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RNA Interference: Applications in Insect Toxicology

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RNA interference is a defence mechanism seen in various organisms. The mechanism has revolutionary applications in insect toxicology. Improving the sensitivity of acetylcholinesterase by corresponding dsRNA injection, silencing of detoxifying enzymes like cytochrome P450 monooxygenases, carboxylesterases, glutathione S-transferases and others or targeting the genes responsible for upregulation of these enzymes through RNAi reduces the detoxification of corresponding insecticides. Host-induced gene silencing where the plants carry dsRNA for target gene is a breakthrough in the field of advanced insect toxicology.

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. Since its discovery in 1998, RNAi has revolutionized functional genomics due to its relatively easy use and its power as a reverse genetic tool. RNAi-high throughput screening is used in fully genome-sequenced organisms to elucidate gene function related to numerous medically and agriculturally relevant topic. 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 modelled in cell-based assays.

History behind RNAi

Napoli and Jorgensen were the first to report RNAi type of phenomenon in 1990 while studying on the Chalcone Synthase (CHS), a key enzyme in flavonoid biosynthesis in petunia flowers. In 1992, Romano and Macino reported a similar phenomenon in *Neurospora crassa*, noting that introduction of homologous RNA sequences caused "quelling" of the endogenous gene. Guo and Kemphues first recorded the silencing mechanism in *Caenorhabditis elegans.* Fire and Mello in 1998 explained silencing of endogenous genes by "cosuppression, quelling and sense mRNA". In 2006, it was found that trigger for gene silencing was not a single-stranded RNA (ssRNA) but double-stranded RNA (dsRNA) in *C. elegans* (Sen and Blau, 2006).

Applications in insect toxicology

(i) Identification or validation of insecticide target genes/enzymes/proteins: Acetylcholinesterase, a target of organophosphate (OP) and carbamate (CB) insecticides, are important enzymes found in the cholinergic synapses and neuromuscular junctions of most invertebrates and vertebrates. Reduced sensitivity of AChE leads to resistance against OPs and CBs. Lu *et al.* (2012) successfully suppressed ace1 and ace2 transcripts by injecting

corresponding dsRNA in *Tribolium castaneum* larvae where 100% mortality was observed after adult eclosion. Similarly, In *Blattella germanica*, 65–75% of total AChE activity was reduced following ace1 dsRNA treatment, significantly increasing the susceptibility to chlorpyrifos (Revuelta *et al.*, 2009).

(ii) Identifications of genes involved in insecticide detoxification and resistance: Increased detoxification and reduced sensitivity of insecticide target sites leads to resistance development. Increased expression of detoxification genes or increased activity of detoxification enzymes like cytochrome P450 monooxygenases (CYPs), carboxylesterases (CarEs) and glutathione S-transferases (GSTs) results in enhanced detoxification. In *H. armigera*, expression levels of genes CYP6B7, NADPH-dependent cytochrome P450 reductase (CPR), and cytochrome b5 (Cyt-b5), decreased after injection of their corresponding dsRNAs in a fenvalerate-resistant strain (Tang *et al.*, 2012).

(iii) Elucidate the mechanism of insecticide-induced up-regulation of detoxification genes: In insects, detoxification of insecticides and phytotoxins is often enhanced by both qualitative and quantitative changes in detoxification enzymes. In *Aedes aegypti*, several CYP genes have been found to be up regulated in adult mosquitoes by pyrethroid insecticides, including permethrin (CYP6AL1, CYP4J16B), cypermethrin (CYP6AL1, CYP9J32, CYP4J16A and CYP4J16B) and deltamethrin (CYP6AA5, CYP4J16A, and CYP4J16B). When RNAi was performed to silence HR96 (Nuclear receptor gene responsible for upregulation of CYP genes) there was a decrease in cypermethrin-mediated up regulation of CYP4J16B from approximately 15-fold to 5-fold which clearly indicates the role of HR96 in upregulation of CYP4J16B gene in mosquito (Issa, 2014).

(iv) Advances of host induced gene silencing for insect pest management: Utilising host-induced gene silencing (HIGS), RNAi-mediated crop protection offers advantages over chemical insecticides. The concept for HIGS against insect pests was first demonstrated in maize plants expressing dsRNA containing a region of the *Diabrotica virgifera virgifera* V-ATPase A transcript. Similar to this, Mao *et al.* (2007) found that feeding genetically modified tobacco leaves expressing dsRNA encoding a portion of a CYP gene obtained from *H. armigera*, decreased the resistance of *H. armigera* larvae to the phytotoxin gossypol.

Conclusion

RNA interference is a potential tool for managing insect pests and is useful to address numerous issues in insect toxicity. The use of RNAi has practical, economical, technological, regulatory, and safety issues. For many insect species, particularly Lepidoptera, variation in RNAi efficacy at the species, tissue, and gene levels has impeded the development of efficient RNAi procedures. Uptake of RNA into cells and systemic distribution are related to variations in the efficacy of RNA delivery strategies and phenotypic impacts of individual target gene suppression.

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