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Molecular Approaches in Entomological Research Associated with Molecular Basis of Metamorphosis

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Entomology today is a super science, having deviated considerably from the morphological-taxonomical approaches of the first half of the century, embracing an interdisciplinary approach involving such aspects as biological diversity, chemical ecology, molecular biology and biotechnology etc. Molecular biology, integrated with genetics and biochemistry, has provided the necessary tools for transferring and evaluating genetic characteristics not only for a host of insects, but also for related host plants. Such knowledge is vital to devise safe and specific agents for disrupting insect life cycles, thus increasing the efficiency of agricultural pests and vectors.

Insect metamorphosis can be classified into three modalities: ametabolan (no changes), hemimetabolan (progressive changes) and holometabolan (dramatical changes at the end of the cycle). The metamorphic changes are mainly regulated by two hormones: the moulting hormone, which promotes the moults, and the juvenile hormone (JH), which represses the transformation into the adult. The action of these two hormones is mediated by a number of transcription factors, and the molecular mechanisms regulating the expression of these and of the corresponding target genes are finally refined by the action of micro ribonucleic acids.

Ecdysone: Pulses of 20-hydroxyecdysone initiate each of the major developmental transitions, including both larval molting and metamorphosis

Juvenile hormone (JH) prevent precocious metamorphosis allow the larva to undergo multiple rounds of molting until it attains the proper size for metamorphosis

JH is classically viewed as an antimetamorphic hormone a high titer of JH in the early larval instars directs ecdysone to initiate molting, while the absence of JH during the final instar allows ecdysone to trigger the morphological changes of metamorphosis.

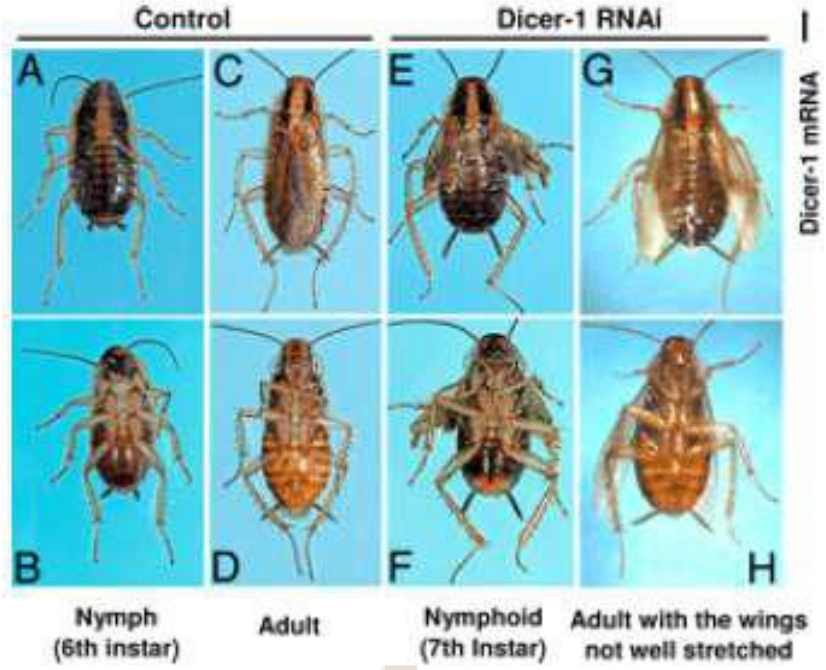
2. Broad-complex transcription factors

- Important group of transcription factors involved in metamorphosis is that known as Broad-Complex (BR-C). The BR-C family of transcription factors is especially interesting because they have different ways of action in hemimetabolans and holometabolans.
- Functional studies in *Drosophila melanogaster* and the lepidopterans *Manduca sexta* and *Bombyx mori* have shown that BR-C factors are essential for the transformation of last instar larvae into pupae

3. MicroRNA-dependent metamorphosis

- MicroRNAs (miRNAs) are small noncoding RNAs, which participate in many biological processes they also involved in insect metamorphosis.
- miRNAs play a critical role in many biological processes, by modulating gene expression at the posttranscriptional level through binding at the 3′-untranslated region of the target mRNA

- Dicer ribonucleases are important in the biogenesis of miRNAs as they are involved in the production of mature miRNAs from miRNA precursors (premiRNAs), and of small interfering RNAs
- Using systemic RNA interference (RNAi) to silence the expression of Dicer-1 in the hemimetabolan insect *Blattella germanica*, by depleted miRNA contents in the last instar nymph. This practically inhibited metamorphosis after the next molt, as the resulting specimens showed nymphoid features.

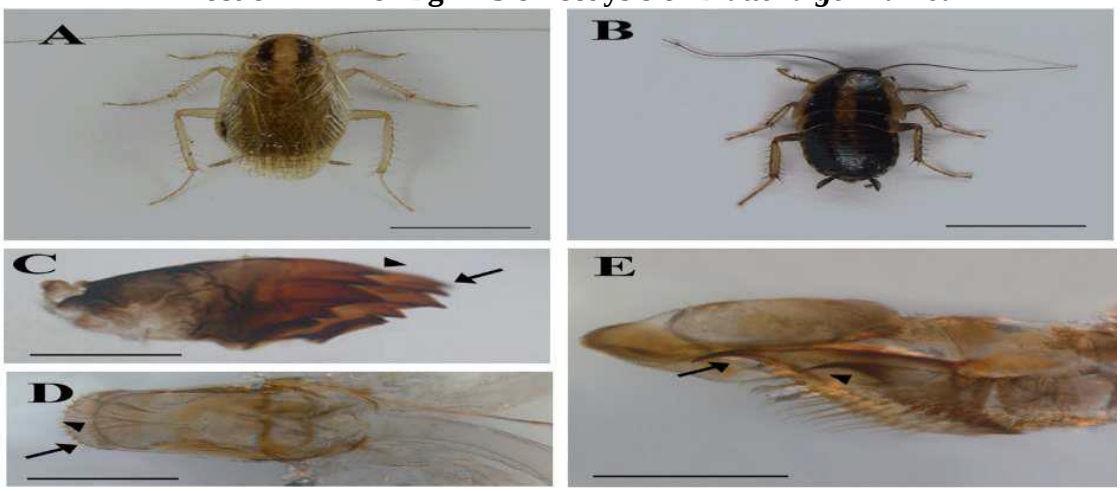


Depletion of Dicer-1 and miRNAs Impairs Metamorphosis

4. Signalling of metamorphosis by Ecdysone

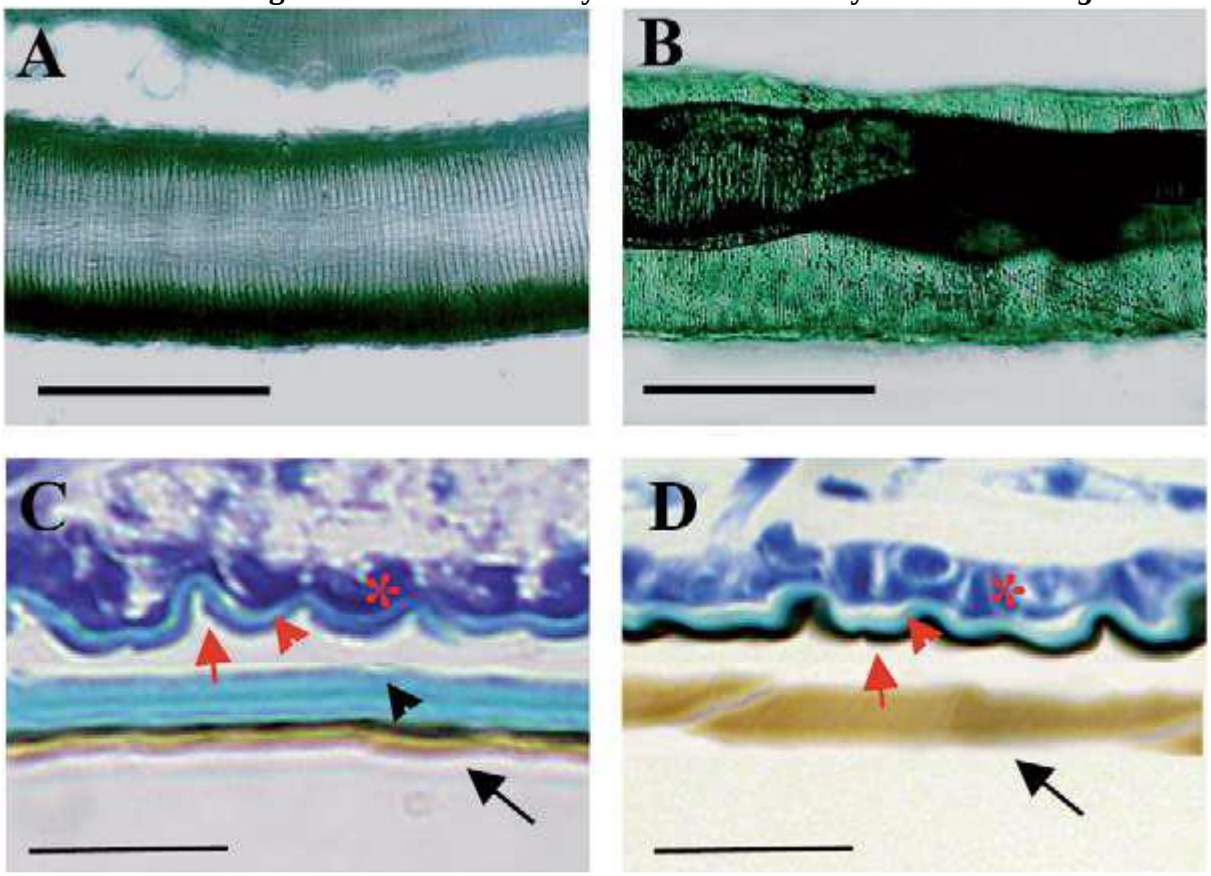
- Ecdysone is a direct regulator of transcription and, like other steroid hormones acts through a nuclear receptor pathway
- The ecdysone receptor is heterodimer of the nuclear receptors EcR and USP that binds to the ecdysone response element (EcRE)
- Upon binding to their heterodimeric receptor composed by two nuclear receptors, the ecdysone receptor (EcR) and the ultraspiracle (USP) activate the expression of a hierarchy of transcription factors (HR3, HR4, HR39, E75, E78, FTZF1, etc.) that regulate the expression of the target genes underlying the cellular changes associated to moulting and metamorphosis

Effect of RNAi of BgHR3 on ecdysis of *Blattella germanica*



(A) Control specimen (B) dsBgHR3-1-treated specimen of the same age (C) mandibles (D) hypopharinge (E) lacinia

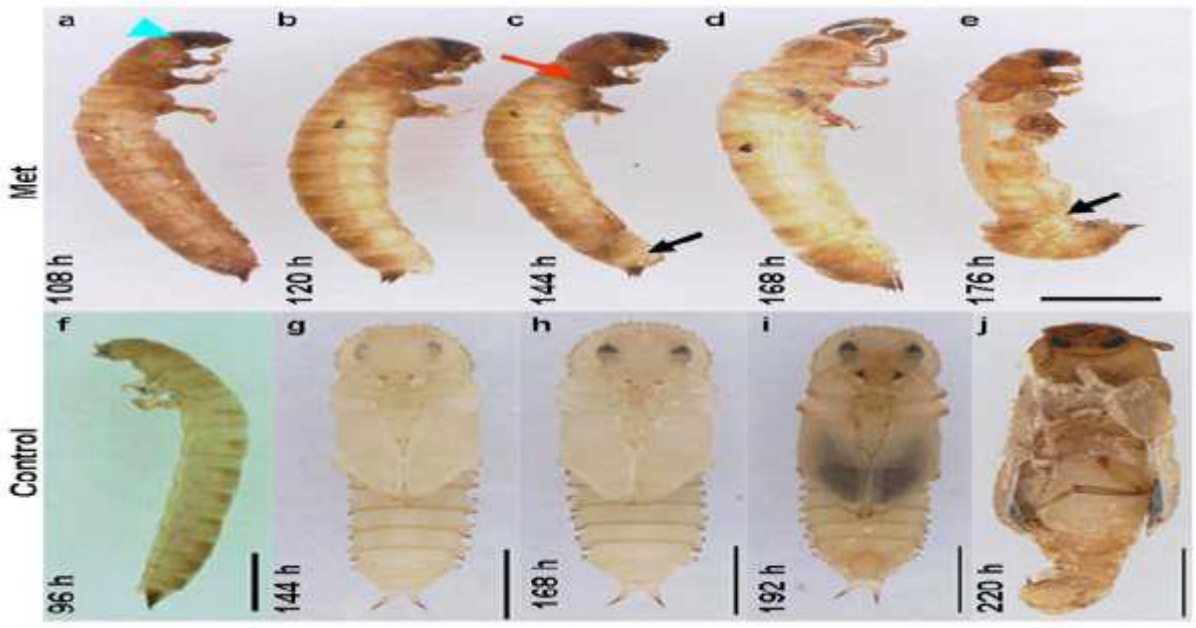
Effect of RNAi of BgHR3 on the tracheal system and cuticle layers of *Blattella germanica*



(A) Trachea from a dsControl specimen (B) Double trachea from a dsBgHR3-1-treated specimen (C) Cuticle layers of a ds Control specimen (D) Cuticle layers of a dsBgHR3- 1-treated specimen

5. Signalling of metamorphosis by Juvenile hormone (JH)

- The multifunctional nature of JH has impeded the characterization of its signaling mechanisms.
- JH is pleiotropic and controls not only development but also sexual behaviour, pheromone production, caste determination, diapause, migration, and the synthesis of female yolk proteins and male accessory gland proteins
- In regulating these diverse processes JH appears to utilize multiple pathways, some of which involve the activation of gene expression while others are transcription-independent
- Activity of JH in many insects influences larval development so mutation of JH receptor would have severe consequences for larval development
- In *Drosophila, JH is essential to preadult development as* its removal by genetic ablation of the corpora allata results in lethality around the time of head eversion and defects in larval development including precocious apoptosis of the fat body
- Methoprene-tolerant (MET) is identified as a potential JH receptor
- This was confirmed in the red flour beetle *Tribolium castaneum, where removal of Met expression produces a premature and* lethal initiation of pupation



6. Conclusion:

Applied Biosystems TaqMan Advanced miRNA Assays enable highly sensitive and specific quantification of mature miRNAs using qPCR. Together with the TaqMan Advanced miRNA cDNA Synthesis Kit, this solution is designed to provide a streamlined workflow that allows for exceptional detection of multiple miRNA targets from a single sample.

7. Reference

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