Virus Anatomists

John E. Johnson and his colleagues are peering into the tiny machinery of a virus to understand how it works. They are trying to figure out how a virus packs its DNA, a key process for the replication of some viruses. Johnson, a professor of molecular biology at The Scripps Research Institute, says his detailed studies of viral structure can help locate potential drug targets to keep a virus from replicating.

A focus of Johnson's research is the bacteriophage P22, which infects the food-borne pathogen *Salmonella*. The P22 consists of little more than a sphere-like shell, called a capsid, its DNA, and a tail used to attach to *Salmonella* cells prior to infection.

By using a cryo-electron microscope (cryo-EM), Johnson, graduate student Gabriel Lander, and their colleagues have determined the method by which the P22 switches off the process of packing its DNA into the capsid. "A structure in the interior of the virus acts as a pressure sensor that tells the virus when it's full of DNA," says Johnson. When the capsid is full, the molecular configuration changes at the opening of the capsid's base, which triggers a halt to the loading of DNA into the capsid. Assembly of the tail then begins, completing the virus.

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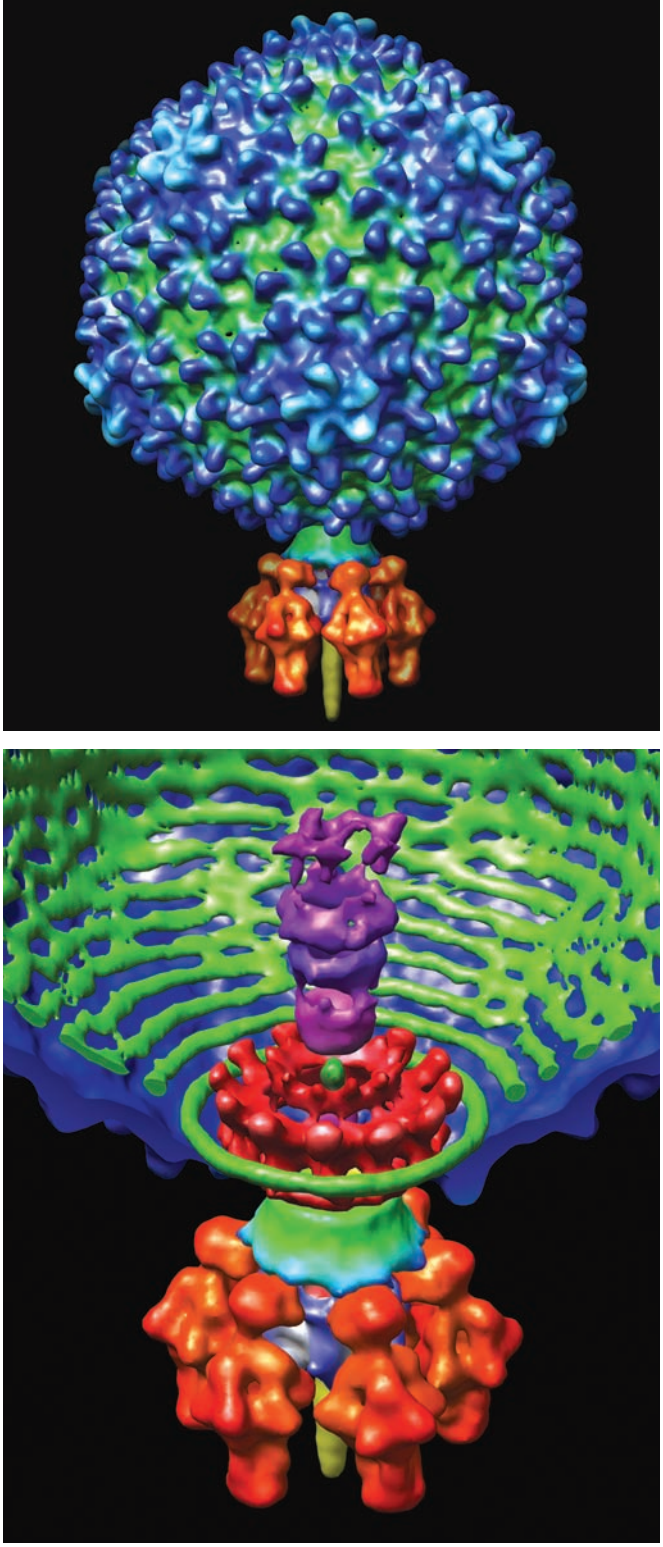
Understanding how the P22 replicates was possible through use of the cryo-EM at the National Resource for Automated Molecular Microscopy (NRAMM) in La Jolla, California. Over the past three years, the NCRR-funded NRAMM has developed innovative tools that automate the process of image collection, speeding results and reducing labor for scientists. "Using cryo-EM typically requires a repetitive task of acquiring and processing thousands of images from different viruses in order to average them into one 3-D image," says Bridget Carragher, director of the NRAMM. "We have developed technology that performs many of the tasks a microscopist would do, including image selection, to automate this process."

"If the cryo-EM were not automated, it would have taken many months to obtain all the necessary data. With the automated system it took only a week," says Lander. "This opens the possibility of doing studies that would previously not be feasible."

Lander hopes this research will open the door to future clinical applications on similar viruses that affect humans, such as the herpesvirus, which causes oral and genital herpes, chicken pox, and mononucleosis. "If we could manufacture a drug that targets the pressure sensor during assembly, the herpesvirus



The P22 virus in full view (top). A cross section (below) reveals the mechanism that packs DNA into the virus. Targeting this mechanism could stop the virus from replicating.

IMAGES BY GABRIEL LANDER, THE SCRIPPS RESEARCH INSTITUTE

would not package its DNA properly, rendering the virus unable to infect cells," he says. **—AL STAROPOLI**

NCRR RESOURCES: The cryo-EM at the National Resource for Automated Molecular Microscopy has helped to conduct more than 50 studies to date and is open for use by external scientists. Researchers can request use of the cryo-EM resource by completing an online application found at http://nramm.scripps.edu/resource/resource.php. Applications are accepted year-round and reviewed within one month of receipt.

Fertility Clues

Trying to understand the causes of infertility is the job of biologist Diana Chu, assistant professor at San Francisco State University. Chu and her colleagues hope to shed some light on male infertility by examining proteins associated with sperm production in the tiny worm *Caenorhabditis elegans*. By studying sperm from worms obtained through the NCRR-funded Caenorhabditis Genetics Center, Chu has discovered dozens of proteins that could be associated with various aspects of infertility.

"These findings could have strong implications on human fertility," says Barbara Meyer, who led Chu as she began her postdoctoral research. Meyer is a professor of genetics and development at the University of California, Berkeley.

Chu suspected that some of the hundreds of proteins found in sperm would have essential functions in fertility, but conducting detailed studies of so many proteins was not practical. To trim down the number proteins to a critical few, Chu and her colleagues designed an ingenious multiphase experiment.

In the first phase, Chu used proteomics to identify only those proteins likely to play a role in spermatogenesis. To assist this effort, Meyer facilitated a collaboration between Chu and two proteomics experts—John Yates and research fellow Hongbin Liu—at The Scripps Research Institute. Yates and Liu drew on the technology of the Yeast Resource Center at the University of Washington to perform detailed mass spectrometry analysis on Chu's worm samples. At this NCRR-funded resource, Liu used a type of mass spectrometry analysis called MudPIT (multidimensional protein identification technology), coupled with custom software, to identify proteins specific only to sperm or eggs. The analysis revealed more than 1,000 potential proteins to study.

Certain proteins, such as the ones stained in blue in the *C. elegans* worm (below), are linked to male infertility. Similar proteins are also found in humans.

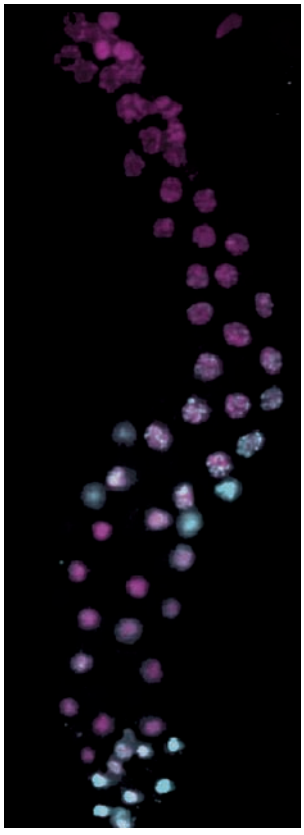


IMAGE BY DIANA CHU, SAN FRANCISCO STATE UNIVERSITY AND JESS GUNTHER, UNIVERSITY OF CALIFORNIA, BERKELEY

Thinking that more important proteins also would be more abundant, Chu and Liu searched for proteins that appeared frequently in samples. Only 132 proteins were found to be abundant in and unique to sperm. This small group of proteins was more likely to have key functions in spermatogenesis, Chu suggests.

In the second phase of the experiment, Chu worked with Meyer's lab to perform RNA interference and eliminate the expression of each of these 132 proteins, thus determining their effect on fertility. Their resulting effects were assessed by worm brood counts, level of sterility, embryo death, and abnormalities in chromosomes or sex glands.

Of the 132 proteins studied, RNA interference determined that sterility or embryonic lethality was related to blocking protein production in 50 genes. In addition, 70 proteins in *C. elegans* were found to have human homologues that have not yet been tested for their fertility function. "Our hope is to supply scientists with a short list of proteins to determine if their counterparts in humans or mammals have a role in fertility," says Chu.

Chu and Meyer say that analyzing additional homologous proteins in humans may aid in the development of diagnostic tests to assess causes of male infertility, sperm competence, or human reproductive potential. "There's a wealth of proteins to be explored that could have implications," says Meyer.

Chu agrees. "Men who face infertility often have few options," she says. "Looking at the human counterparts of the identified worm proteins can help determine the causes of male infertility. Identifying the problem can help to eventually define new options for treatment." (*Nature* 443:101-105, 2006.)

—AL STAROPOLI

NCRR RESOURCES: The Yeast Resource Center at the University of Washington is one of 52 Biomedical Technology Resource Centers supported by NCRR around the nation. The center offers access to five advanced technologies: mass spectrometry, yeast two-hybrid assays, deconvolution fluorescence microscopy, protein structure prediction, and computational biology. For more information or to submit a proposal, visit http://depts.washington.edu/yeastrc/pages/contact.html.

The Caenorhabditis Genetics Center (CGC), located at the University of Minnesota, is responsible for collecting, maintaining, and distributing stocks of *C. elegans*. The center also coordinates genetic nomenclature and maintains a *C. elegans* bibliography, genetic map, and Web server. For more information or to request a *C. elegans* strain, visit www.cbs.umn.edu/CGC.