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# RNAi-Mediated Post-Transcriptional Gene Silencing Strategies in Honey Bees

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**Abstract**

Gene silencing technology (knock-down) via RNA interference (RNAi), a contemporary molecular method, represents an RNA-based, sequence-specific, post-transcriptional approach. RNAi has demonstrated its efficacy in reducing gene expression across a wide array of organisms, encompassing plants, mammals, and insects. Studies conducted on diverse organism groups have significantly contributed to comprehending both the operational mechanism of RNAi and the roles of genes within these organisms' biological processes. Among the organisms benefiting extensively from RNAi is the honey bee *Apis mellifera*. This technology, serving as a crucial natural antiviral defense mechanism in bees, has proven highly effective in probing genes associated with the immune system and in combatting pests and pathogens. Consequently, this study undertakes a review of the existing literature to delineate the potential applications, usage areas, and strategies of RNAi technology in honey bees.

***Keywords:*** *Biotechnology, Gene silencing, Honey bee, RNAi*

## Introduction

Honey bees play a critical role in plant pollination, thereby contributing to increased agricultural production and supporting the diversity and balance of natural ecosystems (Gallai et al., 2009). However, in recent years, several stress factors affecting honey bees have posed serious threats to hive health and productivity (Olate-Olave et al., 2021). Due to these challenges, scientists and beekeepers have displayed significant interest in developing new strategies involving molecular genetic techniques. Modern molecular biology and genetic research are indispensable for regulating gene expression and understanding gene functions in honey bees. Cells employ various mechanisms to control gene expression, and comprehending these mechanisms is pivotal for grasping the underlying causes of diseases and designing treatments. Among the array of mechanisms, RNA-mediated interference (RNAi) has emerged as a prominent post-transcriptional gene silencing strategy, gaining prominence in recent years. This approach provides valuable insights into gene regulation and holds potential for addressing diverse challenges in honey bee health and productivity.

## RNA interference (RNAi)

RNA interference (RNAi) is a sequence-specific post-transcriptional gene silencing mechanism mediated by RNA molecules (Fire et al., 1998). The groundbreaking study conducted by Fire et al. (1998) provided the initial clear demonstration of the biochemistry underlying gene silencing inducers, achieved by directly introducing purified double-stranded RNA (dsRNA) into the body of Caenorhabditis elegans. The natural function of RNA interference encompasses the regulation of various genes and acts as a defense mechanism to safeguard the genome against mobile genetic elements, including viruses and transposons (Agrawal et al., 2003). RNAi has been extensively shown to efficiently reduce gene expression across a diverse spectrum of organisms, spanning plants, mammals, insects, and ticks (Hannon, 2002; Fuente et al., 2007). The silencing pathway initiates with the presence of endogenous or exogenous double-stranded RNA, leading to the generation of small (21-26 base pair) silenced RNA fragments known as small interfering RNAs (siRNA), which are cleaved by the Rnase III-like enzyme. These siRNAs are short dsRNAs characterized by two nucleotide overhangs at their 3' hydroxyl terminals and 5' monophosphate ends (Wilson and Doudna, 2013). Subsequently, the siRNAs are captured by Argonaute 2 (AGO2), an endoribonuclease and a catalytic component of the multiprotein RNA-induced silencing complex (RISC). One strand of the siRNA (the passenger strand) is liberated (the double strand is cleaved into a single strand by the RISC complex), while the other strand, known as the guide strand, targets homologous RNA sequences resulting in the silencing or cleavage of these homologous sequences (Wilson and Doudna, 2013). Notably, siRNAs possess the capability to direct RNA-directed DNA methylation or chromatin remodeling, alongside RNA degradation. They accomplish this by guiding protein complexes to RNAs carrying homologous sequences, thereby governing gene expression and inducing epigenetic modifications (Figure 1) (Hannon, 2002).

## RNAi Applications in Honey Bees

RNA interference (RNAi) has emerged as a valuable method for enabling the screening and characterization of honey bee genes, facilitating the characterization of pathogen interactions and studying the functions of Acari genes (Fuente et al., 2007).

RNAi has been widely employed in honey bees to study gene functions. By selectively silencing specific genes through the introduction of double-stranded RNA (dsRNA), researchers can assess the impact of gene knockdown on various biological processes. Antonio et al. (2008) investigated role of vitellogenin in transition from within-hive activities to foraging behavior in honeybee workers, traditionally attributed to juvenile hormone (JH) regulation. By injecting vitellogenin double-stranded RNA (dsVg) into newly emerged Africanized worker bees, researchers observed that RNA interference-mediated silencing of vitellogenin gene function led to a shift in the onset of long-duration flights to earlier in life (by 3-4 days). Utilizing the RNA interference (RNAi), Barchuk et al. (2008) demonstrated the involvement of the Ultraspiracle (Usp) gene in the regulatory mechanism governing the progression of pupal development in *Apis mellifera*. This conclusion is supported by the observed pupal developmental delay in honeybees with Usp gene knockdown. Elias-Neto et al. (2010) characterized the Amlac2 gene responsible for encoding laccase2 in *A. mellifera*. They found that Amlac2 is prominently expressed in the adult integument of pharate adults and is present before cuticle coloring and sclerotization intensify. When the Amlac2 gene was post-transcriptionally knocked down, structural defects in the exoskeleton significantly impacted adult eclosion, the process of emerging from the pupal case.

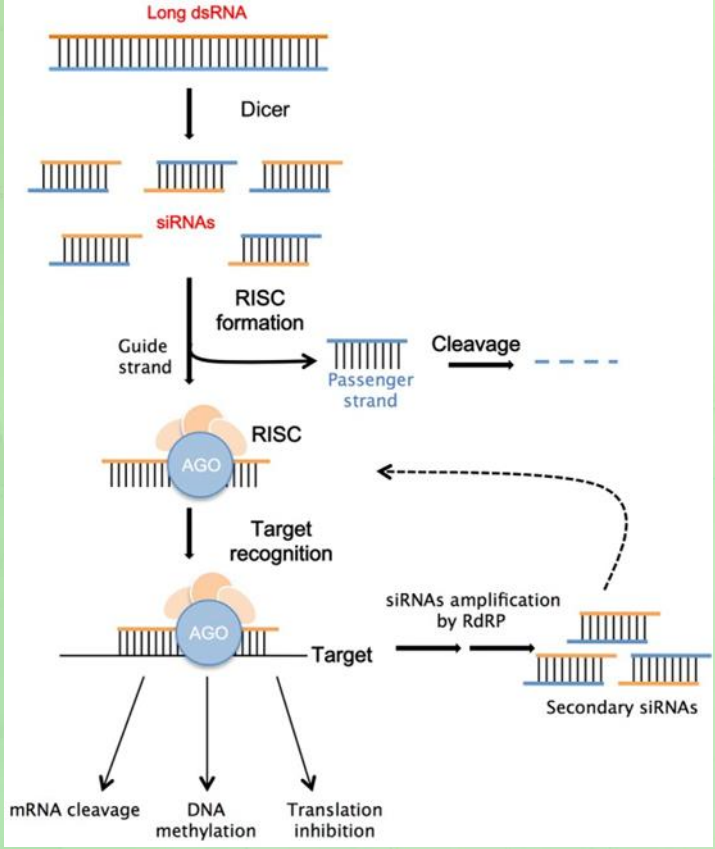


**Figure 1.** Mechanism of RNAi (Limera et al., 2017)

Understanding the interactions between honey bees and pathogens is crucial for mitigating the impact of diseases on bee colonies. RNAi has been instrumental in characterizing the mechanisms underlying honey bee-pathogen interactions. By targeting key genes in pathogens or the bee immune system, researchers can gain insights into disease susceptibility and immune responses. In their study, He et al. (2021) investigated the therapeutic potential of RNA interference (RNAi) for silencing two *Nosema ceranae* encoded spore wall protein (SWP) genes. The researchers found that oral ingestion of double-stranded RNAs (dsRNAs) corresponding to SWP8 and SWP12, either used separately or in combination, resulted in a substantial reduction in spore load, enhanced immunity, and extended the lifespan of *N. ceranae*-infected bees. Desai et al. (2012) aimed to reduce deformed wing virus (DWV) infection in honeybees (Apis mellifera) by feeding both first-instar larvae and adult bees with a specific double-stranded RNA (dsRNA) construct called DWV-dsRNA, which targets DWV in DWV-inoculated bees. The researchers mixed the DWV-dsRNA construct with the bees' food to deliver it. When the larvae were fed with DWV-dsRNA before being inoculated with the virus, it led to a reduction in DWV viral levels and a decrease in wing deformities compared to larvae fed with DWV or DWV mixed with green fluorescent protein-dsRNA.



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Surprising results have been obtained in recent years with the use of RNAi-mediated gene silencing in the struggle against Varroa and attracted great interest. In a pioneering study, Campbell et al. (2010) conducted the first-ever test of RNA interference (RNAi) feasibility in Varroa mites, targeting a mu-class glutathione S-transferase gene (VdGST-mu1). This research laid the foundation for exploring RNAi as a potential approach for controlling Varroa mite infestations, a major threat to honeybee colonies worldwide. Campbell et al. (2010) successfully achieved the suppression of the VdGST-mu1 gene in Varroa mites 48 hours after injection, and this gene suppression was observed to last for at least 72 hours. In their study, Campbell et al. (2010) also explored alternative methods for the transfer of dsRNA to Varroa mites, as they observed that the injection method led to high mortality rates. These researchers sought to find a less invasive and more practical approach to deliver dsRNA to the mites effectively. By investigating and comparing various delivery methods, they aimed to optimize the RNAi application and reduce any adverse effects on the mites' survival, thus advancing the potential use of RNAi as a safe and efficient method for Varroa mite control. When attempting to transfer dsRNA through topical application, Campbell et al. (2010) encountered difficulties due to the inability of dsRNA to penetrate the mites' cuticle effectively. However, the researchers found success with an alternative method by immersing the mites in a dsRNA solution. This immersion method proved to be effective in achieving gene silencing while causing the least damage to the mites during application. This significant finding demonstrates the importance of optimizing the delivery method in RNAi-based studies and highlights the potential of immersion as a practical and efficient approach for gene silencing in Varroa mites.

In a subsequent study conducted by Campbell et al. (2016), the researchers revealed that two neural genes, namely the B-type allatostatin gene and a crustacean hyperglycemic hormone (CHH)-like gene, could be targeted as potential candidates for mite control. By identifying and investigating these specific genes, the researchers aimed to explore their potential roles in mite physiology and develop new avenues for controlling Varroa mite infestations. This study further underscores the significance of RNAi as a valuable tool in understanding the molecular mechanisms involved in mite biology and its potential application for effective mite management strategies. They utilized the immersion method to avoid injection trauma while conducting double-stranded RNA interference (dsRNAi) experiments. By targeting the B-type allatostatin gene and a crustacean hyperglycemic hormone (CHH)-like gene through dsRNAi, they achieved significant knockdown effects on these neural genes in Varroa mites. The knockdown of the allatostatin gene resulted in an impressive 85% mortality rate among the mites, while the CHH-like gene knockdown led to a 55% mortality rate.

In their study aimed at investigating the potential use of RNAi to struggle Varroa mites, Huang et al. (2017) explored the effects of silencing various genes that play crucial roles in the survival and reproduction of Varroa mites. In the study conducted by Huang et al. (2017) using the micro-injection method, significant mortality was observed in Varroa mites when the Da and Pros26S genes were silenced. This result indicated that these genes played crucial roles in the survival and viability of the mites. Additionally, the researchers found that the silencing of RpL8, RpL11, and RpS13 genes did not impact the overall survival of Varroa mites. However, it was noted that the silencing of these genes resulted in a reduction in the number of offspring produced by the Varroa mites. This finding suggested that these genes were involved in regulating Varroa mite reproduction, and their manipulation through RNAi had an inhibitory effect on their reproductive capacity. The study's results shed light on specific genes that could be targeted to disrupt the life cycle and reproductive processes of Varroa mites, offering potential opportunities for the development of RNAi-based strategies for effective Varroa mite control in beekeeping practices.

RNA interference (RNAi) has emerged as a powerful tool in pest control strategies, with applications in various organisms, including insects and nematodes. One common approach is the transfer of RNAi molecules from genetically engineered plants to insects or nematodes that feed on these plants. This method involves the production of specific double-stranded RNAs (dsRNAs) in the plants, targeting essential genes in the pests. When the pests consume these plants, the ingested dsRNAs trigger the RNAi process, leading to the silencing of the targeted genes and eventually causing the pests' mortality or reduced reproductive capabilities (Steeves et al. 2006; Baum et al. 2007; Rechavi et al. 2011). The discovery that double-stranded RNA (dsRNA) can be systematically spread through ingestion in honey bees has sparked the idea that dsRNA can also be horizontally transmitted from honey bees to Varroa mites. This finding suggests the possibility of using bees as RNAi vectors, where bees can carry and deliver the dsRNA molecules to Varroa mites during their normal interactions (Hunter et al. 2010). The study conducted by Garbian et al. (2012) demonstrated that double-stranded RNA (dsRNA) ingested by honey bees can be efficiently transferred to Varroa mites and even to other bees that are subsequently parasitized by the mites. This dsRNA interchange between the bee and the Varroa mite resulted in the targeted silencing of vital genes in the mite, leading to a significant reduction of over 60% in the Varroa mite population. Furthermore, the researchers investigated the stability of dsRNA under hive conditions and found that it could remain viable in a sugar water solution for up to 6 days. This stability is crucial as it ensures the effectiveness of the dsRNA transfer method over a reasonable period. The study conducted by Cedeño et al. (2015) presented an innovative and cost-effective approach for dsRNA transfer into honey bee and Varroa mite tissues using bacteria. In this method, the

researchers engineered bacteria to express specific dsRNAs, eliminating the need for laborious RNA purification steps. The dsRNAs produced by the engineered bacteria were successfully delivered to both honey bee and Varroa mite tissues, offering an efficient in vivo alternative for gene silencing. They reported that this strategy could be used not only to control viral diseases and Varroa infestation in honey bees, but also for functional studies in bees by gene silencing.

## Conclusion

In conclusion, the applications of RNAi in honey bees have significantly broadened our comprehension of gene functions, interactions with pathogens, and prospective strategies for managing parasites. The ongoing research in this domain holds the potential to further enhance honey bee health and make valuable contributions to the sustainable management of bee populations, especially in the face of persistent challenges like colony collapse disorder and Varroa mite infestations.

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