We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

6,600
Open access books available

177,000
International authors and editors

195M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
RNAi in Agriculturally-Important Arthropods

Katherine Aronstein¹, Brenda Oppert² and Marcé D. Lorenzen³

¹Honey Bee Research Unit, USDA-ARS, Weslaco, TX 78596,
²USDA ARS Center for Grain and Animal Health Research, Manhattan, KS 66502, Raleigh, NC 27695
³Department of Entomology, North Carolina State University, USA

1. Introduction

This chapter was inspired by rapid developments in the field of RNA interference (RNAi), an evolutionarily-conserved cellular mechanism that directs protection against nucleic-acid invaders (e.g., viruses and repetitive DNA sequences including transposable elements) in plants, animals, protozoans and fungi. It is now evident that prokaryotes also possess an RNA-based defense system, though completely distinct from that in eukaryotes. The recent discovery of post-transcriptional gene silencing (PTGS) has generated tremendous interest in basic and applied research, including the development of in vitro and in vivo therapeutic approaches to reduce expression of disease-associated genes. This cutting-edge technology has already been successfully used in genomic manipulations of insect disease vectors, such as development of Dengue-resistant mosquitoes (Blair et al., 2006; Franz et al., 2006; Mathur et al., 2010).

Our aim in this chapter is to provide an overview of the profound knowledge accumulated in recent years from invertebrate RNAi studies, but with a focus on agriculturally important arthropods. We start with a brief discussion of the RNAi mechanism to introduce readers to key concepts that underlie the practical application of RNAi discussed in the remainder of the chapter. Our discussion will include a number of important issues that should be carefully considered when working with multi-cellular organisms, including bidirectional transport of silencing signals, processing of dsRNA, homology-driven mRNA degradation and subsequent gene silencing.

We will look at examples of gene silencing in different arthropod systems to illustrate commonalities and differences found in their silencing machinery. Arthropods are a diverse group of organisms, including ticks, mites, spiders, crabs, and insects, many of which are economically significant pests of agricultural crops, parasites of commercially managed pollinators and vectors of livestock diseases.

Considering the huge economic impact of honey bees on crop production, and particularly on pollination of specialty crops, a substantial part of this chapter will be dedicated to honey bee research. Recently, catastrophic losses of honey bee colonies drew national media attention and ignited a renewed interest in basic and applied bee research (Aronstein et al., 2006; Campbell et al., 2010; Dearden et al., 2009; Maori et al., 2009a, 2009b; Nelson et al., 2007). The latest achievements in basic honey bee research provide a better understanding of bee physiology and behavior. Analysis of gene function by selective gene silencing has been

www.intechopen.com
a powerful tool to dissect the complex mechanisms regulating biological processes involved in bee development, immunity, olfaction, learning and memory. Applied studies have focused on practical implementation of RNAi for control of honey bee diseases and parasites that cannot be achieved using conventional management techniques (Liu et al., 2010; Maori et al., 2009a, 2009b; Paldi et al., 2010). Examples will include: 1) development of RNAi-based control for Nosema ceranae, an intracellular parasite infecting adult bees, and 2) development of an antiviral treatment to protect bees against Israeli Acute Paralysis Virus (IAPV) and Chinese Sacbrood Virus (CSBV).

The success of RNAi technology as a control method for agricultural pests depends heavily on target specificity, gene-silencing efficiency and systemic spread of silencing. Originally, systemic RNAi was thought to be unique to plants and nematodes, however recent research revealed that systemic transfer of a silencing signal occurs in many arthropods. The first commercial use of RNAi in agriculture was demonstrated via oral RNAi in transgenic plants (in planta RNAi) toward several economically-important coleopteran pests, including the Western corn rootworm, Diabrotica virgifera virgifera and Colorado potato beetle, Leptinotarsa decemlineata (Baum et al., 2007). Oral RNAi has also been reported in Diptera, Hemiptera, Hymenoptera and Lepidoptera (Araujo et al., 2006; Aronstein et al., 2006; Lehane et al., 2008; Turner et al., 2006; Walshe et al., 2009). Our discussion will focus on the successful use of this strategy, as well as the challenges encountered by researchers seeking to use this sensitive, targeted approach to pest control.

We include an overview of the development of RNAi in the coleopteran model and stored product pest, the red flour beetle, Tribolium castaneum. The fact that the T. castaneum genome has been sequenced, coupled with the beetle’s ability to mount a robust, systemic RNAi response to injected dsRNAs, makes it an excellent model for RNAi. Of particular interest is the discovery of new pest control targets through a candidate gene approach to RNAi. While much can be learned from small-scale RNAi studies, we will also discuss a large-scale RNAI-based screen in this beetle.

The first high-throughput RNAi screens in insects were performed in D. melanogaster cell lines (Belles, 2010; Boutros & Ahringer, 2008; D’Ambrosio & Vale, 2010). Since then, genome-wide screens have revealed the function of genes involved in phenotype (physiology/morphology), neurobiology, signal transduction, ion transport, pathogen response, as well as metabolic and gene-processing pathways, among others (Mummery-Widmer et al., 2009). Currently efforts are underway to apply whole-genome RNAi screens in economically important arthropods, such as the cattle tick, Rhipicephalus microplus (Kurscheid et al., 2009) and T. castaneum (Angelini et al., 2009; Lynch et al., 2009). These and other RNAi-based studies will enable functional analysis of homologous genes in different arthropod species, as well as provide insight into the function of vertebrate homologs that could possibly aid in the identification and validation of drug targets.

2. RNAi mechanisms in arthropods

Like other organisms, arthropods, have evolved efficient homology-driven gene silencing mechanisms for protection against nucleic acid invaders (Dedouche et al., 2008; Gaines et al., 1996; Lu et al., 2004; Olson et al., 1996). Since its discovery, significant efforts have been made to unravel the molecular mechanisms of RNAi. While the core components of RNAi appear to be well conserved across phyla, molecular mechanisms underlying signal amplification and systemic spread of silencing are highly diverged. Improvements in
dsRNA design algorithms and delivery methods triggered an avalanche of new research projects in over 30 insect species (Orthoptera, Dictyoptera, Isoptera, Hemiptera, Coleoptera, Neuroptera, Hymenoptera, Lepidoptera, and Diptera). The most extensive work has been facilitated by sequenced genomes and performed in model insects, including *D. melanogaster*, *T. castaneum* and the silkworm, *Bombyx mori*. The use of RNAi in honey bee research has attracted enormous interest, driven by their economic importance and the sharp increase in colony losses in recent years. Below, we’ll discuss some of these issues in attempt to decipher the ambiguities in research findings that, in some cases, generate more questions than answers. Understanding the critical steps in the RNAi process will facilitate the transfer of this technology to additional arthropod species.

### 2.1 Core RNAi components

The post-transcriptional silencing of gene function is a very rapid process where double-stranded RNA (dsRNA) directs sequence-specific degradation of mRNA. In general, this complicated chain of reactions can be viewed as a two-step process. First, a long dsRNA is cleaved into small interfering RNAs (siRNA), and second, siRNAs are incorporated into silencing complexes (RISC). Following RISC assembly, siRNAs guide degradation of homologous mRNAs (Hammond et al., 2000).

The core components of the RNAi machinery have been thoroughly studied using a combination of biochemical, genetic, and bioinformatic approaches (Hamilton et al., 2002; Hammond et al., 2000; Rolff & Reynolds, 2009; Tomoyasu et al., 2008; Zamore et al., 2000). When dsRNA is introduced into insect cells, it is recognized by a dsRNA-specific RNase-III type ribonuclease called Dicer and cleaved into siRNAs that are 21–23 nt in length. Unlike most animals, insect genomes encode two Dicer-like proteins. One of them, Dcr-2 is involved in RNA interference in *Drosophila*, whereas Dcr-1 recognizes precursors of miRNAs. With the assistance of dsRNA-binding motif proteins (dsRBM), the next phase in the RNAi pathway involves the loading of siRNAs into RISCs. Most evidence indicates that RISCs contain only one siRNA strand, specifically the “guide ssRNA”. Therefore, careful selection of siRNA sequences that favor incorporation of the antisense strand into the RISC may improve efficacy and specificity of RNAi. Another essential member of the RISC complex is the RNase H enzyme Argonaute (AGO) that mediates recognition of the target mRNA. Using the siRNAs as a guide, AGO finds complimentary sequences and cleaves homologous mRNA, consequently leading to its degradation.

While most core RNAi components were characterized in *D. melanogaster*, many have been identified in other insects, nematodes and chelicerates. Recently analyzed genomes of the honey bee, red flour beetle, and silkworm (Consortium, 2008; Consortium, 2006; Richards & Consortium, 2008) indicate they each encode the core components of the RNAi machinery, including Dicer enzymes, Ago1 and 2, dsRBMs and other members of the cell-autonomous RNAi machinery.

### 2.2 Mechanism of dsRNA up-take

In the model nematode, *Caenorhabditis elegans*, systemic RNAi can be induced by dsRNA injection, ingestion or immersion. However, microinjection of dsRNA in *Drosophila* failed to induce systemic RNAi, resulting in the erroneous conclusion that RNAi is a cell-autonomous process in insects (Boutla et al., 2001; Kennerdell & Carthew, 1998, 2000; Roignant et al., 2003). Since then systemic gene silencing has been demonstrated in many
insect species (Amdam et al., 2003; Aronstein et al., 2006; Bucher et al., 2002; Hughes & Kaufman, 2000; Miller et al., 2008; Posnien et al., 2009). Injection, and in some cases ingestion of long dsRNA, produces very robust silencing effects both locally and in tissues distant from the site of introduction in arthropods, suggesting that the systemic nature of RNAi is conserved among the Arthropoda. This discovery promoted the use of RNAi technology in chelicerates and in a wider range of insect species, especially those for which transgenic protocols have not been developed. However, questions remain about the longevity of this effect in different arthropod species. Most studies report a time or stage when gene silencing was confirmed, but the effect was short-lived and did not necessarily coincide with the observed phenotypic change. In general, RNAi in arthropods is transient. However, in some cases the trans-developmental effects can persist long enough to be observed over several developmental stages (e.g., embryonic, larval and pupal) (Grossmann et al., 2009; Liu & Kaufman, 2004; Ronco et al., 2008; Tomoyasu & Denell, 2004). In the honey bee, injections of vitellogenin (Vg) dsRNA into newly emerged workers knocked-down Vg expression and dramatically affected the behavior of aged bees, causing a premature shift from nesting tasks to those of foraging (Amdam et al., 2003; Nelson et al., 2007). Injection or oral administration of Am18w dsRNA into 5-day-old bee larvae resulted in silencing of this Toll-like receptor transcript and produced significant morphological defects (Fig. 1) in both pupae and adults (Aronstein et al., 2006; Aronstein & Saldivar, 2005).

Fig. 1. RNAi-mediated silencing of the honey bee (Apis mellifera) Am18w encoding Toll-like receptor: 5-day-old-larvae were injected A) or fed/soaked B) with Am18w dsRNA as described by Aronstein and Saldivar (2005) and Aronstein et al. (2006). Silencing of Am18w resulted in severe morphological defects observed in pupae (C - E) and adult bees (F - G). The abnormalities were most evident in the thorax and head of pupae (C, red arrow), such as complete absence of or shortened appendages, as well as fused body segments.

In the Indian mealmoth, Plodia interpunctella, and T. castaneum, silencing of a gene encoding tryptophan oxygenase in embryos resulted in loss of eye-color pigmentation in 1st instar
larvae (Fabrick et al., 2004; Lorenzen et al., 2002). Interestingly, the loss-of-function phenotype persisted until pupation in the beetle, approximately four weeks, while the phenotypic changes were not tracked past the initial larval stage in the mealmoth. In addition to trans-developmental effects, trans-generational RNAi has also been observed in insects. For example, zygotic expression of multiple target genes has been silenced by injecting dsRNA into female pupae or adults (i.e., parental RNAi) in *T. castaneum*, a parasitic wasp, *Nasonia vitripennis*, crickets, *Gryllus bimaculatus* or milkweed bugs, *Oncopeltus fasciatus* (Bucher et al., 2002; Lynch & Desplan, 2006; Mito et al., 2011; Ronco et al., 2008; Tomoyasu et al., 2008; Werren & Loehlin, 2009). Recent *Drosophila* studies demonstrated that flies too have the ability to activate systemic RNAi, albeit in response to viral infection (Saleh et al., 2009).

The ability of RNAi signals to move cell-to-cell within the organism suggests the existence of molecular mechanisms for transporting signals bidirectionally across cell membranes, and from the site of introduction to distant tissues. Moreover, to support the long lasting effect of silencing, there may be an additional mechanism for RNAi amplification. It appears that arthropods can efficiently import and export silencing signals, although molecular mechanisms underlying these processes are still debated. Below we discuss recently proposed models explaining mechanisms of import and export of silencing signals in different arthropod systems.

### 2.2.1 Sid-dependent up-take

Organisms exhibit substantial differences in their ability to take up and distribute dsRNA. Some are capable of both up-take and systemic spread (Araujo et al., 2006; Dong & Friedrich, 2005; Saleh et al., 2006; Soares et al., 2005; Walshe et al., 2009), while others apparently readily take up dsRNA, but have significant difficulties with systemic distribution (Dietz et al., 2007; Roignant et al., 2003; Van Roessel et al., 2002). However, the inability of an organism to foster systemic effects of dsRNA could be due to shortcomings in methodology which may be mitigated by improved applications, such as extended hairpin RNA-based transgenic RNAi (Artymovich, 2009; Carthew, 2003; Kennerdell et al., 2002), as well as other improvements in RNAi protocols discussed in Section 4.

In nematodes, a two-step model has been proposed to explain the complex mechanism of large (~500 bp) dsRNA transport (Winston et al., 2007). According to this model, ingested dsRNA does not require Sid-1 (a systemic-interference-defective) to pass through the gut lumen in *C. elegans*. Instead, endocytosis of dsRNA by Sid-2, an intestinal luminal transmembrane protein, is proposed for the import of dsRNA across the midgut lining. Vesicles containing dsRNA bud off from the gut lumen and release their content into the body cavity, where they are taken up by cells in different tissues via Sid-1-assisted passive diffusion in a concentration dependent manner (Feinberg & Hunter, 2003; Jose & Hunter, 2007). Several other genes (*Rsd-2*, *Rsd-3* and *Rsd-6*) are involved in the systemic spread of dsRNA in *C. elegans*, which was previously attributed to endocytosis, suggesting that transport of dsRNA in *C. elegans* can also use a Sid-1-independent mechanism.

While some core processes remain common among different arthropod phyla, it appears that mechanisms of dsRNA up-take and transport are largely diverged. A single *sid-1* gene has been identified in most sequenced insect genomes, with the exception of three *sid-1* homologs found in *T. castaneum* and *B. mori* genomes (Aronstein et al., 2006; Aronstein & Saldivar, 2005; Huvenne & Smagghe, 2010; Tomoyasu et al., 2008). However, the correlation
between the presence of sid-1, or sid-1-like (sil), in insect genomes and systemic RNAi is uncertain (Tomoyasu et al., 2008). One reason for this uncertainty is the lack of one-to-one orthology between the sil genes and sid-1. It has been proposed that the three sil genes in T. castaneum are orthologous to C. elegans tag-130, rather than sid-1 (Tomoyasu et al., 2008). While tag-130 is not required for systemic RNAi in nematodes, it is possible that the sil genes in T. castaneum are orthologous to tag-130, but still function in systemic RNAi. On the other hand, it is also possible that the sil genes are orthologous to sid-1, but play no role in systemic RNAi. However, the most satisfying explanation to date is that the sil genes are functional sid-1 orthologs. This observation correlated with the fact that flour beetles and honey bees are proficient in systemic RNAi, and dipterans (which lack sil genes) are extremely poor. The existence of a Sid-2-mediated mechanism is also questionable, as sid-2 orthologs have not been found in any animal genomes other than C. elegans, but this may be due to the rapid evolution of sid-2 homologs.

2.2.2 Sid-independent transport
While substantial progress has been made towards understanding dsRNA up-take in nematodes, the molecular mechanisms for the transport of silencing signals in other arthropods are still mostly unknown. A new model explaining dsRNA entry and initiation of RNAi silencing in arthropods has been proposed (Saleh et al., 2006; Ulvila et al., 2006). According to this model, dsRNA up-take relies on receptor-mediated endocytosis followed by an active spread of the silencing signal by vesicle-mediated intracellular trafficking (Tomoyasu et al., 2008; Ulvila et al., 2006). The role of SR-CI and Eater in the up-take of dsRNA has been recently tested in Drosophila S2 cells, demonstrating that more than 90% of dsRNA up-take depends on these two receptors (Ulvila et al., 2006). However, silencing of 8 Toll and 19 scavenger receptors did not significantly affect the inhibition of RNAi, indicating that other members of pattern recognition receptors must be tested to evaluate the receptor-mediated endocytosis model (Saleh et al., 2006). Furthermore, systemic transport of endogenous silencing signals may be stimulated by specific physiological conditions, such as exposure to viruses. Saleh et al. (2009) demonstrated that receptor-mediated endocytosis is involved in an antiviral response in Drosophila. Nevertheless, specific mechanisms associated with dsRNA up-take and transport throughout the body in other insects continues to be the subject of ongoing research.

2.2.3 Mechanism of signal amplification
To sustain silencing, some organisms may employ a strategy for signal amplification. In C. elegans, primary siRNAs are amplified through an RNA-directed RNA polymerase (RdRP)-dependent mechanism leading to generation of secondary siRNAs and amplification of silencing (Hamilton et al., 2002). While RdRP activity has not been demonstrated in other organisms, molecular components of this amplification mechanism have recently been identified in the cattle tick genome (Kurscheid et al., 2009). Although insect genomes do not encode a canonical invertebrate RdRP homologue, there is a possibility that RdRP-like activity may occur via other enzymes (Lipardi & Paterson, 2009). The existence of the amplification mechanism in nematodes and possibly in the chelicerate arthropods provides an interesting perspective on the existence of alternative RNAi mechanisms in evolutionarily-diverged groups of animals.
3. Applied research projects using RNAi

Understanding the mechanisms involved in the different phases of gene silencing is absolutely essential for the development of effective RNAi-based applications to control pests of agricultural crops, vectors of livestock disease, and predators and parasites of beneficial insects. RNAi also holds considerable potential as a therapeutic approach to silence disease-causing genes in beneficial insects, particularly important in arthropods deficient in protein-based adaptive immune responses. The identification and utilization of these new approaches in different insect systems may provide more effective control applications. For example, receptor-mediated endocytotic machinery could offer a starting point for novel dsRNA delivery strategies. Below, we describe some of the most recent research findings that harness RNAi technology and hold promise for the development of a new class of therapeutic drugs and pest-control applications.

3.1 RNAi in beneficial arthropods and other non-pests

As honey bees are primary pollinators for most agricultural crops, substantial resources have been devoted to solving recent problems with honey bee health. Therefore, we begin our discussion of RNAi as it relates to agriculture with an overview of the success of the technology related to applications in honey bee research.

3.1.1 Disease control in Honey Bee colonies

The unprecedented loss of honey bee colonies over the past several years has endangered not only the honey bee industry, but also threatens to wipe out agricultural production of crops dependent on pollination. Since 2006, significant colony losses have been reported in many countries around the world, challenging the beekeeping industry to meet pollination demands. The worldwide economic value of the pollination service mainly provided by bees is estimated at $217 billion USD, particularly in added value of specialty crops such as nuts, berries, fruits, and vegetables (Gallai et al., 2009). Among numerous threats, honey bees face diverse parasites and pathogens, some of which are implicated in the massive colony losses, termed Colony Collapse Disorder (CCD) (Genersch, 2010). Microbial pathogens and parasites in the honey bee colonies are traditionally controlled by antimicrobial drugs and pesticides. Although necessary, these activities, often result in the over exposure of bees to synthetic chemicals that ultimately affect the bee’s health and behavior. Misuse of chemicals has also been suspected in some colony losses reported by beekeepers. Therefore, development and implementation of RNAi technology holds great potential for new non-toxic applications for disease control in bee hives. This approach has rapidly emerged as a genetic tool for combating microsporidial and viral diseases in bees. Some bee pathogens, such as Nosema ceranae (Higes et al., 2008; Higes et al., 2009) and Israeli Acute Paralysis Virus (IAPV) or a combination of the two (Bromenshenk et al., 2010), were recently implicated in world-wide losses of bee colonies. Analysis of the N. ceranae genome demonstrated the presence of RNA silencing machinery in this species, suggesting that RNAi can be exploited for control of infection within the host (Cormican et al., 2009). Indeed, in vivo experiments targeting expression of N. ceranae ADP/ATP transporter genes demonstrated inhibiting effects on Nosema development, as well as the level of pathogen in the host when fed dsRNA (Paldi et al., 2010). However, activity against Nosema in these experimental treatments declined sharply within two to three weeks post treatment.
RNAi has also emerged as an important antiviral defense in insects. Since most honey bee viruses are positive-stranded RNA viruses that generate dsRNA in the process of viral replication, they are particularly vulnerable to the insect’s silencing machinery. RNAi applications based on silencing the internal ribosome entry site (IRES) of IAPV were recently tested and showed great potential for developing a novel antiviral drug for use in bee colonies (Maori et al., 2009a, 2009b). One dsRNA product, “Remebee,” is currently being tested in a large-scale field trial (Hunter et al., 2010). This product is delivered to bees during routine feeding and is recommended for multiple applications in bee colonies, subject to FDA approval. To control another bee virus, Chinese Sacbrood Virus (CSBV), second instar *Apis cerana* larvae were fed dsRNA targeting VPI structural protein (Liu et al., 2010). Silencing of the target gene (VPI) was observed 12 h post feeding, but long-term effects of this treatment on the level of CSBV in *A. cerana* have not been evaluated.

We are currently assessing an RNAi-based approach for the control of sexual reproduction in the most prevalent bee fungal pathogen, *Ascosphaera apis* (Aronstein, unpublished). In this study, dsRNAs target newly identified *A. apis* mating type transcription factors (MAT-1 and MAT-2) (Aronstein et al., 2007). If successful, RNAi-based control methods could potentially fill the current void (i.e. no chemical treatments are available for the prevention and/or control of chalkbrood disease in bee colonies).

### 3.1.2 Silkworm and other beneficials

In *B. mori*, RNAi has been used primarily as a tool to investigate gene function at different developmental stages (Goldsmith et al., 2005). Specifically, RNAi has been used to delineate a number of physiological processes in silkworm, including cocoon pigmentation (Tabunoki et al., 2004), the sex pheromone biosynthetic pathway (Ohnishi et al., 2006), segmentation and appendage formation (Masumoto et al., 2009), and programmed cell death (Lee et al., 2009). Transgenic *B. mori* have been developed with a heat shock inducible and inheritable RNAi system to further probe gene function (Dai et al., 2007). RNAi is also being used to address problems in silkworm culture. For example, transgenic *B. mori* larvae expressing dsRNA targeting a baculovirus gene, *baculoviral immediate early-1* (*ie-1*), induced strong protection against infection by the *B. mori* nucleopolyhedrovirus (Kanginakudru et al., 2007).

Another beneficial, *Nasonia* wasps, primarily parasitize large pest flies, making these four closely related parasitoid species a useful tool for biocontrol. After the honey bee genome, *N. vitripennis* is only the second species of Hymenoptera with a sequenced genome (Rütten et al., 2004; Werren et al., 2010), and is rapidly emerging as a powerful model organism for functional studies. Rapid advances in *Nasonia* genomics have already helped to delineate basic mechanisms of embryonic development (Lynch & Desplan, 2010) and sex determination (Verhulst et al., 2010) in this species, and will help to further improve our understanding of the systemic nature (Werren & Loehlin, 2009) and trans-generational effects of gene silencing (Lynch & Desplan, 2006).

### 3.2 Using RNAi for pest control

RNAi applications have already demonstrated great success in silencing essential biological functions of many arthropod-pests (Price & Gatehouse, 2008). However, current *in vivo* applications will require substantial improvements in silencing efficiency, stabilization of dsRNAs and improved formulations to be implemented in the field.
3.2.1 Red flour beetle

The red flour beetle has been associated with human agriculture for at least four thousand years. It is a major pest of stored grains worldwide, causing millions of dollars in damage annually. However, *Tribolium* also has become one of the best understood model organisms in biology in the past few decades, and is the first beetle to have a sequenced genome (Richards & Consortium, 2008). As previously mentioned, injection of dsRNA elicits a robust systemic RNAi response in *T. castaneum*, and can be performed during any life stage (Fig. 2).

Fig. 2. RNAi-mediated gene silencing in the red flour beetle, *Tribolium castaneum*. RNAi can be performed at any life stage in flour beetles by injecting dsRNAs into A) embryos, B) larvae, C) pupae or D) adults. Injected dsRNAs are generally dyed to provide confirmation of injection (in this case a green dye was used). Effect of *T. castaneum* scarlet (*Tcst*) dsRNA on eye pigmentation. Last-instar larvae were injected with *Tcst* dsRNA and observed as adults; E) un.injected individual with wild-type eye color, F) wild-type individual that was injected with *Tcst* dsRNA during last larval stage. Note absence of eye pigmentation in the ommatidia (black “mascara” around eye is from a different pigment pathway). Effect of *T. castaneum* laccase-2 (*TcLac2*) dsRNA on body color. G) wild-type body color, H) “black” body color mutant, I) “black” mutant that was injected with *TcLac2* during last larval stage.

From its initial use in 1999 to phenocopy null mutations of the *T. castaneum* Deformed gene (Brown et al., 1999), to its wide use in functional genomic screens, RNAi has become an extremely valuable tool both to elucidate gene function and to identify potential pest control targets. Here we will discuss key *Tribolium*-based RNAi studies with significance to pest control.

Arthropods are known for their hard chitinous exoskeleton. The chitin/cuticle pathway that gives rise to this arthropod-specific structure has been the object of much interest due to its relevance as a pest control target. While researchers have studied this pathway for many
years, RNAi has revolutionized studies in this field. For example, a group in Manhattan, Kansas (USDA-ARS and Kansas State University) has been using RNAi to identify *T. castaneum* genes that encode proteins responsible for exoskeleton biosynthesis and degradation. Candidate genes from other insects have been selected and orthologs identified via blast analysis of the *T. castaneum* genome database, BeetleBase (http://beetlebase.org/). Prior to RNAi analysis, the spatiotemporal pattern of gene expression is determined via RT-PCR to ensure RNAi is performed at appropriate time points. Chitin/cuticle pathway genes, including those required for chitin synthesis (Arakane et al., 2005b; Arakane et al., 2008), molting, survival and fecundity (Arakane et al., 2010b; Arakane et al., 2009; Arakane et al., 2008; Broehan et al., 2010; Hogenkamp et al., 2007; Zhu et al., 2008) and tanning of the epidermal cuticle (Arakane et al., 2010a; Arakane et al., 2009; Arakane et al., 2005a) have been functionally characterized, revealing a wealth of potential biotargets for arthropod-specific pest control.

While the candidate gene approach has been of enormous value in *T. castaneum*, it imposes limitations due to reliance on sequence conservation. Therefore a genome-wide RNAi screen is underway (Lynch et al., 2009). This project, known as iBeetle, targets all genes (identification based on gene predictions, as well as expression data) at two life stages. Specifically, dsRNAs are injected into 5th-instar larvae (analysis of injected animal), as well as into female pupae (analysis of offspring). Alterations in cuticular phenotypes, fluorescently marked muscles, melanotic stink glands, metamorphosis control and fertility are being scored (G. Bucher, personal communication). Approximately 5,500 genes will be screened by fall 2011, with completion of the project scheduled for fall 2014. The genome-wide collection of PCR templates for dsRNA synthesis in the iBeetle-Library will be made available to the community (http://ibeetle.uni-goettingen.de/resources.html). This invaluable resource will facilitate subsequent RNAi screens for additional processes.

### 3.2.2 Plant-mediated RNAi in crop pests

Although a comprehensive review of RNAi in plants is beyond the scope of this chapter, it is important to highlight transgenic approaches for generating RNAi-based insect-resistant plants (Artymovich, 2009; Baum et al., 2007; Mao et al., 2007). RNAi applications have been effective in silencing target genes in some insects upon oral administration of dsRNA. Our examples detail how this approach has enhanced plant resistance to economically important agricultural pests, such as the cotton bollworm, *Helicoverpa armigera*, and Western corn rootworm (WCR), *D. v. virgifera*, in commercially produced crops (e.g., corn, cotton, and tobacco).

The key to successful *in planta* RNAi depends not only on the identification of suitable gene targets, but also on the expression and delivery of sufficient amounts of intact dsRNA for up-take by the insects. Examples of successful gene silencing by oral delivery have been described in insect species from different orders (Table 1). With few exceptions, most oral RNAi assays in insects have targeted mRNAs in the gut. The first report of oral RNAi in an insect was in a lepidopteran, the light brown apple moth, *Epirphias postvittana*, with temporary knockdown of a gut target, carboxylesterase 1, and an adult antennae target, pheromone binding protein 1 (Turner et al., 2006). Since then, additional reports of ingested dsRNA leading to knockdowns in Lepidoptera (Bautista et al., 2009; Mao et al., 2007; Whyard et al., 2009), Coleoptera (Baum et al., 2007; Whyard et al., 2009), Diptera (Walshe et al., 2009; Whyard et al., 2009), Hemiptera (Price & Gatehouse, 2008), and Isoptera (Zhou et al., 2008), suggest that knockdown of specific targets may be feasible in most insects.

www.intechopen.com
RNAi in Agriculturally-Important Arthropods

| Order   | Insect                        | Gene Target                                      | LC50          | Percent Mortality | Reference     |
|---------|-------------------------------|--------------------------------------------------|---------------|-------------------|---------------|
| Coleoptera | Diabrotica virgifera virgifera | subunits of vacuolar ATPase and others | 2.5 ng/cm² | 80-95             | Baum et al., 2007 |
| Coleoptera | Diabrotica undecimpunctata howardii | subunits of vacuolar ATPase | 780 ng/cm² | 45                | Baum et al., 2007 |
| Coleoptera | Leptinotarsa decemlineata | subunits of vacuolar ATPase | 52 ng/cm² | 90                | Baum et al., 2007 |
| Coleoptera | Tribolium castaneum | vacuolar ATPase E | 0.003 mg/g diet | 70 | W hyard et al., 2009 |
| Diptera | Drosophila spp. | vacuolar ATPase E and tubulin γ | 0.2-0.6 mg/ml | 40-70             | Whyard et al., 2009 |
| Diptera | Glossina morsitans morsitans | I tsese-EP, a major midgut protein | 435 ng/µl | 40 | W alshe et al., 2009 |
| Hemiptera | Acrithosiphon Pism | vacuolar ATPase E | 0.003 mg/g diet | 60 | Whyard et al., 2009 |
| Hemiptera | Rhodnius prolixus | nitroporin 2 | 1 µg/µl | not reported | Price & Gatehouse, 2008 |
| Isoptera | Reticulitermes flavipes | cellulase hexamerin² | 15.3 µg/cm² | 75 | Zhou et al., 2008 |
| Lepidoptera | Epiphyas postvittana | carboxylesterase 1 pheromone binding protein 1 | 1 µg | not reported | Turner et al., 2006 |
| Lepidoptera | Helicoverpa armigera | cytochrome P450 (CYP6AE14) | 3 mg/g diet | not reported | Mao et al., 2007 |
| Lepidoptera | Manduca sexta | vacuolar ATPase E | 0.01 mg/g diet | 50 | Whyard et al., 2009 |
| Lepidoptera | Plutella xylostella | cytochrome P450 (CYP6BG1) | 662-824 µg/µl | 90 | Bautista et al., 2009 |

1 Values are the maximum observed mortality and are approximate (exact values were not given).
2 Administered in combination with juvenile hormone.
3 Decrease in resistance to permethrin was 1-2.6-fold in dsRNA fed larvae compared to control.

Table 1. Reports of oral delivery of dsRNA that reduce the levels of target RNA in insects.

RNAi directed against a number of gene targets (e.g., β-tubulin, v-ATPase A and v-ATPase E) was effective in several economically important coleopteran pests (Baum et al., 2007). Oral RNAi targeting v-ATPase subunits and others resulted in significant larval mortality in perhaps the most economically important pest in the U.S., *D. v. virgifera*. It was further demonstrated that *D. v. virgifera* is highly sensitive to knockdown of a gene encoding the delta subunit of the coatamer complex (COPI) that is involved in intracellular protein trafficking. Similar results were obtained with orthologs for v-ATPase in Southern corn rootworm, *D. undecimpunctata howardii*, and Colorado potato beetle, *Leptinotarsa decemlineata*, but not in the cotton boll weevil, *Anthonomus grandis*. However, increased sensitivity to the cotton metabolite gossypol was reported in a lepidopteran, the cotton bollworm, *Helicoverpa armigera*, with a silenced cytochrome P450 gene, CYP6AE14 (Mao et al., 2007).

Accordingly, transgenic plants are being engineered to express a variety of dsRNAs to silence important biological functions in insects (Artymovich, 2009). Transgenic corn
expressing a v-ATPase subunit A hairpin RNA demonstrated significant protection to damage by WCR larvae (Baum et al., 2007), while cotton engineered to express dsRNA targeting cytochrome P450 CYP6AE14 caused knockdown of the transcript in the H. armigera gut and reduced larval growth (Mao et al., 2007). Thus far, initial laboratory tests of transgenic RNAi approaches to plant protection appear to be as successful as those based on the expression of insecticidal toxins against coleopteran and lepidopteran herbivores. However, further development and refinement of this technology, as well as large-scale field tests will likely be required to know the true potential of in planta RNAi.

3.2.3 Chelicerates (ticks, mites, spiders)

Double-stranded RNA has been successfully delivered to ticks via injection (various developmental stages and tissues), infection (viral vector), ingestion (oral), and incubation (whole body soaking) (de la Fuente et al., 2007). Oral RNAi was used to knockdown an anticomplement gene (isac) in the blacklegged tick, Ixodes scapularis. Affected nymphs weighed less than those fed a control dsRNA (lacZ) and had lower spirochete loads when infected with Borrelia burgdorferi (Soares et al., 2005). Another medically relevant study demonstrated that dsRNA can be transmitted through the blood-brain barrier in I. scapularis (Karim et al., 2008). Cy3-labeled dsRNA specific for either β-actin or Na+-K+-ATPase was injected into the abdomen of unfed adult females, and β-actin protein or Na+-K+-ATPase activity was measured after supplying a partial blood meal. Labeled dsRNAs were detected in the synganglia (CNS), and effective knockdown was confirmed via tissue-specific RT-PCR.

R. microplus is an economically significant tick ectoparasite that transmits a variety of pathogens, such as Anaplasma marginale, thereby increasing cattle exposure to vector-borne infectious diseases. Silencing of a defensin gene, varisin, by injection of dsRNA into male ticks reduced their ability to infect calves with A. marginale (Kocan et al., 2008). Additional RNAi targets, such as those affecting tick mortality and fecundity, have been identified through RNAi screens in R. microplus (Kurscheid et al., 2009).

Parental RNAi was used to knock-down expression of a homeobox gene, Distal-less (Dll), in the two-spotted spider mite, Tetranychus urticae (Khila & Grbic, 2007). Injection of either Tu-Dll-specific dsRNA or siRNA into adult female mites resulted in offspring with truncated and fused leg segments. This experiment demonstrated the conserved nature of Dll function and illustrates the power of RNAi in mites.

Efforts to use RNAi to control a major mite parasite of the honey bee are underway. The gene encoding glutathione S-transferase, involved in detoxification of pesticides used to control mites in the hive, was targeted successfully in Varroa destructor with dsRNA (Campbell et al., 2010). Two different dsRNA delivery methods were evaluated, microinjection and soaking mites in dsRNA solution. Although injection of dsRNA produced up to 96% silencing of the target (VdGST-mu1) gene for over 72 h, there were problems with high mortality in controls. However, encouraging results were obtained by soaking mites in a solution of dsRNA in 0.9% NaCl. The method enables high-throughput screening to identify the best targets for control of this devastating pest of honey bees, even though the exact mechanism of dsRNA up-take is unknown.

3.2.4 Improvements to other insect control methods

Current insect control methods benefit from RNAi studies. For example, the symbionts Heterorhabditis bacteriophora (a nematode) and, Photorhabdus luminescens (a bacterium) are a
lethal combination in some insects. Using genes described in C. elegans as a model, various phenotypes were disrupted in H. bacteriophora soaked in dsRNA (Ciche & Sternberg, 2007). These studies facilitate the discovery of genes involved in symbiosis and/or insect pathogenesis.

RNAi has also been used to explore host-pathogen interactions in mosquitoes. One study explored the role of an antibacterial peptide encoded by a defensin family member (DEF) in Anopheles gambiae (Blandin et al., 2002). Targeting this A. gambiae defensin with gene-specific dsRNA resulted in DEF knock down (up to 12 days) and increased the mosquito’s vulnerability to Gram-positive bacteria (Blandin et al., 2002). However, DEF knock down had no effect on the malaria parasite, Plasmodium berghei. To identify genes involved in mosquito immunity to P. berghei, researchers (Michel et al., 2005; Osta et al., 2004) targeted a number of candidate genes in A. gambiae. They discovered that reduction of transcripts encoding a type-C lectin, a leucine-rich protein or a serpin (SRPN2) were sufficient to make the mosquito refractory to infection by P. berghei in the midgut. Such studies may inspire the development of new methods to control protozoan infections in humans (Lehane et al., 2008; Solis et al., 2009; Walshe et al., 2009).

Researchers are also using RNAi to create mosquitoes that are resistant to dengue fever. Franz and colleagues made transgenic A. aegypti that express an inverted-repeat specific for the dengue type 2 (DEN-2) virus (Franz et al., 2006 ). Careful promoter selection resulted in transgenic A. aegypti that mount an RNAi response to DEN-2 virus in midgut epithelial cells immediately after a bloodmeal. Moreover, the authors demonstrated that after viral infection, transgenic mosquitoes expressing the hairpin RNA had reduced viral loads and DEN-2 virus-derived siRNAs in the midgut compared to control insects.

In agriculture, RNAi applications are being developed as control strategies for the Asian citrus psyllid which vectors Citrus Greening disease (Hunter et al., 2008), the Colorado potato beetle (Zhu et al., 2011), and Varroa mites as described above in section 3.2.3. Results such as these bode well for the future of RNAi in pest control strategies.

4. Future studies for practical implementation of RNAi technology

The success of RNAi depends both on the biology of the organism, and the method of dsRNA delivery. Intrinsic factors, such as the efficiency of dsRNA up-take, relative turnover of target mRNA/protein, signal amplification and systemic spread are important for robust RNAi. Extrinsic factors amenable to manipulation include dsRNA concentration, target gene selection, transcript localization, dsRNA synthesis and route of introduction. One of the most important considerations is determining if the observed phenotype is gene-specific or an “off target” effect. Another critical requirement is the stability and intracellular availability of the RNAi signal. Often such requirements can be met by improved delivery systems, such as stable, transgenic expression of hairpin RNAs (Kennerdell & Carthew, 2000; Tavernarakis et al., 2000), or alternatively, by expression of dsRNA in non-pathogenic bacteria that can be fed directly to the target organism, as demonstrated in nematodes (Timmons & Fire, 1998) and arthropods (Tian et al., 2009; Zhu et al., 2011).

Injection of dsRNA can elicit a stress response in some insects and potentially compromise the study. Moreover, mechanical damage to the cuticle and underlying tissue can stimulate innate immunity (Aronstein et al., 2006; Aronstein & Saldivar, 2005; Brey et al., 1993; Han et al., 1999), complicating interpretation of gene expression data. While appropriate controls
(buffer and/or “control” dsRNA) can address some of these issues, alternative approaches for non-viral and virus-mediated dsRNA delivery have demonstrated great potential (Leng et al., 2009; Yuan et al., 2011). Direct delivery of dsRNA (soaking and/or feeding) is particularly popular in arthropod studies and appears to be feasible in at least some hemipteran, coleopteran, lepidopteran, and hymenopteran insects (Araujo et al., 2006; Aronstein et al., 2006; Baum et al., 2007; Eaton et al., 2002; Mao et al., 2007; Turner et al., 2006). However, most currently used methods reveal significant impediments preventing their application in large-scale in vivo trials.

To increase the efficiency of RNAi, current and future studies are directed at improving existing methodologies and adapting innovative technologies. A new bacterially-expressed dsRNA delivery technology, TransKingdom RNAi (tkRNAi) (Keates et al., 2008; Tian et al., 2009; Xiang et al., 2006; Xiang et al., 2009), recently emerged as a powerful tool for the control of parasites and disease agents in mammalian systems. This method utilizes attenuated, non-pathogenic bacteria that are safe, effective, and inexpensive vectors for delivering RNAi to target cells. Similar methods have been used successfully in nematodes (Newmark et al., 2003; Timmons & Fire, 1998), trophozoites (Entamoeba histolytica) (Solis et al., 2009) and other organisms (Keates et al., 2008; Nguyen & Fruehauf, 2008). Bacteria-based RNAi is technically suitable for production of large quantities of dsRNA, and therefore opens interesting perspectives for mass screening of novel gene targets and development of environmentally-safe pest control applications.

The application of RNAi to pest control, while still at a formative stage, is already taking different forms; 1) in planta dsRNA expression for the direct protection of crops; 2) bacteria-based dsRNA expression for the indirect control of parasites and pathogens of beneficial organisms; and 3) in vivo dsRNA expression for generating disease refractoriness in arthropod vectors of disease. Unlike in planta and bacteria-based RNAi, the use of RNAi for the spread of disease refractoriness requires genetic drive to push inheritance of the effector gene (i.e. hairpin RNA) through target populations.

Among the most promising gene-drive candidates is a synthetic “Medea” element (Chen et al., 2007) which is based on the genetic principles observed in the Maternal-Effect Dominant Embryonic Arrest (Medea) factors found in Tribolium (Beeman et al., 1992; Lorenzen et al., 2008). Heterozygous (M/+*) females transmit dominant-lethal activity to hatchlings by maternal action, but the lethal effect is manifested only in those progeny that fail to inherit an M allele from either parent. Thus, each M allele is bifunctional, encoding both a maternally loaded “poison” and a zygotically expressed “antidote”. Current and future efforts need to focus on both the development of improved RNAi effector genes, as well as on improved methods for driving population replacement if the goal of controlling arthropod vectors of disease by in vivo dsRNA expression is to be realized.

5. Acknowledgment

We thank Eduardo Saldivar (USDA-ARS; Weslaco, TX), William Klobasa (North Carolina State University, Raleigh, NC) and Yasuyuki Arakane (Chonnam National University, Korea) for providing photographs of honey bee microinjection, Tribolium microinjection and laccase2 RNAi phenotypes, respectively. We are also grateful to Gregor Bucher for providing information about the iBeetle project.
6. Disclaimer

Mention of trade names or commercial products in this (article) (publication) is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

USDA is an equal opportunity provider and employer.

7. References

Amdam, G.V.; Simoes, Z.L.; Guidugli, K.R.; Norberg, K. & Omholt, S.W. (2003). Disruption of vitellogenin gene function in adult honeybees by intra-abdominal injection of double-stranded RNA. *BMC biotechnology*, Vol. 3, No. Journal Article, pp. 1 ISSN 1472-6750; 1472-6750

Angelini, D.R.; Kikuchi, M. & Jockusch, E.L. (2009). Genetic patterning in the adult capitate antenna of the beetle *Tribolium castaneum*. *Developmental biology*, Vol. 327, pp. 240-251

Arakane, Y.; Muthukrishnan, S.; Beeman, R.W.; Kanost, M.R. & Kramer, K.J. (2005a). *Laccase* 2 is the phenoloxidase gene required for beetle cuticle tanning. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 102, pp. 11337-11342

Arakane, Y.; Specht, C.A.; Kramer, K.J.; Muthukrishnan, S. & Beeman, R.W. (2008). Chitin synthases are required for survival, fecundity and egg hatch in the red flour beetle, *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology*, Vol. 38, No. 10, pp. 959-962 ISSN 0965-1748; 0965-1748

Arakane, Y.; Lomakin, J.; Beeman, R.W.; Muthukrishnan, S.; Gehrke, S.H.; Kanost, M.R. & Kramer, K.J. (2009). Molecular and functional analyses of amino acid decarboxylases involved in cuticle tanning in *Tribolium castaneum*. *Journal of Biological Chemistry*, Vol. 284, No. 24, (2009 Jun 12), pp. 16584-16594

Arakane, Y.; Dittmer, N.T.; Tomoyasu, Y.; Kramer, K.J.; Muthukrishnan, S.; Beeman, R.W. & Kanost, M.R. (2010b). Identification, mRNA expression and functional analysis of several yellow family genes in *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology*, Vol. 40, No. 3, (2010 Mar), pp. 259-266

Arakane, Y.; Muthukrishnan, S.; Kramer, K.J.; Specht, C.A.; Tomoyasu, Y.; Lorenzen, M.D.; Kanost, M. & Beeman, R.W. (2005b). The *Tribolium chitin synthase* genes *TcCHS1* and *TcCHS2* are specialized for synthesis of epidermal cuticle and midgut peritrophic matrix. *Insect molecular biology*, Vol. 14, No. 5, pp. 453-463 ISSN 0962-1075; 0962-1075

Arakane, Y.; Baguinon, M.C.; Jasrapuria, S.; Chaudhari, S.; Doyungan, A.; Kramer, K.J.; Muthukrishnan, S. & Beeman, R.W. (2010a). Both UDP N-acetylglucosamine pyrophosphorylases of *Tribolium castaneum* are critical for molting, survival and fecundity. *Insect Biochemistry and Molecular Biology*, Vol. 41, No. 1, (2011 Jan), pp. 42-50

Arakane, Y.; Dixit, R.; Begum, K.; Park, Y.; Specht, C.A.; Merzendorfer, H.; Kramer, K.J.; Muthukrishnan, S. & Beeman, R.W. (2009). Analysis of functions of the chitin deacetylase gene family in *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology*, Vol. 39, No. 5-6, (2009 May-Jun), pp. 355-365
Araujo, R.N.; Santos, A.; Pinto, F.S.; Contijo, N.F.; Lehane, M.J. & Pereira, M.H. (2006). RNA interference of the salivary gland nitrophorin 2 in the triatomine bug Rhodnius prolixus (Hemiptera: Reduviidae) by dsRNA ingestion or injection. *Insect Biochemistry and Molecular Biology*, Vol. 36, No. 9, pp. 683-693 ISSN 0965-1748; 0965-1748

Aronstein, K. & Saldivar, E. (2005). Characterization of a honey bee Toll related receptor gene Am18w and its potential involvement in antimicrobial immune defense. *Apidologie*, Vol. 36, pp. 3-14

Aronstein, K.; Pankew, T. & Saldivar, E. (2006). SID-1 is implicated in systemic gene silencing in the honey bee. *Journal of Apicultural Research*, Vol. 45, No. 1, pp. 20-24.

Aronstein, K.A.; Murray, K.D.; de Leon, J.; Qin, X. & Weinstock, G. (2007). High mobility group (HMG-box) genes in the honey bee fungal pathogen Ascosphaera apis. *Mycologia*, Vol. 99, No. 4, pp. 553-561

Artymovich, K.A. (2009). Using RNA interference to increase crop yield and decrease pest damage. *MMG 445 Basic Biotechnology eJournal*, Vol. 5, pp. 7-12

Baum, J.A.; Bogaert, T.; Clinton, W.; Heck, G.R.; Feldmann, P.; Ilagan, O.; Johnson, S.; Plaetinck, G.; Munyikwa, T.; Pleau, M.; Vaughn, T. & Roberts, J. (2007). Control of coleopteran insect pests through RNA interference. *Nature biotechnology*, Vol. 25, No. 11, pp. 1322-1326 ISSN 1087-0156; 1087-0156

Bautista, M.A.; Miyata, T.; Miura, K. & Tanaka, T. (2009). RNA interference-mediated knockdown of a cytochrome P450, CYP6RG1, from the diamondback moth, Plutella xylostella, reduces larval resistance to permethrin. *Insect Biochemistry and Molecular Biology*, Vol. 39, No. 1, pp. 38-46 ISSN 1879-0240; 0965-1748

Beeman, R.W.; Friesen, K.S. & Denell, R.E. (1992). Maternal-effect selfish genes in flour beetles. *Science*, Vol. 256, pp. 89-92

Bellés, X. (2010). Beyond *Drosophila*: RNAi in vivo and functional genomics in insects. *The Annual Review of Entomology*, Vol. 55, pp. 111-128

Blair, C.D.; Sanchez-Vargas, I.; Franz, A.W.E. & Olson, K.E. (2006). Rendering Mosquitoes Resistant to Arboviruses through RNA Interference. *Microbe*, Vol. 1, No. 10, pp. 466-470

Blandin, S.; Moita, L.F. & Kocher, T. (2002). Reverse genetics in the mosquito *Anopheles gambiae*: targeted disruption of the Defensin gene. *EMBO Reports*, Vol. 3 No. 9, pp. 852-856

Boutla, A.; Delidakis, C.; Livadaras, I.; Tsagris, M. & Tabler, M. (2001). Short 59-phosphorylated double-stranded RNAs induce RNA interference in *Drosophila*. *Current Biology*, Vol. 11, pp. 1776-1780

Boutros, M. & Ahringer, J. (2008). The art and design of genetic screens: RNA interference. *Nature Reviews Genetics*, Vol. 9, pp. 554-566

Brey, P.T.; Lee, W.-J.; Yamakawa, M.; Koizumi, Y.; Perrot, S.; Francois, M. & Ashida, M. (1993). Role of the integument in insect immunity: Epicuticular abrasion and induction of cecropin synthesis in cuticular epithelial cells. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 90, pp. 6275-6279

Broehan, G.; Arakane, Y.; Beeman, R.W.; Kramer, K.J.; Muthukrishnan, S. & Merzendorfer, H. (2010). Chymotrypsin-like peptidases from *Tribolium castaneum*: a role in molting
revealed by RNA interference. *Insect Biochemistry and Molecular Biology*, Vol. 40, No. 3, pp. 274-283 ISSN 1879-0240; 0965-1748

Bromenshenk, J.J.; Henderson, C.B.; Wick, C.H.; Stanford, M.F.; Zulich, A.W.; Jabbour, R.E.; Deshpande, S.V.; McCubbin, P.E.; Seccomb, R.A.; Welch, P.M.; Williams, T.; Firth, D.R.; Skowronsak, E.; Lehmann, M.M.; Bilimoria, S.L.; Gress, J.; Wanner, K.W. & Cramer Jr, R.A. (2010). Iridovirus and Microsporidian Linked to Honey Bee Colony Decline. *PLoS One*, Vol. 5, No. 10, pp. 1-11

Brown, S.J.; Mahaffey, J.P.; Lorenzen, M.D.; Denell, R.E. & Mahaffey, J.W. (1999). Using RNAi to investigate orthologous homeotic gene function during development of distantly related insects. *Evolution & Development*, Vol. 1, pp. 11-15

Bucher, G.; Scholten, J. & Klingler, M. (2002). Parental RNAi in Tribolium (Coleoptera). *Current biology* : CB, Vol. 12, No. 3, pp. R85-86 ISSN 0960-9822; 0960-9822

Campbell, E.M.; Budge, G.E. & Bowman, A.S. (2010). Gene-knockdown in the honey bee mite *Varroa destructor* by a non-invasive approach: studies on a glutathione S-transferase. *Parasites & Vectors*, Vol. 3, pp. 73

Carthew, R.W. (2003). RNAi Applications in *Drosophila melanogaster*, in *RNAi: a guide to gene silencing*, H. Gregory, (Ed) Cold Spring Harbor Press, Cold Spring Harbor

Chen, C.H.; Huang, H.; Ward, C.M.; Su, J.T.; Schaefver, L.V.; Guo, M. & Hay, B. (2007). A synthetic maternal-effect selfish genetic element drives population replacement in *Drosophila*. *Science*, Vol. 316, pp. 597-600

Ciche, T.A. & Sternberg, P.W. (2007). Postembryonic RNAi in *Heterorhabditis bacteriophora*: a nematode insect parasite and host for insect pathogenic symbionts. *BMC developmental biology*, Vol. 7, No. Journal Article, pp. 101 ISSN 1471-213X; 1471-213X

Consortium, I.S.G. (2008). The genome of a lepidopteran model insect, the silkworm *Bombyx mori*. *Insect Biochemistry and Molecular Biology*, Vol. 38, No. 12, pp. 1036-1045

Consortium, T.H.G.S. (2006). Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature*, Vol. 443, pp. 931-949

Cornman, R.S.; Chen, Y.P.; Schatz, M.C.; Street, C.; Zhao, Y.; Desany, B.; Egholm, M.; Hutchinson, S.; Pettis, J.S.; Lipkin, W.I. & Evans, J.D. (2009 ). Genomic Analyses of the Microsporidian *Nosema ceranae*, an Emergent Pathogen of Honey Bees. *PLoS pathogens*, Vol. 5, No. 6, (June 2009), pp. 1-14

D’Ambrosio, M.V. & Vale, R.D. (2010). A whole genome RNAi screen of *Drosophila* S2 cell spreading performed using automated computational image analysis. *The Journal of Cell Biology*, Vol. 191 No. 3, pp. 471-478

Dai, H.; Jiang, R.; Wang, J.; Xu, G.; Cao, M.; Wang, Z. & Fei, J. (2007). Development of a heat shock inducible and inheritable RNAi system in silkworm. *Biomolecular Engineering*, Vol. 24, No. 6, pp. 625-630

de la Fuente, J.; Kocan, K.M.; Almazan, C. & Blouin, E.F. (2007). RNA interference for the study and genetic manipulation of ticks. . *Trends in Parasitology*, Vol. 23, pp. 427-433

Dearden, P.K.; Duncan, E.J. & Wilson, M.J. (2009). RNA interference (RNAi) in honeybee (*Apis mellifera*) embryos. *Cold Spring Harbor protocols*, Vol. 2009, No. 6, pp. pdb.prot5228 ISSN 1559-6095

Dedouche, S.; Matt, N.; Budd, A.; Mueller, S.; Kemp, C.; Galiana-Arnoux, D.; Dostert, C.; Antoniewski, C.; Hoffmann, J.A. & Imler, J.-L. (2008). The DElD/H-box helicase
Dicer-2 mediates the induction of antiviral activity in Drosophila. *Nature Immunology*, Vol. 9, pp. 1425-1432

Dietz, G.; Chen, D.; Schnorrer, F.; Su, K.-C.; Barinova, Y.; Fellner, M.; Gasser, B.; Kinsey, K.; Oppel, S.; Scheiblauer, S.; Couto, A.; Marra, V.; Keleman, K. & Dickson, B.J. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature*, Vol. 448, pp. 151-156

Dong, Y. & Friedrich, M. (2005). Nymphal RNAi: systemic RNAi mediated gene knockdown in juvenile grasshoppers. *BMC biotechnology*, Vol. 5, No. 25, pp. 1-7

Eaton, B.A.; Fetter, R.D. & Davis, G.W. (2002). Dynactin is necessary for synapse stabilization. *Neuron*, Vol. 34, pp. 729-741

Fabrick, J.A.; Kanost, M.R. & Baker, J.E. (2004). RNAi-induced silencing of embryonic tryptophan oxygenase in the Pyralid moth, *Plodia interpunctella*. *Journal of Insect Science*, Vol. 4, No. 15, pp. 9

Feinberg, E.H. & Hunter, C.P. (2003). Transport of dsRNA into cells by the transmembrane protein SID-1. *Science*, Vol. 301 No. 5639, pp. 1545-1547

Franz, A.W.E.; Sanchez-Vargas, I.; Adelman, Z.N.; Blair, C.D.; Beaty, B.J.; James, A.A. & Olson, K.E. (2006). Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 103, No. 11, (Mar 14), pp. 4198-4203

Gaines, P.J.; Olson, K.E.; Higgs, S.; Powers, A.M.; Beaty, B.J. & Blair, C.D. (1996). Pathogen-derived resistance to dengue type 2 virus in mosquito cells by expression of the premembrane coding region of the viral genome. *Journal of virology*, Vol. 70, pp. 2132-2137

Gallai, N.; Salles, J.-M.; Settele, J. & Vaissière, B.E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological economics*, Vol. 68, pp. 810-821

Genersch, E. (2010). Honey bee pathology: current threats to honey bees and beekeeping. *Mini-Review Applied Microbiology and Biotechnology*, Vol. 87, No. 1, pp. 87-97

Goldsmith, M.R.; Shimada, T. & Abe, H. (2005). The genetics and genomics of the silkworm, *Bombyx mori*. *Annual Review of Entomology*, Vol. 50, pp. 71-100

Grossmann, D.; Scholten, J. & Prpic, N.M. (2009). Separable functions of wingless in distal and ventral patterning of the *Tribolium* leg. *Development genes and evolution*, Vol. 219, pp. 469-479

Hamilton, A.; Voinnet, O.; Chappell, L. & Baulcombe, D. (2002). Two classes of short interfering RNA in RNA silencing. *The EMBO Journal*, Vol. 21, No. 17, (September 2002), pp. 4671-4679

Hammond, S.M.; Bernstein, E.; Beach, D. & Hannon, G.J. (2000). An RNA-directed nuclease mediates posttranscriptional gene silencing in *Drosophila* cells. *Nature*, Vol. 404, pp. 293-296

Han, Y.S.; Chun, J.S.; Schwartz, A.; Nelson, S. & Paskewitz, S.M. (1999). Induction of mosquito hemolymph proteins in response to immune challenge and wounding. *Developmental & Comparative Immunology*, Vol. 23, pp. 553-562

Higes, M.; Martin-Hernandez, R.; Garrido-Bailon, E.; Gonzalez-Porto, A.V.; Garcia-Palencia, P.; Meana, A.; Nozal, M.J.d.; Mayo, R. & Bernal, J.L. (2009). Honeybee colony
collapse due to *Nosema ceranae* in professional apiaries. *Environmental Microbiology Reports*, Vol. 1, No. 2, pp. 110-113

Higes, M.; Martín-Hernández, R.; Botías, C.; Bailón, E.G.; González-Porto, A.V.; Barrios, L.; Nozal, M.J.d.; Bernal, J.L.; Jiménez, J.J.; Palencia, P.G. & Mañes, A.M. (2008). How natural infection by *Nosema ceranae* causes honeybee colony collapse. *Environmental microbiology*, Vol. 10, No. 10, pp. 2659-2669

Hogenkamp, D.; Arakane, Y.; Kramer, K.J.; Muthukrishnan, S. & Beeman, R.W. (2007). Characterization and expression of the β-N-acetylhexosaminidase gene family of *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology*, Vol. 38, No. 4, (2008 Apr), pp. 478-489

Hughes, C.L. & Kaufman, T.C. (2000). RNAi analysis of *Deformed*, *proboscipedia* and *Sex combs reduced* in the milkweed bug *Oncopeltus fasciatus*: novel roles for Hox genes in the hemipteran head. *Development*, Vol. 127, pp. 3683-3694

Hunter, W.; Ellis, J.; vanEngelsdorp, D.; Hayes, J.; Westervelt, D.; Glick, E.; Williams, M.; Sela, I.; Maori, E.; Pettis, J.; Cox-Foster, D. & Paldi, N. (2010 ). Large-scale field application of RNA interference (RNAi) technology to reduce impact of Israeli Acute Paralysis Virus (IAPV) induced disease in honey bees (*Apis mellifera*). *Hymenoptera: Apiidae. PLoS pathogens*, Vol. 6, No. 12, pp. e1001160

Hunter, W.B.; Shelby, K.; Dowd, S.; McKenzie, C. & Shatters Jr, R. (2008). Gene Expression in Asian Citrus Psyllid Adults Feeding from Florida Citrus: Application to biology and vector control. *IRCHLB Proceedings*, (December), pp. 233-237

Huvenne, H. & Smagghe, G. (2010). Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: a review. *Journal of insect physiology*, Vol. 56, No. 3, pp. 227-235 ISSN 1879-1611; 0022-1910

Jose, A.M. & Hunter, C.P. (2007). Transport of sequence-specific RNA interference information between cells. *Annual Review of Genetics*, Vol. 41, pp. 305-330

Karginakudru, S.; Royer, C.; Edupalli, S.V.; Jalabert, A.; Mauchamp, B.; Chandrashekaraiyah; Prasad, S.V.; Chavancy, G.; Couble, P. & Nagaraju, J. (2007). Targeting i-e-1 gene by RNAi induces baculoviral resistance in lepidopteran cell lines and in transgenic silkworms. *Insect molecular biology*, Vol. 16, No. 5, pp. 635-644 ISSN 0962-1075; 0962-1075

Karim, S.; Kenny, B.; Troiano, E. & Mather, T.N. (2008). RNAi-mediated gene silencing in tick synganglia: a proof of concept study. *BMC biotechnology*, Vol. 8, pp. 30

Keates, A.C.; Fruehauf, J.; Xiang, S. & Li, C.J. (2008). TransKingdom RNA interference: a bacterial approach to challenges in RNAi therapy and delivery. *Biotechnology and Genetic Engineering Reviews*, Vol. 25, pp. 113-128

Kennerdell, J.R. & Carthew, R.W. (1998). Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell*, Vol. 95, pp. 1017-1026

Kennerdell, J.R. & Carthew, R.W. (2000). Heritable gene silencing in *Drosophila* using double-stranded RNA. *Nature biotechnology*, Vol. 18, No. 8, pp. 896-898

Kennerdell, J.R.; Yamaguchi, S. & Carthew, R.W. (2002). *Genes & Development*, Vol. 16, pp. 1884-1889

www.intechopen.com
Khila, A. & Grbic, M. (2007). Gene silencing in the spider mite *Tetranychus urticae*: dsRNA and siRNA parental silencing of the Distal-less gene. *Development genes and evolution*, Vol. 217, pp. 241-251

Kocan, K.M.; Fuente, J.d.l.; Manzano-Roman, R.; Naranjo, V.; Hynes, W.L. & Sonenshine, D.E. (2008). Silencing expression of the defensin, varisin, in male *Dermacentor variabilis* by RNA interference results in reduced *Anaplasma marginale* infections. *Experimental & applied acarology*, Vol. 46, pp. 17-28

Kurscheid, S.; Lew-Tabor, A.E.; Valle, M.R.; Bruyeres, A.G.; Doogan, V.J.; Munderloh, U.G.; Guerrero, F.D.; Barrero, R.A. & Bellgard, M.I. (2009). Evidence of a tick RNAi pathway by comparative genomics and reverse genetics screen of targets with known loss-of-function phenotypes in *Drosophila*. *BMC molecular biology*, Vol. 10, (26 March), pp. 26

Lee, K.S.; Kim, B.Y.; Choo, Y.M.; Yoon, H.J.; Kang, P.D.; Woo, S.D.; Sohn, H.D.; Roh, J.Y.; Gui, Z.Z.; Je, Y.H. & Jin, B.R. (2009). Expression profile of cathepsin B in the fat body of *Bombus mori* during metamorphosis. *Comparative biochemistry and physiology Part B, Biochemistry & molecular biology*, Vol. 154, No. 2, pp. 188-194 ISSN 1879-1107; 1096-4959

Lehane, M.J.; Gibson, W. & Lehane, S.M. (2008). Differential expression of fat body genes in *Glossina morsitans morsitans* following infection with *Trypanosoma brucei brucei*. *International journal for parasitology*, Vol. 38, No. 1, pp. 93-101 ISSN 0020-7519; 0020-7519

Leng, Q.; Woodle, M.C.; Lu, P.Y. & Mixson, A.J. (2009). Advances in Systemic siRNA Delivery. *Drugs Future*, Vol. 34, No. 9, pp. 721

Lipardi, C. & Paterson, B.M. (2009). Identification of an RNA-dependent RNA polymerase in *Drosophila* involved in RNAi and transposon suppression. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 106, pp. 15645-15650

Liu, P.Z. & Kaufman, T.C. (2004). *Hunchback* is required for suppression of abdominal identity, and for proper germband growth and segmentation in the intermediate germband insect *Oncopeltus fasciatus*. *Development*, Vol. 131, pp. 1515-1527

Liu, X.; Zhang, Y.; Yan, X. & Han, R. (2010). Prevention of Chinese Sacbrood Virus Infection in *Apis cerana* using RNA Interference. *Current microbiology*, Vol. 61, No. 5, pp. 422-428

Lorenzen, M.D.; Brown, S.J.; Denell, R.E. & Beeman, R.W. (2002). Cloning and characterization of the *Tribolium castaneum* eye-color genes encoding tryptophan oxygenase and kynurenine 3-monoxygenase. *Genetics*, Vol. 160, No. 1, pp. 225-234

Lorenzen, M.D.; Gnirke, A.; Margolis, J.; Garnes, J.; Campbell, M.; Stuart, J.; Aggarwal, R.; Richards, S.; Park, Y. & Beeman, R.W. (2008). The maternal-effect, selfish genetic element *Medea* is associated with a composite *Tc1* transposon. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 105, pp. 10085-10089

Lu, R.; Li, H.; Li, W.-X. & Ding, S.-W. (2004). RNA-based immunity in insects, S. H. Gillespie, G. L. Smith, A. Osbourn, (Eds), Cambridge University Press, ISBN 0 521 84312 X,

Lynch, J.A. & Desplan, C. (2006). A method for parental RNA interference in the wasp *Nasonia vitripennis*. *Nature Protocols*, Vol. 1, pp. 486-494

www.intechopen.com
Lynch, J.A. & Desplan, C. (2010). Novel modes of localization and function of nanos in the wasp Nasonia. Development, Vol. 137, pp. 3813-3821

Lynch, J.A.; Panfilio, K.A. & Fonseca, R.N.d. (2009). As Tribolium matures as a model insect, coleopteran community congregates in Cologne. Development genes and evolution, Vol. 219, No. 9-10, pp. 531-533

Mao, Y.B.; Cai, W.J.; Wang, J.W.; Hong, G.J.; Tao, X.Y.; Wang, L.J.; Huang, Y.P. & Chen, X.Y. (2007). Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nature biotechnology, Vol. 25, No. 11, pp. 1307-1313 ISSN 1087-0156; 1087-0156

Maori, E.; Paldi, N.; Shafir, S.; Kalev, H.; Tsur, E.; Glick, E. & Sela, I. (2009a). IAPV, a bee-affecting virus associated with Colony Collapse Disorder can be silenced by dsRNA ingestion. Insect molecular biology, Vol. 18, No. 1, pp. 55-60 ISSN 1365-2583; 0962-1075

Maori, E.; Paldi, N.; Shafir, S.; Kalev, H.; Tsur, E.; Glick, E. & Sela, I. (2009b). A bee-affecting virus associated with colony collapse disorder can be silenced by dsRNA ingestion. Insect molecular biology, Vol. 18, pp. 55-60

Masumoto, M.; Yaginuma, T. & Niimi, T. (2009). Functional analysis of Ultrabithorax in the silkworm, Bombyx mori, using RNAi. Development genes and evolution, Vol. 219, No. 9-10, pp. 437-444 ISSN 1432-041X; 0949-944X

Mathur, G.; Sanchez-Vargas, I.; Alvarez, D.; Olson, K.E.; Marinotti, O. & James, A.A. (2010). Transgene-mediated suppression of dengue viruses in the salivary glands of the yellow fever mosquito, Aedes aegypti. Insect molecular biology, Vol. 19, No. 6, pp. 753-763

Michel, K.; Pinto, S.; Budd, A. & Kafatos, F. (2005). Anopheles gambiae SRPN2 facilitates midgut invasion by the malaria parasite Plasmodium berghei. EMBO Reports, Vol. 6, No. 9, pp. 891-897

Miller, S.C.; Brown, S.J. & Tomoyasu, Y. (2008). Larval RNAi in Drosophila? Development genes and evolution, Vol. 218, pp. 505-510

Mito, T.; Nakamura, T.; Bando, T.; Ohuchi, H. & Noji, S. (2011). The advent of RNA interference in Entomology. Entomological Science, Vol. 14, pp. 1-8

Mummery-Widmer, J.L.; Yamazaki, M.; Stoeger, T.; Novatchkova, M.; Bhalerao, S.; Chen, D.; Dietzl, G.; Dickson, B.J. & Knoblisch, J.A. (2009). Genome-wide analysis of Notch signalling in Drosophila by transgenic RNAi. Nature, Vol. 458, No. 7241, (Apr 23), pp. 987-992

Nelson, C.M.; Ihle, K.E.; Fondrk, M.K.; Page, R.E. & Amdam, G.V. (2007). The gene vitellogenin has multiple coordinating effects on social organization. PLoS biology, Vol. 5, No. 3, pp. e62 ISSN 1545-7885; 1544-9173

Newmark, P.A.; Reddien, P.W.; Cebria, F. & Alvarado, A.S. (2003). Ingestion of bacterially expressed double-stranded RNA inhibits gene expression in planarians. Proceedings of the National Academy of Sciences of the United States of America, Vol. 100, No. 1, (September 30, 2003), pp. 11861-11865

Nguyen, T. & Fruehauf, J.H. (2008). Bacterial Vectors for RNAi Delivery, in Patho Biotechnology, R. Sleator, C. Hill, (Eds), Landes Bioscience, www.intechopen.com
Ohnishi, A.; Hull, J.J. & Matsumoto, S. (2006). Targeted disruption of genes in the Bombyx mori sex pheromone biosynthetic pathway. Proceedings of the National Academy of Sciences of the United States of America, Vol. 103, pp. 4398-4403

Olson, K.E.; Higgs, S.; Gaines, P.J.; Powers, A.M.; Davis, B.S.; Kamrud, K.I.; Carlson, J.O.; Blair, C.D. & Beaty, B.J. (1996). Genetically engineered resistance to dengue-2 virus transmission in mosquitoes. Science, Vol. 272, pp. 884-886

Osta, M.A.; Christophides, G.K. & Kafatos, F.C. (2004). Effects of Mosquito Genes on Plasmodium Development. Science, Vol. 303 No. 5666 (26 March), pp. 2030-2032

Paldi, N.; Glick, E.; Oliva, M.; Zilberberg, Y.; Aubin, L.; Pettis, J.; Chen, Y. & Evans, J.D. (2010). Effective gene silencing in a microsporidian parasite associated with honeybee (Apis mellifera) colony declines. Applied and Environmental Microbiology, Vol. 76, No. 17, pp. 5960-5964

Posnien, N.; Schinko, J.; Grossmann, D.; Shippy, T.D.; Konopova, B. & Bucher, G. (2009). RNAi in the red flour beetle (Tribolium). Cold Spring Harbor protocols, Vol. 2009, No. 8, pp. pdb.prot5256 ISSN 1559-6095

Price, D.R.G. & Gatehouse, J.A. (2008). RNAi-mediated crop protection against insects. Trends in biotechnology, Vol. 26 No. 7, pp. 393-400

Richards, S. & Consortium, T.G.S. (2008). The genome of the model beetle and pest Tribolium castaneum. Nature, Vol. 452, pp. 949-955

Rognant, J.Y.; Carré, C.; Mugat, B.; Szymczak, D.; Lepesant, J.A. & Antoniewski, C. (2003). Absence of transitive and systemic pathways allows cell-specific and isoform-specific RNAi in Drosophila. RNA, Vol. 9, No. 3, pp. 299-308

Rolff, J. & Reynolds, S. (Eds.) (2009). Insect Infection and Immunity. Evolution, Ecology, and Mechanisms, Oxford University Press,ISBN 978-0-19-955135-4, New York

Ronco, M.; Uda, T.; Mito, T.; Minelli, A.; Noji, S. & Klingler, M. (2008). Antenna and all gnathal appendages are similarly transformed by homothorax knock-down in the cricket Gryllus bimaculatus. Developmental biology, Vol. 313, pp. 80-92

Rütten, K.B.; Pietsch, C.; Olek, K.; Neusser, M.; Beukeboom, L.W. & Gadau, J. (2004). Chromosomal anchoring of linkage groups and identification of wing size QTL using markers and FISH probes derived from microdissected chromosomes in Nasonia (Pteromalidae: Hymenoptera). Cytogenetics and Genome Research, Vol. 105, No. 1, pp. 126-133

Saleh, M.C.; van Rij, R.P.; Hekele, A.; Gillis, A.; Foley, E.; O’Farrell, P.H. & Andino, R. (2006). The endocytic pathway mediates cell entry of dsRNA to induce RNAi silencing. Nature Cell Biology, Vol. 8, No. 8, pp. 793-802

Saleh, M.C.; Tassetto, M.; van Rij, R.P.; Goic, B.; Gausson, V.; Berry, B.; Jacquier, C.; Antoniewski, C. & Andino, R. (2009). Antiviral immunity in Drosophila requires systemic RNA interference spread. Nature, Vol. 458, (Feb 8), pp. 346-351

Soares, C.A.G.; Lima, C.M.R.; Dolan, M.C.; Piesman, J.; Beard, C.B. & Zeidner, N.S. (2005). Capillary feeding of specific dsRNA induces silencing of the isac gene in nymphal Ixodes scapularis ticks. Insect molecular biology, Vol. 14, pp. 443-452

Solis, C.F.; Santi-Rocca, J.; Perdomo, D.; Weber, C. & Guillén, N. (2009). Use of Bacterially Expressed dsRNA to Downregulate Entamoeba histolytica Gene Expression. PLoS One, Vol. 4, No. 12, pp. e8424

www.intechopen.com
Tabunoki, H.; Higurashi, S.; Ninagi, O.; Fujii, H.; Banno, Y. & Nozaki, M. (2004). A carotenoid-binding protein (CBP) plays a crucial role in cocoon pigmentation of silkworm (*Bombyx mori*) larvae. *FEBS letters*, Vol. 567 pp. 175-178.

Tavernarakis, N.; Wang, S.L.; Dorovkov, M.; Ryazanov, A. & Driscoll, M. (2000). Heritable and inducible genetic interference by double-stranded RNA encoded by transgenes. *Nature Genetics*, Vol. 24, pp. 180-183.

Tian, H.; Peng, H.; Yao, Q.; Chen, H.; Xie, Q.; Tang, B. & Zhang, W. (2009). Developmental Control of a Lepidopteran Pest *Spodoptera exigua* by Ingestion of Bacteria Expressing dsRNA of a Non-Midgut Gene. *PLoS One*, Vol. 4, No. 7, pp. e6225.

Timmons, L. & Fire, A. (1998). Specific interference by ingested dsRNA. *Nature*, Vol. 395, pp. 854.

Tomoyasu, Y. & Denell, R.E. (2004). Larval RNAi in *Tribolium* (Coleoptera) for analyzing adult development. *Development genes and evolution*, Vol. 214, pp. 575-578.

Tomoyasu, Y.; Miller, S.C.; Shuichihiro, T.; Schoppmeier, M.; Grossmann, D. & Bucher, G. (2008). Exploring systemic RNA interference in insects: a genome-wide survey for RNAi genes in *Tribolium*. *Genome Biology*, Vol. 9, No. 1, pp. R10.

Turner, C.T.; Davy, M.W.; MacDiarmid, R.M.; Plummer, K.M.; Birch, N.P. & Newcomb, R.D. (2006). RNA interference in the light brown apple moth, *Epiphyas postvittana* (Walker) induced by double-stranded RNA feeding. *Insect molecular biology*, Vol. 15, No. 3, pp. 383-391 ISSN 0962-1075; 0962-1075.

Ulvia, J.; Parikka, M.; Kleino, A.; Sormunen, R.; Ezekowitz, R.A.; Kocks, C. & Rämet, M. (2006). Double-stranded RNA is internalized by scavenger receptor-mediated endocytosis in *Drosophila* S2 cells. *Journal of Biological Chemistry*, Vol. 281, No. 20, (2006 May 19), pp. 14370-14375.

Van Roessel, P.; Hayward, N.M.; Barros, C.S. & Brand, A.H. (2002). Two-color GFP imaging demonstrates cell-autonomy of GAL4-driven RNA interference in *Drosophila*. *Genesis*, Vol. 34, pp. 170-173.

Verhulst, E.C.; Beukeboom, L.W. & Zande, L.v.d. (2010). Maternal Control of Haplodiploid Sex Determination in the Wasp *Nasonia*. *Science*, Vol. 328 No. 5978 (30 April 2010), pp. 620-623.

Walsh, D.P.; Lehane, S.M.; Lehane, M.J. & Haines, L.R. (2009). Prolonged gene knockdown in the tsetse fly *Glossina* by feeding double stranded RNA. *Insect molecular biology*, Vol. 18, No. 1, pp. 11-19 ISSN 1365-2583; 0962-1075.

Werren, J. & Loehlin, D. (2009). Larval RNAi in *Nasonia* (Parasitoid Wasp). *Cold Spring Harbor protocols*, Vol. 10.

Werren, J.H.; Richards, S.; Desjardins, C.A.; Niehuis, O.; Gadag, J.; Colbourne, J.K. & Group, N.G.W. (2010). Functional and evolutionary insights from the genomes of three parasitoid *Nasonia* species. *Science*, Vol. 327, No. 5963, (Jan 15), pp. 343-348.

Whyard, S.; Singh, A.D. & Wong, S. (2009). Ingested double-stranded RNAs can act as species-specific insecticides. *Insect Biochemistry and Molecular Biology*, Vol. 39, No. 11, pp. 824-832 ISSN 1879-0240; 0965-1748.

Winston, W.M.; Sutherlin, M.; Wright, A.J.; Feinberg, E.H. & Hunter, C.P. (2007). *Caenorhabditis elegans* SID-2 is required for environmental RNA interference. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 104, No. 25, (June 19, 2007), pp. 10565-10570.
Xiang, S.; Fruehauf, J. & Li, C.J. (2006). Short hairpin RNA-expressing bacteria elicit RNA interference in mammals. Nature biotechnology, Vol. 24, pp. 697-702

Xiang, S.; Keates, A.C.; Fruehauf, J.; Yang, Y.; Guo, H.; Nguyen, T. & Li, C.J. (2009). In vitro and in vivo gene silencing by TransKingdom RNAi (tkRNAi). Methods in molecular biology, Vol. 487, pp. 147-160

Yuan, X.; Naguib, S. & Wu, Z. (2011). Recent advances of siRNA delivery by nanoparticles. Expert Opinion on Drug Delivery, Vol. 8, No. 4, pp. 521-536

Zamore, P.D.; Tuschl, T.; Sharp, P.A. & Bartel, D.P. (2000). RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell, Vol. 101, pp. 25-33

Zhou, X.; Wheeler, M.M.; Oi, F.M. & Scharf, M.E. (2008). RNA interference in the termite Reticulitermes flavipes through ingestion of double-stranded RNA. Insect Biochemistry and Molecular Biology, Vol. 38, No. 8, pp. 805-815 ISSN 0965-1748; 0965-1748

Zhu, F.; Xu, J.; Palli, R.; Ferguson, J. & Palli, S.R. (2011). Ingested RNA interference for managing the populations of the Colorado potato beetle, Leptinotarsa decemlineata. Pest management science, Vol. 67, No. 2, pp. 175-182

Zhu, Q.; Arakane, Y.; Banerjee, D.; Beeman, R.W.; Kramer, K.J. & Muthukrishnan, S. (2008). Functional specialization among insect chitinase family genes revealed by RNA interference. Proc Natl Acad Sci USA, Vol. 105, No. 18, pp. 6650-6655
RNA functions broadly as informational molecule, genome, enzyme and machinery for RNA processing. While these functions reflect ancient activities, they also remain vital components of contemporary biochemical pathways. In eukaryotic cells RNA processing impacts the biogenesis of RNA molecules of essentially every shape and function. The collection of articles in this volume describes the current state of understanding of the broad array of RNA processing events in animal and plant cells, key unanswered questions, and cutting edge approaches available to address these questions. Some questions discussed in this volume include, how viruses subvert the RNA processing machinery of the host cell, how the coordination of co-transcriptional RNA processing is regulated at the level of chromatin, the status of RNA processing in plant organelles, and how micro RNA machinery is biosynthesized and regulated.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Katherine Aronstein, Brenda Oppert and Marcé D. Lorenzen (2011). RNAi in Agriculturally-Important Arthropods, RNA Processing, Prof. Paula Grabowski (Ed.), ISBN: 978-953-307-557-0, InTech, Available from: http://www.intechopen.com/books/rna-processing/rna-in-agriculturally-important-arthropods
