only a few controversial Cretaceous specimens linked to modern mammals (*Science*, 21 November 1997, p. 1438), although they have hunted intensely. "There's a huge premium on finding a Cretaceous rabbit or other mammal," says Benton.

Instead, most paleontologists argue that the molecular clock is unreliable. Perhaps the mutation rate sped up and slowed down at different times in mammalian history, suggests Benton. For example, a population explosion during the rapid radiation of mammals 65 million years ago might have allowed their DNA to accumulate mutations more quickly, speeding up the clock.

But Hedges and other molecular evolutionists say their clock is reliable, noting that their study agrees with most dates from the fossil record and with other genetic work. "It's entirely consistent with what we found," says Simon Easteal, a molecular evolutionist at Australian National University in Canberra who also found earlier origins for mammals. And if the clock sped up during the early mammalian radiation, then the later dates for divisions between various mammalian species, such as primates, sheep, and cows, wouldn't match those from fossils—unless the clock later slowed down by precisely the same amount in each of many different lineages. "That is really stretching," says Hedges.

For now, the debate goes on. "We have two fairly entrenched positions," says Benton. "The exciting thing is within our lifetimes, we can hope to see which one is right."

–Ann Gibbons

MICROBIOLOGY

New Clue to How Anthrax Kills

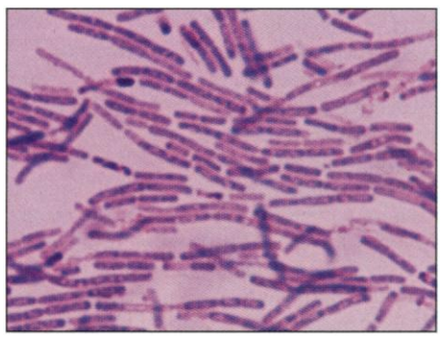
The deadly disease anthrax has been much in the news lately—thanks largely to fears that rogue leaders or international terrorists will attempt to wage germ warfare with the anthrax bacillus. Now, a research team led by George Vande Woude at the National Cancer Institute (NCI) in Frederick, Maryland, has made a serendipitous discovery that may someday give doctors a new countermeasure against the disease.

On page 734, Vande Woude and his colleagues report that they have identified a possible mechanism of action for "lethal factor" (LF), a toxic protein produced by the anthrax bacillus that is thought to be one of the principal causes of death in infected individuals. Scientists have long suspected that LF is a protease, an enzyme that cuts other proteins, but they have not been able to identify its targets. The Vande Woude team has found that LF cleaves and inactivates an enzyme in one of the cell's key signaling pathways, the mitogen-activated protein kinase (MAPK) pathway, which helps control cell growth, embryonic development, and the maturation of oocytes into eggs.

Although it makes sense that disrupting such a critical pathway could kill cells, researchers have not yet shown that this effect is what makes LF so toxic. Regardless, the result may aid the development of new treatments that work by neutralizing the toxin. As Vande Woude puts it, "Conceivably, we could find a drug that would make anthrax as a weapon of destruction as powerful as a water pistol."

He and his colleagues had no intention of studying anthrax pathogenicity. They were interested in the MAPK pathway, named for one of its constituents, a so-called kinase that regulates the activity of other molecules by attaching phosphate groups to them. To find out more about the pathway's role in oocyte maturation, Nick Duesbery, a postdoc in Vande Woude's lab, was seeking compounds that block MAPK's activity. The group knew of one such chemical, PD09859, which is one of more than 60,000 agents the NCI has screened for antigrowth activity on human tumor cell lines. The Vande Woude team asked NCI chemist Ken Paull to search that database for other compounds with effects similar to PD09859's. LF proved to have the highest score.

Working with LF provided by anthrax researcher Steve Leppla at the National Institute of Dental Research in Bethesda, Maryland, Duesbery and colleagues then showed that the toxin prevents frog oocytes from maturing into eggs, indicating possible blockage of the MAPK pathway. The jam apparently happens, the researchers found, because LF clips off a piece of the enzyme responsible for activating MAPK, thereby crippling it. When



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Deadly bug. Lung infections by *B. anthracis* (shown here) can kill rapidly.

they sequenced two forms of this enzyme—MAPK kinase (MAPKK)—from LF-treated cells, for example, they found that the amino terminals of the proteins were missing either seven or nine amino acids.

Biochemist John Collier at Harvard University Medical School in Boston says that killing the host may be a key advantage of targeting this essential molecule. Unlike many microbial pathogens, *Bacillus anthracis* seems to depend on the death of its host to propagate. "As the animal decays, the bacteria are exposed to oxygen; they turn to spores and repopulate the soil," says Collier. "The host has to die for transmission to occur."

The identification of an LF target molecule represents the first step toward developing an antidote to the toxin. Natural anthrax infections, which are usually transmitted by animal products, are rare, but having such an antidote could be a boon if the pathogen were to be used in a terrorist attack. Although antibiotics kill *B. anthracis*, by the time characteristic symptoms appear, the bacteria are already multiplying wildly in the bloodstream and have produced massive amounts of circulating toxin, which can't be eliminated by killing the bacteria. Antibiotic treatment at this point doesn't help. "A drug that inhibits LF enzymatic activity might be able to act very quickly to block any further effects of LF on susceptible cells," says microbiologist Randall Holmes at the University of Colorado Health Sciences Center in Denver.

Still, the researchers aren't certain that LF owes its toxicity to its ability to cleave MAPKK. In fact, it's hard to explain how inactivating MAPKK would result in some of the known physiological effects of LF. In 1993, for example, Collier and Philip Hanna, currently at Duke University Medical Center in Durham, North Carolina, found that immune cells called macrophages mediate the harmful effects of LF in mice at least partly by producing inflammatory cytokines, immune cell–activating molecules that in excessive concentrations can cause some of the toxic reactions, including shock, seen in anthrax. Duesbery is now looking to see whether MAPKK inactivation is linked to overproduction of these compounds.

To try to show directly that MAPKK inactivation is responsible for LF's physiological effects, he is also investigating whether a MAPKK mutant that resists cleavage by LF can protect cells from the toxin. At the same time, the team is looking for other cellular substrates of LF.

As they pursue the many questions their findings have generated, the researchers seem to be enjoying the adventure. "I started out as a virologist and ended up studying oocyte maturation," says Vande Woude. "Now suddenly I'm looking anthrax in the face. It's pretty amazing."

–Evelyn Strauss

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