

MARCH 09, 2007

Sleeping Sickness Parasite Can't Live with Stress

The parasite responsible for African sleeping sickness causes its victims plenty of sleepless nights - but the parasite itself does not cope well with stress. New research from Howard Hughes Medical Institute international research scholar Shulamit Michaeli and colleagues shows that the parasite's natural response to stress is enough to kill it, a weakness that researchers may be able to exploit.

Michaeli and colleagues at Bar-Ilan University in Ramat-Gan, Israel, published their work in the April issue of *EMBO Reports*.

"Triggering this particular stress-induced pathway made the parasites just disappear. It was dramatic, and it was very surprising."

— **Shulamit Michaeli**

Current therapies for sleeping sickness, which is fatal if not treated, have been in use since the 1920s and can cause severe side effects, including changes to the brain's structure and function. In many cases, the parasite can develop resistance to the drugs used to treat the infection. We definitely need better therapies for this disease, said Michaeli.

African sleeping sickness occurs mainly in sub-Saharan Africa. The infection is caused by the parasite *Trypanosoma brucei*, which lives in tsetse flies. After a person is bitten by an infected fly and the parasite crosses into the brain, its presence triggers confusion, disrupted sleep, and other neurological symptoms, leading to death. In 2005, outbreaks in Angola, the Democratic Republic of the Congo, and Sudan infected between 50,000 and 70,000 people, according to the World Health Organization.

Michaeli has spent years studying this parasite, focusing in particular on its RNA metabolism and RNA processing pathways. In the course of these studies, her team discovered that inducing a stress response killed the organism. It started out as an academic question, said Michaeli. But then we saw that triggering this particular stress-induced pathway made the parasites just disappear. It was dramatic, and it was very surprising. When I saw that, I said, 'This could be a potentially new avenue for drug discovery.'

A study conducted by graduate student Yaniv Lustig investigating how proteins move across the internal membranes of the parasite led to the new discovery. In cells, certain proteins have a molecular tag called a signal peptide that indicates that they are destined to cross one of the cell's membranes. The complex that recognizes this tag and directs newly synthesized proteins to the membrane is the signal recognition particle (SRP). During this step, the new proteins remain bound to ribosomes, the protein factories where they were made. Later the SRP interacts with a receptor on the membrane, leading to the protein's translocation through the membrane to reach its final destination.

To study this process, Michaeli's team knocked down the production of the SRP receptor, leaving the SRP complex with no place to dock. When there is no SRP receptor, the SRP has no place to hook. That means the SRP is 'stuck' on ribosomes with the newly synthesized polypeptide, and the organism interprets this as stress. A signal is sent to the nucleus telling it to shut down a small RNA molecule called the spliced leader (SL) RNA, which is responsible for all mRNA production in the cell. This stops protein production, and kills the parasite. In fact, you could say they kill themselves, she said. Michaeli and her colleagues termed this novel stress-induced mechanism spliced leader silencing (SLS).

The team discovered SLS by depleting the SRP receptor from cells. They soon found that a variety of other stressful situations could induce SLS in the same way, ultimately killing the parasites. These included manipulating the pH of the parasites' environment, creating oxidative stress, or causing protein misfolding.

The experiments show that SL RNA is indeed the key molecule in this parasite, said Michaeli. Without it, nothing else works - there is no messenger RNA produced, and no protein being made. That's it.

She continues: The idea for drug discovery is to find compounds that induce this stress response. We now have a way to look for such compounds. Michaeli is working with other teams that have libraries of molecules available for screening.