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Molecular mechanisms underlying plant memory in JA-mediated defence responses

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ABSTRACT

Plants must respond to biotic and abiotic challenges to optimize their Darwinian fitness in nature. Many of these challenges occur repeatedly during a plant's lifetime, and their sequence and timing can profoundly influence the fitness outcome of a plant's response. The ability to perceive, store and recall previous stressful events is likely useful for efficient, rapid and cost-effective responses, but we know very little about the mechanisms involved. Using jasmonate-elicited anti-herbivore defence responses as an example, we consider how 'memories' of previous attacks could be created in (1) the biosynthetic processes involved in the generation of the oxylipin bursts elicited by herbivore attacks; (2) the perception of oxylipins and their transduction into cellular events by transcription factors and transcriptional activators; and (3) the role of small RNAs in the formation of long-term stress imprints in plants.

Key-words: herbivory; insect; jasmonate (JA); JAZ repressors; memory; priming; plant defence; plant fitness; RNA interference (RNAi); stress imprint; tobacco.

INTRODUCTION

Plants, with the exception of potentially immortal, clonally derived organisms, are the life forms that have been documented to live the longest on earth. In the most extreme examples, the Great Basin bristlecone pines (*Pinus longaeva*) can exceed the age of 4000 years, capturing a record of ancient climate changes in their tree rings (Leavitt 1994). In addition to these passive records of environmental change, plants retain information about past experiences and retrieve this information to modify responses to new environmental challenges, capacities that have inspired a lively debate about plant behaviour and intelligence. Some authors advocate that plants should be included among intelligent organisms (Trewavas 2003, 2004, 2005, 2007; Brenner *et al.* 2007); others urge more rigorous studies and experimental approaches be undertaken before new scientific disciplines such as plant neurobiology are established (Alpi *et al.* 2007). In particular, the latter group of scientists critiqued in a collective motion the transfer of terms and concepts developed in animal biology (e.g. neuron, neurotransmitter, brain, synapse) to explain the mechanisms of plant behaviour (Alpi *et al.* 2007). In this review, we examine plant 'memory' to describe how a previous experience and the subsequent modification of a response can be used to improve a plant's fitness in a given environment. To avoid controversy, some authors suggest the use of 'stress imprint' to describe these responses in order 'to avoid anthropomorphic connotations associated with the word memory' (Bruce *et al.* 2007).

Broadly speaking, memory is the capacity organisms have to benefit from their past experience (Tulving 1985). In order to recollect their past, organisms must perceive, store, retain and subsequently be able to retrieve information about these events. Memory is usually associated with higher organisms and most extensively studied in higher animals. Less often, memory concepts are applied to the behaviour of higher plants that seem to lack the central memory organ – the brain – considered essential for cognitive processes. However, Tulving (1985) proposes the existence of three memory systems in a (mono)hierarchical arrangement: procedural, semantic and episodic memory, each characterized by different levels of consciousness. In Tulving's definition, procedural memory is associated with anoetic (non-knowing) consciousness, semantic memory with noetic (knowing) consciousness and episodic memory with autonoetic (self-knowing) consciousness. The lowest level of consciousness characteristic for procedural memory – anoetic consciousness – refers to the ability of organisms to sense and to react to external and internal stimulation, which all plants and simple animals are capable of (Tulving 1985).

Plants show hallmarks of anoetic consciousness: they perceive environmental signals and store information carried by these signals in various forms, including changes in concentration of small molecules, abundance of proteins or modifications of their genetic material (e.g. methylation), or structural scaffold supporting DNA molecules in the cells (histone acetylation and deacetylation) (Trewavas 2003, 2005; Struik, Yin & Meinke 2008). Plants can respond at the level of a single cell to the entire multicellular organism: individual cells are able to store information about the

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orientation of newly formed cell walls during cytokinesis (Lloyd & Buschmann 2007), and whole plants can store permanent memories of cold exposure known as vernalization (Michaels & Amasino 2000). While vernalization usually refers to the acquisition by vegetative biennial plants of the ability to flower by exposure to cold, exposure during zygotic and somatic embryogenesis can also influence the timing of bud set that occurs in the second year of seedling growth after somatic embryo development in Norway spruce (*Picea abies*) (Kvaalen & Johnsen 2008). Finally, transgeneration imprints associated with increased somatic homologous recombination in subsequent generations of plants have been demonstrated in *Arabidopsis thaliana* (Molinier *et al.* 2006).

Examples in the previous paragraph show that memory in plants can have local and short-term character, but it can also last years in individual plants or even across generations of plants. In this review, we concentrate on short-term memory mechanisms in plants that occur after their interaction with biotic and abiotic stressors. In particular, we use examples from plant–insect interactions and concentrate on oxylipins as the main molecular signals involved in these highly dynamic interactions. In the last section, we also briefly summarize the role of epigenetic mechanisms that provide plants with long-term memory and describe in more detail the role of currently identified small RNAs as a possible memory medium in plants.

'MEMORY' FORMATION IN PLANT–HERBIVORE INTERACTIONS

Memory of wound signal in *Bidens pilosus* system

One of the first examples of memory behaviour associated with the wounding of plants has been described in early experiments with the dicotyledonous plant *B. pilosus* L. (Desbiez *et al.* 1984). A few needle puncture wounds to one cotyledon resulted in preferential outgrowth of the opposing bud after decapitation; such 'beheading' made them receptive to the stored signal of the wound to the cotyledon. No outgrowth of buds was observed without removal of the apex; however, just 14 d later, asymmetry in bud growth following decapitation was statistically significant. Interestingly, removing both cotyledons 5 min after the symmetry-breaking signal was introduced to one cotyledon did not abolish asymmetric growth, suggesting that a rapidly elicited long-distance signal mediates storage and retrieval of information about previous wound experience. Several mathematical models were created using the *B. pilosus* system for storage and recall of environmental signals in plants (Demongeot, Thomas & Thellier 2000; Thellier *et al.* 2000, 2004; Demongeot, Thellier & Thomas 2006). According to our current knowledge, a jasmonic acid (JA)-dependent systemic spread of wound signals could explain such behaviour in plants; however, the identity of this long-distance signal still remains unknown.

Application of memory models to plant–herbivore interactions

Plants are sessile, fixed to a particular location in the environment after germinating and establishing a root system. Without the option of changing location, they must rapidly adapt to the surrounding environment and optimize the use of resources, such as water, minerals and light intensity, by adjusting their root length and density, and the growth of their shoots. In addition, plants are often exposed to rapid unpredictable challenges from a spectrum of biotic and abiotic stresses. Because stress conditions often recur in a plant's lifetime – seasonally driven herbivore attacks are an example – memory-based behaviour is likely useful, allowing plant resources to be conserved and plant fitness to be improved by minimizing the costs of delayed, insufficient or even exaggerated defence responses (Heil & Baldwin 2002).

Herbivore attack is often repeated over time and reflects the natural behaviour of insects – feeding followed by digestion (inactivity) – with the feeding increasing in intensity as herbivores grow. In addition, insects can move between and within the plants in unpredictable ways (Bernays & Woods 2000; Bernays, Singer & Rodrigues 2004). Insect behaviour supports the model that bases plant memory on transient and recurrent characters of herbivory-derived signals (summarized in Fig. 1a). In this model, positive memory formation is expressed as a shortened response time to subsequent elicitation events, the increased response amplitude or increasing background (baseline) levels of defence after each elicitation. Alternatively, a negative model of perception memory would display prolonged signal frequency, lowered amplitude or declining baseline levels of defence. Later we will analyse the signalling pathways involved in the oxylipin burst, which are compatible with this model. Extending this model, increasing basal levels of signal molecules (e.g. transcription factors) resulting from previous elicitations may speed up the time required for the plant to respond (Fig. 1b). Even though pathogen attacks do not usually occur as recurrent stress signals with relatively short amplitudes like herbivory, the model shown in Fig. 1b is consistent with priming responses in plant–pathogen interactions discussed below.

PRIMING RESPONSES IN PLANT–HERBIVORE INTERACTIONS

Definition of priming responses

One of the important plant adaptations to complex environment challenges is priming behaviour (Prime-A-Plant Group: Conrath *et al.* 2006; Beckers & Conrath 2007; Frost *et al.* 2008). In principle, priming means that plants that previously experienced abiotic or biotic stress have altered, and most often enhanced, their ability to resist and survive recurring stress conditions. In the current terminology, 'priming' is usually associated with biotic stresses while 'hardening' is used for response adaptations of plants to

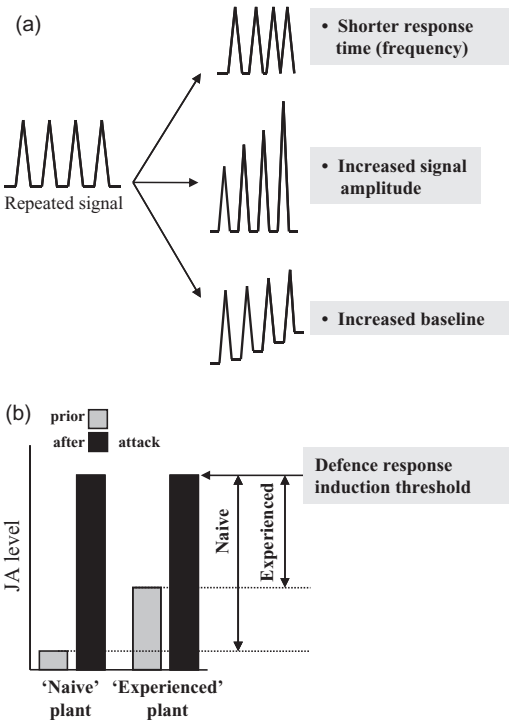

Figure 1. Theoretical model of memory formation in plants based on bursts of oxylipins elicited when herbivore-specific elicitors are introduced into wounds during feeding. Tobacco and other plants respond to herbivore attacks with a precisely defined and highly reproducible burst of jasmonic acid (JA) and other oxylipins. In the proposed model of memory formation, discrete JA bursts would allow plants to 'count' herbivore attacks and modify their response according to the number of herbivores as well as their size – information acquired from the characteristic feeding behaviour of insects. (a) Repeated bursts may result in changes in the frequency, amplitude or baseline levels of response. (b) In the response threshold model, 'experienced' plants could reach the active threshold status earlier and trigger defence faster compared with 'naive' plants.

abiotic factors (Bruce *et al.* 2007). Priming effects span trophic levels: plants can be primed by herbivore attack, pathogen infection, root colonization with micro-organisms, exposure to the metabolites these organisms produce, and even synthetic compounds.

A typical example of priming in plant–herbivore interactions is the vaccination of tobacco plants against herbivores by the cell-content-feeder mirid *Tupiocoris notatus* (Kessler & Baldwin 2004; Voelckel & Baldwin 2004). The combination of herbivore growth-slowing direct defences and predator-attracting indirect defences results in greater hornworm (*Manduca sexta*) mortality on plants attacked by mirids. Priming by airborne signals can boost direct and indirect resistance: exposure of undamaged maize leaves to green leafy volatiles (GLV), which are normally emitted by plants in response to herbivory, primed plants to produce more JA and sesquiterpenes compared with GLV-untreated controls (Engelberth *et al.* 2004). Volatile signalling could overcome the vascular constraints on systemic signalling in hybrid poplar saplings, resulting in primed defence responses in adjacent leaves with little or no vascular connection to wounded leaves (Frost *et al.* 2007). Using a differential display approach, Ton *et al.* (2007) identified a set of genes in maize that are induced by caterpillar feeding. These genes did not respond directly to volatile organic compounds (VOCs) emitted by neighbouring plants; however, a majority of these caterpillar-inducible genes became primed for earlier and/or stronger induction after a subsequent attack, correlating with reduced caterpillar feeding and development. An increased rate of direct defence nicotine accumulation was observed after repeated (one, two or three) elicitations with methyl jasmonate (MeJA) in *Nicotiana sylvestris* plants, suggesting that plants alter the timing and magnitude of their induced defence based on their prior experiences (Baldwin & Schmelz 1996).

The priming effects described earlier were in response to the same or similar groups of attackers. However, interspecies priming involving interactions between organisms from different groups also occurs in nature. A typical example of such multilevel priming is a tripartite interaction involving plant, pathogen and herbivore. Prior infestation of *Arabidopsis* plants with the caterpillars of the herbivorous insect *Pieris rapae* provided various levels of resistance to infections with secondary pathogens (De Vos *et al.* 2006). While induced resistance to *Xanthomonas campestris* and *Pseudomonas syringae* was expressed only in local leaves, resistance to turnip crinkle virus (TCV) was effective in both local and systemic leaves. The anti-viral effect was fully attributable to herbivore feeding because mechanical wounding itself had no effect on TCV performance. Only the application of *P. rapae* regurgitant to the mechanical wounds induced higher levels of protection against pathogens.

Priming imprints mediated by protein accumulation and activation

Conrath *et al.* (2001) proposed that the accumulation of signalling proteins in their inactive form and their rapid activation in new stress situations can contribute to the formation of short-term stress imprints. Protein phosphorylation and dephosphorylation is one of the most important reversible post-translational modifications that causes inactive proteins to become active and vice versa (Van Bentem & Hirt 2007). Members of a diverse class of mitogen-activated protein kinases (MAPK) are known to play important roles in mediating pathogen resistance as well as in JA-dependent signal transduction cascades (Seo *et al.* 2007; Takahashi *et al.* 2007; Wu *et al.* 2007; Zhang & Klessig 2001). A concrete example in *Arabidopsis* shows that the priming competence induced by treatment with salicylic acid analog benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester (BTH), sold under trade name Bion™ (Syngenta AG, Basel, Switzerland) and used commercially for priming and protecting crops against plant pathogens (Iriti, Mapelli & Faoro 2007), can be attributed to the accumulation of inactive MPK3 proteins (Beckers & Conrath 2007);

these are activated in response to pathogen infection, thereby enhancing the expression of defence genes and the accumulation of antifungal metabolites. In tobacco, the role of protein kinases [salicylate-induced protein kinase (SIPK) and wound-induced protein kinase (WIPK)] during herbivory and response to *M. sexta*-specific elicitors – fatty acid amino–acid conjugates (FACs) – has been recently established (Seo *et al.* 2007; Wu *et al.* 2007). A gene-silencing approach in *Nicotiana attenuata* has shown the need for both kinases to be present if JA-biosynthetic gene transcripts are to be accumulated and the JA burst subsequently elicited (Wu *et al.* 2007). The accumulation of both *SIPK* and *WIPK* transcripts in wounded leaves that have been treated with FACs shows some interesting kinetic properties: the peak of *WIPK* transcripts occurs less than 30 min after elicitation and the transcript levels return to initial values in approximately 2 h. In contrast, *SIPK* transcripts accumulate more slowly, peaking at 90 min or when the original JA burst is already completely attenuated. Because Wu *et al.* (2007) demonstrated that SIPK is required for WIPK activation, it is tempting to hypothesize that as in the case of MPK3 in *Arabidopsis*, the ability to delay the decline of *SIPK* transcripts (and presumably proteins) could equip *N. attenuata* plants with an inactive pool of kinase; such a pool could be used when the next bite is inflicted by *M. sexta* larvae or other herbivores.

Lipoxygenases (LOXs) catalyse the first committed step in JA biosynthesis (Fig. 2). *LOX* genes, which are transcriptionally upregulated during the JA burst, may increase active or inactive pools of LOX enzyme in the cells (Hermsmeier, Schittko & Baldwin 2001). In specific cases, activation of the pre-existing plant proteins occurs in a Ca^{2+}-dependent fashion; interestingly all *A. thaliana* LOXs proteins contain Ca^{2+}-binding domains that resemble those allowing the activation of mammalian LOXs (Oldham, Brash & Newcomer 2005; Bonaventure *et al.* 2007). In addition, it has been proposed that LOX enzymes need to be activated through the formation of a protein radical after direct interaction and binding of their own catalytic products – fatty acid hydroperoxides (Jones *et al.* 1996). It is possible that LOX enzyme pools remaining after previous attacks may already exist in the pre-activated oxidized form (ferric oxidation state E-Fe^{3+}), accelerating the production of fatty acid hydroperoxides during subsequent attacks.

Interestingly, the transcript levels of three putative 12-oxophytodienoate-10,11-reductase genes (ZmOPR1/2, ZmOPR5 and ZmOPR8) involved in JA biosynthesis increased after exposure to *cis*-3-hexenyl acetate (Z-3-6:AC), which has been previously shown to induce priming responses in maize (Engelberth *et al.* 2004; Engelberth *et al.* 2007).

Although previous examples show that JA may drive the priming responses, we still need to understand (1) how JA is produced; and (2) which mechanisms are involved in regulating gene expression downstream of JA. In the following section we will attempt to summarize the current progress in this rapidly evolving area.

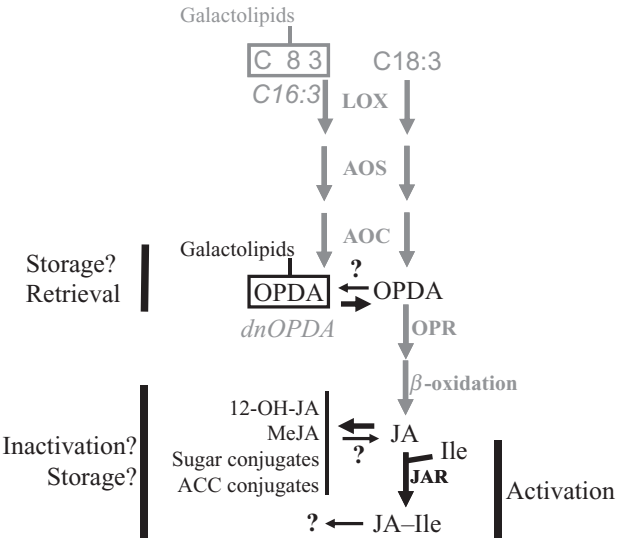

Figure 2. Biosynthesis and fate of jasmonic acid (JA) in plants. JA biosynthesis is initiated when lipases release free linolenic acid (C18:3) from plastidial membranes. Lipoxygenase (LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC) enzymes convert C18:3 to oxo-phytodienoic acid (OPDA). LOX-derived OPDA is converted to JA by OPDA reductase (OPR) and three cycles of β-oxidation in peroxisomes. Esterified OPDA and dinor-OPDA (dn-OPDA) are probably created on the intact galactolipids rather than via the free fatty acid pathway, and it is still unknown if the hydrolysis of these esters could contribute to JA production. JAR ligases mediate the final step in JA activation: the formation of JA–Ile conjugate. Further modifications of JA include conjugation to sugars, amino-cyclopropane carboxylic acid (ACC), methylation (methyl jasmonate, MeJA) and hydroxylation of the pentenyl side-chain in positions C-11 or C-12 (and subsequent sulfatation or conjugation to sugars).

POTENTIAL ROLE OF JA IN MEMORY OF STRESS

A hormonal roller coaster – the JA burst

The leaves of *N. attenuata* respond to wounding by the rapid waxing and waning of JA levels referred to as the 'JA burst' (e.g. Ziegler, Keinänen & Baldwin 2001). The maximum peak of JA is typically reached 30–60 min after elicitation, and JA levels are almost completely repressed as early as 90 min after wounding or simulated herbivory treatment, that is, wounding and application of *M. sexta* regurgitate (Halitschke *et al.* 2001). The JA burst is strongly amplified by the presence of insect regurgitate in the wounds, suggesting that plants are able to recognize herbivore attacks and tailor their defences accordingly. Interestingly, a transient burst of another defence hormone ethylene, which peaks 3 h after an attack or treatment, can be detected in wounded *N. attenuata* leaves, but only in the presence of insect regurgitate (Von Dahl *et al.* 2007). Transience in expression levels is also characteristic of the genes that contribute to JA (-Ile) and ethylene biosynthesis in *N. attenuata* and other species, but these transcriptional changes frequently follow, rather than precede, the

phytohormone burst {NaLOX3, Halitschke & Baldwin 2003; NaAOS (allene oxide synthase), Ziegler *et al.* 2001; AtAOS, AtAOC (allene oxide cyclase), Stenzel *et al.* 2003; NaJAR4, Kang *et al.* 2006; NaACS3a [amino-cyclopropane carboxylic acid (ACC) synthase], NaACO1 (ACC oxidase) and NaACO2a, Von Dahl *et al.* 2007}.

Although rapid activation of JA biosynthesis is known to occur, how the JA signal is efficiently turned off remains poorly understood (Wasternack 2007). Degradation, release as a methyl ester (Seo *et al.* 2001) or *cis*-jasmone (Koch, Bandemer & Boland 1997) into the environment, hydroxylation (Sembdner & Parthier 1993; Swiatek *et al.* 2004) or compartmentalization have been proposed as reasons for the rapid decline of cellular JA levels after its initial burst (Fig. 2). Recently, Miersch *et al.* (2008) provided the first functional proof that ω-hydroxylation could contribute to the inactivation of JA as it does in animal prostaglandins (Kikuta, Kusunose & Kusunose 2002). When JA is converted to 12-hydroxy-JA (12-OH-JA) and 12-hydroxy-JA sulphated forms (Fig. 2), its bioactivity is greatly reduced as evidenced by its inability to inhibit root growth or elicit defence-related genes. In addition, the dramatic accumulation of 12-OH-JA correlates with a decline in free JA levels 1–4 h after tomato plants are wounded, suggesting that this metabolite may mediate the major switch in JA signalling. Interestingly, biologically inactive 12-OH-JA even represses the transcript accumulation of several JA-inducible genes, including those that regulate JA biosynthesis in tomato plants, a result consistent with the rapid 'repolarization' concept of a memory mechanism that monitors the frequency and number of herbivore attacks, as proposed in Fig. 1a.

JA precursors and oxo-phytodienoic acid (OPDA) in the generation of memory

Having examined the dynamics of JAs appearance in plants, we can now investigate mechanisms that have the potential to be memory buffers (e.g. storage forms of JA); such buffers would facilitate elevated or faster responses to recurrent stresses. In plant–pathogen interactions, the leaves of tobacco plants that previously experienced pathogen infection have been demonstrated to contain increased pools of conjugated benzoic acids, precursors of salicylic acid; such pools speed up the accumulation of SA after subsequent elicitations (Chong *et al.* 2001). OPDA and dinor-OPDA (dnOPDA) may be esterified in large amounts to the complex lipids in *A. thaliana*'s membranes. Indeed, the size of this conjugate reservoir dramatically increased between 45 min and 4 h after wounding (Buseman *et al.* 2006). Whether these substrates were formed directly on intact membrane galactolipids or originated from the free fatty acid biosynthetic pathway is not clear (Fig. 2); these OPDA esters, with their folded-back acyl chains, modulate the strength of plastidial membranes. Alternatively, these intermediate OPDA 'stores' could contribute to the faster rate or higher amplitude of JA accumulation during repeated herbivore attacks. Such a mechanism would provide a biochemical foundation for a model of 'positive memory' (Fig. 1). It would be interesting to investigate the timescale in which these enhanced pools can be maintained in plants. In the case of galactolipid-bound OPDA, one can speculate that these esters may survive in cells over prolonged time periods, possibly days or even weeks after an attack.

The recent identification of a biotic stress-inducible acylhydrolase that specifically hydrolyses OPDA-galactolipid complexes has provided the first glimpse into a potential retrieval mechanism of these JA precursors (Yang *et al.* 2007). In addition, chloroplast-targeted lipase with strong galactolipase and weak phospholipase A1 activities *Dongle* (*Dgl*), which is homologous to the previously identified *defective in anther dehiscence1* (*Dad1*) gene (Ishiguro *et al.* 2001), was recently characterized by Hyun *et al.* (2008). The *Dgl* gene is expressed in the leaves, where it plays a specific role in maintaining JA content under normal conditions as well as in the early phases of increased JA production after wounding. If the retrieval of OPDA precursors from the pools that form in response to stress really contributes to priming, DGL and DAD1 would be the most likely enzymes that regulate the hydrolysis of these pools.

Nicotiana attenuata plants silenced by RNA interference (RNAi) in the expression of *WRKY3* and *WRKY6* transcription factors by transformation with inverted-repeat (ir) fragments of the endogenous genes respond differently to multiple elicitations, than to single elicitations. Although *ir-wrky3* and *ir-wrky6* plants have normal JA bursts, they show lower accumulated levels of JA and defence metabolites [trypsin protease inhibitors (TPIs)] after multiple elicitations or after *M. sexta* neonate caterpillars are allowed to feed on the plants; WRKY-deficient plants may not be able to transform their previous experiences into an integrated defence response. These herbivore-induced *N. attenuata* WRKY transcription factors may play an important role in generating priming imprints (JA or JA precursor pools) in tobacco plants (Skibbe *et al.* 2008).

Gene regulation in JA response

We have already demonstrated that the responses of JA and JA–Ile, and their biosynthetic genes oscillate in response to wounding or herbivore attack. Two questions arise from these observations: (1) does the rapid and transient induction of defence-related signals lead to the establishment of quantitative and qualitative information archives in plants? If the answer is yes, (2) how are these responses orchestrated? Certain levels of accuracy are required for a central 'counting clock' to monitor the progression of herbivore attack. Recent investigations into the signalling mechanisms involved in the JA response have suggested the first steps in a plausible model of plant 'memory'. These mechanisms involve the existence of multi-level feedback and feedforward regulatory loops in JA signal transduction (Chini *et al.* 2007; Thines *et al.* 2007).

Transience and accuracy are posited as prerequisites for generating discrete signals essential for a plant to monitor

the progression of herbivory. The example of *N. attenuata* shows how important this might be: in their native habitat, these plants may be situated near sagebrush (*Artemisia tridentata*), plants that emit high levels of oxylipins into the air as volatile MeJA. Not only does this plant-to-air communication channel allow wild tobacco and other plants to eavesdrop on their neighbours (Karban *et al.* 2000, 2003), but it could also 'confuse' these plants by increasing the noise levels in the signal perception machinery; unable to recognize their own JA, these plants would find it difficult to activate their defences.

However, it appears that *N. attenuata* can still distinguish their own signals and elicit adequate defence responses. We propose that this could be accomplished by limiting JA perception to a strictly defined and transiently framed time window. Our current understanding of tightly regulated JA signal perception lends strong support to this hypothesis: the 'Rosetta Stone' in JA signal transduction that was simultaneously discovered by Chini *et al.* (2007) and Thines *et al.* (2007). Both groups demonstrated that dominant mutation caused by a specific C-terminal deletion in a novel class of ZIM domain-containing JAZ proteins can inhibit COI1-mediated, JA-dependent gene expression in *Arabidopsis*. JAZ proteins are repressors that are recognized by the F-box protein COI1 and are specifically targeted for ubiquitination and degradation in 26S proteasome machinery. These JAZ proteins serve as transcriptional repressors of the central MYC2 transcription factor that facilitates downstream transcriptional responses in JA signalling (Dombrecht *et al.* 2007; Fig. 3). The interaction of JAZ and COI1 *in vitro* was specifically stimulated in the presence of JA–Ile, suggesting that this JA-amino acid conjugate might be the 'molecular glue' in the COI1–JAZ complex. If this is the case, then the JAZ–COI1 complex is the actual receptor of JA–Ile (Katsir *et al.* 2008). The resemblance of JA perception to auxin (IAA) is telling; COI1 and auxin receptor TIR1 share significant sequence similarity (Xie *et al.* 1998; Dharmasiri, Dharmasiri & Estelle 2005), and both hormones, JA and IAA, use common components that activate the ubiquitin E3 ligase complex (e.g. AXR1; Tiryaki & Staswick 2002). In summary, the interaction of JAZ, COI1 and JA–Ile leads to the degradation of JAZ proteins, and liberated MYC2 in turn transcriptionally activates downstream genes (Fig. 3). Interestingly, JAZ transcripts accumulate in a MYC2-dependent manner, which is under feedback control, and thus more repressor protein is produced during each induction period (Chini *et al.* 2007; Chung *et al.* 2008). This behaviour may be used to produce sharper peaks and narrower windows of sensitivity, or even to tune the plant's responses to allow for more sensitivity during the plant's life and in accord with previous experience.

Possible tuning of JA response at the JAZ repressor level

Consistent with such a mechanism, the ectopic overexpression of the *AtMYC2* gene under a strong constitutive promoter upregulates the expression of downstream target genes, despite the presence of functional JAZ repressors in AtMYC2 overexpression lines (Chini *et al.* 2007). This result suggests that the interaction of MYC2 with JAZ is a carefully balanced equimolar process that can act as a sensitive and, more importantly, tunable switch. After each induction, the timing and colocalization of proteins in the cells, when the amount of *de novo* synthesized repressor exceeds the amount of degradable JAZ protein, becomes the signal. We emphasize the role of protein turnover, because the transcription of COI1 seems to be constant or even suppressed during elicitation in tobacco (Paschold, Halitschke & Baldwin 2007). At this turning point, the response is quickly attenuated by decreasing the amounts of free MYC2 (titrated out by the excess of JAZ repressor), and the feedback loop for *de novo* synthesis of JAZ proteins is truncated (Fig. 3). It would be interesting to know whether a certain refractory period occurs due to this process that would render plants less sensitive or even insensitive to wound stimulus (JA) during the repression period of the cycle. Results obtained with *N. attenuata* plants suggest that indeed, a refractory period occurs during which a full JA burst cannot be re-elicited after an initial elicitation. This refractory period is limited to this part of the JA burst when the levels of JA are rapidly declining after attaining their maximal levels at 30–60 min and lasts for about 30 min to 1 h (Ziegler *et al.* 2001).

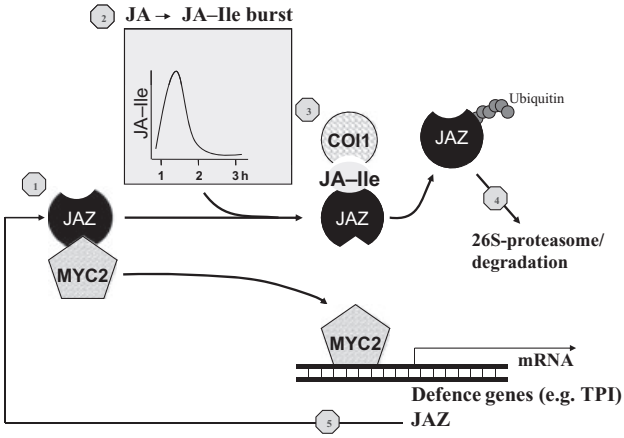

Figure 3. Regulation of jasmonic acid (JA) response includes feedback and feedforward loops. (1) Under non-induced conditions JAZ proteins associate with and inactivate key MYC2 transcription factor in JA responses. (2) Upon wounding or herbivore attack, JA bursts lead to JA–Ile accumulation that is regulated by JAR enzymes. (3) JA–Ile then mediates interaction between the F-box protein – COI1 – and repressors of JA signalling – JAZ – proteins, resulting in ubiquitination of JAZ proteins. (4) Ubiquitin-labelled JAZ proteins are targeted to the 26S-proteasome machinery for degradation, which results in the release of transcription factor MYC2 and activation of transcription of secondary regulators and defence genes. (5) However, MYC2 also regulates the transcription of JAZ repressors, and the actual equilibrium of these proteins, together with the presence of JA–Ile, determines the duration and intensity of response.

According to this induction-repression model, plants could regulate the levels of active JAZ repressor proteins at transcriptional or post-translational levels, depending on their previous experience, defined as encounters with herbivores or other environmental stresses. As such, the difference between 'naive' and 'experienced' plants (as predicted in Fig. 1b) could be found in the action threshold levels of the real-time inducer JA(-Ile), expressed as the proportional activation of COI1-dependent proteolytic activity and degradation of JAZ. Considering the presence of multiple JAZ repressors, plants could even keep different 'memories' (action thresholds) for multiple stimuli controlled by the individual JAZ proteins (Browse & Howe 2008; Chung *et al.* 2008). Future experiments will show how JAZ transcription and protein levels are controlled in plants and whether post-translational mechanisms are used to tune JAZ function in nature during a plant's lifetime.

RESTRUCTURING PLANT CELL WALLS DURING HERBIVORY

While the regulation of JAZ protein levels proposed in the previous paragraph might function as a possible rheostat in plant defence against herbivores, changes in the structure of cell walls have the potential to make plants more resistant to environmental stress signals in a permanent or semi-permanent manner. Cell walls are known to be rapidly remodelled after herbivore attack by a number of enzyme systems that reinforce plant tissues against additional stress conditions, for example opportunistic pathogen infection. However, there may be alternative reasons for plants to invest their limited resources into costly remodelling of their cell walls: remodelling of the rigid cell barriers could mean that plants that recently suffered herbivore attack 'listen' more carefully to the signals and warning signs from the environment.

Pectin methylesterases (PMEs) are enzymes secreted into plant cell walls; they demethylate polygalacturonic acid and produce methanol, contributing to changes in the plasticity of plant cell walls (Micheli 2001). Apart from their role in growth and development, PMEs show some unexpected defence-related functions. For example, PMEs interfere with the process of virus infection in plants by at least two yet unknown mechanisms: (1) PMEs are required for systemic movement of viruses in plants (e.g. tobamoviruses; Chen & Citovsky 2003); and (2) the presence of PMEs enhances RNA-mediated silencing of viruses such as tobacco mosaic virus (TMV) (Dorokhov *et al.* 2006). PME transcripts, as well as enzyme activity, are highly elicited by herbivore attacks, and the methanol they produce dominates the bouquet of volatiles released from herbivore-attacked plants (Von Dahl *et al.* 2006). Previous examples in plant–virus interactions suggest that PMEs, because they are located at the cell boundary and cause profound changes in the structure of plant cell walls and potential signal methanol emission, may play an important role in communication between cells and the surrounding environment.

In addition to PMEs, of xyloglucan endotransglucosylase/hydrolase1 (XTH1) transcripts are rapidly upregulated after *M. sexta* attack in *N. attenuata* leaves (Hui *et al.* 2003). XTH1 functions in the cleavage and concomitant transfer of xyloglucan molecules into plant cell walls; these molecules mediate the loosening of cell walls during growth. It is tempting to speculate that plants that have experienced extensive herbivory might be equipped with a surplus of XTH1 enzymes; these could restructure their cell walls to allow more efficient perception (penetration) of herbivore-derived signals, FACs and 'careful listening' to herbivores to take place. Alternatively, loosening the rigid cell walls by XTH activity may allow volatile compounds to be more easily released from the cells during subsequent attacks.

EPIGENETIC CONTROL OF STRESS IMPRINTS IN PLANTS

Mechanisms of epigenetic control

In the previous sections, we mainly focused on the short-term responses and imprints of plants to stress. However, more permanent but still reversible changes in gene expression – namely those involved in protection from stress – have been described in plants. In particular, DNA methylation (Mathieu *et al.* 2007), histone modifications and changes in small-RNA populations can lead to heritable or transgenerational alterations in plant behaviour, some of which cannot be explained by Mendelian genetics. Readers are directed to examples of several current reviews that analyse the broad areas of DNA and histone modifications in epigenetic control of development and stress responses (Bronner *et al.* 2007; Boyko & Kovalchuk 2008); here we concentrate on understanding the role of small RNAs (smRNAs) in plants. smRNAs are one of the most stress-rearranged classes of molecules in plants (Pandey *et al.* 2008). In animals, smRNAs regulate diverse processes including learning, brain development and memory formation (Rogelj & Giese 2004; Mehler & Mattick 2007). In the next section, we show that smRNAs have several important properties consistent with a role in plant defence-related memory mechanisms.

Biosynthesis and classification of small RNAs

The central dogma of molecular biology places RNA between DNA and proteins, and views it as a direct genetic medium in RNA viruses. However, recent advances have extended the role of RNAs: they are no longer just 'middlemen' but regulators of genetic information. RNA silences or negatively regulates gene expression in a process known as RNA interference or post-transcriptional gene silencing (PTGS) (Chapman & Carrington 2007; Dorokhov 2007; Sunkar & Zhu 2007; Filipowicz, Bhattacharyya & Sonenberg 2008). The role of small double-stranded RNA (dsRNA) in gene activation has also been indicated (Li *et al.* 2006).

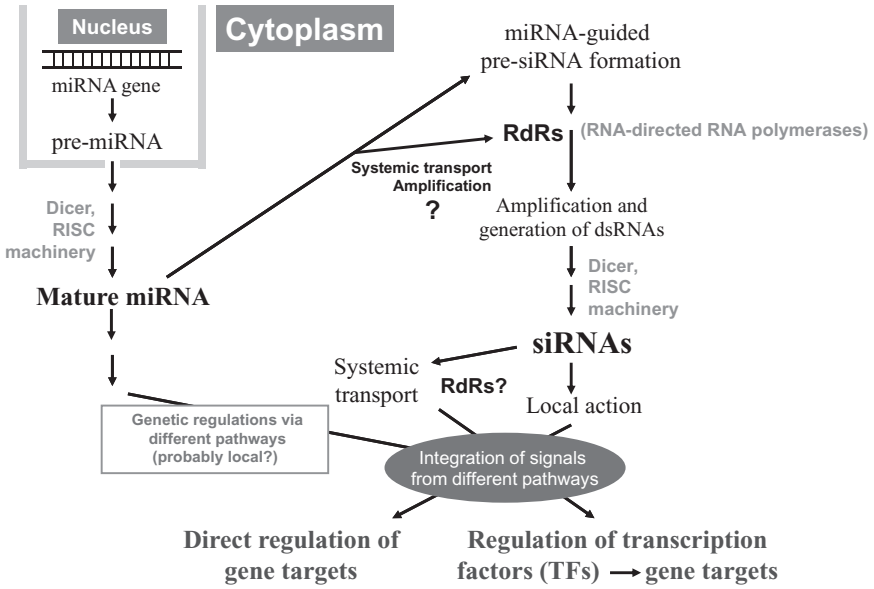

Figure 4. Simplified scheme of small-RNA biogenesis. Two main classes of small RNAs originate from divergent double-stranded RNA templates in nucleus (miRNAs) and cytoplasm (siRNAs). Both classes require Dicer protein and RNA-induced silencing complex (RISC) machinery to cleave and produce mature ~20–24 nucleotide smRNAs.

The original work of Lee, Feinbaum & Ambros (1993) shows how *lin-4* gene regulates development in nematode *Caenorhabditis elegans* not by encoding proteins but by producing an smRNA; as a result of this work, RNAi now occupies a central place in transcriptional regulation. smRNAs facilitate regulation in a sequence-dependent manner by pairing with target mRNA transcripts in perfect or imperfect Watson-Crick base-pairing, resulting in either the degradation of the mRNA transcript (more common in plants) or the inhibition of translation (Dorokhov 2007). Depending on their biogenesis (Fig. 4) and mode of action, smRNAs are classified into two major groups: microRNAs (miRNAs) and small interfering RNAs (siRNAs). miRNAs, generated from the stem-loop pre-miRNAs structures, are transcribed from the 'miR' genes that are present in the inverted-repeat orientation in the genome (Bartel 2004). The pre-miRNAs, transcribed in the nucleus by RNA polymerase II and transported into the cytoplasm, and after processing by Dicer proteins, result in miRNAs (Bartel 2004). In contrast, the siRNAs are generated in the cytoplasm from their double-stranded RNA precursors; these precursors are generated by a specific class of RNA polymerases, namely RNA-directed/dependent RNA polymerases (the RdRs or the RdRps; Wassenegger & Krczal 2006; Pickford & Cogoni 2003).

Small, highly mobile RNAs are involved in short- as well as long-distance signalling (Kalantidis *et al.* 2008). Cell-to-cell movement may occur as a 'relay' of amplification events, occurring within a tissue through the cell plasmodesmal connections. During such systemic transport, the cells respond to the silencing signal in an RdR6-dependent manner (Schwach *et al.* 2005). The long-distance movement of RNA has been reported: the long-distance-systemic spread of RNAs is highly influenced by phloem flow in a source-to-sink dependent manner (Tournier, Tabler & Kalantidis 2006); such transport mechanisms may have evolved as the result of a need for a surveillance system in shoot meristems against viral RNA (Foster *et al.* 2002).

smRNAs as mediators of memory in JA-mediated defence

The process of generating RdR-dependent smRNAs could recycle information, amplifying smRNAs while targeting regulatory pathways in plant defence (Sunkar *et al.* 2007). Recently, plants deficient in RdR1, whose transcripts are strongly elicited by simulated herbivory attack, as well as by the application of SA and JA but not by mechanical wounding, have been shown to be more susceptible to herbivore attacks than are wild-type plants. The compromised defences in RdR1-silenced plants contributed to lower elicited levels of the defence alkaloid nicotine in both glasshouse- and field-grown *N. attenuata* plants (Pandey & Baldwin 2007). In addition to less nicotine, RdR1-silenced plants produced less elicited JA because they have fewer transcripts of essential JA biosynthetic enzymes, LOX and AOS (Pandey *et al.* 2008). Interestingly, the basal levels of defence metabolites were not influenced by silencing the RdR1 gene, suggesting that RdR1 regulates the ability of plants to induce defence responses during herbivory. Unlike RdR1-silenced plants, RdR2-silenced plants are susceptible to ultraviolet (UV) light-induced stress, which only became apparent after these plants were grown in natural habitats (Pandey & Baldwin 2008). The preceding examples suggest that activating defences against biotic and abioitic stresses requires smRNAs and RdR enzymes. Further work is required to establish the role of these highly dynamic molecules in plants and their 'memories' of stressful events and to determine the timescale over which such 'memories' can be maintained during a plant's lifetime.

CONCLUSIONS

All organisms have a sophisticated means of coping with the challenges of living in the real world. While it is clear that plants lack brains and tissues that function as neurons in transmitting action potentials, even though action potentials have been measured in plants (Shimmen 2001), it is apparent that their fitness would benefit from modifying responses in light of past experiences. In this review, we argue that plants possess molecular mechanisms that allow them to memorize previous stress events and generate memory imprints ranging from minutes or hours to days or weeks (by changing the concentration of small molecules and proteins), or even next generations of siblings (by genetic and epigenetic modifications). The ability to recall and use this information, most frequently reflected in various examples of priming effects, has been shown to occur. However, much additional work is required to understand the responsible mechanisms. In particular, real-time studies of plants growing in their natural environment are essential. Add to these, the approaches of the behavioural sciences to our newly emerging mechanistic understanding of plants' signalling pathways, and our appreciation of the sophistication with which plants adapt to their environments will be enhanced. Here we address only a small fraction of the potential mechanisms and forms of memory in plants with the hope of inspiring readers to uncover the behavioural capabilities of plants.

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