News Alert:
Get up-to-date HHMI Science News via E-mail
SIGN UP

RESEARCH NEWS

DOWNLOAD STORY PDF
REQUIRES ADOBE ACROBAT 7.0

FEBRUARY 01, 1996

A New Blood Test for Chagas Disease

Several years ago, Larry Simpson was approached with a project that intrigued him. A colleague had access to tissue from 2,500-year-old Chilean mummies and he wanted Simpson to determine if the preserved tissue showed signs of infection by *Trypanosoma cruzi*, the parasite that causes Chagas disease.

Interested, Simpson told his colleague, Christian Orrega, that he would modify the polymerase chain reaction (PCR) test in such a way that it would tell them whether the mummies had been infected with *T. cruzi.* One might say that Orrega's request was tailor-made for Simpson, who had thought about employing modern science to settle a longstanding debate about whether the legendary evolutionist Charles Darwin had contracted Chagas disease while he was in South America on the voyage of the *Beagle.*

“Minicircle DNA from Leishmania tarentolae.”

But as Simpson, who is currently in the Howard Hughes Medical Institute at UCLA, and his graduate students, Nancy Sturm and Herbert Avila, and his Brazilian collaborators, Wim Degrave, Otavio Thiemann and Carlos Morel, began to adapt the PCR test, they immediately realized how their twist on this powerful assay might transform the current method of diagnosing Chagas disease. At the time, the "gold standard" for diagnosis resembled an ancient torture ritual. The infected person was required to endure a blood feeding by uninfected "kissing bugs," insects that transmit Chagas disease. The bugs were then taken back to the lab, and several weeks later were dissected and examined for the presence of *T. cruzi.* If *T. cruzi* was present in the dissected kissing bugs, the patient had been infected.

As it turns out, the scientists were right because the PCR-based assay for *T. cruzi* detection has revolutionized the diagnosis of Chagas disease. It is rapid, portable and allows medical workers to test people who are far from modern laboratories — something not possible before. Simpson and his Brazilian collaborators continue to make gradual improvements to the PCR test, but, unfortunately, in all the hubbub, the group has had to put the Chilean mummy project on hold. "We might get around to it sometime soon," Simpson said.

The work behind developing the Chagas disease blood test began many years ago as a quest by Simpson and his colleague and wife, Agda, to understand the unique structures of mitochondrial DNA in *Leishmania tarentolae,* a

trypanosomatid parasite that infects lizards. Mitochondria are the energy-producing organelles within cells, and are present in the trypanosomatids such as *L. tarentolae* and *T. cruzi.* Such parasites are also called kinetoplastids because of the presence of a small granule, or kinetoplast, at the base of the flagellum when they were stained with certain dyes. "The kinetoplast actually represents a portion of the single complex mitochondrion of the cell that contains a huge compact mass of DNA," Simpson said.

The Simpsons found that the kinetoplast DNA of trypanosomatid parasites consists of thousands of interlocking minicircles and a few interlocking maxicircles which together form a single giant network of DNA. No one knew the function of minicircle DNA when Simpson and Carlos Morel of the Oswaldo Cruz Institute in Rio de Janeiro, Brazil found that the kinetoplast DNA of various strains of *T. cruzi* differed greatly in their DNA sequence. "This suggested to us," said Simpson, "that minicircle DNA could be used to identify different strains of *T. cruzi* and provide important epidemiological and perhaps even clinical data about the spread of the parasite."

Simpson's laborious analysis of minicircle and maxicircle DNA in *T. cruzi* and *L. tarentolae* led him and several other investigators to a discovery that would come close to shaking the very foundations of modern molecular biology. The central dogma of molecular biology is that DNA makes RNA and RNA makes protein. In 1986, scientists in Amsterdam found an instance of "RNA editing," where an RNA transcript of the maxicircle DNA was modified within the coding region by the insertion of uridine bases to form a translatable message. (Uridine is one of the four bases in RNA.)

Simpson and his graduate student, Janet Shaw, together with Jean Feagin and Ken Stuart at the Seattle Biomedical Research Institute, soon discovered more dramatic examples of sequence modifications of maxicircle transcripts in several trypanosomatid species, involving both the addition and deletion of uridine residues. In essence, the RNAs were being changed into genes that were not found in the DNA. Simpson and Shaw used the term "cryptogenes" to describe these hidden genes. The most astonishing examples of cryptogenes were discovered by Feagin and Stuart in which hundreds of uridines were added at different sites within the entire length of the RNA transcript, a phenomenon known as "pan-editing." "It truly appeared as if 'new genes' were being created from whole cloth," Simpson said.

As evidence for cryptogenes mounted, the Simpson and Stuart labs began to publish their data. But since the matter seemed to be a challenge to the central dogma, this raised much controversy. Cryptogenes were not exactly derided by the scientific community, but their existence was not met with instant acceptance, either, Simpson said. "The data supporting cryptogenes was usually met with astonishment and termed bizarre," he said.

The mystery of cryptogenes was finally explained in 1990 with the discovery of a new class of RNA molecule by Simpson and his postdoctoral fellows, Beat Blum and Norbert Bakalara, which they called guide RNAs (gRNAs). The gRNAs were discovered during a computer search of maxicircle sequences for short stretches of sequence that could pair up with the known edited RNA sequences. The computer search turned up seven short RNA sequences that were scattered throughout the maxicircle. Simpson's team soon showed that these sequences were transcribed into small RNAs of about 50 nucleotides in length that have sequences at one end that allow the gRNAs to anchor to

specific mRNA sequences adjacent to the sites of uridine insertions. And the gRNAs carried an additional length of uridine residues at the other end that were not encoded in the DNA sequence.

"Guide RNAs had come to the rescue of the central dogma," Simpson said. "Of course we were very excited by this discovery, but at the same time a bit chagrined that the answer to the secret of editing was not something completely new and earthshaking, but something that obeyed the simple rules of base-pairing." The only novelty was that guanine seemed to pair frequently with uridine when the gRNAs and edited mRNAs interacted.

Simpson and Sturm and others next showed that most of the gRNAs in the cell were encoded in the minicircle molecules, thus finally providing a function for these mysterious molecules. This also neatly explained the extensive sequence differences in the minicircle DNA molecules, since each minicircle class encoded a different gRNA, and many gRNAs were required for all the different editing events. In recent work, Simpson and Avila have shown that the minicircle sequence differences between different strains of *T. cruzi* are the result of the accumulation of mutations that do not affect the editing process.

The story was made even more complex when the Simpson lab developed a biochemical model whereby the gRNA actually guides the insertion and deletion of uridine residues at specific sites within the mRNA. Soon thereafter, Simpson and Blum discovered yet another class of RNA molecules which consisted of gRNAs chemically linked to mRNA fragments at editing sites. This discovery led them to propose a model for editing which was very similar to the way in which the so-called introns or intervening sequences found in most genes in higher organisms are removed from mRNAs in mitochondria in other organisms, the phenomenon known as RNA splicing.

Soon after Simpson and Blum had discovered the existence of these fused gRNA/mRNA molecules and developed the splicing model, but before they had published their findings, Simpson was invited by Tom Cech, a Hughes investigator at the University of Colorado, Boulder, to give a talk in Colorado. To Simpson's surprise, Cech said that he too had solved RNA editing and proceeded to draw on a board the entire splicing model. "As happens frequently in science, when ideas are ripe, they germinate simultaneously in several gardens," Simpson said.

Prior to the discovery of RNA editing and gRNAs, Simpson had thought that the unique nature of kinetoplast DNA might one day allow it to serve as a molecular target for identifying different strains of *T. cruzi* and other trypanosomatid parasites. This has proven to be true, and the PCR assay developed in the Simpson laboratory is in use around the world — from the steamy Amazon jungle, where Chagas disease is still a deadly threat, to metropolitan Los Angeles where infected immigrants who are selling their blood for money are depositing Chagas disease in blood banks.

Simpson has not given up on his original idea of settling the question of Darwin's illness once and for all. "After all," he mused, "Darwin is buried in a very accessible location — in Westminster Abbey right next to Isaac Newton. And we only need a little piece."

MORE HEADLINES

INSTITUTE NEWS

07.30.10 | Valued Fruit Fly Facility Will Expand with HHMI Support

06.24.10 | Cheryl Moore Named HHMIâ€™s First Chief Operating Officer

06.11.10 | Newsome Receives Champalimaud Vision Award

SEARCH NEWS ARCHIVE

Back to Top

Home | About HHMI | Press Room | Employment | Contact

© 2010 Howard Hughes Medical Institute. A philanthropy serving society through biomedical research and science education.
4000 Jones Bridge Road, Chevy Chase, MD 20815-6789 | (301) 215-8500 | email: webmaster@hhmi.org