

MARCH 17, 2008

## Scrambled Entry Ticket May Fake Out Malaria Cell Gatekeeper

People can understand the meaning of sentences, even if the letters in the words are jumbled. Just try it: Hmunas uednrtsnad wirtetn lganague, evvn if the ltrtees are srcamelbd.

New research shows that some cells may have the same ability. A cellular gatekeeper inside a malaria parasite doesn't care whether the amino acid letters on a protein's entry ticket are out of order, according to new research by Howard Hughes Medical Institute international scholar Geoffrey McFadden at the University of Melbourne in Australia.

In fact, the scientists were able to get their own engineered protein past the gatekeeper using made up tickets with completely different amino acids chains—some even representing English words—as long as the ticket, called a transit peptide, had certain characteristics.

---

**"The potential is there to use this relatively lack of sophistication in this system as a way to confound it. We could predict what would kill the parasite."**

**- Geoffrey McFadden**

---

"Usually in proteins, the order of the amino acids is critical to allowing them inside a compartment in the cell, but these don't have to have any particular order," McFadden said.

The research may lead to new insights into the evolution of this gatekeeper, as well as new ways to fight malaria. It was published in an early online publication March 17, 2008, in *Proceedings of the National Academy of Sciences* and coauthored by Alan Cowman, an HHMI international scholar at the Walter & Eliza Hall Institute, also in Australia.

Inside cells, construction of proteins begins in the nucleus and finishes outside the nucleus, where completed proteins are sent out to do their jobs in the cell. Most of these proteins head to compartments within the cell to perform specific tasks.

Like a fancy club, each compartment has its own exclusive entry requirements, and the transit peptide acts like a ticket that allows the protein inside. The compartment's cellular gatekeeper—McFadden likes to compare him to an usher at a concert—decides which proteins get in.

The Australian researchers wanted to understand what this still mysterious usher looks for when deciding whether to let proteins into a specific compartment of the malaria-causing parasite *Plasmodium falciparum*. The compartment, called a plastid, is essential for the parasite's survival, though researchers are unsure what purpose it serves. It is similar to some plastids found in plants and, although it no longer harvests energy from the sun, appears to be left over from when the parasite used photosynthesis 500 million years ago.

McFadden and his colleagues wondered whether interfering with the plastid might be a good way to kill the parasite because it is relatively simple and isn't present in humans. To do that, they needed to know how proteins get inside the plastid. The team created a computer program to examine the transit peptide ticket of 500 different proteins that are able to get inside the compartment to see if they could determine what about the ticket tells the plastid's usher to let it inside. They found that the peptides had to have certain characteristics: be at least 24 amino acids long or longer, have a positive charge, and include a specific amino acid called asparagine.

The computer program worked so well that the researchers wondered if it would work in reverse, that is, could they create a transit peptide from scratch that would gain entry to the plastid? “The idea was that (the proteins) would respond the way the computer predicted they would. And they did,” McFadden said. “It is kind of creepy, almost.”

Based on their findings, the researchers identified various randomly created strings of amino acids, some that followed the rules and some that didn't. They then made synthetic tickets of their own and attached them to test proteins. If the transit peptide ticket had the essential characteristics—no matter what order its amino acids were in, or even which ones were included—the protein got inside the plastid. If not, it didn't get in.

“I would have thought it would be a very tight sequence that was recognized by a very specific protein,” Cowman said. “Essentially, it is a fairly loose set of instructions.”

The researchers then went even further, trying out strings of English words, with each of the 20 amino acids representing a different letter in the alphabet.

The computer predicted that SKINNYSLINKYKINKYTHING had the correct characteristics and would get into the plastid, and ITWILLNEVERTARGETPLASTID did not and would be left outside. It was right.

The research gives the scientists two important insights. First, evolution could easily have made these transit peptides from scratch, since they follow such simple rules. This could be, in part, because they originally came from plants, Cowman explained.

“That system probably arose fairly early,” he said. “It is still very similar to plants.”

Second, the plastid's simplicity means it should be relatively easy to trick the usher into letting an invader inside, McFadden said. That would kill the plastid and the malaria parasite.

“The potential is there to use this relatively lack of sophistication in this system as a way to confound it,” he said. “We could predict what would kill the parasite.”

Now that they know how to get inside, McFadden and his colleagues are expanding their research focus on a new way to possibly kill the plastid by examining the usher itself, which they know very little about.

“The big quest at the moment is now to find the usher, the person who sees the ticket and lets you through the door,” McFadden said. “If you can disable the usher then you could probably stop the entrance process and kill the parasite.”