FIELD NOTES

CONAN THE BACTERIUM

The world's toughest organism thrives in blistering deserts and radioactive dumps. Now workers are giving it a taste for chemical waste

BY PATRICK HUYGHE

MICHAEL J. DALY PULLS A Pyrex beaker, oddly brown and brittle, off the shelf. It's a memento, he says, of the first time he blasted some bacteria with ungodly amounts of radiation in his laboratory. It happened five years ago. Daly, who is a geneticist at Uniformed Services University of the Health Sciences (a medical school for military personnel) in Bethesda, Maryland, had put a bacterial sample in the beaker. He then stuck it in the laboratory's cobalt 60 irradiator, which looks a bit like a blue cement mixer, and let it "cook" for an hour and a half. When he retrieved it, he was surprised to find that this toughest of glass containers had been discolored and reduced to a fragile weakling.

"This is what happens to glass when it's exposed to 1,750,000 rads, the dose we use in our experiments," Daly now explains. "It comes out brown. That dose of radiation actually begins to break down the molecular structure of Pyrex glass. So you can imagine the sort of damage it inflicts on DNA." Radiation tends to shatter DNA as if it were a pecan in a nutcracker gone berserk. Put *Escherichia coli,* the microbiologist's favorite test bacterium, in the irradiator and within minutes it's history. But *Deinococcus radiodurans,* the bacterium at the center of Daly's attentions, is a different story. It lives.

D. radiodurans is one tough bug. From studies done on the victims of the atomic blasts at Hiroshima and Nagasaki, as well as from the extrapolated results of experiments done with chimpanzees and other mammals, it is known that a person exposed to 1,000 rads will die within a week or two. (A rad is a measure of the energy absorbed from radiation per unit mass of the absorbing material—in this case, a person's body; one rad is equal to .01 joule absorbed per kilogram of body mass.) But *D. radiodurans* can absorb more than 1,000 times that much radiation and survive—easily. Expose a beaker of *D. radiodurans* to a million rads and all the bacteria will live, albeit not happily. "At that level," notes Daly, who now uses plastic containers to hold his bacterial samples in the irradiator, "it's beginning to say to itself: 'I'm not feeling well.'" At 1.75 million rads, 37 percent survive. Even at three million rads, a few somehow manage to survive.

D. radiodurans can withstand doses of radiation that would kill any organism outside of its small family. That is not to say the bacterium is unaffected by radiation. Not at all. In fact, radiation ravages the bacterium's genetic material, breaking each of its chromosomes into more than a hundred pieces. Nevertheless, within twelve to twenty-four hours the bacterial cell manages to repair its DNA completely, as if nothing had ever happened. All living cells can repair their DNA to some extent. Yeast does it. *E. coli* does it. People do it. But *D. radiodurans* does it better than anything else on earth.

SINCE BEING DISCOVERED FORTY YEARS ago, *D. radiodurans* has brought a number of investigators under its spell, posing far more questions than they have been able to answer. How does it repair itself so efficiently? When and how did it come to tolerate such high levels of radiation? Could its special ability be of any practical use? Some investigators have even wondered whether the bacterium might be a kind of superseed: an organism that can disseminate itself by hitching a ride through the most hostile environments—perhaps even through space. As the Martian meteorites discovered in Antarctica make clear, an asteroid or comet can strike a planet so hard that it blasts pieces of rock into space [see "Blast Off," by H. Jay Melosh, page 40]. What if *D. radiodurans* were to hitch a ride on such a rock? Could the bacterium's durability enable it to literally infect another planet?

In the past few years, the answers to some of those provocative questions have begun to emerge. More detailed answers—and with them, undoubtedly, more questions—will soon be forthcoming, when the sequence of all 3.1 million base pairs in the bacterium's genome has been determined. (The Institute for

Christopher Bucklow, Sol Invictus, 3:41 p.m., 22nd December 1994, *1994*

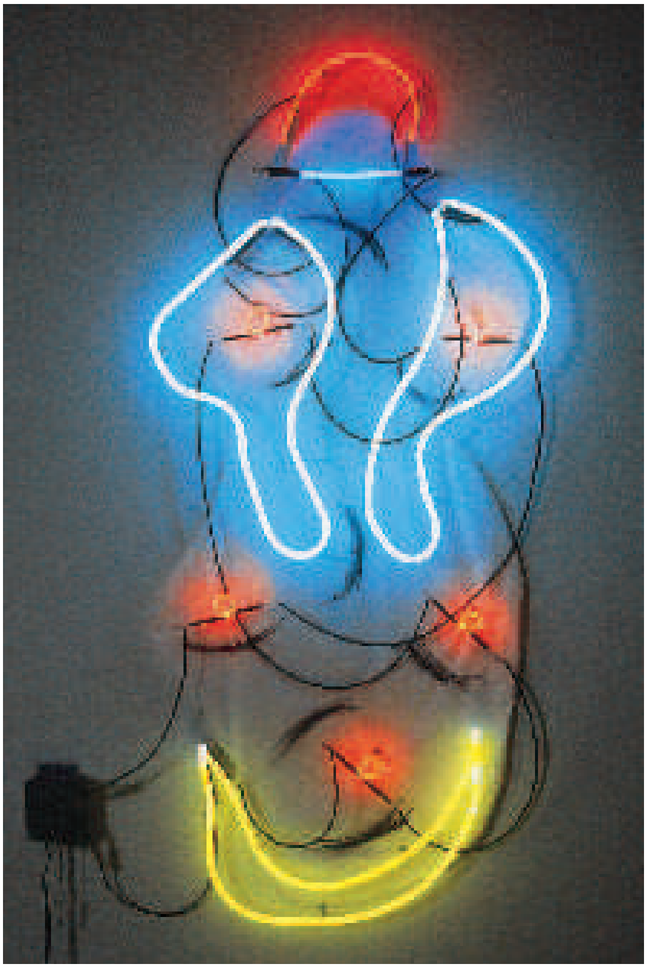
Genomic Research in Rockville, Maryland, has already sequenced more than 99 percent of the genome and has made it available, in blocks of various lengths and overlap, via file transfer protocol, at <ftp://ftp.tigr.org/pub/data/d_radiodurans/>.) Investigators will then have a kind of owner's manual to the inner workings of this remarkable bacterium.

For years *The Guinness Book of Records* has dutifully named *D. radiodurans* the World's Toughest Bacterium. The title is clearly deserved, but *D. radiodurans* is by no means the world's only radiation-resistant organism. It belongs to a family of bacteria known as Deinococcaceae, whose various species are extremely distant, in evolutionary terms, from any other well-characterized bacteria. And whereas many bacterial families include hundreds, if not thousands, of species, Deinococcaceae is made up of only seven. Six of the seven—including *D. radiodurans*—are round or berry-shaped and belong to the genus *Deinococcus;* the seventh is rod-shaped.

Those seven species have been isolated from the most diverse sources throughout the world, from llama feces to weathered granite in Antarctica to the tissue of Atlantic haddock. Most species of bacteria inhabit predictable niches. You can search for *E. coli* in a field all day, for instance, and never find it, but look in any human intestine and there it will be. Not so with the Deinococcaceae. Microbiologists have yet to find the species' natural habitat—the bacteria seem to be both everywhere and nowhere. "This is part of the mystery of this organism," Daly says of *D. radiodurans*. "You can find it everywhere if you look hard enough, even in extremes of environmental habitats, and yet we don't know its natural reservoir."

D. radiodurans was discovered in 1956 by a team of food scientists headed by Arthur W. Anderson at the Oregon Agricultural Experiment Station in Corvallis. The bacteria—large red spheres between one and three microns across—were isolated from a can of ground beef and pork that had spoiled despite having been "sterilized" with radiation. Anderson was astonished to find that the organism was six to eight times more resistant to radiation than a *Staphylococcus* reference bacterium, and more resistant than any spore-forming bacterium that he had ever tested. (A spore is a kind of armored capsule.) Because of its berry shape, Anderson called it *Micrococcus radiodurans,* meaning "small berry that withstands radiation." After further study, Robert G.E. Murray, a microbiologist at the University of Western Ontario in London, realized that the bacterium was different enough from a traditional *Micrococcus* to earn its own name. He rechristened it *Deinococcus,* which means "strange or terrible berry."

D. radiodurans began to shed some of its



Keith Sonnier, Chongaloo, *1996*

mystery in 1972, when Bevan E.B. Moseley, a microbiologist working at the University of Edinburgh in Scotland, discovered that it was not immune to radiation damage. Instead, Moseley found, radiation caused severe damage to the cell, but the bacterium still managed to repair its DNA with such miraculous speed that it looked as if it had been shielded.

Just how the bacterium performs that remarkable feat remained largely unknown until 1992, when the pathologist Kenneth W. Minton invited Daly to join him at Uniformed Services University of the Health Sciences. Daly had earned his doctorate in yeast genetics, and so he was well acquainted with techniques that could help Minton develop genetic systems for studying the repair processes in *D. radiodurans*.

Somewhat to their surprise, Minton and Daly discovered early on that *D. radiodurans* repairs its DNA much the same way all other organisms do. When a chromosome breaks, an enzyme called RecA wraps around one end of a broken strand. Each *D. radiodurans* cell contains between four and ten identical copies of its chromosomes, depending on the cell's stage of growth. The enzyme searches those chromosomes for a stretch of DNA similar to the stretch at the end of the broken strand. If the DNA is found, the bacterial cell uses it as a template to make a replacement part. The process is known as classical, or RecA-dependent, recombination.

It is this "search for homology," as it is known, that *D. radiodurans* seems to do so well. It can put 500 broken fragments of DNA back together flawlessly in just one day. By comparison, *E. coli,* which also applies classical recombination, cannot repair more than two or three breaks to its chromosome. "The logistics of knitting together 500 fragments floating around in a cell, in perfect order, with virtually no mutations, any deletions or rearrangements, is just mind-boggling," Daly says. Minton turns that statement around: Why can't *E. coli* make the same kind of elaborate DNA repairs? Why does *Deinococcus* stand up to 1.5 megarads, whereas *E. coli* is such a cream puff? "Why can't people do this?" he asks, warming to his subject. "If a bacterium can do it, why can't we?"

Minton's fascination with those questions led him, in 1988, to turn his entire laboratory over to the study of *D. radiodurans*. Now, Minton and Daly believe, the answers to those questions may be at hand. One of the bacterium's key characteristics is its two-pronged response to genetic impairment. *D. radiodurans,* the two investigators theorize, does undergo classical recombination, but the process does not get started until about four hours after the bacterium suffers radiation damage. By then, they argue, the bacterium has already begun to repair itself in other ways, notably through a process known as single-strand annealing (SSA). Minton and

Daly admit their hypothesis is speculative, but it is based on some highly suggestive evidence assembled in the past two years. For example, both yeast and *E. coli* may do similar kinds of early repair to breaks in their genomes; the process in *D. radiodurans* is simply much more obvious and much more dramatic.

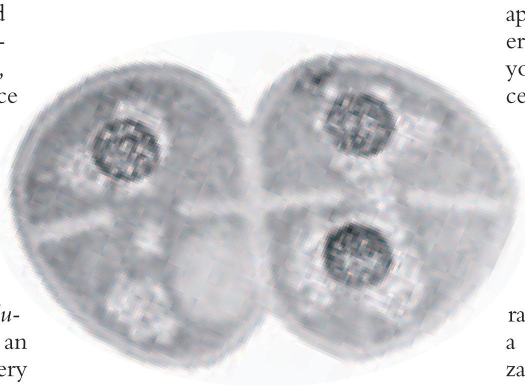
Classical recombination requires two broken ends and an intact template. SSA, by contrast, requires just two overlapping fragments of DNA. The two broken fragments are lined up, one strand of each fragment is nibbled away, and then the two remaining fragments are stuck together to form a new, undamaged fragment. Minton and Daly believe that SSA begins almost immediately after the bacterium's DNA is damaged; it can be detected within an hour and a half. And there is no mistaking it for RecA-dependent recombination; it occurs even in strains of *D. radiodurans* in which RecA has been disabled. But though SSA repairs about a third of all chromosome breaks in *D. radiodurans,* and though it sets up the subsequent process of classical recombination by mending the smaller fragments into bigger ones, that only begins to explain what is so special about the bacterium.

BACTERIAL VARIETIES OF RECA ARE usually interchangeable. But Minton and Daly found that the RecA in *D. radiodurans* is unique. If you put it in *E. coli,* for instance, *E. coli* dies. And if you replace the RecA in *D. radiodurans* with RecA from *E. coli,* the altered *D. radiodurans* can't repair its own radiation damage. Even more surprising to Minton and Daly was the fact that *D. radiodurans,* unlike all other bacteria, shows no traces of RecA until it is damaged.

Could the natural RecA of *D. radiodurans* be a kind of superenzyme? It was an intriguing thought, but another discovery soon sent Minton and Daly down an even more promising path. In 1993, at Louisiana State University in Baton Rouge, the microbiologist John R. Battista was looking at *D. radiodurans* under a light microscope when he noticed something odd: the bacterium's genetic material was concentrated in a tight little wad, known as a nucleoid. At first glance, the nucleoid just looked like a bright little spot. But when Battista increased the magnification, he began to see a pattern within it: from certain angles the spot looked like a ring with a hole in the center—like a doughnut, or a tire, or, as Minton and Daly later put it, like a Life Saver.

Then, as now, only a handful of microbiologists were funded to work on *D. radiodurans*, and they often shared their findings. When Battista was unsure what the nucleoid's shape might signify, he showed his photographs of it to Minton. That was just the prod Minton and Daly needed. Perhaps, they now began to think, *D. radiodurans* owes its incredibly efficient repairs less to its enzymes than to the arrangement of its chromosomes. In other bacteria, DNA is dispersed throughout the cell. As a result, repairing a piece of DNA is like fishing in a large lake stocked with only a handful of fish. For every break in the genome, the two broken ends of DNA must locate an unbroken, homologous stretch of DNA somewhere among the chromosomes floating about in what, from a molecular perspective, is a vast cavity inside the cell. No wonder *E. coli* cannot repair more than a couple of breaks to its DNA.

But what if the chromosomes in *D. radiodurans,* already known to be tightly packed, are also organized into a fixed spatial arrangement? On the basis of Battista's photographs, Minton and Daly went on to develop what they like to call their Life Savers hypothesis. The bacterium's identical chromosomes, they suggest, may be stacked like Life Savers candies, and oriented in such a way that homologous genes on different chromosomes are positioned one atop the other. In that case, the search for homologous DNA is as simple as looking straight up or down. Minton and Daly further speculate that the chromosomes



Cutaway view of two Deinococcus radiodurans *bacteria, each completing a cell division; magnified 20,000 diameters*

may be held in place by mobile structures called Holliday junctions that look like cross-stitches. The junctions connect one strand of DNA to its homologue on another chromosome. Thanks to that arrangement, radiation can break the bacterium's chromosomes without scattering broken DNA all over the cell. Every broken part is always lined up with its replacement part.

MINTON AND DALY'S WORK SEEMS TO ring true. "I think they have a real tiger by the tail there," Battista says. But he thinks the bacterium benefits from more than just good, tight-knit organization. When *D. radiodurans* is hit with a high but sublethal dose of radiation, he points out, its DNA stops replicating almost immediately and doesn't start up again until repairs are complete. Most other bacteria do the same thing, but they stop replicating because they are damaged—their machinery simply breaks down—and they are unlikely to start up again. *D. radiodurans,* Battista believes, may stop and restart its replication deliberately, as a controlled physiological response.

D. radiodurans seems to keep equally strict control over another physiological process as well. Immediately after replication stops, a cell excises any damage to the bases that make up its DNA and to the surrounding areas. The process is known as degradation. "It's like repairing a building that has been knocked over or damaged," Battista explains. "The first thing you have to do is get rid of the rubble and take down everything that's broken—and perhaps a little more—before you can start rebuilding." Organisms such as *E. coli* make use of degradation, but *D. radiodurans* takes the process to another level. The higher the dose of radiation, the more degradation *D. radiodurans* undergoes—and it knows when to stop degrading so as not to clean itself up into oblivion.

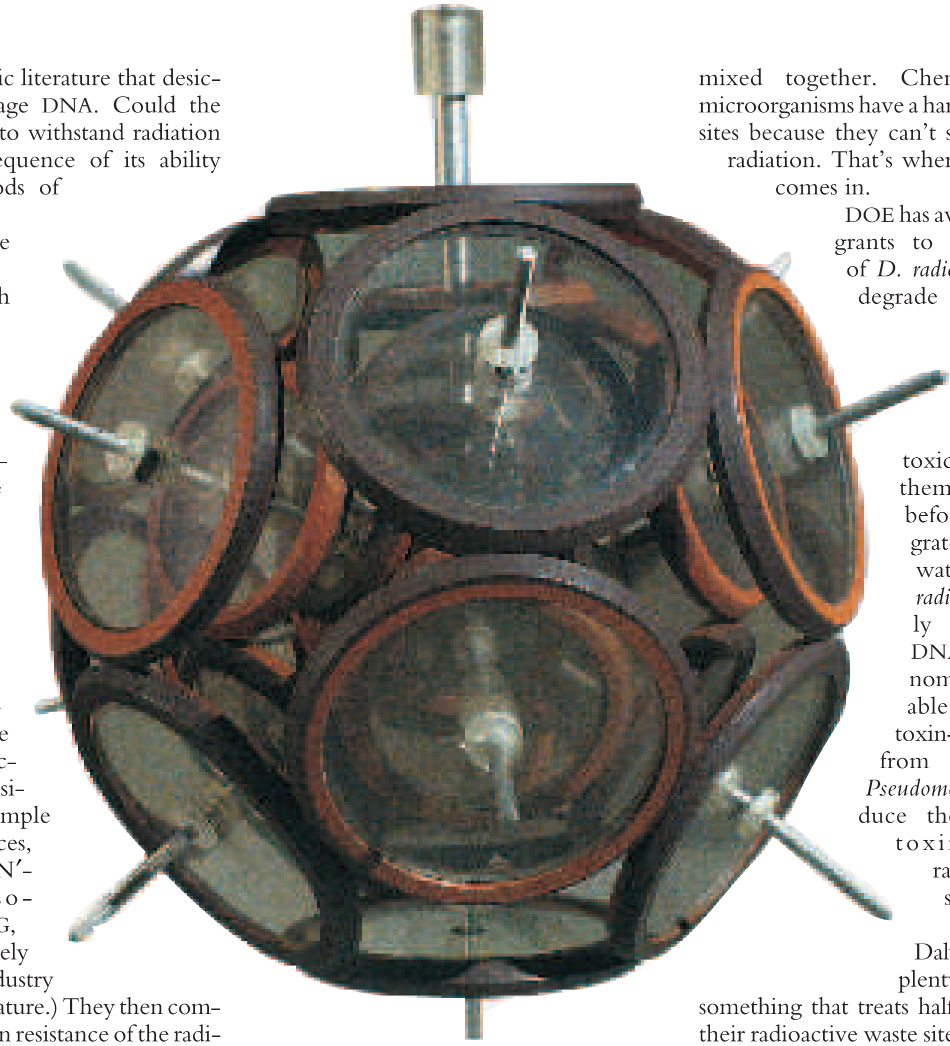
Battista thinks he has found two proteins, unique to *D. radiodurans,* that function as master control switches. One of them appears to stop and start replication; the other seems to turn degradation on and off. "If you inactivate either protein," he says, "the cell becomes sensitive to radiation."

SUCH STUDIES BEGIN TO EXPLAIN why *D. radiodurans* is so durable. But they leave unanswered another, more fundamental question: Why does the organism exist at all? What is the evolutionary advantage of being resistant to radiation, other than to gain protection from a handful of investigators who delight in zapping you with gamma rays? Never in the history of the planet have organisms been bombarded by enough radiation for something like *D. radiodurans* to arise through natural selection. Terrestrial gamma radiation is normally measured in hundredths of a rad per year. Even in areas subject to particularly high levels of radiation—the thorium-rich sands near Guarapari, Brazil, for instance—radiation absorption hovers around twenty rads per year.

Nevertheless, *D. radiodurans* repairs its DNA efficiently for a reason, and Battista thinks he has found one. According to a textbook published nearly twenty years ago, and later cited by Murray, the bacterium is resistant to desiccation as well as to radiation. That would not be unusual in a bacterium that can form a protective spore around itself. But for *D. radiodurans,* which withers into dust when dry, it is quite a feat. Battista recalled some suggestions in the scientific literature that desiccation might damage DNA. Could the bacterium's ability to withstand radiation be a simple consequence of its ability to withstand periods of extreme drought?

Beginning in the fall of 1993, Battista began working with a graduate student, Valerie Mattimore (and later with an undergraduate, Misty Dawn Manuel) to test the hypothesis. They created strains of mutant *D. radiodurans* bacteria that were sensitive to radiation. (Clearly, radiation could not be used to generate mutants of the species, but the bacterium is fairly sensitive to some simple mutagenic substances, such as N-methyl-N′-nitro-N-nitrosoguanidine, or MNNG, that are used routinely in the chemical industry but do not exist in nature.) They then compared the desiccation resistance of the radiation-sensitive strains with that of the wild-type bacterium. "What we found," Battista says, "was that every radiation-sensitive strain that we looked at is also sensitive to desiccation. So our contention is that *D. radiodurans* evolved its DNA-repair ability to survive periods of prolonged drought or dehydration." It just so happened that the same ability also helped it resist radiation. Moreover, that link doesn't appear to be unique to *D. radiodurans*. Other radiation-resistant species, Battista notes, are now beginning to be found in extremely arid environments.

For the moment, however, *D. radiodurans* has the limelight all to itself. And the bacterium is about to join an elite group of organisms, thirteen in number, whose entire genetic structures have been sequenced. Why did *D. radiodurans* earn that honor? "What we are learning as biologists," Daly explains, "is that there is a lot to be gained sometimes by focusing on the oddballs. The oddballs offer you things the normal bugs do not."



Kenneth Snelson,
Atom Magnet Shells, *1976*

NO QUESTION, *D. RADIODURANS* IS AN oddball. Its DNA-repair system is so powerful that it has probably not mutated much over the aeons, which would imply that the bacterium of today could be highly similar to its ancestors a billion years ago. "This appears to be a very ancient organism," Daly notes, "and it also tells us about what we are and where we came from." Its genetic sequence holds a fascination for the evolutionary biologist, because it is a way of looking back in time. "It's really a messenger," Daly says, "a messenger from our past."

It is not impossible, he adds, that *D. radiodurans* has delivered its genetic message to other planets as well. Space travel would freeze, desiccate and irradiate any organism that attempted it, he says. "But in theory, *D. radiodurans* could recover from the genetic damage—provided it somehow survived the heat of reentry and landed in a warm, nutrient-rich environment."

THE DEPARTMENT OF ENERGY (DOE), which is providing the funds for the genetic sequencing, has a vested interest in the little bug. DOE is pouring money into bioremediation, a process that employs microorganisms to gobble up toxic wastes. The most troublesome cleanups, such as the one in progress at the Hanford site near Richland, Washington, are the ones in which chemical and radioactive wastes are mixed together. Chemical-munching microorganisms have a hard time with such sites because they can't survive the high radiation. That's where *D. radiodurans* comes in.

DOE has awarded Daly two grants to develop strains of *D. radiodurans* that will degrade organic toxins in radioactive environments, and that will mineralize toxic metals, forcing them to precipitate before they can migrate into groundwater. Because *D. radiodurans* can easily insert foreign DNA into its genome, Daly has been able to introduce toxin-degrading genes from the bacterium *Pseudomonas,* to produce the world's first toxin-munching, radiation-resistant superbug.

"The DOE," Daly says, "would be plenty delighted with something that treats half the problem at their radioactive waste sites—the chemical half. Trichloroethylene and toluene are the number-one organic chemical contaminants. They are terrible. They give you acne, make you blind and can kill you." Both chemicals were used intensively by the U.S. nuclear weapons industry between 1945 and 1986. Even worse, they were frequently mixed with highly radioactive isotopes, dumped into weak storage containers and simply buried. Those containers are now leaking, and Daly thinks *D. radiodurans* is the key to cleaning up the organic toxins as close as possible to the source of pollution. "What it can't be used for," he says, "is to cure radiation. It doesn't break down isotopes; it doesn't work at the atomic level."

THE IDEA OF TURNING A KIND OF superbug into a fancy "environmental cleaner" may seem fantastic, but then *D. radiodurans* seems to spawn wild ideas in all who encounter it. "When I give a talk about this bizarre organism," Daly says, "it's like someone going to a freak show. They say: 'God, look at that.' It's hard to be taken seriously sometimes. It's a crazy bug. It feeds on the imagination." ●

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