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# Circular RNA in Insects

**Sahana, Sagar D and Ramya R S**
Division of Genomic Resources, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru

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## Introduction

Circular RNAs (circRNAs) are a novel class of non-coding RNA distinguished by their covalently closed loop structures, setting them apart from linear RNAs. First discovered in RNA viruses during the 1970s and subsequently identified in eukaryotic cells. Unlike linear RNAs, circRNAs do not have 5′ caps or 3′ poly (A) tails, which confers them with enhanced stability and resistance to exonuclease degradation. They are considered as the occasional and useless products of RNA splicing errors because traditional RNA sequencing technology could not detect them. Further studies have revealed their important functions regarding regulating gene expression at the transcriptional and post-transcriptional levels. These functions include acting as microRNA (miRNA) sponges, binding to RNA-binding proteins (RBPs), acting as transcriptional regulatory factors, and serving as translation templates (Xie, 2020). The advances in circRNA research have opened researchers' eyes to a new area of research on the roles of circRNAs in the pathogenesis of various diseases, especially at the immune level because of the close relationship between circRNAs and the immune response.

In insects, circRNAs have attracted growing interest because of their potential roles in regulating development, physiology, and adaptation. Research on model organisms like *Drosophila melanogaster* has shown that circRNAs are highly expressed and display tissue-specific and developmental stage-specific expression patterns. These molecules are derived from known genes and can originate from exons, introns, or a combination of both.

## Synthesis of circRNAs

The concept of circular RNAs (circRNAs) was initially introduced in 1976 following their identification in RNA viruses (Sanger, 1976). Subsequent studies by Nigro in 1991, revealed the presence of circRNAs in human cells. Initially, these molecules were thought to be as mere by-products of splicing errors, lacking any significant biological role. This misconception arose due to their unique ring structure, characterized by the absence of the conventional 5′ cap and 3′ poly (A) tails. Consequently, circRNAs eluded detection by

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traditional polyadenylated transcriptome analyses, leading to only sporadic discoveries in earlier research.

Most circRNAs originate from known genes and they are categorized into three types based on their sequences: exonic circRNAs, intronic circular RNAs (ciRNAs), and exon-intron circRNAs. Their formation typically involves reverse complementation of introns and exon skipping. Recent research indicates that circRNAs are generated through back-splicing of pre-mRNAs, a form of alternative splicing that relies on spliceosomal machinery and can be influenced by cis-regulatory elements and trans-acting factors (Zhang, 2014). Despite having low expression levels generally, certain circRNAs exhibit higher abundance compared to their linear counterparts in biological samples (Lasda, 2014), suggesting that circRNAs may have unique and essential biological functions under physiological conditions. It is reasonable to hypothesize that circRNAs are crucial in various physiological and pathological processes.

**Mechanisms Underlying Circular RNA Formation**

The majority of circular RNAs (circRNAs) stem from protein-coding genes and exhibit a diverse exon composition devoid of specific sequence preferences, indicating their transcriptional dependence on RNA Pol II (Chen, 2016). Unlike the conventional mRNA production pathway, the genesis of exonic circRNAs involves backsplicing, wherein a downstream 5´ splice site (splice donor) aligns with an upstream 3´ splice site (splice acceptor), facilitating circular transcript formation (Jeck and Sharpless 2014; Lasda and Parker 2014). Another mechanism contributing to circRNA generation is the "lariat intermediate" or "exon skipping," where a lariat formed by exon skipping undergoes internal splicing, excising intronic sequences and yielding a circular RNA product. It is widely acknowledged that circRNA biogenesis undergoes intricate regulation, both in cis and trans configurations (Salzman, 2016). Regulatory control over circular RNA biogenesis often involves a combination of trans-factors and cis-sequences. For instance, in *Drosophila*, the expression of Laccase2 circular RNA is governed by intronic repeats in cis and influenced by a myriad of heterogeneous nuclear ribonucleoprotein (hnRNP) and serine-arginine (SR) proteins in trans (Kramer *et al*. 2015).

**Defining Traits of circRNAs**

- CircRNAs are generally more stable than their linear counterparts due to the absence of 5′ caps and 3′ poly(A) tails, which makes them resistant to hydrolysis by various cellular endonucleases.
- Another notable feature of circRNAs is their high abundance.

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- They are typically found in the cytoplasm and have also been identified in exosomes present in culture media.
- Additionally, the distribution of circRNAs shows tissue and developmental stage specificity.
- CircRNAs are also evolutionarily conserved.

**Mechanistic Insights into the Functional Roles of circRNAs**

The diverse class of non-coding regulatory RNAs known as circRNAs displays a wide array of functions, localizations, and characteristics (Dong *et al*., 2016). The biological roles of circRNAs are likely determined by their subcellular localization, whether in the nucleus or cytoplasm. Research indicates that circRNAs not only regulate gene expression in the nucleus but also function as miRNA and protein decoys, and serve as scaffolds for circRNA-protein complex formation. Evidence suggests that certain circRNAs may also serve as templates for translation or as sources for the generation of pseudogenes (Hansen *et al*., 2013).

**CircRNAs in insects**

CircRNAs have been observed across a wide spectrum of insect species, particularly those with fully sequenced genomes, with researchers continually uncovering novel circRNAs alongside revisiting existing ones as sequencing technologies advance (Zhang *et al*., 2021). Insects' circRNAs play diverse roles in various aspects of insect biology, including development, reproduction, metamorphosis, insecticide resistance, aging, lifespan, and interactions related to host-pathogen immunity (Liu *et al*., 2022). The implication of these diverse functions suggests that circRNAs may serve as significant regulators of insect physiological processes.

**Exploring the role of circular RNAs in insect innate immunity**

The research on circRNAs in insects remains limited, their potential functions are gradually unfolding. In response to microbial pathogens, insects might generate circRNAs to modulate immune reactions. These circular RNA molecules demonstrate diverse biological roles across various life processes, acting as miRNA and protein sponges, translation regulators, biomarkers, and more (Li *et al*., 2018).

**Unveiling the impact of circular RNAs (circRNAs) on antiviral defense**

Viruses, as obligate intracellular pathogens with limited coding capacity, pose significant medical and economic burdens, especially in insects. While extensive research has elucidated insect innate immunity and its molecules combating viral infections, the role of

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circRNAs remains largely unexplored. Key questions persist regarding how host receptors detect viral pathogens and the pivotal receptor types in antiviral immunity. Although Toll and IMD pathways are activated by viruses, the precise mechanisms and involvement of pattern recognition receptors (PRRs) remain elusive. Notably, only Dicer-2, recognizing viral double-stranded (ds) RNA, has been identified in insects, underscoring the need for further investigation into the regulatory role of circRNAs in innate immunity during viral infections.

**Circular RNAs (circRNAs) in bacterial defense: Unveiling regulatory roles**

CircRNAs have recently been reported to be implicated in the regulation of anti-bacterial immunity. Using *D. melanogaster* as a model organism (Han *et al*., 2020) confirmed the involvement of circRNAs in anti-bacterial immunity. The circular RNAs *viz*., Ect4 and Edis in *D. melanogaster* promote and suppress optimal antimicrobial peptide gene expression in terms of immune regulation in neurons. Ect4 dampens the IMD anti-bacterial signaling in the tracheal epithelium but not in the fat body or digestive tract implying that both circRNAs are actively involved in immune responses against bacteria

**Circular RNAs (circRNAs) in defense against fungi and parasites**

Entomopathogenic fungi infect insects by adhering spores to their cuticles, forming specialized appressoria to penetrate. Inside the insect, filaments transform into yeast-like cells, propagate rapidly, and suppress the immune response. After completing the infection cycle, dead insects either produce asexual spores or form fruiting bodies for the next cycle. While entomopathogenic fungi are used for pest control, they also harm economically important insects, highlighting the need to understand insect resistance mechanisms for effective management. Besides viral and bacterial pathogens, fungal and parasitic pathogens have also influenced the production of circRNAs. Fungal or parasitic infections induce a plethora of circRNAs that are proposed to be involved in anti-fungal immunity (Shang *et al*., 2015). CircRNAs were found to be altered in the *Apis mellifera ligustica* larval guts during the fungus, *Ascosphaera apis* infection and have been linked to host immune responses. These circRNAs in the larval guts are likely to play critical biological roles during an *A. apis* infection, as evidenced by their involvement in a variety of immune pathways, such as apoptosis, autophagy, endocytosis, as well as MAPK, Toll, and IMD signaling pathways.

**Conclusion**

In conclusion, circRNAs have emerged as key players in immune regulation within living organisms, with diverse roles ranging from miRNA sponges to decoys. Despite

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significant strides in understanding their classification, mechanisms of action, and interaction with pathogens, there remains a considerable gap in our knowledge regarding the specific biological functions of many circRNAs, particularly in host-pathogen interactions. Further research leveraging advanced molecular biology techniques is essential to elucidate the precise roles of circRNAs in these interactions and uncover how pathogens manipulate host circRNAs for their survival.

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