

Transgenic alteration of Toll immune pathway affects anti-fungal and anti-*Plasmodium* activity in the female mosquito *Aedes aegypti*.

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Reverse genetics is a powerful tool for understanding gene functions and their interactions in the mosquito innate immunity. We took the transgenic approach, in combination with the RNAi technique, to elucidate the regulation of Toll immune pathway in the mosquito *Aedes aegypti*. Mosquito REL1, a homolog of *Drosophila* Dorsal, is a key factor mediating the Toll immune pathway. By transforming the mosquitoes with Δ REL1-A or a dsRNA construct of REL1 driven by the female fat body-specific vitellogenin (*Vg*) promoter using the pBac[3xP3-EGFP, afm] vector, we generated two different transgenic mosquito strains, one with over-expressed AaREL1, and the second with AaREL1 knock-down. Both strains had a single copy of the respective transgene, and the expression in both transgenic mosquitoes was highly activated by blood feeding. *Vg*- Δ REL1-A transgenic mosquitoes activate Toll immune pathway in the fat body by blood feeding. The over-expression of both isoforms, AaREL1-A and AaREL1-B, in *Vg*- Δ REL1-A transgenic mosquitoes resulted in the activation of *Aedes* Spätzle1A and Serpin-27A, independent of septic injury. The same phenotype was observed in the mosquitoes with RNAi knock-down of an *Aedes* homolog to *Drosophila* cactus, the I κ B inhibitor of *Drosophila* Toll pathway. The effect of the transgenic RNAi knock-down of AaREL1 on mosquito innate immunity was revealed by increased susceptibility to the entomopathogenic fungus *Beauveria bassiana*. In addition, the *Vg*- Δ REL1-A transgenic mosquitoes harbored significantly less oocysts of *Plasmodium gallinaceum* compared to wild-type mosquitoes, showing that the activation of Toll immune pathway restricted the development of the parasites.