

Molecular characterization of insulin-like peptides and ovary ecdysteroidogenic hormone in mosquitoes, but how do they regulate ecdysteroidogenesis in females?

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Investigations into the hormonal regulation of reproduction in female *Ae. aegypti* have demonstrated that an endogenous gonadotropin, ovary ecdysteroidogenic hormone (OEH), and vertebrate insulins stimulate ovaries *in vitro* to secrete ecdysteroid hormones (Brown et al. 1995; Graf et al, 1997; Riehle and Brown 1999). As demonstrated *in vivo* after a blood meal, ovaries of this mosquito secrete ecdysteroid hormones, which stimulate the fat body to produce proteins destined for egg storage. Following the molecular and functional characterization of *Ae. aegypti* OEH, its tissue distribution was described for different life stages of this mosquito and *Anopheles gambiae*. The apparent ortholog OEH gene in *An. gambiae* is known, and its functional characterization of the peptide is being completed. After *An. gambiae* females take a blood meal, the time course for the rise and fall of ovary ecdysteroidogenesis and ecdysteroid hemolymph titer mimics that of female *Ae. aegypti*.

The intracellular pathway of ecdysteroid biosynthesis by insect ovaries or prothoracic glands has yet to be defined, but it is believed to mimic vertebrate steroidogenesis in that precursor steroid molecules shuttle between the endoplasmic reticulum and the inner mitochondrial membrane during processing. Recently, genes encoding proteins involved in this process were characterized after analysis of *Drosophila* ecdysteroid mutants, adding to the list of prospective proteins identified by diverse biochemical and molecular techniques in other insects and vertebrates. Several genes for proteins of interest have been identified in ovaries of *Ae. aegypti*: transport proteins, diazepam-binding inhibitor and steroidogenic acute regulatory protein; an enzyme for shuttling electrons to P450 enzymes, adrenodoxin reductase; and enzymes involved in steroid modification, 3-dehydroecdysone 3b-reductase and 22-hydroxylase. Presently, changes in the transcription of these genes in ovaries are being documented *in vivo* before and after a blood meal, and *in vitro* in response to OEH, bovine insulin, or different intracellular messengers known to stimulate ecdysteroidogenesis. With the *in vitro* experiments, we hope to determine whether these agents exert their effect by activating transcription of a specific gene.

Previously, genes encoding five different insulin-like peptides (ILPs) and many proteins in the insulin signaling pathway have been identified in the *An. gambiae* genome database. A more cautious search of this database and molecular characterization has revealed two other ILP genes that are duplicates. Further studies of the *An. gambiae* ILP genes and their expression will be presented. Multiple ILP genes are known for *Drosophila* and a few lepidopteran species, with the silkworm, *Bombyx mori*, at one extreme having 32 ILP genes (also known as “bombyxins”). All insect ILP genes encode a pre-peptide with a signal peptide and contiguous B, C, and A chains that is processed into active form by linkage of the A and B chains with two disulfide bridges and excision of the C chain. Insect ILPs show limited sequence similarity around structural motifs shared with insulins and related growth factors from vertebrate and other invertebrate animals. In the past few years, numerous studies on *Drosophila* and *Caenorhabditis* have shown through mutation and over expression of genes in the insulin signaling pathway that ILPs likely affect development, longevity, metabolism, and female reproduction through steroidogenesis. How can so many ILPs acting through one signal pathway control this plethora of functions in model invertebrates and maybe even mosquitoes?