MICROBIOLOGY

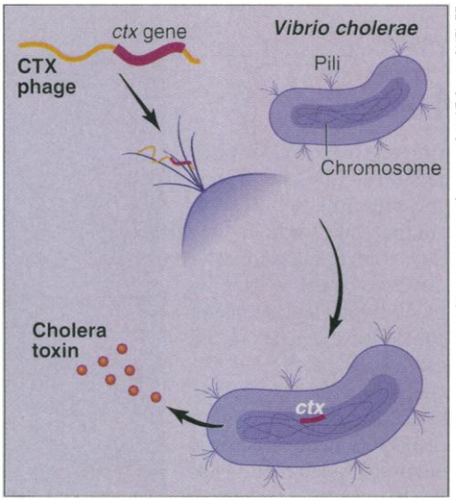
Phage Transfer: A New Player Turns Up in Cholera Infection

Researchers have long puzzled over the natural history of cholera, one of the major human scourges, for cholera bacteria are normally benign, water-dwelling organisms. But sometimes *Vibrio cholerae* turns virulent, and infection is transformed from a mild or even unnoticeable event into a deadly disease, one that has erupted in seven pandemics since the 1870s; the latest outbreak sickened 200,000 people in Southeast Asia in the early 1990s. Although the details of this Jekyll-to-Hyde metamorphosis have remained a mystery, in recent years researchers have recognized a crucial step: Somehow, the disease-causing bacteria appear to acquire genes from other strains and so pick up such menacing characteristics as the toxin responsible for cholera's life-threatening diarrhea. But no one has known just how this gene transfer occurred.

Now, new research reported on page 1910 shows that the gene that codes for the cholera toxin is carried by an unusual bacteriophage, a virus that infects *V. cholerae* itself. And this newly discovered viral pathogen is equipped with a package of other genes to help it spread through the bacterial population. The entry of this new player into cholera infection may force a rethinking of vaccine development and raises fresh questions about the bacteria's evolution and life cycle. The research also suggests that this particular type of bacteriophage may be behind the mysterious transfer of other virulence genes among bacteria. "It's one of the most important findings in cholera research in the last 10 years," says microbiologist Stephen Richardson of the Wake Forest University Medical Center in Winston-Salem, North Carolina. "This looks like an entirely new agent by which virulence genes can be acquired by pathogenic organisms."

The researchers who found the virus, microbiologists Matthew Waldor and John Mekalanos of Harvard Medical School, were originally working on cholera vaccines—an urgent quest because of the disease's toll of suffering and death. But efforts to create an effective vaccine from whole killed bacteria or inert fragments have not produced long-lived immunity. So most researchers now focus on live vaccines, using attenuated bacteria that lack disease-causing genes. Such vaccines raise important safety questions, however, especially if one cholera strain can pick up toxin genes from another.

Waldor and Mekalanos knew that there was plenty of evidence for such horizontal transfer of virulence genes among other groups of bacteria, although there were only hints of it in the case of cholera. Virulence genes are often clustered in "pathogenicity islands," discrete genetic elements within the bacterial chromosome (*Science*, 31 May, p. 1261). In most cases, the mechanism of gene transfer is unknown, but in some, such as the human disease diphtheria, the disease-causing toxin is known to be encoded by a bacteriophage, which can transmit the toxin gene between pathogenic and benign strains of bacteria, says Mekalanos.



SOURCE: J. MEKALANOS ILLUSTRATION: K. SUTLIFF

Deadly acquisition. A phage bearing the CTX gene *(top left)* infects a benign cholera bacteria *(top right)*; the resulting strain can produce cholera toxin and cause disease.

The gene for cholera toxin is also located within a distinct genetic fragment, called the CTX element, which is known to include at least six other genes. Researchers had previously failed to find any evidence that the CTX element can move from pathogenic to benign strains. But the medical implications led Waldor and Mekalanos to keep looking.

To study the transmissibility of the CTX element, the team replaced the cholera toxin gene with a gene conferring antibiotic resistance. Then they cocultured strains bearing the modified CTX element with normal strains, looked for resistant cells, and examined which strain these cells were derived from. "A low but detectable frequency of cells originally lacked CTX but later showed antibiotic resistance—and so somehow acquired the modified CTX element," says Mekalanos.

What's more, when the team studied the mechanism of transfer, they found that CTX could be transferred from the culture liquid to fresh cells with no direct contact between the bacteria, suggesting that a viruslike particle was involved. Waldor and Mekalanos verified this by taking electron micrographs of the virus itself. They saw simple, curved filaments bundled together in a structure resembling a filamentous bacteriophage, a kind of phage never before shown to be involved in the transfer of virulence genes.

But how, exactly, does the phage infect the cholera bacterium? The researchers already had an important clue: Previous work on other filamentous bacteriophages had shown that these viruses use particular molecules that project from the bacterial cell membrane, called pili molecules, as receptors to gain entry into the cell. Many strains of *V. cholerae* also have such pili, which the bacterium may use to adhere to the wall of the gut in its mammalian host. Researchers also knew that the same bacterial gene, *toxR*, regulates both the toxin gene and the gene that encodes the pili, which suggested that the pili have some role in virulence. "It looked as if the pili were one of the viruses' secret weapons," says Mekalanos.

These clues led Waldor and Mekalanos to test mutant strains of bacteria that were unable to express pili. The mutants were completely resistant to infection with the CTX phage, suggesting that the pili do indeed act as receptors for the bacteriophage. Finally, to make sure that their test tube findings apply to bacteria in their natural setting, the pair studied bacteria in the mammalian gut. "It's vital to find out what goes on in the body," says Mekalanos. They found that the phages could be transferred from one bacterial strain to another—and at a significantly higher rate than in test tube cultures, suggesting that other factors in the gut environment help the phage spread.

All this adds up to a compelling though still incomplete picture: The filamentous bacteriophage gains entry to the cell by way of the pili, incorporates its genes into the bacterial chromosome, and, in some manner involving the *toxR* gene, causes the cell to start pumping out cholera toxin. Thus the phage plays a crucial role in the development of virulence in the bacteria. Indeed, researchers speculate that all pathogenic strains of *V. cholerae* may have ultimately arisen from this kind of gene-transfer event from a bacteriophage. "This is an impressive piece of classical microbiology research," sums up bacteriophage expert Marjorie Russel of Rockefeller University in New York. "It's a new way harmless organisms can take up a collection of virulence genes in one swoop," agrees Richardson of Wake Forest.

But the work raises questions about the direction of vaccine research, because of the possibility that the live bacteria used in vaccines could acquire virulence genes via the bacteriophage. "At the theoretical level it's very frightening," says microbiologist Philippe Sansonetti of the Pasteur Institute in Paris. "It looks as if there's always the potential for reversion of harmless strains to toxic strains." Indeed, says Richardson, "there are 150 different strains of *Vibrio cholerae*, but up until recently only strains within one major type have been involved in pandemics. But it looks [now] as if other strains may be able to acquire the toxin gene via the CTX phage."

Still, most forms of *V. cholerae* in the environment are nonpathogenic, suggesting that it is relatively rare for nontoxic strains to acquire the CTX element and then go on to infect people. And Mekalanos and other researchers believe that the new information provides a guide to designing vaccine strains that will resist infection by the bacteriophage. "We now have the knowledge needed to tackle the problem in an informed way," Mekalanos says. For example, classical vaccine strains readily produce pili in vitro and so are vulnerable to infection by the CTX-bearing phage. Strains that don't produce pili in vitro are probably better candidates, he says.

Meanwhile, the discovery that a bacterial gene, *toxR*, coregulates both the cholera toxin gene, from the phage, and the gene for the phage's receptor points to a subtle but complex evolutionary dance between bacteria, bacteriophage, and mammalian host—a dance led by the phage, which exploits the bacterial control of the pili gene to produce toxin. "There was absolutely no reason to understand why the toxin was coregulated with the pili before this work," says microbiologist Staffan Normark of the Karolinska Institute in Stockholm, Sweden. "It shows that pathogenesis cannot be studied apart from the wider life cycle of the pathogen." He wonders if infection by bacteriophages in the normal life cycle of *V. cholerae*, which can survive and reproduce independently of mammalian hosts in freshwater environments, may have implications for human disease.

Whatever the answers to these questions, the new results will stir fresh interest in filamentous bacteriophages as candidates for horizontal transmission of virulence genes in a number of other gram-negative bacteria, such as *Salmonella* and *Yersinia*, which causes bubonic plague, says Mekalanos: "This could be the tip of an iceberg."

–Nigel Williams

GEOPHYSICS

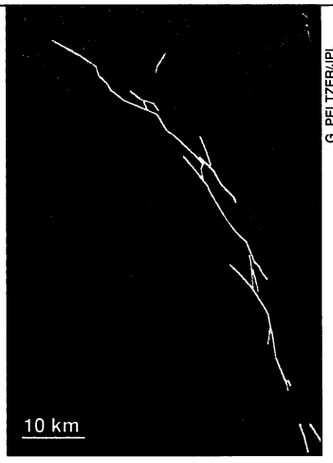
Watching the Earth Move

Geophysicists keep watch over even the most minute changes in Earth's supple crust, because these subtle undulations are driven by the same powerful forces that drive earthquakes. But monitoring these changes along faults extending hundreds of kilometers requires dense networks of strainometers buried below ground or Global Positioning System (GPS) instruments on the surface. Now, however, researchers have perfected a space-based technique that can watch the stirrings of great expanses of crust in one sweep.

Called synthetic aperture radar (SAR) interferometry, this technique measures crustal distortions from slight changes in the travel time of a radar beam played across the surface from a satellite. And while SAR interferometry has already shown its prowess at mapping the large deformations—tens of centimeters—caused in a moment by earthquakes, recent results from the SAR aboard the European Space Agency's ERS-1 satellite show that the radar can also catch localized, centimeter-scale deformation slowly occurring between earthquakes. Preliminary evidence suggests that the technique might also reveal growing strain on a seemingly quiescent fault.

As a result, tectonophysicist Wayne Thatcher of the U.S. Geological Survey in Menlo Park, California, predicts that the growth of SAR interferometry "in the next several years will be at least as explosive as that of GPS in the late '80s and early '90s." The new radar will help probe not just faults straining under growing crustal stress but also volcanoes swelling with magma and land subsiding with the withdrawal of oil or water. "It's going to be the technique of choice for a lot of things," says Thatcher.

Measuring a surface change of a few centimeters from 800 kilometers up is quite a feat, but the principle behind it is simple. Like ordinary radar, a SAR measures the distance to the surface by clocking the travel time of a microwave signal bounced off the surface. A SAR like the one aboard ERS-1 makes such a measurement on every 80-meter-wide spot across a 100-kilometer-wide swath as the spacecraft passes overhead. On its next, slightly different orbit, the radar measures a new distance, which differs because of any intervening deformation and the spacecraft's new perspective. After removing the perspective effect, the remaining tiny difference between passes is measured by letting the two slightly out-of-phase signals interfere with each other to form an image of concentric interference fringes; each fringe in an interferogram represents a displacement of 28 millimeters.



G. PELTZER/JPL

Radar rainbows. Interference patterns gathered by orbiting radar show crustal deformation due to the rupture of the Landers fault.

Thatcher notes that SAR worked "spectacularly well" in mapping out up to 56 centimeters of near-vertical deformation caused by a few seconds of fault rupture in southern California's magnitude 7.3 Landers earthquake in 1992. And at last month's meeting of the American Geophysical Union (AGU), Gilles Peltzer of the Jet Propulsion Laboratory in Pasadena, California, and his colleagues showed detailed interferograms of deformation long after the quake, between August 1992 and September 1995. Where the fault jogs 10 kilometers right or left, the crust rose or subsided about 5 centimeters—a deformation too localized to appear in any GPS survey.

This distortion is in the opposite sense to what the quake would have induced and so may reflect a postquake readjustment as water was sucked into areas stretched by the quake and squeezed out of compressed regions. Fluid flow may also account for measurements reported at last fall's AGU meeting by Didier Massonnet and Hélène Vadon of the National Center of Space Studies in Toulouse, France, and by Thatcher. Using SAR interferometry, they found that a trough that formed along the Landers fault during the quake has since risen, presumably as water flowed into the freshly shattered fault zone. This postseismic flow could be one part of a previously proposed cycle that eventually produces high fluid pressures capable of triggering the next earthquake (*Science*, 6 March 1992, p. 1210).

Peltzer's preliminary interferogram of the Los Angeles basin underscored the value of the technique. Oil fields and aquifers stood out as fringe bull's-eyes where pumping had caused up to 12 centimeters of subsidence. The results also showed apparent deformation along the Newport-Inglewood fault, which is seismically quiet now but in theory is capable of unleashing a devastating magnitude 7 earthquake. Stress may be building there, but ERS-1's next passes should help determine the meaning of this slight crustal shift.

–Richard A. Kerr