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Researchers Unmask Malaria Parasite's Cloaking Mechanism

Scientists are making strides in understanding how the malaria parasite *Plasmodium falciparum* disguises itself to avoid detection by the immune system. The findings could lead to the development of new drugs for a disease that causes more than 300 million acute illnesses and at least one million deaths each year, most of them in developing countries.

Individuals infected with malaria cannot develop an effective immune response because the parasite that causes the disease is a master of disguise. Throughout its lifetime, *P. falciparum* continually changes the version of a protein known as PfEMP1 that it deposits on the surface of infected cells. By the time the immune system learns to recognize the protein and starts making antibodies against it, the parasite has switched to another form of the protein, and the game of hide and seek starts over.

In a new study, scientists led by Alan Cowman and Brendan Crabb, Howard Hughes Medical Institute (HHMI) international research scholars at The Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, set out to test the hypothesis that *P. falciparum* uses gene silencing to mask its presence. Their findings are published in the April 8, 2005, issue of the journal *Cell*. The study also involved researchers from Monash University in Clayton, Australia, the University of Melbourne, and the Institut Pasteur in Paris.

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Since the mid-1990s, researchers have known that a family of genes known as *var* encode PfEMP1. While the parasite's genome contains at least 50 *var*

genes, only one is expressed at any given time, giving rise to a single version of the PfEMP1 protein. Over the course of an infection, expression switches from one *var* gene to another - a phenomenon that until now, scientists have not understood.

The researchers say that teasing out the mechanism by which the *var* genes are switched on or off could lead to the development of novel drugs for malaria. “If you could work out a way of causing the parasite to switch all the *var* genes on, then the body would see all the variations of *var* genes, and the immune system would be able to control the infection,” said Cowman.

To assess whether a region of DNA containing a particular *var* gene was active or silent, the scientists measured expression of a gene that they artificially inserted adjacent to *var* in a population of parasites. The introduced gene encoded resistance to a drug. When the researchers exposed the parasites to that drug, they found that gene silencing was indeed at work. In some parasites, the DNA region was active, and the parasites showed resistance to the drug. In other parasites, the region was not being transcribed, and the drug successfully blocked the biochemical reaction it is meant to block.

After examining the regions around the silent and active *var* genes, the researchers found differences in the way that the DNA was packaged—some of the DNA was wrapped so tightly with proteins that it ceased to be accessible for transcription. This finding implicated a protein called silent information regulator 2 (SIR2), which is already known to play a role in gene silencing in yeast by modifying gene packaging.

To examine the potential role of SIR2 in silencing *var* genes, the scientists created a parasite that lacked a functioning gene for that protein. They found that the genetically-altered parasite expressed a greater number of *var* genes than parasites with normal SIR2.

“Silencing occurs by packaging up the DNA into a tight form and preventing it from being expressed. That tight packaging involves SIR2,” said Cowman. “The question then was how one of those genes is switched back on.”

The researchers knew that, in some organisms, gene regulation is dictated not only by DNA sequence and the way that sequence is folded, but also by physical location. In these cases, the expression of genes located at the edge of the nucleus involves the movement of a gene into certain accessible compartments. They wondered if nuclear compartments came into play in *var* gene activation.

Using a technique called fluorescent in situ hybridization (FISH), which employs fluorescent-labeled probes specific for particular segments of DNA, the investigators gauged the position of *var* genes in on and off states and found that their hunch was correct.

“There does indeed appear to be discrete nuclear compartments that allow gene expression to occur,” said Crabb. “Every now and again, one of the genes moves into the right spot and gets activated.”

Because some *var* genes are located near each other, Cowman said, sometimes more than one *var* gene is moved into a compartment at the same time. Since this does not result in the simultaneous expression of multiple *var* genes, it suggests that there are other tiers of regulation that must be uncovered before scientists can hope to create new drugs.

The Howard Hughes Medical Institute was established in 1953 by the aviator-industrialist. HHMI's principal mission is conducting basic biomedical research, which it carries out in collaboration with universities, medical centers and other research institutions throughout the United States. Its more than 300 investigators, along with a scientific staff of more than 3,000, work at these institutions in Hughes laboratories. The Institute's Janelia Farm Research Campus, now under construction in Loudoun County, Virginia, will be a unique, world-class biomedical research complex where resident and visiting scientists can collaborate on cutting-edge, cross-disciplinary projects. HHMI has a philanthropic grants program that is strengthening science education and training, from elementary school through graduate and medical school. It also supports the work of biomedical researchers in many countries around the globe.

HHMI is one of the largest philanthropies in the world, with an endowment of \$12.8 billion at the end of the 2004 fiscal year. Its headquarters are located in Chevy Chase, Maryland, just outside Washington, D.C.