

## **Comparative Analysis of Promoter Function in Defensin Genes From *Anopheles gambiae* and *Aedes aegypti***

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By facilitating the expression of transgenes that interfere at various stages in the transmission of pathogens through the insect vector, the transformation of blood-sucking insects may ultimately lead to new control strategies for vector-transmitted disease. However, in order to minimise the impact on relative fitness, expected as a result of transgene activity, one of the major requirements in the exploitation of such technology will be control over the timing, location and level of transgene expression. Consequently there is considerable interest in the use of promoters that might direct transgene expression to the key tissues involved in the interaction between insects and the pathogens they transmit, in particular the regulatory sequences of the immune response genes.

Examples of such promoters might include those expressed preferentially in the midgut or the salivary glands. In our laboratory we have focused on the promoters of insect immune response genes, several of which show tissue specific expression patterns in response to infection by pathogens. Of particular interest are insect defensins, which participate robustly in the immune response, and their expression has been shown to be under both temporal and spatial control. To gain a better understanding into how this control is exerted we have focused on a comparative analysis of defensin gene promoters from *Anopheles gambiae* and *Aedes aegypti*. Following our initial description of the *Anopheles gambiae* defensin 1 gene [1] we have cloned and characterized approximately 1kb of regulatory sequence from two *Aedes aegypti* defensin A gene isoforms [2]. Computer assisted sequence analysis has been used to compile detailed promoter maps for all 3 genes, and their comparison has revealed some interesting associations between putative NF- $\kappa$ B and C/EBP factor binding sites, that are conserved between the two species and may therefore reflect functional associations.

Initial comparisons of immune responsive properties of the three promoter regions have used cell transfection experiments, which include full length promoter constructs as well as a series of deletions and mutants, together with a range of immune stimuli. Results for all 3 promoter regions confirm the importance of the region containing the putative NF- $\kappa$ B and C/EBP factor binding sites as well as some differences worthy of further investigation. Current progress in these comparative analyses will be presented.

Work is ongoing to expand the study to include promoter regions from defensin 3 of *Anopheles gambiae* [3] and *Aedes aegypti* defensins B and C [4, 2] and to look more closely at possible associations between promoter structure and tissue specific expression. In the medium term we hope to extend these studies to include *in-vivo* assessments of promoter activity in transgenic mosquitoes. Subsequently, we may be able to develop novel promoters that drive anti-pathogen effector genes with optimal spatial and temporal expression profiles.

## References

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3. Christophides GK, *et al.* (2002). Immunity-related genes and gene families in *Anopheles gambiae*. *Science* 298: 159-165.
4. Chalk R, *et al.* (1995). Full sequence and characterisation of two insect defensins: immune peptides from the mosquito *Aedes aegypti*. *Proc. Roy. Soc. Lond. B. Biol. Sci.* 261: 217-221.

## **Additional Supporting Information**

Research Group Leader:	<b>Professor Paul Eggleston</b>
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**Research Interests:** Molecular entomology, particularly the molecular genetics of mosquitoes that transmit human disease and their interactions with malarial parasites. Because of their medical importance, the focus of the group is on the malaria vector mosquito, *Anopheles gambiae* and the yellow fever mosquito, *Aedes aegypti* although *Drosophila* and other insects are used where appropriate. Current projects include strategies for the targeted integration of transgenes in mosquitoes, the control of transgene expression through characterization of immune gene promoters and gene switching mechanisms, the use of promoter-traps to identify novel cell-signalling pathways, the analysis of fitness associated with parasite refractoriness and transgene flow, development of incompetent transgenic anopheline mosquitoes and the effect of the rat tapeworm on reproductive success of the beetle host. For more details please see Paul Eggleston's web home page:

<http://www.keele.ac.uk/depts/aep/staff/pe.htm>.