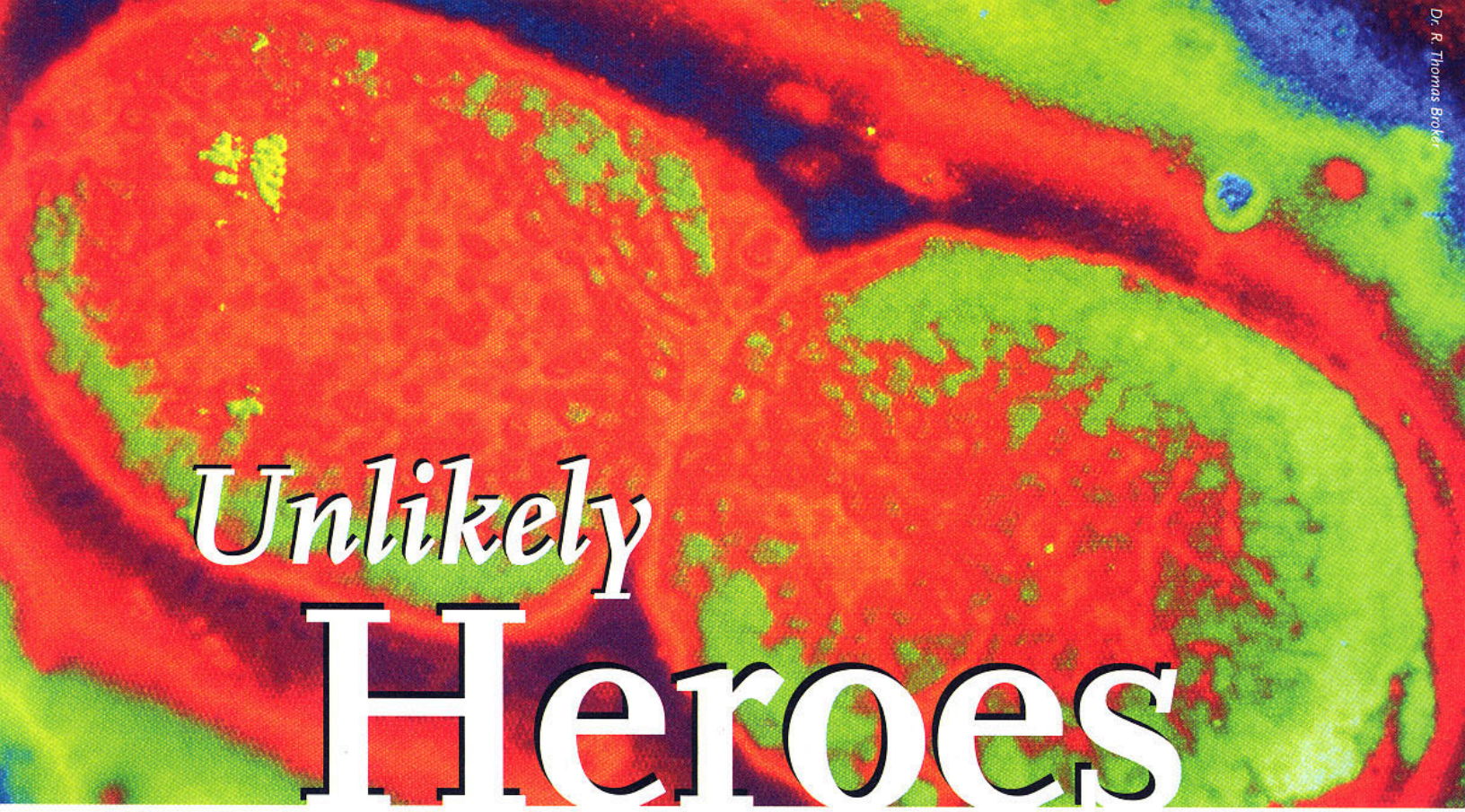


Unlikely Heroes

By Nicole Johnston

Among model organisms, bacteria hold a unique place, as both models of infection and pathogenesis, and as research tools. More to the point, molecular biology was built upon the cell walls of lowly bacteria: The processes of DNA replication, RNA transcription, and protein translation, not to mention gene regulation, were all worked out in bacteria. Those early studies provided the foundations to understanding the more complex processes in eukaryotes.

Despite their relative simplicity (bacterial genomes are about 0.1% the size of mammalian counterparts), bacteria continue to surprise researchers. Nearly 40% of the *Escherichia coli* genome consists of genes encoding proteins of unknown function. Recent evidence shows that some species cheat death, cannibalize their dead neighbors' DNA, and can survive a century in suspended animation. Bacteria's heyday is far from over.

FOUNDATIONS For 100-plus years, scientists have studied the workings of the intestinal bacterium *E. coli*. "We know more about it than any other organism in biology. All molecular biology was done studying *E. coli*," remarks Steve Finkel, University of Southern California, Los Angeles.

Says Roberto Kolter, Harvard Medical School: "Among bacteria, *E. coli* [have] very easy genetics. You could move genes in a number of ways; therefore people could do very good genetic studies. These days, *E. coli* is still an excellent organism to work with and its genetics still the simplest. You can do knockouts anywhere and gene manipulation is still incredibly fast."

Microbial physiology emerged during the late 19th century when simple biochemical tests distinguished *E. coli* from other bacteria. About two decades later, microbiologist Rudolf Massini kick-started bacterial genetics when he discovered *E. coli* strains that could not ferment lactose.

E. coli soon became the organism in which to study environmental sensing. Work by François Jacob, André Lwoff, and Jacques Monod defined the lactose utilization pathways and provided early insights on how organisms cope with their environment; in this case, the presence or absence of sugars. More recently, *E. coli* has contributed to science's understanding of how organisms deal with adverse environmental conditions like elevated temperature and other stresses that result in denatured proteins—the so-called heat-shock response. This mechanism helps the organism cope with environmental stress consequences, either by destroying or refolding the misfolded proteins. Key to that response: RNA polymerase σ factors, which help the polymerase choose which genes to transcribe.

Normally, RNA polymerase associates with σ70, but during the stress response, it switches to either σ32, which helps the organism cope with cytoplasmic heat shock, or σE, which coordinates the periplasmic heat shock defense. The result: heat shock genes like the Hsp70 proteins, nucleoplasmins, and chaperonins become activated. The two chaperonins GroEL and GroES, for instance, form a multimeric safe haven in which denatured polypeptides can refold.

The cytoplasmic heat shock response is characterized. But the periplasmic mechanism is less clear: how does a cytoplasmic protein sense periplasmic events? Recent work offers some insight: The bacterium evidently communicates the periplasmic state to the cytoplasm by the controlled proteolysis of an 'inner membrane antisigma factor,' called RseA.[2] The authors note, however, that other proteins probably are involved as well.

The *E. coli* heat shock response has helped researchers untangle similar reactions in other organisms. These proteins and molecular chaperones are conserved in organisms from *E. coli* to humans, and they could help explain protein-folding problems in diseases such as Alzheimer and Huntington.

NO FOOD? NO PROBLEM More recently, Kolter's group has focused on how *E. coli* survives without nutrients. Its pro-

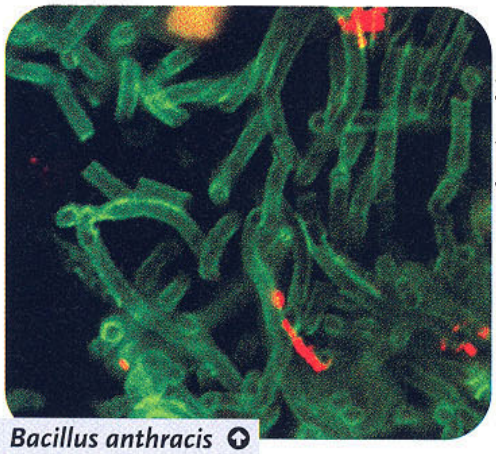
grammed response is to enter 'stationary phase,' a resting state governed by σ^S, the transcriptional regulator that influences the expression or suppression of more than 50 genes. These signals control how the cells respond to the nutrient levels or cell numbers in the environment. However, mutations caused by DNA damage and "SOS-induced" rescue polymerases, give rise to hardy mutants with enhanced fitness. The majority of these survivors are σ^S master regulator mutants.[3,4]

Foregoing commands during stationary phase, these mutant *E. coli* can survive under conditions that halt the growth of their wild-type relatives. These mutations enhance amino acid catabolism, allowing the mutants to grow even faster on the amino acid buffet provided by dying neighbors. Such survivors are said to display a "growth advantage in stationary phase" (GASP) phenotype, enabling them to out-compete the rest of the population.

As a result, they adversely affect survival of wild-type members. Kolter describes the phenomenon in terms of the Prisoner's Dilemma gambit : sometime it's better to defect for selfish reasons than to cooperate with the crowd. Of course, the unanswered question is: Are other organisms biologically predisposed to cheat in their quest to be among the fittest?

Bacteria also can feast on their deceased neighbors' DNA. Finkel and Kolter have shown *that E. coli* can use extracellular DNA, whether homospecific or heterospecific, as their sole source of carbon and energy. Mutant *E. coli* that cannot consume DNA face certain starvation when conventional nutrients are gone. The proteins involved in this quasi-cannibalism are homologs of proteins typically associated with bacteria that take up DNA from the environment. They believe other bacteria probably have this capability, as well.

The spore-forming *Bacillus* species take another approach to starvation. These gram-positive bacteria have, like *E. coli*, long been mainstays in microbiology labs. "It has very easy genetics," explains Peter Setlow, University of Connecticut Health Center, Farmington. Moreover, *Bacillus* species can do particularly nasty things to humans, none more so than *B. anthracis*, which causes anthrax.



Courtesy of CDC/Sherif Zaki, Kathi Tatti, Elizabeth White

Bacillus anthracis

More specifically, "It's infinitely more intelligent than *E. coli*," Setlow explains. While *E. coli* undergo stationary phase adaptation when faced with starvation, *Bacillus* transforms itself into heat- and radiation-resistant spores when nutrients run low. These protective capsules allow the now dormant bacilli to survive for more than a century, until the opportunity to germinate presents itself.

Currently, researchers study *Bacillus* as an infectious diseases model. "It's a very close relative of *Staphylococcus aureus* and streptococci," says A.L. Sonnenshein, Tufts University, Boston. Consequently, bacilli are ideal for studying gene functions of staph and strep: They are naturally competent and are more closely related to the pathogens than is *E. coli*, a Gram-negative bacterium.

THE ANTIBIOTIC ERA During the 1950s, *Bacillus* species weren't the only spore-formers garnering attention. *Streptomyces* species (the name literally means 'chain fungus' because of its fungal-like appearance) took center stage as models of antibiotic production and resistance, and of complex cellular differentiation.

Being soil organisms, *Streptomyces* compete in a microcosm of fungi and neighboring bacteria that would otherwise threaten their survival. Unlike many other organisms, *Streptomyces* are nonmotile, making survival a challenge. Evolution provided a chemical defense: They produce antibiotics to help them compete against other bacteria. Drug manufacturers have capitalized on the microbe's chemical ingenuity for decades, and today, most conventional antibiotics can be traced to the *Streptomyces*.

With each species capable of producing several antibiotics, *Streptomyces* have had to develop mechanisms to protect themselves from their own poisons. As a result, there is particular interest in using *Streptomyces* as a model for developing novel antibiotics by tapping into their biosynthetic and resistance genes. "Drug companies don't seem to be interested in going back into natural product development," says Mark Buttner, John Innes Center, Norwich, UK, describing why major pharmaceutical companies are dropping their antibiotic programs. "Biotech companies [and academic scientists] are trying to develop novel antibiotics through manipulation [of genes] in *Streptomyces*. I'm optimistic that such rational science will be done."

LEARNING THE LANGUAGE In very recent years, *Pseudomonas aeruginosa* has taken its place among the ranks of model bacterial organisms, particularly as a prototypical opportunistic pathogen. Historically, *P. aeruginosa* took a backseat to *E. coli* in the laboratory because it was more difficult to study their genetics at the time. In recent years, however, scientists turned their attention to exploring the organism's intrinsic insensitivity.

The organism is a bioremediator's dream because it can degrade hydrocarbons—it can clean up oil slicks, for instance. But in the clinic, *Pseudomonas aeruginosa* is a clinician's nightmare. First, it produces a slimy biofilm that makes it largely insensitive to antibiotics. But the biofilm, it turns out, is just gravy. "The trouble with *Pseudomonas* is that it is not naturally sensitive to antibiotics, even when not in a biofilm," says E.P. Greenberg, University of Iowa, Iowa City. So, *Pseudomonas* frequently takes its toll on cystic fibrosis patients, burn victims, and immunocompromised persons.

A well characterized cell-cell communication system, known as 'quorum sensing,' has cemented the organism's place as a bacterial model organism. This system turns on antibiotic resistance genes that encode efflux pumps. "Some efflux genes are regulated by quorum sensing," he says. "That has helped us understand efflux pumps and that has been a big contribution in itself." Compounding the problem, quorum sensing controls virulence and virulence factor production, he adds. Devising means of scrambling this communication system may one day allow scientists to halt *P. aeruginosa* in its tracks.

With the genomes for each of these organisms now known, future endeavors will be aimed at deciphering the wealth of newly discovered, but functionally uncharacterized, genes. "What's been really impressive over the last 10 years is the number of different approaches [now available]," says Setlow. "Where are [different] proteins and what are they talking to? It's the beginning of a whole new research area." ⊗

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