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RNAi: An Emerging Technology for pest control

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Introduction

Eukaryotic organisms frequently exhibit the RNAi pathway. Gene expression will be suppressed in this pathway. Since its initial description in the nematode, *Caenorhabditis elegans,* RNA interference (RNAi) has been extensively employed in genetic studies involving insects belonging to many orders such as Diptera, Lepidoptera, Orthoptera, Hemiptera, Blattodea, Coleoptera, Hymenoptera and Neuroptera. This technology is a more effective, precise, adaptable, stable and potent genetic pest management method.

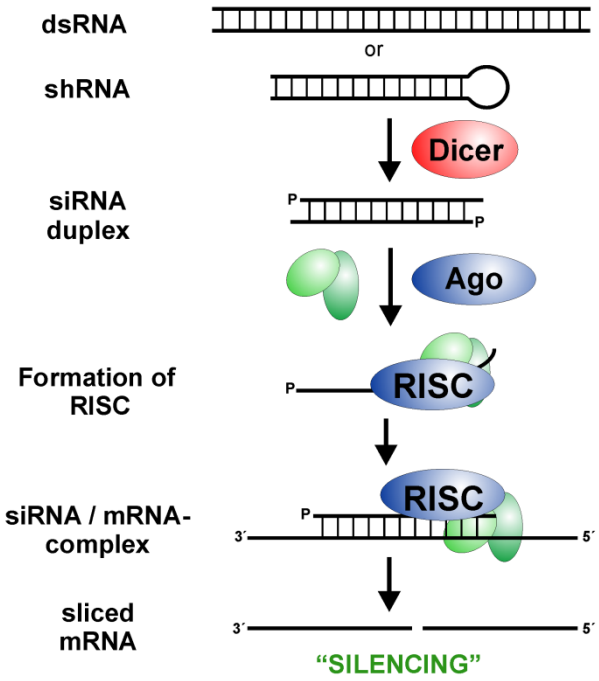
RNAi :

Gene silencing, PTGS (post-transcriptional gene silencing), transgene silencing, and quelling are additional terms for RNA interference (RNAi), which eliminates a specific gene's mRNA and thereby prevents its translation into an active gene product.

Mechanism of RNAi

The phenomena of double stranded RNA (dsRNA) or small interfering RNA (siRNA) controlling gene expression is known as RNA interference (RNAi). RNAi is a post-transcriptional regulatory mechanism that breaks down dsRNA into siRNA which is then

cleaved by endonucleases known as dicers that are specific to dsRNA. SiRNAs have short units of 20–25 bp. Together with the argonaute multi domain protein, which has an RNase H-like domain that degrades targets, proteins found in the cytoplasm known as RNA-induced silencing complex (RISC) will cleave siRNA into a single strand.



Systemic RNAi

RNA interference (RNAi) can have systemic effects on gene expression, allowing genes to proliferate throughout the body and endure throughout development. When dsRNA is administered locally, it amplifies and spreads silencing to neighbouring cells and offspring causing an RNAi response throughout the entire body. This effect is predicated on the existence of an RNA-dependent RNA polymerase (RdRP) which can interact with the RISC complex and use the hybridized siRNA strands as primers to create new dsRNA based on the partially damaged target template. The dicer enzymes function as an amplification step by acting on the produced dsRNA to produce new siRNAs (secondary siRNAs). Ex. A "loss-of-bristle" phenotype was observed throughout the entire epidermis of adult insects (Red flour beetle, *Tribolium castaneum*) when Tc-ASH (a homologue of the Drosophila sensory bristle-forming gene) dsRNA was injected into larvae at a single spot.

Environmental RNAi

The process by which sequence specific gene silencing takes place in reaction to dsRNA encountered in the environment is known as "environmental RNAi." After consuming dsRNA through feeding and soaking, the RNAi impact is seen. Therefore, in multicellular

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organisms, environmental RNA interference (RNAi) entails the uptake of dsRNA by a first group of cells (such as intestinal lumen cells), followed by the systemic dissemination of gene silencing into a secondary group of cells and tissues.

Methodology of dsRNA uptake in insects

The efficacy of gene silencing and the potential of dsRNA as an insect pest control agent are significantly impacted by the wide range of methods of dsRNA uptake in insects.

a) Microinjection

b) Soaking

c) Feeding of artificial diet

d) Developing transgenic insects

e) Virus mediated uptake

Microinjection

One of the best ways to give systemic RNAi types has been to inject dsRNA directly into insect's bodies. The efficiency of RNA interference will depend on the dsRNA's 5' end; a phosphorylated 5' end shows stronger gene silencing than a hydroxylated 5' end. The primary benefit is a high degree of gene expression inhibition efficiency.

Soaking

Gene expression can be inhibited by soaking *Drosophila melanogaster* embryos in a dsRNA solution, this method is similar to injection in that it requires a larger concentration of dsRNA. When *D. melanogaster* cells are soaked in ago dsRNA solutions, the expression of genes related to the cell cycle is efficiently inhibited and protein synthesis is increased.

Feeding of artificial diet

When *Epyphyas postvittana* (light apple brown moth) larvae are fed dsRNA, the expression of the carboxylesterase gene EposCXE1 in the larval midgut is inhibited. It has been shown that oral delivery of dsRNA of two cytochrome P450 genes, CYP6BG1 and CYP6BF1v4 *via* droplet feeding effectively lowers gene expression in *Plutella Xylostella* larvae.

Developing transgenic insects

Transgenic technology has produced transgenic *Aedes aegypti* that produces dsRNA. The transgenic method was initially applied in *D. melanogaster* with the GAL4/UAS transgenic system (a biochemical method used to study gene expression) that results in the expression of hairpin RNA. Cells in *D. melanogaster* can produce short hairpin RNA (shRNA) to suppress gene expression by using a U6 promoter.

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Virus-mediated uptake

This technique entails infecting the host with viruses that target the desired gene in the host and carry dsRNA created during viral replication. When the recombinant Sindbis virus is electroporated into *Bombyx mori* cells, it produces dsRNA that inhibits the expression of the BR-C gene, preventing the larvae from pupating or causing abnormalities in adults.

Five important factors largely influencing the silencing effect and the efficiency of RNAi as insect pest control technique

1. Concentration of dsRNA
2. Nucleotide sequence
3. Length of dsRNA fragment
4. Persistence of the silencing effect
5. Life stage of the target pest.

RNAi research on functional genes in some agriculturally important insects

INSECTS	GENES	METHODS	EFFECTS
Cotton bollworm	Cytochrome P450	Feeding	Inhibition of larval growth
Red flour beetle	TCHT5	Injection	Effects on egg hatching and moulting
Silkworm	Circadian clock gene BR-C	Transgenics Virus-mediated uptake	Disruption of egg hatching rhythm inhibit *BR-C* gene expression (Improper metamorphosis)
Brown plant hopper	Trehalose phosphate synthase	Feeding	Disturbed development, reduction in survival rate
Subterranean termite	Cell-1, Hex2	Feeding	Reduction in in group fitness and increased mortality
Western corn rootworm	Vacuolar ATPase (*v-ATP*)	Feeding	Delayed larval development and increased mortality
Drosophila	*CycE* and *ago*	Soaking	Inhibit gene expression
Beet armyworm	Chitin synthase	Injection	Disorder in insect cuticle
Honeybee	Transcriptor factor gene	Injection	Inhibition of Relish gene expression

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(https://www.gene-quantification.de/siRNA-mechanism.png)