Aedes Relish and Its Regulatory Role in the Mosquito Innate Immunity

Sang Woon Shin*, Vladimir Kokoza, Iouri Lobkov
and Alexander S. Raikhel

Department of Entomology, University of California, Riverside California 92521, USA

The Rel/NF-κB transcription factor Relish performs a central role in the acute-phase response to microbial challenge by activating immune antibacterial peptides. We cloned and molecularly and genetically characterized the gene homologous to *Drosophila* Relish from the mosquito *Aedes aegypti*. Compared to *Drosophila* Relish, The primary structure of *Aedes* Relish has showed three unique features; 1. Three alternatively spliced transcripts encoding different proteins, 2. A death domain in extreme C-terminus, 3. The putative N-terminal transactivation domain. All three *Aedes* Relish transcripts were induced by bacterial injection but not by blood feeding. *In vitro*-translated protein (ΔRel) from the Rel-only construct, C8, specifically binds to the κB motif from *Drosophila* cecropin A1 and *Aedes* defensin genes. PCR and Southern blot hybridization analyses show that these three transcripts originated from the same large inducible mRNA encoded by a single Relish gene.

As a functional analysis of *Aedes* Relish, Vg-ΔRel, the Rel-only construct without the putative transactivation domain following vitellogenin promoter was introduced to *A. aegypti* wild type Rockefeller/UGAL strain by using the piggyBac transposable element vector pBac[3xP3-EGFP afm]. The established line #80 contained the Vg-ΔRel transgene with strong eye-specific expression of the enhanced green fluorescent protein (EGFP) marker gene regulated by the artificial 3xP3 promoter. Southern analyses indicated a single copy incorporation of Δ

Vg-Rel transgene into the mosquito genome. Analysis of ΔVg-Rel transgene expression in #80 transformant demonstrated strong blood-meal activation regulated by the Vg promoter. The transgenic mosquitoes are extremely susceptible to the infection of *Enterobacter cloacae*. This Relish-mediated immune- deficient (RIMD) phenotype activated by blood-meal coincides with the severely reduced induction level of AMP genes, defensin and cecropin, and with the compromised expression of Defensin A peptide. The transgenic mosquitoes showed a marked susceptibility to Gram (-) bacteria, but no more susceptibility to Gram (+) bacteria. This RIMD was dominantly negative phenotype and restored by overexpression of Defensin A peptide.

Our studies with the transgenic mosquitoes show that *Aedes* Relish is specifically involved in the induction of the humoral immune response against Gram (-) bacteria, including the transcriptional activation of two AMP genes, defensin and cecropin. The elucidation of mosquito immune response itself may be a crucial step to understanding of vector-parasite relationship that caused several mosquito-borne diseases including malaria. However, the lack of the genetic approaches like mutagenesis and transgenic techniques, which are ordinary in *Drosophila* studies, has blocked further advance of studies of the mosquito immune response. Generation of transgenic dominant negative Relish mosquito mutant opens the door of utilization of reverse genetic approach to the elucidating immune responses of the mosquito to various pathogenic agents of mosquito- borne human diseases.