Catching the Rhythm of The Bacterial Twist

One bug's failure to divide has multiplied a scientist's insight into how biological structures assemble into complex shapes

THERE WAS LESS THAN HALF AN HOUR TO GO before the 5:00 PM showing of the children's cartoon movie *An American Tail: Fievel Goes West*, so Neil Mendelson didn't waste any time. Pacing in front of the screen at the Loew's Copley Place Cinema in Boston, he motioned to the projectionist and a 16-millimeter, black-and-white short began.

It was clear from the start that this little movie would be no box-office blockbuster. After all, its protagonist had none of the popular appeal of a Julia Roberts or a Kevin Costner. In fact, the star might seem about as unexciting as you can get: a mutant variety of the common bacterial species *Bacillus subtilis*. But it had a special allure, nevertheless. Since Mendelson discovered these mutant bacteria about 15 years ago, they and several of their kin have kept the University of Arizona microbiologist entranced with the intricacies of the threads and helical fibrils they can form. And now, at the annual winter meeting of the Materials Research Society meeting in Boston last month, he was presenting their architectural virtuosity to what at first might seem an unlikely audience.

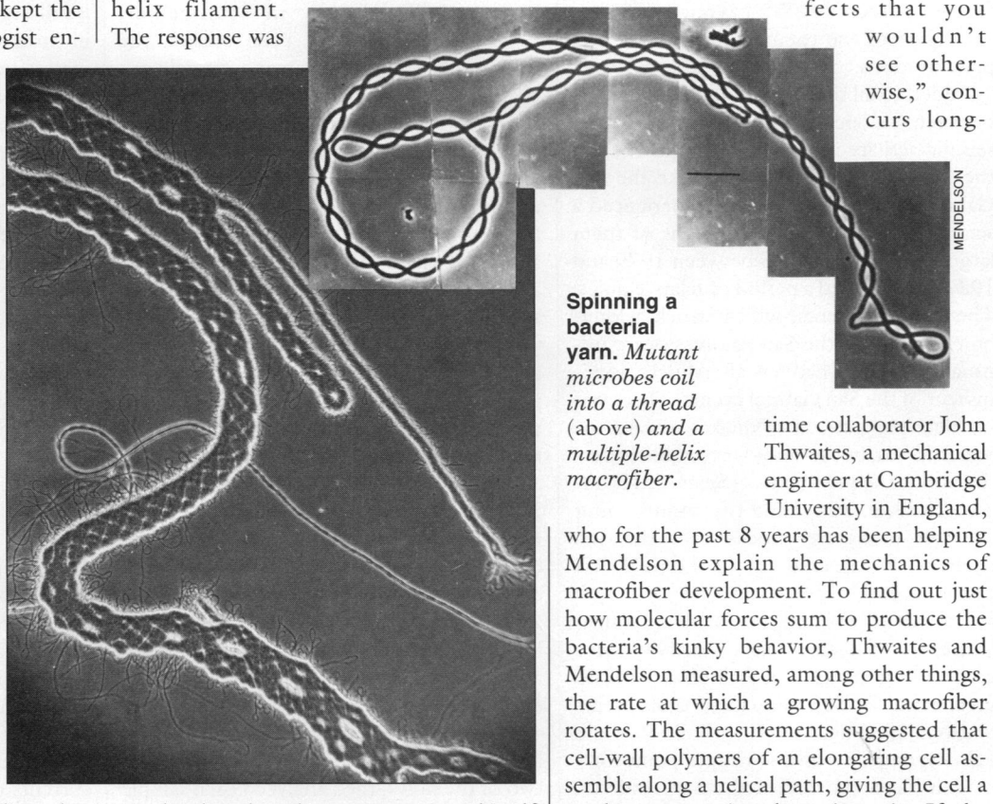
Why should materials scientists care about a mutant bug? The quirky bacteria, Mendelson thinks, hold lessons about how small-scale intermolecular forces can cause simple units to self-assemble into complicated structures—lessons that might be useful in the design of inorganic materials. And he is already testing the possibility that the bacterial fibrils could serve as scaffolding for other substances, possibly opening the way to unconventional drug-delivery systems or biosensors, manufactured on the backs of bacteria.

Indeed, the spectacle unfolding on the screen seemed to be holding the audience of biologically inclined materials scientists rapt—laughing intermittently the way people do when they see something bizarre happening. At first, somewhat below the resolution of the camera, a bacterium elongated on its way to dividing. But though it pinched around its waist, it failed to split completely. Then these Siamese twin daughter cells did the same thing, yielding four granddaughter cells that in turn elongated and pinched into eight great-granddaughters. In a cinematically sped up succession of generations, the chain of bacterial sausage links lengthened at an exponential rate.

Eventually one end of the growing bacterial filament, which had been flailing about like a fire hose on the loose, looped back on itself. The doubled-back end then snaked around the filament, forming a twisted double-stranded structure. A chortle could be heard from the audience when the doubling-back, looping, and twisting action repeated itself to make a four-ply multiple-helix filament. The response was even louder when the process repeated itself yet again to make an eight-ply fibril. Several more iterations led to a ropelike structure—what Mendelson identified in a talk after the film as a macrofiber. The bacterial twist-fest finally stopped when the macrofiber's growing stiffness offset the mechanical forces that had been generated in the growing, twisting bacterial ensemble. "There is no other building plan exactly like this in the biological world that I am aware of," Mendelson told the audience.

The hierarchy of helixes in the bacterial structure explains why symposium organizers asked Mendelson to participate in "Hierarchically Structured Materials," as the session was properly known. (The session had convened in the rented theater because the materials research conference had overflowed the nearby hotels.) And Mendelson's own 15-year devotion to the mutant bacterium is rooted, he says, in its potential for uncovering some of the factors governing biological shape formation. On the molecular level, Mendelson explains, the shape-forming behavior derives from chemical, electrostatic, and other interactions between the biological polymers that cross-link to form the bacterial cell walls. Because the mutant cells fail to separate, these molecular-level forces, which normally would affect an individual cell's shape only subtly, sum into the bending, twisting, and twining observed in growing macroscopic fibers.

The mutation "magnifies certain effects that you wouldn't see otherwise," concurs long-time collaborator John Thwaites, a mechanical engineer at Cambridge University in England, who for the past 8 years has been helping Mendelson explain the mechanics of macrofiber development. To find out just how molecular forces sum to produce the bacteria's kinky behavior, Thwaites and Mendelson measured, among other things, the rate at which a growing macrofiber rotates. The measurements suggested that cell-wall polymers of an elongating cell assemble along a helical path, giving the cell a tendency to twist along its axis. If the twisting is frustrated—for example, when a writhing filament doubles back and touches itself—the stress it generates steers the growing chain into higher-order helical structures.



Spinning a bacterial yarn. *Mutant microbes coil into a thread* (above) *and a multiple-helix macrofiber.*

"Trying to interpret what goes on in single cells by looking at these aggregates is quite clever," says John Galloway, who formerly studied the phenomenon of handedness in biological structures at Oxford University and is now associated with the Nuffield Foundation in London, a trust that supports biological and medical research. "You would never be able to study single cells this way."

Mendelson and Thwaites' strategy has also revealed that whether a fiber twists in a right- or left-hand sense, and how tightly it coils, depends on more than just the genetics of the strain. Temperature, acidity, and the presence of certain amino acids or other chemicals also affect the bacterial architecture, according to Mendelson. In almost all other cases of helical structure in biology, such as snail shells, genetics alone determines the handedness, Galloway says. "With these bacteria, you have this curious [nongenetic] flexibility."

Mendelson and Thwaites' model can successfully describe the coiling of the strands formed by another bacterial strain, the cyanobacterium *Mastitocladus laminosis*, says Edward Stevens, a molecular biologist at Memphis State University. But *Bacillus subtilis* may turn out to be more than a convenient organism for experiments into the genetics, chemistry, and mechanics of biological shape formation—it may also be a template for new materials.

Mendelson has found that the negative charges on the protein and carbohydrate polymers in the cell walls attract positively charged ions such as calcium and iron dissolved in a culture medium. These in turn serve as nucleation sites for salt formations. As a bacterial fibril is drawn from culture, almost the way nylon threads are drawn from a polymer melt, it can form "a brittle [bacterial] thread that looks like a little piece of bone," says Mendelson. He calls such bacteria/crystal composites bionites. By packaging biological material within an inorganic coating, Mendelson thinks, these composites might serve as vehicles for proteins, enzymes, and other products of biotechnology.

To probe this possibility, he grew a bionite using a bacterium genetically engineered to produce abnormally high amounts of an enzyme that can snip a test molecule, yielding a yellow product. When Mendelson dried one of the enzyme-bearing bionites, then rehydrated it and put it into a solution of the clear test molecule, the solution became yellow and then amber. "Enzyme activity was retained," he concludes. And that suggests to him that bionites built around genetically engineered bacteria might serve as implantable drug-delivery devices, or as flow-through gizmos that could perform specific chemical transformations on molecules passing through their porous coatings.

For microbiologist Mendelson, the experience of straying into an unfamiliar discipline like materials science is exhilarating. "I never dreamed that I would contribute something of interest to the materials and engineering world," he says. Of course, it's too early to say whether bionites will amount to more than a laboratory curiosity for Mendelson and Thwaites, who now are studying the strength, stiffness, and other physical properties of the structures. But for now they have every intention of bringing their movie-star bacteria back to a future materials science meeting in a sequel as dazzling as the original. ■ IVAN AMATO

Twin Study Links Genes to Homosexuality

A new study of twins and adoptive brothers has turned up fresh evidence that genes play a strong role in the development of homosexuality. Indeed, the researchers who conducted the study—psychologist J. Michael Bailey of Northwestern University and Boston University psychiatrist Richard C. Pillard—estimate in a paper published in the December *Archives of General Psychiatry* that the genetic component of homosexuality is somewhere between 30% and 70%.

Bailey and Pillard, who recruited subjects through ads in gay publications, studied three groups: identical twins, fraternal twins, and men with adoptive brothers. They interviewed 161 homosexual men and sent questionnaires to their twins or adoptive brothers. Of the 170 relatives whose sexual orientation could be rated, 52% of the identical twins, 22% of the fraternal twins, and 11% of the adoptive brothers were also homosexual.

Although these findings are consistent with the view that homosexuality has a large genetic factor, one finding was not: The researchers determined indirectly through reports from the subjects of the study that only 9.2% of the non-twin biological brothers of homosexuals were themselves homosexual. Bailey and Pillard suspect that this may be a chance finding, however. They believe that the true rate is closer to that shown in an earlier study headed by Pillard, in which brothers of gay men were found to be four to five times as likely as controls to be homosexual. "Obviously, this needs to be replicated," says Bailey.

The investigators were not able to be more precise in their heritability estimates because of uncertainty about the base rate of homosexuality in the population, which is estimated at between 4% and 10% of males. Bailey says that if the 4% figure is more accurate—as he surmises based on recent surveys—then heritability for homosexuality would be at least 50%.

That still leaves a lot of room for environmental influences but, in an interview with *Science*, Bailey argued that these are likely to be predominantly biological ones, in the form of random hormonal variations, rather than psychosocial ones. That, he says, could explain why fraternal twins—who share the same prenatal environment—are more similar in sexual orientation than siblings. He dismisses an alternative explanation, that postnatal environmental influences play a key role: "No one has ever found a postnatal social environmental influence for homosexual orientation—and they have looked plenty," he says.

Other scientists, while endorsing the central finding of the study, are more guarded in speculating about the source of nongenetic influences. Neuroscientist Sandra Witelson of MacMaster University in Ontario says she thinks the paper "shows very conclusively that there is a genetic component in homosexuality." But, "I don't think there are enough data at this point to exclude a postnatal social learning component." Psychiatrist Leonard Heston of the University of Washington in Tacoma says the study confirms the conclusions of earlier twin studies of homosexuality—most of them very small—which "all point in the same direction": that identical twins are concordant for homosexuality about 50% of the time. He agrees that there is no good evidence for postnatal environmental influences but says, "I'm a complete agnostic on that."

Bailey theorizes that the genes implicated in homosexuality are probably those involved in prenatal brain development—specifically in masculinization of the hypothalamus during sexual differentiation. That suggestion is based on animal and human studies that point to the importance of the hypothalamus for sexual behavior, and recently published research by Simon LeVay of the Salk Institute (*Science*, 30 August, p. 1034), who found a certain cell group in the hypothalamus to be smaller in homosexual than heterosexual men. "Our working hypothesis is that these genes affect the part of the brain that he [LeVay] studied," says Bailey. ■ CONSTANCE HOLDEN