# DEATH AND THE MICROBE

**Most people think of bacteria as selfish individualists. But in many microbial colonies, some bugs gladly sacrifice themselves for the greater good of bugkind.**

**WHEN TIMES GET TOUGH, *BACILLUS* GETS PREGNANT. NORMALLY THE** common soil bacterium divides by binary fission; it doubles its chromosomes and builds a septum—a wall—right down its center, dividing itself in half and producing two identical twin cells. But when food starts to run out, and survival becomes paramount, such equality is the first thing to go. Binary fission is still the order of the day, but the precursor cell now places the septum closer to one pole than another, producing two unequal cells—only one of which will survive.

The larger of these cells, now called the mother cell, engulfs its smaller sibling, now called the forespore, creating a cell within a cell. For some ten hours, the mother nurtures that forespore, using most of its energy to stitch together a sturdy protein coat for the life growing within. Once completed, this coat will help make the spore one of the hardiest creatures on the planet.

"There's probably no other life-form that is more tolerant of environmental extremes than the spores of *Bacillus* and related bacteria," says molecular biologist Richard Losick of Harvard. "The center of it is almost like a freeze-dried environment in which water is removed and the DNA and the ribosomes and so forth are all in a semicrystalline state. They can survive for very long periods of time—many years, probably at least hundreds of years." (Other researchers think the bacteria can survive far longer than that: in May a team from the California Polytechnic University in San Luis Obispo described finding and reviving spores of *Bacillus sphaericus* from the abdomen of a bee trapped in amber. The spores, says microbiologist Raúl Cano, were 25 million years old.) But for the mother, time is fleeting. Once the coat is completed, the mother cell will lyse—its membrane will disintegrate or burst open, the contents of the cell will spill out, and the spore will be set free to find greener pastures. It's the ultimate act of maternal sacrifice.

Sacrifice and altruism are concepts not commonly associated with bacteria; nor are bacteria known for going so willingly to their death. Bacteria, in principle, are immortal cells, dividing unchecked until dispatched with a good dose of antibiotic or a stiff swab

BY LORI OLIWENSTEIN

Courtesy James Shapiro

The two large cells of this *Anabaena* colony have given up all hope of reproducing so they can nourish their siblings.

of antiseptic. But researchers are now discovering that everywhere you look in the bacterial world, rather than immortal life, you find death.

Whether you call it suicide or altruism, programmed cell death or "apoptosis," murder or the more euphemistic "terminal differentiation," the death of a single cell that can potentially reproduce forever appears to violate evolutionary good sense. A dead bacterium is nothing but a dead end. "Apoptosis doesn't make adaptive sense for single-celled organisms," notes microbiologist James Shapiro of the University of Chicago. "But it makes lots and lots of sense for multicelled organisms."

If you look at each bacterium not as an individual but as a unit in a much larger organism—the microbial equivalent of a brain cell, a stem cell, a sperm cell—death takes on new meaning. "You tend not to think of bacteria as having differentiated populations, where one part of the population has one function, serves that function, and then dies," says Martin Dworkin, a microbiologist from the University of Minnesota. "But there are populations that behave in a coordinated fashion, that have differentiation."

Biologists have long recognized that groups of microbes can assume a coherent form; a 1920 paper by researchers at the Pasteur Institute in Paris was titled "The Organized State of the Bacterial Colony." And they have long studied how populations of bacteria—often several species working together—carry out chemical processes in the environment: for example, they "fix" nitrogen, gathering it from the air and converting it into a useful form for building protein.

But this view of bacteria has been overshadowed by their identity as pathogens, each cell eager to cause disease single-handedly. This latter view harks back to 1880, when bacteriologist Robert Koch published his criteria for identifying disease-causing bacteria: for the identification to be certain, a culture had to be grown of a single microbial species. And to be absolutely pure, so pure that there was no question the species caused the disease, each culture had to come from the seeding of a single bacterium. "That was the tradition that ultimately led to molecular biology," says Shapiro, "and became the intellectually dominant tradition in the history of microbiology and bacteriology. That's where our concept of the bacteria as single-celled organisms comes from."

For over a decade now, Shapiro and a small cadre of his fellow microbiologists have been working to turn that concept on its head. Shapiro's work with *Escherichia coli*—the common gut bacterium that is in many ways the lab rat of the microbial world—has persuaded at least some microbiologists to take a new and broader view of their subjects. "No matter how you look at an *E. coli* colony," says Shapiro, "you see that it is a highly differentiated structure—that there are many different kinds of cells. They have different sizes, they have different shapes, they have different enzymes in them. And you also see that it's a highly organized structure."

These differences—found in cells that start out as clones of one another, carrying identical DNA—come about because as a colony of bacteria grows, the cells experience different conditions. As conditions change, the bacteria change as well: some will begin to express different genes or will even pick up mutations that enable them to adapt to their new environment. Along the way, however, many cells must die; cell death is, in this way, inextricably linked to life.

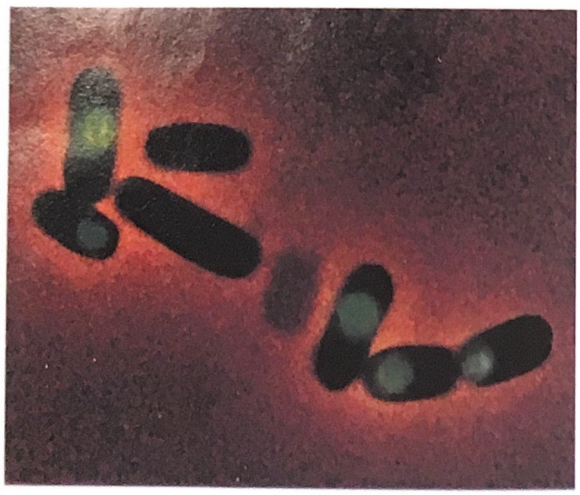
"YOU HAVE A LOT OF ORGANISMS IN WHICH THERE'S A LOT OF WASTAGE, BUT WHAT'S IMPORTANT TO THE ORGANISM IS NOT BEING ECONOMICAL—IT'S SURVIVING."

The death of the mother cell in *Bacillus* is only the simplest example of this link. A more wholesale fatality occurs in another group of soil-dwelling, spore-forming species, the myxobacteria. Myxobacteria, perhaps the most social of all bacteria, live in aggregates of hundreds of thousands of individuals and hold constant biochemical conversations. When nutrients grow scarce, up to 100,000 myxobacteria will join together to create a structure—visible to the naked eye—called a fruiting body. In some species the fruiting body consists of a stalk topped by one or more small balls of spores. But the spores, which make up a mere 10 to 20 percent of the population, are the only part of the fruiting body that survives; the rest of the cells lyse and die.

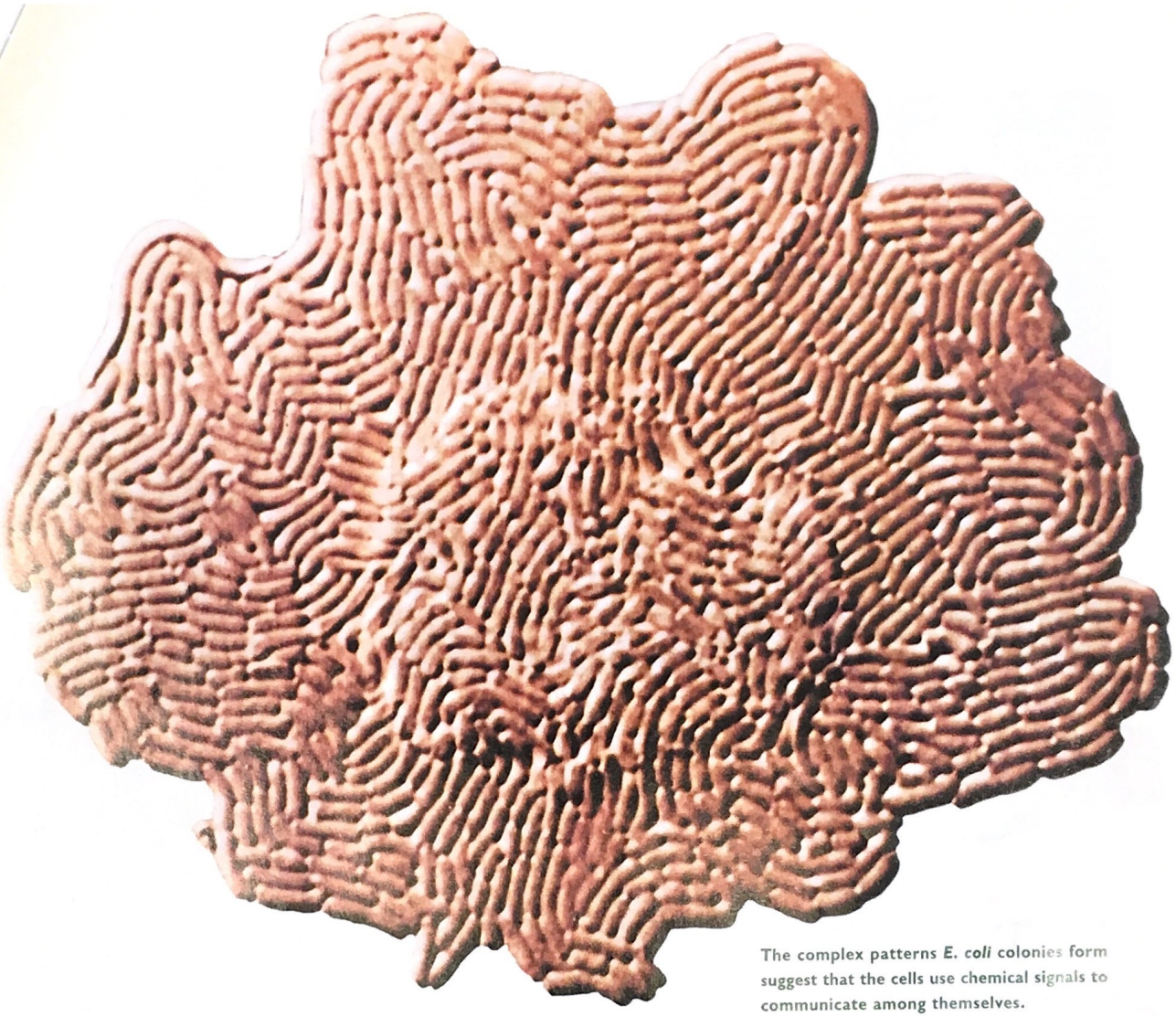
There is no solid explanation as yet for such overwhelming carnage. Dworkin says it's possible that the dead are simply cannibalized for their nutrients, that proteins from the lysed cells might be needed for the spores to build up their protective outer coat.

The blue-green photosynthetic alga *Anabaena* takes this sort of cannibalism to another extreme. It snatches needed nutrients from its "dead" cells while providing them with just enough energy to keep some of their cellular apparatus running but not enough for them to live on their own or to reproduce. Like many other species of cyanobacterium, *Anabaena* comes equipped with the genetic machinery to fix nitrogen. When levels of fixed nitrogen get low, *Anabaena* runs into a basic biological problem. Though

A self-sacrificing *Bacillus* cell turns its daughter cell into a spore by engulfing it, cocooning it in a tough protein coat, and disintegrating. Here, the glowing centers will become spores.



Courtesy Chris Webb; colorized by PixElation/Fran Heyl Assoc.



Courtesy James Shapiro; colorized by PixElation/Fran Heyl Assoc.

**The complex patterns *E. coli* colonies form suggest that the cells use chemical signals to communicate among themselves.**

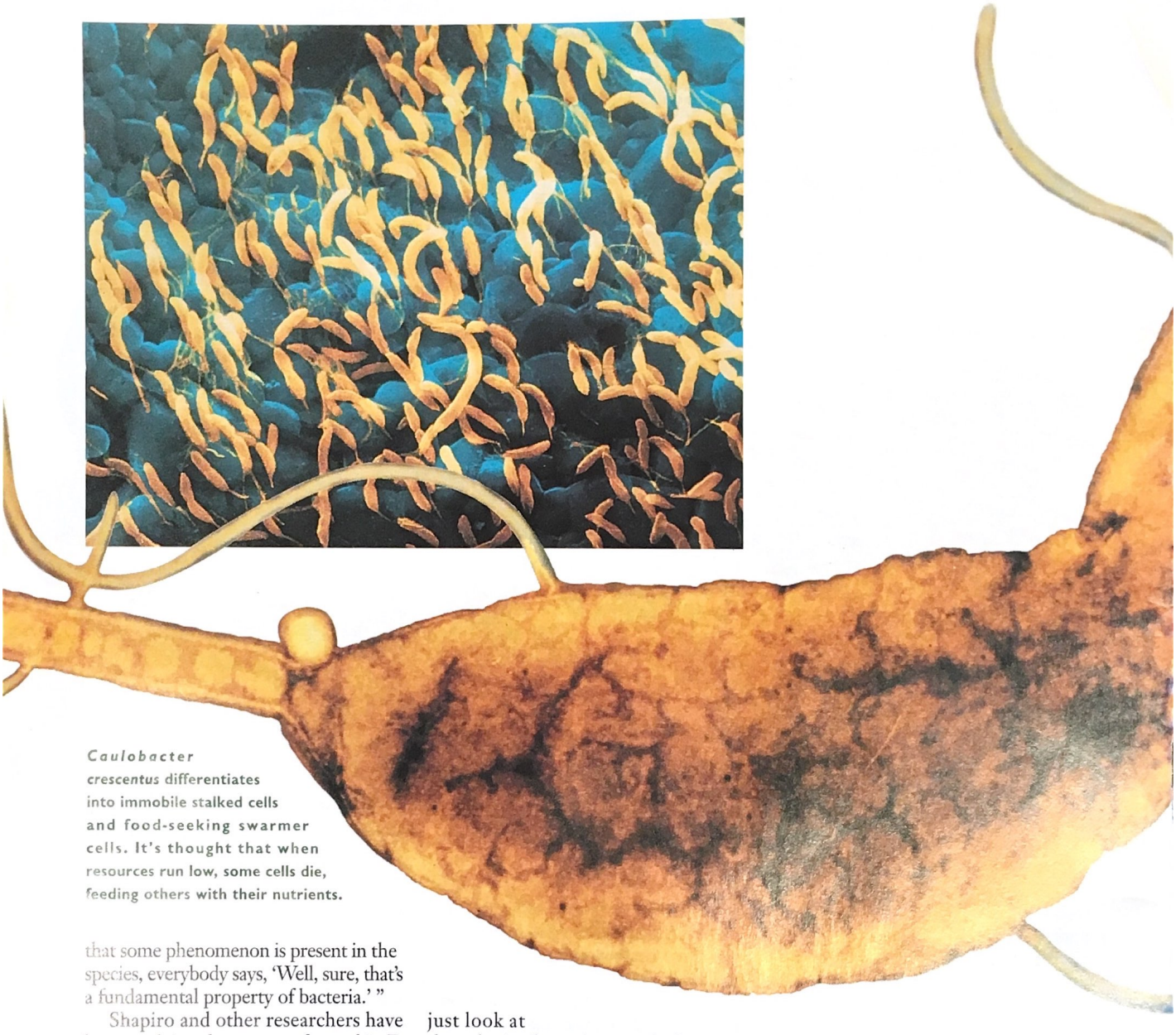
it should fire up its nitrogen-fixing genes and get to work, the cell is filled with oxygen, the end product of photosynthesis, which happens to be poisonous to the main nitrogen-fixing enzyme, nitrogenase. "So one cell by itself cannot carry out both photosynthesis and nitrogen fixation," says Shapiro. "What do these bacteria do? Along the chains they form, special cells differentiate. They lose their photosynthetic apparatus, they develop a nitrogen-fixing apparatus, and they fix nitrogen."

This major internal overhaul comes at a cost. The specialized nitrogen-fixing cells, called heterocysts, can no longer divide to make offspring. In a functional sense, they continue to live—the photosynthetic cells feed nutrients to them through connecting channels—but in an evolutionary sense, they've already died. They will not be passing on their genes. Does this matter? "If your only focus is on the individual cell, then it seems crazy for any cell to do this," says Shapiro. "But if it's on the multicellular population or organism, then you can understand why single cells would become terminally differentiated." After all, the cells in an *Anabaena* filament are clones of one another, so while the heterocyst's genes are goners, these cells are ensuring the survival of all those identical copies carried by the undifferentiated cells.

As Shapiro notes, the multicellular view makes trying to figure out the reason for cell death an empty exercise. "How different is it from a fish or a frog producing zillions and zillions of gametes—sperm and eggs—that are never going to survive?" he asks. "You have a lot of organisms in which there's a lot of wastage, but what's important to the organism is not being economical—it's surviving. You waste a lot of cell mass, that's true, but if you make enough spores and they're properly protected so that they'll ultimately find conditions in which they can germinate, then that's the goal that has to be achieved."

That's all very well on the larger level, but it's probably hard to recruit volunteers for the suicide missions. How are the doomed chosen? Dworkin thinks it's a matter of chance. "If it were predetermined—genetically, let's say—that some cells would die and some would live, you'd imagine there'd be a gradual selection over time for those cells that didn't die, and eventually the so-called altruistic ones would be selected against. So it almost has to be a stochastic thing: if they are in the right place or the wrong place at the right time or the wrong time, then that determines what happens to them."

It's tempting to write off *Anabaena*, myxobacteria, and even *Bacillus* as quirks of nature, exceptions to the bacterial rule. But when death shows up in *E. coli*, it's much harder to dismiss. "Rightly or wrongly," says Shapiro, "people think of *E. coli* as the prototype, so if you show



*Caulobacter crescentus* differentiates into immobile stalked cells and food-seeking swarmer cells. It's thought that when resources run low, some cells die, feeding others with their nutrients.

that some phenomenon is present in the species, everybody says, 'Well, sure, that's a fundamental property of bacteria.' "

Shapiro and other researchers have been studying the patterns formed in *E. coli* colonies under various conditions, such as when obstructions are introduced into the culture, or nutrients are withheld. Sometimes the colonies form dramatic geometric designs resembling flowers; sometimes the patterns are more subtle, as when the bacteria grow around the obstructions. Shapiro sees these arrangements of cells as evidence that the bacteria have some means, perhaps chemical, of communicating among themselves—that a colony is a multicellular unit.

When he noticed one such pattern—an unusual layer of cells—he knew at once that it was worth investigating. "Normally, as part of these studies, you just look at the colonies from above and take a picture of them or look at them through a microscope," he explains. "But one thing I did was fix them and slice through them to see what they look like in cross section. And the big surprise was to find a whole bunch of cells in the colony, in a layer near the bottom—but not right at the bottom—that don't stain for protein. These must be empty cells."

Dead cells in a culture are a common finding—in old enough colonies there are always plenty of cells that won't reproduce when you transfer them to another nutrient plate. But the position of these dead cells within the colonial organism gave Shapiro pause. "We know that bacteria in these colonies are very resistant to all kinds of antagonistic agents—antibiotics, disinfectants, formaldehyde, and viruses," he says. "My hunch is that these cells must be protecting the cells underneath them, so that if something like a virus attack comes along, at least they can survive." Shapiro found that when a normally virulent bacterial virus, a bacteriophage, invaded one of his *E. coli* colonies, the top layers were quickly destroyed, but the lower layer—the one beneath the dead cells—survived intact.

In general, microbes are no more adept at repelling viruses than we humans are; even in the example above, only the bottom, protected cells survived. But every now and then a phage infection is

Top left: John Smit

Courtesy Yves Brun; colorized by PixElation/Fran Heyl Assoc.

stopped dead in its tracks. This can happen in *E. coli* when a phage called T7 meets a plasmid—one of those tiny bits of DNA that float free in the bacterial cytoplasm—called F. The F plasmid is the gender plasmid of the cell; cells with an F are considered male, cells without it female.

Female cells are fully vulnerable to T7 phage infection; male cells can be infected, but before the phage has a chance to replicate and move on, the cell dies, taking the infection with it. "It's an altruistic situation," says Ian Molineux of the University of Texas at Austin, who believes he is close to identifying the cell's protein contribution to this particular suicide mission. "Let's say you have a population of a million bacteria, and one phage enters that population. If the phage can grow, then those million bacteria will die. But in this situation, one cell would become infected by this phage, and that one cell would die, but no more phage would be produced. So the other 999,999 bacteria would survive."

The ability to abort a phage infection is a gift the F plasmid confers on its host. Plasmids are cellular parasites—it costs a cell energy and lots of raw material to keep on replicating a plasmid's DNA along with its own. Cells expend that energy because plasmids tend to do them a lot of good; plasmids often bring genes for resisting antibiotics, for example. Phage resistance is another possible benefit. "This exclusion system may be what the parasite is giving to the host cell in exchange for the cell's providing an environment for the propagation of that parasite," explains Molineux. "It's protecting the cell from certain other parasites that could be lethal."

But plasmids aren't always so generous. Perhaps the most intriguing—and the most sinister—case of bacterial death is that of the parasitic plasmid that murders any host bold enough to try to get rid of it. It's a necessary, if violent, strategy. All other things being equal, a plasmid-free bacterium has more energy for itself and thus can out-reproduce its plasmid-burdened brethren. So some plasmids have devised what molecular biologist Michael Yarmolinsky of the National Cancer Institute calls a simple time bomb: a pair of genes—which are dubbed the plasmid addiction module—that code for both a toxin and its antidote. "In some modules, the plasmid carries a gene for an agent that destroys DNA from the bacterial chromosome, and a gene for an agent that prevents this destruction," says Ichizo Kobayashi of the University of Tokyo, who recently described just such a module in *E. coli*. "Bacteria without the toxin and the antitoxin live happily. Then one day the gene pair sneaks in."

Once the plasmid's in place, churning out its toxin and the antidote, the bacteria become addicted to it. As long as the plasmid is safe, cells remain unharmed. But if the plasmid disappears, it stops making toxin and antidote, so their concentrations drop. Since the toxin kills a cell at a much lower concentration than that necessary for the antidote to protect the cell, ridding itself of the plasmid spells doom for the cell. In this way, the selfish plasmid guarantees its own survival and makes sure it will be replicated into the next generation. Isn't that what life and death are all about?

**TALK TO THE SCIENTISTS** about bacterial multicellularity, and you'll hear story after gory story about bacterial death. Death in childbirth, death in battle, suicidal cells, murderous genes. But ask them what their research focus is, and most of them will tell you it's life—the ways in which the cells communicate with each other; how they divide; how they form patterns, divvy up the biochemical chores, share the resources.

"There may be a problem of perception," says microbiologist Yves Brun of Indiana University. Brun works with bacteria, such as the water-dwelling *Caulobacter crescentus*, that feed off the nutrients released by their dead siblings. "Death is the end of the process," he explains. "Most people, when they look at a test tube and see that the cells have lysed, think, 'Oh damn, the experiment is lost, or things have gone wrong.' We've become accustomed to thinking about cell death as a failed experiment."

But for those in the business of trying to ward off disease, cell death is the goal. For them, understanding how and why bacteria die isn't a purely intellectual exercise; instead, it's a chance to learn from the masters. "When we look at apoptosis, we find out how the organisms go about the business of killing cells themselves," notes Shapiro. "We're probably going to find some new ways of doing it that we didn't really think about beforehand, and they may suggest new approaches to antibacterial therapy. As we understand more about where apoptosis fits in to the bacterial life cycle, it may tell us something that we didn't know or didn't even know to anticipate about how diseases arise and develop, and so it may present us with unexpected opportunities for intervening in the process." D