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Analysis of Transgenic Anopheline Mosquitoes Overexpressing an Antimicrobial Peptide in Their Salivary Glands*1, *2

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Anopheline mosquitoes transmit malaria parasites. The mosquito has an innate immune system to prevent infection by various pathogens; however, malaria parasites escape from this immune system and develop. It remains unclear how this occurs. Transgenesis in anopheline mosquitoes has been utilized for the analysis of mosquito-malaria parasite interaction and a new strategy of malaria control (Terenius *et al*. 2008). The insect antimicrobial peptide defensin is one of the effector molecules against Gram-positive bacteria in the innate immune system (Cirimotich *et al*. 2010). Overexpressed defensin showed a certain inhibitory effect against the malaria parasite in mosquito midgut or hemolymph using transgenic lines (Kim *et al*. 2004; Kokoza *et al*. 2010). Mosquito salivary glands have been shown to be effective tissue for inactivating malaria sporozoites using transgenesis (Sumitani *et al*. 2013); however, the overexpression of defensin has not been examined in these salivary glands.

The aim of this study was to investigate the effect of overexpressed defensin in *Anopheles stephensi* salivary glands against the malaria parasite. Moreover, we chose defensin derived from the tsetse fly, *Glossina palpalis* (*gpdef*), and aimed to examine the effect of this immune system molecule of a non-malaria vector species against the malaria parasite. We cloned the *gpdef* gene, and produced a transgenic mosquito expressing *gpdef* fused to monomeric *DsRed* gene (*mDsRed-gpdef*) under the control of *anopheline antiplatelet protein* (*aapp*) gene promoter, which drives the salivary gland-specific expression in adult females (Yoshida and Watanabe 2006). The transgenic mosquitoes showed female salivary gland-specific expression of the *mDsRed-gpdef* gene. The mDsRed-gpdef protein in female salivary glands was detected at least 13 days after blood feeding, at which time sporozoites begin to invade into the salivary glands. We are now beginning to investigate the effect of the mDsRed-gpdef protein in the salivary gland against sporozoites using a rodent malaria model.

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