

Analysis of RNAi –based resistance in *Aedes aegypti* to dengue viruses.

Irma Sanchez-Vargas, Alexander Franz, Barry J. Beaty and Ken E. Olson

Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, Colorado State University , Fort Collins, CO 80523 USA.

The maintenance and transmission of arbovirus in nature is, in part, dependent on genetically competent mosquito populations that support virus infection. Dengue viruses (DENVs) are the most medically important arthropod-borne viral pathogens affecting humans. *Aedes aegypti* mosquitoes are the principle vectors of DENV serotypes 1-4. DENVs enter the mosquito when the adult female takes a blood meal from a viremic individual. Virus replicates in the infected vector for approximately 10 days before it can be transmitted through saliva while feeding on a new host. We have hypothesized that the triggering of the RNA interference (RNAi) pathway in relevant tissues of DENV vectors may be an important way of stopping the progression of the virus and profoundly affecting virus transmission. RNAi-based resistance in mosquitoes requires the triggering of the silencing pathway in tissues that are critical for vector competence and the silencing trigger (dsRNA) must probably be expressed prior to virus infection.

To successfully use an RNAi approach we must first determine the infection patterns and transmission potential of DENV2 Jamaica 1409 virus in competent vectors. The Rexville D Puerto Rico (Rex D) strain is a competent vector of DENVs and the RexD mutant strain Higgs White Eye (HWE) is currently used in our laboratory to develop transgenic mosquitoes. In either strain, we see DENV2 amplification begin following ingestion of a blood meal containing 10^7 pfu/ml. The infection appears to be unidirectional in the midgut epithelium entering the luminal side and exiting the basal side. We can detect new viral RNA in midguts at 48 h post infection by Northern blot analysis and DENV2 envelope (E) antigen by IFA as early as 96 h post infection. Following replication of virus in the epithelial cells, virus escapes from the midgut and disseminates to secondary target organs such as fat body and salivary glands. Generally DENV2 E antigen is detected in fat body and salivary glands by IFA at 7 and 10 d after infection, respectively. Infection of fat body appears to be transient but usually precedes salivary gland infection. Further replication occurs in the salivary glands. The distal lateral lobes are the first site of virus infection and virus E antigen accumulates in salivary glands where IFA signal appeared most intense at 18-21 days post infection. DENV2 E antigen was not detected in the proximal region of the lateral lobes. The HWE strain is, in general, less competent than the RexD strain, but still supports DENV2 infection and is a convenient strain for transgenesis.

With the success of transformation in *A. aegypti*, we can now genetically engineer DENV-resistant mosquitoes. We have hypothesized that genetically modified mosquitoes can be generated that transcribe a DENV-specific dsRNA (prM and NS5) that triggers the RNAi response soon after ingestion of a blood meal and/or in critical sites in the vector such as fat body and salivary glands. *A. aegypti* promoters such as *carboxypeptidase* (midgut), *Vg* (fat body) and *D7* (distal lobes, salivary glands) should allow tissue-specificity and sex-specificity to promote RNA silencing of virus replication. The ultimate goal is to genetically modify mosquitoes that are profoundly impaired for transmitting DENVs. Unfortunately, there are no small animal models for conveniently studying DENV transmission; however, we are developing high-throughput methods for testing the transmission potential of new mosquito lines. A combination of virus antigen (IFA), genome detection (qRT-PCR), and high-throughput transmission assays should allow rapid assessment of transmission for potentially virus-resistant lines.