Teasing out malaria's genetic secrets

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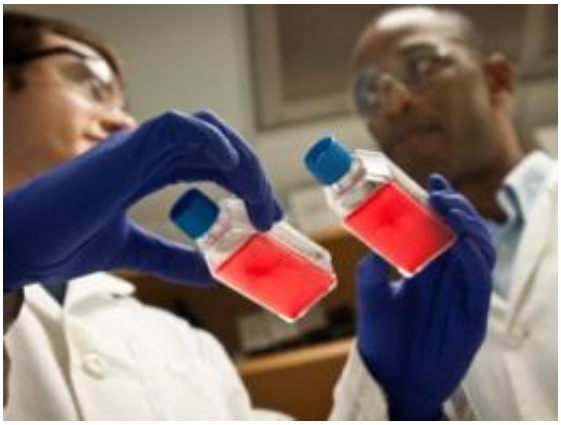

Niles talks with graduate student James Abshire, who holds a flask containing human red blood cells that have been infected with the parasite that causes malaria. Photo: Patrick Gillooly

Every year, malaria infects more than 250 million people, and more than 1 million die from the parasitic disease. For decades, doctors have treated malaria with chloroquine — an effective, inexpensive remedy. But now, the parasite is becoming resistant to chloroquine. Resistance has also begun to develop against artemisinin, a newer drug that is also widely used.

That worries many people, including Jacquin Niles, MIT assistant professor of biological engineering. “Outside of artemisinin and chloroquine, there really isn’t a huge arsenal for effectively treating malaria,” he says.

Developing new drugs is essential, but the parasite that causes malaria is notoriously difficult to study because of its bizarre system of gene regulation. However, Niles has developed a new technique that he believes will make it easier to pin down gene functions and identify potential drug targets.

“Once we have a map of where the vulnerabilities are in the parasite’s arsenal, we can think about targeting them for therapeutics — whether a vaccine or new drugs,” he says.

Niles, graduate student Brian Belmont and their colleagues described the new technique in a recent issue of the journal *ACS* Chemical Biology. Last month, Niles won a $1.5 million, five-year New Innovator grant from the National Institutes of Health to further and broadly develop the technology.

‘Bizarre’ genetics

Plasmodium falciparum, the parasite that causes malaria, has a 48-hour life cycle inside human blood cells. During that time period, the parasite reproduces itself inside the blood cells, causing them to swell and eventually burst, releasing new parasites into the bloodstream. Freed from the cell, those parasites infect more blood cells and start the cycle over again.

Throughout this cycle, P. falciparum genes are turned on and off at specific times, in a sequence that scientists believe guides the parasite’s development. If scientists had a good way to block or activate these genes as the parasite develops, they could figure out which ones are most critical to survival.

In most laboratory organisms, such as bacteria or yeast, or even human cells, it’s relatively simple to turn genes on and off, using molecules that

inhibit or promote transcription — the copying of DNA into messenger RNA (mRNA), which carries DNA's instructions to the rest of the cell.

That is usually a straightforward task, because each gene has an identifiable "promoter" region where transcription starts. However, it's much harder to identify these regions in the malaria parasite. "Plasmodium has a really bizarre strategy for controlling transcription," says Niles. "There are few clear-cut regulatory components."

"So far, we have very limited tools in directing the expression of essential genes," says Liwang Cui, professor of entomology at Pennsylvania State University. Cui, who also studies malaria parasite genetics, calls Niles' new approach, which involves disrupting mRNA activity, "quite innovative."

Blocking protein production

After DNA is copied into mRNA, mRNA travels to cell structures called ribosomes, which assemble proteins according to the mRNA instructions — a process called translation. Niles' new technique controls translation by using proteins that attach themselves to mRNA and prevent it from locking onto the ribosome.

For this to work, the researchers must first identify the gene they want to disrupt. They then tag the gene with a sequence of DNA that codes for a small RNA loop or "aptamer." When the gene is transcribed into mRNA, this loop is also copied. After the mRNA is released into the cell in search of a ribosome, the loop binds to a protein called tetracycline repressor protein (TetR), which blocks the mRNA from being translated. (The gene for TetR must also be inserted into the cell being studied.)

The researchers can reverse the effect by adding an antibiotic, tetracycline or any of its analogs, to the cell, which binds to TetR and

drags it off the mRNA, allowing it to latch onto a ribosome.

In principle, this system could be used to control any known gene. So far, the researchers have tested it in bacteria and yeast, and are now adapting it for the malaria parasite.

Niles also hopes to use this technology to study RNA localization, a recently discovered phenomenon. The classical picture of mRNA is that it floats freely inside the cell, waiting to encounter a ribosome. But now, "many researchers are finding that there are very discrete subcellular regions and patterns in which RNA will localize," says Niles.

RNA localization has already been shown to be significant in neurons, in which certain types of mRNA cluster in the axons (long extensions of the neuron), because that's where the proteins they code for are most needed.

With this tool, researchers should be able to direct mRNA to places it wouldn't normally go, allowing them to see how the cell is affected when mRNA goes to the wrong location. "We like to think of this as a platform for us to gain control over RNA translation or any other RNA activity in any cell type of interest," says Niles.

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