

**USING RNA INTERFERENCE TO DEVELOP DENGUE VIRUS RESISTANCE IN GENETICALLY MODIFIED *Aedes aegypti*.** By Ken E. Olson, Zach N. Adelman†, Emily A. Travanty, Alexander Franz, Kim M. Keene, Barry J. Beaty, Carol D. Blair, and Anthony A. James†. Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523 USA.

The mosquito-borne dengue (DEN) viruses (serotypes 1-4; Flaviviridae) cause DEN fever and DEN hemorrhagic fever in human populations throughout the tropics. *Aedes aegypti*, the principal DEN virus (DENV) vector, has now spread throughout much of the tropical world and the viruses are hyperendemic in many of these regions. New approaches to DEN control are needed to complement traditional disease control strategies. The underlying hypothesis of our research is that one method of achieving DEN disease control is to introduce anti-DENV genes into DENV competent mosquito populations and profoundly alter the vector competence of the population. A key step in testing the validity of this hypothesis is to develop transgenic *Ae. aegypti* mosquitoes that specifically block replication and transmission of the viruses. The block occurs by expression of specific anti-DENV effector molecules throughout the whole mosquito or specifically in the salivary glands or midgut of the transgenic mosquito, preventing replication and transmission of the viruses.

What type of anti-DENV effector molecule may affect vector competence? We now know that *Drosophila melanogaster*, *Caenorhabditis elegans*, many plants, and even humans have an ancient anti-viral pathway, termed RNA interference (RNAi) that is triggered by the presence of double stranded RNA (dsRNA) in cells. dsRNA is an early warning signal in cells of RNA virus invasion and induces RNAi to destroy any mRNA having sequence identity with the dsRNA. Many RNA viruses normally generate dsRNA in infected cells as a byproduct of replication and these replicative intermediates serve as potent recognition patterns for inducing the RNAi intracellular response. We now know that at least two mosquito species, *Ae. aegypti* and *Anopheles gambiae*, elicit an RNAi response very similar to that found in *D. melanogaster*, because they can silence gene expression or virus replication after introducing dsRNA targeted to a specific gene. Some arboviruses appear to trigger the RNAi response in mosquito cells and we now have evidence that *An. gambiae* has a strong antiviral response to the arbovirus O'nyong-nyong (Togaviridae). If RNA viruses trigger RNAi, why are mosquitoes such efficient vectors of arboviruses? We do not know for sure, but DEN viruses may escape the antiviral effects of RNAi in competent mosquitoes by either failing to form a sufficient threshold of dsRNAs molecules required for triggering the response or by encoding a viral protein that suppresses the RNAi response. However, it may be possible to induce an RNAi response to DENVs in the midgut of the mosquito as the mosquito ingests an infected blood meal thus destroying the virus genome before the virus can evade the RNAi response. The midgut is a likely target for mounting this line of defense because it is the first tissue the virus encounters in the vector and is the major determinant of vector competence in the mosquito.

To test this hypothesis, we are using molecular biological and transgenesis techniques to engineer strains of *Ae. aegypti* (Higgs white-eye, Puerto-Rican Rexville D, DENV2 competent) that transcribe an anti-DEN effector molecule that forms dsRNA in mosquito cells and triggers the RNAi response. This effector RNA molecule contains a 300 base sense RNA fragment derived from the premembrane (prM) coding region of DENV2 (New Guinea C), followed by an *Ae. aegypti* sialokinin intron sequence, and a 300 base antisense RNA that has exact complementarity with the prM sense RNA. The effector RNA is driven by the *Ae. aegypti* carboxypeptidase promoter so that a burst of transcription occurs in midgut epithelial cells within hours after ingestion of a blood meal.

The anti-DEN transcription unit has been inserted into the *Mos1* (Mariner) transposable element transformation system containing the 3PAX3-eGFP eye marker to facilitate screening for transformants. We are currently using a binary system of transformation that involves co-injection of two plasmids into mosquito embryos. One plasmid contains the anti-DENV2 and eye color selection genes flanked by the *Mos1* inverted-repeats and the second plasmid contains the transposase. This allows stable integration of the anti-DEN gene and prevents mobilization of the transposon. We currently have a number of transgenic mosquito families that have the anti-DENV2 gene stably inserted in the mosquito genome and transcribe the anti-DEN RNA. Several families have now been challenged with DENV2 (Jamaica 1409). The results of these challenges will be discussed.