this produces networks that are stiff and unable to undergo large shape changes.

Ware *et al.* demonstrate an approach to produce complex director orientations in soft, elastic LCEs, and, as a result, effect complex shape changes. First, photoalignment defines a complex liquid crystal pattern on a surface. Next, this pattern is imprinted into an LCE with a liquid crystal fluid that polymerizes slowly to form a polymer network. Ware *et al.* implement chemistry that proceeds without added solvent to form a soft, elastic polymer network. In an analogy to the tens of thousands of pixels in an LCD screen that produce images on a screen, they produce LCEs with 3D patterned elements known as "voxels." Their light-patterning and polymerization technique can produce more than 20,000 voxels that dictate how the LCE changes shape. By changing the pattern in the alignment layer and within these voxels, they produce a conical actuator, a polymer hinge, and a self-foldable Miura Ori pattern, all from an initially flat film.

The work of Ware *et al.* is an important step toward realizing materials that can assume arbitrary and programmable shapes, but a number of challenges remain, especially the incorporation of smart, real-time control over the shape of the LCE. This capability would require integration of the LCE with an electronic system capable of turning the liquid crystal orientation within each voxel. Adding nanomaterials could produce a faster and more sensitive shape-response to a variety of signals (*10*), and reversible chemistry may enable materials that can be reprogrammed to assume different shapes (*11*). Eventually, we might have access to implantable biomaterials that can respond to their surrounding environment or self-folding devices that can disassemble and shrink to small sizes for storage and transport. ■

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PLANT SCIENCE

Insecticidal RNA, the long and short of it

Transgenic plants that express insect-specific RNA in chloroplasts kill pests through RNA interference

By **Steve Whyard**

Insects cost the agricultural sector billions of dollars every year in lost crop yields and insecticide expenditures. The continued use of chemical insecticides has inadvertently selected for more resistant pest strains, prompting higher doses and more frequent applications to control them. The advent of transgenic plants, such as those expressing insecticidal *Bacillus thuringiensis* (Bt) toxins, reduces the use of chemicals while offering protection to some crops (*1*), but not all insects are affected by Bt toxins, and continued use of Bt technologies will eventually see the rise of Bt-resistant insects. To stay ahead of the pests will require additional technologies. On page 991 of this issue, Zhang *et al.* (*2*) describe a clever modification to an existing transgenic plant technology that produces insecticidal RNAs. The trick is to express lethal RNA in the plant's photosynthetic organelles, the chloroplasts.

RNA interference (RNAi) is a mechanism that suppresses gene expression through the presence of double-stranded RNA (dsRNA). Within a eukaryotic cell, long dsRNA is cleaved by the enzyme Dicer into short interfering RNAs (siRNAs), which are about 21 nucleotides in length. The siRNAs, in conjunction with an RNA-induced silencing complex, scan the cell's RNA molecules until a complementary match is found. Once recognized, the target RNA is destroyed. This means of RNA repression therefore silences the corresponding gene.

Sequence-specific gene silencing may enable the development of a new generation of pesticides. Feeding insects dsRNAs that target essential genes through the RNAi mechanism can result in death of the insect, and the in-built gene specificity of this process offers the potential to design dsRNAs that kill one or a few species but have no effect on nontarget species (*3*). Indeed, plants have been engineered to express dsRNAs that greatly reduce insect damage (*4*–*8*), although in most of these studies, not all insects were completely eradicated or protection of the plant was incomplete.

Zhang *et al.* compared transgenic potato plants engineered to produce insecticidal dsRNAs either within chloroplasts or in the cell's cytoplasm. As such, transgenes encoding anti-insect dsRNA were incorporated either into the chloroplast DNA (the organelle harbors its own genome) or the plant's nuclear genome. The results were dramatic: All of the potato beetles feeding on the chloroplast-transformed plants died after 5 days, whereas beetles feeding on plants with dsRNA in the cytoplasm were not affected. Previous dsRNA-feeding studies (*8*, *9*) indicated that ingested long dsRNAs were much more effective than ingested siRNAs at initiating the RNAi response in insects. Chloroplasts lack the cellular RNAi machinery and therefore, long dsRNA produced in these organelles are protected from being cleaved by Dicer. Thus, beetles that fed on the chloroplast-transformed plants ate almost entirely long dsRNA, whereas beetles that ate nuclear-transformed plants consumed mostly siRNAs. The beetles that fed on the chloroplast-derived long dsRNA showed substantial reduction of the target RNAs and ultimately died from lack of the critical RNA and corresponding protein. By contrast, beetles that fed on the plants with dsRNA expressed in the cytoplasm showed only weak RNAi responses—gene expression in these insects was unaffected.

> ***"... the potential for RNAi to control some of the most costly pest insects is great."***

RNAi induced by ingested dsRNA was first observed in the nematode *Caenorhabditis elegans* (*10*), where it was found that the dsRNA did not remain in the worm's gut cells but spread systemically throughout most of the organism. The uptake and systemic spread of dsRNA in *C. elegans* is

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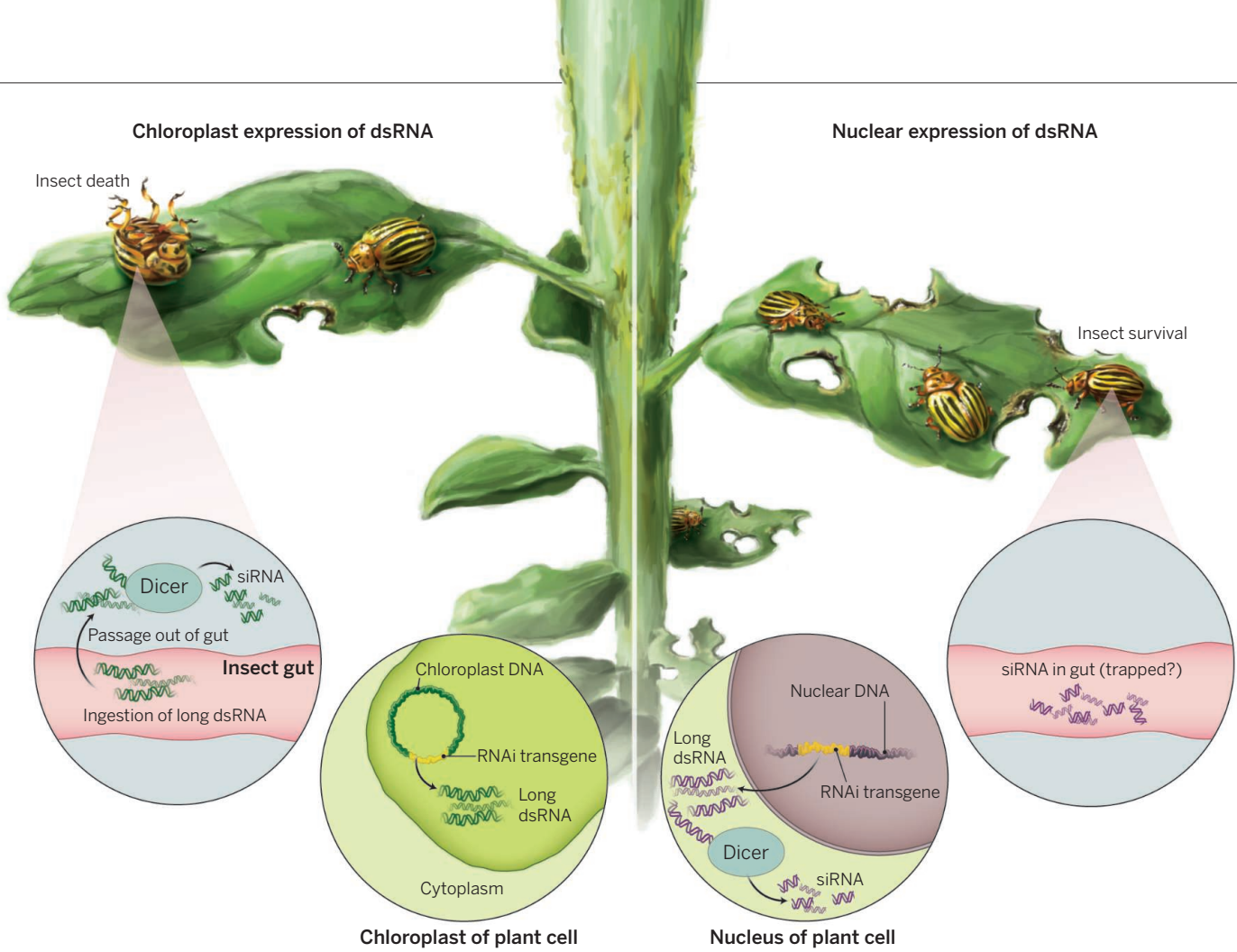

Anti-insect RNA. Transgenic potato plants engineered to express insect-specific long dsRNA in chloroplasts kill potato beetles, whereas those plants expressing dsRNA in the plant cell cytoplasm do not. Chloroplasts do not process dsRNA into siRNA. Therefore, beetles that feed on the chloroplast-transformed plants ingest almost entirely long dsRNA, whereas beetles feeding on nuclear-encoded dsRNAs consume mostly short interfering RNAs (siRNAs). Long dsRNAs are readily absorbed by the beetle's gut cells, and a strong RNAi response is elicited, resulting in high mortalities of the feeding pests. The siRNAs either may not be readily absorbed in the gut or are not in a form suitable to induce RNAi effectively.

facilitated by two transmembrane proteins called systemic RNA interference–defective protein 1 (SID-1) and SID-2 (*11*). Most insects have proteins that share some similarity to the nematode's SID proteins (which function like channels), but their role in dsRNA uptake has not been adequately defined. In the few studies that have examined SID-like proteins in insects, loss of function of the SID-like proteins did not have a large impact on RNAi. Other dsRNA uptake mechanisms, such as receptor-mediated endocytosis (an engulfment process), may play a more important role in dsRNA uptake in insects (*12*). Long dsRNAs, rather than siRNAs, are selectively taken into the gut cells of feeding corn rootworm beetle larvae, but the mechanism of uptake and its curious selectivity for long over short dsRNAs were not identified (*12*). It will be interesting to determine whether different insects use different dsRNA uptake mechanisms, and whether all such mechanisms preferentially select long dsRNAs. Systemic RNAi has also been observed in many insects after ingestion of dsRNA (*12*), but nothing is known of how the RNAi signal is propagated from cell to cell.

It is also unclear whether all crop plants process dsRNA with the same efficiency as the potato plant studied by Zhang *et al.* Indeed, in the few other studies where other plants (corn, cotton, rice, tobacco) were engineered to express anti-insect dsRNAs, the dsRNAs were synthesized in the cytoplasm and presumably were subjected to the host plant's RNAi machinery (Dicer). Each of these studies reported either more pronounced RNAi or better efficacy at controlling pest insects using a cytoplasm-derived dsRNA than did Zhang *et al.*, which could indicate that these plants do not process long dsRNAs as effectively as the potato plants.

Despite the many uncertainties surrounding how insects acquire dsRNAs from their diet and whether all insects will be equally affected by insecticidal dsRNAs, the potential for RNAi to control some of the most costly pest insects is great. With continued improvements in optimizing delivery of the dsRNAs to insects, combined with the specificity that they can confer regarding gene silencing, dsRNA-based insecticides could provide a new generation of environmentally safe insect control technologies to keep crops safe from the hungry mouths of our insect competitors. ■

ILLUSTRATION: V. ALTOUNIAN/*SCIENCE*

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