

DECEMBER 28, 2005

Scientists Lift Malaria's Cloak of Invisibility

The world's deadliest malaria parasite, *Plasmodium falciparum*, sneaks past the human immune system with the help of a wardrobe of invisibility cloaks. If a person's immune cells learn to recognize one of the parasite's many camouflage proteins, the surviving invaders can swap disguises and slip away again to cause more damage. Malaria kills an estimated 2.7 million people annually worldwide, 75 percent of them children in Africa.

Howard Hughes Medical Institute (HHMI) international research scholars in Australia have determined how *P. falciparum* can turn on one cloaking gene and keep dozens of others silent until each is needed in turn. Their findings, published in the December 28, 2005, issue of *Nature*, reveal the mechanism of action of the genetic machinery thought to be the key to the parasite's survival.

A DNA sequence near the start of a cloaking gene, known as the gene's promoter, not only turns up production of its protein, but also keeps all other cloaking genes under wraps, according to Alan Cowman and Brendan Crabb, HHMI international research scholars at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, and their co-authors. "The promoter is all you need for activation and silencing," Cowman said. "It's the main site of action where everything is happening."

"This is the first time anyone has actually been able to infiltrate an antigenic variation program."

- Alan F. Cowman

Malaria parasites enter human blood from infected mosquitoes. The organisms invade and promptly remodel red blood cells. They decorate the surface of the cells they occupy with a protein called PfEMP1, made by the *var* gene family.

Using this versatile surface protein, the parasite evades the host's immune system using two basic strategies. First, the protein sticks infected red blood cells to the blood vessel lining, removing the infected cells from circulation, where they would probably be destroyed.

But the protein cannot protect the parasite from patrolling immune cells, which eventually detect the invader and recruit troops to fight it. So, during a malaria infection, a small percentage of each generation of parasites switches to a different version of PfEMP1 that the body has never seen before. In its new disguise, *P. falciparum* can invade more red blood cells and cause another wave of fever, headaches, nausea, and chills.

“It's like a leopard being able to change its spots,” Cowman said. “New forms come up, and the immune system beats them down again. Because of this a lot of people think you need five years of constant exposure to malaria in its different disguises to gain immunity.”

Many children do not survive malaria long enough to develop immunity. And without continuous exposure, hard-won immunity may disappear. For example, adults in Papua New Guinea who move to work in the mining industry, which is in mountainous regions that are mosquito-free, lose their immunity within a short time, he said.

The diverse genetic sequences of the 60 *var* cloaking genes all code for remarkably similar protein structures, the malaria researcher added. The genes are generally found at the ends of *P. falciparum*'s 14 chromosomes, although some of them cluster in internal regions.

In April 2005, Cowman, Crabb, and colleagues showed that *var* genes are regulated by the chromosome packaging, which unwraps one gene to be expressed at a time and literally packs away the inactive genes. In chromosomes, DNA can be encased so securely by some proteins that other proteins cannot access the nucleic acid for transcription, a process known as epigenetic silencing.

In their new paper, the researchers show that the activation of a *var* gene promoter is all it takes to trigger both the production of that gene's protein and the epigenetic silencing of the 59 other *var* genes. As in a previous study, they found that the physical location of the promoter within the nucleus seems to make a difference. The genetic activity occurred at the edge of the nucleus, with the activated promoter surrounded by chromosome ends containing silenced *var* genes.

To do this research, the scientists had to master the difficult technique of cloning large DNA sequences with a *var* promoter attached to various genes, inserting them into plasmid vectors, and introducing them into red blood cells infected by malaria parasites.

In one experiment, they set up a system where *var* gene expression could be studied using drug selection rather than the immune pressure that is normally needed to select variants in the field. Using this system they found that the information required for switching *var* genes on and off was contained within a promoter and that when active this could silence all of the *var* genes in the parasite.

“This is the first time anyone has actually been able to infiltrate an antigenic variation program,” Cowman said. “We forced the cell to switch our gene on and others off.” Their system can be used to study blood samples from people in the field to determine how they gain immunity over time.

Kirk Deitsch's lab at Cornell University found that a piece of shared DNA—discarded in the process of translating the protein from its genetic instructions—was a key regulator of *var* gene silencing and activation. The HHMI researchers confirmed that this gene segment caused tighter packaging for the silenced genes, but they also showed that it wasn't vital.

The researchers are continuing to disassemble the *var* gene machinery, piece by piece. They want to identify the proteins that unpack and activate the promoter region and learn more about the other proteins in the nuclear compartment that make it the prime spot for *var* gene activation. Eventually, they think their work may lead to new types of therapies that interfere with the parasite's immune evasion strategies.