

## FEMALE-SPECIFIC PROMOTER ACTIVITY IN DIPTERAN LARVAE

Umesh K. Jinwal, Oksana V. Litvinova, Shashank Jain, Neeraj K. Sharma and Helen Beneš

Department of Neurobiology and Developmental Sciences  
University of Arkansas for Medical Sciences, Little Rock, AR, U.S.A.

Successful implementation of transgenic approaches for novel strategies of insect control will require identification of gene regulatory sequences conferring stage, tissue, sex and other physiological specificities to transgene expression. Targeting transgene activity to the female larva offers novel opportunities for control, manipulation of reproductive fitness, genetic sexing or driving transgenes into natural populations. Work in our laboratory is focused on elucidating the molecular basis for regulation of a mosquito hexamerin gene in order to define a sex- and tissue-specific promoter.

Late in the larval stage, holometabolous insects produce and store hexamerins in the fat body, to serve as an important source of amino acids during metamorphosis. As the nutritional status of the late larva dictates its ability to complete metamorphosis and become a reproductively competent adult, it is likely that nutrient signalling plays an important role in the regulation of female-specific hexamerin expression. The mosquito, *Ochleratatus atropalpus* (an aedine mosquito, previously called *Aedes atropalpus*), synthesizes one hexameric storage protein, Hexamerin 1.2, which is unique to females. *Hex-1.2* activity is fat body-specific, and restricted to the late fourth-instar larva and young adult female.

In eukaryotes, nutritional status (in particular amino acid levels) is signaled through the target of rapamycin (TOR) pathway. Recent studies in *Drosophila* indicate that the fat body acts as a nutrient sensor in the pre-metamorphic larva and may control its gene activity via the TOR pathway. We are exploring the possibility that *Hex-1.2* activity is nutritionally regulated through the insulin response pathways and TOR.

In order to investigate the molecular basis for its activity, we have cloned and sequenced the *Hex-1.2* gene. Putative binding sites for specific transcription factors, including Doublesex (DSX), suggest that this gene may share mechanisms for sex-specific transcription in the fat body with other insects. We have shown in *Drosophila* transformants that as little as 300 bp of the *Hex-1.2* 5'-flanking region, including the DSX binding sites, confer female-specificity to a *lacZ* reporter gene and may function as a sex-specific, tissue-specific enhancer. However, as repression of *Hex-1.2* activity in the male *Drosophila* larvae is not as pronounced as in the mosquito, other sequences may be critical for complete female specificity.

We have also demonstrated that the *Hex-1.2* DSX binding sites are indeed functional. Using electrophoretic mobility shift assays (EMSA) and oligonucleotide probes for each of three DSX sites, we established a gradation of binding affinities for the *Drosophila* DSX protein. In transgenic *Drosophila* also carrying the *transformer* mutation (*tra*<sup>1</sup> which cannot produce the activating DSX isoform in females), we observed in pseudo-females a low expression level of the reporter gene more typical of males. These results suggest that the male form of *Drosophila* DSX is able to repress gene activity through the *Hex-1.2* DSX sites.

As we define a female-and fat body-specific enhancer for *Hex-1.2*, and its responsiveness to nutritional status, we plan to apply it to the development of novel strategies for mosquito control. Such strategies are likely to be extensions of the SIT or RIDL approaches studied in other laboratories.

Address correspondence to: [beneshelen@uams.edu](mailto:beneshelen@uams.edu)