

RNAi for insect-proof plants

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RNA interference induced in insects after ingestion of plant-expressed hairpin RNA offers promise for managing devastating crop pests.

Plants and the insects that feast on them have waged war for hundreds of millions of years. Insect pests cost billions of dollars in the form of crop losses and insecticides, and farmers face an ever-present threat of insecticide resistance, fueling a continual search for alternative pest-control strategies. Two papers in this issue^{1,2} show the potential of using RNA interference (RNAi) induced by hairpin RNAs as a new line of defense against coleopteran and lepidopteran pests.

Among transgenic approaches to insect-pest management, *Bacillus thuringiensis* (*Bt*) toxin has shown spectacular success, replacing chemical insecticides in a range of crops. But many important insect pests are not amenable to *Bt* protection, and there is an imminent danger of at least some species developing *Bt* resistance. RNAi has been exploited in plants for applications ranging from functional genomics to provision of valuable crop traits, such as resistance against viruses, bacteria and nematodes^{3,4}. However, it has not yet been harnessed to defend crops against insects.

In animals and especially in insects, RNAi is usually accomplished by injection of double-stranded RNA (dsRNA)⁵, but in nematodes it may also be triggered by a diet containing dsRNA⁶. This latter observation, combined with the demonstration of transgene-encoded RNAi in plants⁷, has long prompted speculation that plants could be protected from herbivorous insects by engineering them to express dsRNAs targeting vital insect genes. However, the passage of years without reports of success using this

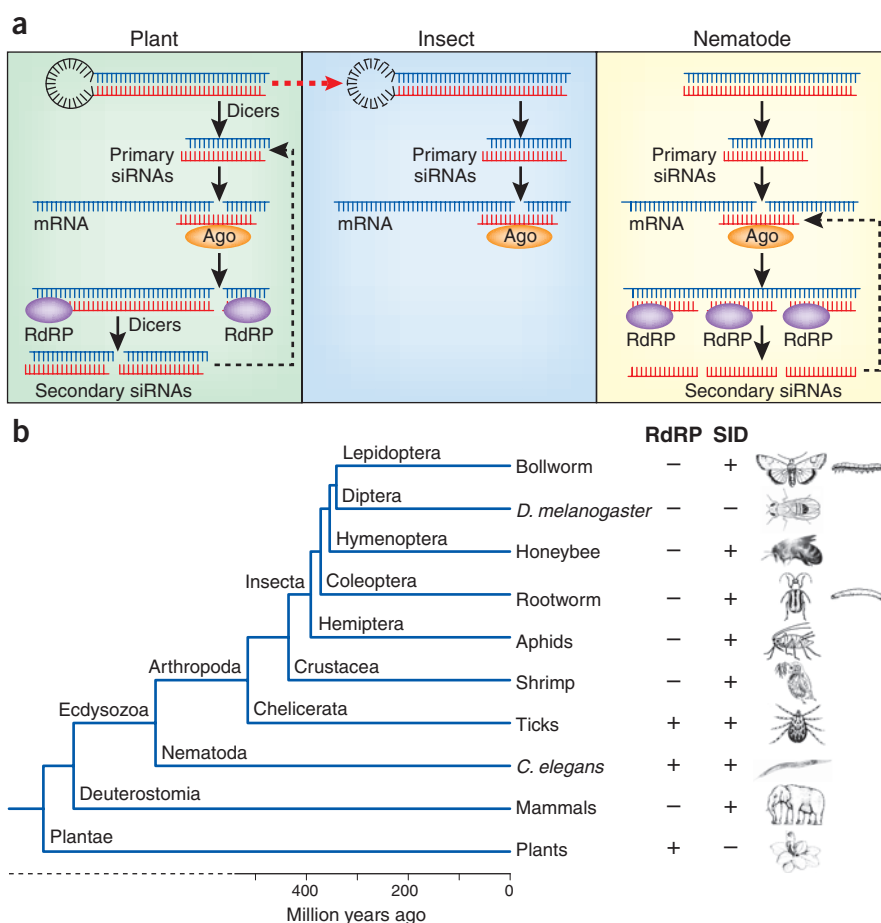


Figure 1 RNAi and the genes that control it in plants and animals. (a) Comparison of RNAi in plants, insects and nematodes. Hairpin RNA or dsRNA delivered into cells of each of these species is processed by Dicer enzymes into primary siRNAs, which in turn direct the endonuclease Argonaute (Ago) to their target mRNAs. The silencing amplification circuits that produce secondary siRNAs in both plants and nematodes are driven by RNA-dependent RNA polymerase (RdRP). As insects apparently lack RdRP activity (b), Ago-mediated RNA cleavage is probably directed solely by primary siRNAs. Silencing of vital or detoxifying genes of herbivorous insects after ingestion of hairpin RNA seems to be triggered by long dsRNAs (red arrow) and not plant siRNAs. (b) Evolutionary relationships of insects, higher animals and plants, indicating the presence or absence of genes encoding RdRP and the RNA channel transporter SID¹². Thus far, SID genes have been found in all animal genomes except those of dipterans. In arthropods, RdRP is restricted to the basal tick lineage, consistent with observations of systemic RNAi in these animals (as found in nematodes). Larval stages of the two pests discussed in the text are shown for emphasis. *D. melanogaster*, *Drosophila melanogaster*; *C. elegans*, *Caenorhabditis elegans*.

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approach seemed to suggest that simply expressing hairpin RNA in plant material to be ingested by an insect would not provide sufficient levels of intact dsRNA to trigger potent RNAi in the pest.

Experimental validation of this strategy, however, has now been achieved by Mao *et al.*¹—working with cotton bollworm—and Baum *et al.*²—working with a range of insects that includes western corn rootworm (WCR), southern corn rootworm and Colorado potato beetle. Their results suggest that transgene-encoded ingestible dsRNA may one day stand alongside *Bt* transgenes in insect management programs.

Baum *et al.* first used feeding assays, in which larvae were fed an artificial diet supplemented with specific dsRNAs, to screen a large number of essential insect genes. These assays identified 14 genes whose knockdown by low levels of dsRNA killed WCR larvae. Transgenic corn expressing one of these dsRNAs, targeted to a subunit of the midgut enzyme vacuolar ATPase (V-ATPase), showed protection against WCR infestation comparable to that provided by a *Bt* transgene. When dsRNAs designed to target WCR genes were tested in larvae from two other insect pests, mortality declined with decreasing sequence identity between the WCR genes and their orthologs in the other species, indicating that gene silencing was potentially very selective.

In an imaginative twist on the strategy, Mao *et al.* first identified the cotton bollworm's antidote against one of the cotton plant's natural insecticides—an indiscriminately toxic compound called gossypol—and then used RNAi triggered by ingested hairpin RNA to weaken the insect's tolerance to it. As the antidote, a cytochrome P450 monooxygenase, is induced by gossypol, and appears to neutralize it, silencing of the gene encoding this protein by RNAi exposes the insect to the full force of the insecticide.

Working with *Arabidopsis thaliana* rather than cotton, Mao *et al.* used a mutant line to show that the molecules inducing the RNAi in the feeding insect are probably the long hairpin RNAs—not their short interfering RNA derivatives that are normally generated by the plant's dicer-like (DCL) enzymes (Fig. 1). *A. thaliana* triple mutants lacking DCL2,

DCL3 and DCL4 produced more intact, long hairpin RNA and caused more pronounced silencing of the P450 gene. The efficacy of plant-produced long dsRNA in silencing insect genes may indicate that the success of the approach depends on transgenic plants expressing hairpin RNAs at rates faster than they can be diced. Perhaps earlier attempts involving similar approaches failed because they generated transgenic plants with insufficient hairpin RNA transcription.

An important question arising from both reports is whether the plant-induced RNAi amplifies and spreads within the insect. Minute amounts of dsRNA ingested by nematodes can trigger systemic silencing that may even be transmitted to a proportion

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of the next generation⁸, and amplification and spread of RNAi are well documented in plants⁹. Although it is plausible that a small amount of dsRNA ingested by the insect could be processed, amplified and mobilized throughout its body, insect genomics indicates that cotton bollworm and WCR lack the RNA-dependent RNA polymerase needed to drive this RNAi amplification (Fig. 1). Perhaps unamplified RNAi is effective against the P450 and V-ATPase genes because they are mainly expressed in the midgut and the gut cells of the feeding insect receive a continuous supply of hairpin RNA. The hairpin RNA would be diced into siRNAs in these cells and may also spread into surrounding cells and tissues via the intercellular dsRNA transport SID proteins, whose genes are present in almost all animals, including arthropods (Fig. 1). It would be interesting to discover whether phenotypic silencing can be observed for genes not expressed in the midgut.

Gossypol is only one of the thousands of allelochemicals produced by plants to protect themselves against insects. In turn, insects have several, often large families of

enzymes (for example, the P450s, glutathione S-transferases and carboxylesterases) able to detoxify plant chemical cocktails. Some of these have been implicated in the evolution of pesticide resistance¹⁰, for example through tissue-specific transcriptional upregulation¹¹. Such genes are attractive targets for applying the ingestible dsRNA approach to managing resistance against plant allelochemicals or insecticides. Indeed, Mao *et al.* have corroborated the conclusion of Baum *et al.* that the strategy works for multiple midgut-expressed genes in rootworms by demonstrating silencing of a glutathione S-transferase gene in bollworm. These findings strongly suggest that plant-delivered silencing of insect-detoxifying genes could be a powerful strategy for controlling insect pests.

Before these exciting possibilities can be realized, some significant challenges remain. Are all detoxifying genes equally amenable to this approach? Will this technology extend to other insect groups? Will ingestible RNAi be circumvented by sequence polymorphisms in pest populations? And will the successes seen in the lab translate into effective pest control in the field? For WCR at least, there is reason for optimism, as the assay involved measuring root damage on the crop itself. Nonetheless, the long-term effectiveness of the approach will need to be established. Likewise, the bollworm studies await rigorous assessment of how effectively cotton plants are protected. Despite these questions, there is no doubt that researchers and farmers have grounds to look forward to a new era in insect pest control.

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