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Mini Review

RNA interference: Applications and advances in insect toxicology and insect pest management

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ABSTRACT

Since its discovery, RNA interference (RNAi) has revolutionized functional genomic studies due to its sequence-specific nature of post-transcriptional gene silencing. In this paper, we provide a comprehensive review of the recent literature and summarize the current knowledge and advances in the applications of RNAi technologies in the field of insect toxicology and insect pest management. Many recent studies have focused on identification and validation of the genes encoding insecticide target proteins, such as acetylcholinesterases, ion channels, *Bacillus thuringiensis* receptors, and other receptors in the nervous system. RNAi technologies have also been widely applied to reveal the role of genes encoding cytochrome P450 monooxygenases, carboxylesterases, and glutathione *S*-transferases in insecticide detoxification and resistance. More recently, studies have focused on understanding the mechanism of insecticide-mediated up-regulation of detoxification genes in insects. As RNAi has already shown great potentials for insect pest management, many recent studies have also focused on host-induced gene silencing, in which several RNAi-based transgenic plants have been developed and tested as proof of concept for insect pest management. These studies indicate that RNAi is a valuable tool to address various fundamental questions in insect toxicology and may soon become an effective strategy for insect pest management.

1. Introduction

RNA interference (RNAi) is a sequence-specific post-transcriptional gene silencing process elicited by double stranded RNA (dsRNA) that occurs widely among plants, animals, and microorganisms [1,2]. Since its discovery in 1998, RNAi has revolutionized functional genomics due to its relatively easy use and its power as a reverse genetic tool [2–6]. RNAi-high throughput screening is being used in fully genome-sequenced organisms to elucidate gene function related to numerous medically and agriculturally relevant topics [7]. In addition, RNAi can be applied to organisms lacking genetic tools as it does not require transformative methods, and it can be performed *in vivo*, allowing for the study of tissue specific and life-stage specific phenotypes associated with gene expression, as well as complex phenotypes that cannot be modeled in cell-based assays [7,8].

Detailed descriptions of the history, universal applications, and mechanisms of RNAi have been reviewed elsewhere [1,7,9,10]. In this review, we focus on recent applications and advances of RNAi in the field of insect toxicology and host-induced gene silencing as it is related to insect pest management. Specifically, our review summarizes the current knowledge and progress in the applications of various RNAi technologies to: 1) identify and validate the genes encoding major insecticide target enzymes or proteins; 2) reveal the role of the genes encoding detoxification enzymes and transporters; 3) elucidate the mechanism of insecticide-induced up-regulation of detoxification genes; and 4) develop RNAi-based transgenic plants as proof of concept for insect pest management [11].

2. Applications in insect toxicology

2.1. Identification or validation of insecticide target genes

RNAi technology has been widely applied for identification or validation of the genes encoding insecticide target proteins (Table 1). Many recent studies have focused on acetylcholinesterase (AChE, EC 3.1.1.7), an essential enzyme at the cholinergic synapses and neuromuscular junctions of most invertebrates and vertebrates [24], and the target of organophosphate (OP) and carbamate (CB) insecticides. Reduced sensitivity of AChE has also been reported as one of the major insecticide resistance mechanisms against OPs and CBs. Two different *ace* genes (*ace1* encoding AChE1, *ace2* encoding AChE2)

Abbreviations: AChE, acetylcholinesterase; APN, aminopeptidase N; *APP*, aminopeptidase P-like gene; Cad, cadherin; CarE, carboxylesterase; CB, carbamate; CPR, NADPH-dependent cytochrome P450 reductase; dsRNA, double-stranded RNA; GABA-R, γ-aminobutyric acid receptor; GST, glutathione *S*-transferase; HIGS, host-induced gene silencing; nAChR, nicotinic acetylcholine receptor; OP, organophosphate; RNAi, RNA interference; RyR, ryanodine receptor.

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Table 1
Summary of studies to identify or validate insecticide target genes by RNAi.

Insecticide target	Insect	Suppression of transcript (%)	Insecticide treatment	Remarks	Reference
AChE 1 & 2	*Plutella xylostella*	7–34	NT*	AChE1 has major effects on larval growth.	[12]
AChE 1 & 2	*Helicoverpa armigera*	NA[a]	NT	siRNA is designed at a conserved region of the two AChE genes. High mortality, growth inhibition.	[13]
AChE 1 & 2	*Tribolium castaneum*	92–95	Carbaryl, carbofuran, dichlorvos, malathion	AChE1 is a major target of OPs and CBs. AChE2 plays non-cholinergic role.	[14]
AChE 1 & 2	*Blattella germanica*	95–97	Chlorpyrifos, lambda-cyalothrin	AChE1 is the predominant enzyme and major target of OPs.	[15]
AChE 1 & 2	*Chilo suppressalis*	50–70	NT	AChE1 has major effects on larval growth.	[16]
nAChR-α6	*Tribolium castaneum*	Approx. 50	Spinosad	Reduced expression of nAChR-α6 by RNAi is insufficient to alter spinosad susceptibility.	[17]
nAChR-α6	*Drosophila melanogaster*	25–44	Spinosad	Reduced expression of nAChR-α6 by RNAi is insufficient to alter spinosad susceptibility.	[17]
$GABA_A$-R	*Drosophila melanogaster*	50	NT	GABAA receptors negatively modulate olfactory associative learning.	[18]
RyR 1 & 2	*Leptinotarsa decemlineata*	35–55	Chlorantraniliprole	RyR is a target of chlorantraniliprole.	[19]
RyR 1 & 2	*Sogatella furcifera*	78–82	Chlorantraniliprole	RyR is a target of chlorantraniliprole.	[20]
APP	*Ostrinia nubilalis*	38	Cry1Ab	APP is associated with *Bt* resistance.	[21]
APN	*Spodoptera litura*	95	Cry1C	APN is a receptor of Cry1C.	[22]
Cad	*Spodoptera exigua*	Approx. 80	Cry1Ca	Cad is a receptor of Cry1Ca.	[23]

* Insects were not treated with insecticides.
[a] Information not available.

have been characterized in various insect species [25–31]. AChE1 is proposed to be the major catalytic enzyme and target of OPs and CBs, because AChE1 generally has higher expression levels and shows higher frequencies of point mutations associated with insecticide resistance than those of AChE2 [25,26,29,30,32–36].

Lu et al. [14] successfully suppressed *ace1* and *ace2* transcripts by injection of corresponding dsRNA in *Tribolium castaneum*. They observed 100% mortality after adult eclosion when larvae were treated with *ace1* dsRNA; however, the injection of *ace2* dsRNA did not lead to a significant mortality. The effects of exposure to OPs and CBs were then investigated in larvae injected with *ace1* or *ace2* dsRNA. Larval susceptibility to each of the four insecticides increased significantly when *ace1* transcript level was suppressed by RNAi but did not differ significantly from the control when the *ace2* transcript level was suppressed. If an *ace* gene encodes the AChE targeted by these insecticides, suppression of its transcript level by RNAi followed by insecticide exposures would be expected to increase the insect susceptibility to the insecticides. Thus, these results indicate that AChE is a major target of OP and CB insecticides. Although the RNAi of *ace* gene acts at the transcriptional level whereas the inhibition of AChE by insecticides acts at the enzymatic level, both ultimately reduce the amount of AChE for insects to function normally. Thus, RNAi of an *ace* gene encoding AChE targeted by these insecticides followed by exposures of the insects to the insecticides is expected to work additively to reduce the amount of AChE, leading to increased insect mortalities (Fig. 1A).

In *Blattella germanica*, 65–75% of total AChE activity was reduced following *ace1* dsRNA treatment. Moreover, a significant increase in susceptibility to chlorpyrifos was observed after *ace1* dsRNA injection [15]. These findings strongly support that AChE1 plays a major role in cholinergic functions, and is the major target of anticholinesterase insecticides. However, more recent studies revealed that some insects express AChE2 as the major catalytic enzyme, rather than AChE1 [28,37,38]. Although the ability of AChE2 to function as a major catalytic enzyme does not necessarily indicate either its major function in cholinergic neurotransmission or as a major target for OP and CB insecticides, it would be interesting to investigate if AChE2 is a major enzyme responsible for cholinergic neurotransmission and serves as a major target for these insecticides by RNAi.

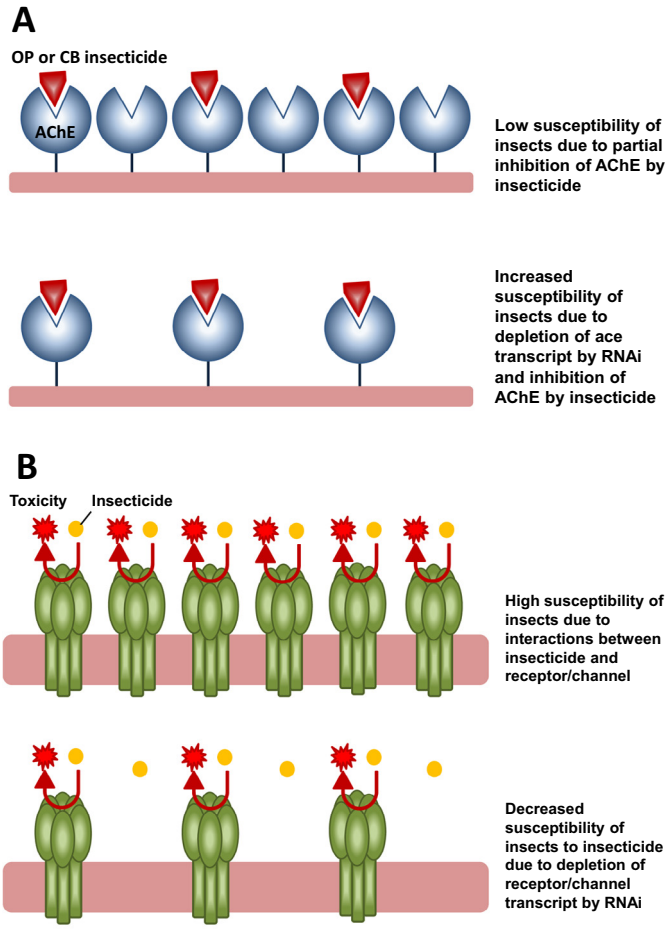

Fig. 1. Schematic illustrations of (A) increased insect susceptibility to an organophosphate or carbamate insecticide as a result of additive-like effects of the RNAi-mediated depletion of the *ace* transcript and the insecticide-mediated inhibition of AChE encoded by the same *ace* gene, and (B) decreased insect susceptibility to an insecticide as a result of the RNAi-mediated depletion of the transcript encoding a receptor or ion channel protein that serves as a target of an insecticide.