tions in *M. tuberculosis*. By now, Bloom, Jacobs, and their colleagues have generated thousands of *M. tuberculosis* mutants, some of which have affected the pathogen's survival and virulence. And they are just getting started.

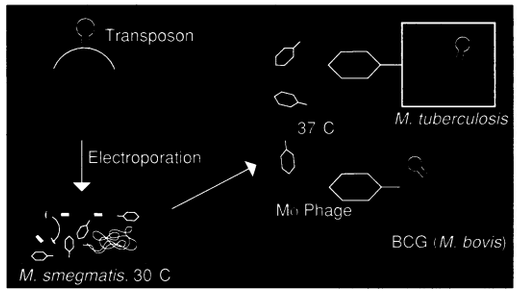
"With these techniques, researchers should be able to make mutations in virtually every gene of *Mycobacterium tuberculosis*," says Ann Ginsberg, program officer for tuberculosis at the National Institute of Allergy and Infectious Diseases. "Once you have mutants, you can understand gene functions. It allows you to answer a lot of questions about the pathogenesis of the disease." Along with pointing to targets for new drugs, the mutants might also lead to new ways to protect against TB: vaccines based on the avirulent strains Bloom and his colleagues are generating.

The Einstein team has been on the trail of better ways of producing *M. tuberculosis* mutants for more than a decade. Early on, Jacobs reasoned that the barrier posed by the microbe's waxy coat might be circumvented by taking advantage of viruses that naturally infect mycobacteria. With few such viruses available commercially, Jacobs turned to a handy source—his own backyard in the Bronx.

Bacterial viruses, or phages as they are called, are common soil dwellers, and so Jacobs screened soil from his yard looking for any that infect *M. tuberculosis* efficiently. He won the "prokaryotic Lotto," he says, in 1987 when he found a mutant bacteriophage that infects *M. tuberculosis* at a frequency seven orders of magnitude higher than its parent.

It's difficult to package transposons into a phage, so Jacobs combined the phage DNA with a plasmid from another bacterium, *Escherichia coli*—a creation he christened a "phasmid." Besides accommodating transposons, the plasmid DNA tricks *E. coli* into copying the entire recombinant molecule, churning it out in large quantities. The researchers then introduce the phasmid DNA into *M. smegmatis*, a mycobacterium that doesn't have its pathogenic cousin's waxy coat, so its cell wall can be breached with a jolt of electricity. There the phasmid replicates, forming phage particles that will infect *M. tuberculosis*.

In an added twist, the researchers mutated the phage itself so that it replicates only at 30 degrees Celsius. As a result, it doesn't kill infected cells kept at higher temperatures but merely transfers in the phasmid DNA, along with its transposon, which can then jump into the mycobacterial DNA, causing mutations. This procedure has proved very efficient at making *M. tuberculosis* mutants; the researchers have so far collected 10,000. "We keep making them, and hopefully we will statistically accumulate enough to cover all the genes," says Bloom.



SOURCE: W. R. JACOBS AND B. SHAPIRO

Transposon shuttle. After being grown in *E. coli*, the phasmid, including both phage (red) and plasmid (white) DNA plus a transposon (yellow), replicates in *M. smegmatis*. This produces phage particles that infect mycobacteria with transposon-carrying DNA.

But the mutants are already providing insights into the biochemical machinery that mycobacteria need to cause disease. For example, the Albert Einstein researchers traced loss of virulence in one mycobacterial mutant to a transposon interrupting the gene encoding a sigma factor, a protein that helps turn on other genes. Another mutant lost its virulence because it could no longer synthesize the amino acid leucine. "The various mutants tell us what typical *Mycobacteria* need to survive and grow," says Jacobs. That should make *M. tuberculosis* much less of a mystery to researchers looking to develop better TB drugs and vaccines.

–Carol Potera

Carol Potera is a free-lance writer in Great Falls, MT.

ARCHAEOLOGY

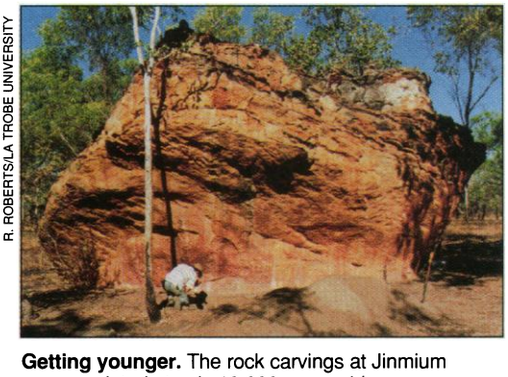
Young Ages for Australian Rock Art

Two years ago, archaeologists caused an international stir with their dates for a remote rock shelter called Jinmium in the Northern Territory of Australia. The dates of 116,000 to 176,000 years ago made the shelter by far the earliest trace of humans in Australia, and its circular carvings the oldest known rock art in the world. But archaeologists questioned the dates, partly because they were obtained with a method that has yielded spectacularly early dates at other sites (*Science*, 10 October 1997, p. 220). Now the results are in from a painstaking effort to redate Jinmium, and the doubters have been vindicated.

In this week's issue of *Nature*, a team headed by geochronologist Richard Roberts of La Trobe University in Melbourne, Australia, reports that Jinmium's age is a completely unremarkable 10,000 years. The new dates "nail the coffin shut" on the claim that humans have been in Australia two to three times longer than previously thought, says geochronologist Jack Rink of McMaster University in Hamilton, Canada.

The early dates for Jinmium came from a team led by archaeologist Richard Fullagar of the Australian Museum, who is also a coauthor on the new paper. For the early dates, he used a method called thermoluminescence dating (TL), which relies on a clock driven by natural radiation in common minerals like quartz. As long as the mineral remains in the dark, the radiation bumps electrons from their normal positions in the minerals' crystal lattice into defects, or "traps," at a regular rate. But exposure to sunlight or heat empties the electrons from the traps and sets the clock to zero. The traps refill over time, and scientists can read the clock by emptying them in the lab, either by heating the sample (TL) or by tickling it with light (optically stimulated luminescence, or OSL). The material glows as the electrons drop back into the lattice; the more intense the glow, the more time has passed since the sediments last saw daylight.

But those dates can be contaminated. In the paper in *Nature*, Roberts's team notes that pebbles of older rock—crumbly sandstone from the boulder wall and the bedrock below—were jumbled into the sediments being dated. When Roberts used OSL to tease dates from individual mineral grains, he was able to distinguish old grains of bedrock from the grains of sediment that would reveal the true age of the shelter. His conclusion: The base of the deposit at Jinmium is no more than 10,000 years old, and some of the quartz grains were laid down more recently. That means humans were at the site "no more than 10,000 years ago," says Roberts.



R. ROBERTS/LA TROBE UNIVERSITY

Getting younger. The rock carvings at Jinmium are now dated at only 10,000 years old.

These ages agree with radiocarbon dates from the upper layers of the deposit. Besides removing a puzzle in Australian prehistory, says Rink, the new dates should restore confidence in luminescence dating, which is a powerful tool when applied correctly.

–Ann Gibbons