New Phage Image Analysis Unveils Structural and Mechanistic Surprises

A powerful new imaging analysis method provides very high-resolution pictures of intact bacteriophage, revealing details about how they infect their bacterial hosts, according to Wen Jiang, now of Purdue University in West Lafayette, Ind., Wah Chiu of Baylor College of Medicine in Houston, Tex., Jonathan King of the Massachusetts Institute of Technology in Cambridge, Mass., and their collaborators, whose findings appear in the February 2 issue of *Nature*. Although several other imaging methods do well at showing viral components, isolating those pieces often casts doubt about how closely they match with their shapes in intact viruses.

In this instance, such doubts were justified. Previous images of the isolated portal structure of phage Epsilon 15, which infects *Salmonella*, portrayed it as containing either a 12-, 13-, or 14-member ring. However, in the intact phage particle, that ring is unmistakably 12-membered.

The portal is the site through which the phages inject their DNA into host cells, and it is considered a prototype nanomachine. The images also reveal that this phage unexpectedly contains a core structure, which can be found in many other types of virus. Although its function in this case is unknown, the researchers speculate that the Epsilon 15 core takes part in injecting viral DNA into host cells.

The analysis also reveals clear images of the six tail spikes on the phage surface, or capsid, which is made of protein. Before, researchers thought that all six spikes were used to attach phage particles to the surfaces of host bacterial cells during the first stage of an infection. “Because they’re so off-kilter,” Jiang says, “only two of the spikes actually grasp the... surface.”

Developing these spectacular views not only requires good hardware for producing the cryoelectron micrographs that were used in the analysis. It also depends on specialized software. Indeed, Jiang developed an algorithm for constructing three-dimensional models based on data extracted from about 15,000 two-dimensional images, each taken from slightly different angles. These compiled images enable the researchers to view phage DNA and core structures that lie beneath the capsid surface. “While before we could only see virus parts that were symmetric, we can now see those that have nonsymmetric structures,” he says.

“This is the most complete, detailed structural view we have of any complex virus,” says Roger Hendrix of the University of Pittsburgh. “The non-symmetric parts of the virus are particularly interesting; they include parts of the DNA packaging machinery and proteins that help the DNA get through the cell envelope during infection.”

More generally, the phage research
“sets a new standard” for determining virus structures by cryoelectron microscopy, says virologist Dwight Anderson of the University of Minnesota, Minneapolis. Further, he adds, the methods appear to be broadly applicable to “other multisubunit nanomachines of mixed symmetry that synthesize, fold, assemble, translocate, or degrade macromolecules.”

Still, there are technical limits to overcome, according to Jiang. “We remain limited to observing those viruses that are identical from one individual particle to the next—which, sadly, is only a small portion of the viral species that are out there,” he says. “But this is a major step toward our goal of seeing them all.” These new bacteriophage images also could prove valuable in other ways, he adds. “The properties of bacteriophages have frequently paved the way for major discoveries in biology and medicine.” Indeed, researchers are approaching phage with renewed interest, seeking to use them in agriculture and also to treat human infections, including as a means for overcoming antibiotic resistance (ASM News, October 2005, p. 453).

David Holzman
David Holzman writes from Lexington, Mass.

Protective Mask Shortages Worrying Officials Anticipating Pandemic Flu

As they contemplate an influenza pandemic, officials at the Department of Health and Human Services (HHS) and other federal agencies worry not only about developing a vaccine and stockpiling antiviral drugs but also about possible shortages of key items such as masks and respirators that could hinder the spread of flu viruses. At the request of HHS officials, the Institute of Medicine (IOM) in Washington, D.C., convened a panel whose members met in January to begin reviewing this issue. They are asking, among other questions, whether simple design changes could enable reuse of masks to overcome anticipated shortages.

This inquiry will not be so easy, panel members quickly learned. For one thing, redesign is complicated because of differences in construction and application among different kinds of face masks, including those called respirators and those used in ordinary surgical or other health care settings. Other complications arise because of differences among mask users—ranging from infants with small faces to men with big beards, or firefighters and ambulance drivers responding to emergencies.

Officials from the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., and elsewhere in HHS are not recommending that members of the general public don face masks to avoid either pandemic or seasonal flu viruses. In general, the flu virus appears to spread not so much by aerosols but mainly in respiratory secretions, which are generally transferred by hand-to-surface and hand-to-face contacts. Although masks may sometimes be effective when they keep people from touching their own faces, they are not a particularly practical public health measure for controlling flu outbreaks. Even so, they may well come into wider use because wearing masks makes it look as if something special is being done.

Wearing a surgical mask mainly protects those nearby from someone who is infected or might be carrying pathogens. Thus, surgeons and other medical staff in an operating room (OR) wear masks mainly to protect their patients after incisions expose tissues to intrusive microbial pathogens. Only to a limited extent do those masks also protect personnel in the OR and in other settings against infections from patients.

Meanwhile, health care staff may wear filter-equipped respirators when doing intrusive procedures, such as intubations or endoscopies, to protect themselves from aerosols of infectious agents that patients may be carrying. Here again because of the primary way in which flu viruses spread through secretions, health care workers are not likely to use respirators routinely when caring for such patients. In a high-volume outbreak of

Polio Eradication Program May Be Back on Track

The global polio eradication program appears to be back on track, according to officials of the World Health Organization in Geneva, Switzerland, Rotary International, UNICEF, and the Centers for Disease Control and Prevention in Atlanta, Ga. Early this year, they declared two more nations, Egypt and Niger, free of this viral disease, leaving only four—Nigeria, Afghanistan, India, and Pakistan—where reservoirs of wild strains of the virus continue to cause cases locally. For these and other “mop-up” campaigns, officials are switching to use of monovalent vaccines. For example, a vaccine targeting type-1 poliovirus in Egypt was used during the successful vaccination campaign there last year, officials say. Similar strategies reduced cases of polio in India and Pakistan during the last quarter of 2005 by more than half compared with the previous year. Between 1988 and 2004, these global efforts reduced polio cases from 350,000 annually to a low of 1,189. In 2005, however, the number of cases rose to 1,831, during an outbreak that moved from northern Nigeria to 21 previously polio-free countries.

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flu during which many patients might also be ill with bacterial pneumonias, however, respirators could well come into wider use.

In general, surgical masks are simpler in design and cheaper to make than are respirators. Complex, pricier models of respirators are more readily used for extensive periods, and some high-end models may be reused after being decontaminated. However, more-effective models do not lend themselves to being made cheaply for mass use.

Current production of such products is near full capacity, as manufacturers fulfill large orders from several countries in Europe and Asia as well as from the U.S. military, according to representatives of several companies. When a company decides to expand production capacity, it takes about six months to do so. Even with expanded production capacity, however, a rampaging pandemic still might disrupt supplies of materials needed for making masks and respirators.

Representatives of emergency workers such as firefighters and others who work in public settings express concern that changing the design of such devices to make them more widely available could lead to a sacrifice in their effectiveness, endangering those who rely on them. Indeed, some emergency workers insist that respirators of higher quality should be made more widely available.

Recommendations from the IOM panel are expected later this year.

Jeffrey L. Fox
Jeffrey L. Fox is the Microbe Current Topics and Features Editor.

Progress Developing, Using Various Vaccines

Amid laments about difficulties developing antimicrobial drugs, investigators evince some frustration but often more celebration regarding vaccine development. Despite unmet challenges, progress can be measured on many fronts, according to experts who described vaccines to protect against malaria, influenza, and other viral or bacterial pathogens during the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) last December in Washington, D.C. Here are some highlights.

Although malaria vaccines fall short of the ideal, one such product for protecting children against Plasmodium falciparum is now on an accelerated development and clinical testing track, according to W. Ripley Ballou of GlaxoSmithKline Biologics (GSK) in Rixensart, Belgium, who spoke during the symposium, “New Vaccines: New Approaches to Disease Prevention.” This disease is responsible for as many as 3,000 deaths per day in sub-Saharan Africa, he says.

The GSK malaria vaccine reduces clinical signs of malaria by about 30% among children older than two years of age and severe cases of malaria by 50%, according to Ballou, who summarized findings from a recent clinical trial in Mozambique. Despite significant progress, plans call for modifying this vaccine in several ways, including by adding an adjuvant to improve immune responses and other specific antigens to elicit immune responses to the form of the parasite that circulates in red blood cells, he says. Optimizing vaccine schedules, vaccinating very young children in poor countries with multiple doses, convincing regulatory authorities to approve vaccine use abroad, and finding affordable ways to distribute the vaccine are among the looming challenges.

Influenza represents another pressing issue for vaccine developers. No matter what strategy proves effective, at least 900 million doses of vaccine—about threefold more doses than typically are available each year—will be needed worldwide to cope with an influenza pandemic, according to Klaus Stohr of the World Health Organization in Geneva, Switzerland, who spoke during the ICAAC Keynote Session. Several efforts now are aiming to develop a “universal” vaccine, including some efforts that focus on viral antigens other than hemagglutinin (HA) and neuraminidase that are standard components of the widely used, seasonal flu vaccine.

For example, the “highly conserved” M2 antigen is part of the “ectodomain” of the flu virus, and is the key component of an experimental vaccine that protects mice against viral challenges.

Malaria Parasites Found in Mammalian Lymph Nodes

After Plasmodium-carrying mosquitoes bite mice, some of the parasites remain in the skin and about 25% of the parasites invade nearby lymphatic vessels and nodes, according to Howard Hughes Medical International scholar Robert Ménard of the Pasteur Institute of Paris, France. Although immune system cells in those nodes destroy many parasites, some escape damage and develop into forms that were thought to occur only in the liver. By 52 hours after a mosquito bite, no parasites remain in those lymph nodes, which suggests that the parasites neither develop nor cause symptoms from these sites, he says, adding: “But even partially developed or destroyed parasites could significantly affect how the immune system responds to infection.” These findings are detailed in the January 22, 2006, issue of Nature Medicine.
according to William Ernst of Molecular Express in Los Angeles, Calif., and his collaborators there, at Cal Poly Pomona in nearby Pomona, and at the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. Because of its high degree of conservation, these researchers are testing whether a “limited number” of M2 variants could provide broader protection. Drawbacks to this approach are that antibodies to this viral antigen are difficult to detect among humans following bouts of flu, and that the experimental vaccine being tested only partly protects inoculated mice.

Another approach to protect against flu involves a DNA-based vaccine that encodes a HA antigen, which apparently confers “cross-protective” activity in humans against several similar flu strains whose HA antigens “drifted” apart, according to John Beadle of PowderMed of Oxford, United Kingdom, who presented findings during a late-breaker poster session. This approach, being evaluated in a phase-I clinical trial, might be useful not only against seasonal variants of the flu virus but also against the pandemic threat, he says.

Other types of vaccines to protect against pandemic flu are being considered, including newer versions of FluMist, which is administered intranasally to protect against seasonal flu, and also vaccines administered intradermally with adjuvants, according to John Treanor of the University of Rochester in Rochester, N.Y. A key goal is to deliver adequate protection with lower doses of antigens, particularly if demand for vaccine during a pandemic challenges production capacities.

Jeffrey L. Fox

Technique Could Help To Identify Novel Antibacterial Targets

A new “predator-prey” system can help in identifying infection-critical genes of bacterial pathogens such as Klebsiella pneumoniae and also which mutants are less capable of producing pneumonia in mice, according to Mohammed Benghezal and his colleagues from the company Athelas and the University of Geneva, both in Geneva, Switzerland. They presented their findings during the poster session “Pathogenesis I” at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) last December in Washington, D.C. Their poster, “New Targets for Antibacterial Drugs,” received the ICAAC Program Committee Award for research excellence.

In this test system K. pneumoniae, which are gobbled up, are considered the prey, while cells of the amoeba Dictyostelium discoideum, which phagocytose such bacteria, are the predator. “To establish an infection in a host such as a mammal, bacteria have to face host defenses requiring the activation of survival mechanisms,” Benghezal says. “We developed a simple prey-predator model system with the goal to identify... particular mechanisms... essential to bacterial survival within a host.”

The assay involves spreading amoebae on a “lawn” of bacteria grown to confluence on a solid medium. When the cells of D. discoideum engulf susceptible cells of K. pneumoniae, they leave plaques on the surface of the medium. If those bacteria are virulent, they can short-circuit growth of D. discoideum, preventing its disturbance of the lawn. However, after Benghezal and his collaborators mutagenized K. pneumoniae and used the mutants to form such lawns, many plaques appeared, indicating that the mutation had disrupted the virulence of many of the pathogens.

Among some of these mutants, changes occurred in genes known to be important for bacterial virulence, including genes involved specifically in producing lipopolysaccharide or the amino acid tryptophan, or in regulating transcription—thus providing evidence that the model system can peg virulence-related genes. Addition-
Bacteria, Plant Viruses Unexpectedly Plentiful, Active in GI Tracts

Despite being bathed by acids, *Helicobacter pylori* thrives in the stomach, and RNA viruses such as Norwalk virus and rotavirus, which both cause gastroenteritis, withstand this inhospitable chamber and their passage through the lower gastrointestinal tract (GI). After probing this niche for bacteria or viruses, two separate teams of researchers were surprised to find abundant plant viruses in one case, and a broad variety of bacteria in the other.

Viral ecologist Forest Rohwer and his collaborators at San Diego State University in California and the Genome Institute of Singapore expected to find mostly bacteriophages in fecal samples from healthy human volunteers, he says. Instead, 97% of the RNA viral sequences that they identified from such samples correspond to intact viruses that infect edible plants such as peppers, corn, oats, and grapes. “We were really surprised to see that the viral community was not bacterial viruses but plant viruses,” says Rohwer, referring to findings summarized in the January 2006 issue of *PLOS Biology*.

The most abundant viral sequence is that corresponding to the pepper mild mottle virus (PMMV), a pathogen of chili peppers. The researchers measured PMMV in foods eaten by the volunteers, whose diets were rich in chili peppers and sauces. Although they did not detect PMMV in fresh peppers, they detected high levels in samples of chili powders and hot sauces. “PMMV clearly is coming from food,” says Rohwer, who expects to find other kinds of plant viruses in feces of people who eat other foods. These plant-associated viruses do not appear to harm humans through whom they pass, according to Rohwer and his collaborators. They suspect that the viruses are carried within plant seeds, and thus are well protected when passing through GI tracts.

To assess whether those viral nucleic acids derive from intact PMMV, the researchers inoculated pepper plants with suspensions made from PMMV-positive fecal samples. Within a week, the inoculated plants showed telltale signs of being infected with PMMV, while uninoculated plants did not. Thus, applying feces or sewage sludge as fertilizer on crops could spread diseases to them, Rohwer cautions. He speculates that germinating plant seeds in feces or sludge release intact viruses that infect seedlings.

Meanwhile, medical microbiologist David Relman at Stanford University in Stanford, Calif., and his collaborators set their sights slightly higher anatomically by focusing on bacteria in the human stomach. Except for *Helicobacter pylori* being associated with ulcers, the stomach “is a missing habitat in the microbial continuity of the gut,” Relman says, with the lower GI considered microbially rich but the stomach, impoverished.

However, following a nucleic acid-based analysis of endoscopic samples from 23 volunteers, the researchers identified 128 bacterial phylotypes, dominated by five major phyla, namely *Proteobacteria, Firmicutes, Bacte-
roidetes, Actinobacteria, and Fusobacteria, as well as Deinococcus, an extremophile never before reported in humans. “The organisms we detected in the stomach clearly are not simply a random subset of those found in the mouth and esophagus,” Relman says. Detailed findings appear in the January 17, 2006, Proceedings of the National Academy of Sciences.

Although many of those bacteria are nonpathogenic, judging from their taxonomic assignments, some of them might account for stomach ailments that often prove difficult to diagnose, according to Relman. This bacterial community, which is notably heterogeneous, “gives us a window into the normal and abnormal physiology of the stomach,” he says. Although patients sometimes undergo endoscopies to try to find a cause for nonspecific symptoms such as nausea, seldom are microbial culprits other than H. pylori detected. However, he points out, other types of previously unsuspected bacteria might be affecting such patients and may account for their symptoms.

At sites in the body where microbial communities are better characterized, such as the mouth, “we find that they are more important for health and disease than previously thought,” Relman says. Particular profiles of microorganisms in the stomach may protect some people from being infected by H. pylori or could hinder digestion of particular nutrients. Further analyses of stomach-situated bacteria among larger numbers of people and how those bacteria change over time could help us to better understand gastric physiology, he says.

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MRSA, Multidrug-Resistant A. baumannii Stirring Similar Concerns

The methicillin-resistant Staphylococcus aureus (MRSA) epidemic continues to shift, driving a search for novel antibiotics with which to slow its progress. And, although considerably more restricted, infections caused by multidrug-resistant (MDR) Acinetobacter baumannii—called “the gram-negative MRSA” in some circles—are stirring similar concerns, according to researchers who spoke during the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) last December in Washington, D.C.

A. baumannii is a jack-of-all-trades pathogen, primarily infecting the respiratory tract but found in the bladder, in skin and soft tissues, in wounds, and in the bloodstream, according to Harald Seifert of the University of Cologne in Cologne, Germany, one of several participants during the ICAAC symposium “Multiresistant Acinetobacter: a Worldwide Pandemic.” The microbe may be picked up following natural disasters, such as earthquakes, or following unnatural disasters, including bomb blasts in battles and terrorist attacks. Once it reaches health care settings such as hospitals, A. baumannii tends to enconce itself as a nosocomial agent, spreading with epidemic ease, he says.

Drug-resistant infections caused by A. baumannii rose sharply during the past decade, according to Anthony Harris of the University of Maryland Medical Center in Baltimore. Unlike many other gram-negative pathogens, this one is “more hardy” in environmental settings, making it particularly difficult to eliminate and sometimes enabling it to move between sites, perhaps traveling with patients who are inadvertently carrying organisms they acquired during past visits to other health care units, he notes.

Dealing with patients infected with A. baumannii can be the equivalent of practicing medicine during the “preantibiotic” era, says Yehuda Carmeli of Tel Aviv Sourasky Medical Center in Tel Aviv, Israel. “The U.S. is relatively spared, except for the Northeast. But in other countries, it’s becoming the number one or two [nosocomial] problem. In Israel, for example, it’s second to Pseudomonas in causing bacteremia, and the most important cause of pneumonia for ventilated patients.” Moreover, death

FDA Approves CDC-Developed, PCR-Based Test for Identifying Avian Flu

Officials at the Food and Drug Administration (FDA) early in February approved a new test for identifying H5 strains of influenza from patients, and thus more quickly determining to test further for whether the strains are of the H5N1 subtype. The test, developed at the Centers for Disease Control and Prevention (CDC), is called the “Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set,” and it provides preliminary results within four hours of a sample arriving at a lab. The test is being distributed to members of the Laboratory Response Network (LRN), a system of about 140 labs across the United States whose personnel are mandated to conduct surveillance of important infectious disease agents. The H5N1 influenza subtype, which by mid-February proved deadly in half of about 170 human cases so far, early this year moved from Asia into countries in both Europe and Africa but had not been detected in the Americas.
rates are high from such infections because few treatments prove effective. He says that case rates rose dramatically at his hospital, and now lead to about 150 deaths per 500 cases each year.

In some cases, Carmeli and others say, physicians are using colistin (polymixin), a drug that otherwise is seldom used systemically because it can damage kidneys, to treat patients with such infections. Even this option has problems because resistance to colistin is being seen in Greece, where this drug is more widely used, he points out. And, says Harris of the University of Maryland, “We desperately need new antibiotics for gram-negative bacteria, but the ones in the pipeline are unlikely to solve the Acinetobacter [challenge].”

Meanwhile, shifting MRSA patterns are keeping infectious disease control specialists hopping, particularly because of diverging trends in hospital and community settings, varied geographic patterns, and evidence of exchanges of drug-resistant pathogens between humans and animals, such as horses or other companion animals, according to participants at the symposia, “MRSA Control: Controversies and Worldwide Differences,” and “Community-Associated MRSA.”

“The MRSA epidemic is not even all over the world,” says Vincent Jarlier of the Groupe hospitalier Pitie-Salpetriere in Paris, France. Countries such as Spain, France, and Italy have “large-scale epidemics,” whereas Denmark and other Scandinavian countries have controlled outbreaks effectively, he says. In the Netherlands, health care workers “never let the epidemic start” and continue to follow aggressive “search-and-destroy” tactics to purge MRSA from their hospitals. Inspired by such approaches, France instituted tougher infection-control measures about a decade ago, and has cut the MRSA rate in a major hospital network by about one-third, he points out.

U.S. hospitals are not dealing with so severe an epidemic and are tending to take a more-measured approach to MRSA, first adopting administrative approaches and then becoming increasingly aggressive if conditions dictate, according to Michele Pearson of the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. “There is great variability within and between systems in the U.S.,” she says. Although about 50% of U.S. hospitals are dealing with MRSA to some extent, the problem has “flattened out but not diminished in the last few years,” she adds.

One of the more perplexing puzzles revolves around community-acquired MRSA strains now appearing in U.S. hospitals, according to Pearson. They differ considerably from those usually found in hospitals and tend to be more virulent because of toxins that they produce, she says.

Similar community-acquired strains appear to arise from “multiple clones,” and they are occurring “locally” but are detected globally, according to Keryn Christiansen of Royal Perth Hospital in Perth, Australia. The community-acquired cases tend to be more sensitive to antibiotics than their hospital-acquired counterparts, but multidrug resistance is an “increasing problem,” she adds.

MRSA outbreaks vary greatly in intensity, ranging from mild skin infections to very severe disease, including pneumonia and sepsis, says Daniel Jernigan of CDC. Some of that severity is attributable to strains that produce toxins. In some cases, adding agents to neutralize such toxins can help to reduce disease severity, Christiansen points out. Another wrinkle with community-acquired outbreaks is that horses and other pets often carry MRSA, according to Scott Weese of the Ontario Veterinary College and University of Guelph in Guelph, Ontario, Canada.

Although exchanges of such strains between animals and humans appear to occur often, these exchanges apparently seldom lead to disease among humans—at least, so far, he says.

Jeffrey L. Fox