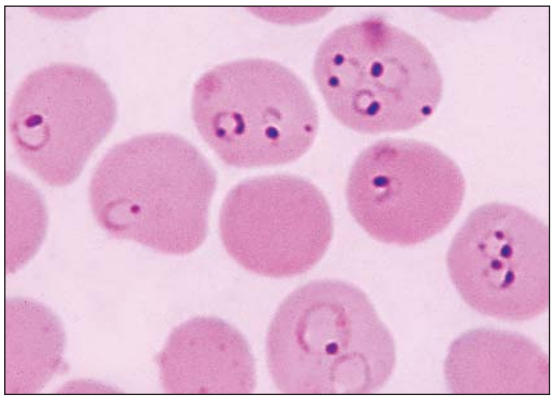
MALARIA RESEARCH

Parasite Genome Sequenced, Scrutinized

Plasmodium falciparum packs a powerful punch. The protozoan parasite causes malaria in hundreds of millions of people, most living in Africa. Thus far it has ducked every vaccine attempt and shaken off most of the drugs developed to knock out the disease. But now *P. falciparum*'s opponents have three new genome sequences in their corner, making them hopeful that they will put up a better fight in the next round. (See The Mosquito Genome, a special section that begins on page 77.)

This week, an almost complete DNA sequence of *P. falciparum*, as well as a draft of the genome sequence of a related *Plasmodium* species that infects rodents and is used to learn more about its human counterpart, appears in *Nature*. And on page 129 of this issue of *Science*, other researchers are reporting the DNA sequence of *Anopheles gambiae*, the mosquito that most efficiently transmits *P. falciparum* to humans in Africa. Together with the human genome sequence, researchers now have in hand the genetic blueprints for the parasite, its vector, and its victim. This "will provide the ability to take a holistic approach in understanding how the parasite interacts with the human host," says Alan Cowman, a molecular parasitologist at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia. With that approach, researchers say, new antimalarial strategies should be possible.



Malarial menace. Researchers have sequenced the genome of *Plasmodium falciparum* (dark spots inside red blood cells).

CREDIT: CDC

Just as *P. falciparum* has shown no mercy in its long association with people, it also proved a tough opponent for researchers trying to decipher its 23-million-base genome. They encountered "surprisingly difficult problems," says David Roos, a malaria expert at the University of Pennsylvania in Philadelphia. Indeed, it took dozens of people from four organizations—the Sanger Centre in Hinxton, U.K. (now called the Wellcome Trust Sanger Institute), The Institute for Genomic Research (TIGR) in Rockville, Maryland, the Naval Medical Research Center (NMRC) in Silver Spring, Maryland, and the Stanford Genome Technology Center in California—to get the job done.

When the project began in 1996, the sequencers were immediately stymied by the parasite's unusually high proportion of adenine and thymine, two of the bases that make up the DNA code. The chemical nature of these bases hindered efforts to chop the DNA into large snippets that make wholesale sequencing and reassembling of each chromosome easier. Early on, the preponderance of adenine and thymine clogged up the computer programs designed to evaluate data quality and piece the DNA back together. And in another complication, the researchers were able to isolate the DNA for just 11 of the parasite's 14 chromosomes. They eventually treated the three remaining chromosomes as a "blob" that proved even more difficult to decipher.

Chromosomes 2 and 3 were finished in 1998, and now another eight are complete, with four more—6, 7, 8, and 13—in the final stages. Even the incomplete code provided ammunition for malaria fighters. Now, with the sequence essentially known, as well as a draft of the *P. yoelii* genome sequence by a team led by TIGR, whole-genome analyses are providing a "much better appreciation for the complexity [of *P. falciparum*] and also its Achilles' heels," says NMRC molecular biologist Daniel Carucci, a physician who helped coordinate the project.

One Achilles' heel could be an odd subcellular component, called the apicoplast, found only in *Plasmodium* and its relatives. It seems to be derived from a chloroplast that had been appropriated from algae consumed by the parasite's ancestor. Ever since the apicoplast's discovery by Roos in 1997, malaria experts have been eyeing its proteins as possible drug targets. Researchers knew that the apicoplast was involved in lipid metabolism, "but we didn't know how," says Roos.

Now, thanks to the genome sequence, "we've been able to put together a complete metabolic pathway," he says, and show that about 12% of all the parasite's proteins, once made, head for the apicoplast. This structure also appears to be the only place where the parasite makes the fatty acids it needs to survive. Thus, Carucci explains, "if one [could] target this biochemical pathway, one would have a drug-target strategy that would be highly effective against the parasite and would not affect humans."

Malcolm Gardner and his colleagues at

ScienceScope

Caught Plagiarizing Indian physicists are demanding an inquiry into a case of plagiarism involving a paper co-authored by the vice chancellor of a prominent regional university. Balwant Singh Rajput, a particle physicist and vice chancellor of Kumaun University in the Himalayan state of Uttaranchal, has acknowledged that he failed to properly oversee a student—S. C. Joshi—who has admitted that he plagiarized a 6-year-old paper (www.geocities.com/physics_plagiarism). Joshi's tainted paper was published in the March 2002 issue of *Europhysics Letters*; it borrowed extensively from an article on the properties of black holes published in *Physical Review D* by Renata Kallosh of Stanford University.

Rajput says that Joshi never told him about the paper and that he has asked the journal editor to delete his name from it. An apologetic Joshi admits to having erred but says that it is the university's "usual practice" to credit superiors as co-authors.

The Society for Scientific Values, an independent think tank in New Delhi that monitors scientific misconduct, is asking authorities to investigate. Indira Nath, an immunologist and past secretary of the group, says Rajput's response "is too flimsy."

North Korean Glasnost? Western experts might soon get a glimpse of North Korea's shadowy network of defense research labs. Speaking at a nuclear security meeting in London this week, Representative Curt Weldon (R–PA), a senior member of the House Armed Services Committee, revealed that U.S. and North Korean officials are discussing how to redirect North Korean weapons scientists to peaceful, commercially oriented projects. The potential U.S.-sponsored effort could be modeled on the Department of Energy's Initiatives for Proliferation Prevention program, which now retrains defense scientists in the former Soviet Union.

The possibility of working with North Korean researchers is intriguing, say some scientists. The nation's science community is a "black box," says Abel Julio Gonzalez, a nonproliferation specialist at the International Atomic Energy Agency in Vienna, Austria. Weldon says he hopes to lead a congressional delegation to Pyongyang later this year.

TIGR are still trying to make sense of the sequence. *P. falciparum* appears to have about 5300 genes. The researchers are not yet able to identify the function of some 60% of these genes, they report. In addition, genes with related functions appear to be clustered on the genome, suggesting that they might share the same regulatory DNA.

Even with unanswered questions, researchers are using the sequence to build a catalog of *Plasmodium* proteins and to make gene chips for molecular studies of different points in *P. falciparum*'s life cycle. In the proteomics arena, at least two research groups report in *Nature* that they are using sophisticated mass spectrometry techniques to look at thousands of proteins and determine when in the parasite's life cycle they are active. For vaccine developers, who want to create defenses against all of the parasite's alter egos, "that's very valuable information," says Gardner.

In one proteomics study, Laurence Florens and John Yates of the Scripps Research Institute in La Jolla, California, and their colleagues examined more than 2400 proteins. They found that the protein complement of the sporozoite—the form of the parasite a mosquito injects when it feeds on human blood—was quite different from that of other stages of the life cycle. Almost half of the sporozoite's proteins were found nowhere else, they report. But there were also a few unexpected genes in common. Researchers had thought that the parasite made var proteins—used to evade the immune system—only while in human blood, but these studies have now shown that "they are expressed before it even gets to the host," says Carucci.

And in a separate evaluation of 1289 proteins, Edwin Lasonder and Matthias Mann of the University of Southern Denmark in Odense found 315 that are unique to the immature male and female gametes that enter the mosquito and 226 in the asexual stages. "Intellectually, it's very exciting to think we have a total catalog of the relevant genes for all the parts of the life cycle," says Roos, who has set up a database (PlasmoDB.org) to compile the onslaught of genomic data on *Plasmodium*.

Harvard's Sarah Volkman and her colleagues, among others, are using the *P. falciparum* sequence to expand studies of drug resistance. As described on page 216, working with Elizabeth Winzeler of the Genomics Institute of the Novartis Research Foundation in La Jolla, California, Volkman's team built gene chips to detect genetic changes—or polymorphisms—between *P. falciparum* strains. "Drug resistance is bred by polymorphisms," Winzeler explains. "So being able to actually determine where [the polymorphisms] exist allows you to study the spread of drug resistance."

Whether with more gene chips, proteomic studies, gene searches, or comparative genomics, other malaria experts are eager to make use of the newly sequenced mosquito and *Plasmodium* genomes. "There are going to be fantastic strides in the years to come," says Thomas Wellems, a malaria expert at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. "I have no doubt that a deeper understanding of the biology of the parasite is going to lead us to better therapies." **–ELIZABETH PENNISI**

CLINICAL RESEARCH

Gene Therapy a Suspect In Leukemia-like Disease

A French gene-therapy team that was hailed in 2000 for its breakthrough in curing children of a lethal immune deficiency reported a serious adverse event this week. One of 10 children they treated has developed a blood disorder resembling leukemia. Concerned that the therapy might have caused the problem, researchers Alain Fischer and Marina Cavazzana-Calvo of the Necker Hospital in Paris have halted the trial and urged others who use similar methods to hold off until the risks are assessed. At press time, French regulatory officials were preparing a public advisory.

Setback. Alain Fischer and Marina Cavazzana-Calvo announcing successful gene-therapy treatment in April 2000.

Fischer says he and his group recognized the importance of the case "exactly 1 month ago" but decided to study it and explain it to their patients before going public. The French team quickly sent advisory letters to investigators in charge of similar gene therapy trials using gene transfer "vectors" made from a retrovirus called the mouse Moloney leukemia virus. When the warning reached the U.S. National Institutes of Health (NIH) in Bethesda, Maryland, a clinical group immediately cancelled a six-patient trial due to begin in September.

This French trial was designed to identify children with a type of severe combined immunodeficiency (SCID) caused by a mutation on the X chromosome and to treat them early (*Science,* 28 April 2000, p. 669). So far, the team has treated nine infants and one teenager. All faced the prospect of lethal infections or harsh therapy such as bone marrow transplantation, which itself often has fatal consequences. Gene therapy offered a way out; in most cases it restored the immune system without toxicity.

During a routine check of their fourth patient last spring, however, the French researchers noted that the child had a high number of γδ T cells in his blood. The import didn't hit home until late August, Fischer says, when the T cell count climbed "very high"—to 200,000 cells per microliter. Other symptoms also appeared, including mild anemia, and the child was hospitalized.

Molecular studies revealed that the T cells were monoclonal: All had come from a single cell. Furthermore, Fischer explains, all the cells contained the same DNA signature, a sequence reflecting the site where the retrovirus vector had integrated itself into the host's genome. "Unfortunately," Fischer says, that site is in the coding region of a gene on chromosome 11 that's "aberrantly expressed" in a form of childhood acute lymphoblastic leukemia.

Fischer believes that the vector triggered "an insertional mutagenesis event"—splicing itself into a dangerous gene and stepping up its production. "Everyone was aware" of a theoretical risk that retrovirus vectors might do this, he says, but the risk seemed very small. The phenomenon did not turn up in animal experiments or in other clinical data.

Although gene therapy probably contributed to the patient's T cell response, Fischer says, other factors probably played a role, too. For example, the child might have been predisposed to disease, as other members of his family have had childhood cancers. And an infection might have been important as well; the child got chickenpox shortly before his T cell count spun out of control. But at the moment, Fischer acknowledges, it's not clear whether this was a "very unlucky" random event or a sign that the risk of using retrovirus vectors has been "underestimated" in the past.

Researchers at the Necker Hospital are collaborating with Christof von Kalle of the Institute for Molecular Medicine in Freiburg, Germany, to try to create a map of all known human DNA integration sites for this retrovirus vector. They hope this will enable them to estimate the risks better. Meanwhile, the

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