#24: Viral directed microRNA manipulation within *Anopheles gambiae*

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Anopheles gambiae is the major vector of the deadliest human malaria parasite, Plasmodium falciparum, in sub-Saharan Africa. In recent years, this mosquito vector has been found to express over 66 unique microRNA (miRNAs). These short (~22 nucleotide) segments of noncoding RNA have been found to alter gene expression levels through post-transcriptional regulation processes known as RNA activation and RNA interference. Differential expression of miRNAs has been reported in various mosquito vectors during important biological processes such as malaria infection, egg development, and sugar absorption, yet the role of these endogenous miRNAs in *An. gambiae* has not been extensively investigated. Manipulation of specific miRNAs within *An. gambiae* may identify novel targets for control strategies and further our understanding of mosquito gene regulation processes. Transgenic viral agents such as the recently discovered *An. gambiae* densovirus (AgDNV) have the potential to stably infect *An. gambiae* and alter miRNA levels in vivo. AgDNV is species specific and has been shown to have nominal effects on the *An. gambiae* transcriptome. We have developed an AgDNV co-transfection system to manipulate *An. gambiae* miRNA levels by expressing endogenous pre-miRNA sequences or developed miRNA sponge sequences from a created viral intronic region. In vitro expression of mature *An. gambiae* miR-375 has been validated via qPCR, indicating proper pre-miRNA recognition and processing. AgDNV-based expression of miR-375 in vitro led to a decrease in mRNA transcripts encoding REL1, an important mosquito immune gene and a predicted target of miR-375. While other miRNAs have yet to be fully tested, this AgDNV system represents a novel molecular tool with which the role of endogenous *An. gambiae* miRNAs can be studied. Future studies using transgenic AgDNV may lead to innovative vector control methods and aid in basic An. gambiae miRNA function investigations.