

Damaged-self recognition in plant herbivore defence

Martin Heil

Departamento de Ingeniería Genética. CINVESTAV – Irapuato, Km. 9.6 Libramiento Norte, CP 36821, Irapuato, Guanajuato, México

Feeding by herbivores induces plant defences, but we still do not know all the signals that mediate this response. Here, I argue that a general principle in this mediation is ‘damaged-self recognition’, that is, the perception of motifs by the plant that indicate disintegrated plant cells. Most defence-inducing molecules are (or contain) plant-derived motifs or disintegrate plant cells and thereby release defence elicitors. By perceiving the ‘damaged self’, plants can retain evolutionary control over their interactions with herbivores rather than allowing herbivores to dominate the interaction. The concept of ‘damaged-self recognition’ provides a paradigm for plant responses to herbivory and helps the search for the currently unknown elicitors of those defence responses, which have so far only been described at the phenotypic level.

Inducing plant defence against herbivores

Plants have an array of resistance traits to protect themselves against pathogens and herbivores. Many of these traits are expressed in response to attack, and no single resistance trait can fend off all types of enemy. Therefore, to mount a quantitatively and qualitatively adequate response, plants require mechanisms for reliably detecting whether or not they are being attacked and, if so, by which type of enemy.

Attack by piercing–sucking herbivores, such as aphids, or by cell-content feeders, such as thrips and spider mites, inflicts little physical damage and harms a limited number of plant cells. By contrast, folivory (see Glossary) by chewing insects or mammals inevitably damages large portions of the plant tissue. Resulting changes in the defensive status of the plant have been named the ‘plant wound response’ [1,2], and the first studies aimed at identifying the inducing signal found that mechanical damage is enough to elicit such a response [3,4]. However, subsequent experiments with specific plant–herbivore combinations often revealed that mechanical damage inflicted by punching holes into leaves or cutting them off elicited either no detectable defence response or only a subset of those responses that could be observed after natural herbivory [5–10]. Four classes of specific insect-derived defence elicitors have been identified: (i) fatty acid–amino acid conjugates (FACs), such as volicitin and chemically related analogues from insect oral secretions [7,11–13]; (ii) bruchins, benzyl cyanide and other as yet unidentified elicitors from insect oviposition fluids that are associated with ‘early alert’ defence against insect eggs

and first-instar larvae [14–16]; (iii) caeliferins from grasshopper (*Schistocerca americana*) oral secretions, which induced defence responses in maize (*Zea mays*) plants [17]; and (iv) *b*-glucosidases from caterpillar (*Pieris brassicae*) regurgitant, which can induce the release of volatile organic compounds (VOCs) that attract parasitic wasps to plants [18].

Intriguingly, most of these elicitors have been found and proven active only in certain plant–insect interactions. By contrast, responses to herbivory are known at the phenotypic level from a wide taxonomic range of plant–herbivore interactions and comprise, among others, the induction of

Glossary

Aminopeptidases: enzymes that cleave N-terminal residues from proteins and peptides [86].

Damaged-self recognition: the paradigm that perception of elicitors that indicate the presence of damaged plant cells underlies general plant responses to herbivory.

Effectors: molecules used by pathogens to modulate or suppress plant defence responses to infection [34].

EFN (extrafloral nectar): plant nectar that serves as an indirect defence against herbivores via tritrophic interactions with carnivores [23], now described from >300 plant genera (<http://www.biosci.unl.edu/emmeriti/keeler/extrafloral/worldlistfamilies.htm>).

Elicitors (of plant defence): all types of molecule that indicate attack and induce defence responses when being perceived by plant receptors (Box 2).

ETI (effector-triggered immunity): an evolutionarily advanced level of plant resistance to pathogens that is induced by the perception of specific effector molecules or of their effects on plant metabolism [34].

Folivory: feeding by chewing animals on plant leaves.

HAMPs (herbivore-associated molecular patterns): herbivore-derived molecules that serve as signals in plant defence [10,38].

Herbivory: any type of animal feeding on plant material; comprises different feeding strategies, such as folivory, sap-sucking, cell-content feeding, wood boring, leaf mining and feeding on spores, fruits and other plant parts.

MAMPs (microbe-associated molecular patterns): comprise molecules that are specific for microbes and thus absent from a healthy plant cell [34–36].

Modified-self recognition: describes a perception of metabolic changes that are inflicted by pathogens and that can be used by plants to perceive their status of infection [34,37].

Non-self recognition: the general paradigm that a perception of molecules that are absent from a healthy plant cell (i.e., MAMPs or PAMPs) induces plant defence responses to infection [34,37].

PAMP–ETI model: describes the resistance response of plants to pathogen infection that is achieved by combining the evolutionarily ancient perception of PAMPs with the more derived ETI [34].

PAMPs (pathogen-associated molecular patterns): in the context of plant disease resistance, this term is synonymous to MAMPs [34–36].

Peritrophic membrane: an extracellular matrix composed of chitin microfibrils, attached to which is a gel-like mixture of proteins, glycoproteins and proteoglycans. The membrane forms a thin sheath around the midgut lumen of most insects and prevents particularly large molecules and polyanions from being absorbed into the ecto-peritrophic space and, finally, the gut cells [64].

PIs (proteinase inhibitors): plant molecules that inhibit protein digestion in insects and thereby reduce the nutritive value of plant tissues for herbivores [3].

Wound response: the general defensive response of plants that can be observed after herbivore feeding and other types of wounding [1–4].

Corresponding author: Heil, M. (mheil@ira.cinvestav.mx).

toxic or repellent secondary compounds [19]; the synthesis of proteinase inhibitors (PIs) [3]; the formation of trichomes and thorns that function as physical barriers against herbivores [20,21]; the secretion of extrafloral nectar (EFN) [22,23]; and the increased formation of extrafloral nectaries [24,25]. In short, although there are hundreds of known plant responses to herbivory [19,23], there are only four known classes of animal-derived defence elicitors. These elicitors have been described from specific plant–insect systems, leaving the question open as to how plants perceive feeding by non-insect herbivores, such as nematodes, mites and mammals. Thus, gaining a more complete picture of induced plant responses to herbivory requires searching for further and taxonomically more widespread elicitors.

Here, I highlight a currently underestimated source of plant defence elicitors that is common to all types of herbivory: the damaged plant cell itself. The concept of ‘damaged-self recognition’ (Box 1) is based on the observation that a general principle underlying animal feeding on plant tissues is the disruption and disintegration of plant cells. This damage results in the occurrence of plant molecules outside the compartments to which they are bound in the intact cell and releases fragments from such molecules because they become exposed to enzymes that, in the intact cell, are localized to different compartments. These chemical motifs are indicative of the ‘damaged self’ and can principally serve as elicitors of plant defence responses.

Box 1. The concept of damaged-self recognition

Most elicitors of plant responses to folivory represent, or contain, parts of plant-derived molecules that are degraded, digested or localized outside their original cell compartment. To the plant, these elicitors indicate the ‘damaged self’. Such elicitors are released from disrupted cells and are probably perceived by receptors that monitor the extracellular chemistry. Thus, the information on the ‘damaged self’ is transported into the inner compartments of intact and metabolically active cells, which react through metabolic responses such as the synthesis of systemic signals and defence compounds.

Elicitors *sensu stricto* should be distinguished from elicitor-forming compounds and from wound hormones because perception mechanisms are likely to differ among these three classes of defence-inducing compound (Box 2). Elicitors that are released directly from the damaged plant cell require neither coevolution among plants and certain herbivores nor a specific physiological interaction with the metabolism of the insect. These elicitors are, thus, probably evolutionarily ancient and seem to be most suitable for evoking general defences against a broad range of herbivore species. By contrast, elicitors whose synthesis requires an interaction with the metabolism of the herbivore represent evolutionarily derived layers of the wound response and mediate responses to attack by specialist herbivores.

Perceiving elicitors that are released from their own damaged cells enables plants to respond quickly with general defensive responses to attack by herbivores, without depending on specific signals that are derived from (and thus can be evolutionarily controlled by) the animal. Therefore, damaged-self recognition seems to be a reliable and indispensable part of the overall plant wound response to animal feeding, although the coevolutionary arms race between plants and herbivores has led to numerous modifications of the general scheme. Focusing research on the damaged plant cell will help to find the perception mechanisms that underlie all those defensive plant responses to herbivore feeding for which no elicitors have been identified so far.

The plant wound response

In a seminal publication [3], it was reported that feeding by Colorado potato beetles induced the accumulation of PIs in both damaged and undamaged leaves of tomato (*Lycopersicon esculentum*). The same response was elicited by punching holes into (or crushing) leaves or by applying leaf extract to intact leaves [3,4]. Similarly, ‘corn juice’ (a liquid obtained by mortaring maize seedlings and filtering the resulting homogenate) and ‘leaf juice’ from cabbage (*Brassica oleracea*) leaves elicited the release of herbivore-induced VOCs in the respective species [18,26]. Release of such VOCs attracts parasitic wasps and acts as an indirect defence of many plant species against herbivores [19,23,27,28]. Follow-up studies into the nature of the systemic signal found that both electrical and chemical signals can induce PIs in tomato. Tomato plants dispose of two types of electrical long-distance signal (reviewed in Ref. [29]): (i) directionally propagated action potentials; and (ii) slow-wave potentials following hydraulic pressure changes. Both types of depolarization event are elicited by local wounding [30], occur after folivory and induce PI synthesis in tomato [31]. The search for the chemical signal identified an 18-amino-acid peptide, systemin, which is released upon wounding and locally potentiates the synthesis of jasmonic acid (JA) [32]. This hormone then forms the mobile signal in the systemic wound response of tomato and, probably, of most other higher plant species (see Box 3 in Ref. [33]).

Perceiving pathogens and herbivores

Infection by pathogens initially affects only limited numbers of cells rather than physically destroying tissues, but it features intimate contact between the host cells and the infecting agent. Because microbes contain specific compounds that are absent from the healthy plant cell, infection can be detected by the perception of ‘microbe-associated molecular patterns’ (MAMPs, synonymously called PAMPs for ‘pathogen-associated molecular patterns’) [34–36]. During the evolutionary arms race between pathogens and hosts, pathogens evolved specific effectors to overcome plant resistance strategies, whereas plants evolved to perceive these effectors (or their effects on the plant metabolism) to mount a second level of more specific resistance, named ‘effector-triggered immunity’ (ETI). Because effectors cause specific changes in the host cell metabolism, ETI is achieved, at least in part, by ‘modified-self recognition’ [34]. In short, the evolutionarily ancient mechanism and parts of the derived mechanisms by which plants detect infection depend on perceiving foreign molecules that are absent from the healthy plant, in other words by ‘non-self recognition’ [34,37].

In analogy to MAMPs, herbivore-derived molecules that serve as signals in plant defence responses have been termed HAMPs for ‘herbivore-associated molecular patterns’ [10,38]. Several HAMPs can induce plant defences at pmolar or even fmolar concentrations, and classic HAMPs, such as FACs from tobacco hornworm (*Manduca sexta*), have been reported to be ‘necessary and sufficient to induce herbivore-specific plant defence responses’ [7]. Thus, HAMPs have an important role in plant defence induction and are likely to mediate the specificity in the response of

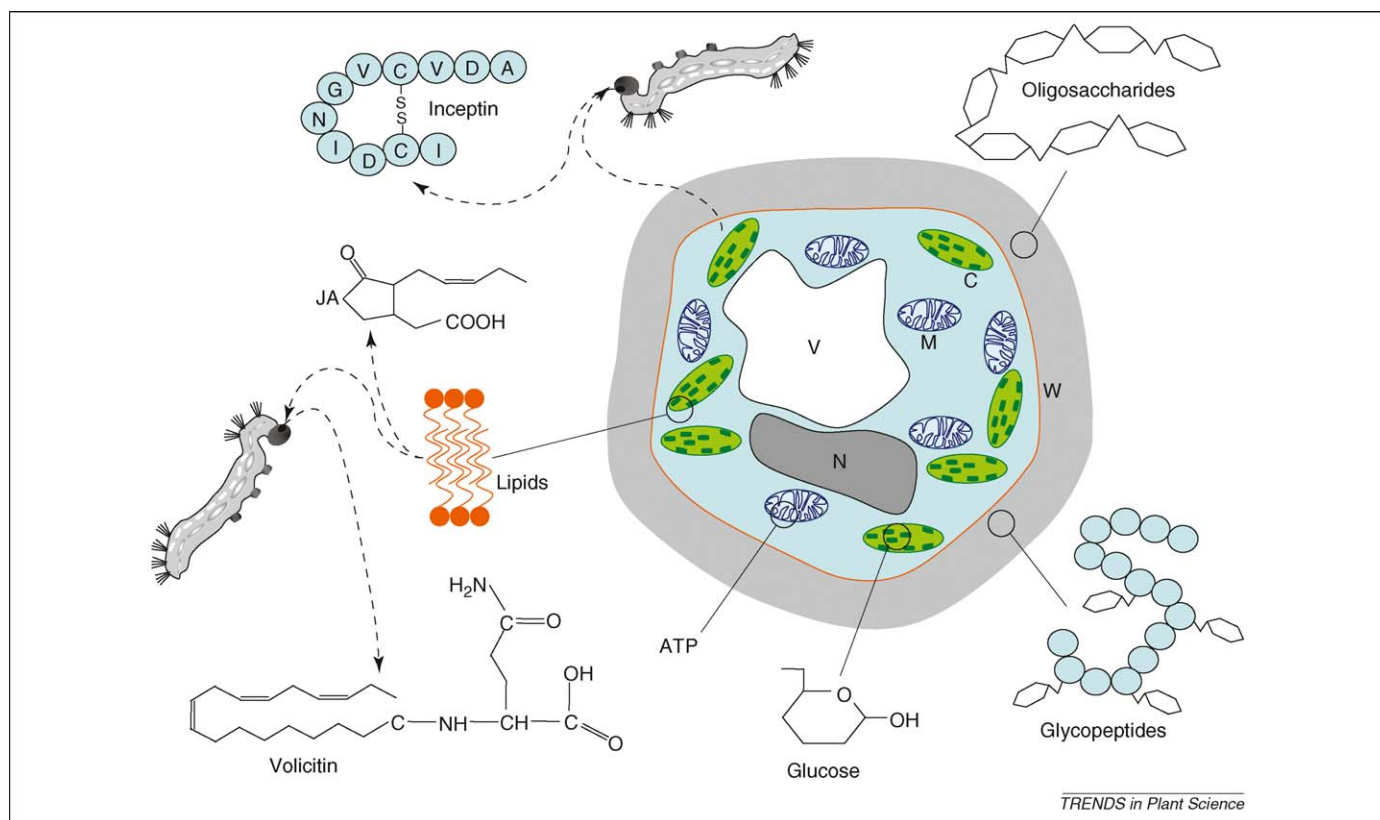


Figure 1. Origin of elicitors of the wound response. The damaged plant cell is the source of most of the elicitors that have been described so far to induce the wound response. Several organelles (W: cell wall, C: chloroplast and M: mitochondrion) form the metabolic origin of these elicitors. The central signalling hormone, JA, is processed by plant-derived enzymes from lipids that are localized in the chloroplast membrane and thus is derived completely from the plant metabolism. The same is true for other elicitors, such as cell-wall-derived glycopeptides, oligosaccharides and extracellular glucose or nucleotides (ATP). All these compounds are created or released by cell disruption, with or without the action of plant-cell-derived enzymes. By contrast, the formation of FACs, such as volicitin, and of defence-eliciting peptides, such as inceptin, requires uptake of plant cell-derived molecules into the insect gut and their processing by insect-derived enzymes. No elicitors derived from the vacuole (V) or the nucleus (N) have yet been reported to my knowledge.

a plant to feeding by different herbivore species [39–41]. Unfortunately, the mechanisms by which HAMPs induce plant defence remain to be investigated in most cases. Receptor proteins have been predicted and a specific receptor has been reported for systemin [32]. However, recent studies question the relevance of this finding [42] and no other receptors for any elicitor of plant defence to herbivores have yet been identified. FACs have surfactant, amphiphilic properties and can wet hydrophobic surfaces [43]. This detergent-like function could also depolarize cell membranes. Such depolarization events are among the earliest events that can be observed in the tissue surrounding a feeding site and might induce electrical defence signals, which appear to be a taxonomically widespread wound response [31].

In short, plant cells are likely to use different mechanisms to perceive (or interact with) different elicitors. Although the action of individual elicitors in individual plant species thus needs to be investigated to achieve a complete picture at the physiological and molecular level [44], this complex situation makes the search for underlying general principles very attractive.

The metabolic origin of plant defence elicitors

In spite of the specific functions of HAMPs, other studies have found that mechanical damage was enough to elicit the same responses that are observed after herbivore feeding

[3,4,22,45–47]. Mechanical damage of *Arabidopsis thaliana* (Brassicaceae), maize, lima bean (*Phaseolus lunatus*), *Vicia faba* and several *Acacia* species (Fabaceae), *Macaranga tanarius* (Euphorbiaceae), poplar (*Populus trichocarpa* × *P. P. deltoides*, Salicaceae) and tomato, among other species, induced JA synthesis in the damaged tissue and/or the expression of defensive phenotypes [3,6,21,22,26,46–49]. Because JA regulates most of the genetic or phenotypic changes that are observed after herbivore feeding [1–3], these results suggest that JA-dependent responses can, in principle, be activated via damaged-self recognition.

The above results hint at elicitors that stem from the damaged plant itself. What then, is the role of animal-derived elicitors? Answering this question requires a more detailed look into the chemical nature of HAMPs (Figure 1). FACs have been isolated from insect oral secretions [11,12]; however, their fatty acid portion derives from the plant, whereas the hydroxylation reaction and the conjugation with the amino acid occur in the insect gut [50]. These conjugates therefore contain directly plant-derived motifs.

Fragments of plant cells were among the first described defence elicitors and include pectines [51], oligogalacturonide fragments [52], oligosaccharides [52,53] and peptides [54]. Unfortunately, although screenings for further elicitors have successfully been extended to non-model plant species [54–56], the search for further plant-derived elicitors and for

Box 2. Three classes of defence-inducing compounds and their predicted receptors

To achieve a paradigm for understanding plant wound responses, three major classes of defence-inducing compound should be distinguished, based on their origin and their mode of action. A receptor has so far been described only for the peptide hormone systemin [32], and the mode by which elicitors affect plant metabolism remains, therefore, to be discovered for all other cases. However, here I briefly discuss why the perception mechanisms are likely to differ among these three classes of defence-inducing compound.

Elicitors *sensu stricto*

Elicitors *sensu stricto* are compounds that characterize the initial attack and whose perception by the plant induces a defensive response. Examples include fatty acid–amino acid conjugates [11,12], cell wall fragments [51–54], peptides [32,57], herbivore-induced VOCs (reviewed in Ref. [33]) and extracellular sugars or nucleotides [74,75]. These compounds represent (or contain) plant-derived units that are degraded or that occur outside their normal compartment; they thus indicate a disrupted, heavily damaged or partly digested plant cell. Elicitors *sensu stricto* are predicted to

interact locally and directly with receptors that monitor their occurrence outside the cell or the cell compartment of origin (as described in Ref. [32] for the systemin receptor).

Elicitor-forming compounds

Elicitor-forming compounds are compounds whose activity produces the elicitors *sensu stricto*. Important elicitor-forming compounds that activate damaged-self recognition are, for example, cell-wall degrading and other digestive or hydrolytic enzymes [18,52,65–67]. Elicitor-forming compounds are not elicitors themselves because they are unlikely to be interacting directly with specific receptors in the plant cell.

Hormones and other types of mobile signal

Hormones and other types of mobile signal (such as electrical long-distance signals) induce defence responses when they are released from the damaged tissue and reach systemic tissues [1,22,48]. They are, however, not true elicitors because they serve to process and distribute information on the status of attack rather than its original perception.

their receptors has slowed because most research has focused on only a few model plants and on the few chemically well-defined elicitors. However, even highly specific defence-eliciting peptides, such as inceptin [57], systemin [32] and other hydroxyproline-rich glycopeptides from solanaceous plants [56,58] and the *AtPep* peptide from *Arabidopsis* [59], represent fragments of plant proteins. Inceptin is a fragment of a chloroplastic ATP synthase [57], a protein that fulfils a vital function in the intact leaf. Systemin originates from a larger precursor of as yet unknown function [32] that is also expressed in species in which systemin is not active [60], and its release from the precursor occurs when prosystemin becomes exposed to proteolytic enzymes from other compartments [61].

The presence of such compounds indicates a disrupted, heavily damaged plant cell. Moreover, the molecular weights and physicochemical properties of most of these compounds make it unlikely that they can pass across the peritrophic membrane of insects, which serves as an ultrafilter, preventing macromolecules, polyanions and micelle-forming lipophilic and amphiphilic compounds from being taken up from the gut lumen [62–64]. Most of these indicators of the ‘damaged-self’ are therefore unlikely to have ever entered (and been metabolized in) an insect cell and thus represent molecules of plant origin only.

Another class of resistance-inducing molecules comprises enzymes or other compounds that cleave plant molecules, disintegrate the cell or depolarize the membrane potential [31]: examples include β -glucosidases [18], polygalacturonases [52], channel-forming peptaibols [65], cellulysin [66] and ionophores [67]. β -Glucosidases from insect regurgitate and from almonds likewise induced the release of VOCs from cabbage plants, which underlines that it is the activity of the enzyme rather than its specific identity that is responsible for the inducing effect [18]. These molecules should not be termed elicitors *sensu stricto* (Box 2) because it is unlikely that they represent perceived signals. Instead, they cause the release of defence elicitors. However, their action produces compounds that are indicative of the ‘damaged self’ and thus channels into the same general perception mechanism.

VOCs, JA and extracellular molecules as indicators of the ‘damaged self’

Further indicators of the ‘damaged self’ that are not usually discussed in this context are VOCs, extracellular sugars, extracellular ATP and the wound hormone JA (Figure 1). VOCs are mainly discussed as defensive factors repelling herbivores [68,69] or attracting carnivores [23,27,70], but green-leaf volatiles and other VOCs can also trigger herbivore resistance in systemic but undamaged tissues of the same plant [71–73] or in its neighbours (reviewed in Ref. [33]). Green-leaf volatiles are synthesized when membrane-bound lipids become exposed to pre-existing enzymes during cell disruption and thus represent another class of indicators of the ‘damaged self’. Similarly, extracellular sugars and nucleotides can elicit defence responses [74,75], and the most parsimonious explanation of their defence-inducing activity might be that compounds that are bound to the cell in intact tissues appear in the apoplast: hence, their extracellular localization indicates the ‘damaged self’.

Finally, JA is the main hormone in the wound response, and experimental application of JA and its derivatives induces most of those plant defences that are observed after folivory [1–3]. As described for green-leaf volatiles, in response to cell disruption JA metabolizes from a precursor that is already present in the intact cell: the octadecanoid pathway, which terminates in JA synthesis, starts with linolenic acid released by phospholipase activity from chloroplast membranes [76]. Although elicitors such as systemin induce the expression of the genes that are involved in this pathway [32], the JA burst that can be observed after damage occurs too fast to depend entirely on induced gene expression, indicating that the enzymes involved exist already in the undamaged cell [77]. Furthermore, transgenic overexpressers of enzymes of the octadecanoid pathway did not have increased resting levels of JA in undamaged tissue but showed increased JA production after wounding [78,79]. Therefore, because control of wound-induced JA synthesis seems to occur at the level of substrate availability [79], mechanical wounding might expose membrane-bound linolenic acid to compartmen-

tally separated phospholipases, thus triggering JA synthesis. By being synthesized from a plant-derived precursor by enzymes that get access to it as soon as the cell becomes disintegrated, JA represents *per se* a signal of the 'damaged self'.

To follow this thought, wound-induced JA synthesis would depend quantitatively on the number of damaged cells, enabling the plant to respond in a dose-dependent manner [3,22,46]. Many of the apparently contradictory results that led to the discussion of whether mechanical damage suffices to mimic the general wound response can be explained by the use of different damaging regimes: crushing leaves, puncturing many holes into the leaf blade or applying leaf extract usually elicited the full response [3,18,21,22,49], whereas cutting off parts of the leaves or inflicting little damage with pattern wheels resulted in less apparent or undetectable responses [6,7,9,80]. Different damaging regimes even cause the same plant species to respond differently [46]. For example, removing 50% of the leaf area of *Prunus avium* with a punching treatment (hence, inflicting intensive mechanical damage) induced a stronger defensive response than did removing the same amount of leaf area by cutting off leaf blades and thereby destroying a much lower number of cells [25].

Evolutionary scenario

In summary, elicitors *sensu stricto* of plant defences represent, with few exceptions, plant-derived compounds (Figure 1) that indicate the presence of heavily damaged, disintegrated cells. If the concept of damaged-self recognition holds true, how can plants mount specific responses to feeding by herbivore species that are characterized by different feeding types or differing host ranges [39–41]? What are the benefits of perceiving plant-derived versus animal-derived elicitors?

The answers probably lie in the evolutionary arms race between plants and herbivores: herbivores obtain fitness benefits from avoiding detection by the plant or suppressing its defence responses, whereas plants benefit from an early and specific detection of herbivore attack. This requirement poses a conflict on plants, which need to reliably and specifically detect herbivore damage without allowing the animal to control the interaction. Although perceiving animal-derived molecules can enable plants to detect herbivores at the species level, these molecules would be under the full evolutionary control of the herbivore. Therefore, plants can retain significant control when monitoring their status of damage via the perception of their own, plant-derived elicitors. Thus, in analogy to the PAMP-ETI model, the most probable scenario features a damaged-self recognition that forms the evolutionarily ancient part of the wound response. Depending on the specific type of plant-herbivore interaction, the general wound response might or might not be modulated by the recognition of evolutionarily derived and more specific elicitors.

Piercing-sucking herbivores often enter their stylets through the apoplast, inflicting minimal damage on living plant cells and thus avoiding the activation of plant defences that depend on damaged-self recognition. Other insects use 'biochemical crypsis' [81] or chemically sup-

press defensive plant responses [82–85]. These observations are likely to indicate situations in which the herbivore has gained an advantage in the evolutionary arms race. By contrast, mechanisms evolved by plants to pre-empt future damage already at the oviposition state [14,15] or via monitoring VOCs released from damaged organs (reviewed in Ref. [33]) can be interpreted as being advantageous to the plant.

Heuristic benefits of the concept of damaged-self recognition

Research over the past few years has revealed a high specificity of plant responses to herbivore feeding that goes far beyond a simple 'wound response'. Plants can respond specifically to different herbivores [39–41], external application of the wound hormone, JA, often elicits responses that are overlapping with (but not identical to) plant responses to herbivory [5–8,10], and the JA-independent action of intermediates of the octadecanoid signalling cascade [12] points to signalling pathways that function in parallel to, or downstream of, JA. Insect-derived elicitors probably have a crucial role within this specificity because they can accelerate or repress the response of a plant to mechanical damage.

However, recognition of the 'damaged self' is an inevitable part of all these responses and is likely to be the main trigger for the induction of the wound response. Focusing on this principle will help researchers to: (i) find the as yet unknown elicitors of widespread plant defence responses that have been described so far only at the phenotypic level; (ii) search for the receptors of these elicitors; and (iii) understand how the general principle has been modified through the ongoing coevolutionary arms race between plants and herbivores.

Hypothesis testing

Several hypotheses can be formulated that enable experimental testing of the concept of damaged-self recognition.

First, the number of damaged cells should correspond directly to the resulting concentration of JA and the intensity of the induced defensive response. Supporting observations have been made with various plant species [3,22,25,46,49]. To test this central hypothesis, future studies will need to control carefully the spatiotemporal patterns of mechanical damage and the resulting plant responses.

Second, every enzyme, compound or physical event that causes intensive cell disruption or the disintegration of cell compartments or plant molecules should activate or intensify the wound response. Interestingly, aminopeptidases have recently been suggested to have a role in plant herbivore defence, and *leucine aminopeptidase A* (*LAP-A*)-silenced plants were more susceptible to herbivore damage [86]. Being bound to specific compartments, wounding brings these aminopeptidases into contact with proteins and peptides from which they are separated in the intact cell. This event could cause the release of protein fragments that modulate damaged-self recognition in a similar manner to the plant ATPase fragment, inceptin [57], or systemin [61]. Recent evidence demonstrates that *LAP-A* is inducible by JA and positively regulates the

expression of JA-dependent defence genes in tomato [87]. Thus, LAP-A is likely to modulate or accelerate rather than initiate the original wound response.

Third, plant chemistry should have a crucial role in the synthesis of the elicitors: if the precursor is missing, no elicitor can be produced. Indeed, volicitin was absent from oral secretions of *Heliothis* caterpillars fed on *Physalis* fruits but was present when the caterpillars fed on leaves of the same species: *Physalis* fruits lack linolenic acid, whereas the leaves contain this component of volicitin [81].

Fourth, the evolutionary scenario developed above predicts that ‘damaged-self’ signals should be taxonomically widespread, elicited by generalist herbivores and induce responses that act against many types of herbivore. Elicitors whose synthesis involves the metabolism of the herbivore should, by contrast, represent the evolutionarily derived layer(s) of the defence response, hence being taxonomically less widespread and active in interactions with specialist herbivores. Several cases confirm this prediction. At the general end of the spectrum, EFN functions by attracting ants, which defend plants against a taxonomically wide spectrum of herbivores [22,48]. EFN is inducible by herbivory, and studies of 11 genera from six families (*Prunus* [Rosaceae], *Acacia*, *Phaseolus*, *Prosopis*, *Vicia* and *Leucaena* [Fabaceae], *Macaranga* and *Sapium* [Euphorbiaceae], *Populus* [Salicaceae], *Gossypium* [Malvaceae] and *Paulownia* [Scrophulariaceae]) found this induction to be elicited by mechanical damage [22,24,25,45,48,88–90]. Similarly, in a study on phytohormonal responses of five plant species, endogenous JA levels increased in all species 1 h after mechanical damage [44]. At the opposite end of the spectrum, activity of four elicitors (volicitin, N-linolenoyl-glutamine, caeliferin and inceptin) was highly specific: eggplant (*Solanum melogena*, Solanaceae) and maize responded to the same two elicitors and the other three species to only one elicitor, whereas in all other elicitor–plant species combinations tested, no significant differences between elicitor treatment and damage alone became obvious [44]. For example, volicitin induces defence expression in maize but was inactive in lima bean [12], cowpea (*Vigna unguiculata*) and *Arabidopsis* [44]; systemin functions in a group of closely related solanaceous species but not in another member of the same family, *Solanum nigrum* [60]; bruchins are active only in certain pea (*Pisum sativum*) genotypes [14,91]; and inceptin induced higher JA levels than those induced by mechanical damage in cowpea but not in soybean (*Glycine max*), *Arabidopsis*, eggplant or maize [44].

Fifth, elicitors of the wound response are formed from precursors that are present in the intact cell and are thus easily overlooked, particularly in screens of genes that are expressed only after damage. Pharmacological studies testing fractions or single compounds obtained from the damaged plant tissue for their defence-eliciting activity will, therefore, form a necessary part of the search for new elicitors.

Finally, it can be predicted that receptors of elicitors indicating the ‘damaged self’ must monitor the extracellular chemistry and transport information to the inner compartments (Box 2): although destroyed cells release many molecules into the extracellular space [8] and cell content

outside its normal compartment provides the elicitors, intact cells must perceive and process these signals to synthesize signalling hormones and defence compounds. Indeed, the putative systemin receptor protein SR160 is membrane-localized and binds to apoplastic systemin [32]. JA synthesis underlies a positive-feedback regulation [1,2,77], making it tempting to speculate that JA released from disrupted cells then induces JA synthesis in neighbouring, still intact tissues. Further studies should actively search for mechanisms and cell-membrane-bound receptors that monitor the extracellular chemistry.

Conclusions

The concept of damaged-self recognition arose from the observation that most elicitors *sensu stricto* of plant responses to herbivory represent or contain plant-derived molecules that indicate the presence of disintegrated plant cells (Box 1). This concept can help researchers to find the elicitors of those defence responses for which no elicitors are known so far and to understand how and why elicitors affect defence expression. Chemically well defined and apparently independent examples of defence elicitors, such as FACs, peptides, cell wall components and extracellular sugars and ATP, represent cases of the same general scheme: the occurrence of (fragments of) plant molecules outside the intact plant cell. That some elicitors occur in insect oral secretions should not detract attention from their origin in the destroyed plant cell: most HAMPs (with the exception of caeliferins, bruchins and other elicitors that are derived from insect oviposition fluids [14–17]) are unlikely to have been metabolized in an insect cell. Studies searching for new elicitors should, therefore, consider elicitors that stem directly from the plant and that are derived from precursors that are present in the intact cell.

A damaged-self recognition that perceives these elicitors is sufficiently fast to mount an appropriate general wound response of plants to herbivory and is independent of the specific biochemistry of the attacking animal. Thus, damaged-self recognition enables the plant to retain evolutionary control over its interaction with herbivores. The wound response of plants to herbivore feeding will be completely understood only when the full set of plant- and herbivore-derived defence elicitors has been identified. Because these elicitors are likely to differ among plant species, drawing attention to the damaged plant cell as a source of elicitors will significantly improve our understanding of all the different mechanisms by which plants perceive and react to herbivory.

Acknowledgements

I thank Wilhelm Boland, Jurriaan Ton, Regina Moritz, Peter M. Campbell and three anonymous referees for discussion and stimulating comments on earlier versions of this article. Financial support from CONACyT (Consejo Nacional de Ciencia y Tecnología de México) and the German Research Foundation (DFG-grant He 3169/4–2) is gratefully acknowledged.

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