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Molecule Allows Malaria Parasite to Commandeer Red Blood Cells

The Life cycle of Malaria Part 1: Human Host When a malaria-carrying mosquito bites a human host, the malaria parasite enters the bloodstream, multiplies in the liver cells, and is then released back into the bloodstream, where it infects and destroys red blood cells.

Video: HHMI Biointeractive

Two groups of Howard Hughes Medical Institute (HHMI) scientists working independently have identified a critical enzyme that allows the malaria-causing parasite, *Plasmodium falciparum*, to take over and thrive in human red blood cells.

The enzyme plasmepsin V (PMV) is a gatekeeper inside the malaria parasite that allows the parasite to export its own proteins into a human red blood cell. Once PMV opens the gate into the red blood cell, the parasite moves hundreds of the proteins into cell, which remodels it and, eventually, annihilates it. The new observations demonstrate that PMV is critical to survival of the malaria parasite and suggest that drugs targeting PMV may be able to kill the parasite before it develops inside red blood cells. This research was published by HHMI international research scholar Alan Cowman and HHMI investigator Daniel Goldberg in two articles in the February 4, 2010, issue of *Nature*.

Malaria affects between 350-500 million people worldwide each year and kills between 1 to 3 million of them, according to the Centers for Disease Control and Prevention. Malaria parasites go through a series of steps on their way to causing disease in humans. When a malaria-carrying mosquito bites a human host, the malaria parasite enters the bloodstream, multiplies in the liver cells, and is then released back into the bloodstream, where it infects and destroys red blood cells. Parasites invade red blood cells in an attempt to evade the immune system and to remodel them for their own use.

When the parasite first enters a red blood cell, it is enclosed in a membrane, and scientists weren't sure how it took the next critical step out of the membrane and into the cell itself. The *Nature* papers demonstrate that PMV

scans the proteins inside the parasite for a molecular barcode called the PEXEL motif, which Cowman co-discovered in 2004. When PMV detects that signal, it trims off the protein's end and then feeds the processed protein into a molecular machine called a translocon, which pushes the protein into the red blood cells. "I think of PMV as the ticket-taker," says Goldberg, who is at Washington University School of Medicine in St. Louis. "If you go to the theater, someone at the door looks at your ticket to make sure you belong in the audience—that's what PMV does."

After parasite proteins are ushered into human red cells, they begin destroying oxygen-carrying hemoglobin and disfigure the cell surface so that the blood cells stick to blood vessels rather than circulating freely. This allows the parasite to use the red blood cell as a shield to hide from the host immune system. All of this mischief causes the fatigue, fever and neurological symptoms characteristic of this sometimes fatal form of malaria.

Malaria researchers have been focused for a long time on identifying the proteins responsible for wreaking this cellular havoc. "We knew that approximately 300 parasite proteins were exported into the red blood cell," says Cowman, who is at The Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia. "If (the proteins) were being cleaved at the PEXEL site, and if you could inhibit that activity, you could block all of those proteins—including the ones essential for parasite survival—from entering the cell."

Cowman and Goldberg credit a 2008 paper from former HHMI investigator Michael Marletta for pointing the way to PMV as the key protein involved in the malaria parasite taking over red blood cells. Marletta's lab at the University of California, Berkeley showed that proteins with a PEXEL motif were cleaved by an unknown factor housed inside a network of tubules inside the parasite called the endoplasmic reticulum (ER). Researchers also knew that protease inhibitor drugs used to treat human immunodeficiency virus were moderately effective against malaria. Those drugs block a class of protein-cutting enzymes called aspartyl proteases. Based on these hints, Cowman compiled a list of candidate *P. falciparum* aspartyl proteases. He chose PMV for further analysis, in part, because it was expressed at a stage when parasites were infecting blood cells and he suspected he might find it in the ER.

"Our group came from a different angle," says Goldberg, who has a long-term interest in the role of proteases in *P. falciparum* biology. "We had been studying aspartyl proteases because they make very good drug targets—as in the case of HIV protease inhibitors," he says. Plus Goldberg knew that PMV was found in the parasite ER, where Marletta had placed the mysterious PEXEL-cleaving enzyme.

With PMV as prime suspect, both groups showed that PMV cleaved exported proteins marked by the PEXEL motif, both in the test tube and in parasite-infected human red blood cells. Cowman's group also eliminated

other proteases as candidates— most notably signal peptidase, which like PMV is expressed in the ER. Goldberg’s group did a critical experiment that proved PMV was essential for the malaria parasite’s survival. The scientists inserted a dead form of PMV into the parasite’s genome and found that all of the parasites with the broken PMV protein died, presumably because they could not propagate themselves in red blood cells.

Finally, using different approaches, both groups present evidence in their *Nature* articles that suggests PMV uses helpers to move parasite cargo into red cells. Cowman’s lab played a trick on the parasite by engineering proteins that display a “faux-PEXEL” barcode. These proteins were snipped by a protease other than PMV but retained a stump resembling a PMV cleavage site. Significantly, those proteins were not exported—meaning that a protein somehow *knows* it was clipped by PMV and not by an imposter. This suggests that when PMV clips a protein it immediately hands the protein off to a set of dedicated ushers who guide it through the translocon into red cells.

Developing effective novel therapies for malaria is high on the global health agenda because the parasite has a talent for acquiring drug resistance. “Some current malaria treatments rely on an ancient Chinese herbal medicine called artemisinin,” says Goldberg, referring to the drug of choice to treat *P. falciparum*-mediated malaria. “But recent reports indicate that the parasite is becoming resistant to artemisinin—as happened with chloroquine and sulfa drug treatment. We worry that the same thing will happen with artemisinin.”

Both of these papers will help researchers narrow the search for drugs capable of inhibiting the *P. falciparum* growth. “The race is on to develop inhibitors of PMV,” says Cowman, who is now collaborating with chemists and structural biologists on drug development effort.

Goldberg will likely stay focused on basic parasite biology. “We will to continue to define new drug targets in *P. falciparum* and show whether they are good ones,” he says, adding that there is still a lot to know about how enzymes like PMV work. “If potent inhibitors can be found, blocking the entire parasite virulence program with one stroke will be a promising new strategy for combating this nefarious organism.”