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## Dengue Virus Reveals Its Circular Secret

The first step in the transmission of mosquito-borne viruses is no mystery: it's the pesky insect's bite that allows the virus to enter its victim's bloodstream. But for some of the most dangerous insect-borne viruses, details of what happens next have been unclear.

In a finding that could help scientists develop ways to prevent or treat certain infections, researchers led by a Howard Hughes Medical Institute (HHMI) international scholar in Argentina have identified a genetic element that the dengue virus uses to replicate, triggering the potentially fatal illness known as dengue hemorrhagic fever.

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- **Andrea V. Gamarnik**

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In the August 15, 2006, issue of the journal *Genes & Development*, published online August 1, 2006, virologist Andrea Gamarnik and colleagues at Leloir Institute Foundation in Buenos Aires, describe how a viral enzyme recognizes and amplifies the genetic material needed to assemble new dengue viruses. Their findings provide the first model for RNA replication in the family of viruses that includes West Nile, St. Louis encephalitis, and hepatitis C. These viruses, known as flaviviruses, cause millions of cases of human illness each year, but no vaccines or antiviral drugs exist to control most of the infections. Dengue fever is endemic in many tropical and subtropical regions, causing a severe, flu-like illness that sickens more than 50 million people and kills 25,000 each year.

Once a virus enters a host cell, its top priority is to copy its genetic code so that it can make more virus. Flaviviruses are so efficient at this task that they can churn out tens of thousands of copies of their genome—which is composed of ribonucleic acid, or RNA—within hours of infecting a cell.

For dengue and other flaviviruses, the first step is to produce viral proteins, including an enzyme that can copy RNA. But the viral RNA is not the only RNA in an infected cell. So once the enzyme, called RNA-dependent RNA polymerase (RdRp), is produced, it finds itself surrounded by cellular RNA, creating a dilemma: How does RdRp distinguish viral from cellular RNA, to replicate the right molecule?

Last year, Gamarnik got her first hint when her group identified two RNA sequences located at the ends of the dengue virus genome. These short sequences interact during RNA replication, shaping the viral RNA genome into a circle. Gamarnik's team published those findings in the June 2005 issue of the *Journal of Virology*.

Further studies of the dengue virus life cycle revealed another piece of the virus's RNA that recruits the enzyme RdRp. Found at one tip of the genome, that sequence adopts a characteristic stem-loop structure that the scientists suspected might be important to its function.

To test whether RdRp was relying on that stem-loop shape to recognize the viral RNA, the scientists created copies of the dengue genome with minor changes that would alter its structures. The mutated RNAs were then inserted into mosquito cells or hamster cells to see if the viral RNA would be copied.

To their surprise, the scientists found that the stem-loop or SLA sequence is essential for viral replication. Changes in even one or two building blocks in this structure were enough to halt the replication process. "That told us that RdRp probably discriminates the viral RNA by recognizing SLA," Gamarnik said.

To confirm the vital link between RdRp and SLA, the researchers allowed virus particles that couldn't replicate to evolve in cells grown in lab dishes. Spontaneous mutations that occurred in the SLA often restored RdRp's activity and full viral replication capacity.

The scientists didn't expect to find that RdRp activity relies on a sequence at the far end of the genome, thousands of nucleotides away from the end where the enzyme begins copying the RNA.

The new discovery makes sense, Gamarnik said, because the circular shape adopted by the virus brings the distant ends of its genome together. "At first we were puzzled by the cyclization feature of this virus," said Gamarnik. "We now recognize that it serves a role in bringing the SLA promoter near the initiation site."

Paul Ahlquist, an HHMI investigator at the University of Wisconsin-Madison and an expert on RNA viruses, said that the Gamarnik team's findings explain prior observations from her lab and others that binding between the 5-prime and 3-prime ends of the viral genome is required for replication of dengue and several other medically important flaviviruses. "These insights suggest possible mechanisms by which flaviviruses may regulate some distinct replication steps, and might ultimately provide foundations for new ant flavivirus strategies," Ahlquist said.