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Review

Programmed cell death in the plant immune system

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Cell death has a central role in innate immune responses in both plants and animals. Besides sharing striking convergences and similarities in the overall evolutionary organization of their innate immune systems, both plants and animals can respond to infection and pathogen recognition with programmed cell death. The fact that plant and animal pathogens have evolved strategies to subvert specific cell death modalities emphasizes the essential role of cell death during immune responses. The hypersensitive response (HR) cell death in plants displays morphological features, molecular architectures and mechanisms reminiscent of different inflammatory cell death types in animals (pyroptosis and necroptosis). In this review, we describe the molecular pathways leading to cell death during innate immune responses. Additionally, we present recently discovered caspase and caspase-like networks regulating cell death that have revealed fascinating analogies between cell death control across both kingdoms.
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Immune Surveillance Systems in Plants and Animals

The interaction between a pathogen and its host is sophisticated and dynamic. Disease develops when the pathogen is able to evade the multiple layers of host defenses. The immune system of an organism has been tailored through evolution by a long history of warfare with its invaders. In contrast to most animals, plants are sessile organisms, they lack a circulatory system and their cells are framed with a rigid cell wall. These evolutionary constraints have resulted in the evolution of a primary cell-autonomous immune system. Despite these fundamental differences between the two kingdoms, plants and animals share striking similarities in their innate immune systems, some of which tell a story of likely convergent evolution.[1] Immune systems discriminate self from non-self, and activate tightly regulated pre- and post-invasion defense responses to minimize the damage inflicted by harmful agents. The first line of defense in both plants and animals is provided by pattern recognition receptors (PRRs), which recognize microbe- or danger-associated molecular patterns (MAMPs and DAMPs, respectively), and trigger immune signaling (Figure 1). Plant PRRs are transmembrane receptors.[2,3] The best-studied class of plant PRRs are receptor-like kinases (RLKs), which feature an ectodomain of leucine-rich repeats (LRRs) involved in MAMP perception, and an intracellular kinase domain, involved in signal transduction relay via MAPK cascades, resulting in MAMP-triggered immunity (MTI).[4]

Typical animal extracellular PRRs, called Toll-like receptors (TLRs) possess an intracellular Toll-interleukin-1 (IL-1) receptor domain (TIR) that recruits the kinases IRAK or RIP via adaptor proteins, inducing expression of antimicrobial defense molecules.[5] These kinases belong to the same functional class of non-RD kinases as plants, and they are linked to innate immune responses in both kingdoms.[6] Although plants and animals evolved under very different selective pressures, both evolved similar sensors that converge onto the same generic function: to alert the organism about the presence of non-self.[7]

By definition, pathogens are microorganisms and viruses that are able to evade or suppress PRR-based defenses. They do so by deploying various effectors, determinants of virulence on susceptible hosts via MTI suppression, into the host cell.[4,8] Successful pathogens are then faced with another hurdle, evolved by hosts to recognize the presence of their effectors and of intracellular MAMPs. In plants, a second

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Abbreviations: ASC, apoptosis-associated speck-like protein containing a caspase-activating recruiting domain; AtMC, Arabidopsis metacaspase; CARD, caspase activation recruitment domain; CC, coiled-coil domain; cFLIP, cellular FLICE-inhibitory protein; DD, death domain; EDS1, enhanced disease susceptibility 1; ETI, effector-triggered immunity; HR, hypersensitive response; ICE, interleukin-1β-converting enzyme; IG, immunoglobulin; IL, interleukin; JA, jasmonic acid; LOL, LSD-one-like; LRR, leucine-rich repeat; LSD, lesion simulating disease; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MAMP, microbe-associated molecular pattern; MAPK, mitogen-activated protein kinase; MTI, MAMP-triggered immunity; NB-LRR, nucleotide-binding domain, leucine-rich repeat; NDR1, non-race specific disease resistance 1; NLR, nod-like receptor; NOI, nitrogen oxide intermediates; PAD4, phytoalexin deficient 4; PAMP, pathogen-associated molecular pattern; PCD, programmed cell death; Pro, proline-rich domain; PRR, pattern recognition receptor; rcd, runaway cell death; RLK, receptor-like kinase; ROS, reactive oxygen species; SA, salicylic acid; SAG101, senescence-associated gene 101; TIR, Toll-interleukin-1 receptor domain; TLR, Toll-like receptor; VPE, vacuolar processing enzyme
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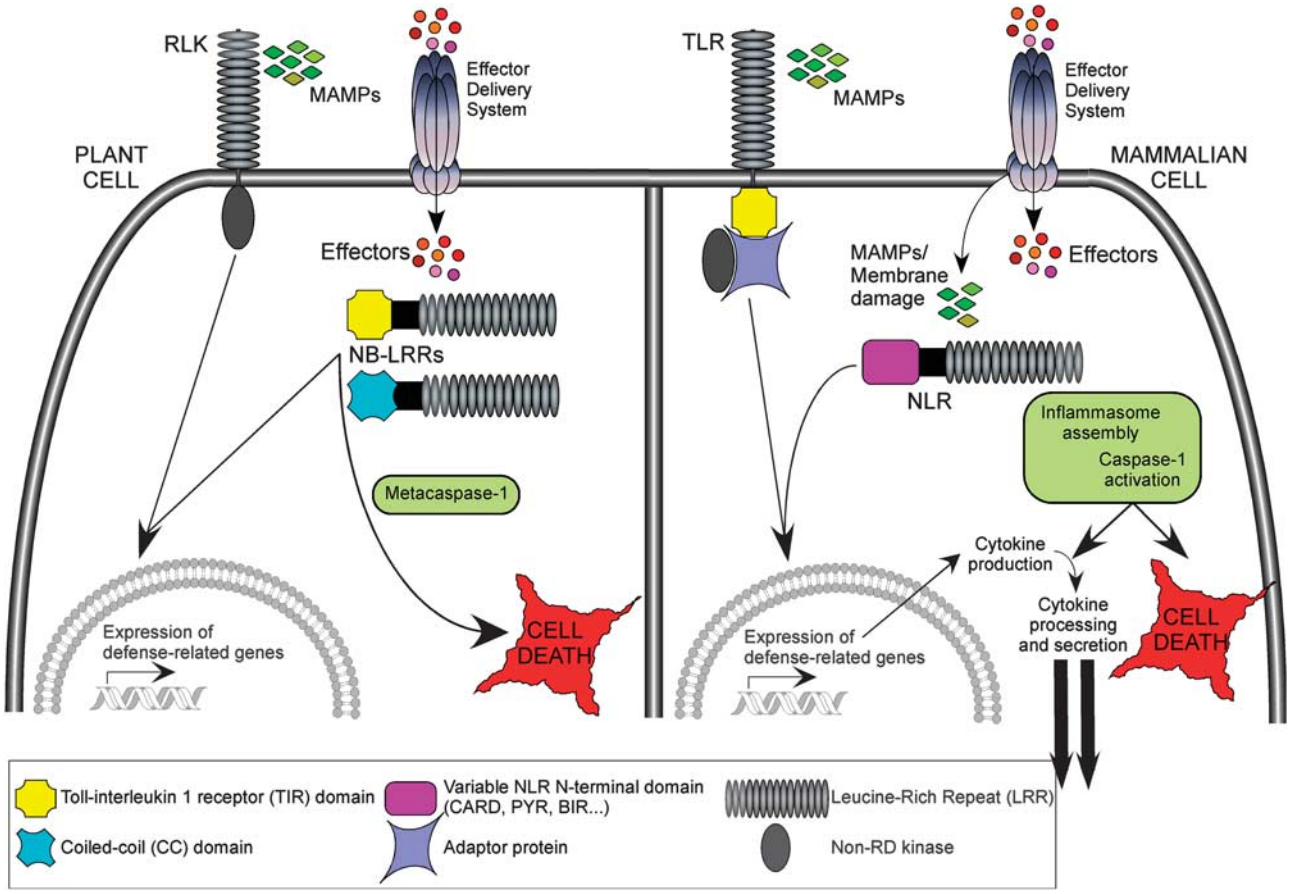

Figure 1 Innate immune pathways in plants and mammals. In both plants (left) and mammalian (right), cells pathogen detection by membrane and intracellular innate immune receptors leads to signaling cascades that culminate in expression of defense-related genes. Defense mechanisms eventually result in programmed cell death in both kingdoms. This diagram exemplifies hypersensitive response cell death mediated by metacaspase-1 in plants and caspase-1-mediated cell death in animals

intracellular class of innate immune receptors is activated via recognition of pathogen effectors, resulting in effector-triggered immunity (ETI). ETI is mediated by nucleotide-binding domain, LRR (NB-LRR) disease resistance proteins. Their structure is very similar to Nod-like receptors (NLRs) in mammals.[9] Both are intracellular proteins that contain a central nucleotide-binding domain involved in activation and multimerization[9,10] and an LRR domain. In addition to structural similarities, NLRs and NB-LRRs have shared function and their stability is regulated by a chaperone complex containing HSP90 and SGT1.[11–13] Both NB-LRRs in plants and NLRs in mammals are classified according to their N-terminal domain architecture. Two main classes of plant NB-LRRs have been described: CC-NB-LRRs contain a predicted coiled-coil N-terminal domain and TIR-NB-LRRs carry N-terminal homology to the intracellular TIR domain of TLRs. In mammals, the NLR family is divided into five subfamilies with different N-terminal effector domains.[14] The N-termini of NLRs mediate protein–protein interactions with downstream signaling partners.

Both NB-LRRs and NLRs act as intracellular immune sentinels. NB-LRRs have evolved to recognize specific pathogen effector proteins, which are delivered into the host cytosol by a broad range of pathogens using various delivery systems. An effective recognition system must be able to sense and respond to a multitude of effectors, since each pathogen delivers its unique repertoire.[15] In plants, effector recognition can occur by direct binding of the NB-LRR protein or indirectly, via an intermediate protein. The guard hypothesis[16,17] explains indirect recognition, which occurs after an effector modifies a particular host protein (guardee) that is monitored by a particular NB-LRR (guard). Plants do not appear to express somatic recombination-based diversity generation in their immune system, as do animal cells to generate the familiar T- and B-cell antigen receptors. Therefore, sensing of 'modified-self' accounts for a powerful recognition system, that can manage with a limited set of receptors an effective defense response.

In animals, the mechanism by which NLRs sense MAMPs or DAMPs to trigger an appropriate immune response is not fully understood. *In vivo* direct recognition has not been proven and recent models suggest that NLR activation could occur indirectly as a result of the membrane damage inflicted by pathogens that are either able to reach the cytoplasm, or that accidentally deliver MAMPs via their secretion systems along with effector proteins.[18,19] In this sense, NLRs could be conceived as guard proteins similar to plant NB-LRRs.[20]

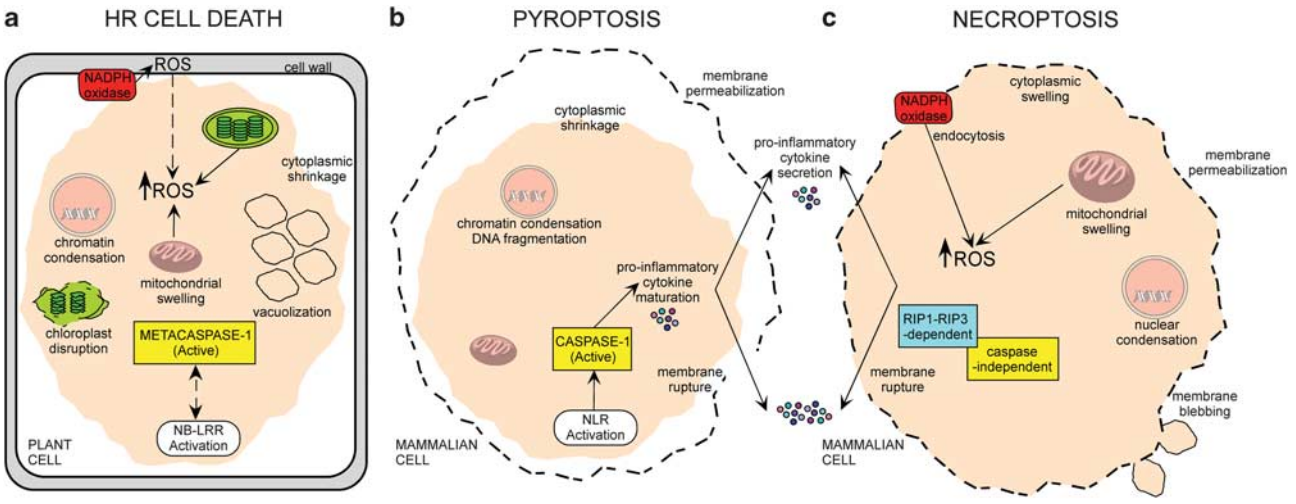

Figure 2 Cell death modalities in response to infection. Diagram representing some of the characteristic features of different types of programmed cell death that can occur in response to infection in plants and mammals. HR cell death in plants (**a**) and pyroptosis (**b**) and Necroptosis (**c**) in mammalian cells. See the text for details

Cell Death at the Center of Immune Responses

Pathogen recognition via NLRs in animals and NB-LRRs in plants leads to inhibition of pathogen growth, which is often, but not always, accompanied in plants by the *hypersensitive response* (HR), a form of programmed cell death localized at the site of attempted pathogen invasion (Figure 2a). The first observations of HR date back to 1902 in the wheat-*Puccinia glumarum* pathosystem,[21] and the counter-intuitive term 'hypersensitiveness' was coined in 1915,[22] to describe a pathogen-triggered cell death reaction that correlated with disease resistance in wheat infected with *Puccinia graminis*. Morphologically, HR is a specific and unique type of cell death. Its hallmarks, some of which are typical for different forms of animal cell death, include cytoplasmic shrinkage, chromatin condensation, mitochondrial swelling, combined with other characteristics that are plant specific, such as vacuolization and chloroplast disruption during the final stages.[23]

The chloroplast has a central role in defense responses and HR in plants. First, it constitutes a very important source of defense signaling molecules such as reactive oxygen species (ROS), reactive nitrogen oxide intermediates (NOI) and the defense hormones salicylic acid (SA) and jasmonic acid (JA). Second, in many cases, light is required for HR development. Third, several pathogen effectors have chloroplast localization signals,[24] and in some cases they have been shown to suppress immunity.[25,26]

In plants, the molecular events that lead to HR during ETI are partly overlapping with those associated with MTI, including accumulation of SA, ROS and NOI, activation of MAPK cascades, changes in intracellular calcium levels, transcriptional reprogramming and synthesis of antimicrobial compounds.[23] Compared with MTI, ETI is typically an accelerated and amplified response, suggesting that quantitative rather than qualitative differences account for HR induction.[4]

In animals, naturally occurring cell death was first reported in the 19th century[27] and many years later the term *apoptosis* was defined.[28] Caspases, a family of cysteine proteases that cleave their substrates after an aspartic acid residue, emerged as the orchestrators of this cell death process. Remarkably, the first caspase discovered in mammals was the IL-1β-converting enzyme (ICE), later known as caspase-1, which does not participate in apoptosis, but does control inflammation and pyroptotic cell death (see below) downstream of NLR activation.[8,29] During the last decade, an increasing number of cell death morphologies with mixed features have been described in mammals, potentially offering a parallel to plant HR, and programmed cell death classification has become a complex task. A thorough compilation of the morphological/biochemical/functional criteria to define various sorts of cell deaths has recently been published.[30]

In mammals, several types of cell death have been reported in response to infection. Because these are often associated with NLRs, it may be instructive to view them as possible analogs of pathogen-dependent HR in plants (Figure 2). *Pyroptosis* is a pro-inflammatory form of cell death initially described as caspase-1-dependent necrosis in macrophages[31] (Figure 2b). Pyroptosis has been reported in response to infection with several bacteria[32] and viruses.[33] Caspase-1 activation occurs within molecular platforms known as inflammasomes.[34] The best studied to date are the NLR inflammasomes, which sense mostly MAMPs and DAMPs.[14] These supramolecular complexes are assembled via NLR N-terminal domain homotypic interactions. Once activated, the NLRs within the inflammasome bind the N-terminal caspase activation recruitment domain (CARD) of caspase-1 directly or via the adaptor PYD-CARD protein ASC (apoptosis-associated speck-like protein containing a caspase-activating recruitment domain). Once recruited to the inflammasome, caspase-1 is activated by induced proximity and processes the inactive precursors of IL-1β and IL-18 into their mature forms. Caspase-1 also regulates the release of these and other pro-inflammatory cytokines into the extracellular millieu.[35] These secreted molecules are instrumental for

inflammation, cytoprotection and tissue repair. Interestingly, cytokine maturation is genetically separable from pyroptotic cell death: a recent report has shown that ASC-independent inflammasomes can activate caspase-1 without autoproteolysis, promoting cell death without processing IL-1β/IL-18.[36] In contrast to ASC-containing inflammasomes, which form a single large cytoplasmic speckle, no such large structure is generated by ASC-independent inflammasomes.[36]

The precise mechanism by which caspase-1 leads to cell death has also been investigated by looking at caspase-1 substrates during infection and inflammation. The caspase-1 digestome includes chaperones, cytoskeletal maintenance components and proteins involved in energy metabolism,[37] as well as caspase-7,[38] which has been shown to be activated downstream of NLRC4 inflammasome during bacterial infection.[39] However, it is still not clear why pyroptotic cells die. Permeabilization of the plasma membrane, which presumably participates in protein secretion at early stages of pyroptosis, can be the cause of later cell death due to ruptures caused by cytoplasmic swelling.[40] This feature of pyroptosis is shared with another cell death modality: programmed necrosis or *necroptosis*[41] (Figure 2c). This alternative form of programmed cell death is in most cases initiated by stimulation of the extrinsic apoptotic pathway when caspases are absent or inhibited.[42] It can also be triggered after PRR activation, by a mechanism not yet characterized.[42] Generally, necroptosis is mediated by RIP1–RIP3 kinase complex formation.[43,44] RIP1 is a pleiotropic protein that can mediate both pro-survival (via NF-κB activation) and pro-cell death pathways (apoptosis or necroptosis).[45] During apoptosis, active caspase-8 can cleave RIP1 and RIP3 and abolish their kinase activity, preventing them from initiating necroptosis.[43] When apoptosis is blocked, necroptosis becomes the predominant form of cell death.[42]

Increased ROS levels are a hallmark of necroptosis and may be one of the main causes of necroptotic cell death. Enhanced ROS production during necroptosis can be mediated by mitochondria, due to a RIP3-dependent increase in energy metabolism,[46] and/or by the NADPH oxidase NOX1, which is recruited to the plasma membrane by RIP1.[47] In plants, apoplastic ROS (superoxide) generated by the plasma membrane NADPH oxidases are essential for HR development and activation of systemic immunity,[48] drawing a possible mechanistic connection between these two types of cell death. ROS produced in other plant organelles as the chloroplast, mitochondria and peroxisomes also contribute to the HR response and, in fact, compartmentalization might be essential for ROS signaling functions during defense.[49]

Necroptosis has a pivotal role in inflammation and immunity. Similar to pyroptotic cells, necroptotic cells secrete a broad array of pro-inflammatory molecules that signal through PRRs.[50] Necroptosis has been reported to occur in response to infection by certain viruses that block apoptosis in the host cell as a colonization strategy.[51] Because of the pro-inflammatory nature of necroptosis, it may constitute not only a backup mechanism for virus clearance when apoptosis is inhibited, but also a way to engage the immune system leading to a systemic response.

Caspase-independent necroptosis and caspase-1-dependent pyroptosis constitute two pro-inflammatory, explosive cell death modalities. In contrast, apoptosis, mediated by apoptotic caspases, is in most cases an immunologically silent process, since cell corpses are cleared by phagocytes. In the context of infection, it might be beneficial to minimize tissue damage during the immune response. Apoptosis can be triggered upon pathogen attack, and several lines of evidence indicate that it is essential for clearance of certain pathogens.[52]

Pathogen Strategies to Evade Cell Death

The fact that many pathogens have evolved strategies to inhibit different types of cell death further underscores its fundamental role in fighting infections. In mammals, apoptosis can be efficiently blocked by several pathogens via inhibition of apoptotic caspases, prevention of cytochrome c release or activation of pro-survival pathways.[53] Necroptosis has been shown to be inhibited by viral inhibitors during infection[51] and pyroptosis can be blocked through caspase-1 inhibition by pathogenic bacteria and viruses.[32] In some instances, suppression of pyroptosis by a pathogen leads to activation of autophagy,[54,55] highlighting the complex circuitry involved in cell death processes leading to pathogen clearance.

Plant (hemi)biotrophic pathogens feed on living cells, therefore they must evade host detection and death of the invaded plant cells. Thus, they have evolved mechanisms to suppress HR using specific effectors delivered into the cell via diverse secretion systems. Several *Pseudomonas syringae* pathovar *tomato* DC3000 effectors are capable of suppressing HR in tobacco and Arabidopsis.[56,57] HR in tobacco can also be suppressed by *Xanthomonas campestris* pv. *vesicatoria* effectors.[58] Oomycete effectors can also inhibit HR in plants.[59–61] The mechanisms by which HR is suppressed remain unknown, but systematic characterization of the increasing number of effectors identified will help us understand how they interfere with plant defenses, including the control of HR.

In contrast to (hemi)biotrophs, necrotrophic pathogens take their nutrients from dead or dying cells. Necrotrophs have developed mechanisms to induce cell death in their hosts by secreting phytotoxins and cell wall degrading enzymes, resulting in the formation of expanding necrotic lesions in the infected plant tissue.[62,63] While (hemi)biotrophs have evolved strategies to suppress HR, some necrotrophs use the plant HR machinery as a strategy to promote virulence.[64] The necrotrophic fungus *Cochliobolus victoriae*, originally described as the causal agent of Victoria blight in oats,[65] secretes the toxin Victorin, required for pathogenicity.[66] This fungus hijacks HR via activation of a CC-NB-LRR protein LOV1, which confers sensitivity to victorin and susceptibility to *C. victoriae* in Arabidopsis.[67] In oats, loss of function mutations that eliminate toxin sensitivity and susceptiblility to *C. victoriae* also eliminate specific recognition and resistance to a biotrophic fungus, *Puccinia coronata*.[68] Thus, as selection favors resistance to the biotrophic fungus, susceptibility to the necrotrophic pathogen is assured. It would be of interest to study the allele frequency of this gene in wild oats and their progenitors.

Regulators of Plant Cell Death

The chain of events leading to cell death in plants after effector recognition via NB-LRR receptors is not fully elucidated. Two separate signaling modules regulate NB-LRR proteins: non-race-specific disease resistance 1 (NDR1) regulates in most cases immune responses mediated by CC-NB-LRR proteins, whereas the enhanced disease susceptibility 1 (EDS1)/phytoalexin deficient 4 (PAD4)/senescence-associated gene 101 (SAG101) complex mediates TIR-NB-LRR signaling.[69] These two systems integrate redox signals downstream of NADPH oxidase[49] leading to SA accumulation,[70,71] which has a central role in defense responses. ROS and SA act synergistically to drive HR.[72]

Mutants exhibiting HR-like phenotypes have been long described in many plant species, including corn,[73,74] tomato,[75] barley[76] and Arabidopsis.[77] These mutants, also known as lesion mimic mutants, are classified into initiation and propagation mutants; initiation mutants inappropriately induce PCD and form localized, necrotic spots, whereas propagation mutants cannot stop it, once it has been initiated.[78] A forward genetic screen for mutants with HR-like lesions and characteristics of defense responses, including molecular and biochemical markers and enhanced disease resistance, revealed the *l*esion *s*imulating *d*isease resistance (*lsd*) class of mutants.[79] Two of these genes have been cloned: LSD4, an FtSH protease (PE, Jürg Schmid and JLD, unpublished data; see ref. [80] for details) and the zinc-finger protein LSD1,[81] a negative regulator of superoxide-induced cell death.[82] LSD1 protects plants from ROS-induced stresses and consequently, *lsd1* mutant plants are characterized by runaway cell death (rcd).[79,83] Therefore, *lsd1* can be regarded as a sensitized mutant with respect to cell death initiation, and it has been instrumental in identifying other components of the signaling pathway leading to programmed cell death. For example, EDS1 and PAD4 functions are required for *lsd1* rcd induced by abiotic stress.[83] EDS1, PAD4 and NDR1 are also required for full *lsd1* rcd in response to pathogen infection.[84] EDS1 and PAD4 regulate a ROS- and SA-dependent signal amplification loop, which in turn is modulated by LSD1.[84]

The LSD1 protein contains three internally conserved zinc-finger domains of the C2C2 class (consensus: CxxCRxxLMYxxGASxVxCxxC) (Figure 4a).[81] This zinc-finger motif is found in plants, algae and protozoa, but not in animals. Only six other Arabidopsis proteins contain one or more LSD1-like zinc-finger domains: the LSD-one-like proteins LOL1 (At1g32540)[85] and LOL2 (At4g21610), the metacaspases AtMC1 (At1g02170), AtMC2 (At4g25110) and AtMC3 (At5g64240; although the zinc-finger motif is non-canonical) and LOL6 (At1g79350) (Figure 4a). Yeast-two-hybrid assays demonstrating interaction between the zinc-finger domains of LSD1, LOL1 and AtMC1 (Figure 3) have been validated by genetic approaches: both *LOL1* and *AtMC1* are required for full *lsd1* rcd. Thus, *LOL1* and *AtMC1* are positive regulators of PCD.[85,86] Surprisingly, *AtMC2* functions as a negative regulator of cell death[86] (see below). Furthermore, *AtbZIP10* (At4g02640) function is required for *lsd1* rcd and both R-gene mediated and basal defense responses. Intriguingly, AtbZIP10–LSD1 interaction *in planta* prevents AtbZIP10 translocation to the nucleus.[87] A yeast-two-hybrid screen for LSD1 interactors revealed 10 additional putative LSD1 interaction partners (Mike Richberg, Hironori Kaminaka and JLD, unpublished data; Figure 3). It is conceivable that LSD1 acts as a scaffold protein in the cytoplasm: sequestering positive regulators of cell death (LOL1, AtMC1, AtbZIP10) prevents their function, thereby inhibiting PCD.

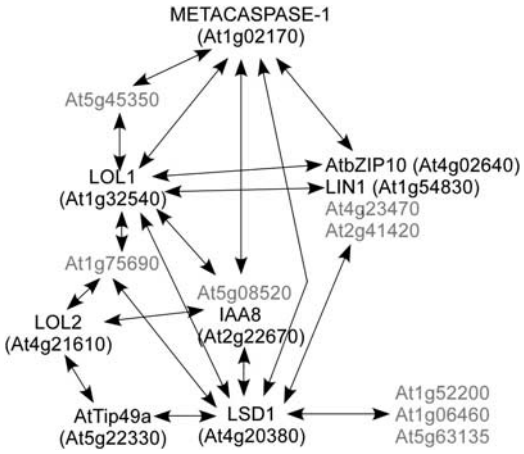

Figure 3 The LSD1 'deathosome'. Diagram depicting interactions between known cell death regulators and their yeast-two-hybrid interacting partners. The genes without annotated function are shown in gray

The Type I Metacaspase Regulatory Module in HR

Despite the lack of close caspase homologs in plants, several studies using caspase-specific peptide inhibitors suggested the presence of HR-induced caspase-like protease activities in plants.[88–91] The vacuolar processing enzyme VPE in *Nicotiana benthamiana* and its homolog VPEgamma in Arabidopsis have caspase-1-like activity during HR.[89,91] Additionally, vacuolar fusion to the plasma membrane mediated by a caspase-3-like activity of PBA1, a plant proteasome subunit, was suggested to be a functionally relevant early event in NLR-mediated HR.[88]

More than a decade ago, two new families of caspase-like proteins, metacaspases and paracaspases, were identified *in silico*[92] (Figure 4b). Similar to caspases, they contain a conserved histidine-cysteine catalytic dyad and homology modeling predicts a caspase-hemoglobinase fold.[92,93] Paracaspases have been found in animals and slime molds, whereas metacaspases are present in plants, fungi, protozoa and cyanobacteria.[92] These cysteine proteases differ from caspases in their substrate specificity; caspases cleave their targets after an aspartate residue, while paracaspases are arginine specific[94,95] and metacaspases can cleave both after an arginine or a lysine.[96] The human paracaspase, also known as mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) has an N-terminal extension containing a death domain (DD), which is present in several proteins involved in apoptotic signaling. However, MALT1 seems to act as an anti-apoptotic scaffold protein, bridging several pathways that converge into NF-κB activation during innate and adaptive immune responses.[97]

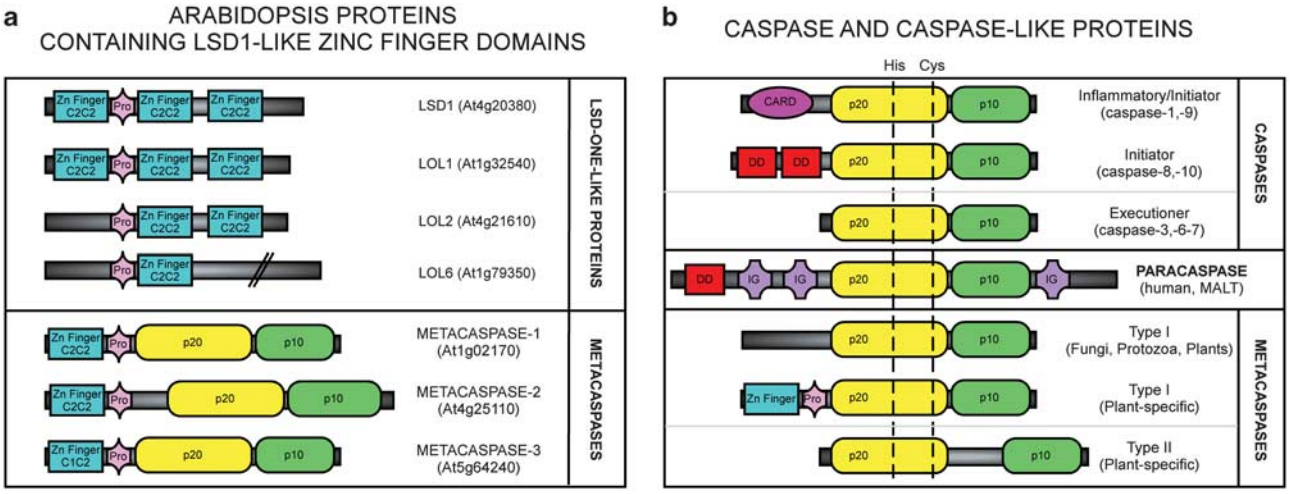

Figure 4 Domain structures of (**a**) Arabidopsis proteins containing LSD1-like zinc-finger domains and (**b**) caspases and caspase-like proteins. CARD, caspase activation recruitment domain. DD, death domain; IG, immunoglobulin domain; Zn Finger, LSD1-like zinc-finger domain (C2C2 Class); Pro, prolin-rich domain; p20 and p10, caspase (-like putative) catalytic subunits

Species	Group	TYPE I		TYPE II
		Total	LSD1-like Zn finger	
Manihot esculenta	Dicotyledoneous	11	3	2
Ricinus communis	Dicotyledoneous	7	1	2
Populus trichocarpa	Dicotyledoneous	8	5	4
Medicago truncatula	Dicotyledoneous	4	-	2
Glycine max	Dicotyledoneous	8	4	6
Cucumis sativus	Dicotyledoneous	4	3	2
Prunus persica	Dicotyledoneous	5	3	2
Arabidopsis thaliana	Dicotyledoneous	3	2	6
Arabidopsis lyrata	Dicotyledoneous	4	1	7
Carica papaya	Dicotyledoneous	5	3	2
Vitis vinifera	Dicotyledoneous	4	2	2
Mimulus guttatus	Dicotyledoneous	5	2	12
Aquilegia coerulea	Dicotyledoneous	1	1	3
Sorghum bicolor	Monocotyledoneous	6	2	6
Zea mays	Monocotyledoneous	8	5	3
Setaria italica	Monocotyledoneous	6	2	6
Oryza sativa	Monocotyledoneous	4	1	4
Brachypodium distachyon	Monocotyledoneous	2	-	4
Selaginella moellendorffii	Lycopodiophyte	1	1	2
Physcomitrella patens	Bryophyte	2	1	2
Chlamydomonas reinhardtii	Algae	1	-	1
Volvox carteri	Algae	1	-	1

Figure 5 Classification of all the metacaspases found in the Viridiplantae phylum into type I (with or without the LSD1-like zinc-finger domain) or type II metacaspases, according to Phytozome (http://www.phytozome.net/)

Eukaryotic metacaspases have been classified into type I if they bear an extension in their N-terminal domain, and type II if there is no such extension and they have a long linker region between the putative catalytic subunits.[92] Type II metacaspases are present in algae and land plants, but not in protozoa or fungi. A single metacaspase present in yeast, YCA1, can serve both pro- and anti-cell death functions,[98,99] as well as other functions unrelated to cell death regulation.[100,101] In contrast to a single protein with dual functions, two different Arabidopsis type I metacaspases, AtMC1 and AtMC2, have opposing roles during cell death control (detailed below).[86]

The N-terminal extension of metacaspases varies among species. Fungi, protozoa and algae generally have a proline-rich domain. Some plant type I metacaspases do not have any recognizable motif in their N-termini, while others feature the conserved, plant-specific LSD1-like zinc-finger domain before the proline-rich domain (Figure 5). These motifs usually participate in protein–protein interactions, and could indicate that oligomerization is important for type I metacaspase activation, analogous to initiator/inflammatory caspases. Recruitment of a limited set of N-terminal extensions through evolution could have driven diversification and functional specialization of this protein family.

Several lines of evidence suggest a function for metacaspases in plant HR. Infection was shown to induce the expression of a metacaspase in tomato[102] and *N. benthamiana*[103] and

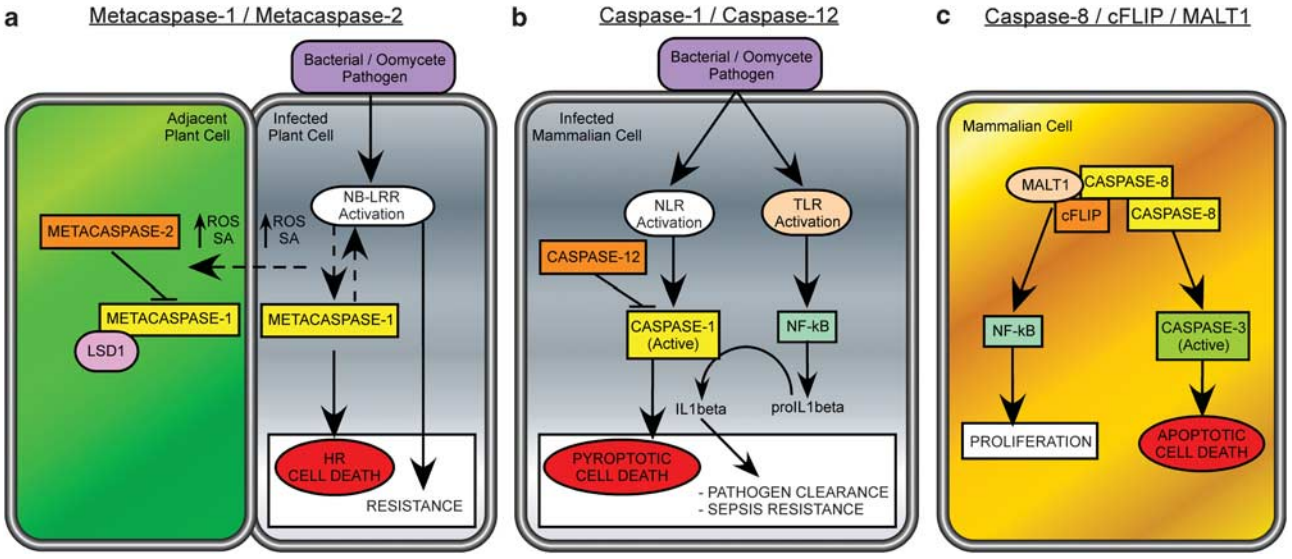

Figure 6 Metacaspases/caspases networks in plants and animals. (**a**) In plants, metacaspase-1 (*AtMC1*) positively regulates HR cell death mediated by NB-LRR recognition of the invading pathogen at the site of infection. LSD1 negatively regulates cell death propagation in cells surrounding the infection site presumably by binding to AtMC1 and holding it inactive in the cytoplasm. *AtMC2* negatively regulates *AtMC1* function by an unknown mechanism. (**b**) In mammals, caspase-12 negatively regulates caspase-1 functions in pathogen clearance and sepsis resistance and (**c**) cFLIP is a negative regulator of caspase-8 function in the apoptotic extrinsic pathway. Caspase-8 function in lymphocyte proliferation is regulated by both cFLIP and MALT1

several metacaspase genes are pathogen inducible in Arabidopsis.[104] Analysis of metacaspase function using knockout or knockdown mutants indicated roles in susceptibility to necrotrophic or hemi-biotrophic pathogens.[103,105] We recently demonstrated that AtMC1 and AtMC2 antagonistically control HR downstream of NB-LRR activation[86] (Figure 6a) using Arabidopsis as a model plant to study the immune system in plants.[106]

AtMC1 is a positive regulator of cell death. It interacts via its N-terminal prodomain with the second and third zinc fingers of LSD1. *atmc1* knockout mutants suppress cell death in *lsd1* and also bacterial- and oomycete-triggered HR. HR mediated by both CC- and TIR-NB-LRR intracellular immune receptors is severely attenuated in *atmc1* plants, indicating convergence of the two pathways into a single cell death output. Interestingly, pathogen growth restriction is not affected by HR suppression, indicating that disease resistance and cell death can be uncoupled. AtMC2 acts genetically as a negative regulator of AtMC1. AtMC2 over-expression mimics *atmc1* mutant phenotypes, whereas the lack of AtMC2 results in enhanced HR and accelerates cell death in an *lsd1* background. Similar to some animal caspases, the function of both AtMC1 and AtMC2 is negatively regulated by their N-terminal domain. Since AtMC1 interacts with LSD1, prodomain removal could result in release of the putative active form from the LSD1-anti-cell death scaffold into the cytoplasm.

The mechanism by which AtMC2 regulates AtMC1 remains enigmatic. AtMC2 does not interact with LSD1 or AtMC1 in yeast-two-hybrid or in planta co-immunoprecipitation assays. While AtMC1 activity requires caspase-like catalytic residues, AtMC2 function is independent of its putative catalytic cysteines. In mammals, there are several examples of atypical caspases or caspase-like proteins modulating the activity of a caspase independent of their protease activity.[107–113]

Caspase-12 has recently emerged as a negative regulator of immune responses in mammals, causing higher susceptibility to colitis, bacterial infection and sepsis.[110,112–114] Mechanistically, caspase-12 can either inhibit caspase-1, dampening the production of pro-inflammatory cytokines[112,114] or suppress the NF-κB pathway, independent of caspase-1[110,115] (Figure 6b). Cellular FLICE-inhibitory protein (cFLIP) is a proteolytically inactive caspase-8 homolog that acts as a dominant-negative inhibitor of caspase-8 in the apoptotic extrinsic pathway of mammals.[108,111] cFLIP also regulates caspase-8 function in lymphocyte survival and proliferation.[107] This non-apoptotic function of caspase-8 can also be mediated by the paracaspase MALT1, independent of its proteolytic activity[109] (Figure 6c).

In line with observations using the plant AtMC2, and animal cFLIP and MALT1, the catalytic activity of caspase-12 is not required to exert its regulatory function.[110,112,113] Caspase-12 inhibition of NLR-mediated innate immunity in mammals [110] recapitulates the role of AtMC2 inhibiting AtMC1-dependent HR, mediated by the analogous NB-LRR proteins in plants.[86] The sum of these studies suggests that cell death control mediated by the caspase/metacaspase superfamily is coupled to intracellular innate immune receptor function in both animals and plants.

The HR: Cause or Consequence?

In plants, a fundamental question remains unanswered: why does HR occur? Traditionally, HR was envisioned as the plant mechanism that prevented pathogen growth in incompatible plant–pathogen interactions and therefore causal to disease resistance. This notion was first challenged by Kiraly *et al*.[116] in a study showing that it is not plant cell death that inhibits pathogen proliferation. Since then, several natural examples

of plant–pathogen interactions resulting in resistance without cell death have been reported, in particular the potato *Rx* and barley *Rrs1* disease resistance genes.[117–124] Additionally, suppressing caspase-like activities (unrelated to metacaspases) in plants inhibits pathogen-induced cell death without affecting disease resistance.[89,125] As described above, elimination of the metacaspase AtMC1 results in drastically reduced HR after infection with incompatible pathogens, but bacterial growth restriction remains unaffected in this mutant.[86] These studies have added new components to the sparsely populated signaling pathways that translate NLR/NB-LRR recognition of pathogens into downstream activation of cell death. The mechanisms by which plants stop pathogen growth require further analysis.

Cell death and restriction of pathogen growth leading to disease resistance are genetically separable in both animals[36] and plants,[86] at least for the pathogens tested in these studies. In plants, HR cell death may occur simply as a consequence of the escalated signaling at the interface of plant–pathogen interactions, and the consequent rise in toxic intermediates that lead to both host and pathogen cell death. If HR is not adaptive in restricting pathogen growth, it may be adaptive for the generation of long range signals, mediated by ROS and SA, that induce the systemic acquired resistance that primes a plant for secondary infection.[126–128]

Conflict of interest

The authors declare no conflict of interest.

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