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Metabolic analysis reveals changes in the mevalonate and juvenile hormone synthesis pathways linked to the mosquito reproductive physiology

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ABSTRACT

Juvenile hormone (JH) regulates reproductive maturation in insects; therefore interruption of JH biosynthesis has been considered as a strategy for the development of target-specific insecticides. The *corpora allata* (CA) from mosquitoes is highly specialized to supply variable levels of JH, which are linked to ovarian developmental stages and influenced by nutritional signals. However, very little is known about how changes in JH synthesis relate to reproductive physiology and how JH synthesis regulation is translated into changes in the CA machinery. With the advent of new methods that facilitate the analysis of transcripts, enzymes and metabolites in the minuscule CA, we were able to provide comprehensive descriptions of the mevalonic (MVA) and JH synthesis pathways by integrating information on changes in the basic components of those pathways. Our results revealed remarkable dynamic changes in JH synthesis and exposed part of a complex mechanism that regulates CA activity. Principal component (PC) analyses validated that both pathways (MVAP and JH-branch) are transcriptionally co-regulated as a single unit, and catalytic activities for the enzymes of the MVAP and JH-branch also changed in a coordinate fashion. Metabolite studies showed that global fluctuations in the intermediate pool sizes in the MVAP and JH-branch were often inversely related. PC analyses suggest that in female mosquitoes, there are at least 4 developmental switches that alter JH synthesis by modulating the flux at distinctive points in both pathways.

1. Introduction

Juvenile hormone (JH) controls insect female reproductive physiology (Goodman and Cusson, 2012). The *corpora allata* (CA) interfaces between the brain and reproductive tissues, producing JH at rates proportional to female nutrient reserves (Clifton and Noriega, 2011, 2012; Perez-Hedo et al., 2014). In *Aedes aegypti* mosquitoes, four stages can be defined in the development of the ovaries: females emerge with 40 μm immature previtellogenic follicles that grow into 100 μm mature previtellogenic oocytes in the next 24–48 h. Oocytes remain in a dynamic "state of arrest", and will enter vitellogenesis after a blood meal (Hagedorn, 1974; Klowden, 1997) (Fig. 1). JH directly controls nutrient allocation into the ovaries in the previtellogenic phases, and indirectly influences the fate of vitellogenic follicles after a blood meal (Clifton and Noriega, 2011, 2012; Noriega, 2004).

JH is synthesized through the mevalonate pathway (MVAP), an ancient metabolic pathway present in the three domains of life (Lombard and Moreira, 2010), responsible for the synthesis of many essential molecules required for cell signaling, membrane integrity, energy homeostasis, protein prenylation and glycosylation (Goldstein and Brown, 1990; Holstein and Hohl, 2004; McTaggart, 2006; Vranova et al., 2013). The MVAP consists of a main trunk followed by sub-branches that generate a diverse range of biomolecules. Insects lack the cholesterol-synthetic branch present in vertebrates (Bellés et al., 2005), but in the CA the MVAP branches into the synthesis of JH. The main trunk of the MVAP consists of multiple enzymatic steps through which acetyl-CoA is gradually transformed into the 5-carbon compound isopentenyl-pyrophosphate (IPP), and later on to the 15-carbon molecule farnesyl-pyrophosphate (FPP) (Klowden, 1997). In the CA of mosquitoes, FPP is sequentially transformed to farnesol (FOL), farnesal (FAL), farnesoic acid (FA), methyl farnesoate (MF) and JH III (hereafter, JH) (Nouzova et al., 2011) (Fig. 2).

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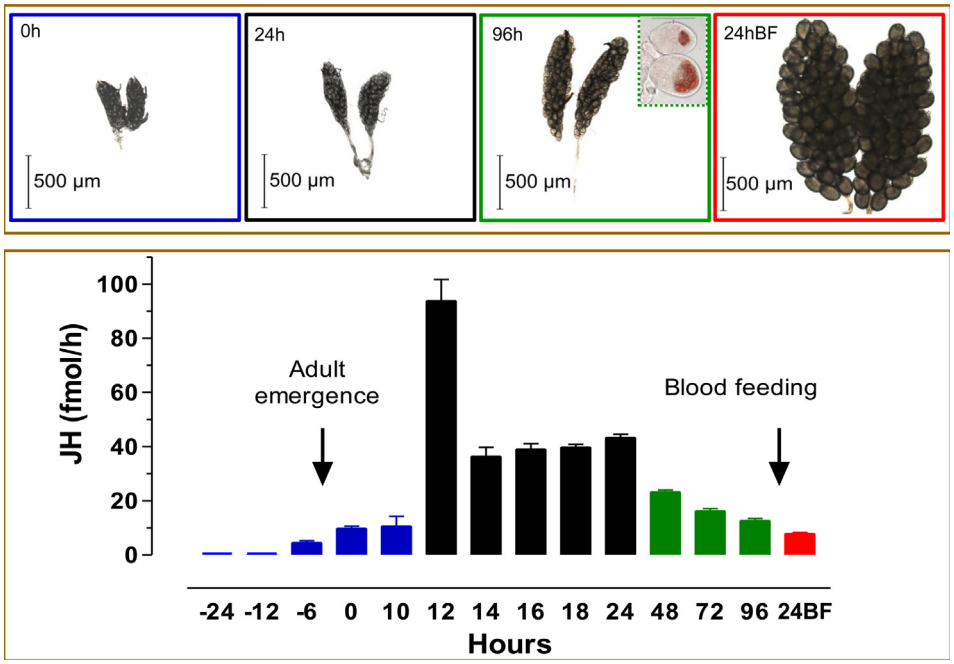

Fig. 1. JH biosynthesis and ovarian development in female mosquitoes. Top panel: representative images of the progression of ovary development from emergence to 24 h after blood feeding. The inset in 96 h shows the lipid content of follicles from females fed 3% sugar (top) and 20% sugar (bottom). Colors for the panels match colors for the CA physiological phases described in the bottom panel. Bottom panel: JH biosynthesis by CA dissected from pupa, sugar-fed and blood-fed adult females. Hours represent times before (pupa) and after adult emergence (sugar-fed), or after blood feeding (BF). *Y* axis: JH biosynthesis expressed as fmol/h. Bars represent the means ± SEM of three independent replicates of three groups of 3 CA. Colors represent the four distinct CA physiological phases identified: inactive CA (blue), active CA (black), modulated CA (green) and suppressed CA (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Regulation of CA activity occurs at three different levels (Applebaum et al., 1991; Unnithan and Feyereisen, 1995): developmental maturation to synthesize JH which normally proceeds in conjunction with developmental changes, such as the transition from pupa to adult (Goodman and Cusson, 2012), long-term modulation such as variations in enzyme levels during cycles of CA activity (Applebaum et al., 1991; Nouzova et al., 2011) and short term responses such as the inhibition of JH synthesis by allatostatins (Unnithan and Feyereisen, 1995; Li et al., 2003a). In all these instances, the rate of JH biosynthesis is controlled by the rate of flux of isoprenoids in the pathway, which is the outcome of a complex interplay of changes in precursor pools, enzyme levels and external regulators (Li et al., 2004, 2003a,b, 2006; Nouzova et al., 2011; Nyati et al., 2013; Rivera-Perez et al., 2013). A coordinated expression of most JH biosynthetic enzymes has been previously described in mosquitoes and silkworm (Kinjoh et al., 2007; Nouzova et al., 2011; Ueda et al., 2009). Increases or decreases in transcript levels for all the enzymes are generally concurrent with increases or decreases in JH synthesis (Kinjoh et al., 2007; Nouzova et al., 2011; Rivera-Perez et al., 2013; Ueda et al., 2009). Previous studies have proposed that regulation of JH synthesis occurs upstream of the acetyl-CoA pool (Sutherland and Feyereisen, 1996), as well as by rate limiting bottlenecks at different enzymatic steps in the pathway, including the activities of HMG-CoA reductase (Kramer and Law, 1980; Monger and Law, 1982), farnesol dehydrogenase (Mayoral et al., 2009), farnesal dehydrogenase (Rivera-Perez et al., 2013) or juvenile hormone acid methyltransferase (Shinoda and Itoyama, 2003; Minakuchi et al., 2008; Sheng et al., 2008). Studies in several insects demonstrated that exogenous additions of precursors stimulate JH biosynthesis in a developmental-dependent mode (Feyereisen et al., 1981a,b; Nouzova et al., 2011), indicating that as a general rule, enzymes of the JH pathway are in excess. However, studies evaluating how changes in metabolite levels and enzyme activities influence the flux of precursors on the JH synthesis pathway and how these changes relate to the different stages of reproductive physiology are lacking. With the development of new strategies that facilitate the analysis of transcripts, enzymatic activities and metabolites in the minuscule CA of insects (Nouzova et al., 2011; Nyati et al., 2013; Rivera-Perez et al., 2012, 2013); it is now possible to provide comprehensive descriptions of the MVA and JH pathways by integrating information on changes in the three basic components of those pathways. In the present studies, we integrated the analysis of changes in mRNA levels for the 13 enzymes of the MVA and JH-branch pathways at 10 different developmental or physiological states during pupae and adult female mosquito, with studies of the catalytic activities of 8 of the enzymes at 5 critical times in pupae and adult female and finally, we measured changes in all the intermediates of the MVAP from acetyl-CoA to FPP, as well as all metabolites of the JH-branch during 9 distinct physiologically-relevant time points in female pupal and adult stages. We studied the complex mechanisms that control CA activity and revealed that the MVAP is well adapted for delivering adjustable levels of isoprenoid precursors to the JH synthesis metabolic branch, with both pathways playing key roles on the regulation of JH titers and reproduction in insects.

2. Materials and methods

2.1. Insects

A. aegypti from the Rockefeller strain were reared as previously described (Nouzova et al., 2011).

2.2. JH synthesis assays

JH III quantification by High Performance Liquid Chromatography coupled to a Fluorescent Detector (HPLC-FD), and mass

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