The spirochete that causes this longtime scourge is almost impossible to study in the laboratory, but modern genetic research has finally revealed its secrets

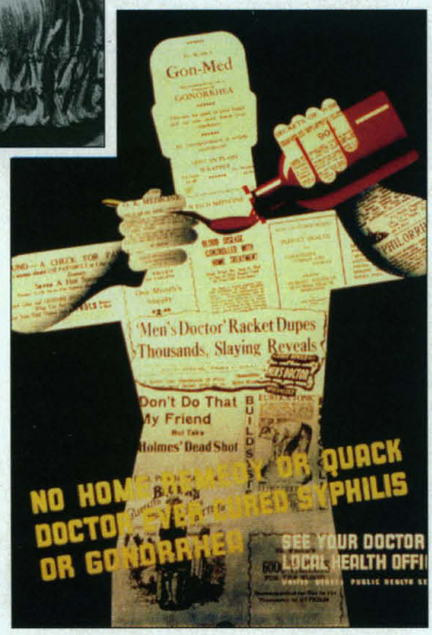
# Genome Reveals Wiles and Weak Points of Syphilis

When Steven Norris first started studying *Treponema pallidum* more than 20 years ago, the microbe quickly defeated him. A microbiologist at the University of Texas Health Science Center in Houston, Norris wanted to understand how this bacterium causes the many different symptoms of syphilis, a sexually transmitted disease that, if left untreated, can ultimately lead to insanity, cardiovascular problems, or death. His first step had been to try to grow the organism in a laboratory dish so that he could harvest its proteins. But as countless microbiologists before him had already learned, the spiral-shaped bacterium, or spirochete, so tenacious in the body, doesn't survive in the culture dish. It died within days no matter what the mix of nutrient media and growing conditions.

Now Norris has made a new assault on the syphilis pathogen using the tools of modern genetic research, and this time it has yielded its secrets. On page 375, Norris, Texas microbiologist George Weinstock, and a team led by Claire Fraser at The Institute for Genomic Research (TIGR) in Rockville, Maryland, describe the sequence of the 1.14 million base pairs that make up this microbe's genetic code. The microbiology community at large is thrilled. "[The sequence] is a big step forward," says Patricia Rosa, a molecular biologist at the National Institute of Allergy and Infectious Diseases' Rocky Mountain Laboratories in Hamilton, Montana. Although complete gene sequences are known for perhaps 14 other microbes,* including a half-dozen that cause disease, none has been so difficult to study with the traditional tools of microbiology.

And few pathogens have presented a medical conundrum as durable as syphilis, which once raged across Europe, decimating the ranks of royalty and forcing physicians to rely on extreme, often dubious treatments such as mercury vapors and arsenic. Penicillin ended the need for such measures, but even now the disease is common in developing countries, and there is no vaccine. One reason is that "it's a bug that knows very well how to evade the immune system," says Sheila Lukehart, a microbiologist at the University of Washington, Seattle, who has studied *T. pallidum* for more than 20 years. "Once you are infected, you are infected for life" if the disease is not treated, says Lukehart.

This genome sequence, released bit by bit over the past year, is already revealing clues to what makes the spirochete so tenacious and how it might be defeated with a vaccine. The sequences of individual genes are giving Rosa and others instant access to more than 1000 potential proteins—quite a leap from the two dozen or so known when the sequencing began in earnest. In the works are new diagnostic tests and potential vaccines based on the newly identified proteins. "[The genome] has saved us several years of work," notes Lukehart. "Because the organism is so difficult to work with, it is one that should have been sequenced first." The genome may also help investigators solve a long-standing mystery: where syphilis originated.




**When syphilis raged.** A 19th century French illustration and a later U.S. poster reflect attitudes toward this serious public health problem.

Norris and Weinstock's team began characterizing the genetic makeup of *T. pallidum* about 8 years ago. Their progress was slow, however. Because the microbe couldn't be grown in the lab, they had to get DNA by inoculating rabbits and then, weeks later, harvesting tissue and extracting the microbes. And they had to repeat this expensive and tedious procedure for each step of mapping and sequencing. A break came in 1995, when TIGR showed that it could rapidly sequence microbes in just a single step, by breaking their genomes up into many smaller, overlapping pieces, sequencing the pieces separately, and then matching up the overlapping ends to "assemble" the pieces into the complete genome (*Science*, 28 July 1995, p. 496; 20 October 1995, p. 397). Under Fraser's direction, the TIGR-Texas team was able to finish the sequence.

SYPHILIS: POÈME EN QUATRE CHANTS AUGUSTE MARSEILLE BARTHÉLEMY 4TH ED. PARIS, MARTINON, 1851
NATIONAL LIBRARY OF MEDICINE

### Portrait of a parasite

When the team took a look at the organism's complete collection of genes, they realized right away that "this was a metabolically crippled organism," says Norris. This spirochete has very few sets of enzymes for building complex molecules such as enzyme cofactors, fatty acids, or even nucleotides, the building blocks of DNA, and no genes for some of the proteins key to transporting electrons. Instead, it steals molecules essential to life from its host. Toward this end, 5% of its genes seem to code for transport proteins, which ferry in amino acids and carbohydrates, for example. This strong reliance on the host is what has made the organism so hard to culture, Norris says.

The genome also revealed possible clues to the spirochete's virulence, among them genes coding for proteins that may help the organism attach to and infiltrate skin, bone, heart, and other host tissue. It contained genes for 22 different lipoproteins, which serve a variety of functions—many unknown—in the spirochete but also provoke a very strong in-

* See www.tigr.org/tdb/tdb.html

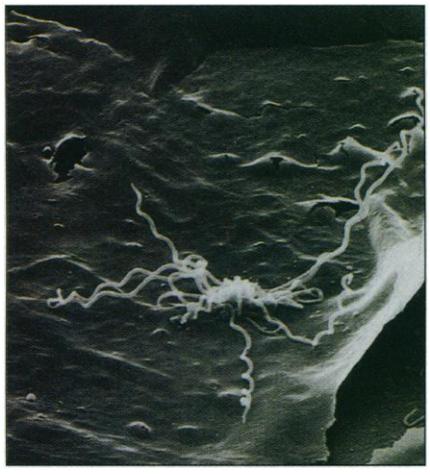
flammatory response in infected people.

For Lukehart, the most exciting features of the genome are some unexpected repetitive sequences. These repetitions slowed the sequencing by making it difficult to match up the ends of the sequence fragments in the right order. But they also represent a family of very similar genes that may explain the organism's ability to evade the immune system—and they could hold the key to an effective vaccine.

Previously, her group had found two of these genes. She was intrigued because the makeup of the proteins they code for suggested that the spirochete carries them on its surface. As the genome data became available, Lukehart and her colleagues were able to tap into the sequence database and find genes for similar proteins—a total of 10 more members of this protein family. These proteins seem to be the face the spirochete presents to the immune system, as Lukehart found when she exposed the organism to antibodies made to shortened recombinant versions of these proteins. The antibodies coated the spirochete, as if preparing it to be gobbled up by macrophages, cells that are part of the immune system's first line of defense.

This immune response sometimes does not defeat the spirochete, however, as the course of the disease shows. If the infection is not treated with antibiotics, the initial genital sores still heal, but months later the disease reemerges, causing a full-body rash, and years later it can surface again with its array of crippling, even fatal, symptoms. The variety of surface proteins revealed by the new sequence may aid this insidious behavior, Lukehart says. She speculates that the spirochete easily recombines the genes for these proteins, mixing up their amino acid makeup so that the immune system does not recognize them, allowing some spirochetes to survive the immune system's initial assault and reemerge later.

G. WEINSTOCK



**Elusive culprit.** The 12-micrometer-long spirochete, shown here in rabbit epithelium, thrives only in mammalian tissue.

The spirochete's ability to change its coat will complicate the development of an effective vaccine, as a vaccine that evokes immunity against a spirochete bearing one set of surface proteins would be blind to another variant, bearing a different set of proteins. But Lukehart and Norris think that these so-called TPR proteins still provide the best targets for a vaccine, because the antibody experiments suggest they stimulate a powerful immune reaction. "This is the only family of molecules with any evidence of showing protection," says Lukehart.

> **"Because the organism is so difficult to work with, it is one that should have been sequenced first."**
>
> —Sheila Lukehart

To get around the variability of these proteins, she and others hope to identify pieces of the proteins that are common to all strains and use one of those pieces to immunize against multiple strains. Alternatively, vaccine manufacturers could make cocktails of peptides that contain fragments of many different TPR variants—enough to protect against the spirochete in spite of its variations. Lukehart, Norris, and several other research teams are working feverishly to try out these and other strategies.

But even before these efforts yield a vaccine, the new sequence may help public health workers battle the disease. Syphilis is not very common in the United States, infecting about 9000 people a year, but the genital sores it causes make infected people more vulnerable to a still deadlier infection, HIV. For that reason, and because the disease reemerges as a minor epidemic once every decade or so, public health officials are considering a campaign to eliminate syphilis in the United States (see p. 353).

To do so, says Michael St. Louis, a physician at the Centers for Disease Control and Prevention in Atlanta who specializes in sexually transmitted diseases, health workers need a way to track the various strains of *T. pallidum*. "That's a very important part of understanding the transmission of infection, of how cases are linked in time and space, and why [*T. pallidum*] is able to persist in the population," St. Louis points out. Both Lukehart's group and a CDC team have already come up with schemes to track the strains based on the TPR genes, which vary from one strain to another.

### Genealogy of a plague

Weinstock, for his part, hopes to learn not where the microbe is going but where it came from. Syphilis first spread across Europe in the 16th century, and many experts have long assumed that *T. pallidum* came from the newly discovered Americas. "In retrospect, that isn't clear," says Weinstock.

He notes that the few records describing native people at the time of the Spanish conquest say little about a syphilislike disease. But the records do suggest that indigenous people in the tropical Americas had a skin condition called yaws, which is caused by a treponeme that is related to *T. pallidum*. Weinstock wonders whether it was yaws, rather than syphilis, that made the passage back to Europe, either from South America or from Africa, where it was also endemic. Facing a new environment in Europe—chilly weather and heavy clothing—it might have somehow mutated into a new, more virulent species, he speculates.

To test the idea, he is already comparing the *T. pallidum* genome to that of the yaws pathogen. His group is using the polymerase chain reaction to make many copies of both genomes, then chopping up the DNA with restriction enzymes, which cut DNA at specific sequences. Where the two genomes are the same, these molecular shears yield pieces of the same size. Thus far, the syphilis and yaws genomes seem incredibly similar, Norris says, much more so than, say, two strains of *Escherichia coli*. But he expects to find enough differences between syphilis and yaws so that he can double-check whether syphilis really was absent from the Americas. He hopes to examine pre-Columbian mummies from Latin America for DNA with the distinctive features of *T. pallidum*.

For Norris, the genome has revived an old interest: finding a way to grow the spirochete. That would open a more direct route to studying the genes revealed by the new sequence. Growing spirochetes in culture will also make it easier for pharmaceutical companies to identify new drugs or compounds that inhibit spirochete growth.

Having the genome in hand "is a very good place to start" learning what *T. pallidum* might need to survive outside mammalian tissue, says Rosa. Besides indicating that the organism has to import building blocks such as amino acids from its host, the genome also suggests that the spirochete lacks genes that protect it from damage by reactive oxygen molecules. It presumably can flourish only in low-oxygen environments. Based on these clues, says Norris, "I'm betting that we can figure out a way to culture it." And that, says Rosa, "would be wonderful." **–ELIZABETH PENNISI**