Identification of the cell membrane receptors of insect juvenile hormone

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Abstract: Insect development is regulated by various hormones, including insulin/insulin like growth factor/insulin like peptide (IN/IGF/ILP) promoting cell proliferation and body growth, 20-hydroxyecdysone (20E) promoting metamorphosis, and juvenile hormone (JH) maintaining larval status. JH plays roles by defusing into cell and binding to the intracellular receptor methoprene-tolerant (MET) to regulate gene transcription to prevent larval–pupal transition. Some studies suggest that cell membrane receptors, including G protein-coupled receptor (GPCR) and receptor tyrosine kinase (RTK), also play essential roles in JH signaling, suggesting the cell membrane receptors of JH exist, however, the cell membrane receptor of JH is not identified for a long time. Using Helicoverpa armigera, the cotton bollworm, as a model, we observe that JH via MET1 upregulates JH pathway gene Krüppel homolog 1 (Kr-h1) expression and represses 20E pathway gene Brz7 and Hr3 expression to maintain the larval status, by regulating JH transcription complex MET1-USP-HSP90-p formation and repressing 20E transcription complex EcR-USP-p-HSP90 formation. JH regulates the rapid phosphorylation of transcription factor BRZ7 via a GPCR-, phospholipase C-, and protein kinase C-triggered signaling axis. The phosphorylated BRZ7 promotes calponin expression that interacts with USP to activate the JH pathway and antagonize the 20E pathway. JH III induces threonine-phosphorylation of MET1 at threonine 393 (Thr393) in the Per-Arnt-Sim (PAS) B domain. Thr393-phosphorylation is necessary for MET1 binding to the JH response element (JHRE) to promote the transcription of Kr-h1. Thr393-phosphorylated MET1 increases its interaction with Taiman (Tai) and prevents the Met1-Met1 association. We determine that receptor tyrosine kinases (RTKs) cadherin 96ca (CAD96CA) and fibroblast growth factor receptor homologue (FGFR1) function as JH cell membrane receptors by their roles in JH-regulated gene expression, larval status maintaining, rapid intracellular calcium increase, phosphorylation of JH intracellular receptor MET1 and cofactor Taiman, and high affinity to JH III. Gene knockout of Cad96ca and Fgfr1 by CRISPR/Cas9 in embryo and knockdown in various insect cells, and overexpression of CAD96CA and FGFR1 in mammalian HEK-293T cells all supported CAD96CA and FGFR1 transmitting JH signal as JH cell membrane receptors. Together, we identify JH cell membrane receptors CAD96CA and FGFR1 and demonstrate their relationship with the intracellular receptor MET1, which presents target for developing insecticides. This work is supported by the National Natural Science Foundation of China (grant nos. 32330011 and 32270507).
Keywords: Insect development;juvenile hormone;cell membrane receptor

The transcription co-factor Taiman modulates wing vein formation through suppressing the Hh pathway

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Abstract: The Hedgehog (Hh) pathway plays diverse roles in cellular processes by activating the transcription factor Cubitus interruptus (Ci). Abnormal regulation of this pathway has been linked to various human diseases. While previous studies have focused on how Ci is regulated in the cytoplasm, the control of nuclear Ci remains poorly understood. In this study, we have discovered that the transcriptional cofactor Taiman (Tai) functions as an inhibitor of the Hh pathway. Tai interferes with the response of Hh signal, rather than Hh secretion. Our epistatic analyses reveal that Tai works in parallel with Ci to reduce its activity, thereby counteracting organ overgrowth and the activation of target genes caused by Ci overexpression. Specifically, Tai interacts with Ci to decrease its binding to target gene promoters. The Hh signal weakens the interaction between Ci and Tai, releasing the inhibition on Ci. Importantly, this regulatory mechanism is conserved from Drosophila to mammalian cells. Moreover, NCOA1-3 are the mammalian ortholog of Drosophila protein Tai, but only NCOA2 plays a similar role in inhibiting the Hh pathway. These findings reveal a new way to modulate the transcriptional activity of nuclear Ci/GLI.

Keywords: Wings;Hh pathway;Taiman;Wing vein

Chronologically inappropriate morphogenesis (Chinmo) is required for maintenance of larval stages of fall armyworm

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Abstract: Broad complex (Br-C) and eip93F (E93) transcription factors promote insect metamorphosis from larva to pupa and from pupa to adult, respectively. Recently, chronologically inappropriate morphogenesis (Chinmo) has been proposed as a larval specifier in Drosophila melanogaster. However, whether Chinmo is required for larval maintenance in lepidopteran insects, the underlying mechanisms involved in maintaining the larval stage, and its interactions with the JH signaling pathway are not well understood. Here, we used a binary transgenic CRISPR/Cas9 system to knockout Chinmo and Kr-h1 (primary response gene in the JH signaling pathway) in the fall armyworm. Kr-h1 knockout induced premature metamorphosis only after L5 (penultimate), whereas Chinmo and Kr-h1 double knockout induced premature metamorphosis in L3. Sequencing and differential gene expression analysis of RNA isolated from mutants and single-cell multiome ATAC analysis of Chinmo, Kr-h1, and Chinmo and Kr-h1 double knockout Sf9 cells revealed that Chinmo participates in chromatin modifications that prevent the promoter accessibility and expression of metamorphosis promoting genes. These results suggest that Chinmo is a larval specifier that plays a major role in preventing metamorphosis in early larval stages by controlling chromatin accessibility near the promoters of genes such as Br-C and E93 required for pupal and adult development.

Keywords: Chinmo; Krüppel homolog 1; Metamorphosis; Chromatin accessibility

SoxN-Vvl complex predominantly controls juvenile hormone biosynthesis in Drosophila corpus allatum

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Abstract: Juvenile hormone (JH) produced by the corpus allatum (CA) maintains juvenile status and promotes adult reproduction in insects. Despite recent breakthroughs in JH signaling studies in Drosophila, little is known about how JH biosynthesis is precisely regulated by transcription factors (TFs) partially due to the complexity of JH biology in this insect species. Here, by performing single-cell RNA sequencing of 7,919 cells isolated from the larval ring glands, we identified three major cell types, including CA, prothoracic gland, and corpora cardiaca. We obtained detailed transcriptional information of CA cells and validated the authenticity using known JH biosynthetic enzymes and newly identified marker genes. Significantly, SoxNeuro (SoxN), a CA-enriched TF, is indispensable for JH biosynthesis and thus functionally important for both metamorphosis and reproduction. Moreover, SoxN interacts with Ventral veins lacking (Vvl) to form a TF complex for controlling JH biosynthesis, which determines the expression of the rate-limiting enzyme gene Jhamt via a promoter region. This study yields a single-cell transcriptomic atlas of the ring gland and identifies a master regulator for JH biosynthesis, advancing our understanding of insect endocrinology.
Keywords: scRNA-seq;Ring gland;Corpus allatum;SoxN;Juvenile hormone

V-ATPase subunit M9.7-d is essential for sperm motility in Drosophila melanogaster

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Abstract: V-ATPases are crucial for animal development and survival, but their functions in fertility is largely unknown. Here, we found that knockdown of *VhaM9.7-d*, coding for a subunit of V-ATPase, in germ cells induced complete sterility in male *Drosophila melanogaster*, but had no effects on female fertility. Depletion of *VhaM9.7-d* did not severely impair spermatogenesis, as the mature sperm appeared in the seminal vesicles (SVs) of the testes. However, the sperm released from the SVs of the *VhaM9.7-d*-knockdown males rapidly lost their motility and were unable to move to longer distances. These sperm could be transferred to female' s uterus during copulation, but failed to be stored in the seminal receptacle (SR) and fertilize the egg. Tandem mass tag (TMT) proteomic analyses of SVs, including their contents, identified 434 differentially expressed proteins (DEPs) when comparing the control group to the *VhaM9.7-d*-knockdown group. Many downregulated proteins were enriched in phagosome and oxidative phosphorylation (OxPHOS) pathways. Subsequent experiments, encompassing the LysoSensor Probe assay, CMXRos staining, and ATP measurement, confirmed that the knockdown of *VhaM9.7-d* significantly disrupted phagolysosomal and mitochondrial functions, leading to diminished acidity, heightened levels of reactive oxygen species (ROS), and a decrease in both mitochondrial membrane potential and ATP contents in the SVs. These results suggest that VhaM9.7-d plays an essential role in maintaining the homeostasis of sperm energy metabolism by regulating phagolysosomal activity and mitochondrial OxPHOS. Our data provide valuable insights for the further study of the mechanisms of related diseases such as human asthenospermia.

Keywords: Drosophila melanogaster;VhaM9.7-d ;Sperm motility

Targeting KARS and LAP1 Disrupts Mosquito Reproduction for Population Suppression

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Abstract: Controlling mosquito populations by regulating their reproduction is critical for minimizing devastating pathogen transmission. While transcriptional regulation of mosquito reproduction is well understood, the underlying translational mechanisms remain unclear. Our study reveals that lysyl-tRNA synthetase (KARS) plays an essential role in translational regulation, governing mosquito fecundity in a hormone-dependent manner. Silencing of KARS disrupts ovarian maturation and impairs fecundity, with dysregulated mRNA translation. Further analysis of ribosome profiling sequencing indicates the occurrence of ribosome stalling, which ultimately suppresses vitellogenin synthesis. Additionally, we identified LAP1 (Leucine aminopeptidase1) as a key protein in spermathecal proteomics, where its levels dynamically change across different mating states. CRISPR/Cas9-mediated LAP1 deficiency severely reduces female fecundity and fertility due to defective sperm mitochondria and abnormal autophagy in males. Importantly, LAP1-/- males can efficiently propagate genetic changes despite producing fewer viable offspring, highlighting LAP1 as a promising target for gene drive systems to suppress mosquito populations. Together, these findings underscore the significance of both translational control (via KARS) and spermathecal regulation (via LAP1) in mosquito reproduction, thereby offering novel strategies for population suppression and disease control.

Keywords: mosquito; reproduction; control

Male-specific lethal-3 gene is critical for survival and fecundity in rice brown planthopper, Nilaparvata lugens

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Abstract: Male-specific lethal-3 (MSL3) is a component of the dosage compensation complex in Drosophila melanogaster, where its mutation leads to male-specific lethality. However, the function of MSL3 in hemipteran insects remains unclear. This study investigated the role of the MSL3 homolog in a major rice pest, the brown planthopper (Nilaparvata lugens). We cloned and characterized the gene NlMSL3 from N. lugens, which is 1467-bp long and encodes a protein of 488 amino acids. Phylogenetic analysis revealed that MSL3 is conserved across various insect orders, with high conservation in the chromo-barrel domain. Quantitative real-time polymerase chain reaction indicated differential expression levels of NlMSL3 between male and female insects during development, with the highest expression in the testes. RNA interference-mediated knockdown of NlMSL3 in N. lugens resulted in significant mortality in later instar nymphs and adults compared with the control group. In females, NlMSL3 knockdown impaired feeding behavior, leading to decreased body weight, notably reduced honeydew excretion, flat abdomens, decreased vitellogenin expression, and defective ovarian development. When dsNlMSL3-treated males were mated with control females, the number of eggs laid was similar to that laid by the females mated with control males; however, none of the eggs laid by the former hatched into nymphs. These results highlight the crucial role of NlMSL3 in the development and fecundity of N. lugens.

Keywords: Male-specific lethal-3;Nilaparvata lugens;mortality;fecundity

Mechanism of RNA methylation modification in reproductive development in Bactrocera dorsalis

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Abstract: RNA N6-methyladenine (m6A) modification represents a pivotal epigenetic modification that facilitates the remodeling of gene expression and regulates a variety of biological processes via certain post-transcriptional mechanisms. However, the specific function of RNA m6A modification in insect reproduction remains unclear. In this study, we show that gut commensal bacteria promote host reproduction by providing amino-acid methionine, which controls the RNA m6A modification level of insulin receptor (InR) in the ovary of the invasive insect Bactrocera dorsalis. Antibiotic-treated B. dorsalis showed reduced RNA m6A methylation levels and methionine content, resulting in arrested ovarian development and decreased fecundity. Gut commensal bacteria Enterobacter hormaechei-derived metabolite methionine restored the decreased RNA m6A level and the reproductive defects. Notably, knockdown of METTL3 and METTL14, two genes encoding the RNA m6A methyltransferases, reduced InR mRNA and protein levels, and impaired ovarian development of in B. dorsalis. Interestingly, we also found that METTL3/METTL14-mediated RNA m6A modification regulates male reproduction in B. dorsalis. Knockout of METTL3 or METTL14 decreased the expression level of m6A and reduced the titer of 20E as well as the mRNA m6A level of Dismbodied (one of 20E synthesis genes) in males , resulting in testicular deformities and a significant reduction of viable sperm number. These data suggested that RNA m6A modification regulates testis development and fecundity by modulating 20E synthesis. Taken together, this study further expand the functional landscape of m6A modification to include nutrient-dependent control of reproduction an highlight the essential role of epigenetic regulation in microbe-host interactions and reproduction, suggesting strategies for pest control and treating reproductive pathologies by targeting gut microbiota and the reproduction-related genes.
Keywords: RNA m6A methylation;reproduction;gut commensal bacteria;methionine ;20E;Bactrocera dorsalis

Oppsing insulin signaling activities regulates the same wing-morph switching across species

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Abstract: An emerging pattern in the evolution of signalling pathways is their ability to produce similar developmental outcomes or switches despite showing a remarkable degree of evolutionary flexibility across species. Yet, the molecular mechanisms underlying this process remain unknown. Here we address this question using wing polyphenism (a switch between long or short wing development in response to environmental cues) across hemipteran insects as a model. We discovered that genes within the insulin/insulin-like signalling (IIS) pathway evolved opposite roles in wing growth, such that the same developmental switch to long wings is produced by either activating or inactivating IIS. This flexibility is integrated by targeting different genes required for ecdysteroids biosynthesis in each species, resulting in low ecdysteroid titers that induce a similar switch to long wings. Our findings may reflect a general mechanism for how a pathway integrates inputs from rewired signalling pathways across species to produce similar developmental switches.
Keywords: Wing polyphenism;Insulin signaling;Ecdysteroids;Signaling flexibility

The Drosophila histone variant H2Av facilitates Notch signaling activity in a two-tier regulatory fashion

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Abstract: H2Av is an evolutionarily conserved H2A variant protein involved in the regulation of transcription. The Tip60 complex is recruited by different transcription factors to facilitate the incorporation and acetylation of H2Av, thereby influencing target gene expression. The Tip60-H2Av axis is involved in various developmental processes, though its precise roles are not yet fully understood. Here we report that H2Av is required for Notch signaling activation during Drosophila wing development. H2Av depletion disrupts the expression of Notch target genes, resulting in wing marginal defects. Unexpectedly, we find that H2Av regulates the expression of the Su(H) gene which encodes for the transcription factor of the Notch signaling cascade. We further demonstrate that the Tip60 complex modulates the transcription of both Notch targets and Su(H) likely through H2Av. Based on these observations, we propose a model that the Tip60-H2Av axis facilitates Notch pathway activation by simultaneously promoting the expression of both the target genes and the transcription factor.
Keywords: H2Av;Notch;Su(H);Drosophila;Tip60

ERK-activated CK-2 triggers blastema formation during appendage regeneration

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Abstract: Appendage regeneration relies on the formation of blastema, a heterogeneous cellular structure formed at the injury site. However, little is known about the early injury-activated signaling pathways that trigger blastema formation during appendage regeneration. Here, we provide compelling evidence that the extracellular signal– regulated kinase (ERK)–activated casein kinase 2 (CK-2), which has not been previously implicated in appendage regeneration, triggers blastema formation during leg regeneration in the American cockroach, Periplaneta americana. After amputation, CK-2 undergoes rapid activation through ERK-induced phosphorylation within blastema cells. RNAi knockdown of CK-2 severely impairs blastema formation by repressing cell proliferation through down□regulating mitosis-related genes. Evolutionarily, the regenerative role of CK-2 is conserved in zebrafish caudal fin regeneration via promoting blastema cell proliferation. Together, we find and demonstrate that the ERK-activated CK-2 triggers blastema formation in both cockroach and zebrafish, helping explore initiation factors during ap□pendage regeneration.

Keywords: Periplaneta americana; leg regeneration; regeneration initiation signal

Unveiling the Molecular Mechanism of Royal Jelly's Antibacterial Action: Insights from Proteomic Analysis and Host Regulatory Networks

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Abstract: Royal Jelly (RJ) is a natural substance produced by honeybees, serving not only as nutrition for bee brood and queens but also as a functional food due to its health-promoting properties. Despite its well-known broad-spectrum antibacterial activity, the precise molecular mechanism underlying its antibacterial action has remained elusive. In this study, we investigated the impact of RJ on the bacteria model MG1655 at its half-maximal inhibitory concentration, employing LC-MS/MS to analyze proteomic changes. The differentially expressed proteins were found to primarily contribute to the suppression of gene expression processes, specifically transcription and translation, disrupting nutrition and energy metabolism, and inducing oxidative stress. Notably, RJ treatment led to a marked inhibition of superoxide dismutase and catalase activities, resulting in heightened oxidative damage and lipid peroxidation. Furthermore, through a protein-protein interaction network analysis using the STRING database, we identified CRP and IHF as crucial host regulators responsive to RJ. These regulators were found to play a pivotal role in suppressing essential hub genes associated with energy production and antioxidant capabilities. Our findings significantly contribute to the understanding of RJ's antibacterial mechanism, highlighting its potential as a natural alternative to conventional antibiotics. The identification of CRP and IHF as central players highlights the intricate regulatory networks involved in RJ's action, offering new targets for developing innovative antimicrobial strategies.

Keywords: Royal Jelly;Antibacterial activity;Oxidative stress

Dimorphic histone methylations turn on dichotomous spermiogenesis

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Abstract: The process of spermatogenesis is similar across species, while sperm morphology exhibits diversity. Butterflies and moths with dimorphic sperm (eupyrene and apyrene sperm) are typical representatives among organisms. However, the mechanism of dichotomous spermatogenesis is still unclear. Here, we explored the histone dynamic change and its regulatory mechanisms during dichotomous spermatogenesis. We found that core histones were lost in the elongated eupyrene sperm but reserved in the apyrene sperm. The histone retention micronucleus also showed high levels of H3K4me2/3 and H3K9me2/3, which were undetectable in the elongated eupyrene sperm nucleus. The knockout of the demethylase gene BmLid increased the level of H3K4me2/3 and H3K9me2/3, leading to the repressed removal of core histones in the eupyrene sperm nuclei. Furthermore, ChIP-seq analysis suggested that high levels of H3K4me2 and H3K9me2 inhibit lysine degradation and the arginine biosynthesis pathway. Our results indicate that dimorphic histone methylations control the core histone removal, resulting in dimorphic sperm.

Keywords: Histone removal;histone methylation;dichotomous spermatogenesis;BmLid;Bombyx mori

Identification and functional analyses of novel immune cells in Drosophila kidney

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Abstract: Immune cells provide defense against non-self in the immune system and have been shown to play roles in diverse processes such as development, metabolism, and tumor progression. The *Drosophila* kidneys (Malpighian tubules or MTs) act as autonomous immune sensing organs, which secrete antimicrobial peptides in response to invading microbial pathogens. Yet the heterogeneity of *Drosophila* immune cells (kidneys) remains an open question. Here, we identify a migratory and immune responsive renal cell population (lower segment principle cells, LSPCs) expressing many genes involved in immune response and inflammation through *Drosophila* renal single-nucleus RNA sequencing. We show that the LSPCs of adult MTs sense immune challenge and reacts to it quickly through the NF-κB transcription factor, *Relish*, a key component of the IMD innate immunity pathway. In addition, LSPCs enhances the cell-cell communication with other cell clusters and induce renal immune response in Yki gut tumor flies. Notably, suppression of the IMD pathway in the kidney LSPCs partially rescue organ damage and survival in gut tumor model. Collectively, our data define a population of novel immune cells that is essential for regulate *Drosophila* kidney development and provide some insights to study the biology of *Drosophila* immune cells in physiological and pathological conditions.
Keywords: Drosophila;Immune cells;Malpighian tubules;scRNA-seq analysis;LSPCs;Relish

Germline Cas9 promoters show improved performance for homing gene drive and transgene knock-in

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Abstract: Gene drive systems could be a viable strategy to prevent pathogen transmission or suppress populations of pests, vector insects, and invasive species by propagating drive alleles with super-Mendelian inheritance through populations. CRISPR-based homing gene drive is perhaps the most powerful gene drive strategy, able to convert wild-type alleles into drive alleles in the germline of heterozygotes after cleavage by Cas9 and gRNA. However, homing suppression drives require high performance to achieve success. Thus far, high-performance promoters have only been found in vector mosquito Anopheles gambiae. In fruit flies, the nanos promoter avoids fitness costs from leaky somatic expression, but still suffers from high embryo resistance due to maternally deposited Cas9. To improve homing drive efficiency, we tested eleven Drosophila melanogaster germline promoters in several configurations. Some achieved high drive conversion efficiency with significantly less embryo resistance, but none could completely avoid somatic expression like the nanos promoter. In cage experiments with a 4-gRNA suppression drive, which previously failed with the nanos promoter, we found that the rcd-1r promoter failed due to somatic expression, but the CG4415 promoter showed superior performance, successfully eliminating cage populations. The process of homology-directed repair for transgene knock-in is similar to homing gene drive. We thus assessed some of our germline promoters for transgene knock-in in fruit flies, either by injecting into Cas9 lines or adding germline Cas9 plasmids into the injection mix. Compared to injection with ubiquitously expressed hsp70-Cas9 plasmid, transformation rates were increased with both methods, both in the rate of injected founders and the number of transgenic progeny per founder. Overall, these novel Cas9 promoters could be advantageous for homing drives and transgene knock-in for Drosophila species and may have useful homologs in other organisms.
Keywords: gene drive;transgene knock-in;germline promoter;fitness cost

Draper-ATG3 Interaction Positively Regulates Autophagy to Mediate Silk Gland Degradation in Bombyx mori

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Abstract: Autophagy plays a pivotal role in developmental cell death during insect metamorphosis, yet the regulatory mechanisms governing autophagy-dependent cell death remain poorly understood. Here, we uncover the essential role of the engulfment receptor Draper in tissue clearance and remodeling, demonstrating its evolutionary conservation across insect species. Initially, we showed that Draper is evolutionarily conserved in most insect species. Using CRISPR/Cas9-mediated gene editing in Bombyx mori, a model lepidopteran insect, we show that Draper loss impedes autophagy activation and delays middle silk gland degradation during metamorphosis, while its overexpression enhances autophagy induction. Proteomic analyses reveal that Draper deficiency disrupts the ubiquitin system, silk protein metabolism, and autophagic substrate degradation. Furthermore, liquid chromatography–tandem mass spectrometry and coimmunoprecipitation assays identify a direct interaction between Draper and autophagy-related protein 3 (ATG3), which enhances autophagic activity. These findings bridge a critical gap in understanding how developmental signals mechanistically interface with core autophagy machinery to ensure precise tissue remodeling. Our study challenges the current paradigm of autophagy initiation by identifying Draper as an evolutionarily conserved regulator, providing a unifying framework that integrates developmental timing, phagocyte signaling, and metabolic clearance during metamorphosis.

Keywords: Autophagy; Metamorphosis; Draper; ATG3; Silk gland; Bombyx mori

Phase Separation of an Endocytic Factor EPS15 Orchestrates the Egg Maturation in the Fall Armyworms

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Abstract: Successful reproduction in most female insects critically depends on vitellogenesis, a key process involving the rapid, massive accumulation of the primary yolk protein precursor, vitellogenin (Vg), via clathrin-mediated endocytosis. However, the regulatory mechanisms governing Vg uptake by maturing ovaries remain poorly understood. In this study, we demonstrate that coordinated hormonal control of the endocytic factor EPS15 is essential for egg maturation in the fall armyworm, Spodoptera frugiperda. EPS15 exhibited high expression and localized to the developing oocyte membrane. Functional depletion of EPS15 disrupted Vg accumulation within vitellogenic ovaries, consequently impairing egg maturation. GFP-tagged EPS15 formed liquid-like condensates both in Sf9 cells and in purified protein solutions, with Fluorescence Recovery After Photobleaching assays confirming liquid-liquid phase separation. Further investigation revealed that EPS15 phase separation was significantly potentiated at the protein level by the juvenile hormone pathway and finely modulated at the post-translational level by trace amounts of 20-hydroxyecdysone through arginine symmetric demethylation. Corpora allata deactivation and methyltransferase depletion in the moths compromised EPS15 phase separation and further disrupted endocytic uptake of fluorophore-tagged native Vg in ovaries. This study elucidates an interplay between two major insect hormone pathways that relies on phase separation of an endocytic initiator protein to regulate reproduction in a significant agricultural pest.
Keywords: 20-hydroxyecdysone; endocytosis; juvenile hormone; phase separation; vitellogenin

Functional divergence of NR5 nuclear receptors during metamorphosis and reproduction in the migratory locust

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Abstract: The NR5A nuclear receptor family, including Ftz-f1 and its paralog HR39, plays pivotal roles in insect developmental transitions and reproduction. In the migratory locust Locusta migratoria, Ftz-f1 generates two isoforms, LmαFtz-f1 and LmβFtz-f1, via alternative splicing that differ only at their N-terminus, while LmHR39 represents a related but structurally distinct NR5A member with a single transcript. Spatiotemporal profiling showed that LmαFtz-f1 is expressed at significantly higher levels than LmβFtz-f1 throughout both nymphal and adult stages, whereas LmHR39 exhibits broader but moderate expression across both stages and tissues. RNA interference (RNAi) reveals nonredundant roles, LmβFtz-f1 is essential for nymphal metamorphosis, while LmαFtz-f1 is required for vitellogenin (Vg) expression and ovarian maturation in adults. LmHR39 knockdown causes molting defects and mildly impairs adult oocyte development. To uncover underlying mechanisms, RNA-seq was conducted following RNAi targeting the conserved regions of LmFtz-f1 and LmHR39. In nymphs, both knockdowns disrupted genes involved in 20-hydroxyecdysone (20E) and juvenile hormone (JH) biosynthesis. In adults, LmFtz-f1 RNAi suppressed cell cycle–related genes, suggesting roles in fat body cell fate determination and remodeling, while LmHR39 RNAi reduced ribosome- and translation-related gene expression, indicating a limited role in reproduction. These findings highlight isoform- and stage-specific functions of NR5A factors in coordinating developmental and reproductive transitions, providing potential targets for stage-specific insect control.

Keywords: Ftz-f1 isoforms; HR39; metamorphosis; reproduction; NR5A nuclear receptor

miR-7-GABA influences female Aedes aegypti reproduction by modulating midgut homeostasis

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Abstract: The robust reproductive plasticity of female Aedes aegypti mosquitoes following blood-feeding exacerbates the transmission of mosquito-borne viruses, imposing a heavy burden on global public health. The blood-feeding-initiated post-blood meal (PBM) phase activates coordinated changes in the midgut-fat body-ovary axis, storing nutrients for female reproductive behaviors. MicroRNAs (miRNAs) precisely control gene expression through a post-transcriptional regulatory mechanism that targets mRNAs. To explore the impact of endogenous miRNAs on the digestion kinetics of exogenous hemoglobin (HGB), this study combined digestive physiology assays, sRNA-seq, CRISPR-Cas9, RNA interference (RNAi), in vitro neurotransmitter delivery to the digestive tract, and molecular biology assays. The results demonstrated that the miR-7-glutamate decarboxylase (GAD)-γ-aminobutyric acid (GABA) signaling axis mediates the reproductive cascade of the midgut-fat body-ovary axis by regulating midgut homeostasis. Through high-throughput sRNA-seq combined with digestion rate measurements, we identified miR-7 as a key regulator of the HGB digestion rate. Dual-luciferase reporter gene assays and fluorescence in situ hybridization (FISH) co-localization experiments confirmed that GAD is a target gene of miR-7. CRISPR-Cas9-mediated miR-7 knockout combined with GAD RNAi rescue experiments demonstrated that miR-7 deficiency disrupts the midgut glutamate-GABA homeostasis by derepressing GAD. High levels of GABA inhibit the secretion of digestive proteases by promoting midgut cell apoptosis. This endocrine imbalance is directly manifested as delayed blood-meal processing, secondary to impaired lipid storage and arrested ovarian development, ultimately reducing reproductive fitness. The in vitro experiment of delivering GABA to the digestive tract further verified the tissue-specificity of the above-mentioned view. Our study identified an important miRNA that affects reproductive output by regulating digestion, providing key targets for interventions aimed at blocking the transmission of arboviruses.

Keywords: miR-7;glutamate decarboxylase;gamma-aminobutyric acid;digestion;reproduction

CRISPR/Cas9-mediated knockout of serpin15 impacts reproduction and immunity in Plutella xylostella Linnaeus

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Abstract: Plutella xylostella is a destructive pest of cruciferous crops worldwide. The excessive reliance on synthetic insecticides leads to ecological pollution and and the development of resistance, prompting scientists to explore eco-friendly biopesticides. Serine protease inhibitors (serpins) play pivotal roles in melanization in innate immunity, reproduction, and metamorphic development. Based on our laboratory proteomic analyses conducted across the developmental stages of P. xylostella, serpin15 was identified as a critical member of the typical inhibited serpin family, although its specific function in P. xylostella remains unclear. In the present study, RT-qPCR analyses of gene expression patterns across different tissues and developmental stages revealed that the serpin15 gene is highly expressed in the gonads of male adults and exhibits the highest abundance in hemolymph. Notably, serpin15 mRNA levels displayed dynamic regulation in the midgut following infection with Serratia marcescens (PS-1) showing an initial decrease followed by subsequent upregulation. CRISPR/Cas9-mediated knockout of serpin15 in homozygous lines resulted in a reduced oviposition rate and embryonic hatching rate of offspring. Functional assays confirmed that serpin15 inhibits phenoloxidase (PO) activity, with the exogenous supplementation of recombinant serpin15 protein effectively suppressing hemolymph melanization, thereby establishing its regulatory role in counteracting PS-1 via immune melanization. Collectively, these findings underscore serpin15's dual functionality in modulating both fecundity and immunity against PS-1 in P. xylostella. This discovery provides a theoretical foundation for the development of biocontrol strategies targeting insect immune and developmental systems.
Keywords: Plutella xylostella;Serine protease inhibitors;CRISPR/Cas9;Melanization

N6-methyladenosine modification of RNA (m6A) regulates the diapause of cotton bollworms，Helicoverpa armigera

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Abstract: Diapause is a complex physiological adaptation that enables insects to survive adverse environmental conditions, involving coordinated regulation by numerous genes. Although N6-methyladenosine (m6A) RNA modification plays a critical role in epigenetic gene regulation, its function in insect diapause remains poorly characterized. In this study, we observed that m6A levels were downregulated during pupal diapause but upregulated during development in the cotton bollworm (Helicoverpa armigera). Inhibition and knockout of the mettl3, the key catalytic enzyme for m6A modification, significantly delayed pupal development and induced diapause-like phenotypes. Integrated MeRIP-Seq and RNA-Seq analyses revealed that key response factors (including HR3, HR4, and Ftz-f1) in the 20-hydroxyecdysone (20E) signaling pathway are regulated by m6A modification. These findings suggest that mettl3-mediated m6A formation modulates ecdysteroid signaling to control diapause in cotton bollworms. Our work provides pivotal insights into the role of m6A RNA modification in regulating pupal diapause in insects.

Keywords: Diapause;N6-methyladenosine;RNA methylation;Helicoverpa armigera

The nuclear receptor gene E78 regulate the molting process of the two-spotted spider mite，Tetranychus urticae

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Abstract: The two-spotted spider mite, Tetranychus urticae, is an important pest mite in agriculture worldwide. E78, as a member of the nuclear receptor superfamily and a downstream responsive gene of ecdysteroids, plays a crucial role in regulating physiological behaviors such as development and reproduction in insects. However, its function in mites remains unclear. In this study, RT-qPCR experiments to analyze the expression pattern of TuE78 during the development of T. urticae, demonstrated that the expression level of TuE78 was higher during the molting state than that after the completion of molting, and it reached a peak expression level when the deutonymph mites entered the molting stage. RNAi-mediated gene-silencing of TuE78 resulted in 95% deutonymph mite molt failure. A series of analysis under the light, and scanning and transmission electron microscope revealed that RNAi mites died within the exuvium without ecdysis, and that apolysis had started but the new cuticle was thin and the typical cuticular lamellae were absent, indicating blockage of the post-apolysial processes and explaining molt failure. Hence, transcriptome sequencing confirmed that the expression of cuticle protein and lipid metabolism-related genes was significantly affected after TuE78 silencing. This study demonstrated that TuE78 participates in the molting process of T. urticae by regulating the post-apolysial processes with the formation of new cuticle and successful ecdysis. This in turn suggests the potential of TuE78 as a target for pest mite control and provides a theoretical basis for further exploration of the molecular regulatory mechanism of spider mite molting.
Keywords: Tetranychus urticae;nuclear receptor E78;cuticle formation

Temperature-dependent embryonic diapause in the endoparasitoid wasp Macrocentrus cingulum (Hymenoptera: Braconidae)

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Abstract: Parasitoid wasps serve as crucial biological control agents, and their diapause characteristics significantly impact not only their own survival and reproduction but also the efficacy of pest control. *Macrocentrus cingulum* (Hymenoptera: Braconidae) is a larval endoparasitoid of the Asian corn borer *Ostrinia furnacalis*, a major crop pest in China. However, the factors, stage, and mechanisms underlying its diapause remain unclear. Morphological observations and cell proliferation assays revealed that its development arrests at the late blastoderm stage, prior to germ band formation. This early embryonic diapause stage is relatively rare within the insect class, and to date, no reports exist of embryonic diapause in parasitoid wasps. This study investigated the effects of abiotic (photoperiod, temperature) and biotic (host diapause status, maternal effects) factors on diapause induction. Temperature was identified as the primary factor regulating diapause, and an asynchrony was observed between parasitoid diapause and host diapause. Diapause termination experiments demonstrated normal biological parameters, including eclosion rate, proportion of females, female longevity, and parasitism rate. These findings elucidate the autonomous diapause characteristics of *M. cingulum* within its host, laying the groundwork for research into its diapause mechanisms and provide both theoretical foundations and practical guidance for artificially regulating the wasp's development and for the application of natural enemy products in field-based pest management.

Keywords: Parasitoid wasp;Embryonic diapause;Temperature;Host diapause asynchrony