“Peculiar” *T. vaginalis* Parasites Are Jam-Packed with Genes

When ranked with other pathogens of humans, *Trichomonas vaginalis* sets few records for effectiveness. Although tenacious among those it infects, this flagellated protist and sexually transmitted parasite of the human urogenital tract is generally more irritating than damaging—except in cases where it leads to pelvic inflammatory disease, increases susceptibility to HIV, or contributes to other complications. However, despite its poor showing as a pathogen of humans, this parasite may set records insofar as it carries an unusually complex genome, one that is extraordinarily rich in numbers of genes and gene copies.

Indeed, it has “many more genes”—possibly 60,000 or more—compared to other parasites, and its genome of about 160 Mb distributed amid six chromosomes is about “10-fold bigger than expected,” says Jane Carlton of New York University School of Medicine in New York, N.Y., leader of a group of researchers from two dozen institutions on four continents who are involved in analyzing this parasite’s massive genome. The study was funded by the National Institutes of Health, and a report about its draft genomic sequence appears in the 12 January 2007 issue of *Science*.

Notwithstanding its biochemical and genetic versatility, *T. vaginalis* is not free-living. Although the World Health Organization estimates that this parasite is responsible for some 170 million cases of human infection each year, the symptoms of such infections typically are so mild that its hosts often remain unaware of being infected, and this mild-mannered behavior tends to put this parasite toward the low-priority end of public-health threat lists. A majority of men and about half the women who become infected are asymptomatic, according to Carlton. *T. vaginalis* is kinder to men than to women because the parasite cells are readily flushed through the male urethra, whereas they prove more adept at colonizing the vagina and female urethra.

The parasites are facultative anaerobes, again making them well suited to their favored anatomic niche, which contains little oxygen, she continues. Moreover, the parasites are equipped with plenty of means for resisting oxidative stress, and they generate hydrogen and other gases—a behavior that can be a clinical telltale when it produces a frothy and malodorous discharge, she notes.

Although the parasites lack mitochondria, they do carry an organelle called the hydrogenosome, which may be a mitochondrial relic, that produces hydrogen and is also the target of drugs used for treating *T. vaginalis* infections, according to Patricia Johnson of the University of California, Los Angeles.

A competing hypothesis is that the hydrogenosome derived from an anaerobic bacterial symbiont instead of the mitochondrion, she continues. Although the hydrogenosome contains amino acid-metabolizing proteins similar to those found in mito-
Warnings Being Sounded on Extensively Drug-Resistant Tuberculosis

First noted two years ago in South Africa, extensively drug-resistant (XDR) tuberculosis is found widely in sub-Saharan Africa, Eastern Europe, and other parts of the world. Attributable to strains of Mycobacterium tuberculosis that resist isoniazid, rifampin, any fluoroquinolone, and at least one of three specific injectable antibiotics, XDR tuberculosis is raising alarms in public health circles. XDR tuberculosis strains are also of serious concern in clinical lab settings where staff members could become exposed to them. “The most basic requirement [for combating XDR] is an effective disease-control infrastructure, starting with much-strengthened laboratory capacity,” note Mario C. Raviglione and Ian M. Smith of the World Health Organization in Geneva, Switzerland, who urge stepped-up control measures in the February 15, 2007 issue of the New England Journal of Medicine.

“Diagnosis based on sputum-smear microscopy and rapid liquid-culture methods followed by . . . appropriate support for patients and strict supervision of treatment until cure are the basis of tuberculosis control.”

West Nile Virus Settled in, but Perhaps No Longer Expanding in U.S.

The population growth of West Nile virus (WNV) in North America declined recently, perhaps indicating that the virus has passed its peak prevalence, according to Edward C. Holmes and undergraduate first author Katherine W. Snapinn, who are both at the Pennsylvania State University, University Park. Their findings are published in the March Journal of Virology (81:2531–2534).

“This article presents compelling phylogenetic evidence that the West Nile virus epidemic in North America has undergone several important dynamic shifts, and that it may recently have reached an epidemiological plateau,” says Tony L. Goldberg of the University of Illinois (UI) College of Veterinary Medicine, Urbana, who is not a collaborator. Their findings and his further indicate that WNV in North America “shows little geographic substructure” but has “evolved rapidly as it has . . . become established.” Although the death rate for those infected with it is low, WNV infections can lead to serious disease in humans. For instance, about 25% of the 1.4 million Americans infected so far with the virus have been incapacitated for several months. Their symptoms typically include severe headache and extreme fatigue. Moreover, among the roughly 9,000 WNV-infected patients in the U.S. who developed neuroinvasive disease, long-term neurological effects, including paralysis, persisted on average for at least 1.5 years, according to a forthcoming report from public health officials at the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga.

To track the population of WNV, the Penn State researchers used statistical methods to estimate several parameters—such as the rate of evolutionary change, the divergence time of sequences, and the rate at which viral populations are growing—from changes in viral gene sequence data, according to Holmes. This modeling also estimates numbers of new infections in all species that nurture the

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microbe. “The epidemiological history of viruses is written in their gene sequences,” he says.

However, not everyone agrees that WNV populations have peaked in North America. “I think the jury is still out,” says Lyle Petersen of CDC. One problem is that the estimates by the Penn State researchers are based on viral samples that were collected only from birds in the northeastern United States rather than from across the entire country, he points out.

“Although reliance on viruses drawn largely from birds raises the possibility that our sampling is not representative, the results of the coalescent analyses [analysis of how phylogenetic lineages join together on a phylogenetic tree] are highly concordant with epidemiological and epizootiological records, indicating that the approach is robust,” Holmes and Snapinn note in defense of their estimates. They also find no evidence of host-dependent evolutionary patterns, a finding that they say further supports their conclusions about a leveling off of WNV population growth.

Another problem is that their conclusions do not appear to jibe with the death rate among humans who are infected by WNV, says Alan Barrett of the University of Texas Medical Branch in Galveston. That rate, he says, is the best surrogate for the state of the epidemic, as other measures such as the infection rate can be skewed by differences in reporting among states and through time. Human deaths from viral infections, which were first reported as infecting humans, birds, and other species in the eastern United States in 1999, peaked in 2002 and 2003 at more than 260, then fell to 87 in 2004. They climbed again thereafter, rising to 161 in 2006.

The complex ecology of the disease could help to account for these fluctuating rates, according to Petersen. For instance, he says, “Weather is critical. In the more northern parts of the United States, these outbreaks tend to occur during abnormally hot summers.” Mosquito populations, which harbor the virus, and the number of WNV-susceptible birds in a particular locale also play important roles. A substantial snowpack this winter in Colorado could boost infections there next summer by furnishing more runoff water for irrigation, thus providing better conditions for mosquitoes to breed, he says.

“West Nile virus is here to stay, but its initial phase of rapid spread and rapid genetic evolution may be over,” says UI’s Goldberg. “As these [Penn State] authors point out, however, future epidemics may still occur, but they are likely to be driven by changes in host populations and environmental conditions more so than by rapid evolutionary changes in the virus.”

David Holzman
David Holzman is the Microbe Journal Highlights Editor.

Lentivirus-Carried Antisense Appears Safe, Effective for Treating HIV

A one-time, novel “antisense” treatment carried on an engineered lentiviral vector successfully reduced HIV loads and partly restored damaged immune functions for up to a three-year period for five HIV-infected patients. This approach is “a potential breakthrough for HIV and other chronic conditions,” says Carl June, a professor of pathology at the University of Pennsylvania (UP) Cancer Center in Philadelphia, who headed the phase I clinical trial. Moreover, the trial has turned up no obvious side effects so far.

The lentiviral vector, known as VRX496, carries an unusually long antisense insert of 937 nucleotides that targets the gene encoding the HIV envelope protein. Researchers at VIRxSYS Corporation in Gaithersburg, Md., are producing the vector. They designed the antisense segment to be extremely long as a way of blocking sites where mutations might arise to confer resistance. “We have not seen any resistance develop to our therapy in vivo or in vitro,” says Gary McGarrity, executive vice president of scientific and clinical affairs at VIRxSYS.

In the clinical trial, UP researchers used apheresis to collect CD4+ T cells (the main site of HIV replication) from each of the patients. Those cell
samples were separately purified, genetically modified with the vector, and reinfused into each patient, all of whom had become resistant to standard antiviral drugs. Patients entered the study with viral loads ranging from 19,970 to 188,500 copies per ml (cpm), which dropped dramatically between 6 to 12 months, then stabilized. For instance, the baseline viral load of 54,100 cpm for one patient fell to 8,627 cpm at 6 months and to 1,063 cpm at 12 months.

Meanwhile, the CD4+ T cell counts for four of the five patients were stable or increased since the infusions, and all five patients showed increased immune responses to HIV antigens and other pathogens. The results are reported in the November 7, 2006 issue of the Proceedings of the National Academy of Sciences. VIRxSYS recently started a phase 2 study that will include 40 patients to establish the best dose for those who are chronically infected with HIV.

VIRX496 apparently interferes with HIV replication in T cells and “makes the virus wimpy,” says June. He speculates that HIV mutants that do continue to replicate after the antisense treatment are attenuated and act as a vaccine. “It’s an exciting thought that could lead to new vaccine strategies,” he says. If true, this approach might also be used against other chronic viral infections such as hepatitis C.

People with chronic HIV infections typically need to take more than a half-dozen antiviral drugs daily—a regimen designed to circumvent problems from the virus mutating and developing resistance to those drugs if taken singly. This experimental alternative approach of treating infected individuals with a lentivirus-borne antisense segment could help to simplify those antiviral drug regimens. Indeed, June is now testing several early-stage AIDS patients with VIRX496 to see if several infusions over four months will allow them to stop antiviral drug treatments.

VIRX496 is the first and only lentiviral vector so far approved for human trials by the U.S. Food and Drug Administration. It derives from HIV, but its infectious elements are gone. Unlike the Moloney leukemia virus-derived vector that was used for other gene therapy clinical experiments on several children in Europe with severe combined immunodeficiency disease, this lentiviral gene vector apparently does not cause insertional mutagenesis. “Many animal models show that lentiviruses are not oncogenic,” says McGarrit of VIRxSYS. The company plans to follow all patients treated with VIRX496 for 15 years to monitor for oncogenesis and other side effects.

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### Energy Department Expanding Microbial Genome Efforts

“Take off your ties,” decreed one official from the Department of Energy (DOE), mandating both informal dress and mood for the 2007 Joint Genomics: GTL Contractor-Grantee Workshop V and USDA-DOE Plant Feedstock Genomics for Bioenergy Joint Program Meeting that were held in Bethesda, Md., last February. This year marks the 10th anniversary of the DOE Joint Genome Institute (JGI), whose programs continue to expand. Specifically of interest to microbiologists, JGI is plowing plenty of resources, enthusiasm, and energy into the analysis of microbial genomes.

The main JGI user facility is in Walnut Creek, Calif., where the pace of genomic sequencing is accelerating, according to Paul Richardson from the JGI Microbial Program, who spoke during the meeting in February. The facility now accounts for “about 25% of the world capacity for sequencing microbial genomes,” he says. “We sequenced about 80 microbial genomes last year, amounting to about 316 megabases of microbial DNA. Our five-year plan is to sequence 2,000 microbial genomes.” Last year, for example, the 270 staff
members at JGI turned out more than 30 gigabases worth of DNA sequencing data, relying on two different analytic platforms.

In general, the team at JGI works on genomic-sequencing projects that come to the center through several expanding programs from the broader research community, which in 2006 submitted about 500 proposals. Despite the apparent emphasis on size and scope of these genomic sequencing projects, however, “the role of the individual researcher is becoming more important as they feed their ideas into this and other sequencing centers,” says David Thomassen, who is chief scientist if the DOE Office of Biological and Environmental Research. Proposals for regional centers were under review last February, and three new ones will likely be funded soon, he says. “We are urging individual scientists to take advantage of those new centers.”

At JGI, about 20% of those projects are microbial, another 60% are split between mission-related microbial and eukaryotic projects, another 15% comes from research groups working within the National Laboratory system, and the rest the director selects on a “discretionary” basis, according to Richardson. Recent energy-related genomic-sequencing projects extend to a wide array of organisms, including the poplar tree, microbes found in the hindgut of termites, and the white-rot fungus.

The JGI, like other facilities doing high-throughput sequencing, is “on the brink of a dramatic change in DNA-sequencing capabilities,” Richardson says. Several companies “are competing in this ‘space,’ and a number of platforms are being developed.” These newer instruments and methods generate a great deal of data faster and more cheaply than do current systems, albeit with some drop in “signal-to-noise,” he says. “We will need to develop software to improve the reads because this technology was designed for resequencing human genomes, but we’re using it for novel organisms. The capacity of this new technology could be up to 1,000 gigabases or higher within one year or so.”

A microbial test case for JGI’s emerging resequencing capabilities is the genome of the yeast *Pichia stipitis*, which has the highest known native capacity for fermenting xylose of any microbe, according to Richardson. It took only a few weeks to resequence portions of the genome of a high-ethanol-producing mutant. This rapid analysis not only uncovered an anticipated point mutation but also a number of other unexpected changes along the genome that may be involved in increasing ethanol productivity, including an altered gene encoding an aldehyde dehydrogenase, he says. “It’s very early, and you can’t even buy this machine yet, and we’re not there yet either, but we will be soon and plan to use this approach on other projects.”

Jeffrey L. Fox

Urey Instruments To Test for Life on Mars

For the first time in more than 30 years, the National Aeronautics and Space Agency (NASA) plans to send a new instrument to Mars to address the question of life there. Called the Urey organic and oxidant detector to honor the late Nobel Laureate in chemistry Harold C. Urey, the compact instrument is scheduled to travel in 2013 aboard the Pasteur Rover as part of the ExoMars mission that is sponsored by the European Space Agency.

Although the 1976 NASA Viking Lander mission was equipped with scientific instruments to test for evidence of life on Mars, its results proved inconclusive. Specifically, its gas chromatograph/mass spectrometer (GC/MS) did not find organic molecules at levels consistent with even scarce microbes being there.

The core of the Urey instrument is the Mars organic detector (MOD), a microfabricated capillary electro-
Bacterial Signaling Molecule also “Speaks” Directly to Host Immune Systems

The small molecule c-di-GMP, already recognized as an intracellular signaling molecule for a wide range of bacterial species, can serve as an immunostimulatory agent, modulating immune responses in mammalian hosts that c-di-GMP-carrying bacteria infect, according to David Karaolis of the Intragenics Research Institute in Havre de Grace, Md., and Karagen Pharmaceuticals in nearby Baltimore, and his collaborators there and at several institutions in the United States, Canada, Belgium, and Japan. They propose that this molecule belongs to a “new class of immunotherapeutic molecules” that “might have broad clinical use in humans and animals.” For instance, administering c-di-GMP to mice before they are exposed to a bacterial challenge prevents infections, they note. Moreover, c-di-GMP, when administered by itself, is immunostimulatory and immunoprophylactic. Further, when combined with vaccines, it has adjuvant properties, and it also activates human dendritic cells in vitro. Unlike CpG, another small molecule from bacteria that affects immune systems, c-di-GMP does so independently of host toll-like receptor responses. Details appear in the February 15 issue of the Journal of Immunology (178:2171–2181, 2007).

phoresis (CE) device for measuring amino acids or other organic compounds. It uses sublimation to purify and concentrate compounds, a dye called fluorescamine, and a fluorescence spectrograph to analyze organics that react with that dye. Its sensitivity is in the parts-per-trillion range, making it 1 million times more sensitive than the Viking GC/MS.

MOD can detect amino acids, nucleotide bases, and polycyclic aromatic hydrocarbons in samples. “If any Martian biota either extinct or extant is present in the upper 2 m of Mars, Urey should be able to detect this, providing the biota uses amino acids [or similar organics] in its biochemistry,” says Jeffrey L. Bada from the Scripps Institution of Oceanography in San Diego, Calif., who is the MOD principal investigator.

Urey is the first instrument to be sent to Mars capable of detecting and identifying amino acids. It also can determine their chirality or “handedness,” thus providing a means to distinguish between biotic, if chiral, and abiotic, if racemic, sources for any amino acids it might detect. Earth-bound organisms use left-handed (L) amino acids in proteins and, in rarer instances such as in bacterial cell wall components or other metabolites, right-handed (D) amino acids. “Finding a large excess of D-amino acids would be absolute proof of the existence of a unique Martian biology distinct from Earth life,” Bada says.

The Urey instrument package will also contain the Mars oxidant instrument. One of the Viking biology instruments appeared to detect oxygen and carbon dioxide being released from several soil samples after being dropped into a nutrient broth. Taken together with the negative GC/MS findings, many on the Viking science team postulated that an oxidant such as hydrogen peroxide (recently discovered in minute quantities in the Martian atmosphere) in the dry Martian soil would destroy all traces of organics. Then once any liquid water is added, any oxygen molecules attached to the soil grains would be released on contact.

That hypothesis remains unproved. Another hypothesis asserts that Martian microbes themselves produce hydrogen peroxide that this same Viking experiment inadvertently helped to release, thereby liberating carbon dioxide from organic compounds while also destroying further evidence of those microbes. Gathering fresh evidence about oxidizing agents in Martian soil samples could prove important for addressing questions about life there. Specifically, MOI will determine reaction rates of chemically coated films with different sensitivities to various oxidants and will attempt to measure the reactivity of free radicals and oxidants in the atmosphere and soil.

With a report in the November 2006 issue of Science showing that newly formed Martian gullies are releasing liquid water, questions about life on Mars once again seem tantalizingly within reach. The Urey exploration will be the next best chance to address and perhaps resolve some of those questions.

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ASM-NIH Report Emphasizes Importance of Basic Research

Although basic research involving bacteria has had a “significant impact” over many decades, the discipline is now “at a crossroads” and very much in need of a renewed investment, according to the report “Basic Research on Bacteria, the Essential Frontier.” Moreover, despite a recent trend that favors large projects undertaken by interdisciplinary consortia, there remains a “major role for research by individual groups” throughout microbiology, the report notes.
That report, which was released in early March, is based on a workshop that was convened jointly by the ASM Public and Scientific Affairs Board (PSAB) and officials of the National Institute of Allergy and Infectious Diseases (NIAID), and the National Institutes of General Medical Sciences (NIGMS), part of the National Institutes of Health (NIH). The workshop, which was attended by several dozen prominent microbiologists, was held in November 2005 at the NIH campus in Bethesda, Md.

The workshop and report emphasize “the fundamental importance of basic research,” says former ASM President Stanley Maloy of San Diego State University in San Diego, Calif., who spearheaded this PSAB-coordinated effort. Because basic research in microbiology extends beyond the health sciences, he adds, representatives of several other federal agencies that support such research also participated in that workshop, including the National Science Foundation (NSF), the U.S. Department of Agriculture (USDA), and the Department of Energy (DOE).

“We believe we got our message across,” Maloy says. “The real question is, will [this message] be parlayed into more funds for supporting that research? It’s a little too early to tell because the report is just beginning to reach people.”

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Another component of this education-based approach is inner-directed—aimed squarely at enticing successive generations of graduate students into pursuing basic research in microbiology, according to Maloy. “We want them to understand the foundations of this field because there is a need for a cadre of students from other disciplines like physics, math, and engineering being attracted to this field, who could have an important future doing research in microbiology,” he says. “We would also like to see ASM members become better at answering questions about why microbiology is important, and to be more aware of current and future challenges. The report can help them answer questions about microbiology’s contributions and its prospects.”

The report outlines an “education-based” strategy for building broad-based support for advancing basic research in microbiology. “One appeal is for the research community to tell stories in ways that make [the value of such research] clear to the newspaper-reading public, [for whom] the concept of bacterial physiology is not foreign,” Maloy says. “The public cares not so much about how it’s done as about the value of the end product and the time frame for getting it done. If the public thinks something is important and critical, then funding will flow that way.”