

Development of transgenic *Aedes aegypti* that display resistance to dengue-2 virus infection by triggering RNA interference to the virus in the midgut. Ken E. Olson, Alexander Franz, Irma Sanchez-Vargas, Zach Adelman, Barry J. Beaty, and Anthony A. James. Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523 USA. Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697 USA

We have previously shown that dengue virus serotype 2 (DENV2) can be silenced in cultured mosquito cells by triggering RNA interference (RNAi) with virus-specific, double stranded RNA (dsRNA). We hypothesized that *Aedes aegypti*, the principle vector of DENVs, can be genetically manipulated to express a dsRNA that blocks DENV2 infection of the midgut, the first tissue that the virus encounters after the mosquito ingests a blood meal. To test this strategy in transgenic mosquitoes, we developed transgenic lines using the non-autonomous *Mos1* transformation system. Transgenic lines contained the donor *Mos1* transposable element with the 3xP3-EGFP eye marker gene for selection of transformants and a transcription unit that expressed either EGFP or an effector dsRNA from the inducible *Aedes aegypti* carboxypeptidase (*AeCP*) promoter (Moreira et al., 2000. PNAS, 97:10895-10898). Two transgenic lines, AeCP.GFP#52 and AeCP.GFP#112, displayed high levels of EGFP throughout the midgut within 24h of ingesting a blood meal identifying *AeCP* promoter as a potentially excellent promoter for expressing effector RNAs in a tissue-specific manner. Two transgenic lines, Carb51.2i2 and Carb77.2i2 were then developed that used *AeCP* to express an effector gene containing a 580 bp non-translatable DENV-2 prM sense sequence, a 64 bp intron sequence from the *Aedes aegypti* sialokinin gene, an inverted repeat prM (antisense) sequence, and a transcription terminator. Northern blot analysis showed that only the Carb77.2i2 line expressed a transcript of the predicted size (~1200bp) following ingestion of a blood meal. The heterozygous Carb77.2i2 line had Mendelian ratios of 1:1 at G₄ following outcrosses (G₃ GFP+ males with wild-type females). We infected EGFP +, G₃ Carb77.2i2 mosquitoes by feeding them with a blood meal containing 10⁷ pfu/ml of DENV2. Carb77.2i2 mosquitoes showed reduced accumulation of DENV2 genomic RNA at 48h post infection (pi), but genomic DENV2 RNA was readily detected in midguts of parental non-transgenic control mosquitoes (HWE strain). Furthermore, immunofluorescence analysis detected significantly less DENV2 envelope (E) antigen in Carb77.2i2 midguts than in control midguts at 7, 10, and 14 days pi and virus titers were 100-1000 fold less in whole Carb77.2i2 mosquitoes at 7 and 10 days pi than in control mosquitoes. Transmission analysis confirmed that the Carb77.2i2 line was significantly less likely to transmit DENV2 than the non-transgenic control at 14 days pi. Future work will analyze the fitness of the Carb77.2i2 line in cage studies and determine the stability of the resistance phenotype over time. We are also testing the specificity of resistance (other DENV2 genotypes and DENV serotypes) and whether RNAi resistance can be triggered in additional tissues (fat body and salivary gland) to add more barriers to DENV infection in the vector.