GENETICS

Can Organisms Speed Their Own Evolution?

Intriguing hints from cell and molecular biologists suggest that they might, but evolutionary biologists are not yet convinced

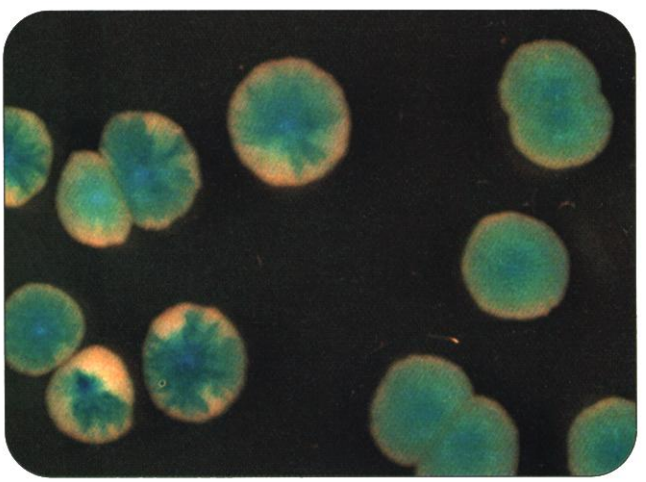
In November 1970, Miroslav Radman, a molecular geneticist now at the Université René Descartes in Paris, stunned his colleagues with a heretical proposal: that bacteria harbor a genetic program to make mutations. Through this program, Radman suspected, bacteria can crank up their mutation rates in stressful situations, helping accelerate their own evolution. Virtually no one believed him.

But 3 decades later, with the discovery of a new family of DNA-synthesizing enzymes, or polymerases, Radman feels vindicated. Unlike regular polymerases, this new family is prone to make mistakes. And recently, two independent groups led by molecular geneticist Susan Rosenberg of Baylor College of Medicine in Houston, Texas, and Patricia Foster of Indiana University, Bloomington, fingered one of them—polymerase IV—as a generator of mutations in times of stress. Says Radman: "These are the polymerases that I was dreaming about 30 years ago."

Based on these and other recent findings, the idea that organisms have ways of speeding their evolution by boosting their genetic variability is generating increasing excitement among a group of cell and molecular biologists. Within the past 2 years, for instance, researchers have unearthed molecular clues that could help explain apparent increases in genetic variability not only in bacteria but in eukaryotes as well. "I think it's very cool stuff that, amazingly, not enough people have really gotten the scoop on," says Rosenberg.

But a number of evolutionary biologists are trying to put the brakes on this mounting enthusiasm. Although the critics say the new molecular findings are intriguing, they question their origins and role in evolution. Specifically, these biologists say it is uncertain whether these processes were selected for their ability to generate variability in the first place. Nor is it clear whether they accelerate long-term evolution. Because most mutations are harmful, increased variability may often be costly to individuals and species. "It is hard to see how selection would directly favor a process that generated random variation or even one that just preserved it," says evolutionary biologist Jon Seger of the University of Utah in Salt Lake City.

Thus, a spirited tussle has ensued as researchers from these two camps put forth their interpretations of the new molecular findings. With roots dating back to Charles Darwin in the mid-1850s, the question of whether organisms harbor systems for adjusting their own rate of evolution remains open.



Bacterial lunch. Adaptive mutations allow a strain of *E. coli* to feed on lactose assessed by a blue indicator dye (solid blue colonies on right). Bacteria can also acquire this ability by amplifying the lactose-digesting genes, a temporary phenomenon (left).

CREDIT: SUSAN ROSENBERG

Error-prone enzymes

For decades, most biologists have worked under the assumption that mutation rates are constant and that individual organisms passively submit to the forces that shape evolution. Yet the idea that organisms may modulate their genetic variability has surfaced from time to time. Even Darwin suggested in *The Origin of Species* that environmental changes resulting from animal domestication affect variability.

But that didn't prepare members of the biological community for the jolt they received in 1988, when molecular biologist John Cairns and his colleagues at the Harvard School of Public Health published in *Nature* an even more shocking idea than Radman's. Cairns proposed that, depending on their environmental conditions, bacteria might be able to direct mutations to particular genes. The dogma-shattering idea that mutations might not be completely random "touched a raw nerve," says Cairns. It smacked of "Lamarckism"—a reference to Jean-Baptiste Lamarck's now-discredited theory that species evolve through the inheritance of characteristics acquired during an organism's lifetime. Outraged, a number of evolutionary biologists quickly embarked on their own studies to test the notion. The flurry of studies ultimately revealed that Cairns's original proposal was untenable, and the community, including Cairns, now at the Radcliffe Infirmary in Oxford, United Kingdom, discarded it.

Thanks to the commotion it ignited, however, Cairns's article prompted the study of a new phenomenon: the increased mutation rate observed in *Escherichia coli* during times of stress—in particular, starvation. Most of the evidence for this phenomenon comes from studies of a strain of *E. coli* that carries a mutation inactivating the lac operon, a group of genes that allow bacteria to digest lactose. When cells have plenty of food choices, they rarely acquire mutations that counteract the lactose deficiency. Yet, as Cairns and Foster described in the journal *Genetics* in 1991, when lactose is the only choice on the menu, rates of these compensating mutations skyrocket.

Over the past decade, researchers have been dissecting the molecular underpinnings of these so-called adaptive mutations. And within the last 2 years, they have made impressive strides. They have found, for example, that although these mutations are not directed to particular genes, as Cairns originally suggested, they don't uniformly pepper the bacterial genome either. "There are hot and cold regions for hypermutation," says Rosenberg, who is now working on defining these regions. "All regions are not equal."

One of the most exciting findings has been the discovery of the error-prone polymerases. "It's a great novelty," says Radman. "We knew of these *E. coli* genes for over 20 years but couldn't recognize them as polymerases." That changed in 1999, when researchers found that these proteins could copy lesioned DNA in a test tube.

Microbiologists already knew that when bacteria suffer DNA damage, they switch on a response, called SOS, that arrests the cell cycle and turns on genes that repair DNA and allow its duplication. They suspected that these genes might help regular polymerases avoid getting stuck when they run into a damaged stretch of DNA. But by monitoring the activities of the SOS-

induced proteins in a test tube, three independent groups discovered that instead of helping regular polymerases, these proteins were polymerases themselves, capable of copying less-than-perfect DNA.

These SOS polymerases appear to help cells produce DNA when high-fidelity enzymes can't. In an article published in 1995 in the *Proceedings of the National Academy of Sciences* (*PNAS*), Radman and colleagues provided evidence suggesting that starvation could activate the SOS response. And last year, Rosenberg reported in *PNAS* that efficient adaptive mutation requires RecF, a protein that helps induce the SOS response, as well as other proteins produced during the SOS response. So researchers began to suspect that starvation might activate the SOS response, turning on error-prone polymerases, which results in an increase in the number of mutations. Foster cautions that this scenario has yet to be unequivocally proven. But her results, published last spring in the proceedings of the 65th Cold Spring Harbor Symposium on Quantitative Biology, and Rosenberg's study in the March 2001 issue of *Molecular Cell* show that the error-prone polymerase IV is indeed responsible for many adaptive mutations.

This is important, says Rosenberg, because it provides the molecular basis for a potential path for the rapid evolution of new traits. "Everybody in the field [of adaptive mutation] is really excited," says Foster.

In a 1999 *Nature* News and Views article, Radman, one of the chief proponents of this view, enthusiastically described the role—at the time, merely suspected—of the error-prone polymerases in adaptive mutation and dubbed them "mutases." These mutases, he said, are "enzymes designed to generate mutations"—implying that they had been selected for this explicit purpose during evolution.

Not so fast, said a number of evolutionary biologists, including Joe Dickinson of the University of Utah. Dickinson was particularly critical of Radman's assertion because he failed to distinguish between the purpose of the enzymes and their effects. "There's a sad history in evolutionary biology of people not making careful distinctions and therefore getting lured into sloppy thinking," he says.

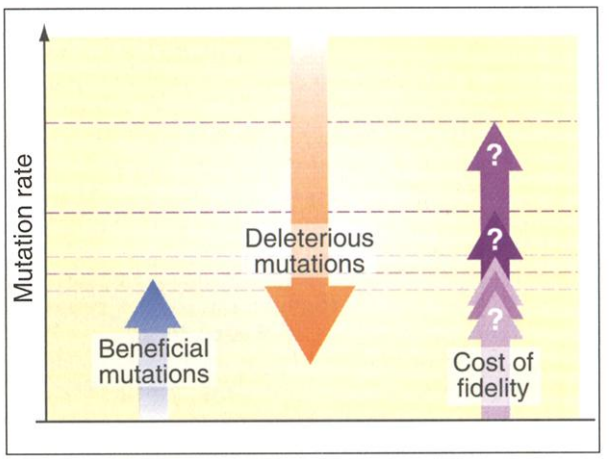
Radman now agrees with Dickinson that whether the error-prone polymerases were selected during evolution for their ability to generate mutations remains far from certain. Still, Dickinson chides Radman and others studying adaptive mutation for giving scant attention to the alternatives. For instance, error-prone polymerases may have been selected for their ability to allow cells to cope with damaged DNA; the generation of variability may be simply a nonselected byproduct. It's also possible that when cells are stressed, they can't afford the cost of high-fidelity DNA synthesis. "The cells may be turning the lights off to keep the whole system from crashing, just trying to hang on," says evolutionary geneticist Paul Sniegowski of the University of Pennsylvania in Philadelphia. What's more, say Sniegowski and others, conjuring scenarios in which evolution selects and maintains elevated mutation rates is not easy (see sidebar on p. 1826).

The molecular biologists counter that even if increased mutation rates did not evolve for the purpose of tuning evolution, the diversity it generates could nevertheless act as an engine of change. And if this is the case, increased mutation rates could allow organisms to accelerate evolution when times get tough.

Evolutionary fast track

Susan Lindquist, a cell and molecular geneticist at the University of Chicago, shares this view. She recently discovered processes in eukaryotes—not just simple bacteria—that she proposes could provide evolutionary fast tracks in times of stress. "The main point is that, no matter how they arose, [these processes] provide a plausible route to the evolution of new traits," she says.

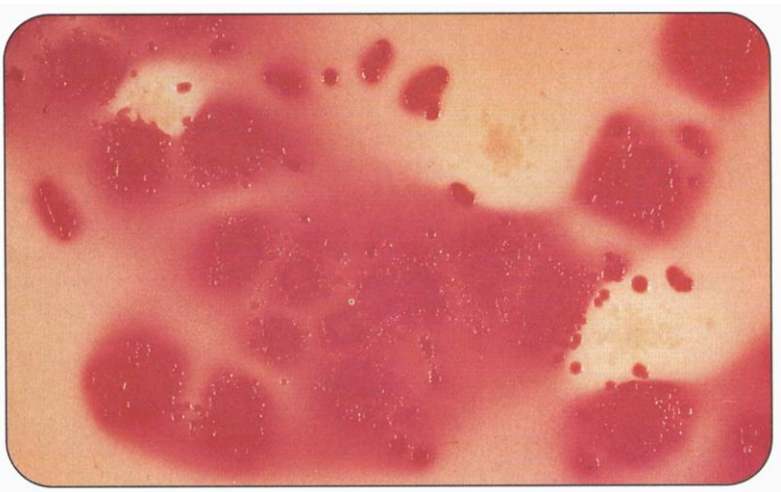
One of these processes involves a yeast protein, Sup35, that helps terminate protein translation—the process by which proteins are generated using messenger RNA (mRNA) as a template. Researchers have known that Sup35 sometimes changes its shape and turns into a prion—a protein that self-propagates by causing other Sup35 proteins to misfold and that can be passed from parent to daughter cells. In this prion form, known as the [PSI$^+$] prion, the protein fails to perform its job properly. That, in turn, causes the translation machinery to miss stop signals in the mRNA and create proteins with extra segments.



Evolutionary forces. Little is known about the cost of fidelity in DNA synthesis. If it is high, it could be an important force shaping the evolution of mutation rates.

Lindquist and her Chicago colleague Heather True wanted to see whether this increased variability could help the organism cope with a stressful environment, as others have proposed for the elevated mutation rates of starving *E. coli*. To find out, they compared the growth of yeast harboring either the normal protein or the prion under a wide variety of conditions, including different food sources, a range of temperatures, and exposure to toxic drugs. As they reported in the 28 September 2000 issue of *Nature*, in nearly half of 150 tests, the prion affected growth—boosting it in over a quarter of these cases.

Given that random increases in variability, such as the random disruption of single genes, usually squelch growth, the prion's effects were surprisingly beneficial. And Lindquist thinks they may be an evolutionary boon. Nobody knows exactly what triggers the Sup35 protein to switch to its prion conformation. But in a typical yeast population, roughly one cell in a million does. So in large yeast populations, there are probably always a few members that sport new, heritable



Multiple mutations. Increased mutation rates also crank up nonadaptive mutations. In a population of *E. coli* that have acquired adaptive mutations for feeding on lactose (red and white cells), some cells (white) also bear mutations in genes required for feeding on maltose.

CREDITS: (TOP TO BOTTOM) MODIFIED FROM P. SNIEGOWSKI *ET AL.*, *BIOESSAYS* 22:1057 (2000); SUSAN ROSENBERG

Why Evolution Might Not Favor Increased Genetic Variability

Evolutionary biologists are often stymied when they try to imagine the evolution of mechanisms designed to increase genetic variability. One reason is that such mechanisms can only be selected indirectly, through the beneficial mutations they create.

For example, if a population of bacteria encounters a deadly antibiotic, only those that have mutations allowing them to survive the toxic effects of the drug will thrive—and those aren't necessarily the same bacteria as those with increased mutation rates, explains Richard Moxon of the John Radcliffe Hospital in Oxford, United Kingdom. Admittedly, bacteria with increased mutation rates may have a better chance of acquiring resistance mutations, but selection acts directly only upon the resistance mutation, not the mutation generator. In other words, mutator mechanisms can only persist by hitchhiking with the beneficial variants they produce.

An elevated mutation rate can't be maintained in a population simply because of its promise to provide beneficial mutations in the future. "Evolutionary selection lacks foresight," says Joe Dickinson of the University of Utah in Salt Lake City.

And in sexual organisms such as yeast, generators of variability face an additional obstacle. In asexual bacteria, a genetic variation coding for an elevated mutation rate can travel for several generations with the beneficial mutation it generated; the two genes are said to be linked. By contrast, in sexual organisms, linked genes can be separated through a process called recombination, which reshuffles the genetic deck every generation. Consequently, any two given genes are not necessarily inherited as a pair, just as two adjacent cards may or may not remain together after shuffling.

So, in sexual organisms, the chances are low that generators of variability will remain associated with their beneficial mutations for several generations. And once a generator is separated from favorable mutations, natural selection is likely to act against it because of the more common deleterious mutations it causes. "That's a problem that anybody who thinks about the theoretical concept of a mutator, and the proposal that it can be advantageous, has to deal with," says Moxon. "And that's a very serious objection." **–M.C.**

traits, says Lindquist. If the environment is static and does not favor these traits, these few anomalous organisms will die out. But in a fluctuating environment—say, a vineyard where food and warmth are plentiful in summer but not in winter—the prion could be a source of useful variations, enabling at least a few of the organisms to survive. Those, in turn, would be selected for in classic Darwinian style, the researchers propose. The population wouldn't be ultimately overrun by prions, however, because the prion spontaneously flips back to its nonprion shape.

Evolutionary biologist Nicholas Barton of the University of Edinburgh, for one, questions that interpretation. "It is not surprising that the prion should sometimes increase growth rates in environments to which the yeast is not well adapted," says Barton. But "without knowing how the yeast lives in nature, it is hard to assess the significance of this one intriguing example."

But there's a bigger problem, Barton and other evolutionary biologists say. Most random mutations are deleterious, so how could processes that boost variability help organisms survive overall? In fact, says Barton, "a major problem in evolutionary biology is to explain why genetic variation is so abundant in nature."

One researcher who has examined the benefits and costs of genetic variation is Richard Lenski, a microbial ecologist at Michigan State University in East Lansing. In a series of experiments, his team created high-mutating bacteria and low-mutating bacteria in identical environments to see which adapted faster. To create populations with different mutation rates, Lenski's team inserted gene variants encoding deficient DNA repair enzymes into repair-proficient bacteria. They then monitored the bacteria's increase in fitness over thousands of generations.

In a few circumstances, elevated mutation rates provided their owners with an adaptive edge, the group reported (*Science*, 15 January 1999, p. 404). Members of very small populations, for example, sometimes fared better when undergoing high mutation rates, presumably because their chances of acquiring a beneficial mutation at normal mutation rates were exceedingly low. But in other cases, higher mutation rates did not accelerate the pace of evolutionary adaptation. "What they found is that strong benefits will be observed only under special circumstances," says Penn's Sniegowski.

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Two research teams led by François Taddei at the French biomedical research agency INSERM in Paris and Michel Fons at the French Institute for Agronomy Research in Jouy-en-Josas have performed competition experiments to examine the costs and benefits of increased mutation rates. They inoculated mice with control *E. coli* and with a strain sporting a high mutation rate due to a defective DNA-repair enzyme. Even when the control bacteria outnumbered the mutators 50 to 1 in the initial inoculum, the mutators quickly outgrew the controls within a few days, they reported (*Science*, 30 March, p. 2606). Yet over time, the mutators lost their edge and could not keep pace with the controls when nutrients were scarce, the in vitro experiments showed. And when the researchers monitored the transmission of these bacteria between hosts, the controls outperformed the mutators. The researchers speculate that the high mutators accumulated deleterious mutations that weakened their chances of survival as they encountered nutrient-poor environments in their travels between hosts. In short, elevated mutation rates seem to provide benefits only under certain circumstances, and mostly in the short term.

But these tests are far from definitive on the evolutionary benefits and drawbacks of enhanced genetic variability—in either starving bacteria or prion-carrying yeast. Barton and evolutionary biologist Linda Partridge of University College London think more competition experiments, such as those performed by Taddei, are needed.

To gain a full picture of the evolutionary implications of these processes, however, theoretical studies will also be necessary, asserts Partridge: "There's actually a huge

pedigree of theory that would enable one to analyze this formally."

Working up estimates of the costs and benefits of mutation in bacteria under various conditions is one important step in that direction. Previous studies have suggested the frequencies at which beneficial and deleterious mutations arise. Now Sniegowski is planning to factor in the cost of DNA synthesis fidelity to see how much this could contribute to the selection and maintenance of baseline mutation rates. This approach might also help researchers studying adaptive mutation. Enhanced variability is not the only potential benefit of increased mutation rates; another benefit might be a reduced cost of maintaining high fidelity. So systems that crank up their mutation rates may persist because of their cost-reducing benefits rather than their variability-generating abilities.

"One way to think of the cost of fidelity is that its impact depends on the current economics of a population," says Sniegowski. If maintaining high-fidelity DNA synthesis is pricey, then cells under stress might be unable to afford it.

Drawing on the combined wisdom of theory and experiment, such approaches might help sort out some of these unresolved questions. And the increasing interest in evolvability may spark additional approaches. Evolutionary biologist Christopher Wills of the University of California, San Diego, for one, is enthusiastic about the possibilities: "I'm very glad that the evolution of evolvability is finally starting to catch people's attention." **–MARINA CHICUREL**

Marina Chicurel is a writer in Santa Cruz, California.

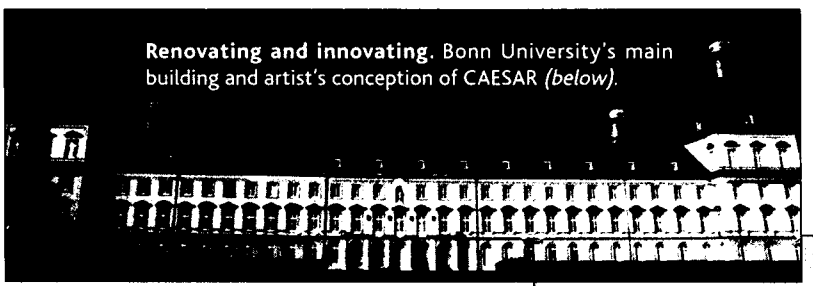
GERMANY

A Former Capital Stakes Its Future on Science

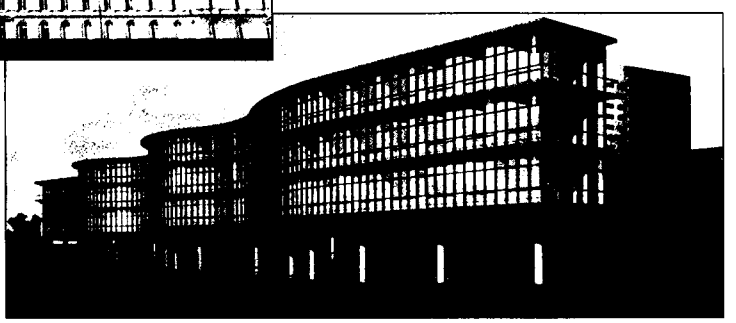
Most of the politicians have left for bustling Berlin, but scientists are hoping to keep Bonn from becoming a post–Cold War backwater

BONN—When reunified Germany moved its capital back to Berlin a few years ago, this placid city seemed poised to fade into obscurity. Instead, Bonn is seeking to redefine itself as one of the country's foremost science cities. Last week, the cornerstone was laid for a $100 million edifice, the Center of Advanced European Studies and Research (CAESAR), which is now rising on the banks of the Rhine. Bonn's 183-year-old university, once known primarily for its law and liberal arts faculties, has been pouring resources into its medical and natural sciences departments. The federal government's science and education ministry and the main research granting agency, the Deutsche Forschungsgemeinschaft (DFG), have remained in Bonn rather than joining the exodus to Berlin, and the city is now home to several international science secretariats. Bonn is also busy refurbishing a collection of unique science museums. All this is intended to breathe more scientific life into a place that, even in the depths of the Cold War, novelist John le Carré dismissed as "a small town in Germany." But Bonn's ascension is by no means assured: It faces stiff competition from established science bastions like Heidelberg and Munich, as well as upstarts in the east such as Dresden and Leipzig.

Bonn's venerable university is spearheading the basic research end of this attempt to take on the rest of Germany. The university now houses Europe's most advanced center for epilepsy research, and it is considering a proposal to build a $35 million "Life and Brain Center" that would promote collaborative neurobiology projects between academia and industry. "The idea is to build on a U.S.-style model that would bring together basic researchers, the neurological medical sciences, and the biotech industry," says Bonn University neuropathologist Oliver Brüstle, who has become one of Germany's most controversial and outspoken scientists since his research team submitted the first application in Germany to import embryonic stem cells for nerve-cell regeneration research (see p. 1811).



Renovating and innovating. Bonn University's main building and artist's conception of CAESAR *(below)*.



CREDITS: (LEFT TO RIGHT) UNIVERSITY OF BONN; CAESAR RESEARCH CENTER

Brüstle, who spent 4 years at the U.S. National Institutes of Health, says he sees great potential for Bonn as a research center—as long as Germany's political and scientific establishments permit the sort of research that needs to be done. The political turmoil over Brüstle's stem cell research—which the DFG has approved in principle, but the German government is now debating—is in some ways emblematic of the problems that Bonn and other German research centers face. University research is often impeded by excessive bureaucracy, and the research and education ministry has been stymied in some of its efforts to make research more flexible.

CAESAR is trying to buck these hidebound research traditions. "We want to become Germany's leading example of high flexibility in research," says CAESAR's scientific director, applied mathematician Karl-Heinz Hoffmann. Even though the center is government-funded, it does not operate under rules that bog down research and clog turnover at most German institutes. Scientists are hired on 5-year contracts, for example, and are required to finish their research projects within that time.

For now, CAESAR's 70 scientists are housed on the upper floors of an office building in Bonn's old town, not far from the birthplace of composer Ludwig van Beethoven. Over the next 2 years, after the center's new headquarters and labs are finished, CAESAR will expand to about 300 researchers in fields ranging from biology to computer science.

CAESAR, whose acronym recalls the Roman general who invaded the Rhineland 2000 years ago, is aiming to conquer a far different territory: emerging markets in fields such as nanotechnology, "smart materials," and biosensors. For example, CAESAR has