Review

RNAi-mediated crop protection against insects

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Downregulation of the expression of specific genes through RNA interference (RNAi), has been widely used for genetic research in insects. The method has relied on the injection of double-stranded RNA (dsRNA), which is not possible for practical applications in crop protection. By contrast, specific suppression of gene expression in nematodes is possible through feeding with dsRNA. This approach was thought to be unfeasible in insects, but recent results have shown that dsRNA fed as a diet component can be effective in downregulating targeted genes. More significantly, expression of dsRNA directed against suitable insect target genes in transgenic plants has been shown to give protection against pests, opening the way for a new generation of insect-resistant crops.

Introduction: RNAi in insect genetics and crop protection

A decade has passed since the initial discovery of RNA interference (RNAi) in the nematode *Caenorhabditis elegans* [1], and it is now clear that double-stranded RNA (dsRNA)-mediated gene silencing is a conserved mechanism in many eukaryotes [2,3] (Box 1, Figure 1). Since its initial description the technique has become a valuable tool for functional genomics in insects, particularly in studying gene function in the model insect *Drosophila melanogaster* [4–6]. The preferred delivery methodology in the majority of insect studies has been microinjection of nanogram amounts of long dsRNA, synthesized *in vitro*, into the insect haemoceol [7]. This method of delivery contrasts with the situation in *C. elegans*, where RNAi effects can be produced by feeding bacteria expressing dsRNA [8,9], or even by soaking nematodes in dsRNA solution [10]. Microinjection of dsRNA in insects was considered to be necessary to produce RNAi effects because the complete genome sequence for *D. melanogaster* (and, subsequently, for other insects) has shown that they lack genes encoding RNA-dependent RNA polymerase (RdRP). RdRP is the enzyme necessary for the siRNA amplification step that leads to persistent and systemic RNAi effects [11]. The RdRP function is defined by a characteristic domain, designated PF05183 in the PFAM database (http://pfam.sanger.ac.uk), that has been identified in gene products of eukaryotic microorganisms, fungi, plants, nematodes and a primitive vertebrate (*Branchiostoma floridae* – a cephalochordate) but not in insects, molluscs or other vertebrates. The absence of RdRP in insects predicts that any effects of RNAi will be limited to cells that have taken up dsRNA and will require continuous input of dsRNA to persist. Injection of dsRNA into the body cavity, where it can circulate through the haemolymph, allows short-term effects on gene expression in most cells to be assessed.

The possibility of using RNAi effects to protect plants against insects by downregulating essential gene functions in the herbivore, thus resulting in its death, has been recognized for many years, but the method was considered unfeasible. The absence of dsRNA amplification implies that gene-knockdown effects produced by feeding RNAi to insects would be limited. Effects would only be expected in cells exposed to the nucleic acid; these cells would be those of the midgut and associated structures because these are the only regions of the insect not covered by the chitin exoskeleton (Box 2). Degradation of dsRNA in the gut would require continuous administration of high levels of dsRNA; production of sufficient dsRNA in a transgenic plant and its delivery in a sufficiently undegraded state to the insect would provide another significant technical problem, if a role in defence against insect pests was required. However, recent results have shown that many of these preconceptions were unduly pessimistic and that viable levels of insect resistance can be achieved by producing dsRNAs in plants [12,13].

RNAi in insects; cellular dsRNA uptake and export

RNAi-mediated gene knockdown in *Drosophila* is localised to the site of dsRNA delivery and effects are temporally limited; indeed, a systemic long-lasting RNAi response has never been observed in *Drosophila*, in contrast to *C. elegans* [1]. The systemic RNAi effect in *C. elegans* is a multistep process that requires the amplification and spread of the silencing signal [11,14]. If a similar system was present in insect pests, it would enable targets to be selected from the whole insect (not just gut-specific targets). In addition, the RNAi amplification step would negate the need for a continuous supply of high levels of dsRNA, and thus could avoid many of the problems associated with the instability of dsRNA in the insect gut.

What lessons can be learned from the use of RNAi in model organisms in relation to a 'real-life' biological problem, such as crop protection against insect pests? Uptake of dsRNA in *C. elegans* has been studied by genetic analysis. A mutant has been identified that is impaired in its ability to mediate a systemic RNAi response when dsRNA is delivered orally [15]. The gene identified, *systemic RNA interference deficient-1* (*sid-1*), is essential and sufficient to mediate systemic RNAi effect in *C.elegans*. When expressed in *Drosophila* S2 cells, *sid-1* enhanced the

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Box 1. RNA interference – a basic outline

RNA interference (RNAi) is the specific downregulation of gene expression by double-stranded RNA (dsRNA). The specificity is sequence-based and depends on the sequence of one strand of the dsRNA corresponding to part or all of a specific gene transcript (for recent RNAi reviews see [56–58]). RNAi is a post-transcriptional control mechanism involving degradation of a target mRNA. This degradation is mediated through the production of small interfering RNAs (siRNAs) from the dsRNA, which is cleaved by dsRNA-specific endonucleases referred to as dicers (from the *dicer* gene identified in *Drosophila melanogaster* [59], reviewed in [60,61]). The siRNAs are 21 bp dsRNA fragments carrying two base extensions at the 3′ end of each strand; one strand of the siRNA is assembled into an RNA-induced silencing complex (RISC) in conjunction with the argonaute multi-domain protein, which contains an RNaseH-like domain responsible for target degradation [62,63] (see Figure 1 in main text). The process is closely related to post-transcriptional gene regulation by microRNAs (miRNAs), where the end-result is inhibition of translation initiation, and shares many of the same components. In plants and nematodes, RNAi can have systemic effects on gene expression, so that gene knockout spreads throughout the organism and persists over development. The basis of this effect is thought to lie in the presence of an RNA-dependent RNA polymerase (RdRP) that is able to interact with the RISC complex and generate new dsRNA based on the partially degraded target template by using the hybridised siRNA strands as primers. The synthesized dsRNA is then acted on by the dicer enzymes to generate new siRNAs (secondary siRNAs), thus acting as an amplification step. In this way, once a dsRNA is introduced into a cell, its effect can persist over development; in addition, the dsRNAs can be exported to neighbouring cells and thus spread the gene knockout effect through the organism.

ability of S2 cells to uptake dsRNA at sub-optimal dsRNA concentrations. The gene is predicted to encode an eleven-helix transmembrane channel protein that is expressed on the cell surface and enables uptake of dsRNAs, thereby mediating a systemic RNAi effect. Further potential mechanisms for RNA transport have been suggested by the recent identification of a further *C. elegans* dsRNA uptake mutant, *sid-2* [16]. *sid-2* mutants are unable to mediate an RNAi response when fed bacteria expressing specific dsRNAs. The *sid-2* gene product has been identified as a gut-specific transmembrane protein with a single transmembrane region. To demonstrate functionality, a related nematode, *Caenorhabditis briggsae*, which is defective in uptake of dsRNA from the gut lumen, was transformed with *C. elegans sid-2,* and a systemic RNAi phenotype was restored [16]. This demonstration of the complexity of RNAi-uptake mechanisms and the systemic spread of an RNAi signal in a single organism needs to be bourne in mind when considering RNAi in insects.

Could the absence of RNA transport mechanisms explain why *Drosophila* cannot manifest a systemic RNAi response? Homologues of the *C. elegans sid-1* gene have been identified in insects such as *Tribolium castaneum*, *Bombyx mori* and *Apis mellifera* but not in the *Drosophila* genome. *sid-2* homologues have only been detected in nematodes closely related to *C. elegans*. A *sid-1* homologue has also recently been identified in aphids [17]. However, recent evidence suggests that dsRNA uptake into cultured *Drosophila* S2 cells does not involve a *sid-1*-based mechanism but takes place by receptor-mediated endocytosis [18,19] because pharmacological inhibition of endocytosis also inhibited RNAi effects. Endocytosis of dsRNA also seems to occur in *C. elegans* because knockdown of components of the endocytotic pathway by RNAi results in worms with a 'loss-of-RNAi-function' phenotype [18]. These results suggest that receptor-mediated endocytosis is a widespread mechanism for dsRNA uptake and might well occur across different insect orders. If this is the case, herbivorous insect pests from different orders can be effectively targeted by oral delivery of dsRNA. Further understanding of the complexities of insect dsRNA-uptake mechanisms might facilitate the targeting of specific insect pests.

Systemic RNAi in insects

To evaluate the potential for systemic RNAi effects in insects, an experimental approach using species other than *Drosophila* has been pursued. Insect systemic RNAi was first documented in the coleopteran *Tribolium castaneum* (flour beetle) by two independent studies. In the first, a homologue of the *Drosophila* sensory bristle-forming gene *Tc-achaete-scute* (*Tc-ASH*) was identified and targeted. Injection of *Tc-ASH* dsRNA into larvae at a single discrete site resulted in a 'loss-of-bristle' phenotype over the entire epidermis of adult insects [20]. In the second study, a parental RNAi effect transmissible between generations was demonstrated by identifying and targeting developmental genes. Injection of dsRNA specific to (i) *Distalless* (leg development gene), (ii) *maxillopedia* (homeotic gene) and (iii) *proboscipedia* (encoding a homeotic protein required for the formation of labial and maxillary palps) was used to produce an RNAi effect in both mother insects (injected) and developing progeny embryos after egg hatch [21]. Thanks to its well-documented, robust systemic RNAi response and the recent completion of its genome sequence, *Tribolium* is becoming an accepted model for the study of systemic RNAi in insects. Intriguingly, a recent genome comparison of *C. elegans* and *Tribolium* revealed a lack of conservation of a systemic RNAi mechanism [22]. For example, *Tribolium* lacks a *C. elegans*-like RdRP, so the signal amplification observed in *Tribolium* must be based on a different gene with a similar activity, or possibly even a different mechanism. RdRP-like activity has been demonstrated in cell-free extracts from *Drosophila* embryos, even though the RdRP gene is not present in insects [23].

Future research aimed at elucidating the mechanism of systemic RNAi in insects is likely to broaden the range of insects amenable to systemic RNAi and of genes that can be regarded as targets for a knockdown effect on expression. RNAi-mediated gene knockdown has been reported in several insect orders, including Diptera, Coleoptera, Hymenoptera, Orthopetra, Blattodea, Lepidoptera and Hemiptera [6,7,20,21,24–32], although most of these studies have used injected dsRNA.

dsRNA feeding in insects

Development of a robust dsRNA feeding methodology in insects that mimics the results obtainable with *C. elegans* (where efficient suppression of gene expression by orally delivered dsRNA is routine) is a prerequisite for utilization of RNAi for crop protection against insect pests. Turner *et al.* [31] provided a convincing demonstration of RNAi effects after dsRNA feeding in larvae of the light brown apple moth (*Epiphyas postvittana).* dsRNAs

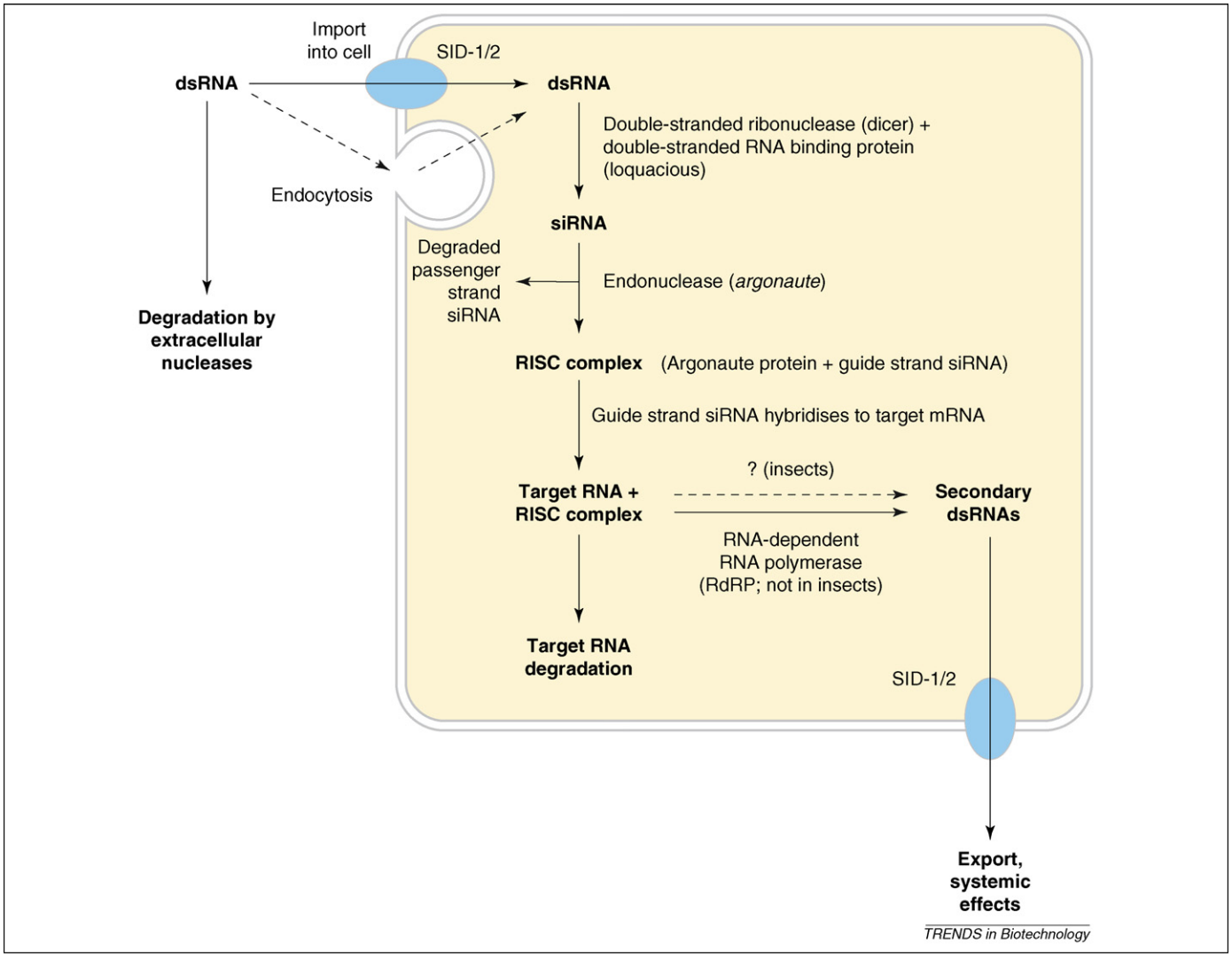

Figure 1. Functional stages of gene silencing with double-stranded RNA (dsRNA) in cells of lower animals. The figure shows steps involved in local and systemic gene silencing. Exogenous dsRNA is imported into cells, processed by *dicer* into small interfering RNA (siRNA; 21 bp + 2-base 3′ extensions on each strand) and assembled with the argonaute protein into the RNA-induced silencing complex (RISC). The RISC complex targets and degrades specific mRNAs based on the siRNA sequence. Systemic RNAi effects are mediated through the production of new dsRNAs by RNA-dependent RNA polymerase (RdRP), which uses the target RNA as a template and is primed by siRNA strands. The secondary dsRNAs can be exported from the cell to spread the RNAi effect to other cells. Gene names in italics have been identified in *Drosophila melanogaster*. The transport proteins SID-1 and SID-2 have been identified in *Caenorhabditis elegans*, as has the RdRP enzyme. Transport mechanisms might differ between diffeent organisms.

directed against carboxyesterases were incorporated into an artificial diet. Gene repression was observed after two days of feeding, and maximal repression occurred after seven days. These genes are thought to be gut-expressed, and thus only a local RNAi effect was required for repression. However, in the same investigation, knockdown of a gene expressed in the adult antenna could be achieved through feeding dsRNA to larvae, demonstrating a persistence of the RNAi signal throughout the larval and adult stages and a systemic spread of RNAi signal from the gut to the antennae. In contrast to these positive results, an earlier report showed that midgut aminopeptidase-N gene in larvae of the lepidopteran *Spodoptera litura* was efficiently downregulated by microinjection of dsRNA into the insect haemoceol but stated that attempts to feed dsRNA were unsuccessful in generating an RNAi response [28], although no details of methodology were given. An RNAi response after feeding dsRNA has also been reported in the bug *Rhodnius prolixus* (Hemiptera), where a salivary gland transcript encoding nitroporin 2 (NP2) was targeted both by oral delivery of dsRNA and by microinjection [32]. Both treatments produced downregulation of NP2 expression; however, microinjection was more effective (75% reduction in gene expression) than dsRNA feeding (42% reduction).

Variation in the midgut environment between different species might dictate whether a feeding approach will be successful. However, comparisons based on existing data are difficult because the susceptibilities of different targets to RNAi effects show considerable variation in model species. Some targets have proved to be completely refractory to suppression; for example, most of the neuronally expressed genes of *C. elegans* [33].

Lessons learned from development of RNAi for plant parasitic nematodes

Plant expression of dsRNAs directed against genes in pathogens has become an established technique, and

Box 2. The insect gut

The insect gut is divided into three regions; foregut, midgut and hindgut. Of these the first two are continuations of the 'outside' of the insect and are chitin-lined, so that their surfaces do not present areas of exposed cells (although receptors and transporters are present to allow processes such as taste recognition in the mouth cavity and water transfer in the hindgut to occur). The midgut region is the only part of the gut that contains surfaces of exposed cells, and it is the main site of exchange between the circulatory system (haemolymph) and the gut contents. The midgut itself is responsible for nutrient absorption, whereas excretion and water balance take place primarily in the Malpighian tubules attached to the hind end, which carry out a function similar to that of the kidney in higher animals. RNAi effects occurring in insects as a result of oral delivery of dsRNA are presumably mediated by the midgut surfaces through exposure of cells of the midgut epithelium and the Malpighian tubules to dsRNA in the gut contents.

Conditions in the gut vary considerably between insect orders. Gut pH is an important factor in insect digestion and can vary from predominantly acidic (coleopteran larvae) to strongly alkaline (up to pH 10.5 in some species of Lepidoptera). In addition, within a single insect the pH changes along the gut and with distance from the gut epithelium. The stability of ingested dsRNA in the insect gut could be affected both by chemical hydrolysis (which increases with increasing pH) and by enzymes present in the gut contents.

plants that show increased resistance to a plant virus [34–36] and bacteria [37] through an RNAi effect have been described. The use of dsRNA approaches for the control of plant parasitic nematodes has been recently reviewed in detail [38–40]; however, it is worth highlighting some of the key developments in the application of this technology. Transgenic plants expressing dsRNAs specific to genes encoding a root knot nematode (*Meloidogyne* spp.) splicing factor and integrase (a chromatin remodelling protein) successfully knocked down transcripts in the pest, resulting in almost complete resistance [41]. In another study, a nematode secretory peptide (16D10) that stimulates root growth was successfully downregulated in four closely related species of root knot nematode by transgenic plants expressing dsRNAs, resulting in levels of resistance that varied between 63% and 90% [42]. A further study demonstrated the feasibility of downregulating a root knot nematode transcription factor with plant-expressed dsRNAs; however, in this case loss of function did not result in a deleterious phenotype [43]. To date, there is only one report of the successful downregulation of a cyst nematode transcript via similar approaches [44]; this might reflect the poor uptake of dsRNAs by cyst nematodes, in which the feeding tube has a lower exclusion limit than in root knot nematodes [40].

Although the nematode system clearly differs from insects, it highlights several important points that must be considered in developing an RNAi approach in insect pest species. RNAi effects are species-specific because knockdown experiments and identification of lethal phenotypes in *C. elegans* has not resulted in a universal set of 'nematode target genes' that are useful for protection against plant parasitic nematodes. Therefore, the success of the RNAi approach is dependent on careful target selection (which takes into account differences in specificity between different species) and the ability of the target nematode to mount a systemic RNAi response.

Using RNAi to produce insect-pest-resistant plants

Despite having been considered for many years, application of RNAi technology to give resistance to herbivorous insects has only just been realised. Two recent papers have described transgenic plants producing dsRNAs directed against insect genes. These plants showed enhanced resistance to the economically important agricultural pests cotton bollworm (*Helicoverpa armigera*; Lepidoptera) and Western corn rootworm (WCR; *Diabrotica virgifera virgifera* LeConte; Coleoptera). The key to the success of this approach is: (i) identification of a suitable insect target and (ii) dsRNA delivery, which includes *in planta* expression of dsRNA and delivery of sufficient amounts of intact dsRNA for uptake by the insect. Although different approaches were used for the generation of insect-resistant plants, careful target selection was common to both (see Figure 2).

Baum *et al.* [12] utilised a screening approach where genes from WCR were identified in cDNA libraries, and genes encoding polypeptides predicted to provide an essential biological function were classified as 'targets'. A total of 290 potential targets were identified, and corresponding dsRNAs were synthesized *in vitro*; their effects on larval performance were determined by delivery in artificial diet feeding trials. Using this approach a total of 14 genes from the initial list demonstrated specific downregulation of target sequences at low dsRNA concentrations and resulted in insect stunting and mortality. The most effective dsRNA, directed against a gene encoding V-type ATPase A, demonstrated rapid knockdown of endogenous mRNA within 24 h of ingestion and triggered a specific RNAi response with low concentrations of dsRNA. The orally delivered dsRNA could produce systemic silencing of genes (encoding both V-type ATPase subunits and β-tubulin) throughout the insect.

The specificity of RNAi-mediated insecticidal effects is an important consideration for the use of this technology in a practical application; effects on non-target insects should be minimised. dsRNAs directed against three target genes (β-tubulin, V-ATPase A and V-ATPase E) demonstrated an effective RNAi response in WCR that resulted in high larval mortality. These dsRNAs were also delivered to three other coleopteran plant pests: Southern corn rootworm (SCR; *Diabrotica undecimpunctata howardii*), Colorado potato beetle (CPB; *Leptinotarsa decemlineata)* and cotton boll weevil (*Anthonomus grandis* Boheman). The dsRNAs demonstrated significant larval mortality in SCR and CPB, although only at higher concentrations than those used for WCR. The sequence identities between WCR and CPB were only 83% and 79% for V-ATPase A and V-ATPase E, respectively. As expected, synthesis of gene-specific dsRNAs for CPB V-ATPase A and V-ATPase E showed increased effectiveness in feeding trials compared with the WCR orthologues. Cotton boll weevil was not only completely insensitive to the three WCR-directed dsRNAs, but was also insensitive to dsRNAs directed against orthologous boll weevil genes, emphasising the differences between insect species in susceptibility to orally delivered RNAi strategies.

To demonstrate the practical application of this technology, transgenic corn was engineered to express dsRNA directed against WCR V-ATPase A. The plants were sub-

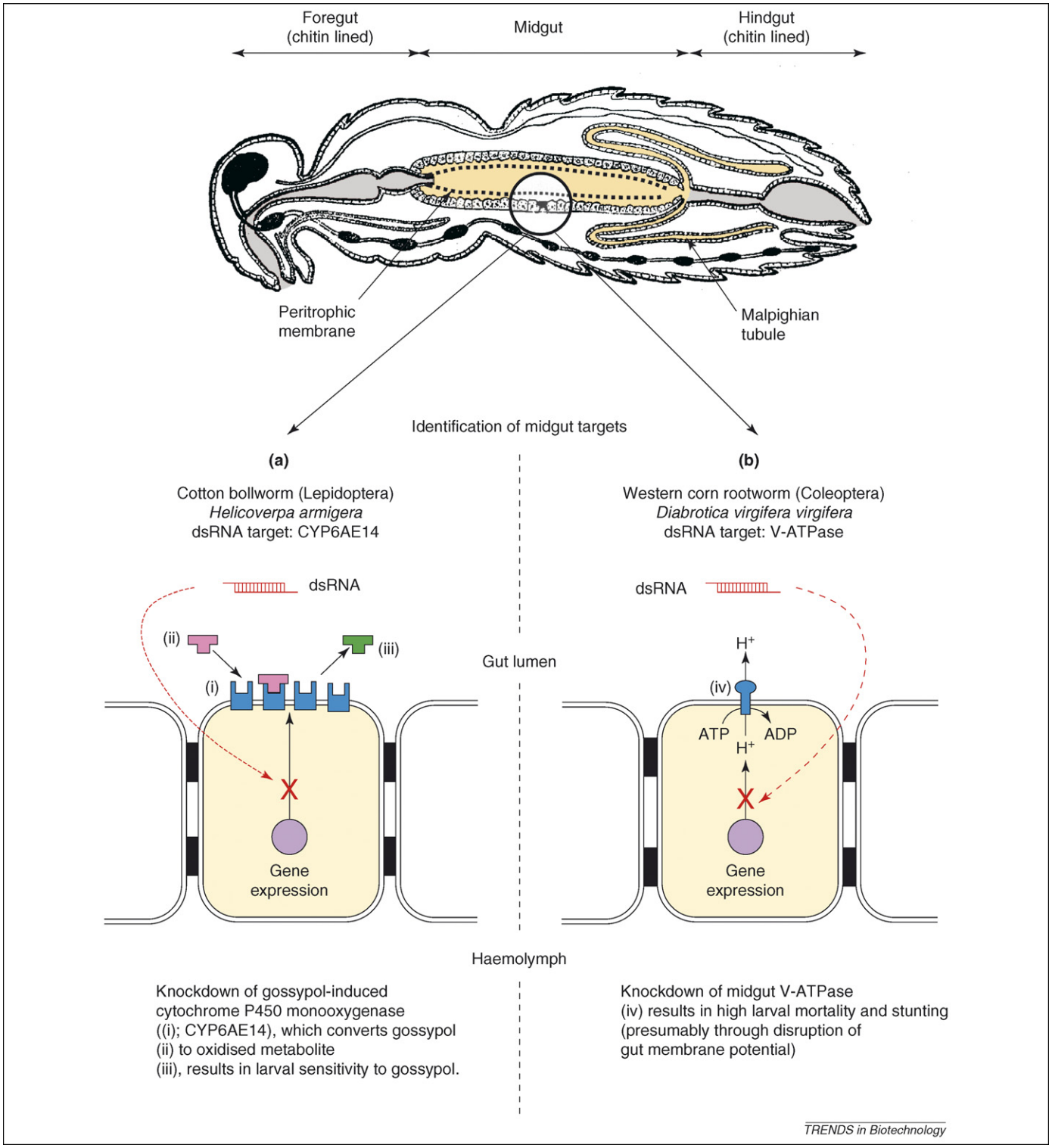

Figure 2. Overview of RNAi approaches for insect-resistant transgenic plants. Double-stranded RNA (dsRNA) produced *in planta* can lead to targeted gene silencing in Lepidoptera and Coleoptera pest species [12,13]. dsRNAs corresponding to specific insect targets are expressed *in planta* and are cleaved by endogenous plant Dicer enzymes to produce short interfering RNAs (siRNAs) of around 21 nucleotides. Large dsRNA and siRNA cleavage products are expressed throughout plant tissues and are orally delivered to insect herbivores feeding on transgenic plant material. For gene-silencing to initiate in targeted insect pests, large dsRNAs and siRNAs must persist in the insect gut, and sufficient quantities must be present for uptake into cells in contact with RNAs (the exact uptake mechanism in target insects remains unknown). Approach **(a)**: a gut-specific cytochrome monooxygenase, CYP6AE14, has been identified (i) whose expression correlates with larval growth on diets containing gossypol (ii), a cotton secondary metabolite. CYP6AE14 is presumably involved in detoxification of gossypol (iii) because specific knockdown of this gene product by dsRNAs delivered in artificial diet and by transgenic plant material increases larval sensitivity to gossypol [14]. Approach **(b)**: a related study [13] used a screening approach to identify a lethal phenotype in *Diabrotica virgifera virgifera* when midgut V-type ATPase A (V-ATPase) (iv) was downregulated by dsRNAs delivered in artificial diet feeding trials and transgenic corn. Although no direct evidence was presented for the deleterious effects observed in larvae, it is tempting to speculate that knockdown of V-type ATPase A results in disruption of electrochemical gradient across the gut epithelia, which results in high larval mortality.

jected to WCR infestation and demonstrated a significant level of protection compared to controls; that is, they showed reduced damage from WCR feeding.

A different approach was used by Mao *et al.* [13]. By studying the interaction between cotton bollworm and cotton, they identified a cytochrome P450 gene, *CYP6AE14*, which is highly expressed in the insect midgut and whose expression is correlated with larval growth when gossypol, a cotton secondary metabolite, is added to artificial diets. The authors concluded that expression of *CYP6AE14* is causally related to gossypol tolerance, presumably via detoxification of this compound, and that suppression of the expression of this gene could increase the sensitivity of the insect larvae to the plant's endogenous defence. Tobacco and *Arabidopsis* plants were engineered to produce dsRNAs directed against the bollworm *CYP6AE14* gene. When plant material of both species was fed to larvae, effective repression of the endogenous *CYP6AE14* transcript was observed, and the insects showed increased sensitivity to gossypol when transferred to artificial diets. Interestingly, expression of *CYP6AE14*-directed dsRNA in an *Arabidopsis dicer* mutant (knockout of *Arabidopsis dicer* genes *DCL2*, *DCL3* and *DCL4*) resulted in the production of longer dsRNAs in the plant that were more effective in gene repression of *CYP6AE14*. This result shows that optimal efficiency of repression of targeted genes in pests might require stabilization of dsRNAs. The group of Mao *et al.* [14] has recently reported that they have engineered cotton to express the cotton bollworm *CYP6AE14* dsRNA and that the plants show partial resistance to *Helicoverpa armigera*, as expected[*].

Future prospects for RNAi-based control of insect pests

The feasibility of using RNAi in the protection of crops against insect herbivores has been demonstrated. This approach holds great promise for the future because it allows a wide range of potential targets for suppression of gene expression in the insect to be exploited. However, at the moment the method compares unfavourably with existing transgenic technologies giving resistance to coleopteran and lepidopteran herbivores. From the limited data currently available for whole-plant bioassays in laboratory trials, protection of maize against corn rootworm, even in the best-performing RNAi-expressing plants, is not as effective as in transgenic maize engineered to produce a modified Cry3Bb *Bacillus thuringiensis* (Bt) toxin [45]. Although it is unfair to compare the resistance of non-optimised research material with a commercial product, RNAi-expressing maize is unlikely to replace Bt-maize in the short term, especially as the effectiveness of the new crop-protection strategy at the field level remains to be determined. However, recent reports of resistance to Bt toxins being observed in field populations of insects exposed to transgenic plants [46,47] will provide an additional impetus for the development of alternative crop-protection strategies.

Which insect genes should be targeted? The screening approach used by Baum *et al.* has already identified a series of potential targets in corn rootworm, of which a gene encoding the β-subunit of a COPI coatomer complex was the most effective in terms of LC_{50} for RNAi in artificial diet. The COPI complex is involved in translocation of proteins from endosomes to the cytoplasm, as well as other potential roles in protein trafficking in the cell, but it is not obvious why interference with this function should be lethal. The screening approach can thus identify targets that would not necessarily be predicted from functional considerations but has the drawback of being very labour-intensive if large numbers of insect bioassays are required. However, the demonstrated efficacy of targeting V-type ATPase A could easily be extended to other insect species. The approach of Mao *et al.*, in which insect detoxification mechanisms towards plant secondary metabolites are targeted, has the advantage of being predictable and specific to pests that feed on a crop producing a defined defensive chemical [48,49]. It can be readily extended to detoxification mechanisms in other plant–insect interactions. Further development of RNAi biotechnology could also seek to complement existing crop protection strategies; for example it might be possible to use technologies in combination to counter broad-range, protein-degradation-based resistance to Bt toxins (observed in highly polyphagous insect pests such as *Heliothis virescens*, which gain resistance through the upregulation of specific proteinase genes [50]).

Further increases in the effectiveness of RNAi strategies might be achieved by utilizing multiple targets. The feasibility of pyramiding multiple targets by RNAi has been demonstrated in *Drosophila* [51] but has yet to be applied to crop-protection strategies. The development of an understanding of the specificity of RNAi gene knockdown in insects should allow crops to be produced that express a cocktail of dsRNAs that are highly effective against target insect pest species. The sequence specificity of dsRNAs can be maximised by a careful bioinformatic approach, although multiple gene knockdown events might be achieved with a single dsRNA by targeting genes belonging to large families with high sequence similarity. However, care must be taken to avoid the possibility that loss of function is compensated for by another untargeted gene.

Although RNAi is unlikely to have an immediate effect on crop protection against lepidopteran and coleopteran plant pests, for which Bt-based strategies offer a high degree of protection, the technology is likely to be taken up for applications where Bt-based approaches have proved difficult, for example protection against flies (dipterans), or where no effective Bt toxins are known, for example protection against sap-sucking homopteran pests such as aphids, leafhoppers and whitefly. Targeting these phloem-feeding insect pests would require *in planta* expression of dsRNAs and transport of dsRNAs in phloem sieve elements. The transport of RNA in plant phloem is well documented; viral RNA genomes, endogenous cellular mRNAs and small noncoding RNAs are known to be transported in plant phloem elements [52–55]. However, there is no evidence for phloem transport of dsRNA; even though systemic RNAi-based gene silencing occurs in plants, recent evidence suggests that siRNAs are transported as single stranded sense and antisense molecules

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[54] and that all RNA in phloem is single stranded. It is possible that dsRNA expressed in phloem cells could be converted to single-stranded RNA (ssRNA) for transport in the phloem by the plant endogenously, but the stability and uptake of ssRNA into insect cells after feeding might then prove a problem. Further experimentation will be required to determine whether dsRNAs can be introduced into plant phloem sap to make targeting specialist phloem feeders by RNAi feasible with current technology.

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