swapping in Nature

Genes travel between independent bacteria more often than once was assumed. Study of that process can help limit the risks of releasing genetically engineered microbes into the environment

by Robert V. Miller

In the early 1980s, as scientists were perfecting techniques for splicing foreign genes into bacteria, some investigators began suggesting ways to use the technology to benefit the environment. For instance, they proposed that genetically engineered bacteria might be deployed for such tasks as cleaning oil spills or protecting crops from predation and disease. But the enterprise, known as environmental biotechnology, soon came under fire.

Then, as now, the proposals elicited concern that the altered microbes might run amok or that their genes would hop unpredictably to other organisms—a phenomenon termed “horizontal” gene transfer (to distinguish it from the “vertical” transfer occurring between a parent and its offspring). Such activities, it was feared, might somehow irreparably harm the environment, animals or people. Some observers even issued dire warnings that the unnatural organisms would destroy the earth. No longer were tabloids worried about attacks by “killer tomatoes” from outer space; now the danger was home-grown—genetically altered microorgan-

isms that would eat the environment.

Unfortunately, at the time, biologists had little solid information on which to base responses. They knew almost nothing about the fate of genetically engineered microbes in nature and about the propensity of innate or introduced bacterial genes to migrate to new hosts. That paucity of data is now being remedied, thanks to unprecedented cooperation between genetic researchers and microbial ecologists, who study microorganisms in their normal habitats.

Today at least two strains of genetically engineered bacteria have gained approval (for agricultural use) by the U.S. Environmental Protection Agency, and dozens of field trials have been conducted. Those trials and more general investigations of gene transfer between bacteria in their natural habitats indicate that genetically manipulated bacteria themselves are unlikely to proliferate out of control. They tend to be fragile and to die out relatively quickly instead of persisting indefinitely; for that reason, their genes probably do not have much opportunity to spread.

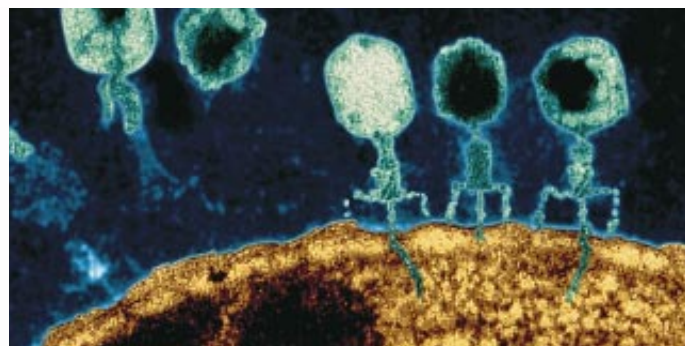
Yet under certain circumstances, the genes can potentially find their way into

other bacteria or even into other types of organisms. A key to the safe release of the microbes, then, is to identify the conditions that will encourage or deter specific bacteria from transferring their genes to other organisms—a challenge my laboratory at Oklahoma State University and others are pursuing vigorously. With such information in hand, biologists can select bacteria that will be least likely to exchange genes with other organisms in the particular site being “treated.” As an example, for release into a lake, biotechnologists might be able to choose a bacterial species that does not readily exchange its genes in water.

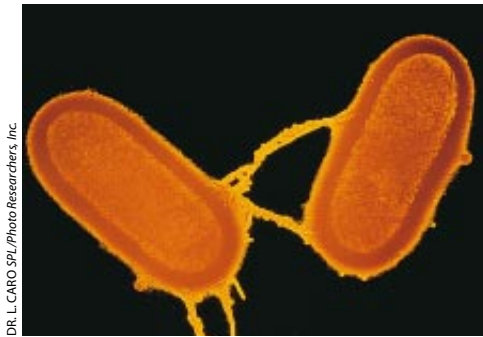
Scientists cannot yet compile an exacting list of which bacteria are best for any given application. The combined research has revealed a great deal, however, about the propensity for the three most common forms of horizontal gene transfer—transduction, conjugation and transformation—to occur in nature.

Those findings will be the focus of this article, but I should note that improved understanding of the conditions that facilitate horizontal gene transfer in bacteria has a bearing on another modern

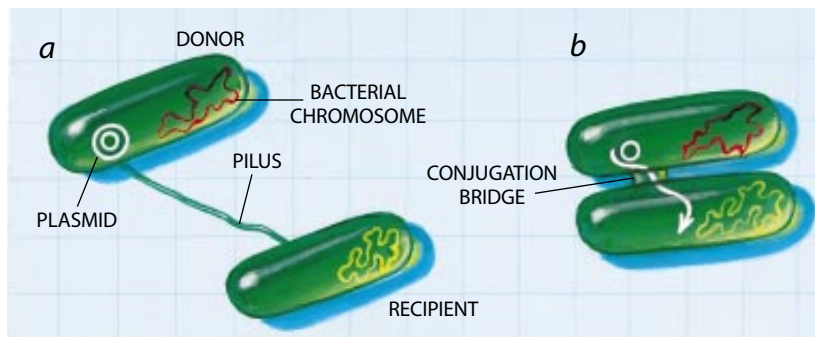
GENE TRANSFER by a process called transduction has been found to occur between bacteria living in the slimy layer, or epilithon, of neighboring, submerged rocks (left) and elsewhere. Transduction takes place after a bacteriophage (bacteria-infecting virus) attaches to a bacterial cell and injects its DNA into the cell (a and b at left; micrograph at right). Inside the bacterium, the injected DNA replicates (c), and the bacterium's chromosome breaks up (d). Normally, the viral DNA is packaged into new viral particles, which burst from the host (e). But during transduction, some particles instead take up bacterial DNA (containing bacterial genes) and deliver it to a second bacterium (f), which incorporates the hijacked DNA into its chromosome (g).



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BACTERIA CAN TRANSFER PLASMIDS, circles of DNA, through conjugation. In gram-negative bacteria, a donor cell extends one or more projections—pili—that attach to a recipient cell and pull the two bacteria together (*micrograph* and *a*). Next a bridge (essentially a pore) forms between the cells. Then one

strand of plasmid DNA passes into the recipient bacterium (*b*), and each single strand becomes double-stranded again (*c*). With the transfer complete, the bacteria separate (*d*). Conjugation in gram-positive bacteria (*not shown*) is similar, but the cells are drawn together by chemical signaling instead of by a pilus.

concern: rising resistance to antibiotics in disease-causing bacteria. It turns out that bacteria, which are single-cell organisms, often donate antibiotic-resistance genes to other species of bacteria in the human body. Understanding when and how this transfer occurs should help investigators develop strategies for blocking it.

On a more theoretical level, the discovery that horizontal gene transfer is fairly common in nature suggests that, over evolutionary time, the process could have contributed to the great genetic diversity now evident in bacteria. Some findings even indicate that genes have been exchanged among the three major groups of biological life: bacteria, eukaryotes (animals, plants, fungi and protozoa) and archaea (ancient microbes having some properties of both bacteria and eukaryotes). Current information suggests that gene transfer has occurred from bacteria to eukaryotes, from bacteria to archaea and especially from eukaryotes to bacteria. Horizontal gene exchange may thus have influenced the evolution of any number of life-forms.

A Fateful Fishing Trip

My own involvement in exploring horizontal gene transfer in nature dates back to the spring of 1976, when I was an assistant professor at the University of Tennessee at Knoxville. I was then strictly a geneticist interested in how living cells work. I did realize that certain bacteria could naturally transmit genes from one mature bacterial cell to another. From my perspective, though, horizontal gene transfer was of interest only insofar as it provided a practical way to introduce new genes,

and thus new traits, into cells being studied in the laboratory.

A fishing trip one Saturday afternoon with Gary Saylor, another young assistant professor at the university, suddenly altered my narrow view. As we sat in our boat, Saylor, a microbial ecologist, asked me whether I thought much genetic exchange was occurring among the bacteria in the lake below. I assumed bacterial cells would be dispersed in the water and would have relatively little contact with one another. I therefore guessed that the rate of gene transfer was low. When pressed, though, I had to admit that I was not well versed in the scientific papers on horizontal gene transfer in nature.

The following Monday, confident that the literature would be extensive, I strolled to the library in search of a more authoritative answer. Several hours later I emerged shocked and disappointed; virtually nothing was known.

Saylor, however, was elated. He had just developed a chamber for studying organisms living in freshwater. We could test the device and begin to fill a scientific gap by measuring the amount of transduction taking place at our fishing hole. Over the following fall and spring seasons, we carried out the first studies demonstrating that transduction can occur in freshwater.

When we published the results, in 1978, we were certain others would be as intrigued as we were and that our paper represented the first in a long series of research projects on bacterial gene exchange in nature. Yet at the time, no funding agencies shared our vision. By 1985, though, worry over the release of genetically engineered bacteria in the environment had changed all that. Hence, Saylor and I—and others—began play-

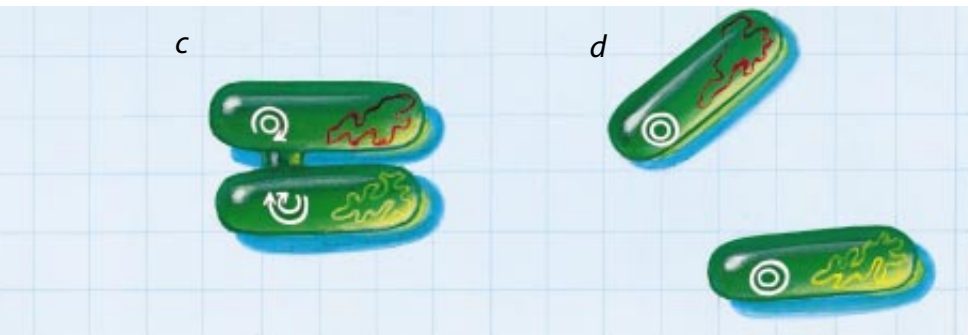
ing catch-up and exploring the potential for horizontal gene transfer to occur in a variety of settings.

Conjugation Is Confirmed

Conjugation was the first mechanism of gene transfer studied extensively as a way bacteria might disseminate genetic material in nonlaboratory arenas. It was identified in 1946, when Joshua Lederberg and Edward Tatum of Yale University found that the intestinal bacterium *Escherichia coli* uses a process resembling sex to exchange circular DNA elements that are now called plasmids.

Plasmids contain genes but are separate from the bacterial chromosome, which is larger and contains the genes needed for bacterial reproduction. (Chromosomes can sometimes be exchanged by conjugation as well but only in extremely rare circumstances.) Plasmids often carry genes that enhance the chances of survival in hostile circumstances. For example, in addition to including the genes needed for their own replication and transfer, they often harbor genes for proteins that enable bacteria to evade destruction by antibiotics, to degrade toxic compounds such as polychlorinated biphenyls (PCBs) or to transform mercury or other heavy metals into less noxious forms.

For historical reasons, microbiologists divide bacteria into gram-negative and gram-positive types, depending on whether the bacteria retain a particular stain. Laboratory work has shown that in gram-negative bacteria, which do not keep the stain, conjugation begins when a donor bacterium attaches an appendage called a pilus to a recipient bacterium that displays a receptor for the pilus; then the pilus retracts, drawing togeth-



er the donor and recipient. Generally, many donors extend pili at about the same time, and several donor cells can converge on a recipient at once. Consequently, extension of pili causes bacterial cells to aggregate into clusters. After aggregation occurs, bridges, or pores, form between donor and recipient cells, and plasmids pass through the bridges from the donors to the recipients.

Some pili can promote aggregation of bacterial cells in liquid and on solid surfaces; others can stimulate aggregation efficiently only on solid surfaces. Such differences imply that researchers who wanted to introduce a genetically engineered gram-negative bacterium into an aquatic environment might be wise to select a species having pili that induce aggregation on solid surfaces only.

Conjugation in gram-positive bacteria does not involve pili. In advance of conjugation a would-be recipient of new genes secretes substances that prompt potential donors to produce proteins, often called clumping factors, able to bring bacterial cells together. When the cells associate, they form the pores needed for DNA transfer. Hence, if investigators were to choose a recombinant gram-positive bacterium for release into an area containing other gram-positive bacteria, they might reduce the risk of gene transfer in the setting by altering the bacterium so that it was unable to manufacture any clumping factor.

In general, gram-negative and gram-positive bacteria, which can occur together in natural aquatic and terrestrial environments, exchange plasmids exclusively with members of their own group; many restrict exchange to their own species. But some “promiscuous” plasmids can transfer DNA between very unrelated species: between gram-negative and gram-positive bacteria and even from bacteria to yeast cells and plants. Obviously, then, bacteria that carry promiscuous plasmids would be poor choices for use outside the laboratory.

But does conjugation, in fact, occur frequently enough in nature to justify the precautions suggested by bench research? Since the advent of environmental biotechnology in the 1980s, researchers have demonstrated that conjugation does take place in many natural spheres, including in water, on land and in various plants and animals.

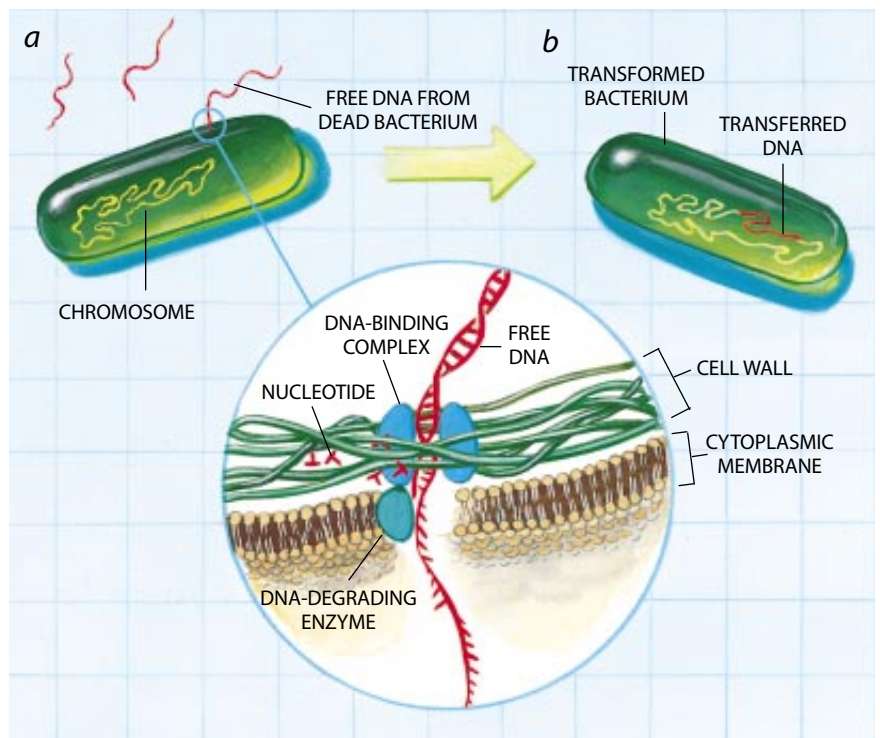
Key Conjugation Studies

Notably, in one series of studies, John C. Fry, Martin J. Day and their colleagues at the University of Wales demonstrated that gene transfer through conjugation can occur between

bacteria in freshwater environments. The researchers found that conjugation can enable a laboratory strain of *Pseudomonas aeruginosa* to pick up a plasmid that naturally provides resistance to mercury toxicity in bacteria that inhabit the polluted river Taft, near Cardiff, Wales. *P. aeruginosa* is a common soil and freshwater bacterium that can cause respiratory and urinary tract infections in humans whose immune defenses are weakened.

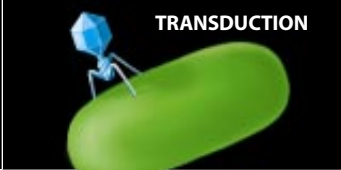





The investigators began by mutating a *P. aeruginosa* gene; this manipulation caused the gene to generate an abnormal version of the protein specified by the intact gene. The altered protein would later serve as a marker for keeping track of any bacterial cells put into the river. Having revised the *P. aeruginosa* gene, the team introduced the marked bacteria into the nutrient-rich, slimy layers, or epilithons, covering submerged stones in the river. (The stones were wrapped in a very fine filter material to prevent the bacteria from escaping.)

After 24 hours, the group retrieved the stones and examined the epilithons for marked *P. aeruginosa* cells that had received a mercury-resistance plasmid



BACTERIUM UNDERGOING TRANSFORMATION (a) picks up free DNA released from a dead bacterial cell. As DNA-binding complexes on the bacterial surface take up the DNA (*detail*), enzymes break down one strand into nucleotides; meanwhile the other strand may integrate into the bacterium’s chromosome (*b*). Transformation, shown here in a gram-positive bacterium, can also occur in gram-negative species, but the process is not especially common in either group.

Some Environments Where Horizontal Gene Transfer Has Been Documented

	 TRANSDUCTION	 CONJUGATION	 TRANSFORMATION
 TERRESTRIAL ENVIRONMENTS	Soil, plant surfaces	Soil, plant surfaces	Soil
 WATERY ENVIRONMENTS	Lakes, oceans, rivers, sewage in treatment facilities	Lakes, oceans, marine sediments, rivers, epilithon (slime layer) on river stones, sewage in treatment facilities	Marine sediments, rivers, epilithon on river stones
 INSIDE ORGANISMS	Shellfish, mice	Plants, insects, chickens, mice, humans	Plants, insects, mice

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from the naturally occurring *P. aeruginosa* population on the rocks. Only between one in 10,000 and one in a billion of the introduced *P. aeruginosa* had acquired a plasmid, but such transfer had undeniably taken place. The work also yielded the useful information that such factors as water temperature, acidity level and concentration of nutrients affect conjugation frequency.

In many studies, environmental factors have been found to modulate conjugation differently in nature than in the laboratory. For example, in the experiments of Fry, Day and their colleagues, conjugation occurred at six to 18 degrees Celsius—temperatures too low to support conjugation in laboratory strains of bacteria. These unexpected results mean that conjugation has to be studied in nature if researchers are to specify accurately the conditions that keep it to a minimum.

Based on the work of Fry, Day and others, scientists now conclude that although bacteria do use conjugation to transfer genetic information in many different environments, genetically altered plasmids probably pose little danger. Plasmids slow the growth rates of bacteria and are usually eliminated if there is no advantage to an organism in keeping them. For instance, if a genetically manipulated plasmid carrying the mercury-resistance trait found its way into an organism living outside of a mercury-polluted locale, the new host would soon get rid of the plasmid.

Further, plasmids are seldom, if ever, integrated into bacterial chromosomes. Thus, even if they travel to a new bacterial host, they do not become a stable part of that host's genome; chromosomes are invariably copied and distributed to new generations of bacterial cells whenever a parent cell reproduces itself, but plasmids are not reproduced consistently when cells divide. Nevertheless, to virtually eliminate the chance that a gene put into a genetically engineered bacterium will spread via conjugation, biotechnologists considering using recombinant bacteria in nature have opted to insert the genes into chromosomes instead of plasmids.

Transformation Risk Is Minimal

Although conjugation was the first mechanism of bacterial gene transfer to be studied extensively in the environment, it was not the earliest to be identified. The study of gene transfer among bacteria began in 1928, when British bacteriologist Frederick Griffith observed that nonvirulent pneumococcal bacteria became virulent when injected into mice along with dead virulent pneumococcus. Griffith concluded that the initially nonvirulent bacteria picked up a "transforming" agent from the dead virulent bacteria and thus became potent enough to kill the mice. That transforming agent is now known to be DNA that was released into the surrounding medium when the dead

bacteria fell apart. A gene is said to be successfully exchanged through transformation if it is taken in as part of a full plasmid or if a fragment of DNA containing the gene becomes integrated into a recipient's chromosome.

Natural transformation in both gram-negative and gram-positive bacteria requires that the freed DNA remain stable and that potential recipient cells become competent to take it up. That is, the recipients must display specialized surface proteins that bind to the DNA and internalize it.

Until recently, researchers assumed that transformation would not occur in most places, because free DNA would not be stable in soil or water. But studies by Michael Lorenz and Wilfried Wackernagel of the University of Oldenburg in Germany, Guenther Stotzky of New York University and others have demonstrated that free DNA can become stable by associating with soil components and that this DNA can be taken up by competent cells. Newer investigations indicate that plasmid DNA has at times been transferred by transformation in river water and in the epilithon on river stones. (I know of no observations, however, indicating that chromosomal genes have been transferred by transformation in aquatic or terrestrial environments.)

Still, few researchers believe gene exchange by transformation is likely to ensue readily if genetically engineered bacteria are put into the environment.

Natural transformation seems to occur only between cells of the same species, and relatively few bacterial species are capable of becoming competent for transformation; biotechnologists can avoid using these species for genetic engineering. Further, although dead bacteria may at times release large quantities of DNA that is absorbed by certain other bacteria, the DNA often is not assimilated as intact genes. John Paul of the University of South Florida and his colleagues have demonstrated that high concentrations of cell-free bacterial DNA can appear in estuarine waters after dawn, when many bacteria typically die and release their genetic material. Yet in laboratory experiments the researchers found that most released DNA salvaged by living bacteria is broken down promptly into its constituent parts for use in the synthesis of new DNA; the genes contained in the free DNA are rarely kept intact.

From Bacteria to Virus and Back

Unlike transformation, the third form of horizontal gene transfer—transduction—can occur in a wide range of bacteria. In transduction, bacteriophages (viruses that infect bacteria) pick up genetic material from one bacterial cell and deposit it in another.

As part of their life cycle, bacteriophages attach to bacteria and inject their DNA. This DNA then serves as a blueprint for making more copies of the bacteriophage, which burst from the infected bacterium and go on to infect other cells. At times, however, some of the new particles carry bacterial instead of viral DNA. Indeed, bacteriophages are capable of transferring whole plasmids and pieces of chromosomes between hosts. (Full chromosomes are too big to fit into bacteriophages.) Labora-

tory experiments indicate that some bacteriophages can apparently infect several species and even genera of bacteria, suggesting they might broadcast bacterial genes well beyond the locale where they first took up the genes.

Because transduction could potentially lead to broad dispersion of a foreign gene, my colleagues and I concentrate on its study. Initially, we looked for transduction by collecting bacteria in the kind of environmental containment chamber invented by Saylor. That chamber consisted of a clear plastic tube capped at both ends with filters that allowed water and nutrients in but prevented bacteria from leaking out. These days we use gas-permeable plastic bags for our experiments.

On the basis of our studies, we have proposed a model for the transduction-mediated dispersal of genetic material from an introduced bacterium to other bacteria in nature. Simply put, our model states that when a bacterium carrying a new gene enters a habitat, bacteriophages infect that cell and create more bacteriophage particles. If any particles end up containing the new gene, that gene can be passed on to the indigenous bacterial population. This model is equally applicable to the transduction of chromosomal and plasmid DNA. Recently we have sought to prove that this scenario is in fact carried out in freshwater. We have isolated bacteria and bacteriophages from various lakes and have demonstrated that bacteria do share genetic information by transduction in those settings.

Many microbiologists originally thought transduction would not be an important means of gene exchange in the environment, because it requires viruses and bacteria—both of which were thought to be present in low concentrations—to interact. But my co-workers

and I have recently found bacteriophages in very high concentrations (often 100 billion virus particles per milliliter) in fresh and marine waters. These observations have caused a reevaluation of the frequency of interactions, including transduction, that occur between bacteriophages and their hosts.

Even so, current understanding suggests that transduction of genes carried by genetically engineered bacteria in the environment is probably severely limited by a number of factors. One is that most bacteriophages infect only one species of bacteria, not many different species. Another is that most bacteriophages in the wild infect only bacteria that are native to the bacteriophage's habitat—not the laboratory strains of bacteria used in genetic engineering. Eventually, molecular biologists should also be able to equip genetically altered bacteria with traits that limit the ability of the bacterial DNA to move to and survive in another species; such restrictions are already under development.

Biologists can now manipulate the genetic makeup of almost any organism. In addition to being applied to the creation of recombinant bacteria, the technology is being used by farmers to grow genetically altered crops that resist various ills [see "Making Rice Disease-Resistant," by Pamela C. Ronald; *SCIENTIFIC AMERICAN*, November 1997]. The collected studies of bacteria in their native habitats suggest that genetically engineered organisms can be put into the environment safely and that the most important consideration is whether the genetically altered organism will do the job asked of it. Still, caution is warranted. As understanding of horizontal gene transfer expands, environmental biotechnologists should gain the information needed to reduce the risks to the barest minimum. SA

The Author

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Further Reading

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