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Review

Diet-delivered RNAi in *Helicoverpa armigera* – Progresses and challenges

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ABSTRACT

Helicoverpa armigera (the cotton bollworm) is a significant agricultural pest endemic to Afro-Eurasia and Oceania. Gene suppression via RNA interference (RNAi) presents a potential avenue for management of the pest, which is highly resistant to traditional insecticide sprays. This article reviews current understanding on the fate of ingested double-stranded RNA in *H. armigera*. Existing *in vivo* studies on diet-delivered RNAi and their effects are summarized and followed by a discussion on the factors and hurdles affecting the efficacy of diet-delivered RNAi in *H. armigera*.

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1. Introduction

Helicoverpa armigera (the cotton bollworm) is a major agricultural pest globally, the larvae of which feeds on >180 plant species (Pawar, 1998; Sithanantham, 1986), causing an estimated US$2 billion in annual crop damage (Sharma, 2001). Indiscriminate usage of insecticides has rendered almost all strains of the pest resistant through adaptive mutation (Fournier et al., 1992; Hsu et al., 2006; Yoo et al., 2002). Transgenic plants expressing *Bacillus thuringiensis* (*Bt*) toxins are being increasingly adopted to reduce reliance on insecticides, but the technology remains limited to a handful of crops (ISAAA, 2015).

RNA interference (RNAi) presents a potential strategy for insect control via silencing of gene expression. The RNAi mechanism is initiated by recognition and cleavage of dsRNA by a ribonuclease

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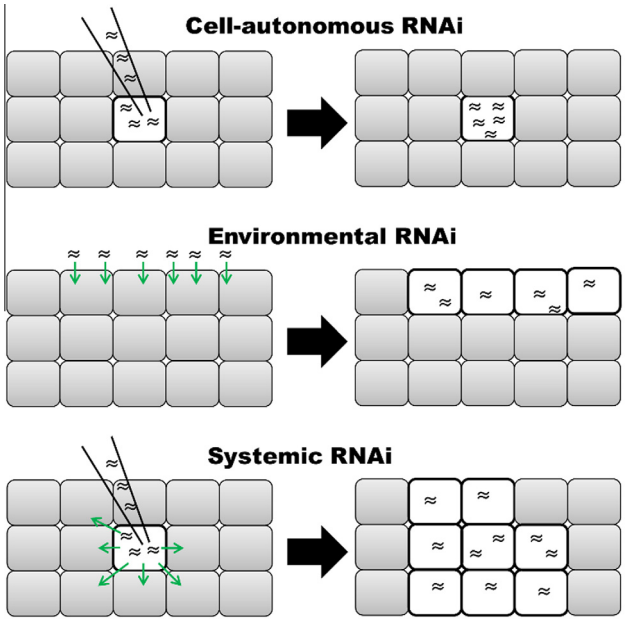

Fig. 1. Types of RNAi response in *H. armigera*.

III (Dicer-2) into small interfering RNAs (siRNA) of ~20–21 nucleotides in length (Bernstein et al., 2001). These siRNAs are loaded into the RNA-induced silencing complex (RISC), which contains an Argonaute-2 (Ago-2) protein with an RNase H-like domain (Hammond et al., 2001). Here one of the siRNA strands (passenger strand) is degraded while the other (guide strand) remains associated with Ago-2, which mediates recognition and cleavage of mRNA sequences complementary to the guide strand by RISC (Meister and Tuschl, 2004; Tomari and Zamore, 2005).

Much progress has been made in recent years with regard to diet-delivered RNAi due to its potential for insect control (Baum and Roberts, 2014; Burand and Hunter, 2013; Gu and Knipple, 2013; Zhang et al., 2013). The aim of this article is two-fold: (i) to summarize current understanding on the fate of ingested dsRNA in *H. armigera*, and (ii) to review existing literature on diet-delivered RNAi in the pest.

2. Mechanism of dsRNA uptake and spread

For the purpose of clarity, nomenclature for the three types of RNAi response outlined in this article will be defined according to Whangbo and Hunter (2008) and is illustrated in Fig. 1: (i) cell-autonomous RNAi, in which RNA silencing is restricted within cells where dsRNA is constitutively expressed or has been exogenously introduced; (ii) environmental RNAi, which involves uptake of dsRNA by midgut cells from the lumen; and (iii) systemic RNAi, where RNAi spreads from cells in which the dsRNA was originally produced or imported to other cells in which silencing consequently occurs.

The core machinery for RNAi within the cell, e.g. Dicer, is conserved among insects and other eukaryotes, where it plays a role in post-transcriptional regulation of gene expression through the miRNA pathway (Asgari, 2013; Lucas and Raikhel, 2013) and as an antiviral mechanism through the siRNA pathway (Gammon and Mello, 2015). Sensitivity to diet-delivered RNAi, however, has been shown to be highly variable between insect species (Baum and Roberts, 2014; Bellés, 2010; Katoch et al., 2013; Swevers and Smagghe, 2012), with less derived species demonstrating a more robust RNAi response compared to more derived ones, e.g. Lepidoptera (including *H. armigera*) (Bellés, 2010). This disparity in terms of RNAi sensitivity has partly been attributed to the presence or lack of certain cellular import and export mechanisms for dsRNA, as well as RNAi signal amplification among insect orders (Bellés, 2010; Gatehouse and Price, 2011; Gu and Knipple, 2013; Terenius et al., 2011).

Current understanding of dsRNA uptake in insects is based on studies using *Caenorhabditis elegans* (although not an insect) and *Drosophila melanogaster* S2 cells, characterization of which has identified two mechanisms for dsRNA uptake to date. The first mechanism involves the presence of two transmembrane proteins, SID-1 and SID-2, responsible for dsRNA transport. SID-1 functions in systemic spreading of RNAi by serving as a channel for dsRNA uptake and release among cells (Jose and Hunter, 2007; Winston et al., 2002), while SID-2 is a gut-specific transmembrane protein required for initial import of dsRNA into intestinal cells (Whangbo and Hunter, 2008; Winston et al., 2002, 2007). The second mechanism implicates involvement of receptor-mediated endocytosis in dsRNA uptake. Screening of a dsRNA library performed by Saleh et al. (2006) in *D. melanogaster* S2 cells identified 23 genes coding for components of endocytosis and vesicle-mediated trafficking that were necessary for uptake and processing of exogenous dsRNA. These included genes for clathrin heavy chain and its adaptor AP-50, which mediate early endocytic uptake, and vacuolar H^+-ATPase (*V-H-ATPase*), involved in controlling endocytic vesicle trafficking and protein sorting. Both fluorescent dsRNA uptake and dsRNA-initiated silencing were strongly inhibited when S2 cells were pretreated with poly-inosinic acid and fucoidin prior to dsRNA administration (these are ligands of the scavenger-receptor family and compete with dsRNA for binding (Peiser et al., 2002)). However, individual downregulation of 19 genes coding for scavenger receptors did not result in significant inhibition of RNAi, which suggests that dsRNA uptake is mediated by multiple scavenger receptors with overlapping functions. Ulvila et al. (2006) further showed that two *Drosophila* scavenger receptors, SR–CI and Eater, together account for >90% uptake of dsRNA fragments (500 bp) into S2 cells. Receptor-mediated endocytosis has been demonstrated to also play a dominant role in cellular uptake of dsRNA in *Tribolium castaneum* (Xiao et al., 2015) and *Schistocerca gregaria* (Wynant et al., 2014), with both species demonstrating significant attenuation of RNAi effect upon silencing of key components of the endocytosis pathway or pharmacological inhibition of scavenger receptors.

In the case of *H. armigera*, ingested dsRNA is presumably exposed and imported into epithelial cells in the midgut (Price and Gatehouse, 2008; Zha et al., 2011). The presence of at least 2 *sid-1* homologs in *H. armigera* has been reported (unpublished data, Xue et al. (2012)), but analysis of published insect genomes thus far has not produced clear correlation between the presence of *sid-1* homologs and systemic RNAi response in insects (Tomoyasu et al., 2008). *Sid-1* homologs have been shown to be dispensable with regard to the robust systemic RNAi observed in *Locusta migratoria* (Luo et al., 2012) and *T. castaneum* (Tomoyasu et al., 2008). Furthermore, homologs of genes necessary for systemic RNAi in *C. elegans* have also been found to be absent in *T. castaneum* (Tomoyasu et al., 2008), which suggests the existence of an alternative mechanism for systemic RNAi in insects.

A similar scenario applies to RNA-dependent RNA polymerase (RdRp). RdRp utilizes RISC-cleaved mRNA as template for production of secondary dsRNA, enabling persistent amplification of RNAi effect (Ghildiyal and Zamore, 2009). However, homologs for *RdRp* have yet to be identified in insect genomes sequenced to date, even in species demonstrating robust systemic RNAi, e.g. *T. castaneum* (Tomoyasu et al., 2008). The same study surmised that secondary amplification in *T. castaneum* is likely based on a different gene with similar activity to *RdRp* or another mechanism entirely.