Adult specifier E93 takes control of reproductive cyclicity in mosquitoes

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The females of adult hematophagous mosquitoes are vectors for deadly viral pathogens that cause devastating human diseases such as dengue, yellow fever, West Nile fever, chikungunya, or Zika. They can also transmit *Plasmodium* parasites that cause malaria. The reason behind this is that the female must feed on vertebrate blood to initiate and complete egg development. In addition, the maturation of successive batch of eggs during each gonadotrophic cycle entails different blood meals, increasing the chance of female mosquitos to incorporate and transmit the pathogens. Successful reproduction as well as acquisition and transmission of pathogens are, therefore, linked processes that render mosquitoes the deadliest animals on earth, killing approximately 1 million people each year. It is evident, therefore, that understanding the molecular processes underlying the transition between successive gonadotrophic cycles not only can provide interesting insights into how reproduction is regulated in blood-sucking insects but can be used also to design rational methods for controlling pathogen transmission. In PNAS, Wang et al. (1) shed light on this process by reporting the identification of transcription factor ecdysone-induced protein 93 (E93) as a key regulator of gonadotrophic cyclicity in adult females of the yellow fever mosquito *Aedes aegypti*.

The helix–loop–helix transcription factor E93 was first identified as a dedicated regulator of cell death in *Drosophila melanogaster* pupae (2). Later, it was characterized as a determinant of target gene responsiveness during the pupal phase of the fly (3). Work carried out in hemimetabolous and holometabolous insects led finally to the characterization of E93 as the critical stage identity factor that is responsible for the metamorphic transition to the adult, a function for which it was dubbed as the master "adult specifier" (4). However, although the role of E93 seems thus to be restricted to the metamorphic transition, recent results in the coleopteran *Tribolium castaneum* have shown that the expression and function of E93 extend to the adult stage (5). Consistent with this finding, transcriptome analysis of fat bodies (a key reproductive organ that is equivalent to the vertebrate liver and adipose tissue) of *A. aegypti* adult females revealed the presence of E93 in this tissue (6, 7). This evidence, therefore, pointed to the possibility that E93 might also exert important regulatory functions in mosquito reproduction.

The first gonadotrophic cycle of *A. aegypti* adult females can be divided into previtelogenic and vitellogenic stages, which are regulated by the sesquiterpene Juvenile hormone (JH) and the steroid 20-hydroxyecdysone (20E), respectively (8) (Fig. 1). The previtellogenic period begins after adult emergence and is regulated by JH and its receptor Methoprene tolerant (Met). During this period, JH-Met initiates the growth of the ovaries and renders the fat body competent to synthesize yolk proteins in response to the impending blood feeding. The vitellogenic period starts after blood ingestion and is characterized by a strong pulse of 20E that promotes in the fat body the production and secretion of yolk protein precursors, which are incorporated into growing oocytes. After completion of egg development, the expression of yolk protein precursor ceases, making the female mosquito ready for a new gonadotrophic cycle to start after a second blood meal.

In their study, Wang et al. (1) start with the interesting observation that *E93* expression in the fat body follows a clear cyclic pattern during the first two gonadotrophic cycles of adult *A. aegypti* females. This timing of expression is consistent with the known regulatory effects that the two main hormones exert on *E93* during postembryonic development, repressed by JH and activated by 20E (reviewed in ref. 9). By combining different in vitro techniques, the authors confirmed the repressive activity of JH on *E93* expression in previtellogenic females. Remarkably, they demonstrate that this repression is mediated by Krüppel homolog 1 (Kr-h1) and Hairy, two transcriptional repressors that are induced by JH in mosquitoes (10). Importantly, this result was confirmed by in vivo experiments, as RNA interference (RNAi)-mediated depletion of

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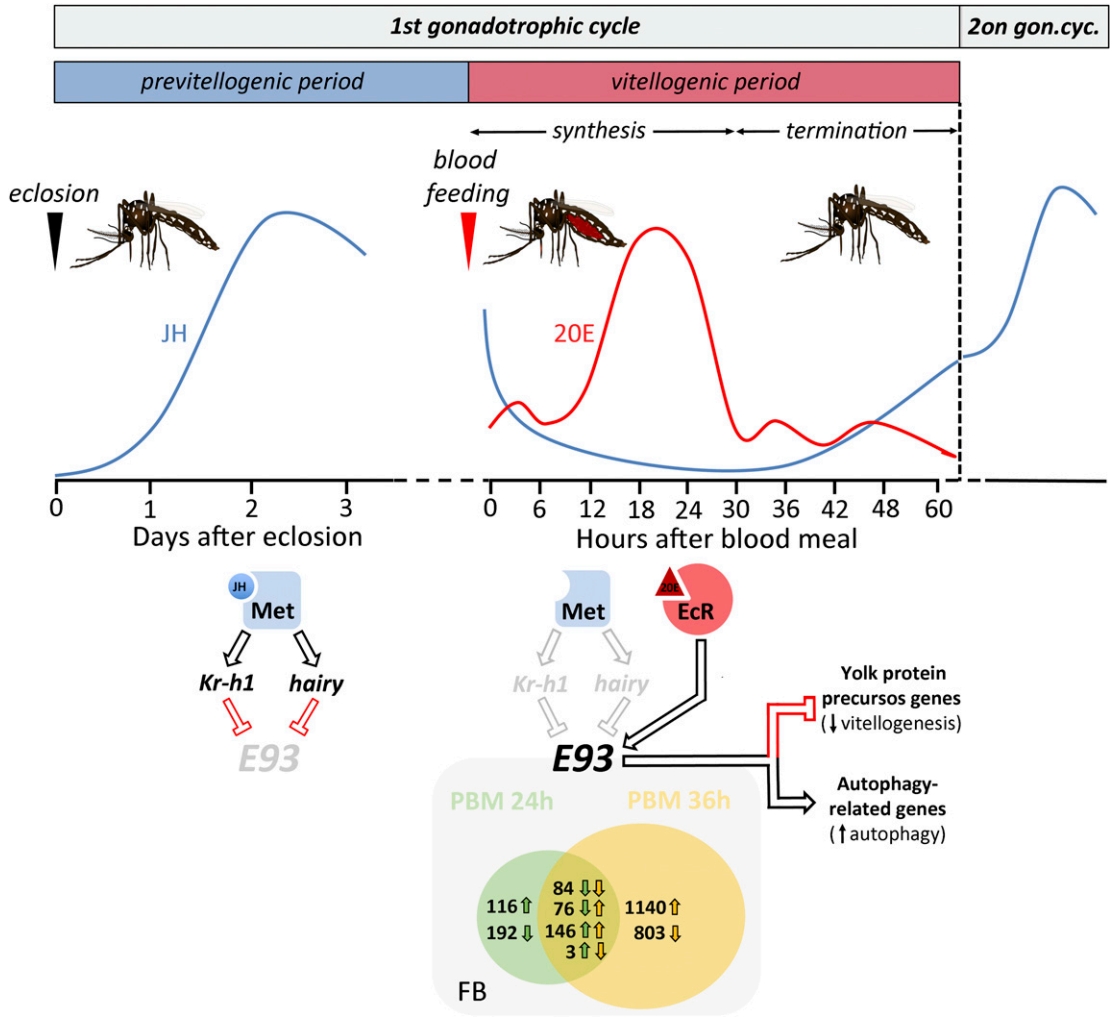

Fig. 1. The adult specifier E93 controls vitellogenic cyclicity in *A. aegypti* adult female mosquitoes. *E93* expression is inhibited during previtellogenesis by JH–Met-induced transcriptional repressors Kr-h1 and Hairy, whereas it is activated by 20E and its receptor EcR during the vitellogenic period. Black arrows represent inductive effects, red lines represent repressive effects, and gray shades represent genes and transcriptional regulatory events that are absent during this particular period. During vitellogenesis, E93 temporally controls the expression of thousands of genes in the fat body (FB), especially in the last part of vitellogenesis, as revealed by a transcriptome analysis (data are from ref. 1). During the termination of vitellogenesis, E93 promotes gonadotrophic cyclicity by repressing *yolk protein precursor* genes and inducing *ATGs*. Levels of JH (blue lines) and 20E (red line) are depicted in *Upper*.

Kr-h1 or Hairy in previtellogenic female mosquitoes led to a significant increase in *E93* expression. The induction of *E93* was even higher when both repressors where knocked down simultaneously, clearly indicating that they act synergistically, a feature that has been observed in other JH-repressed genes in adult mosquitoes (11). Since only Kr-h1 has been implicated in the repression of *E93* in larval development, it would be interesting to investigate whether the activity of Hairy is also important during this period. On the other hand, the authors, using again the same in vitro methodologies, confirmed the direct inductive effect of 20E and its receptor ecdysone receptor (EcR) on *E93* expression during the vitellogenic period. Taken together, these results revealed that in a gonadotrophic cycle, *E93* is sequentially suppressed by JH during the previtellogenic period (indirectly through the synergistic action of Kr-h1 and Hairy) and directly induced by 20E during the vitellogenic stage (Fig. 1).

The expression and regulation data presented in Wang et al. (1) were strongly suggestive of an involvement of E93 in the regulation of reproductive cycles. Using in vivo RNAi, the authors show that this is indeed the case. Thus, when *E93* was depleted in previtellogenic females, ovarian development (measured by the length of the follicles, the number of eggs deposited, and their hatchability) was severely reduced and retarded in the first and second gonadotrophic cycles. Importantly, they found that the expression of *vitellogenin* (*Vg*), the main yolk protein precursor gene, was neither properly induced in early to midvitellogenesis nor fully repressed in late vitellogenesis of E93-depleted females. Likewise, the same deregulation was observed in the expression of the 20E-dependent nuclear receptor *HR3*, a critical regulator of the transitions between reproductive cycles (12). Altogether, these remarkable results confirmed the fundamental role of E93 in the control of vitellogenesis and gonadotrophic cyclicity in *A. aegypti* adult females. It is interesting to note that some regulatory aspects of E93 in reproduction appear to be conserved in the coleopteran *T. castaneum*, for *E93* knockdown adult females also show decreased *Vg* synthesis as well as impaired egg development (5). Whether the role of E93 in reproduction is conserved in other insect orders with different hormonal requirements remains to be investigated.

Having established the crucial role for E93 in the control of gonadotrophic cyclicity, the authors then sought to determine how this factor exerts its regulatory functions at the molecular level. To this aim, they performed transcriptome analysis of *E93*-depleted adult female fat bodies from midparts and late parts of the first gonadotrophic cycle. The main conclusion reached by the authors was that E93 is implicated in a global control of temporal-specific gene expression, especially during late vitellogenesis. In fact, 1,943 genes were differentially expressed at the end of vitellogenesis (86% of all affected genes at this stage), whereas only 308 genes were affected at midvitellogenesis (50% of the genes altered at this stage) (Fig. 1). The ontology of the identified genes, mostly belonging to translation, yolk protein precursors, and metabolisms categories, suggested that E93 is important for basal metabolism and proper energy homeostasis during vitellogenesis.

Among the differentially expressed genes, two groups were of especial interest. The analysis of the first group, consisting of yolk protein precursor genes such as *Vg*, *vitellogenin like*, *vitellogenic carboxypeptidase*, and *cathepsin B-like protease*, supported the model of a dual regulatory role of E93, from being inductor during early to midvitellogenesis to act as a repressor at the end of vitellogenesis. Remarkably, the timely shutdown of these genes by E93 is of particular importance as yolk protein precursor repression has been shown to be critical for the proper transition to the second reproductive cycle. It is important to note that the dual role of E93, as transcriptional activator and repressor, is conserved in *D. melanogaster*. Thus, in the fly pupae, E93 has been shown to promote adult differentiation by modifying chromatin accessibility in temporally dynamic enhancers, acting either as a conventional activator, as a pioneer transcription factor, or as a repressor (13, 14). The second interesting set of E93-dependent genes that showed up in the transcriptome analysis is formed by a number of *autophagy-related genes* (*ATGs*). The analysis showed a significant reduction in the expression of *atg* genes in midlate vitellogenic fat bodies of *E93*-depeleted adult females, especially *atg8*, a key autophagic gene involved in autophagosome biogenesis. That a process like autophagy is halted in *E93*-depeleted adults is especially interesting, for the termination and progression of vitellogenic cycles in *A. aegypti* depend on the autophagy of the fat body (15). In the absence of autophagy, the fat body maintains high levels of nutrition-dependent target of rapamycin signaling, and vitellogenesis is significantly extended, thus hindering the proper transition to the following gonadotrophic cycle. Interestingly, this is an example of E93-dependent autophagy that is not associated with the degeneration of the tissue. Until now, the causal relation between E93 and autophagy has been always associated with the death larval structures, such as *D. melanogaster* mushroom body neuroblasts, midgut, and salivary glands (16, 17). Altogether, the transcriptome analysis revealed that two key processes for the proper transition between gonadotrophic cycles, the repression of yolk protein precursor genes and the induction of developmentally regulated autophagy, are tightly regulated by E93 (Fig. 1).

In summary, the study by Wang et al. (1) expands the function of E93 from the control of the metamorphic transition to another type of transitions, those occurring between successive gonadotrophic cycles during adulthood. It is now clear that E93 is not only critical for the formation but also, for the proper functioning of an adult insect. The fact that this work significantly advances our knowledge of how reproduction in mosquitoes is regulated is also of paramount importance for developing efficient strategies for mosquito control. The importance of this fact becomes clear when one realizes that during the time it took to read this commentary, say 5 min if you got a good look at the figure that illustrates it, seven people will have died from mosquito-transmitted diseases.

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