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MicroRNA functions in insects

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ABSTRACT

MicroRNAs (miRNAs) are small non-coding RNAs that are generated in all eukaryotes and viruses. Their role as master regulators of gene expression in various biological processes has only been fully appreciated over the last decade. Accumulating evidence suggests that alterations in the expression of miRNAs may lead to disorders, including developmental defects, diseases and cancer. Here, I review what is currently known about miRNA functions in insects to provide an insight into their diverse roles in insect biology.

1. Introduction

It was not a very long time ago that the non-coding regions in the genomes of living organisms were considered to be junk DNA. Advances in new approaches in molecular biology in the last two decades have tremendously changed our understanding of genomes and their expressed non-coding RNAs, which play significant roles in various aspects of cell and organismal biology. One of these non-coding RNAs, which becomes further processed into small RNAs of about 22 nucleotides, is microRNA (miRNA) and it was first reported to regulate timing of development in *Caenorhabditis elegans* (Lee et al., 1993). Since then, thousands of miRNAs have been reported from plants and animals and their viruses and are now databased on miRBase (www.mirbase.org) (Kozomara and Griffiths-Jones, 2011). The main function of miRNAs is regulation of gene expression at the post-transcriptional level adding a new layer of control to the complex pathways that exists in cells. In many instances, miRNAs are essential for target tuning and optimal expression levels of genes (Bartel and Chen, 2004), and what is found common among miRNAs is their pleiotropic role in regulating transcripts of target genes at different times and locations during development. After two decades of research, there are still many unknowns about the miRNA biogenesis and the dynamics of miRNA interactions with targets. New discoveries, for example, have shown that there may exist several non-canonical pathways of miRNA biogenesis (Miyoshi et al., 2010); besides down-regulation of target transcript levels, miRNAs may also up-regulate the transcript levels of their targets (Hussain et al., 2011, 2012); and may bind to locations other than the 3′UTR (Bartel, 2009; Rigoutsos, 2009); etc. These new findings continuously challenge previous mainstream views of miRNAs and their function, which in turn influence the molecular and bioinformatics approaches used in miRNA research.

Over the years many publications have reported the miRNA profiles of various insects and their role in diverse functions, such as development and host–microorganism interactions (reviewed in Aravin et al., 2003; Chawla and Sokol, 2011; Mead and Tu, 2008; Yu et al., 2008). One of the main obstacles in miRNA research in insects has been the absence of complete genome sequences of several insects. For this reason, most studies have focused on model insects with genome sequences available (e.g. *Drosophila* and *Bombyx*).

Viruses infecting insects have also been shown to encode miRNAs, which play important roles in host–virus interactions, especially subverting host defense responses to assist the replication of viruses (Asgari, 2011; Asgari and Sullivan, 2010). Encoding miRNAs is ideal for viruses since they take up little spaces on the viral genome. In addition, miRNAs are not immunogenic, can rapidly evolve by small changes in their sequences to regulate new targets and one miRNA can potentially regulate transcripts of several host genes. In this review, the latest key findings on miRNAs involved in various insect functions will be presented and discussed.

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2. miRNA biogenesis and interaction with the target

miRNAs may be encoded from non-coding transcripts, introns or even coding regions. miRNA genes, that are mostly independent transcriptional units, are predominantly transcribed by RNA polymerase II as a primary miRNA (pri-miRNA), which may contain one or more stem-loops. Like mRNAs, pri-miRNA transcripts are 5′ capped and polyadenylated (Bracht et al., 2004). The stem-loop is further processed by Drosha, in association with Pasha (equivalent to DGCR8 in mammals), into a short hairpin of around 70 bases known as the precursor miRNA (pre-miRNA; Fig. 1) (Bartel, 2009). Pre-miRNA hairpins may also be directly processed from primary mRNA transcripts by splicing and debranching of short introns which are referred to as mirtrons (Flynt et al., 2010). Pre-miRNA is then transported into the cytoplasm by Exportin-5, where the terminal loop is removed by the ribonuclease enzyme Dicer-1 (Dcr-1) producing a miRNA:miRNA* duplex with 2 nucleotide overhangs on both ends (Hutvágner et al., 2001). The duplex becomes incorporated into the RNA Inducing Silencing Complex (RISC) in which Argonaute-1 (Ago-1) protein is the main component (Miyoshi et al., 2009). The miRNA* strand (=passenger strand) is then degraded and the miRNA strand (=guide strand) guides the RISC complex to the target mRNA. In certain instances, the miRNA* may also have a regulatory function(s), as shown in *Drosophila* (Czech et al., 2009; Okamura et al., 2008). In addition to their role in silencing, Ago proteins have been shown to increase miRNA stability (Winter and Diederichs, 2011).

Initially, it was thought that the target sequences of miRNAs only reside in the 3′UTR of target mRNAs. However, accumulated data suggest that target sequences may exist in the open reading frame and the 5′UTR as well as the 3′UTR (Bartel, 2009; Rigoutsos, 2009). In plants, miRNA sequences are perfectly complementary to their target sequences (Naqvi et al., 2012), but in animals the complementarity is imperfect with several mismatches (Bartel, 2009). In miRNA-target base-pairing, complementarity of the miRNA seed region (nucleotides 2–8 from the 5′ end) with the mRNA is believed to be important, although there are several examples showing that this is not a general rule (Didiano and Hobert, 2006). Each miRNA may target mRNAs from one to several genes and sometimes multiple target sites may reside in one mRNA (Brodersen and Voinnet, 2009; Shin et al., 2010). The outcome of the miRNA interaction with a target varies from mRNA degradation (Hussain and Asgari, 2010), repression of translation (Zdanowicz et al., 2009) through to increases in the target transcript levels (Hussain et al., 2012). Interestingly, different species of an mRNA with variable 3′UTRs could be produced in different regions in the embryo with different sets of miRNA target sites allowing differential regulation of a gene in different tissues (Thomsen et al., 2010). For more details on the biogenesis and target–miRNA interactions, particularly as they apply to insects, readers are referred to Chawla and Sokol (2011).

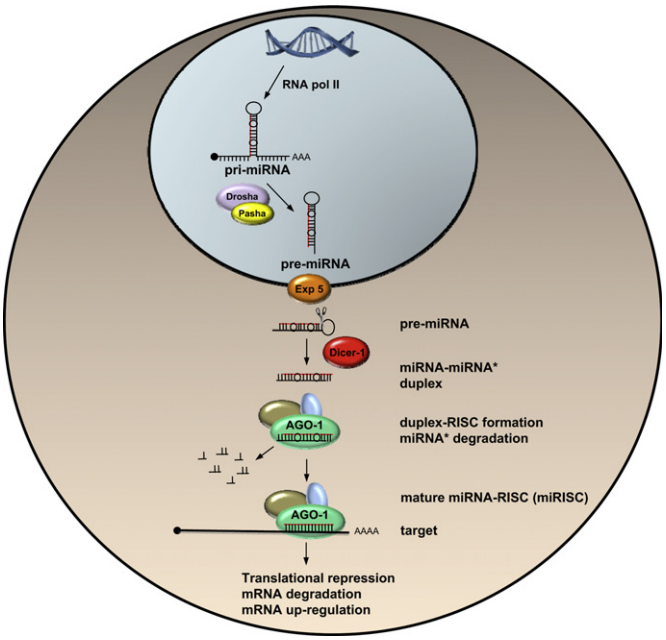

Fig. 1. Canonical miRNA biogenesis pathway. miRNA genes are expressed in the nucleus by RNA polymerase II with the primary miRNA (pri-miRNA) transcript containing one or more stem-loop structures. The pri-miRNA is processed by an RNase III type enzyme, Drosha, in association with Pasha, cutting the stem-loop from the base and producing a 70 nt hairpin structure known as precursor miRNA (pre-miRNA). Pre-miRNA is then transported into the cytoplasm by Exportin 5 where the hairpin terminal loop is diced by Dicer-1 forming the miRNA–miRNA* duplex which becomes incorporated into the RISC complex. miRNA* is normally degraded and the mature miRNA–RISC complex (miRISC) interacts with the target sequences in the mRNA target. The outcome of the interaction is variable and includes translational repression, mRNA degradation and in certain instances target up-regulation.

3. Role of miRNAs in development

Compared to other areas in insect biology, the role of miRNAs in insect development has attracted the most attention mainly because of their conserved functions in development in animals. Several studies have shown stage-specific or tissue-specific expression of miRNAs during insect development in *Drosophila melanogaster* (Aravin et al., 2003), *Bombyx mori* (Yu et al., 2008), *Blattella germanica* (Cristino et al., 2010), *Spodoptera litura* (Rao et al., 2012), *Apis mellifera* (Behura and Whitfield, 2010), *Aedes aegypti* (Behura et al., 2011), *Aedes albopictus* and *Culex quinquefasciatus* (Skalsky et al., 2010) and the brown planthopper *Nilaparvata lugens* (Chen et al., 2012). Let-7, which was the second miRNA discovered from *C. elegans* (Reinhart et al., 2000), was subsequently discovered in all other animal groups which makes it one of the most conserved miRNAs known. One of the main functions of let-7 is to regulate developmental timing (Ambros, 2011). Expression of the let-7-complex (let-7-C) locus in *Drosophila*, which comprises of miRNAs let-7, miR-100 and miR-125, is induced by 20-hydroxyecdysone (20E) with 20E responsive elements being present in the locus (Chawla and Sokol, 2012). Broad-complex (Br-C), which is an early 20E induced transcription factor that first appears at the larval–pupal molt, and ecdyson are both required for the expression of let-7-C, which appears during the larval–pupal stage at the beginning of metamorphosis (Sempere et al., 2002, 2003). Loss of Let-7-C is not lethal but causes reduced mobility, juvenile characteristics of neuromusculature, and reduced fecundity in the adult flies (Caygill and Johnston, 2008; Sokol and Ambros, 2005). Let-7-C is also involved in wing development and neurogenesis (see Sections 3.2 and 3.4). In addition to let-7, a number of miRNAs have been studied in details in regard to their specific functions in various developmental processes (see below). Interestingly, like let-7, many of them are deeply conserved across the animal kingdom and regulate various developmental processes, suggesting that they usually target transcripts of several genes.

3.1. Germ cell development

Experimental evidence suggests that proteins which are involved in regulating the development of germ cells function in association with the miRNA pathway to control key signaling pathways that determine the fate of progenitor cells. Some

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