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Plant–insect interactions: molecular approaches to insect resistance

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Recent advances in our understanding of induced responses in plants and their regulation, brought about by a revolution in molecular biology, have re-focused attention on the potential exploitation of endogenous resistance mechanisms for crop protection. The future goal of crop biotechnology is thus to engineer a durable, multimechanistic resistance to insect pests through an understanding of the diversity of plant responses to insect attack.

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Abbreviations

Bt *Bacillus thuringiensis*
PI proteinase inhibitor
VOC volatile organic compound

Introduction

With a projected increase in world population to 10 billion over the next four decades, an immediate priority for agriculture is to achieve maximum production of food and other products in a manner that is environmentally sustainable and cost-effective. Losses due to insect herbivores, estimated at 10–20% for major crops, are a significant factor in limiting food production. Engineering crop plants for endogenous resistance to insect pests has been one of the real successes of GM technology, following the commercial introduction of genetically modified maize, potato and cotton plants expressing genes encoding the entomocidal δ -endotoxin from *Bacillus thuringiensis* (*Bt*) in the mid-1990s. Currently, over 10 million hectares are planted to *Bt* crops globally, mainly with plants expressing toxins effective against lepidopteran pests (butterflies and moths), although toxins for insects in the orders Diptera (flies and mosquitoes), Coleoptera (beetles and weevils), and Hymenoptera (wasps and

bees) have also been identified [1]. Although plants expressing *Bt* toxins have been successful in protecting agricultural crops against insect pests, there is still a need to develop further strategies for engineering insect resistance. This review considers some current research interests in this area.

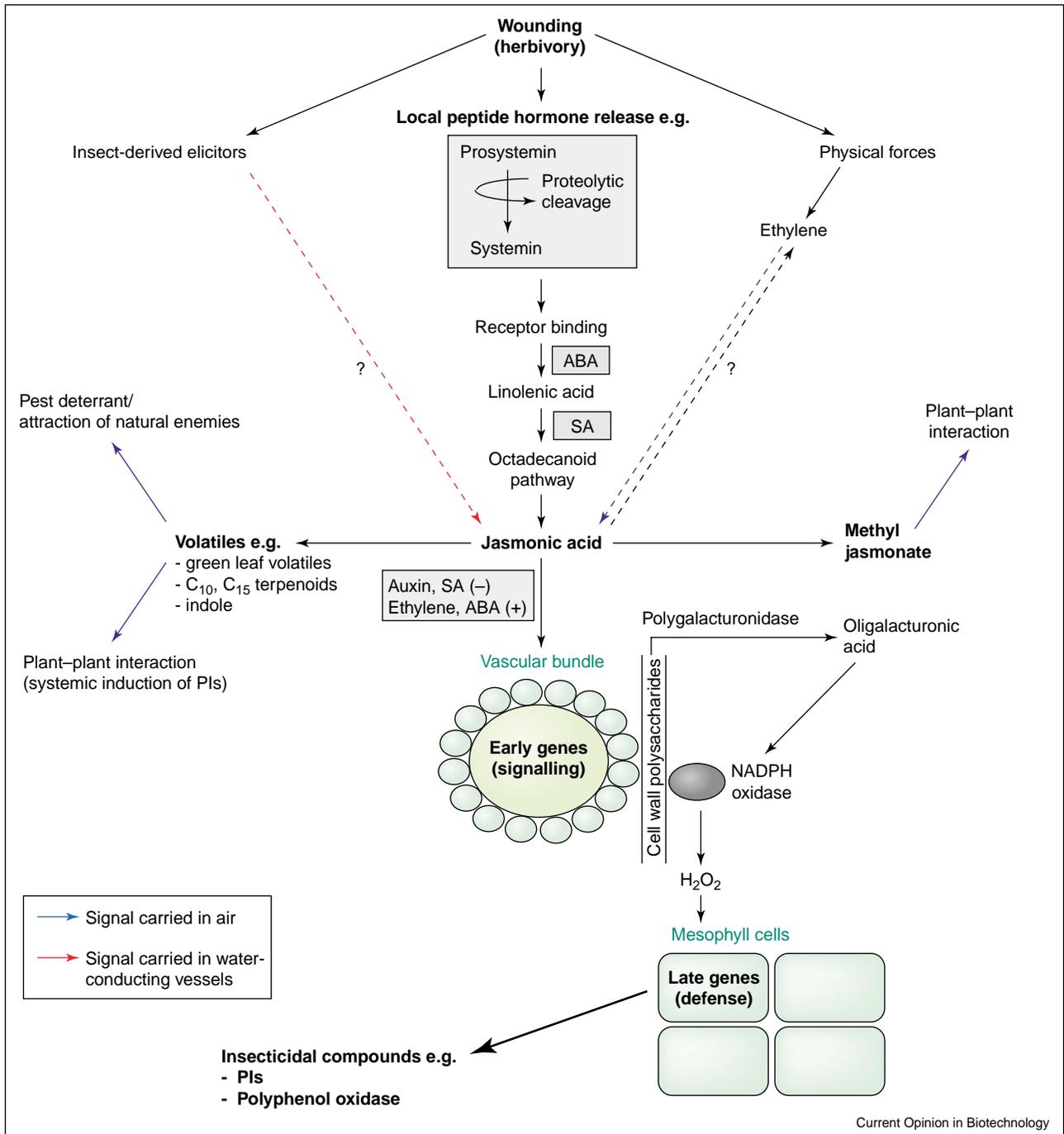
Future strategies in biotechnology

Although the development of pest resistance has been predicted to limit field durability of *Bt* crops [2], the use of management practices, such as provision of refugia (which provides conditions suitable for survival of *Bt*-susceptible pests) has so far been successful in preventing this. Pyramiding (stacking) of genes encoding different *Bt* toxins has been developed as a method for preventing the development of pest resistance, and for conferring greater levels of pest control [3–5]. For example, corn lines have recently been developed [6,7] co-expressing two δ -endotoxins from *Bt* for resistance to corn rootworm. Expressing insecticidal proteins from sources other than *B. thuringiensis* in crop plants should reduce the likelihood for development of insect resistance to toxins. Therefore, other strategies based on the expression of toxins produced by foreign genes from plants (e.g. enzyme inhibitors and lectins) [8–11] and animal sources including insects (e.g. biotin-binding proteins [12], neurohormones [13[•]] and enzyme inhibitors [14]) are being developed. Toxins from other insect pathogens are also opening new routes to transgenic pest control. Recently, a highly toxic 283 kDa protein from the bacterium *Photorhabdus luminescens* was expressed in *Arabidopsis* and shown to confer insect resistance [15]. The use of fusion proteins to increase the spectrum and durability of resistance is also actively being pursued [13[•], 16[•], 17], as is the development of hybrid Cry proteins [18] with increased activities against insect pests from different orders. A key challenge still facing the biotechnologist, however, is the identification of novel genes that will produce insecticidal products with suitable characteristics for use in transgenic crops.

Endogenous defence

Alternative strategies for protecting crops from insect pests seek to exploit the endogenous resistance mechanisms exhibited by plants to most insect herbivores, through a greater understanding of induced defences in plants. Induced defences are exemplified by the wounding response, first identified as the local and systemic synthesis of proteinase inhibitors (PIs), which block insect digestion in response to plant damage [19^{••}].

Figure 1



The wounding response. Generalised overview of the plant wounding response and signalling molecules that can modulate it. The pathways necessary for both local and systemic induction of insecticidal proteins are shown. Abbreviations: ABA, abscisic acid; SA, salicylic acid.

However, more recent research has shown that induced defences also involve the plant's ability to produce toxic or repellent secondary metabolites as direct defences, and volatile molecules that play an important role in indirect defence [20]. Insect herbivores activate induced defences both locally and systemically via signalling pathways

involving systemin, jasmonate, oligogalacturonic acid and hydrogen peroxide (Figure 1).

Molecular responses

Ecologists have long understood that plants exhibit multimechanistic resistance towards herbivores, but the

molecular mechanisms underpinning these complicated responses have remained elusive [21^{••}]. However, recent studies investigating the plant's herbivore-induced transcriptome, using microarrays and differential display technologies, have provided novel insights into plant-insect interactions. The jasmonic acid cascade plays a central role in transcript accumulation in plants exposed to herbivory [22]. A single microarray-based study revealed that the model plant *Arabidopsis* undergoes changes in levels of over 700 mRNAs during the defence response [23]. By contrast, only 100 mRNAs were upregulated by spider mite (*Tetranychus urticae*) infestation in lima bean (*Phaseolus lunatus*), although a further 200 mRNAs were upregulated in an indirect response mediated by feeding-induced volatile signal molecules [24]. The insect herbivore has an additional effect on the plant's response over that caused by mechanical tissue damage [25]. Analysis of timing, dynamics and regulation of the expression of 150 genes in leaves of *Arabidopsis* showed that many genes strongly induced by mechanical damage were induced less, or not at all, when the plant was attacked by the lepidopteran pest *Pieris rapae*.

The studies of Baldwin and his group on the interaction between insect herbivores and tobacco (*Nicotiana attenuata*) have provided new insights into the molecular basis of plant defence. They estimate that approximately 500 mRNAs constitute the insect-responsive transcriptome in tobacco [22]. However, many of these genes are of unknown function, and many changes in gene expression do not represent induction of defence-related proteins. Photosynthetic genes, for example, are downregulated in tobacco plants in response to insect attack. Further microarray analysis [26^{••}] has demonstrated putative upregulation of defence-associated transcripts and downregulation of growth-associated transcripts. This analysis provided evidence for the simultaneous activation of salicylic acid, ethylene, cytokinin and jasmonic acid-regulated pathways during herbivore attack. Similar co-activation of numerous signalling cascades in response to various stresses has been found in *Arabidopsis* [27] and supports the idea of a network of interacting signal cascades. Microarray analysis also identified direct defensive responses in dramatic increases in PI transcripts, and increases in transcripts encoding putrescine *N*-methyl transferase, which catalyses the first committed step of nicotine biosynthesis, as well as metabolic commitment to terpenoid-based indirect defences.

Signalling

Deciphering the signals that regulate herbivore-responsive gene expression will afford many opportunities to manipulate the response. Signalling molecules such as salicylic acid, jasmonic acid and ethylene do not activate defences independently by linear cascades, but rather establish complex interactions that determine specific responses. Knowledge of these interactions can be exploited

in the rational design of transgenic plants with increased disease/insect resistance [28].

Further signalling pathways might remain to be elucidated. For example, the discovery of electrophilic cyclopentenone oxylipins (oxygenated fatty acids; ably reviewed by Farmer *et al.* [29^{••}]) provided evidence for another signalling molecule involved in local and systemic responses [30[•]].

Insect-specific elicitors

The primary method used by plants to 'sense' the presence of insect herbivores has been shown to be insect oral secretions. Schittko *et al.* [31] demonstrated that *Manduca sexta* regurgitant extensively modifies the tobacco wound response, eliciting jasmonic acid and ethylene bursts [20], and confirmed the identity of the elicitors as fatty acid conjugates [32]. Volicitin, a fatty acid conjugate present in lepidopteran oral secretions, also causes upregulation of expression of genes involved in the biosynthesis of both indole [33] and terpene volatiles in maize [34].

Sap-feeding insects

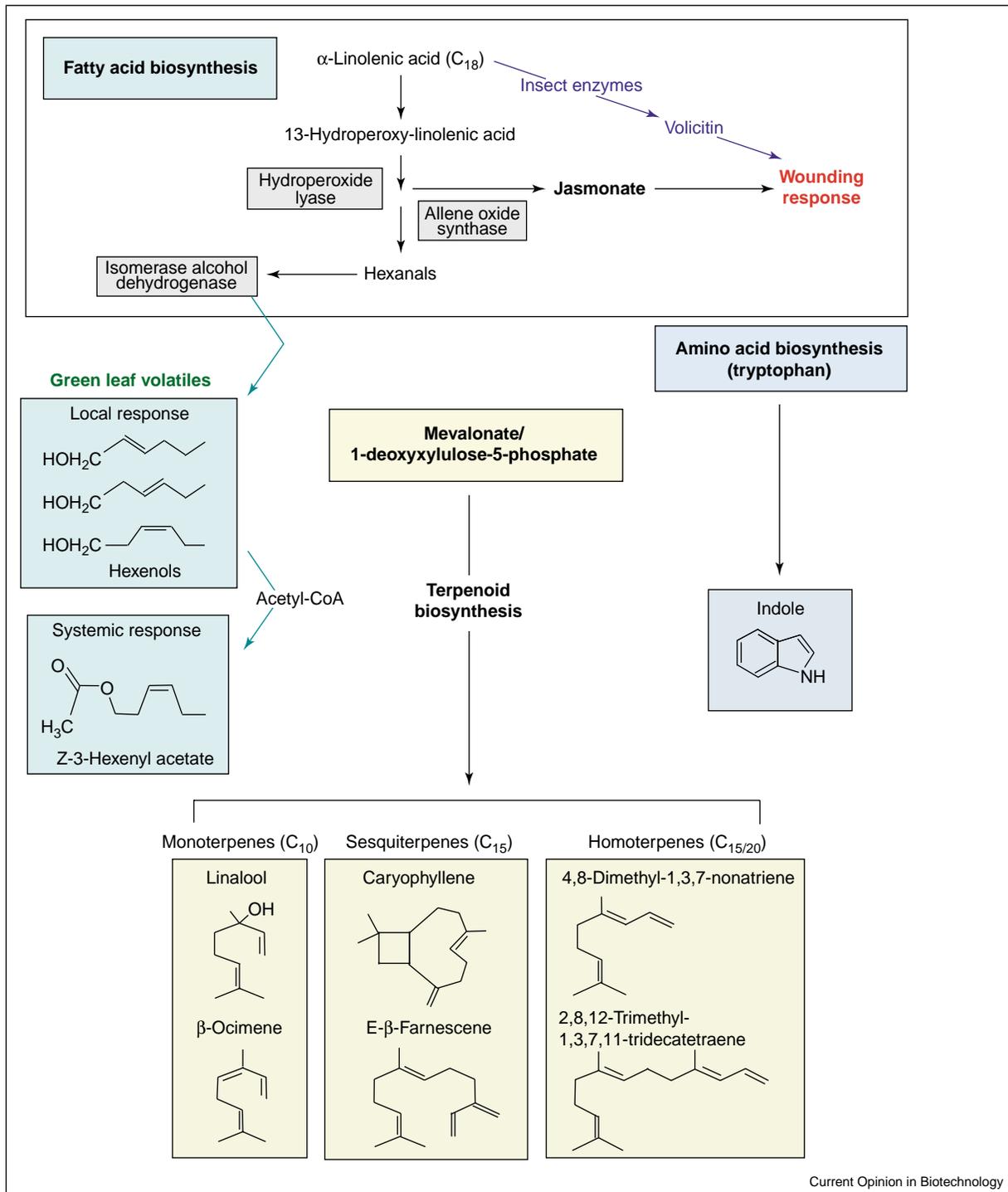
Whereas most herbivorous insects cause extensive damage to plant tissues when feeding, many insects of the order Homoptera feed from the contents of vascular tissues by inserting a stylet between the overlying cells, thus limiting cell damage and minimising induction of a wounding response. In contrast to wounding, plant responses following attack by these insects have been shown to be typical of pathogen attack, with examples of gene-for-gene interactions being known (i.e. genetic systems determining virulence in the pathogen are paralleled by genes conferring resistance in the host) [35,36^{••}]. However, these pathogen-induced pathways can induce expression of many of the genes upregulated by wounding owing to pathway cross-talk.

Moran and Thompson [37] demonstrated that phloem-feeding by the green peach aphid (*Myzus persicae*) on *Arabidopsis* induced expression of genes associated with salicylic acid responses to pathogens, as well as a gene involved in the jasmonic acid mediated response pathway. These results suggest stimulation of response pathways involved in both pathogen and herbivore responses. Microarray and macroarray data have identified genes involved in oxidative stress, calcium-dependent signalling, pathogenesis-related responses, and signalling as key components of the induced response [36^{••}].

Indirect defence (volatile production)

The role of plant volatiles in indirect defence has been described as 'top-down' defence [21^{••}]. Some volatiles (Figure 2) appear to be common to many different plant species, including C₆ aldehydes, alcohols and esters (green leaf volatiles), C₁₀ and C₁₅ terpenoids, and indole,

Figure 2



Volatile compound biosynthesis. General overview of plant volatiles synthesised in response to insect attack (both locally and systemically).

whereas others are specific to a particular plant species. Many volatiles are preformed and act in herbivore deterrence; furthermore, the wounding response also includes the formation of volatile compounds. Top-down control of herbivore populations is achieved by attracting pre-

dators and parasitoids to the feeding herbivore, mediated by these volatile organic compounds (VOCs). For example, genes involved in the biosynthesis of the maize VOC bouquet are upregulated by insect feeding [33,34]. In addition, herbivore oviposition has been shown to induce

VOC emissions, which attract egg parasitoids [38]. Herbivore-induced VOCs can also elicit production of defence-related transcripts in plants near the individual under attack [24,39]. Exposure to herbivore-induced volatiles in lima bean results in the transcription of genes involved in ethylene biosynthesis [24].

The manipulation of VOC biosynthesis can affect insect resistance. Transgenic potatoes in which production of hydroperoxide lyase (the enzyme involved in green leaf volatile biosynthesis) was reduced were found to support improved aphid performance and fecundity, suggesting toxicity of these volatiles to *M. persicae* [40]. In a current review of the topic Degenhardt *et al.* [41] discuss the potential of modifying terpene emission with the aim of making crops more attractive to herbivore natural enemies.

Responses in herbivores to plant defences

The optimal use of plant protection strategies against insects depends on pest responses to insecticidal compounds produced by plants. As a result of co-evolution, herbivorous insects have adapted to plant defences that they normally encounter [19,42]. For example, coleopteran and lepidopteran herbivorous insects are able to adapt to dietary PIs by the production of proteinases insensitive to inhibition [43,44–47]. Novel proteases insensitive to inhibition are selected from a repertoire of digestive enzymes available in the insect genome. Despite the abilities of some insects to adapt to PIs, attempts to produce transgenic plants resistant to insect pests via expression of these proteins are continuing (e.g. Rahbé *et al.* [9]). De Leo *et al.* [48] show that the effectiveness of a PI against a given insect pest is related to its expression level in plants, its activity towards the targeted insect proteinases, and the adaptive capacity of the target insect. The ability of pests to adapt to specific PIs is species-specific; for example, expression of a mustard trypsin inhibitor (MTI-2) in oilseed rape resulted in high mortality and significantly delayed larval development of diamond-backed moth (*Plutella xylostella*), but not of armyworm (*Spodoptera littoralis*). Detailed knowledge of enzyme–inhibitor interactions and the response of insects to exposure to PIs is important if this strategy for crop protection is to succeed.

Detoxification and insect modulation of the wounding response

Many insects are able to detoxify potentially toxic secondary metabolites, using cytochrome P450 monooxygenases and glutathione *S*-transferases. These enzymes are induced by exposure to toxic plant secondary compounds; for example, xanthotoxin (a furanocoumarin) induces P450 expression in corn earworm [49]. Recently, Li *et al.* [50] have shown that corn earworm uses signalling molecules from its plant host, jasmonate and salicylate, to activate four of its cytochrome P450 genes, thus making the induction of detoxifying enzymes rapid and specific.

Insects can effect subtle changes in plant defence responses, resulting in an ability to tolerate the defences. Although insect oral components (saliva and regurgitant) act as elicitors recognized by the plant to upregulate the wounding response, a recent report suggests that a salivary glucose oxidase produced in corn earworm actually suppresses the production of the defensive compound nicotine, normally induced upon wounding in tobacco [51]. By contrast, glucose oxidase is known to induce PIs in tomato [52]. These subtle shifts in defensive responses highlight the complexity apparent in the wounding response.

Tritrophic interactions

Agro-ecosystems consist of complex trophic interactions [53] and any pest control strategy must recognize that the impact of insecticidal compounds extends beyond the herbivore at the second trophic level. The impacts of insect-resistant transgenic plants on predators and parasitoids, which play an important role in suppressing insect pest populations, have been considered in recent research.

Insect predators are exposed to the insecticidal compounds used for protection against phytophagous pests [54–56] by consumption, giving rise to potentially deleterious tritrophic effects if the predator is susceptible to the toxin. There is an extensive history of safe use relating to *Bt* toxins [2] and, recently, important insect natural enemies (ladybirds and stinkbugs) have been shown to be equally able to adapt to PI exposure as the pests that they prey upon [55,56].

Conclusions

Advances in our understanding of induced responses in plants and their regulation, brought about by a revolution in molecular biology, has re-focused attention on the potential exploitation of endogenous resistance mechanisms for crop protection. Although this strategy is an integral component of integrated pest management strategies, it does not afford similar levels of protection as those provided by the use of ‘direct’ protective methods such as the expression of *Bt* toxins. The goal of the plant breeder, and now the biotechnologist, is to engineer durable multimechanistic resistance to insect pests in crops, and increased knowledge of induced defence mechanisms and their molecular control is likely to play an important role in realising this aim.

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