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How are comparative genomics and the study of microRNAs changing our views on arthropod endocrinology and adaptations to the environment?

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ABSTRACT

As the last few decades of work has shown, precise regulation of biosynthesis and release of arthropod hormones is essential to cope with environmental stresses and challenges. In crustaceans and insects, the sesquiterpenoids methyl farnesoate (MF), farnesoic acid (FA) and juvenile hormone (JH) regulate many developmental, physiological, and reproductive processes. In this review, we discuss how comparative genomics has and will impact our views on arthropod endocrinology. We will also highlight the current knowledge of regulation of genes involved in arthropod hormone biosynthesis by microRNAs, and describe the potential insights into arthropod endocrinology, evolution, and adaptation that are likely to come from the study of microRNAs.

1. Introduction

All multicellular animals produce hormones (from the Greek "hormon": excite) to control their developmental, physiological, and reproductive activities. The phylum Arthropoda (Greek: jointed leg) is highly speciose and comprises the majority of described extant animal species including arachnids, crustaceans, and insects. The diversity of the habitats that they have conquered perhaps is partly a result of the unique sesquiterpenoid hormonal system that has coevolved with them, which has been shown to be responsive to environmental stresses such as anoxia, temperature, salinity, and even environmental contaminants (e.g. Borst et al., 2001; LeBlanc, 2007; Lovett et al., 1997, 2001; Nagaraju and Borst, 2008).

Hormone production in bilaterians shares a conserved mevalonate biosynthetic pathway derived from simple acetate molecules. Different final products are generated in different species (i.e. cholesterol in vertebrates, juvenile hormone (JH) in insects, and methyl farnesoate (MF) and farnesoic acid (FA) in crustaceans, Fig. 1). Details of the pathway have been extensively reviewed, and will not be repeated here (Bellés et al., 2005; Hui et al., 2013; Tobe and Bendena, 1999). This article will focus on the comparison of JH in insects with the MF and FA in crustaceans. These hormones are thought to be produced in structurally similar organs (MF and FA in the mandibular organ and JH in the corpora allata), and are believed to serve similar functions in the regulation of gametogenesis/reproduction, metabolic activities, metamorphosis and ecdysteroid secretion for moulting (Laufer et al., 1987; Le Roux, 1968; Tiu et al., 2009, 2012; Tobe and Stay, 1985; Tobe et al., 1989). As the chemical structure of MF lacks the epoxide group found in JH-III, and as no JH has ever been identified in crustaceans, MF or FA are commonly thought to be the crustacean equivalents of "JH", and to date, JH is generally considered to be an evolutionary derivative of MF and a hormone unique to insects (see section 3.3).

One rate-limiting step in the biosynthesis of these hormones is thought to be the final step, the conversion to JH or MF through a *S*-adenosyl-methyltransferase (SAM)-dependent methylation (for details, see Hui et al., 2010, 2013; Tobe and Bendena, 1999). In insects, the methylation occurs by way of the juvenile hormone methyltransferase (JHAMT), which is involved in the conversion of JH acids or FA to JH (through MF) in a range of insects (Kinjoh et al., 2007; Marchal et al., 2011; Minakuchi et al., 2008; Niwa et al., 2008; Shinoda and Itoyama, 2003). Studies of knockdown and overexpression of JHAMT all suggest an essential role in maintaining normal development and metamorphosis (Kinjoh et al., 2007; Minakuchi et al., 2008; Niwa et al., 2008).

Abbreviations: JH, juvenile hormone; MF, methyl farnesoate; NGS, next generation sequencing; JHAMT, juvenile hormone methyltransferase; FAMeT, farnesoic acid *O*-methyltransferase; SAM, *S*-adenosyl-methyltransferase; RNAi, RNA interference.

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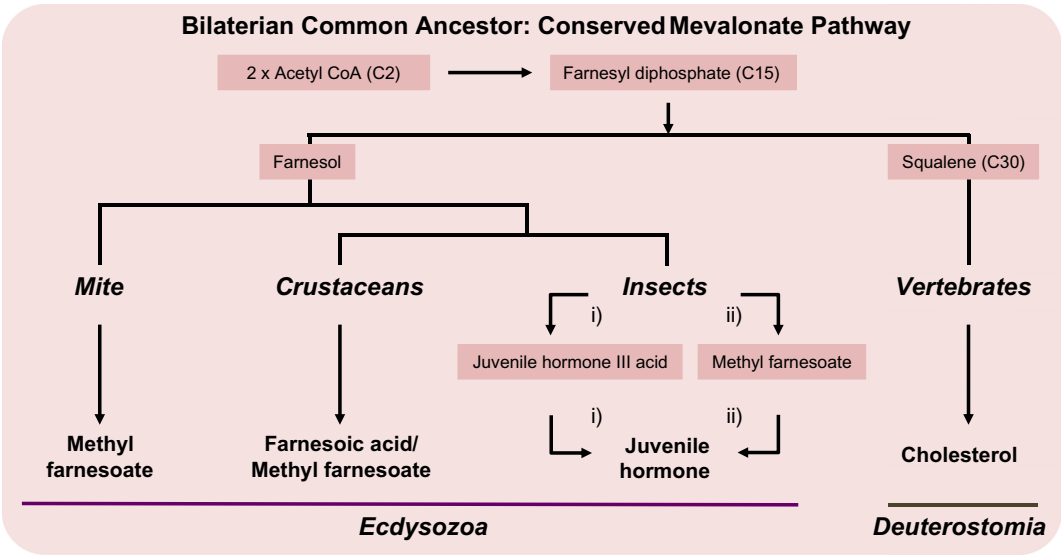

Fig. 1. Comparative endocrinology of the Mevalonate pathway within the Bilateria modified from Bellés et al. (2005), Hui et al. (2013), and Tobe and Bendena (1999). Superphyla and subphyla where the mevalonate pathway has been studied are shown. Intermediate molecules within the pathway are shown in boxed darker coloration. Note crustacean water flea *Daphnia pulex* (Hui et al., 2010) and mite *Tetranychus urticae* (Grbic et al., 2011) contain JHAMTs in their genomes, but may not produce JH.

However, the corresponding situation in crustaceans is not as clear. For the past decade, the putative farnesoic acid *O*-methyltransferase (FAMeT) in crustaceans was assumed to play this role (e.g. Burtenshaw et al., 2008; Gunawardene et al., 2002; Holford et al., 2004; Hui et al., 2008; Wainwright et al., 1998). With the first systematic phylogenomic analysis of FAMeT and JHAMT across bilaterians (Hui et al., 2010) this received view of crustacean endocrinology has been completely revised (for full details, see review (Hui et al., 2013). In summary, the finding of a true JHAMT in the water flea *Daphnia pulex* reveals that, contrary to earlier opinion, crustaceans do possess the JHAMT enzyme and JHAMT is not unique to insects. This discovery of the first *bona fide* JHAMT in a crustacean also opens new avenues for research. Examples of questions raised by this discovery include those related to the expression, role and function of JHAMT in crustacean endocrinology. Further, *JHAMT* is known to be one of the most up-regulated genes in presence of kairomone signals in water fleas (a typical ecotoxicological model organism) (Miyakawa et al., 2010). Study of the interactions of JHAMT and biosynthesis of sesquiterpenoids in different arthropods under different environmental conditions may also provide useful information on ecotoxicology.

With the recent rapid advancement in sequencing technologies, and a simultaneous reduction in costs, the feasibility of applying comparative genomic approaches to the questions above is now a reality, even for smaller laboratories. Here we will discuss how these comparative genomic approaches have been made possible, how they may be performed, and how they might change our view of arthropod endocrinology as a whole. These studies are likely to focus on the clade Ecdysozoa within the animals, since it is already is clear that no JH biosynthetic pathway specific components have been observed in the lophotrochozoans or deuterostomes (Fig. 1).

In the present review, we will focus our discussion on the relatives of insects and crustaceans that can be used to test evolutionary hypotheses within these clades. Also, recent studies show that non-coding microRNAs have huge impact on many areas of biology by regulating genes at the post-transcriptional level, and one which is largely unexamined in this field (Hui et al., 2013). We will therefore also review the latest findings on the role of non-coding RNAs in arthropod endocrinology, and discuss how the study of microRNAs may contribute to our understanding of arthropod hormones and the adaptations of arthropods to their environment.

2. Comparative genomics

Recent years have seen an explosion in the amount of genomic data available from across the animal kingdom. This increase in data availability has revolutionised comparative biology, allowing inferences into the evolution and diversification of traits at a molecular level. Whereas initial sequencing efforts were concentrated on traditional model organisms such as flies and nematodes, increased sampling across the metazoan 'tree of life' has quickly shed much light on previously intractable problems in assessing the course of evolution, as we will illustrate with particular reference to the evolution of the hormonal system within the Arthropoda.

2.1. Next generation sequencing and assembly

Until recently, genome projects have depended on traditional Sanger-based shotgun or clone-by-clone sequencing. While effective, these methods have always been slow, labour intensive and expensive, and with recent technological advances, this approach has been almost entirely supplanted by next-generation sequencing (NGS) technologies Shendure and Ji, 2008. A range of NGS platforms are available for purchase or commercial use. Perhaps the most common providers are those utilizing Illumina or Roche 454 chemistry, although others (such as PacBio, Ion Torrent, SOLiD and Helioscope) are also available. Each source of NGS data has its own merits and drawbacks, and these have been covered in depth elsewhere (Glenn, 2011; Henson et al., 2012). Nanopore sequencing technology is likely to appear commercially in the near future, and the long read length provided by such an approach might further simplify genome assembly (Hayden, 2012).

A major difference between traditional Sanger approaches and NGS methods is the amount of DNA sequence generated. For example, a single DNA sequencing run on the most advanced Sanger platform (the ABI 3730XL machine) can yield up to 70 kb of DNA sequence derived from 96 reactions each generating ~700 bp. In contrast, a single lane run on an Illumina HiSeq generates over 35 Gb of sequence: a staggering half a million times as much data. The vast amount of data provided by a typical NGS project has necessitated the use of novel algorithms for assembling these data,

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