Trends in Immunology

CellPress REVIEWS

Review

Extracellular DAMPs in Plants and Mammals: Immunity, Tissue Damage and Repair

Giulia De Lorenzo,[3,*] Simone Ferrari,[3] Felice Cervone,[3] and Eitan Okun[1,2]

Innate immune receptors, well known mediators of response to non-self-molecules and inflammation, also act as mediators of immunity triggered by 'damage-associated molecular patterns' (DAMPs). Pathogen-associated molecular patterns (PAMPs) cause inflammation in mammals and a rapid immune response in plants, while DAMPs trigger more complex responses, including immunity, tissue maintenance and repair. DAMPs, their receptors and downstream transduction mechanisms are often conserved within a kingdom or, due to convergent evolution, are similar across the kingdoms of life. Herein, we describe the dynamics and functionality of specific extracellular DAMP classes and their receptors in immunity, inflammation and repair of tissue damage in plants and mammals.

Highlights

Vertebrates and plants harbor an innate-type immune system that shows striking similarities.

Structural similarities and conservation seem to characterize DAMP signaling across the evolutionary tree.

Animals and plants share remarkably similar regulatory mechanisms that involve the ECM; some of these might be mediated by evolutionarily conserved elements, whereas others might derive from convergent evolution.

Danger Recognition across Kingdoms

A key function of the innate immune response is the recognition of exogenous pathogen-derived (nonself) or endogenous (self) danger signals by pattern-recognition receptors (PRRs) (Box 1). Unlike mammals, plants lack circulating cells and an adaptive immune system and, instead, rely entirely on the ability of individual cells to recognize and mount responses to pathogens [1], as well as to repair mechanical damage [2,3]. Nevertheless, mammals and plants show several similar characteristics of innate immunity. Here, we focus on the commonalities and disparities in signaling and response cascades activated by endogenous danger molecules in plants and mammals. We highlight the differences in terminology between self-molecular patterns originating from sterile inflammation or injury (DAMPs) and non-self-molecular patterns originating from microbes (microbe-associated molecular patterns), also referred to as pathogen-associated molecular patterns (PAMPs). These are different from the danger molecules originating from nematodes [4] and herbivores [5] (Box 1). DAMPs, a term coined by Land in 2003 [6], applies to endogenous molecules that are released, activated, or secreted by cells and tissues upon stress, damage, or even cell death, and that are perceived by the organism as a danger, with consequent activation of the immune system [1,5]. DAMPs act in defense against infection and are important players in pathogen-independent processes such as tissue damage and repair [2,7,8], and at least in plants, developmental processes [9]. However, while it is well known that release and activation of DAMPs occur during tissue damage, the role played by these molecules in other biological events is less well studied. DAMPs likely play roles still uncovered as regulatory signals in the broader context of both physiological and pathological processes. We describe here a subset of extracellular DAMPs that are conserved or are functionally equivalent in plants and mammals and discuss how their receptors work in the context of immunity and tissue damage and repair. New concepts are arising that suggest that molecules acting as danger signals under pathological conditions may display physiological regulatory functions at lower concentrations (Box 2). Knowledge of their mechanisms of action and expression may lead to the exploitation of these molecules in plant and animal biotechnology to improve resistance to diseases.

[1]The Leslie and Susan Gonda Multidisciplinary Brain Research Center, Bar-Ilan University, Ramat Gan, 5290002, Israel
[2]The Paul Feder Laboratory for Alzheimer's Disease Research, The Mina and Everard Goodman Faculty of Life sciences, Bar-Ilan University, Ramat Gan, 5290002, Israel
[3]Dipartimento di Biologia e Biotecnologie 'C. Darwin', Sapienza – Università di Roma, Piazzale Aldo Moro 5, Rome, 00185, Italy

*Correspondence:
giulia.delorenzo@uniroma1.it
(G. De Lorenzo).

© 2018 Elsevier Ltd. All rights reserved.

Box 1. Definition of Different Types of Danger Signals

Pathogen-associated molecular patterns (PAMPs): molecular structures present in pathogenic microorganisms, like LPS, flagellin, and peptidoglycan. These molecules are recognized by pattern recognition receptors (PRRs) expressed on both immune and nonimmune cells.

Microbe-associated molecular patterns (MAMPs): conserved molecular patterns that have been shown to act as PAMPs are also present in commensal microorganisms, which are part of the normal flora. In plant immunology, the terms PAMPs and MAMPs are often treated interchangeably.

Damage-associated molecular patterns (DAMPs): endogenous molecular structures that can normally be either outside the cell as part of a polymeric molecule or contained inside the cell and are liberated upon tissue damage. Examples include ATP and HMGB1 in both animals and plants, and low-molecular-weight fragments of hyaluronic acid and homogalacturonan in animals and plants, respectively. These molecules are recognized by a number of receptors, including PRRs and are capable of inducing inflammation and immune responses in the absence of infection.

Nematode-associated molecular patterns (NAMPs): a class of small molecules that is made only by nematodes and function as pheromones in these organisms. NAMPs are recognized by a wide range of plants.

Herbivore-associated molecular patterns (HAMPs): molecules deriving from herbivorous insects that trigger plant defense responses. They can be present in saliva, ventral eversible gland secretions, oral secretions (regurgitant), digestive waste products, ovipositional fluids as well as herbivore-associated endosymbionts.

Box 2. DAMPs in Health versus Inflammation

The current view is that damage-associated molecular patterns (DAMPs) are released actively or passively by cells following exposure to either a physical stress or an infective agent. We would like to suggest that low levels of DAMPs can be released from cells to regulate physiological processes, and high concentrations of the same DAMPs are accumulated during infection or tissue damage. We hypothesize that under physiological conditions these DAMPs act on receptors with high affinity that are different from the lower affinity receptors active during infection and/or inflammation.

DAMPs in Tissue Damage and Sterile Inflammation

Sensing and reacting to the danger provoked by tissue damage is a crucial trait for survival of organisms across the kingdoms of life. Upon tissue damage due to a mechanical insult or infection, organisms can activate defenses upon sensing endogenous signals. Disruption of the homeostatic cellular processes *per se* is sensed as a dangerous event leading to induction of sterile inflammation in mammals [5,10], immune responses in plants [1,5,9], and tissue repair in both [2,3,11]. Sterile inflammation may respond to the release into the extracellular space of molecules that are normally encased in larger polymers or confined in compartments inside the cell and are perceived by PRRs as DAMPs. The recognition of damaged self has evolved as a mechanism in all eukaryotes, providing similar solutions to the same problem. Because damage is recognized independently of an invading pathogen, the activated response is not specific against a given threat [5].

To date, a plethora of DAMPs have been described in mammals, including oligonucleotides, uric acid, ATP, large fragments of DNA and RNA, oligosaccharide fragments of hyaluronic acid (HA), and heparan sulfate (HS), as well as intracellular proteins such as heat shock proteins and high mobility group box (HMGB)1 [12]. Although fewer DAMPs, such as cell wall fragments, ATP, and DNA have been described in plants [1], the commonalities existing across these biological kingdoms suggest that many more plant-derived DAMPs are expected to be discovered.

Extracellular DAMPs

Similarity and conservation characterize DAMP signaling in vertebrates and plants. So far, different types of extracellular DAMPs have clearly emerged as triggers of immunity. Among these, several have been extensively reviewed elsewhere, including extracellular ATP [13–15]

Glossary

Abscission: natural separation (shedding) of flowers, fruit, or leaves from plants at a special separation cell layer. It is a specific developmental process involving a spatially localized degradation of the cell wall.
Apoplast: in a plant, the space in between cells comprising cell wall and intercellular material, including air containing CO_2, O_2, and water vapor.
Berberine-bridge enzyme-like (BBE-like) proteins: family of flavoenzymes (28 in *Arabidopsis*), apoplastic or putatively apoplastic and still poorly characterized. The name of the family derives from a distant homolog, the (*S*)-reticuline oxidase or berberine bridge enzyme from California poppy (*Eschscholzia californica*). It catalyzes an oxidative ring closure reaction, through a C–C bond (the so called berberine bridge), in the conversion of (*S*)-reticuline to (*S*)-scoulerine, a branch point in the biosynthesis of benzylisoquinoline alkaloids.
Biglycan: belongs to the small leucine-rich proteoglycan family and may be involved in collagen fiber assembly.
Callose: extracellular plant polysaccharide, mainly in the form of β-1,3-glucan, made at the outer plasma membrane. It is a normal component in the dividing cell or the pollen tube wall and in transitional stages of wall development of certain cell types. Its synthesis is induced by pathogens, at the site of infection and by treatment with PAMPs and DAMPs.
Camalexin: main phytoalexin (see below) in *Arabidopsis*.
Chitin: polysaccharide made of N-acetylglucosamine and a primary component of the cell walls in fungi. It is a well-known PAMP.
Conspecifics: organisms belonging to the same species.
Egg box: conformational state induced in HGA and OGs by calcium-mediated intermolecular bonds. This conformation is intermediate between single-isolated chains and calcium-associated multimeric chains, which are responsible for the gelation properties of pectin.

and the nuclear protein HMGB1, which have a role in both mammals [16] and plants [1,5]. Other types of ubiquitous DAMPs are represented by endogenous extracellular double-stranded (ds) DNA, released either passively or actively from damaged or infected cells, and by functionally equivalent polysaccharide DAMPs released from the extracellular matrix (ECM) in both animals and plants, as discussed below.

Extracellular DNA (exDNA)

Many receptors are responsible for sensing DNA in mammals, as recent studies have demonstrated; however, nothing is known about DNA sensing by plant cells, in which, accumulating evidence points to involvement of dsDNA in immunity [17]. For instance, in root tips of the model plant *Arabidopsis thaliana*, root border cells rapidly export newly synthesized DNA to the extracellular space (exDNA) [18]. Its degradation by DNase 1, leads to loss of resistance to fungal infection, while slower degradation by exonuclease BAL31 delays the loss of resistance [19]. These results provide supportive evidence that exDNA might form a sort of extracellular trap in the **root border cells** (see Glossary), analogous to the **neutrophil extracellular traps** (NETosis) used by immune cells to immobilize and kill invading microbes [20]. The observation that extracellular DNases can contribute to the virulence of the phytopathogenic bacterium *Ralstonia solanacearum*, likely due to degradation of the extracellular traps, corroborates the hypothesis [21]. It must be noted that NETosis in mammals is activated following tissue damage, while release of exDNA in plants appears to be a constitutively active system; most likely because the root tip is continuously exposed to mechanical stress. Thus, while exDNA released by border cells at the root tip of plants is not a DAMP *per se*, its function in plant immunity and functional resemblance to mammalian NETs merit further attention. High concentrations of extracellular fragmented DNA from **conspecifics** can inhibit growth and eventually induce cell death in *Arabidopsis*, which is intriguing because heterologous DNA does not so do [22]. A similar effect has been observed on growth of a wide taxonomic range of organisms, including bacteria (*Bacillus subtilis*), protozoa (*Physarum polycephalum*), green algae (*Scenedesmus obliquus*), fungi (*Trichoderma harzianum*) and insects (*Sarcophaga carnaria*; the common flesh fly) [22,23]. Conspecific but not heterologous exDNA has also been reported to induce plasma membrane depolarization and calcium signaling in lima beans and maize [24]. This apparently general species-specific biological effect of exDNA might be related to the capability of extracellular DNA to act as a DAMP [17,22,23,25]. Research on how exDNA triggers responses in plants, and determining the nature of these responses, may gain more attention in the future, with implications in the fields of pathology and ecology.

ECM-Derived DAMPs

Certain animal cells such as epithelial cells and endothelial blood vessel cells have a glycocalyx that is considered similar to the ECM of plants, that is, the plant cell wall. Both the glycocalyx and the plant cell wall contain a complex and dynamic array of polysaccharides, proteoglycans, and (glyco)-proteins; the function of which goes beyond mechanical/structural support. The components of the animal and plant ECM mediate cell–cell adhesion and communication, providing the spatial context for many signaling events that govern cell functions, differentiation, developmental patterning, and growth [26].

Animals and plants share regulatory mechanisms involving the ECM, some of which are mediated by evolutionary conserved elements, whereas others derive from convergent evolution. Examples of the latter are the vertebrate HA and the plant homogalacturonan (HGA). Both molecules are high-molecular-weight (HMW) acidic linear polysaccharides. HA is formed by the repetition of a disaccharide formed by glucuronic acid and acetylglucosamine bound via alternating β-1,4 and β-1,3 glycosidic bonds (Figure 1) [27]. HGA is a structurally simpler

Growth-defense trade-offs: it is the balance that in plants, reflects the allocation of the limited metabolic resources between defense responses and growth.
Innate immune memory: an enhanced reactivity in innate immune cells previously exposed to a stress stimulus. It is different from the adaptive memory in T and B lymphocytes.
NETosis (neutrophil extracellular traps): networks of extracellular fibers, primarily composed of DNA from neutrophils, which can bind and kill invading pathogens.
Peptidoglycan: also known as murein; structural polymer of the bacterial cell envelope made of sugars and amino acids. It is a well-known PAMP in both plant and mammals.
Phytoalexins: antimicrobial substances synthesized *de novo* by plants that accumulate rapidly at areas of pathogen infection. They are broad-spectrum inhibitors and are chemically diverse with different types characteristic of particular plant species.
Preconditioning: term utilized in animal biology to define a phenomenon whereby exposure to a small but potentially harmful stimulus is able to induce a prompter and stronger protection against a subsequent insult.
Priming: adaptive mechanism that improves the capacity of plants to defend themselves. Treatment with pathogens, beneficial microbes, as well as chemicals and abiotic cues activates, upon subsequent challenge with pathogens, a faster and/or stronger defense response and increased resistance to a stress.
Root border cells: cells that separate from plant root tips and disperse into the soil environment. In most species, each root tip can produce thousands of metabolically active border cells daily.
Sentinel cells: refer to cells representing the first line of defense in tissues such as skin or to specific antigen-presenting cells, such as macrophages.
Trained immunity: property that allows cells such as macrophages, monocytes, and natural killer cells to show enhanced responsiveness when they re-encounter pathogens.

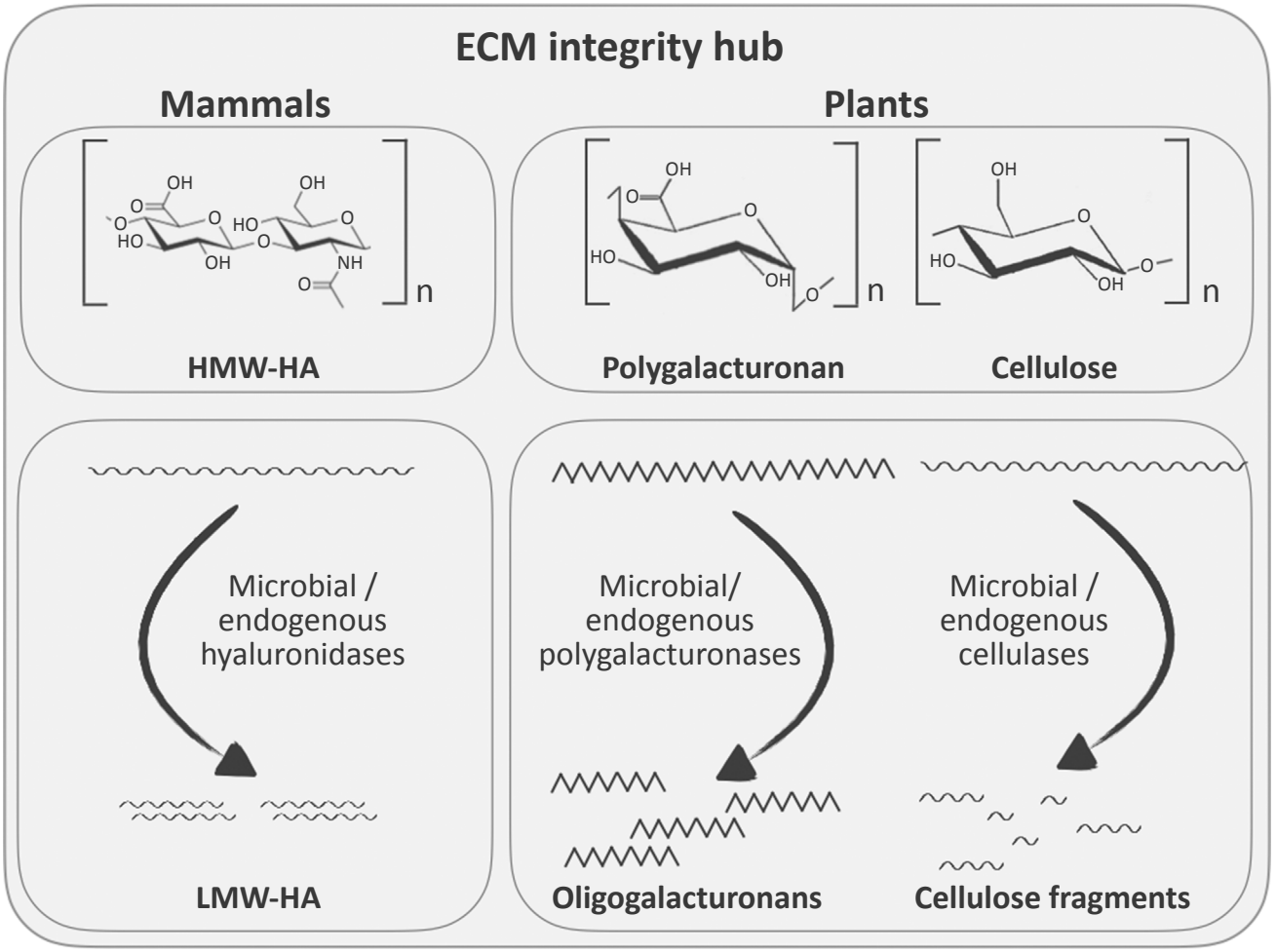

Figure 1. ECM Components Function as an Integrity Hub in Tissues. In mammals, HMW-HA can be degraded by either microbial or endogenous hyaluronidases to form LMW-HA, which act as DAMPs. In plants, homogalacturonan can be degraded by either microbial or endogenous polygalacturonases to form homogalacturonan fragments. Oligomers derived from cellulose, such as cellobiose are other oligomeric ECM polysaccharides described to act as DAMPs in plants. Abbreviations: DAMP, damage-associated molecular pattern; ECM, extracellular matrix; HMW-HA, high-molecular-weight hyaluronic acid; LMW-HA, low-molecular-weight hyaluronic acid.

polymer of α-1,4 d-galacturonic acid only. The structure of HA and HGA is crucial for health and survival of animals and plants, respectively, and is continuously and strictly monitored as a major indicator of ECM integrity [9,27–29]. The structure is challenged in the synthesis and remodeling that occurs during organ formation, growth, and development [30,31]. Mechanisms that sense the state of HA and HGA might counteract the consequences of loss of ECM structural integrity [32]. It has been confirmed that breakage products of HA in animals, similarly to HGA fragments, that is, oligogalacturonides (OGs) in plants [33], are perceived as DAMPs [28] (Figure 1). In plants, OGs may originate during pathogen infection from the fragmentation of HGA accomplished by microbial polygalacturonases (PGs) [29,34,35], whereas, upon wounding and mechanical damage, OGs may be produced by plant-derived endogenous PGs [2,36] (Figure 1). The mechanisms of release and homeostasis of these molecules may modulate their functions during infection, wounding, growth, and developmental processes that involve cell wall remodeling.

For both HA and HGA, the size of fragments is a major factor dictating their biological action [28,37]. In plants, OGs that are composed of ten to 15 sugar residues ($OG_{10–15}$) appear to be the most biologically active in terms of induction of immune responses [9], which includes production of reactive oxygen species (ROS) [38–40], accumulation of **phytoalexins** [41], expression of defense-related genes [42], and synthesis of **callose**. The deposition of the latter at the site of microbial infection is a typical plant immune response that retards the spread of pathogens in the tissue [43]. Shorter OG oligomers (2–6 residues long) show less pronounced elicitation activity than $OG_{10–15}$. In tomatoes, they induce expression of proteinase inhibitor genes effective against insects [44], and in *Arabidopsis*, they regulate numerous defense genes, producing a response qualitatively similar to that of $OG_{10–15}$ but of lower amplitude [45]. Compared to $OG_{10–15}$, however, OG_3 does not elicit an ROS burst and is more effective in causing growth retardation [45]. The activity of OGs is maximal when they have a degree of polymerization (DP) >8; likely because this length promotes the formation of the so-called **egg boxes** [46]. While modification of the reducing end of OGs does not affect the formation of egg boxes, the degree of methylation and/or acetylation of HGA, which varies in different organs during plant development, may influence the nature of OGs released during infection or upon wounding [29], and consequently, their biological activity. Direct experimental evidence indicates that OGs produced on command *in vivo* by engineering *Arabidopsis* plants to express, under an inducible promoter, a protein fusion comprising a fungal PG and its plant-derived specific inhibitor (polygalacturonase-inhibiting protein or PGIP), activate immunity and confer protection against fungal and bacterial pathogens [33,47]. However, hyperaccumulation of OGs may affect growth of the whole plant, eventually leading to cell death [33], and pointing to OGs as players in the well-known plant behavior referred to as **growth-defense trade-off** [48]. A mechanism that may control the homeostasis of OGs, avoiding their deleterious hyperaccumulation, has been recently discovered. A battery of at least four *Arabidopsis* enzymes, belonging to the family of the so-called **berberine-bridge enzyme-like** proteins [49], specifically oxidize OGs and produce oligosaccharides that display reduced ability to induce expression of defense genes, ROS burst, and deposition of callose [50]. Whether oxidation of DAMPs is a general mechanism by which their hyperaccumulation is kept under control deserves further investigation.

Additional ECM polysaccharides act as DAMPs. In plants, for example, cellulose-derived oligomers including cellobiose and cellotriose induce immune responses and are suggested to play a role in surveillance of cell wall integrity [51–53] (Figure 1). In *Arabidopsis*, cellobiose triggers a signaling cascade that shares similarities to that triggered by $OG_{10–15}$ [52], whereas cellotriose induces ROS and cytosolic Ca^{2+} accumulation, and upregulation of defense genes such as *RBOHD* that encodes NADPH oxidase necessary for the oxidative burst and callose deposition [53]. To date, no potential receptors for cellulose-derived oligomers have been identified.

Inulin and fructo-oligosaccharides (FOSs), which are plant fructans composed by fructose monomers connected via β(2, 1) glycosidic bonds linked to a terminal glucose residue, differ mainly in chain length, with a DP >10 for inulin and <10 for FOS. Extracellular FOS from the plant *Arctium lappa* (burdock) are reported to act as DAMPs in tomatoes and grapes, and confer protection against fungal pathogens by inducing the stress hormone salicylic acid [54]. FOSs are also perceived by human gut cells in a TLR2/TLR4/MYD88-dependent manner [55,56]; in this case FOSs are considered to be PAMPs as they are also present in bacteria [54].

In mammals, HS fragments, produced by heparanase-1, a tightly regulated enzyme, activate the innate immune response by several mechanisms including sequestration of cytokines/

chemokines in the ECM, modulation of leukocyte interactions with endothelium and ECM; as well as TLR4 activation [57]. HS is also a component of some proteoglycans and consists of repeating disaccharide units, constituted by glucuronic or iduronic acid and hexosamine residues with various degrees of sulfation. HS maintains the structural integrity of the ECM and can inhibit cellular invasion by promoting cell–cell and cell–ECM interactions (including leukocytes), sequestering cytokines/chemokines in the extracellular space, and initiating innate immunity through interactions with TLR4 [58]. Specifically, heparanase can promote the release of proinflammatory cytokines by immune cells generating fragments of HS that can activate either TLR4 or TLR2 in an MyD88-dependent manner. This manipulation of the ECM is thought to promote efficient migration of leukocytes to sites of inflammation. In this respect, HS as a DAMP may promote tissue damage repair in response to inflammatory cues [58,59].

DAMP Receptors

Plants and mammals often utilize convergent solutions to defend themselves and use PRRs for the recognition of PAMPs and DAMPs. Unlike mammalian TLRs, plant PRRs do not harbor a Toll-interleukin receptor (TIR) domain in their cytosolic portion and exhibit either leucine-rich repeat (LRR) or non-LRR sensor ectodomains [60]. Many of them carry a monophyletic kinase domain and share a common evolutionary origin with the human IRAK and *Drosophila* Pelle group of soluble Ser/Thr kinases [61]. Plant PRRs carry either an arginine–aspartate (RD) or, less frequently, a non-RD kinase domain [62], both with Ser/Thr specificity [63] and, in the case of *Arabidopsis* CERK1 involved in sensing the PAMPs **chitin** and **peptidoglycan**, dual specificity (Ser/Thr and Tyr) [64]. In plants, a TIR domain is found at the N terminus of several members of the superfamily of nucleotide-binding oligomerization domain (NOD) LRR-containing proteins, which are similar to the NOD-like receptors (NLRs) in animals. Similar to mammalian NLRs, most plant NLRs interact, either directly or indirectly, with pathogen-derived effector molecules in a genotype-specific manner [65].

Recently discovered PRRs are PEPR1 and PEPR2, LRR-containing receptor kinases, which in *Arabidopsis* are sensors of the endogenous peptides AtPeps [66,67]. These are considered DAMPs by several authors [66–68]; however, since their accumulation is induced by both PAMPs and DAMPs [42,52], they might be considered damage-induced signals rather that damage-associated patterns, analogous to cytokines in mammals [1]. Perception of AtPeps enhances the resistance of *Arabidopsis* and maize to bacteria, fungi, and herbivores, showing their receptors as amplifiers of innate immunity [69]. In addition to their roles in response to biotic stresses, AtPeps and their receptors might be involved in the regulation of plant development [68]. Similarly, mammalian PRRs such as TLRs are reported to expand their role beyond immunity towards development-related processes including that of the central nervous system [70–74]. Unlike LRR-containing Pep receptors, the *Arabidopsis* wall-associated kinase (WAK)1 carries an ectodomain characterized by epidermal growth factor-like motifs and can act as a receptor for OGs [75]. WAK1 belongs to a family of five members that collectively play a role in immunity and development [76]. Alterations of the expression of WAK1 and of its interactors glycine-rich protein-3 (extracellular) and kinase-associated protein phosphatase (cytoplasmic) alter the response to wounding, supporting the notion that OGs may act as local signals in response to mechanical injury [77]. The analysis of several mutants shows that subsets of responses to OGs require different immunity-related co-receptors and receptors [39], indicating that OGs are sensed through multiple and partially redundant perception/transduction systems, with a complexity that is unprecedented in plant immunity and might resemble that of the systems sensing HA/HA fragments in vertebrates (see below).

The perception of HA fragments in mammals depends on several factors including the size of the fragments, receptor activation state, and the distribution of the receptor throughout the cell membrane [78]. Specifically, a length of approximately 20 monosaccharides is the minimum that differentiates between monovalent and divalent interactions of HA with CD44 [78]. These considerations are critical to determine whether CD44 mediates downstream signaling. HA fragments >1 million Da, that is, the minimal native HA, are termed HMW-HA [79]. The fragments with intermediate size are termed low-molecular-weight (LMW)-HA [28]. During pathological events such as physical tissue damage, exposure to ROS, bacterial infection, or UV irradiation, ECM homeostasis may be disrupted. Under these conditions, endogenous HMW-HA may be concomitantly degraded by hyaluronidases and ROS into LMW-HA, which can be further depolymerized to oligomeric HA [78]. Extensive research conducted mostly in rodents has revealed that HMW-HA and its degradation products LMW-HA and oligomeric HA can bind several cell surface receptors. These include CD44, RHAMM (receptor for HA-mediated motility), HARE (human hyaluronan receptor for endocytosis), LYVE1 (lymphatic vessel endothelial hyaluronan receptor 1), layilin, and the PRRs TLR2 and TLR4 [80–88]. These receptors are reported to mediate different processes such as inflammation, cellular migration, and angiogenesis; all of which are involved in wound healing [80–88].

Examples of Signaling Cascades Activated by Extracellular DAMPs

DNA

Both exDNA and mitochondrial DNA (mtDNA) can activate intracellular innate immune pathways. Specifically, mtDNA is rich in unmethylated CpG dinucleotides due to its evolutionary bacterial origin [89]. In mammals, mtDNA activates the TLR9, NLRP3 inflammasome, and STING signaling pathways, as shown in various mouse immune cells such as B lymphocytes and macrophages, as well as in fibroblasts [90–93]. In unstimulated cells, TLR9 is located in the endoplasmic reticulum (ER) and, upon stimulation by CpG DNA, is translocated to endosomal membranes, where it recognizes its ligands and initiates cellular activation [91]. This causes secretion of inflammatory mediators such as matrix metalloproteinase-8, nuclear factor (NF)-κB signaling, and increased expression of other proinflammatory cytokines, including tumor necrosis factor-α interleukin (IL)-6 and IL-1β [89,94]. mtDNA released in the cytoplasm is dependent on the release of mtROS triggering the NLRP3 inflammasome [90,92]. It is not clear whether mtDNA and mtROS mediate their effects in a sequential manner, or if mtDNA oxidized by mtROS directly binds NLRP3 and activates the inflammasome [90]. Moreover, NLRP3 activates the release of mtDNA into the cytosol, further potentiating the activation of inflammasomes [95]. Interaction of the NLRP3 inflammasome complex with the adaptor protein ASC and procaspase-1 enables the recruitment and activation of caspase-1, leading to the maturation of IL-1β and IL-18 and the induction of proinflammatory cell death of **sentinel cells** [95]. mtDNA is also capable of exerting noncanonical TLR9 signaling in cultures of primary cardiomyocyte and neuronal cells, leading to AMPK activation through inhibition of SERCA2 in mouse neonatal cardiomyocytes [96]. As cardiomyocytes possess abundant mitochondria, it is likely that higher amounts of mtDNA are released during damage of heart tissues rather than from other tissues [96].

Another signaling cascade activated by mtDNA in mammalian cells is the STING pathway. Fragmented mtDNA induces mitochondrial cGMP-AMP synthase to generate the second messenger cGMP-AMP dinucleotide and activates STING in the ER [93]. STING then activates TANK-binding kinase 1, which phosphorylates interferon (IFN) regulatory factor (IRF)3 and induces IRF3-dependent expression of type I IFN and other IFN-stimulated genes. Both responses can promote innate antiviral defenses to dampen viral propagation [93].

In plants, the importance of mitochondria as well as ROS production and programmed cell death in innate immunity is well established [97]. For example, the bacterial pathogen *Pseudomonas syringae* can exert its virulence through effectors such as HopG1 that impairs mitochondrial function [98]. It is therefore likely that release of mtDNA during pathogen infection also occurs in plants, especially when a localized cell death occurs. However, direct evidence of a signaling role of mtDNA in plants has not been reported.

Extracellular Fragments of HA/HGA

Perception of OGs by plants triggers a transduction pathway that shares several components with those induced by PAMPs. For example, the transcriptional profiles of *Arabidopsis* seedlings treated with OGs and flagellin, which is a well-studied PAMP of bacterial origin, are almost identical at the early stages of the response. Transcript changes diverge later in timing of expression and number of genes involved [9]. Typical early signaling events involved in both OG- and PAMP-triggered defense responses include: (i) plasma membrane depolarization and alkalization of extracellular pH; (ii) calcium fluxes and activation of specific calcium-dependent protein kinases; (iii) production of ROS and nitric oxide; and (iv) activation of specific MAPK cascades [2,9,99]. In *Arabidopsis*, MPK6 has a crucial role in gene expression and enhanced resistance against the necrotrophic fungus *Botrytis cinerea*, induced by OGs as well as flagellin [9,100], while the mitogen-activated triple kinases ANPs act in the cascades activated by OGs and elf18 – another PAMP of bacterial origin [40]. Moreover, simultaneous loss of the *Arabidopsis* CPK5, CPK6, and CPK11, important for flagellin-mediated responses, affects the production of the stress hormone ethylene during infection with *B. cinerea* and the duration of gene expression induced by OGs [99].

A rapid and robust oxidative burst is a hallmark of both OG- and PAMP-triggered immunity. Plasma membrane NADPH oxidases release superoxide anions in the **apoplast** that are rapidly converted into hydrogen peroxide by extracellular superoxide dismutases [101]. The functional role of the oxidative burst is dependent on the activated downstream responses. For instance, the oxidative burst mediated by the NADPH oxidase RBOHD in *Arabidopsis* is necessary for callose deposition but not for protection against *B. cinerea* induced by OGs and flagellin; presumably because callose is not important for resistance against this pathogen, although this remains to be verified. In contrast, the study of loss-of-function mutants has shown that *Arabidopsis* RBOHD is required for flagellin-induced resistance against *P. syringae* infection [38], whereas silencing of expression of the apoplastic peroxidase (PRX)33 and 34 blocks the oxidative burst in response to a fungal elicitor and OGs, and causes enhanced susceptibility to a broad range of fungal and bacterial pathogens [102,103].

With respect to HA, HMW-HA displays anti-inflammatory and immunosuppressive properties [104], whereas LMW-HA is a potent proinflammatory molecule [105]. Specifically, LMW-HA is a potent activator of macrophages and airway epithelial cells, by driving the expression of the proinflammatory cytokines and matrix-modifying enzymes such as MME, inducible nitric oxide synthase, and plasminogen activator inhibitor-1. These transcriptional effectors enhance inflammatory responses in a positive feedback manner [105].

Understanding downstream signaling outcomes by HA is complex, in part, due to the large repertoire of receptors that interact with HA. CD44, the main receptor for HA, consists of ten constant and ten variant exons in its extramembrane site, resulting in multiple splicing variants, which may provide higher functional flexibility [106]. In the context of a mouse LPS-induced lung inflammation model, CD44 is responsible for cellular internalization of HA, and blocking CD44 on the surface of macrophages causes impaired clearance and delay in the healing process

[107]. In an *in vitro* wound model, CD44 can promote fibroblast migration [108]. Following binding of oligomeric HA, CD44 may mask cell death receptors and consequently prevent the cell from reaching apoptosis, by coating the cell membrane, whereas smaller HA fragments might not induce this effect, as tested in an *in vivo* LPS-induced ear inflammation model [109].

RHAMM-induced signaling is complex and dependent on its localization. RHAMM can be located either in the cytoplasm, nucleus, cell surface, or altogether secreted, where it can interact with CD44 and participate in many cell functions, including cell motility, wound healing, and modification of signal transduction of the Ras signaling cascade [85]. RHAMM activation and downstream signaling are intimately linked with cellular migration. This has been extensively studied in the context of cancer [85]. Thus, depending on the context of the interaction, cell surface RHAMM interacts with CD44, HA and growth factor receptors to mediate protein tyrosine kinase signaling cascades that activate the ERK1/2 MAPK cascade in a c-Src/Raf-1/MEK-1/ERK1/2-dependent manner [85,110].

Much is unknown regarding the involvement of TLR signaling in LMW-HA-mediated wound healing. One *in vitro* study conducted on a human vaginal epithelial cell line has indicated that LMW-HA favors repair of vaginal epithelial injury involving TLR2 and TLR4, and independently from its classical receptor CD44 [81]. This study demonstrated, using western blotting and immunofluorescence, that upon treatment with LMW-HA, an intracellular pathway involving phosphorylation of Akt and megalencephalic leukoencephalopathy with subcortical cysts 1 (MLC) was required to promote cell migration rather than cell proliferation [81]. The induction of wound-healing activity by LMW-HA signaling may provide additional protective activity in the vaginal compartment, which is constantly exposed to microbiota.

Priming and Preconditioning

The capacity of PAMPs to condition tissue for faster activation of defense responses upon infection, and therefore, conferring long-term protection against microbes has been demonstrated in plants and mammals, and is called **priming** in plants [111] and **preconditioning** in mammals [112,113]. Priming and preconditioning rely on a form of nonspecific memory, indicated as **trained immunity** in mammals or innate immune memory in both mammals and plants [114], which does not depend on activated defense responses but makes an organism capable of responding faster and more efficiently to a secondary insult or infection. Priming and preconditioning likely occur through the accumulation of dormant signaling components and persistent changes in histone modification patterns in defense-related genes and their regulatory regions. In fact, preconditioning has been shown to occur independently of T and B lymphocytes, which mediate adaptive immunity in mammals [115]. Preconditioning was mostly studied in models of focal ischemic brain stroke, where pretreatment with PAMPs was shown to confer protective effects in a TLR2- and TLR4-dependent manner [116]. In *Arabidopsis*, priming leads to higher levels of the flagellin receptor FLS2 and its co-receptor BAK1, and of the chitin receptor and peptidoglycan co-receptor CERK1 [117]. Major signal transduction elements such as MPK3 and MPK6 also mediate priming, and their transcript and protein levels are higher in primed *Arabidopsis* plants. These kinases are kept inactive but ready for activation upon pathogen attack [118]. Moreover, priming can be inherited [119], likely through epigenetic mechanisms, such as DNA methylation and histone modifications that are triggered by different biotic stresses [113]. Priming is also induced by DAMPs such as OGs [9]. OG pretreatment elicits accumulation of the endogenous phytoalexin **camalexin** in *Arabidopsis* plants inoculated with *B. cinerea* [99], despite the fact that expression of genes involved in camalexin biosynthesis is induced only transiently [9]. While priming can be inherited in plants, it is not known whether preconditioning can be inherited in mammals. DAMPs such as HMGB1

have been shown to mediate preconditioning in a rat model of focal cerebral ischemia–reperfusion [120]. Despite *in vitro* evidence for the efficacy of LMW-HA and HMW-HA in preconditioning, there is a lack of evidence for such effects *in vivo* [121].

Thus, despite the parallels that can be found between plant priming and mammalian preconditioning, it is unclear whether the two processes share mechanisms. It will be interesting to see whether DAMPs and PAMPs equally induce preconditioning in mammals.

Tissue Maintenance and Repair

Mounting evidence suggests that DAMPs play a role in immunity and mediate additional physiological processes. An overlooked aspect of DAMP biology is the presence of these molecules in physiological conditions due to remodeling, turnover of cellular structures and compartments, and programmed cell death [122]. For example, OGs in plants are located in key locations that allow them to act as indicators of cell wall integrity; both in adverse conditions and during normal growth [123]. In *Arabidopsis*, hundreds of pectic enzymes are regulated during development and can potentially release OGs [124]. Under physiological conditions microlesions occurring in developmental processes such as cell expansion, lateral root formation, organ **abscission**, tissue maturation, and pollen–stigma interactions may be perceived through the release of low amounts of OGs, while larger ruptures, which generally occur during pathogenic events and mechanical injury, can be sensed because large amounts of OGs are formed [33]. Besides activation of immune responses, OGs can affect growth and developmental processes [123,125], likely through a signaling pathway different from the one that regulates immunity [39], although this remains speculative. It is possible that most of the effects of OGs on growth and development are likely due to their capacity of antagonizing the action of auxin, an important plant hormone, both in the formation of roots and in the regulation of gene expression [123].

Examples of innate immune receptors involved in tissue repair and maintenance are also present in mammals. Endothelial cells, for example, express TLRs that are strategic mediators of the immune response in endothelial cells but can also contribute to regulating angiogenesis, a critical process for tissue repair [126]. For instance, **biglycan**, an important component of the ECM, can lead to an increased interaction between NF-κB and the *HIF1A* promoter in human cells lines [126]. This results in transcription of *HIF1A* mRNA, as well as enhanced activity of the HIF-1α protein, which culminates in VEGF expression in a TLR2- and TLR4-dependent manner. This is reported to lead to enhanced endothelial cell migration, proliferation, and blood vessel formation (endothelial tube formation assay), and is also implicated in gastric cancer [126]. Thus, DAMPs represent a major pathway supporting the survivability of both plants and mammals in the light of unexpected injuries to self.

Concluding Remarks

DAMPs have so far been studied separately in plants and mammals. Our synthesis here indicates that, although numerous questions remain (see Outstanding Questions), the functions and mechanisms of action of DAMPs in plants and mammals share similarities in addition to differences. Thus, it is anticipated that parallel features might be revealed in the future. For example, as discussed above, it is reasonable to hypothesize that graft incompatibility in plants may arise due to the nonspecific release of DAMPs or to a specific mechanism involving DAMPs. Moreover, there is evidence that low levels of regulatory molecules can be released from the ECM during its synthesis, remodeling, and turnover occurring during normal growth. Higher concentrations of the same molecules can accumulate during infection or tissue damage and act as DAMPs, that is, molecules with a physiological function may behave as

Outstanding Questions

What is the mechanistic link between the integrity of the ECM and activation state of the immunity response?

The structural complexity of the ECM could serve as a latent DAMP reservoir. How many different DAMPs derive from the fragmentation of the components of the ECM? How do they activate the immunity responses?

An exaggerated accumulation of DAMPs might lead to detrimental effects on the organisms and hyperimmunity. How many homeostatic mechanisms for maintaining the optimal level of DAMPs exist and may they be exploited for agricultural or therapeutic purposes?

exDNA may act as a DAMP in many organisms. Does it also play a role as a factor for shaping natural plant ecosystems?

DAMPs when their concentration increases, widening the classical definition of DAMPs [123,127]. In this respect, it would be interesting to examine whether the receptor types for DAMPs differ between inflammation/tissue damage versus physiological conditions. One might speculate that under physiological conditions, certain DAMPs that are present at low concentrations could activate DAMP receptors with a low Km, whereas under inflammatory tissue-damage conditions, DAMPs might be perceived by receptors with a higher Km. Such a differential perception that depends on the ligand concentration might be the simplest way by which one single molecule might activate two completely different pathways; on the one hand, related to a physiological response, and on the other hand, related to a pathological one (Box 2).

As demonstrated in plants, DAMP formation and expression can be engineered on command by using inducible promoters [47]. Therefore, one might hope that a deeper knowledge of DAMPs and their mode of action in various organisms might lead to exploit them in such a way as to promptly respond to many insults or to different pathogens. A more in-depth examination of this field is both timely and translational to commercial applications in both plant and pharmaceutical biotech industries.

References

1. Gust, A.A. *et al.* (2017) Sensing danger: key to activating plant immunity. *Trends Plant Sci.* 22, 779–791
2. Savatin, D.V. *et al.* (2014) Wounding in the plant tissue: the defense of a dangerous passage. *Front. Plant Sci.* 5, 470
3. Melnyk, C.W. *et al.* (2018) Transcriptome dynamics at *Arabidopsis* graft junctions reveal an intertissue recognition mechanism that activates vascular regeneration. *Proc. Natl. Acad. Sci. U. S. A.* 115, E2447–E2456
4. Manosalva, P. *et al.* (2015) Conserved nematode signalling molecules elicit plant defenses and pathogen resistance. *Nat. Commun.* 6, 7795
5. Heil, M. and Land, W.G. (2014) Danger signals - damaged-self recognition across the tree of life. *Front. Plant Sci.* 5, 578
6. Land, W.G. (2003) Allograft injury mediated by reactive oxygen species: from conserved proteins of *Drosophila* to acute and chronic rejection of human transplants. Part III: interaction of (oxidative) stress-induced heat shock proteins with toll-like receptor-bearing cells of innate immunity and its consequences for the development of acute and chronic allograft rejection. *Transpl. Rev.* 17, 67–86
7. Venereau, E. *et al.* (2015) DAMPs from cell death to new life. *Front. Immunol.* 6, 422
8. D'Agostino, A. *et al.* (2015) *In vitro* analysis of the effects on wound healing of high- and low-molecular weight chains of hyaluronan and their hybrid H-HA/L-HA complexes. *BMC Cell Biol.* 16, 19
9. Ferrari, S. *et al.* (2013) Oligogalacturonides: plant damage-associated molecular patterns and regulators of growth and development. *Front. Plant Sci.* 4, 49
10. Braza, F. *et al.* (2016) Role of TLRs and DAMPs in allograft inflammation and transplant outcomes. *Nat. Rev. Nephrol.* 12, 281–290
11. Melnyk, C.W. (2017) Plant grafting: insights into tissue regeneration. *Regeneration (Oxf.)* 4, 3–14
12. Feldman, N. *et al.* (2015) DAMPs as mediators of sterile inflammation in aging-related pathologies. *Ageing Res. Rev.* 24, 29–39
13. Cao, Y. *et al.* (2014) Extracellular ATP is a central signaling molecule in plant stress responses. *Curr. Opin. Plant Biol.* 20, 82–87
14. Hernandez-Onate, M.A. and Herrera-Estrella, A. (2015) Damage response involves mechanisms conserved across plants, animals and fungi. *Curr. Genet.* 61, 359–372
15. Idzko, M. *et al.* (2014) Nucleotide signalling during inflammation. *Nature* 509, 310–317
16. Daniels, M.J. and Brough, D. (2017) Unconventional pathways of secretion contribute to inflammation. *Int. J. Mol. Sci.* 18, 102
17. Gallucci, S. and Maffei, M.E. (2017) DNA sensing across the tree of life. *Trends Immunol.* 38, 719–732
18. Wen, F. *et al.* (2017) Visualization of extracellular DNA released during border cell separation from the root cap. *Am. J. Bot.* 104, 970–978
19. Cannesan, M.A. *et al.* (2011) Association between border cell responses and localized root infection by pathogenic *Aphanomyces euteiches*. *Ann. Bot.* 108, 459–469
20. Driouich, A. *et al.* (2013) Root border cells and secretions as critical elements in plant host defense. *Curr. Opin. Plant Biol.* 16, 489–495
21. Tran, T.M. *et al.* (2016) Escaping underground nets: extracellular dnases degrade plant extracellular traps and contribute to virulence of the plant pathogenic bacterium *Ralstonia solanacearum*. *PLoS Pathog.* 12, e1005686
22. Mazzoleni, S. *et al.* (2015) Inhibitory and toxic effects of extracellular self-DNA in litter: a mechanism for negative plant-soil feedbacks? *New Phytol.* 205, 1195–1210
23. Mazzoleni, S. *et al.* (2015) Inhibitory effects of extracellular self-DNA: a general biological process? *New Phytol.* 206, 127–132
24. Barbero, F. *et al.* (2016) Extracellular self-DNA (esDNA), but not heterologous plant or insect DNA (etDNA), induces plasma membrane depolarization and calcium signaling in Lima bean (*Phaseolus lunatus*) and maize (*Zea mays*). *Int. J. Mol. Sci.* 17
25. Duran-Flores, D. and Heil, M. (2018) Extracellular self-DNA as a damage-associated molecular pattern (DAMP) that triggers self-specific immunity induction in plants. *Brain Behav. Immun.* 72, 78–88
26. Tarbell, J.M. and Cancel, L.M. (2016) The glycocalyx and its significance in human medicine. *J. Intern. Med.* 280, 97–213
27. Jiang, D. *et al.* (2011) Hyaluronan as an immune regulator in human diseases. *Physiol. Rev.* 91, 221–264
28. Cyphert, J.M. *et al.* (2015) Size matters: molecular weight specificity of hyaluronan effects in cell biology. *Int. J. Cell Biol.* 2015, 563818
29. Bellincampi, D. *et al.* (2014) Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. *Front. Plant Sci.* 5, 228

30. Bonnans, C. *et al.* (2014) Remodelling the extracellular matrix in development and disease. *Nat. Rev. Mol. Cell Biol.* 15, 786–801
31. Hamann, T. (2015) The plant cell wall integrity maintenance mechanism – a case study of a cell wall plasma membrane signaling network. *Phytochemistry* 112, 100–109
32. Engelsdorf, T. *et al.* (2018) The plant cell wall integrity maintenance and immune signaling systems cooperate to control stress responses in *Arabidopsis thaliana*. *Sci. Signal.* 8, eaao3070
33. Benedetti, M. *et al.* (2015) Plant immunity triggered by engineered *in vivo* release of oligogalacturonides, damage-associated molecular patterns. *Proc. Natl. Acad. Sci. U. S. A.* 112, 5533–5538
34. Cervone, F. *et al.* (1989) Host–pathogen interactions. XXXIII. A plant protein converts a fungal pathogenesis factor into an elicitor of plant defense responses. *Plant Physiol.* 90, 542–548
35. Bravo Ruiz, G. *et al.* (2016) Combined action of the major secreted exo- and endopolygalacturonases is required for full virulence of *Fusarium oxysporum*. *Mol. Plant Pathol.* 17, 339–353
36. Yang, Y. *et al.* (2018) A profusion of molecular scissors for pectins: classification, expression, and functions of plant polygalacturonases. *Front. Plant Sci.* 9, 1208
37. D'Agostino, A. *et al.* (2017) Is molecular size a discriminating factor in hyaluronan interaction with human cells? *Carbohydr. Polym.* 157, 21–30
38. Galletti, R. *et al.* (2008) The AtrbohD-mediated oxidative burst elicited by oligogalacturonides in *Arabidopsis* is dispensable for the activation of defense responses effective against *Botrytis cinerea*. *Plant Physiol.* 148, 1695–1706
39. Gravino, M. *et al.* (2017) Immune responses induced by oligogalacturonides are differentially affected by AvrPto and loss of BAK1/BKK1 and PEPR1/PEPR2. *Mol. Plant Pathol.* 18, 582–595
40. Savatin, D.V. *et al.* (2014) The Arabidopsis NUCLEUS- AND PHRAGMOPLAST-LOCALIZED KINASE1-related protein kinases are required for elicitor-induced oxidative burst and immunity. *Plant Physiol.* 165, 1188–1202
41. Hahn, M.G. *et al.* (1981) Host-pathogen interactions. XIX. The endogenous elicitor, a fragment of a plant cell wall polysaccharide that elicits phytoalexin accumulation in soybeans. *Plant Physiol.* 68, 1161–1169
42. Denoux, C. *et al.* (2008) Activation of defense response pathways by OGs and Flg22 elicitors in *Arabidopsis* seedlings. *Mol. Plant* 1, 423–445
43. Voigt, C.A. (2014) Callose-mediated resistance to pathogenic intruders in plant defense-related papillae. *Front. Plant Sci.* 5, 168
44. Moloshok, T. *et al.* (1992) Oligouronide signaling of proteinase inhibitor genes in plants: structure–activity relationships of di- and trigalacturonic acids and their derivatives. *Arch. Biochem. Biophys.* 294, 731–734
45. Davidsson, P. *et al.* (2017) Short oligogalacturonides induce pathogen resistance-associated gene expression in *Arabidopsis thaliana*. *BMC Plant Biol.* 17, 19
46. Cabrera, J.C. *et al.* (2008) Egg box conformation of oligogalacturonides: the time-dependent stabilization of the elicitor-active conformation increases its biological activity. *Glycobiology* 18, 473–482
47. Cervone, F. *et al.* (2015) Enhancing immunity by engineering DAMPs. *Oncotarget* 6, 28523–28524
48. Huot, B. *et al.* (2014) Growth-defense tradeoffs in plants: a balancing act to optimize fitness. *Mol. Plant* 7, 1267–1287
49. Daniel, B. *et al.* (2017) The family of berberine bridge enzyme-like enzymes: a treasure-trove of oxidative reactions. *Arch. Biochem. Biophys.* 632, 88–103
50. Benedetti, M. *et al.* (2018) Four *Arabidopsis* berberine bridge enzyme-like proteins are specific oxidases that inactivate the elicitor-active oligogalacturonides. *Plant J.* 94, 260–273
51. Aziz, A. *et al.* (2007) Elicitor and resistance-inducing activities of beta-1,4 cellodextrins in grapevine, comparison with beta-1,3 glucans and alpha-1,4 oligogalacturonides. *J. Exp. Bot.* 58, 1463–1472
52. Souza, C.A. *et al.* (2017) Cellulose-derived oligomers act as damage-associated molecular patterns and trigger defense-like responses. *Plant Physiol.* 173, 2383–2398
53. Johnson, J.M. *et al.* (2018) A Poly(A) ribonuclease controls the cellotriose-based interaction between *Piriformospora indica* and its host *Arabidopsis*. *Plant Physiol.* 176, 2496–2514
54. Versluys, M. *et al.* (2016) Fructans as DAMPs or MAMPs: evolutionary prospects, cross-tolerance, and multistress resistance potential. *Front. Plant Sci.* 7, 2061
55. Capitan-Canadas, F. *et al.* (2014) Prebiotic oligosaccharides directly modulate proinflammatory cytokine production in monocytes via activation of TLR4. *Mol. Nutr. Food Res.* 58, 1098–1110
56. Vogt, L.M. *et al.* (2014) Toll-like receptor 2 activation by beta2→1-fructans protects barrier function of T84 human intestinal epithelial cells in a chain length-dependent manner. *J. Nutr.* 144, 1002–1008
57. Goldberg, R. *et al.* (2013) Versatile role of heparanase in inflammation. *Matrix Biol.* 32, 234–240
58. Goodall, K.J. *et al.* (2014) Soluble heparan sulfate fragments generated by heparanase trigger the release of pro-inflammatory cytokines through TLR-4. *PLoS One* 9, e109596
59. Blich, M. *et al.* (2013) Macrophage activation by heparanase is mediated by TLR-2 and TLR-4 and associates with plaque progression. *Arterioscler. Thromb. Vasc. Biol.* 33, e56–e65
60. Pontillo, A. and Crovella, S. (2017) NOD-like receptors: a tail from plants to mammals through invertebrates. *Curr. Protein Pept. Sci.* 18, 311–322
61. Klaus-Heisen, D. *et al.* (2011) Structure-function similarities between a plant receptor-like kinase and the human interleukin-1 receptor-associated kinase-4. *J. Biol. Chem.* 286, 11202–11210
62. Macho, A.P. and Zipfel, C. (2014) Plant PRRs and the activation of innate immune signaling. *Mol. Cell* 54, 263–272
63. Macho, A.P. *et al.* (2015) Importance of tyrosine phosphorylation in receptor kinase complexes. *Trends Plant Sci.* 20, 269–272
64. Suzuki, M. *et al.* (2018) Autophosphorylation site Y428 is essential for the *in vivo* activation of CERK1. *Plant Signal. Behav.* 13, e1435228
65. Williams, S.J. *et al.* (2016) Structure and function of the TIR domain from the grape NLR protein RPV1. *Front. Plant Sci.* 7, 1850
66. Bartels, S. and Boller, T. (2015) Quo vadis, Pep? Plant elicitor peptides at the crossroads of immunity, stress, and development. *J. Exp. Bot.* 66, 5183–5193
67. Tang, J. *et al.* (2015) Structural basis for recognition of an endogenous peptide by the plant receptor kinase PEPR1. *Cell Res.* 25, 110–120
68. Poncini, L. *et al.* (2017) In roots of *Arabidopsis thaliana*, the damage-associated molecular pattern AtPep1 is a stronger elicitor of immune signalling than flg22 or the chitin heptamer. *PLoS One* 12, e0185808
69. Huffaker, A. *et al.* (2013) Plant elicitor peptides are conserved signals regulating direct and indirect antiherbivore defense. *Proc. Natl. Acad. Sci. U. S. A.* 110, 5707–5712
70. Griffioen, K. *et al.* (2018) Deficiency of Toll-like receptors 2, 3 or 4 extends life expectancy in Huntington's disease mice. *Heliyon* 4, e00508
71. Gross, A. *et al.* (2017) Toll-like receptor 3 deficiency decreases epileptogenesis in a pilocarpine model of SE-induced epilepsy in mice. *Epilepsia* 58, 586–596
72. Okun, E. *et al.* (2012) Evidence for a developmental role for TLR4 in learning and memory. *PLoS One* 7, e47522

73. Okun, E. *et al.* (2014) Toll-like receptors 2 and 4 modulate autonomic control of heart rate and energy metabolism. *Brain Behav. Immun.* 36, 90–100
74. Okun, E. *et al.* (2010) Toll-like receptor 3 inhibits memory retention and constrains adult hippocampal neurogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 107, 15625–15630
75. Brutus, A. *et al.* (2010) A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *Proc. Natl. Acad. Sci. U. S. A.* 107, 9452–9457
76. Kohorn, B.D. (2016) Cell wall-associated kinases and pectin perception. *J. Exp. Bot.* 67, 489–494
77. Gramegna, G. *et al.* (2016) GRP-3 and KAPP, encoding interactors of WAK1, negatively affect defense responses induced by oligogalacturonides and local response to wounding. *J. Exp. Bot.* 67, 1715–1729
78. Lesley, J. *et al.* (2000) Hyaluronan binding by cell surface CD44. *J. Biol. Chem.* 275, 26967–26975
79. Jiang, D. *et al.* (2007) Hyaluronan in tissue injury and repair. *Annu. Rev. Cell Dev. Biol.* 23, 435–461
80. Bhattacharya, D. *et al.* (2017) Impact of structurally modifying hyaluronic acid on CD44 interaction. *J. Mater. Chem. B* 5, 8183–8192
81. Dusio, G.F. *et al.* (2011) Stimulation of TLRs by LMW-HA induces self-defense mechanisms in vaginal epithelium. *Immunol. Cell Biol.* 89, 630–639
82. Forteza, R.M. *et al.* (2012) Hyaluronan and layilin mediate loss of airway epithelial barrier function induced by cigarette smoke by decreasing E-cadherin. *J. Biol. Chem.* 287, 42288–42298
83. Johnson, L.A. *et al.* (2017) Dendritic cells enter lymph vessels by hyaluronan-mediated docking to the endothelial receptor LYVE-1. *Nat. Immunol.* 18, 762–770
84. Kim, M.Y. *et al.* (2013) Hyaluronic acid oligosaccharides suppress TLR3-dependent cytokine expression in a TLR4-dependent manner. *PLoS One* 8, e72421
85. Misra, S. *et al.* (2015) Interactions between hyaluronan and its receptors (CD44, RHAMM) regulate the activities of inflammation and cancer. *Front. Immunol.* 6, 201
86. Pandey, M.S. and Weigel, P.H. (2014) A hyaluronan receptor for endocytosis (HARE) link domain N-glycan is required for extracellular signal-regulated kinase (ERK) and nuclear factor-kappaB (NF-kappaB) signaling in response to the uptake of hyaluronan but not heparin, dermatan sulfate, or acetylated low density lipoprotein (LDL). *J. Biol. Chem.* 289, 21807–21817
87. Quero, L. *et al.* (2013) Hyaluronic acid fragments enhance the inflammatory and catabolic response in human intervertebral disc cells through modulation of toll-like receptor 2 signalling pathways. *Arthritis Res. Ther.* 15, R94
88. Tolg, C. *et al.* (2014) Hyaluronan and RHAMM in wound repair and the "cancerization" of stromal tissues. *BioMed Res. Int.* 2014, 103923
89. Zhang, J.Z. *et al.* (2014) Mitochondrial DNA induces inflammation and increases TLR9/NF-kappaB expression in lung tissue. *Int. J. Mol. Med.* 33, 817–824
90. Nakahira, K. *et al.* (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* 12, 222–230
91. Pohar, J. *et al.* (2017) Selectivity of human TLR9 for double CpG motifs and implications for the recognition of genomic DNA. *J. Immunol.* 198, 2093–2104
92. Shimada, K. *et al.* (2012) Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* 36, 401–414
93. West, A.P. *et al.* (2015) Mitochondrial DNA stress primes the antiviral innate immune response. *Nature* 520, 553–557
94. Julian, M.W. *et al.* (2013) Mitochondrial transcription factor A, an endogenous danger signal, promotes TNFalpha release via RAGE- and TLR9-responsive plasmacytoid dendritic cells. *PLoS One* 8, e72354
95. Horng, T. (2014) Calcium signaling and mitochondrial destabilization in the triggering of the NLRP3 inflammasome. *Trends Immunol.* 35, 253–261
96. Shintani, Y. *et al.* (2014) Toll-like receptor 9 protects non-immune cells from stress by modulating mitochondrial ATP synthesis through the inhibition of SERCA2. *EMBO Rep.* 15, 438–445
97. Colombatti, F. *et al.* (2014) Plant mitochondria under pathogen attack: a sigh of relief or a last breath? *Mitochondrion* 19 Pt B, 238–244
98. Block, A. *et al.* (2010) The *Pseudomonas syringae* type III effector HopG1 targets mitochondria, alters plant development and suppresses plant innate immunity. *Cell. Microbiol.* 12, 318–330
99. Gravino, M. *et al.* (2015) Ethylene production in *Botrytis cinerea*- and oligogalacturonide-induced immunity requires calcium-dependent protein kinases. *Plant J.* 84, 1073–1086
100. Galletti, R. *et al.* (2011) *Arabidopsis* MPK3 and MPK6 play different roles in basal and oligogalacturonide- or flagellin-induced resistance against *Botrytis cinerea*. *Plant Physiol.* 157, 804–814
101. Qi, J. *et al.* (2017) Apoplastic ROS signaling in plant immunity. *Curr. Opin. Plant Biol.* 38, 92–100
102. O'Brien, J.A. *et al.* (2012) A peroxidase-dependent apoplastic oxidative burst in cultured arabidopsis cells functions in mamp-elicited defense. *Plant Physiol.* 158, 2013–2027
103. Daudi, A. *et al.* (2012) The apoplastic oxidative burst peroxidase in *Arabidopsis* is a major component of pattern-triggered immunity. *Plant Cell* 24, 275–287
104. Austin, J.W. *et al.* (2012) High molecular weight hyaluronan reduces lipopolysaccharide mediated microglial activation. *J. Neurochem.* 122, 344–355
105. Collins, S.L. *et al.* (2011) Hyaluronan fragments promote inflammation by down-regulating the anti-inflammatory A2a receptor. *Am. J. Respir. Cell Mol. Biol.* 45, 675–683
106. Avenoso, A. *et al.* (2018) Hyaluronan in the experimental injury of the cartilage: biochemical action and protective effects. *Inflamm. Res.* 67, 5–20
107. Liang, J. *et al.* (2007) CD44 is a negative regulator of acute pulmonary inflammation and lipopolysaccharide-TLR signaling in mouse macrophages. *J. Immunol.* 178, 2469–2475
108. Acharya, P.S. *et al.* (2008) Fibroblast migration is mediated by CD44-dependent TGF beta activation. *J. Cell Sci.* 121, 1393–1402
109. Corradetti, B. *et al.* (2017) Hyaluronic acid coatings as a simple and efficient approach to improve MSC homing toward the site of inflammation. *Sci. Rep.* 7, 7991
110. Kouvidi, K. *et al.* (2011) Role of receptor for hyaluronic acid-mediated motility (RHAMM) in low molecular weight hyaluronan (LMWHA)-mediated fibrosarcoma cell adhesion. *J. Biol. Chem.* 286, 38509–38520
111. Mauch-Mani, B. *et al.* (2017) Defense priming: an adaptive part of induced resistance. *Annu. Rev. Plant Biol.* 68, 485–512
112. Bell, J.D. *et al.* (2017) MicroRNA changes in preconditioning-induced neuroprotection. *Transl. Stroke Res.* 8, 585–596
113. Gourbal, B. *et al.* (2018) Innate immune memory: an evolutionary perspective. *Immunol. Rev.* 283, 21–40
114. Boraschi, D. and Italiani, P. (2018) Innate immune memory: time for adopting a correct terminology. *Front. Immunol.* 9, 799
115. Netea, M.G. *et al.* (2016) Trained immunity: a program of innate immune memory in health and disease. *Science* 352, aaf1098
116. Gesuete, R. *et al.* (2014) Toll-like receptors and ischemic brain injury. *J. Neuropathol. Exp. Neurol.* 73, 378–386
117. Tateda, C. *et al.* (2014) Salicylic acid regulates *Arabidopsis* microbial pattern receptor kinase levels and signaling. *Plant Cell* 26, 4171–4187

118. Beckers, G.J. *et al.* (2009) Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. *Plant Cell* 21, 944–953

119. Reimer-Michalski, E.M. and Conrath, U. (2016) Innate immune memory in plants. *Semin. Immunol.* 28, 319–327

120. Wang, C. *et al.* (2016) Preconditioning with recombinant high-mobility group box 1 induces ischemic tolerance in a rat model of focal cerebral ischemia–reperfusion. *J. Neurochem.* 137, 576–588

121. Law, C.H. *et al.* (2013) Hyaluronic acid-dependent protection in H9C2 cardiomyocytes: a cell model of heart ischemia–reperfusion injury and treatment. *Toxicology* 303, 54–71

122. Kabbage, M. *et al.* (2017) The life and death of a plant cell. *Annu. Rev. Plant Biol.* 68, 375–404

123. Savatin, D.V. *et al.* (2011) Oligogalacturonide-auxin antagonism does not require posttranscriptional gene silencing or stabilization of auxin response repressors in *Arabidopsis*. *Plant Physiol.* 157, 1163–1174

124. Senechal, F. *et al.* (2014) Homogalacturonan-modifying enzymes: structure, expression, and roles in plants. *J. Exp. Bot.* 65, 5125–5160

125. Bellincampi, D. *et al.* (1996) Oligogalacturonides prevent rhizogenesis in rolB-transformed tobacco explants by inhibiting auxin-induced expression of the rolB gene. *Plant Cell* 8, 477–487

126. Hu, L. *et al.* (2016) Biglycan stimulates VEGF expression in endothelial cells by activating the TLR signaling pathway. *Mol. Oncol.* 10, 1473–1484

127. Shao, Y. *et al.* (2017) Lysophospholipids and their receptors serve as conditional DAMPs and DAMP receptors in tissue oxidative and inflammatory injury. *Antioxid. Redox Signal.* Published online April 26, 2017. http://dx.doi.org/10.1089/ars.2017.7069