COMMENTARY

Something "hairy" in juvenile hormone signaling for mosquito reproduction

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In PNAS, Saha et al. (1) explain how the sesquiterpenoid juvenile hormone (JH) regulates mosquito reproduction by repressing gene activity. The authors show that by acting via its intracellular receptor Methoprene-tolerant (Met), JH induces expression of a gene called *hairy* whose product forms a transcriptional repressor complex with another protein, Groucho (2). Like Met and JH itself, the repressor function of Hairy and Groucho is essential for reproductive maturation in mosquito females. Throughout the animal kingdom, interaction between Hairy and Groucho or their vertebrate homologs, HES and TLE proteins, respectively, is critical for normal output of major signaling pathways and hence for development and homeostasis. Therefore, interestingly, the arthropod-specific hormonal signaling uses the evolutionarily conserved Hairy–Groucho module to orchestrate a complex genetic program in mosquitoes (Fig. 1). The present study (1) notably extends our knowledge of signaling events downstream of the JH receptor while demonstrating their significance for reproduction of the most dangerous insect vectors of human diseases.

The amazingly effective insect reproduction not only fascinates biologists; it also matters because some insects consume our crops or spread diseases. Particularly, mosquitoes transmit dengue, yellow fever, West Nile, or Zika viruses, and the deadly *Plasmodium* parasites that cause malaria. Like most other insects, mosquitoes rely on JH to stimulate their reproduction. Soon after a female mosquito ecloses from its aquatic pupal form, she begins to secrete JH. While she is still feeding on nectar, the hormone prepares her organs for mass production of eggs, which will be triggered by other endocrine signals once the female ingests blood (3). Within 3 d of the posteclosion (PE) phase, JH induces ovarian growth and makes the fat body (the adipose tissue and liver of insects) of the female competent to synthesize yolk precursor proteins in response to the blood meal.

A previous DNA microarray-based transcriptome analysis of the female fat body in the dengue/yellow fever mosquito, *Aedes aegypti*, has revealed that during the critical PE period the rising JH titer activates or represses thousands of genes (4). Based on the timing of expression, the JH-regulated genes have been classified as early-posteclosion (EPE), midposteclosion (MPE), and late-posteclosion (LPE), respectively. Generally, EPE and MPE genes are highly expressed when JH levels are low to intermediate and repressed later when the JH titer rises; LPE genes are induced by this JH increase (Fig. 1). The EPE and MPE clusters are enriched for carbohydrate and lipid metabolism genes, reflecting the nectar diet and the high energy demand of host seeking that the female mosquito experiences. In contrast, genes associated with protein synthesis dominate within the LPE cluster, reflecting preparation of the fat body for blood intake and nutrient conversion to the egg mass (4).

How the small lipophilic JH molecule exerts its broad genomic effects could begin to be unraveled owing to the identification of an intracellular JH receptor (5). The receptor protein Met is a bHLH-PAS transcription factor (6) whose function depends on its direct binding to JH (7). In response to JH or its agonists, Met has been shown to induce transcription of the *early trypsin* and *hairy* genes in *A. aegypti* (8, 9) and of *Krüppel-homolog 1* (*Kr-h1*) in *A. aegypti* (9) and various other insects (10, 11). As one would expect, both *hairy* and *Kr-h1* rank among LPE genes whose expression in the fat body of *A. aegypti* females rises with JH titer (Fig. 1). Indeed, about one-third of all 1,815 LPE genes identified in the microarray study require Met for their increased expression, and high incidence of Met-binding DNA sequences upstream of these genes suggests their direct transcriptional activation by the JH receptor (4). However, somewhat surprisingly the microarray data have also uncovered a large subset of EPE and MPE genes that are repressed by JH in a Met-dependent manner. Intriguingly, these JH-repressed genes lack the Met-binding DNA elements, leading the authors to postulate unknown transcriptional repressors that should act downstream of the activated JH receptor (4). Both Kr-h1 and Hairy make perfect candidates for such a role.

In their study, Saha et al. (1) identify Hairy as one of the wanted JH-induced repressors. First, they have established that expression of the Hairy protein in

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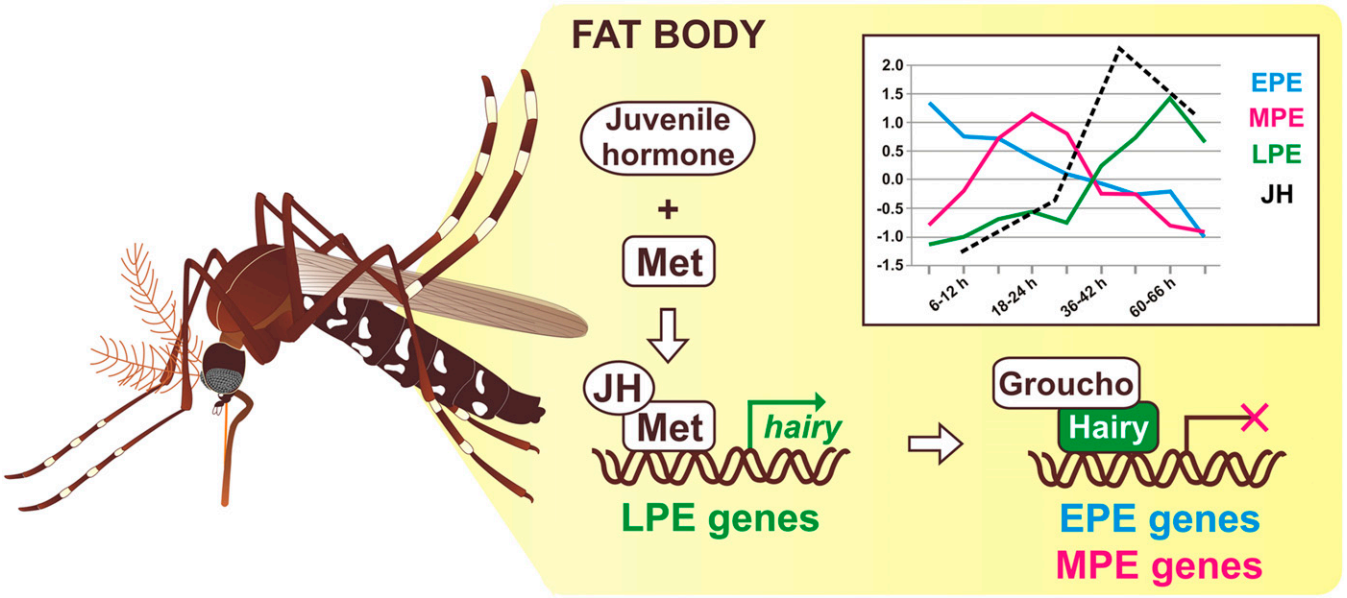

Fig. 1. PE development of reproductive maturity in *A. aegypti* female mosquito involves sequential activation and repression of EPE, MPE, and LPE genes. Their expression in the fat body changes in response to JH acting through its intracellular receptor Met. Repression of some EPE and MPE genes is mediated by a JH-induced Hairy protein conditional on its binding to a corepressor Groucho. Data from ref. 1, graph (*Upper Right*) reproduced from ref. 4, image courtesy of Martina Hajduskova (www.biographix.cz).

the fat body strictly depends on JH and Met, but not vice versa, placing the *hairy* gene downstream of Met (Fig. 1). RNAi-mediated depletion of either Met or Hairy in female mosquitoes early during the PE phase led to retarded ovarian growth, confirming that both proteins are important for reproductive maturation. Using Illumina RNA sequencing on fat bodies deficient either for Met or Hairy, the authors have shown that a vast majority (80%) of some 310 transcripts repressed by Hairy also require Met for their repression. Manual annotation of RNA-sequencing (RNA-seq) data from multiple experiments finally yielded a list of 193 genes that become derepressed when Met and Hairy are removed from the fat body. I have cross-checked this list with the previous microarray data from the same laboratory (4), finding that, as should be expected, 78% of all 122 genes present in both datasets belong to the EPE or MPE clusters.

Here, the authors selected three of those target genes to demonstrate that JH-induced, de novo protein synthesis of Hairy is essential for their repression (1). Moreover, they showed that the bHLH protein Hairy bound to specific DNA sites in the upstream regulatory regions of these genes. In a cell-based assay using reporter constructs, developed from these target genes, the DNA-binding activity of Hairy was necessary but insufficient to block transcription without a specific corepressor. Among several candidates known to form repressor complexes with Hairy in other systems, the authors identified an *A. aegypti* homolog of Groucho (Gro1) as a partner essential for Hairy to repress the reporter constructs. They confirmed that this effect required a physical interaction between Gro1 and the conserved C-terminal WRPW motif of Hairy (2). Importantly, RNAi-mediated knockdown of Gro1 led to the abnormal expression of a target gene (*ornithine decarboxylase*) that is otherwise repressed by JH/Met and Hairy in the mosquito fat body. Finally, the authors showed that the entire JH/Met-Hairy/Gro gene regulatory hierarchy operates in the malaria mosquito, *Anopheles gambiae*.

Collectively, the present findings extend the still unexplored JH signaling pathway to include the negative regulatory components. The action of the Hairy–Groucho repressor complex downstream of the insect endocrine signal is interesting, because Hairy/HES and Groucho/TLE proteins act in highly conserved developmental pathways including Notch, Wingless/Wnt, or Dpp/TGFβ (12, 13). Both *hairy* and *groucho* were originally discovered in *Drosophila* for their role in repressing proneural genes. Their names (Groucho for one of the Marx Brothers with prominent mustache and eyebrows) came from excessive sensory bristles that form at the expense of epidermis in *hairy* or *groucho* mutant flies. Whereas the expression of Hairy occurs in locally restricted patterns in developing *Drosophila* tissues, Groucho is rather ubiquitous. In the mosquito fat body, Gro1 is expressed constitutively throughout the PE phase, whereas Hairy is only present at the time of elevated JH titer (1). Thus, in both flies and mosquitoes, it is the availability of Hairy that brings about the target gene repression.

Nonetheless, the recruitment of Hairy and Gro1 alone does not explain all gene repression occurring in the mosquito fat body downstream of JH and Met. The present RNA-seq data show that out of >1,600 transcripts that rise upon Met RNAi, only 15% overlap with those derepressed by removal of Hairy (1). This discrepancy implies additional repressive mechanisms that must operate downstream of the JH receptor. One obvious candidate is the zinc-finger protein Kr-h1, which plays a critical role as a JH-induced repressor of insect metamorphosis (11, 14). Interestingly, a recently published paper shows that Kr-h1 indeed acts via direct DNA binding to repress transcription of the *Broad-Complex* gene (15), which is absolutely necessary to initiate metamorphosis (larva-to-pupa transition) in holometabolous insects (11). Whether the transcriptional repressor Kr-h1 also regulates genes important for the mosquito PE development remains to be investigated.

In summary, the current discoveries of transcriptional repressors in JH signaling significantly advance our understanding of how this important hormone regulates insect reproduction and development. Facilitated by this work, future studies should address the full repertoire of players downstream in the pathway, and ultimately the biological roles of their target genes.

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