The Life and Death of a Plant Cell

Mehdi Kabbage,[1,*] Ryan Kessens,[1,*] Lyric C. Bartholomay,[2] and Brett Williams[3]

[1]Department of Plant Pathology, University of Wisconsin–Madison, Madison, Wisconsin 53706; email: kabbage@wisc.edu

[2]Department of Pathobiological Sciences, University of Wisconsin–Madison, Madison, Wisconsin 53706

[3]Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Queensland 4001, Australia; email: b.williams@qut.edu.au

Annu. Rev. Plant Biol. 2017. 68:375–404

First published online as a Review in Advance on January 11, 2017

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-043015-111655

*These authors contributed equally to this work.

Keywords

programmed cell death, apoptosis, autophagy, caspase, Bcl-2-associated athanogene, metacaspase, vacuolar processing enzyme, SQUAMOSA promoter-binding protein, sugar metabolism, resurrection plants

Abstract

Like all eukaryotic organisms, plants possess an innate program for controlled cellular demise termed programmed cell death (PCD). Despite the functional conservation of PCD across broad evolutionary distances, an understanding of the molecular machinery underpinning this fundamental program in plants remains largely elusive. As in mammalian PCD, the regulation of plant PCD is critical to development, homeostasis, and proper responses to stress. Evidence is emerging that autophagy is key to the regulation of PCD in plants and that it can dictate the outcomes of PCD execution under various scenarios. Here, we provide a broad and comparative overview of PCD processes in plants, with an emphasis on stress-induced PCD. We also discuss the implications of the paradox that is functional conservation of apoptotic hallmarks in plants in the absence of core mammalian apoptosis regulators, what that means, and whether an equivalent form of death occurs in plants.

Contents

INTRODUCTION

Programmed cell death (PCD): a genetically regulated program of cellular suicide employed by both unicellular and multicellular organisms

What can be more fundamental to biology than life and death decisions? Programmed cell death (PCD) plays an essential role in plant development and responses to abiotic and biotic insults, just as it does in many other eukaryotic organisms. This program directs a given cell to eliminate itself for the overall benefit of the organism. The role of PCD in plant development has been well documented in multiple processes, including gametophyte maturation, degeneration of embryo suspensor cells, formation of xylem tracheary elements, root development, and senescence (172). PCD plays an equally important role in plant responses to abiotic and biotic stress. This is particularly important for plants because of their sessile nature, which prevents physical escape from harsh environmental conditions, pathogens, and predators. Perhaps the most striking examples of plant PCD occur as a function of pathogen invasion; these include the hypersensitive response (HR), cell death–inducing toxins, and responses to necrotrophic pathogens (36).

Despite the critical importance of cell death outcomes in plants, the biochemical pathways that underpin the execution of this fundamental cellular program remain largely unknown. Indeed,

progress in our understanding of PCD in plants has been hindered because many of the core regulators of apoptosis—the main form of PCD in animals—are absent in plants. By contrast, homologs of the canonical elements of autophagy, a different form of PCD, are more obvious in both sequence similarity and function.

Apoptosis has been studied extensively in mammalian systems for its crucial roles in development and disease, including cancer and neurological, cardiovascular, and autoimmune disorders (49). The fundamental understanding of apoptosis in mammals involves one of two pathways, which are triggered by intrinsic or extrinsic cellular cues. The extrinsic pathway is mediated by cell surface receptors that bind various ligands and launch intracellular signaling cascades. By contrast, the intrinsic pathway is mediated by the mitochondria, largely in response to stress cues. The outer mitochondrial membrane contains members of the B-cell lymphoma 2 (Bcl-2) family of proteins. This family contains both anti- and proapoptotic members, the balance between which affects mitochondrial membrane potential. Under apoptosis-inducing conditions, the mitochondrial membrane permeability increases, releasing apoptotic regulators such as cytochrome *c* (47). Ultimately, both intrinsic and extrinsic pathways activate downstream caspases (short for cysteinyl aspartate–specific proteases). Active caspases degrade cellular components, leading to the orderly demise of the cell (127). Given that apoptosis is a terminal event, caspase activation is strongly regulated to prevent PCD in the absence of the appropriate signals. For example, members of the inhibitor of apoptosis (IAP) protein family provide negative regulation of caspase activity.

Although Bcl-2 family members, caspases, and IAP family members are critical regulators of apoptosis in animals, sequence-level conservation does not clearly extend to plants. This led to the argument that the term apoptosis, or apoptotic-like cell death, should not be used in reference to plant PCD and prompted new terminologies to describe plant PCD (170, 171). However, animal apoptosis and plant PCD have many morphological similarities. Plasma membrane blebbing, cytoplasmic and nuclear shrinkage, chromatin condensation and fragmentation, and cytochrome *c* release have all been described in animal and plant cells undergoing developmental or stress-induced PCD (36). Ectopic expression of animal core regulators of apoptosis produces analogous PCD outcomes in plants (20, 37, 83, 103). Furthermore, synthetic caspase substrates are processed by plant cells undergoing PCD, and caspase inhibitors can prevent HR-PCD following pathogen recognition (67, 135, 163). Finally, several protease families that are unique to plants but are operationally or functionally analogous to caspases also function in PCD.

This review aims to provide a broad and comparative overview of cell death processes in plants, including both apoptosis and autophagy, with an emphasis on stress-induced PCD. It also discusses the problem of how to reconcile the ideological conservation of apoptotic features in plants and the lack of direct sequence homology to the canonical elements of apoptosis as they are understood in animals.

Apoptosis: a specific form of PCD characterized by a suite of morphological changes (*apo* = "from"; *ptosis* = "falling")

Autophagy: "self-eating," a highly conserved process for the bulk degradation of cellular components following sequestration in double-membrane vesicles (autophagosomes) and degradation in the lysosome (mammals) or vacuole (plants)

Caspases: a family of cysteine-aspartic proteases responsible for the execution of apoptosis in animals

Inhibitor of apoptosis (IAP): a family of proteins that negatively regulate apoptosis through direct and indirect inhibition of caspases in animals

Initiator caspases: caspases involved in the early stages of apoptosis; they are characterized by an N-terminal prodomain containing a protein-protein interaction motif

THE EXECUTIONERS OF CELL DEATH: CASPASES AND BEYOND

Caspases are the principal proteases responsible for the execution of apoptosis in animals. The first caspase described in humans was caspase-1, which, interestingly, functions as a mediator of the inflammatory response and not of apoptosis (18, 160). Caspase-1 cleaves the cytokine interleukin 1β after a highly conserved aspartic acid residue, a feature that would go on to define this class of cysteine proteases (16). Based on homology to caspase-1, Yuan et al. (193) discovered the *Caenorhabditis elegans* protein CED-3, which plays an essential role in developmental PCD; this study established the first link between caspases and apoptosis.

Caspases involved in apoptosis are classified into two types: initiators and executioners. Initiator caspases are so named because of their apical position in the apoptotic cascade and

are characterized by an N-terminal prodomain containing protein-protein interaction motifs that are absent in executioner caspases. Like all caspases, initiator caspases exist as inactive zymogens that require proteolytic processing to become fully active (5, 133). The N-terminal prodomains serve a dual function in inhibiting initiator caspase activity and facilitating the association between initiator caspases and caspase-activating complexes (97).

Executioner caspases: caspases that are activated by initiator caspases and are responsible for cleaving the cellular substrates necessary for an orderly demise of the cell

Metacaspases: a family of cysteine proteases in plants that are most similar to animal caspases; they cleave specific proteins after an arginine or lysine residue

Initiator caspases can be further divided into two groups based on whether they contain a caspase recruitment domain (CARD) or a death effector domain (DED) (97, 143). Caspases with a CARD are recruited to the apoptosome, whereas those with a DED are recruited to the death-inducing signaling complex (DISC) for activation upon perception of an apoptotic stimulus. Formation of an apoptosome complex is the result of activation of the intrinsic (or mitochondrial) apoptosis pathway. In human cells, the release of cytochrome *c* from the mitochondrial inner membrane space allows cytochrome *c* to associate with apoptotic protease–activating factor 1 (Apaf-1) (197). The cytochrome *c*–Apaf-1 complex then recruits procaspase-9 through homotypic interactions between the CARD of Apaf-1 and the CARD of procaspase-9 (102). The complex comprising cytochrome *c*, Apaf-1, and procaspase-9 is known as the apoptosome complex and functions as a scaffold by which multiple Apaf-1 and procaspase-9 subunits assemble (198). The role of Apaf-1 in procaspase-9 activation is supported by work in *Drosophila melanogaster*, in which DARK (an Apaf-1 homolog) is required for most developmental and stress-triggered apoptosis through its activation of DRONC (a caspase-9 homolog) (117). However, the DARK-DRONC apoptosome complex found in flies does not require cytochrome *c* to assemble, as it does in humans (192). Upon activation, caspase-9 cleaves two effector procaspases, procaspase-3 and procaspase-7, to their active forms (102, 167, 198).

Once activated by initiator caspases, executioner caspases are responsible for cleaving the cellular substrates necessary for the orderly demise of the cell. The principal executioner caspases in humans are caspase-3 and caspase-7, while the principal executioner caspase in *D. melanogaster* is DRICE; these caspases seem to serve the most prominent roles in cell execution compared with other executioner caspases. General targets of caspases are numerous and include negative regulators of apoptosis, cell structure components, and other caspases. Ultimately, caspase activity manifests as an orderly breakdown of cellular integrity.

Plant Proteases Involved in Programmed Cell Death

Although plant genomes do not encode obvious homologs of animal caspases, caspase-like activity has been observed during PCD in plants (67, 135, 163). For instance, caspase-1 and -3 inhibitors prevent HR cell death in tobacco in response to *Pseudomonas syringae*, and a caspase-1 inhibitor suppresses HR in response to tobacco mosaic virus (TMV) infection (30, 68). Research efforts focused on identifying the source of caspase-like activity in plants led to the discovery of many proteases with roles in PCD execution (see **Figure 1**). That said, a core family of PCD proteases, akin to the caspases in animals, has not been discovered in plants. Instead, there appear to be several protease families associated with distinct triggers (abiotic stress, pathogen infection, etc.) of plant PCD.

In terms of domain architecture, metacaspases are the plant family of proteases most similar to caspases (168). The metacaspase family can be subdivided into type 1 and type 2 metacaspases based on the presence (type 1) or absence (type 2) of an N-terminal prodomain (166). Every metacaspase consists of small (p10) and large (p20) catalytic subunits, each of which contains a catalytic dyad of cysteine and histidine residues. Unlike caspases, metacaspases have a strict requirement for cleavage after an arginine or lysine residue at the first position (P1) of the cleavage site.

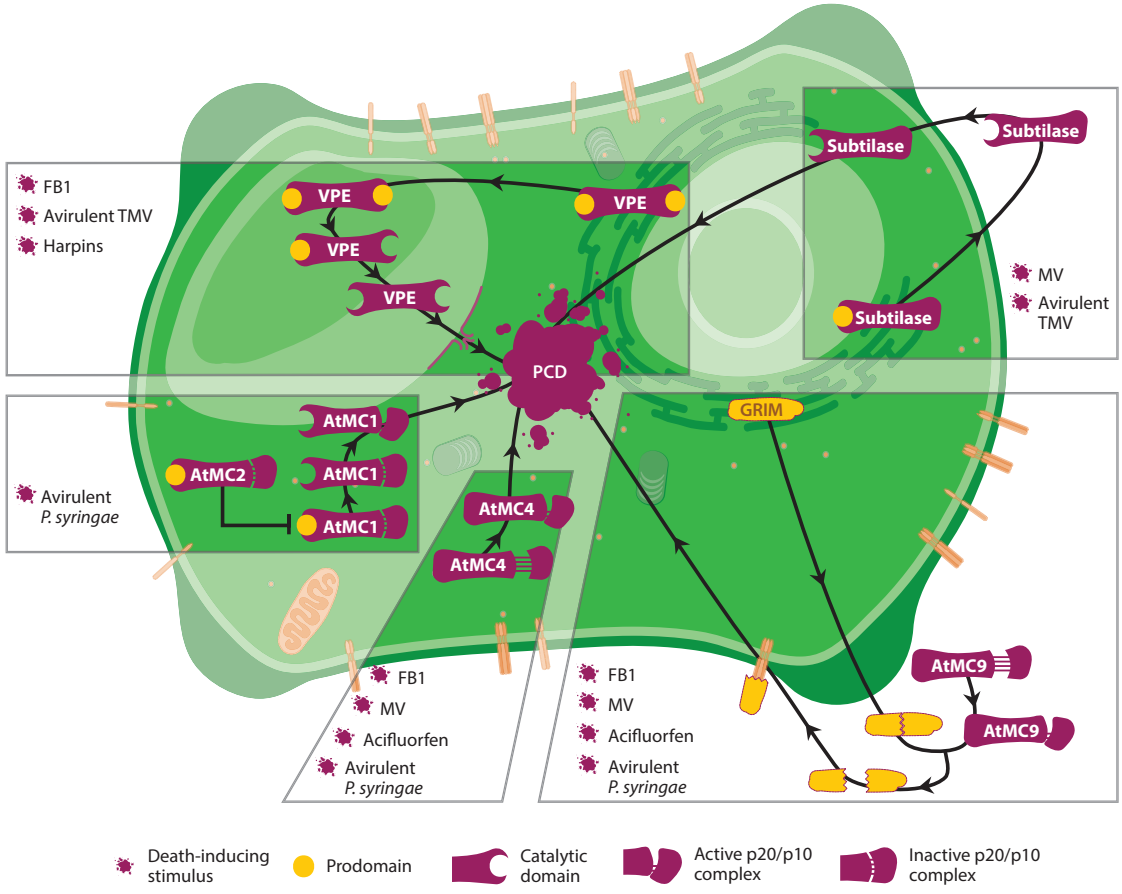

Figure 1

Schematic illustration of the role played by various plant proteases in PCD regulation. Specific examples of PCD regulation by the type 1 metacaspases AtMC1 and -2 and the type 2 metacaspases AtMC4 and -9 are included; more general summaries are shown for VPEs and subtilases. The death-inducing stimuli that lead to the activation of each protease are indicated. The prodomains of type 1 metacaspases, VPEs, and subtilases must be removed for these proteases to be catalytically active. The small (p10) and large (p20) catalytic subunits of type 1 and type 2 metacaspases must be cleaved for these proteases to be catalytically active. Abbreviations: AtMC, *Arabidopsis thaliana* METACASPASE; FB1, fumonisin B1; GRIM, GRIM REAPER; MV, methyl viologen; *P. syringae*, *Pseudomonas syringae*; PCD, programmed cell death; TMV, tobacco mosaic virus; VPE, vacuolar processing enzyme.

One of the most highly expressed type 2 metacaspase genes in *Arabidopsis*, *Arabidopsis thaliana METACASPASE 4* (*AtMC4*), is a positive regulator of cell death induced by numerous abiotic and biotic stresses. *Atmc4* mutants are less sensitive to cell death induced by the mycotoxin fumonisin B1 (FB1), the herbicides methyl viologen (MV) and acifluorfen, and HR caused by avirulent *P. syringae* (176). AtMC1 and -2 are positive and negative regulators of HR-PCD, respectively (23). The prodeath activity of AtMC1 is dependent on the removal of the N-terminal prodomain and an active-site cysteine residue. Interestingly, *Capsicum annuum* metacaspase 9 (CaMC9) seems to play more of a role in cell death caused by disease progression. Silencing of *CaMC9* in pepper plants suppresses the progression of tissue death caused by the bacterial pathogen *Xanthomonas campestris* pv. *vesicatoria* race 3, indicating that CaMC9 is a positive regulator of cell death induced by this pathogen (93).

Fumonisin B1 (FB1): a fungal toxin produced by *Fusarium* species that induces apoptosis in animal cells and PCD in plant cells

Vacuolar processing enzymes (VPEs): a family of cysteine proteases in plants that localize in vacuoles and are related to animal asparaginyl endopeptidases

Subtilases: a family of extracellular serine proteases found in all kingdoms of life

Another family of plant proteases implicated in PCD is the vacuolar processing enzyme (VPE) family of cysteine proteases. VPEs are composed of a signal peptide for trafficking to the central vacuole, N- and C-terminal prodomains that are removed upon processing, and a catalytic active site. VPEs are converted to the mature form through autocatalytic processing after aspartic acid or asparagine residues. The inhibitory C-terminal prodomain must be removed before the N-terminal prodomain can be removed to yield the mature enzyme (73). This VPE is a positive regulator of HR induced by TMV through its role in vacuolar collapse. Interestingly, the caspase-1 inhibitor (Ac-YVAD-CHO) suppresses HR and inhibits VPE activity, thus establishing the first link between caspase-like activity in plants and a specific family of proteases (68).

The *Arabidopsis* δVPE protein is implicated in developmental PCD of the inner integument layer of the seed coat that occurs during embryo development. Cells in the inner integument layer of *δvpe* mutants do not undergo plasma membrane shrinkage or tonoplast rupture, as they do in wild-type cells (122). VPE also functions in stress-induced PCD; for example, FB1 toxin resistance in *Arabidopsis* was achieved by cotreating with a VPE inhibitor or by knocking out all four VPE-encoding genes (98). Features of PCD in wild-type plants, such as plasma membrane blebbing, were absent in mutant plants exposed to FB1. Silencing *VPE1a* and *VPE1b* in *Nicotiana benthamiana* reduced sensitivity to cell death caused by the bacterial elicitor harpin (194) but did not do so for cell death caused by the necrosis- and ethylene-inducing protein Nep1 or the oomycete elicitor boehmerin. Based on these data, VPE1a and -b appear to execute PCD in a context-specific manner.

Subtilisin-like serine proteases (subtilases or saspases) with caspase-like activity have also garnered interest for their potential role in plant PCD regulation. Subtilases contain a signal peptide for secretion into the apoplast, a prodomain for autoinhibition, and a catalytic domain characterized by an active-site serine residue. The first of the subtilases characterized from plants, SAS-1 and -2, were purified from extracellular extracts of *Avena sativa* (oat) challenged with the PCD-inducing fungal toxin victorin (22). In a different study, silencing of a subtilase with caspase-like activity isolated from tobacco resulted in reduced HR-PCD in response to TMV, whereas overexpression resulted in an enhanced HR (21). Additionally, subtilase silencing enhanced resistance to cell death induced by MV and NaCl, whereas overexpression led to enhanced cell death. Notably, treatment with MV and TMV resulted in the relocalization of the subtilase from the apoplast into the cell interior (21).

Recent work by Fernández et al. (50) showed that a subtilase from potato with caspase-3-like activity (StSBTc-3) was present in the apoplast following *Phytophthora infestans* challenge. Tomato cell suspensions treated with purified StSBTc-3 underwent cell death with plasma membrane retraction and cytoplasmic shrinkage, features indicative of a programmed response. Even more recently, Zimmermann et al. (195) demonstrated that the subtilase P69B is a substrate of two matrix metalloproteinases from tomato, Sl2-MMP and Sl3-MMP. Silencing of *Sl2-MMP* and *Sl3-MMP* in tomato resulted in spreading cell death that started in the hypocotyls and progressed to the outer cortical cells, suggesting that these matrix metalloproteinases are negative regulators of epidermal cell death. Silencing *P69B* in the *Sl2/3-MMP* RNA-interference-silenced plants resulted in reduced expression of the cell death marker genes *hsr203j*, *Hin1*, and *tpoxC1*.

Efforts to identify proteases responsible for caspase-like activity in plants have yielded valuable information about the role of VPEs and subtilases in PCD execution. Additionally, many studies have focused on metacaspases because of their shared domain architecture with caspases. Future studies can now focus on identifying the substrates that these plant proteases act upon to reveal more about their role in PCD execution.

Plant Protease Substrates Associated with Programmed Cell Death

Some progress has been made on identifying metacaspase substrates, and the results suggest some commonalities between caspases and metacaspases. For example, the metacaspase mcII-Pa from *Picea abies* (Norway spruce) processes Tudor staphylococcal nuclease (TSN)—an evolutionarily conserved substrate cleaved by caspase-3 in mammals—as a cleavage substrate during developmental and stress-induced PCD (151). Furthermore, degradome analysis of AtMC9 in *Arabidopsis* revealed that AtMC9 cleaves general regulatory factor 5 (GRF5), phosphoenolpyruvate carboxykinase 1 (PEPCK1), nicotinamide adenine dinucleotide (NAD) synthase, citrate synthase 3 (CSY3), GDP-mannose 4,6-dehydratase 2 (GMD2), and pentatricopeptide repeat (PPR)–containing protein (165). An additional study showed that AtMC9 processes a secreted protein called GRIM REAPER into an 11-amino-acid peptide that induces cell death in *Arabidopsis* (183); this is the only example of a plant protease processing a secreted protein that then binds to the extracellular portion of a transmembrane receptor and induces cell death.

Research on apoptosis in animals has been greatly accelerated by the development of antibodies and synthetic substrates that detect active caspases and processed caspase substrates. As more PCD regulators are identified in plants, it will become possible to use biochemical hallmarks to track PCD progression. Lu et al. (108) have made recent advancements in the development of highly specific probes for VPEs and other cysteine proteases. These probes use synthetic substrates optimized for the specific peptide sequences that the proteases bind and cleave. Their VPE probe specifically binds active VPEs in *Arabidopsis*, tomatillo, winter cherry, tomato, tobacco, barley, and maize (108). Probes such as these will allow protease activity to be monitored at different stages of death progression through fluorescence and localization assays. Additionally, proteases could be purified and mass spectrometry employed to determine the composition of protease activation complexes and posttranslational modifications of these proteases.

INHIBITORS OF APOPTOSIS

The IAP family consists of proteins that are important antiapoptotic regulators in animals. The first IAP to be discovered was the baculovirus CpIAP from *Cydia pomonella* granulosis virus (24). A characteristic feature of this IAP, and a defining feature of IAP homologs, is the presence of a zinc-binding domain known as the baculovirus IAP repeat (BIR) domain (42). The BIR domain is responsible for the substrate binding and specificity of IAP proteins (138, 153). Additionally, many IAPs contain a RING domain that functions as an E3 ubiquitin ligase (186). IAPs that contain a RING domain target substrates for degradation via the 26S proteasome (17, 142, 158). IAPs exert antiapoptotic activity by negatively regulating caspase activity, through either direct binding to the caspase or binding to an upstream component required for caspase activation. Although IAP binding alone is enough to inhibit the activity of some caspases, for other IAPs, E3 ligase activity is required (17, 142, 186). IAPs are themselves kept under multiple layers of regulation through autoubiquitination or ubiquitination by other IAP regulators. Additionally, IAP activity can be regulated by antagonists that bind to IAP-binding motifs located within the IAP protein (41, 173, 184). Proapoptotic IAP antagonists bind to and relieve IAP inhibition of caspase activity so that apoptotic cascades commence.

Although plant genomes do not appear to encode IAPs, ectopic expression of animal and viral IAPs in plants confers tolerance to stress-induced cell death. The first IAP shown to function in plants was the baculovirus *Orgyia pseudotsugata* nuclear polyhedrosis virus IAP (OpIAP). Transgenic tobacco plants overexpressing *OpIAP* were resistant to tomato spotted wilt virus infection and the necrotrophic fungi *Cercospora nicotianae* and *Sclerotinia sclerotiorum* (37). Tissue necrosis

typically associated with the systemic spread of tomato spotted wilt virus was suppressed in *OpIAP*-overexpressing transgenic lines, as was tissue death associated with *C. nicotianae* and *S. sclerotiorum*. Interestingly, *OpIAP* overexpression did not suppress HR in tobacco challenged with TMV (188).

Expression of an IAP from *Spodoptera frugiperda* (fall armyworm) in tobacco and tomato prevents cell death associated with infection by the necrotrophic fungus *Alternaria alternata*, heat, salt, and FB1 treatment. Cell death induced by these stresses and suppressed by SfIAP displayed characteristic apoptotic features, including DNA laddering and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)–positive nuclei (103). Suppression of salt-induced cell death in protoplasts was partially dependent on a functional E3 ligase for SfIAP (83). Transgenic rice expressing *SfIAP* have increased tolerance to salt stress. Root cells are less susceptible to cell death caused by excessive salt in the soil, which allows for greater photosynthesis in *SfIAP*-expressing rice under salt stress compared with wild-type and empty-vector control plants. The maintenance of high photosynthetic levels provided the plants with the necessary chemical energy to mitigate stress and survive (75).

Following the discovery of the functionality of IAPs in tobacco and tomato, efforts were made to identify IAP homologs in other plants. Although no homology to a BIR domain has been discovered, simple Basic Local Alignment Search Tool (BLAST) searches revealed *Arabidopsis* proteins with RING domains exhibiting some similarity to those from human and *D. melanogaster* IAPs. Validation of functionality came with the discovery that *Arabidopsis thaliana* IAP-LIKE PROTEIN (AtILP) inhibits tumor necrosis factor alpha (TNFα)–induced cell death in HeLa cells and suppresses caspase and caspase-like activity in HeLa cells and *Arabidopsis* upon treatment with FB1 (94). Overexpression of AtILP also inhibits HR-PCD. The RING domain was not required for the anti-PCD phenotype under any of these death triggers, which indicates that the anti-PCD activity of AtILP in *Arabidopsis* comes from its N terminus, which has no similarity to human or *Drosophila* IAPs. It is important to emphasize that these IAP-like proteins have been identified based strictly on alignments of their RING domains with the RING domains from true IAPs. The *Arabidopsis* genome encodes a total of 477 predicted proteins with a RING domain; of these, 186 have the same C3HC4-type structure as human and *Drosophila* IAPs (150). Of these 186 proteins, 11 contain a predicted RING domain with an E-value below 1×10^{-5}. Although these are provocative leads for novel negative regulators of plant PCD, in the absence of structural and functional analysis, it is premature to conclude that these proteins are true IAP homologs.

Although most research efforts have focused on understanding how IAPs regulate caspase activity, there is increasing evidence that they function in diverse cellular processes and interact with a variety of substrates. The human IAP (XIAP) has been implicated in copper homeostasis because of its ability to bind intracellular copper, which dramatically reduces the half-life of XIAP and results in increased caspase-3 activity in vitro (120). Additionally, XIAP physically interacts with and ubiquitinates MURR1, another regulator of copper homeostasis responsible for copper toxicosis in some dog breeds (14). IAPs have also been implicated in cell division, mitogen-activated protein kinase activation, innate immunity, and nuclear factor κB signaling (148). Further support for a caspase-independent role for IAPs comes from the observation that BIR domains can be found in some fungal proteins, which lack caspases and other animal apoptotic regulators (144).

DNA laddering: apoptotic DNA fragmentation resulting in internucleosomal DNA cleavage, observed as a distinct banding pattern of 180-base-pair multiples on an agarose gel

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay: an assay used to detect double-stranded DNA breaks occurring at the late stages of apoptosis

Bcl-2-associated athanogene (BAG) family: an evolutionarily conserved family of co-chaperones in mammals and plants distinguished by a characteristic BAG domain that mediates direct interaction with HSP70

THE CENTRALITY OF BAG FAMILY MEMBERS IN PLANT CELL DEATH

The Bcl-2-associated athanogene (BAG) family is an evolutionarily conserved group of proteins with homologs across wide evolutionary distances, from single-celled yeasts to metazoans, including humans. The initial discovery of this family came from a yeast-two-hybrid screen aimed

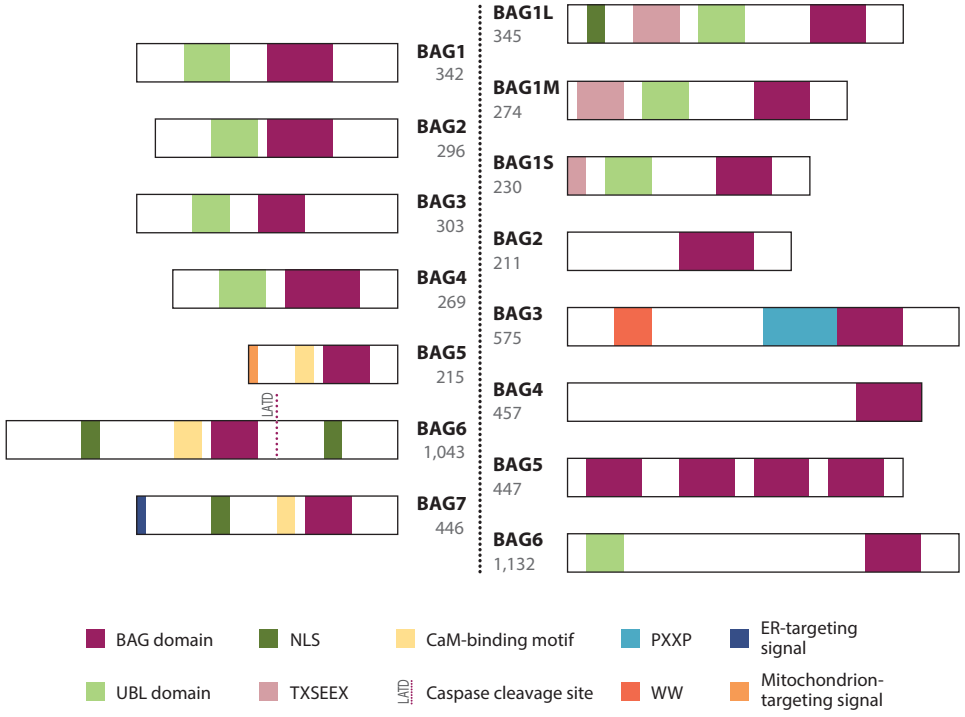

Figure 2

A comparison of BAG protein domains in *Arabidopsis* (*left*) and humans (*right*), showing their characteristic C-terminal BAG domains. The structure of *Arabidopsis* BAG proteins includes a conserved BAG domain, UBL domain, NLS, and plant-specific CaM-binding motif. BAG5 and -7 contain mitochondrion- and ER-targeting peptides, respectively. An operational caspase-1 cleavage site (LATD) is shown for BAG6. The protein length (in number of amino acids) is displayed under each BAG name. Abbreviations: BAG, Bcl-2-associated athanogene; CaM, calmodulin; ER, endoplasmic reticulum; NLS, nuclear localization signal; UBL, ubiquitin-like.

at identifying Bcl-2 partners (155). The gene identified was termed *Bcl-2-associated athanogene 1* (*BAG1*) and was shown to enhance the antiapoptotic activity of Bcl-2, suggesting its involvement in apoptotic pathways (155). A common signature of all BAG proteins is the presence of a protein motif at the C terminus termed the BAG domain (BD) (see **Figure 2**). This domain mediates direct interaction with the heat shock protein 70 (HSP70) chaperone (13). Later studies more accurately characterized BAG proteins as co-chaperones that function as molecular switches acting upon HSP70 and other substrates, both positively and negatively, to maintain protein homeostasis. Several excellent reviews have been published on BAGs and the remarkable array of cellular events they modulate, including cell death regulation (8, 82, 154).

A Concise Overview of Animal BAG Proteins

The human BAG family contains six members, BAG1–6, all of which have a single BD except for BAG5, which contains four such domains (see **Figure 2**). BAG1 is the founding member of this family and has attracted much attention since its discovery largely because of its association with Bcl-2. Four human BAG1 isoforms have been identified, all of which contain a ubiquitin-like (UBL) domain in addition to the BD. The presence of a UBL domain suggests a link to

proteasome-mediated protein degradation. Indeed, BAG1 itself is a substrate of an E3 ligase, C terminus of HSC70-interacting protein (CHIP); BAG1 and CHIP form a ternary complex with HSP70 to target proteins for degradation (1, 31). BAG1 is also involved in a wide range of signaling events through interactions with steroid hormone receptors (52, 95, 96, 105), the Raf1 protein kinase (147, 174), and Siah, a protein involved in p53-mediated apoptosis (110).

BAG2 and -3 were later identified in a screen of HSP70 binding partners, and they too were shown to regulate its function (156). BAG2 has also been linked to CHIP, but unlike BAG1, this association appears to inhibit CHIP-dependent ubiquitin ligase activity (3, 28). In addition to its impact on many major biological processes (reviewed in 8), BAG3 has recently attracted considerable attention because of its potential as a target in cancer therapies (reviewed in 136) and its role in protein quality control during aging (57). It was reported to maintain cell survival in several tumor types and was implicated in resistance to chemotherapy. Interestingly, interplay between BAG1 and -3 affects protein degradation during the aging process (57). The ratio of BAG1 and -3 directly regulates the induction of macroautophagy and the turnover of polyubiquitinated proteins in aging cells.

BAG4, known as a silencer of death domains (SODD), was also identified in a screen for HSP70 binding partners (156). It has been proposed to function as a negative regulator of the TNF superfamily, including TNF1 and death receptor 3 (81). In cooperation with HSP70, BAG4 appears to prevent the aggregation of these death receptors to maintain an inactive monomeric state (164), a process that also confers resistance to cancer therapies (59).

BAG5 is a structurally unique member of this family that contains four BDs. Beyond its capacity to bind HSP70, the significance of having multiple BDs is not well understood. BAG5 has been linked to Parkinson's disease (9, 87), and was found to inhibit both parkin E3 ubiquitin ligase and HSP70 chaperone activity (87). Thus, BAG5 contributes to neurodegeneration and Parkinson's disease development, which is in stark contrast to the prosurvival effect of other BAG family members. However, a cytoprotective role for BAG5 has also been identified: Wang et al. (175) proposed that it stabilizes PTEN-induced kinase 1 (PINK1) and prevents mitochondrial damage as a result of oxidative stress.

Finally, BAG6 (also known as Scythe or BAT3) is the largest member of the BAG family. In *D. melanogaster*, it associates with Reaper and promotes Reaper-induced cytochrome *c* release and caspase activation (161, 162). In mammalian development, BAG6 inactivation is linked to increased lethality and severe developmental defects in the lung, kidney, and brain (34). Other studies have implicated BAG6 in endoplasmic reticulum (ER) stress–induced cell death via its regulation of apoptosis-inducing factor (AIF) (35). Interestingly, a recent study called into question the legitimacy of BAG6 as a BAG family member because of its noncanonical BD (119).

The Emerging Role of Plant BAG Proteins in Cell Death Regulation and Stress Responses

Doukhanina et al. (40) used a combination of computational approaches to identify BAG homologs and found seven BAG-like proteins in *Arabidopsis* (AtBAG1–7) based on structural conservation of the BD (**Figure 2**). Although the functions of several members of this family are still unknown, significant progress has been made in our understanding of BAG function and regulation in plants. Similarly to animal BAGs, plant BAGs appear to function largely in cytoprotection, particularly in response to stress and developmental cues; this also suggests that at least some aspects of animal PCD regulation are conserved between plants and animals. *Arabidopsis* BAG proteins can be divided into two broad groups based on their domain organization: AtBAG1–4 have a domain structure similar to human BAG1, with a UBL motif present in addition to the BD, whereas

AtBAG5–7 contain a calmodulin (CaM)–binding motif near the BD, a feature unique to plant BAG proteins (**Figure 2**). The presence of a CaM domain may reflect divergent plant-specific functions associated with plant BAGs.

The function of UBL-containing *Arabidopsis* BAGs is largely unknown, with the exception of AtBAG4. Overexpression of *AtBAG4* conferred enhanced resistance to several abiotic insults, such as drought, salt, UV irradiation, and oxidant treatment (40, 74). Importantly, AtBAG4 inhibited the development of apoptotic features, such as DNA laddering and TUNEL-positive nuclei (40). Thus, AtBAG4 appears to inhibit apoptotic-like PCD and functions in stress tolerance in plants. Biochemical details that could explain the anti-PCD activity of AtBAG4 are still lacking. BAG proteins are known to bind HSP70/heat shock cognate 70 (HSC70) molecular chaperones, and an association between AtBAG4 and AtHSC70 has been established (40). However, unlike animal BAGs, a functional link between plant BAGs and HSC70s has not emerged, and it is not clear whether AtBAG4 activity is reliant on AtHSC70 or vice versa.

As mentioned, AtBAG5–7 have a unique domain organization, with a CaM-binding motif upstream of the BD. AtBAG5 is the smallest member of this family and has a predicted mitochondrial target peptide (82). This feature is intriguing given the role of the mitochondria in intrinsic apoptosis pathways and that none of the animal BAGs are localized to the mitochondria. Li et al. (101) recently suggested that AtBAG5 forms a complex with Hsc70 and CaM that regulates plant senescence. AtBAG6 is the largest member of this family and was originally identified as a CaM-binding protein (88). This plant BAG member appears to be essential for basal immunity against the fungal pathogen *Botrytis cinerea* (40). Surprisingly, AtBAG6 does not bind AtHSC70, but AtBAG6 expression in yeast and *Arabidopsis* induces PCD (88). A study by Li et al. (104) recently clarified the prodeath function of AtBAG6 and the molecular mechanisms that underpin AtBAG6-mediated basal immunity. This study showed that AtBAG6 is a substrate for an aspartyl protease (AtAPCB1) that cleaves the protein at a caspase-1 cleavage site downstream of its BD. Thus, AtBAG6 is proteolytically activated to trigger basal immunity and induce PCD. Active AtBAG6 triggers autophagy in *Arabidopsis*, which coincides with disease resistance (104). These findings are particularly important because the molecular details of plant PCD involving proteases and their substrates are largely unknown. Finally, AtBAG7 is an ER-localized *Arabidopsis* BAG member that is involved in the maintenance of the unfolded protein response (179). AtBAG7 interacts with *Arabidopsis thaliana* LUMINAL BINDING PROTEIN 2 (AtBIP2) and modulates PCD pathways of cells undergoing ER stress. During ER stress, AtBAG7 also appears to translocate from the ER to the nucleus, where it interacts with WRKY DNA-binding protein 29 (WRKY29) (Y. Li, B. Williams & M.B. Dickman, manuscript in review).

In toto, it is clear that BAG proteins are involved in a remarkable array of important cellular functions, owing in part to their association with HSP70 and the multiplicity of their targets (see **Figure 3**). Their roles in cell proliferation, stress responses, and PCD make them prime targets for therapeutic drugs. The discovery of a homologous family in plants is intriguing and suggests that, despite the lack of conservation of core apoptotic regulators, some aspects of cell death regulation are conserved between animals and plants. Our understanding of BAG function in plants is improving as we continue to position BAG proteins in a biochemical context in plants (**Figure 3**). Many attributes of BAG regulation and properties are conserved in plants and animals, with the exception of a functional link between BAG proteins and HSP70.

CELL DEATH IN PLANT-MICROBE INTERACTIONS

The most striking and well-known examples of plant PCD include HR triggered by biotrophic pathogen invasions, cell death in response to toxins, and cell death induced by necrotrophic

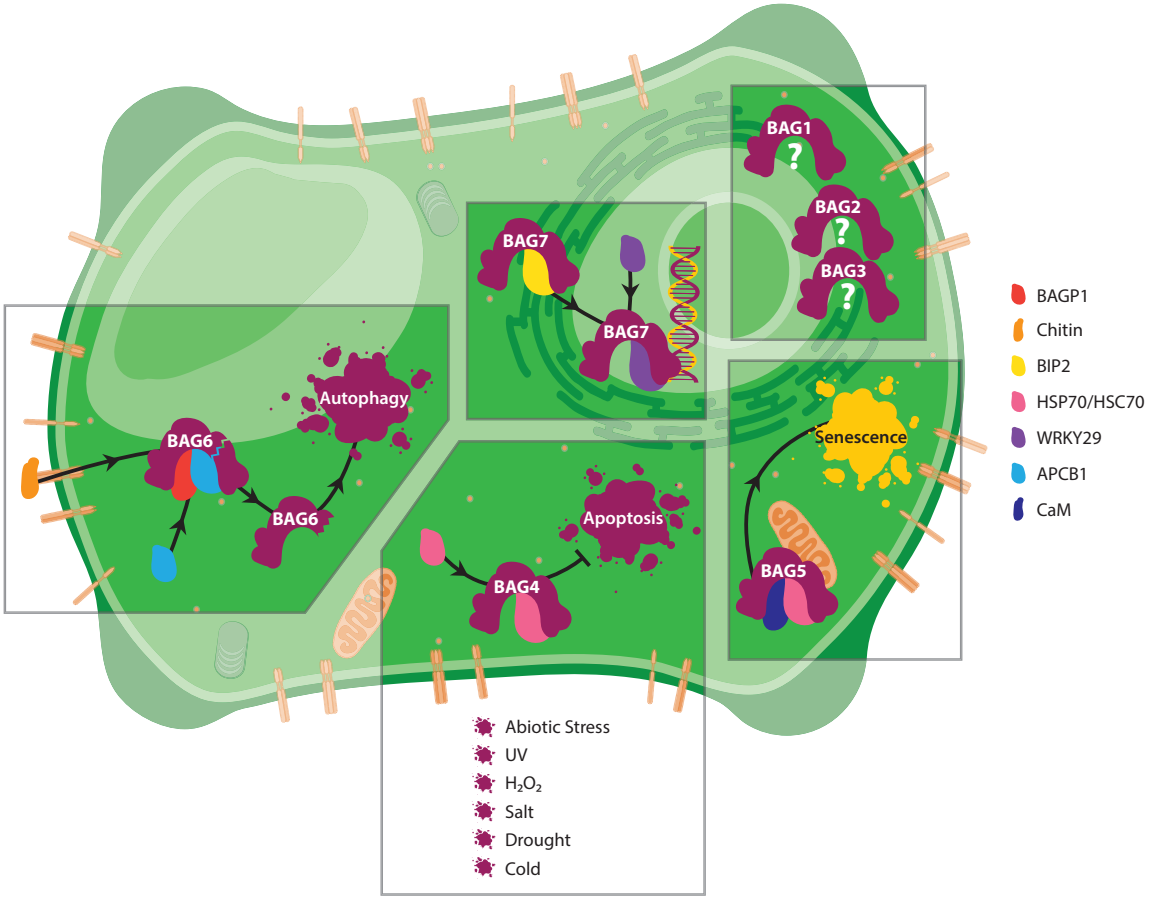

Figure 3

The role of *Arabidopsis* BAG family proteins in abiotic and biotic stress responses and cell death modulation. The functions of BAG1–3 are unknown. BAG4 is involved in cell death inhibition in response to abiotic stress and binds HSP70/HSC70 molecular chaperones. BAG5 forms a complex with CaM/HSC70 and regulates plant senescence. BAG6 is proteolytically activated via aspartyl protease activity and links fungal or chitin perception to the induction of autophagy. The ER-localized BAG7 binds the molecular chaperone BIP2 and is an essential component of the unfolded protein response. Abbreviations: BAG, Bcl-2-associated athanogene; BIP2, LUMINAL BINDING PROTEIN 2; CaM, calmodulin; ER, endoplasmic reticulum; HSC70, heat shock cognate 70; HSP70, heat shock protein 70.

pathogens. PCD as an end point in a plant-pathogen interaction, particularly in the case of HR, begins with a basal immune response—the first line of plant defense against invading pathogens, which is predicated on the recognition of specific microbial molecules commonly referred to as pathogen-associated molecular patterns. This receptor-based defense component relies on the recognition of common microbial molecular signatures, such as flagellin, peptidoglycan, and chitin (159, 196). Pathogens have evolved virulence factors, often in the form of small secreted proteins called effectors, to suppress plants' basal defense responses (159). This, in turn, led to the evolution of a second tier of plant defenses that use resistance (R) proteins. R proteins recognize pathogen effectors directly or indirectly and trigger a strong defense response that culminates in HR (62), a form of PCD. It is now recognized that significant crosstalk occurs between defenses mediated by pathogen-associated molecular patterns and those mediated by R proteins, blurring the distinction

between these responses (159). Although PCD (i.e., HR) is effective against invading pathogens with a biotrophic lifestyle, it can be advantageous to necrotrophic pathogens that require dead host tissue to achieve pathogenic success. Indeed, many necrotrophs induce cell death in their host by hijacking the host cell death machinery for their own benefit (26, 60, 84).

Overall, it is clear that PCD plays a key role in interactions between plants and microbes and often represents the deciding factor in whether a plant is resistant or susceptible to a particular pathogen. Although significant progress has been made in understanding the early events describing the interplay between host and microbes, these early signaling events have not yet been linked to the execution of plant cell death. These missing pieces will be critical for the development of novel control strategies against plant pathogens.

The Hypersensitive Response: Classification and Execution

The term hypersensitive response was coined a century ago to describe the robust localized cell death observed in resistant lines in response to infection by rust fungi. It is now used to describe a programmed disease resistance outcome in plants that is triggered by many pathogens and leads to tissue death and the restriction of pathogen growth. Although HR is generally controlled by a single *R* gene on the host side, corresponding to a cognate effector gene on the pathogen side, this process is highly complex and involves a suite of cellular and morphological events (reviewed in 6, 25, 36, 62, 70).

HR-PCD has been proposed to be distinctly different from developmental PCD (reviewed in 170). In his review, van Doorn (170) argued against the use of the term apoptosis in reference to plant PCD and coined the term vacuolar cell death, described as a combination of autophagy-like processes and the release of vacuolar hydrolases. He classified developmental PCD as vacuolar cell death and argued that HR-PCD encompasses both vacuolar cell death and necrosis features. It is important to note, however, that apoptotic features have been observed following HR-PCD and development processes alike (85, 130). Thus, these observations point to a shared mechanism underpinning the execution of both programs.

One possible key piece of evidence suggesting that at least some aspects of cell death execution are conserved comes from the recent discovery linking an R protein to a SQUAMOSA promoter-binding protein (SBP) transcription factor involved in resistance to TMV and avirulent *P. syringae* (126). The SBP family of plant-specific transcription factors [also referred to as the SQUAMOSA promoter-binding protein–like (SPL) family in some plant species] is characterized by a highly conserved SBP-box DNA-binding domain, and members of this family are present in organisms ranging from single-celled green algae to multicellular plants. They play diverse roles in plant development, including the juvenile-to-adult phase transition, trichome development, apical dominance, and pollen sac development (129). Although SBPs/SPLs have been linked principally to developmental processes, the N immune receptor of *N. benthamiana* associates with NbSPL6 upon activation of HR-PCD in response to the defense-eliciting TMV U1 strain but not the noneliciting TMV Ob strain. Similarly, the SPL6 ortholog in *Arabidopsis* was required for HR-PCD against *P. syringae* mediated by the R protein RESISTANT TO P. SYRINGAE 4 (RPS4) (126). Beyond HR-PCD, another *Arabidopsis* SBP family member, AtSPL14, has been implicated in cell death caused by the mycotoxin FB1 and is required for sensitivity to this toxin (149). FB1 is known to induce apoptotic-like cell death in both plant and animal cells (61). These are provocative leads for a linkage between SBP transcription factors and fundamental processes requiring cell death regulation, such as HR-PCD, development, and abiotic stress. Future studies may prove fruitful for elucidating the importance of SBPs as core regulators in the execution or inhibition of plant cell death programs.

Necrotrophic Fungal Pathogens and Host Programmed Cell Death

Necrotrophic fungi kill host tissue using a plethora of toxins and lytic enzymes to achieve pathogenic success. However, the views associated with necrotrophic pathogenesis are changing, and we now recognize that some of these pathogens do not simply cause direct injury to host cells, but instead co-opt host PCD machinery. A classic example is victorin-induced PCD imposed by the fungal pathogen *Cochliobolus victoriae* (26, 60). Victorin is a host-selective toxin that is required for susceptibility to this pathogen and the development of Victoria blight disease in oats. Victorin sensitivity is genetically conditioned by the *Vb* gene, which paradoxically has been proposed to be inseparable from an *R* gene (*Pc2*) that controls HR-PCD against another fungal pathogen, the biotrophic rust *Puccinia coronata* (26). Thus, it appears that *C. victoriae* hijacks an HR mechanism to induce PCD in its host.

Further investigation showed that this cell death regime has distinct apoptotic hallmarks. These include traditional molecular phenotypes of apoptosis, such as DNA laddering and mitochondrial membrane potential changes, as well as morphological features, such as cell shrinkage and retention of plasma membrane integrity (26). Here, then, is another example where distinct features of animal apoptosis are conserved in a plant cell undergoing PCD. Genetic association between host-selective toxins and plant cell death has also been described in other systems. In *Stagonospora nodorum*, the causal agent of *Stagonospora nodorum* blotch in wheat, several gene interactions between toxin and host have been identified that result in a type of cell death analogous to HR-PCD (51). *S. nodorum* host-selective toxins are often referred to as necrosis-inducing toxins. It is important to note that the term necrosis here simply denotes the visual manifestation of tissue death, rather than a mechanistic interpretation of the cell death regime imposed by these toxins.

The induction of plant PCD has also been discussed in response to broad-host toxins. *S. sclerotiorum* is a broad-host fungal pathogen that secretes a key virulence toxin, oxalic acid. Oxalic acid triggers apoptotic-like PCD in host plants (92), and oxalic acid–deficient mutants cannot trigger host PCD and are nonpathogenic (180). Interestingly, transgenic expression of the antiapoptotic *CED9* gene from *C. elegans* provided resistance to this pathogen, presumably by inhibiting cell death pathways activated by oxalic acid (84). These results suggest a functional conservation of apoptotic cell death between animal and plant cells. Although apoptotic PCD was conducive to disease development, the defense response against this pathogen involves an autophagic response (84). This indicates that different cell death regimes can mediate opposing disease outcomes and that cell death execution is, as mentioned above, context dependent.

REGULATION OF CELL DEATH IN PLANTS

Like animals, plants use a suite of regulatory pathways to program the demise of cells. Importantly, studies have shown that the manner of PCD execution is crucial, with different outcomes occurring depending on the context and strength of the signal (29, 76, 84, 180). Fundamental differences between mammals and plants include the presence of a central vacuole and chloroplasts in plants, both of which can play critical roles in PCD, particularly autophagy (36). Accordingly, emerging evidence suggests that autophagy is key to effective regulation of plant homeostasis and is indispensable in a plethora of processes, including energy metabolism, proper development, aging, and abiotic and biotic stress responses (reviewed in 7). What remains contentious, however, is whether autophagy directly results in cell death or death is an aberration of severe stress and excessive autophagy. Recent studies that have linked autophagy with glycolysis and plant metabolism suggest that autophagy is a prosurvival mechanism that plays significant roles in maintaining homeostasis.

Does Autophagy Carry the Brunt of Cell Death Regulation in Plants?

Autophagy is an intracellular self-eating process that facilitates the bulk remobilization of cytoplasmic constituents, including fragments of organelles, by trafficking in double-membraned vesicles termed autophagosomes for degradation in lysosomes (in mammals) or vacuoles (in plants) (115). This process is regulated by a suite of autophagy genes that are conserved from yeasts to animals to plants (137). In fact, most of the autophagy processes that have been identified in metazoans have been observed in plants (100, 106). Proteins involved in autophagy are termed autophagy-related (Atg) proteins and are highly conserved across broad eukaryotic kingdoms. They can be classified into four main classes or complexes that are found in all eukaryotes: the Atg1 kinase complex, the phosphatidylinositol 3-kinase complex, the Atg9 complex, and the Atg8-Atg12 conjugation complex (187). The Atg1 kinase complex is involved in responses to nutrient deprivation and comprises Atg1, Atg13, and Atg101. The phosphatidylinositol 3-kinase complex is regulated by the Atg1 complex and contains vacuolar protein sorting 34 (Vps34) kinase, Vps15, Atg6, and the autophagy-specific component Atg14 (79). A homolog of the conserved Atg14 has not been identified in plants.

Once generated, the phosphatidylinositol 3-kinase complex is recognized by various phosphatidylinositol 3-phosphate effectors, including members of the Atg9 complex, Atg2, and Atg18. The precise mechanistic details of the function of the Atg9 complex remain unclear, but it appears to play a role in autophagy responses to starvation (191). The Atg8-Atg12 conjugation complex contains numerous proteins, including Atg3, Atg4, Atg5, Atg7, Atg10, Atg8–phosphatidylethanolamine (PE), and Atg12. During autophagosome formation, Atg8 is cleaved by the Atg4 protease to expose a specific glycine that allows conjugation to PE by the E1 (ubiquitin-activating enzyme)–like protein Atg7, the E2 (ubiquitin-conjugating enzyme)–like protein Atg3, and the E3-like Atg12-Atg5-Atg16 complex. A second ubiquitin-fold protein, Atg12, is conjugated to Atg5 by Atg7 and Atg10 (E2). In the absence of active phagocytosis and debate over whether apoptotic-like cell death conservation extends to plants, autophagy may play a large role in plant PCD regulation.

Autophagy and Energy Metabolism

Regulating metabolic and energy homeostasis is a significant challenge for all organisms, including plants, and strong relationships have been observed between nutrient availability, energy status, cell growth, and cell death rates (3). Glycolysis is an essential first step for the breakdown of glucose into available energy for plant metabolism. Recent studies involving *Arabidopsis* glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mutants provide a direct link between GAPDH activity and the regulation or restriction of autophagy. Compared with their wild-type counterparts, *AtGAPHD* mutants demonstrate increased reactive oxygen species (ROS) accumulation, constitutive autophagy, and enhanced pathogen resistance (64, 72). Additional studies have indicated that GAPDH interacts directly with and suppresses the activity of ATG3 (64). This interaction is inhibited by high levels of ROS, demonstrating a possible mechanism by which ROS molecules contribute to autophagy (64, 115). The association of GADPH with both glycolysis and autophagy suggests that GAPDH, via interaction with ATG3, buffers the recycling role of autophagy with the metabolic state of the cell. Further examples demonstrating the tight linkages between autophagy and energy metabolism, particularly sugar signaling, are described below.

Selective Autophagy and Its Use to Regulate Cellular Degradation and Nutrient Recycling

The recent discovery of selective autophagy is important because it identifies the ability of autophagy to remobilize bulk cellular constituents and to selectively target protein complexes and

organelles. It is now understood that autophagy is responsible for the clearing of plant-specific compartments such as chloroplasts via Rubisco-containing bodies and ATG8-INTERACTING PROTEIN 1 (ATI1)–containing plastid bodies (78, 116). The observation of peroxisome clearance during seedling growth in *Arabidopsis* suggests that selective autophagy plays more than a housekeeping role but is also critical for the regulation of proper development and stress tolerance (91). Other forms of selective autophagy include the specific targeting of organelles such as the mitochondria (mitophagy), protein aggregates (aggrephagy), invading pathogens (xenophagy), and chloroplasts (chlorophagy) as well as specific proteins (23, 46).

Resurrection plant: a type of flowering plant that has evolved to tolerate desiccation to levels equivalent to a pressure of −100 MPa in vegetative tissues

The identity of the source of autophagosome membranes remains controversial, but evidence is accumulating that the ER is a prominent source. Studies have demonstrated ER-stress-induced delivery of ER components, including the membrane decorated with ribosomes in plant cells (106). A more fascinating discovery was the observation of 26S proteasome degradation (proterophagy) in nutrient-deprived *Arabidopsis* (109). Further investigation using either knockout mutants or chemical inhibitors of key players of the proteasome degradation pathway demonstrated induction of autophagy (109). These data indicate a potential antagonistic relationship between autophagy and proteasome degradation pathways. Future work should focus on determining whether the cell utilizes this interplay to intimately regulate cellular degradation and recycling pathways to maintain homeostasis.

SWEET DEATH: THE ROLE OF SUGAR METABOLISM IN CELL DEATH DECISIONS

To drive metabolism and growth, plants must photosynthetically convert light into chemical energy (146). This conversion relies on the reception of light energy by photosynthetic machinery as well as gas exchange through leaf stomata. In addition to being the site of gas exchange, stomata are the major sites for leaf water loss, and the water gradient created by open stomata is a significant driver of the transpirational pull that draws water from the soil. Consequently, photosynthesizing plants compromise between energy metabolism and water retention. Prolonged stress often causes decreased photosynthesis and increased ROS accumulation, which can trigger PCD pathways (58). To mitigate stress, plants utilize elaborate antioxidant systems in addition to downregulating chlorophyll synthesis and other components of the photosynthetic pathway (48). To power these mechanisms, the plant must maintain sucrose metabolism to provide reducing power (in the form of NADPH) for the synthesis of nonenzymatic antioxidants, such as ascorbic acid and glutathione (12). If energy metabolism is unable to meet cellular demands, it is logical that autophagy pathways may be triggered to provide both nutrients and energy until photosynthesis can be restored.

Arabidopsis plants infiltrated with 15-mM sucrose solutions display substantial changes in transcriptional activity compared with untreated controls, including expression changes for transcription factors, redox regulators, proteasome-mediated protein degradation, and trehalose metabolism (125). Notably, expression of four trehalose phosphate synthase–encoding genes [*TREHALOSE 6-PHOSPHATASE SYNTHASE S8* (*TPS8*), *TPS9*, *TPS10*, and *TPS11*] and of *ATG8E* was repressed in the presence of sucrose within 30 min; these genes were also suppressed rapidly upon interception of light. Conversely, nutrient deprivation and extended darkness increased expression of the same gene set (169). In combination with studies of the native Australian resurrection plant *Tripogon loliiformis* that also linked trehalose metabolism with autophagy pathways and the shutdown of photosynthesis (discussed below), these findings indicate that small changes in sugar metabolism, particularly trehalose, can result in the induction or suppression of innate cell death pathways (181).

The Influence of Sugars and Cell Cycle Status on Programmed Cell Death Outcomes

Sugar and energy metabolism can play significant roles in controlling cell cycle status, which can also regulate the induction of cell death pathways. Several key observations in mammalian studies have linked the cell cycle and apoptosis, including (*a*) that apoptosis almost always occurs in proliferating cells; (*b*) that the transition from late G1 to S phase is controlled by the oncogene *p53*, among other genes; (*c*) that metabolites present in late G1 phase are required for apoptosis; and (*d*) that retroviral and other artificial manipulations of the cell cycle can either suppress or induce apoptosis (113). Evidence supporting the importance of cell cycle control in plant PCD outcomes was provided by studies conducted by Kadota et al. (86) using synchronized BY-2 tobacco cells, which showed restriction of cell death mediated by the proteinaceous elicitor cryptogein to the G1 and S phases. The detection of cryptogein elicited ROS production in all cell cycle phases, indicating that cryptogein was detected in all cell cycle phases; however, the cell death response was limited to specific phases. Furthermore, there was a clear demarcation in the expression of defense-related genes in the different phases of the cell cycle (86).

Mechanistic target of rapamycin (mTOR): a multidomain serine/threonine protein kinase that forms protein complexes that regulate cellular metabolism; mTOR complexes play roles in cell growth, proliferation, motility, survival, autophagy, protein synthesis, and transcription

The Intimate Relationship Between Energy Status and Programmed Cell Death

Plants must maintain energy stores in different forms to compensate for prolonged periods of inclement environmental conditions. As such, they have evolved effective and proficient means to store resources in the form of proteins, lipids, and starch; efficient mobilization strategies are also required (38). Autophagy is one established method that can play a significant role in the provisioning and recycling of nutrients within a cell. Signaling pathways involving the evolutionarily conserved target of rapamycin (TOR) protein and the energy sensor sucrose nonfermenting 1–related kinase 1 (SnRK1) (4) are key regulators of energy homeostasis and autophagy (**Figure 4**).

TOR promotes both catabolism and anabolism of cellular material as a function of both nitrogen and carbon availability (132). In an antagonistic relationship with SnRK1, TOR regulates nutrient status to drive autophagy responses (38).

Yeast sucrose nonfermenting 1 (SNF1) and mammalian AMP-activated protein kinase (AMPK), which are orthologs of plant SnRK1, play multiple roles in alternative carbon metabolism, including respiration, gluconeogenesis, nutrient transport, and meiosis (71). In mammals, AMPK is activated by stresses that increase the AMP/ATP ratio, suggesting an energy-deprived status, which switches off energy-consuming processes and triggers catabolism; a similar relationship has been observed in plants (66, 111) (**Figure 4**). Rice SnRK1 regulates the activation of the α-amylase gene promoter via MYBS1 (v-myb avian myeloblastosis viral oncogene homolog involved in sugar signaling) (107).

Glucose triggers mechanistic target of rapamycin (mTOR) activity and subsequently glycolysis and raffinose accumulation in response to stress (39) (**Figure 4**). Conditional silencing of TOR led to accelerated senescence; catabolism of chlorophyll; yellowing of leaves; suppression of photosynthesis; and high levels of soluble sugars, amino acids, and starch (33, 132). It has therefore been suggested that TOR is involved in regulating or suppressing senescence and regulating life span. Further evidence linking TOR activity with senescence and energy metabolism was provided by studies in which TOR was inhibited by expression of an artificial microRNA, resulting in increased starch and triacyl glyceride as well as accumulation of tricarboxylic acid cycle intermediates (15).

Relationship Between TOR and SnRK1

A close relationship exists between TOR- and SnRK1-mediated signaling of autophagy pathways (38). In mammals, energy and nutrient deficiency triggers AMPK, which phosphorylates

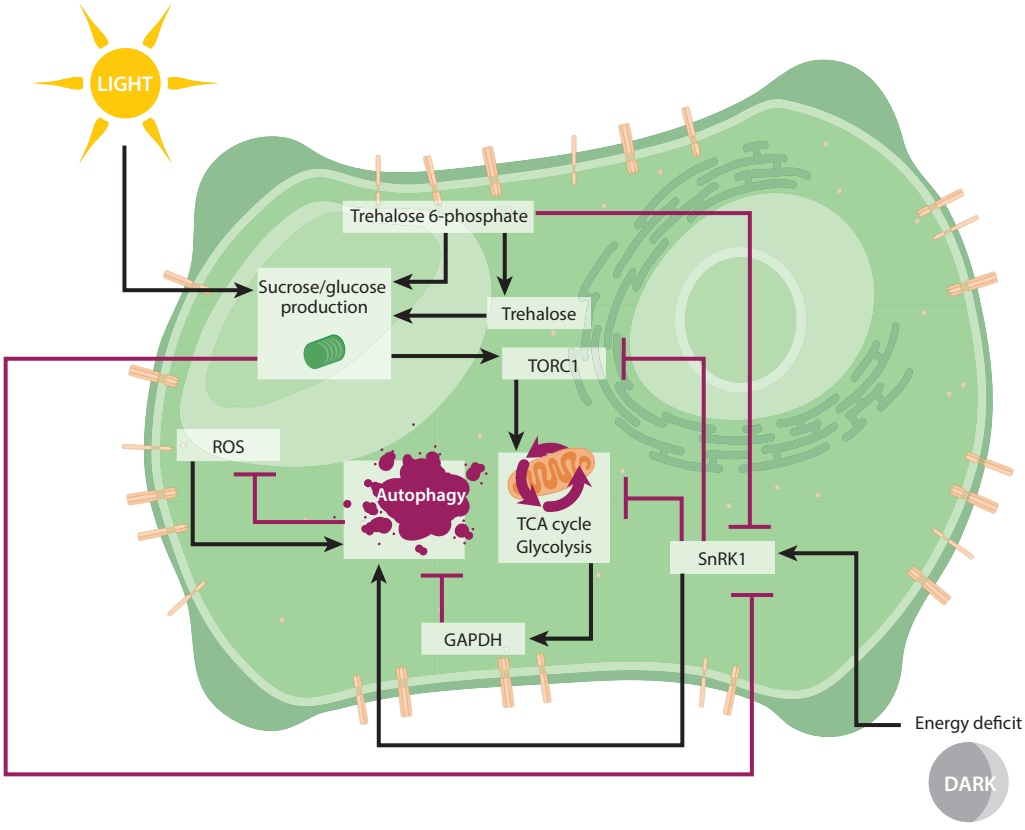

Figure 4

Regulation of energy homeostasis and autophagy through the interplay between TORC1 and SnRK1. Energy deficiency and an increase in the AMP/ATP ratio result in the activation of the SnRK1 pathway. Once activated, SnRK1 in turn activates a range of energy-saving signaling pathways (including autophagy pathways) and suppresses nitrate assimilation, the TCA cycle, and glycolysis. The buildup of the trehalose precursor trehalose 6-phosphate is indicative of energy production and inactivates SnRK1 activity and energy-saving pathways (again including autophagy pathways). The accumulation of sugars represses SnRK1 function and activates TORC1 to trigger energy storage systems and the shutdown of autophagy. Autophagy is induced by the accumulation of trehalose and ROS and suppressed by the accumulation of GAPDH. Autophagy degrades cellular toxins such as misfolded proteins, damaged organelles, and ROS. Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ROS, reactive oxygen species; SnRK1, sucrose nonfermenting 1–related kinase 1; TCA, tricarboxylic acid; TORC1, target of rapamycin complex 1.

ATG1/Unc51-like 1 (ULK1) to initiate autophagy (45, 69, 94). Conversely, the TORC1 complex suppresses autophagy through inhibitory phosphorylation of ATG1/Unc1 and its protein partner, ATG13 (44, 69, 90). Similarly, TORC1 phosphorylates ATG1 in plants; however, the exact mechanistic details of crosstalk between TOR and SnRK1 in autophagy remain to be elucidated (38, 152). Dunlop et al. (43) have suggested that in mammals this crosstalk is based on the potential phosphorylation of regulatory-associated protein of the mammalian target of rapamycin (RAPTOR) by SnRK1. RAPTOR is a well-conserved protein that is orthologous to yeast kontroller of growth 1 (KOG1) and human RAPTOR (40% sequence identity) (16). RAPTOR mutants display phenotypes similar to those observed in TOR knockouts, including reduced growth, delayed flowering, and increased branching (32). Studies have also highlighted plant hormones as significant players in TOR signaling. Auxin activates TOR function, and TOR activity in turn contributes to downstream auxin-signaling-mediated pathway reactions (11, 114, 141).

WHAT CAN EXTREMOPHILES TEACH US ABOUT PLANT CELL DEATH?

As plants became terrestrial and evolved elaborate water transport systems, PCD became a prominent means of maintaining cellular homeostasis (145). Combined with other physiological traits, PCD allowed land plants to promote the survival and demise of select cells, typically in reproductive structures, in order to tolerate adverse environmental conditions, including drying (145). In addition to these traits, a small group of flowering plants termed resurrection plants evolved the capacity to tolerate desiccation to levels equivalent to a pressure of −100 MPa in vegetative tissues (2, 53, 54, 56). Given that these extremophiles, by nature, "rise from the dead," the fate of their tissues and cells provides an interesting foundation for discussion of cell death regulation.

When desiccated, resurrection plants can survive snap freezing in liquid nitrogen or heating for short periods at temperatures above 60°C (55). Additionally, rising global temperatures and subsequent melting of polar ice caps has resulted in the "resurrection" of plants previously frozen for hundreds of years (99). How does this group of angiosperms control innate PCD pathways to survive 95% water loss when the majority of crop plants die after 40% water loss? A key trait of resurrection plants is the revitalization of preexisting tissues without the requirement for new growth (181). Recent studies have suggested that at least some resurrection plants maintain cellular vitality in desiccated vegetative tissue by tightly regulating the interplay of PCD pathways and autophagy with energy metabolism during the drying process (63, 181).

Regulatory-associated protein of the mammalian target of rapamycin (RAPTOR): an mTOR-binding adaptor protein that helps regulate energy metabolism in response to nutrient levels as part of mTOR complex signaling pathways

How Do Resurrection Plants Maintain Cell Vitality?

Resurrection plants are primed for desiccation tolerance in the hydrated state and can slow down the drying process through reduced numbers of stomata on the abaxial surface, thick epicuticular waxes, the presence of large bulliform cells that can act as water reservoirs, and the vitrification and protection of proteins by the accumulation of sugars (27, 55, 123–125, 128, 134, 189, 190). Although these traits are not directly related to cell death pathways, they can play an important role in the regulation of PCD processes (77, 157). Desiccation-sensitive and desiccation-tolerant plants elicit starkly different responses to water deprivation that could result in distinct outcomes in terms of the induction of cell death programs (123, 189, 190). During dehydration, sensitive plants appear to utilize water reserves inefficiently by continuing to photosynthesize well into the dehydrated state, where they lose the ability to tightly regulate water loss with their metabolism (123). Tolerant plants, by contrast, rapidly respond to dehydration by closing their stomata and shutting down photosynthesis at a high relative water content (89, 177, 178). Stomatal closure and the shutdown of photosynthesis have profound effects on plant metabolism and cell death regulation. Although stomatal closure reduces carbon fixation, chlorophyll continues to absorb light, causing photoexcitation and electron transfer to ground-state oxygen ($^3O_2$), which is then converted to singlet oxygen (O_2^-), a toxic ROS molecule that induces PCD pathways (112). In addition to increased ROS production, the inability to convert light into chemical energy predisposes the plant to caloric deficiency, a known trigger of autophagy (65, 118). As water loss ensues, sensitive plants display drought-induced senescence to minimize water loss by reducing canopy surface area (19, 121). Tolerant plants, by contrast, control water loss and energy metabolism through nutrient recycling, potentially via autophagy, and so prevent the induction of senescence-associated cell death (63, 181).

Supporting this hypothesis, *Arabidopsis* plants grown in low light undergo autophagy-dependent caloric restriction and delayed senescence (118). Furthermore, *Arabidopsis* mutants with dysfunctional autophagy pathways display increased sensitivity to nutrient deprivation and accelerated

aging (65). In the absence of autophagy-mediated nutrient recycling, cellular resources are rapidly depleted, resulting in senescence-induced cell death. Similarly, studies in *Saccharomyces cerevisiae*, which is also desiccation tolerant, have shown that methionine deficiency triggers autophagy, vacuolar acidification, and increased longevity (139). In desiccation-tolerant plants, autophagy may facilitate cellular survival in at least two ways: the removal of damaged or unwanted cellular components that induce apoptotic-like signaling pathways, and the maintenance of energy homeostasis during caloric or nitrogen starvation. Transcriptome studies of *T. loliiformis* during dehydration and desiccation showed significantly increased and decreased accumulation of transcripts associated with autophagy and apoptosis, respectively (181). Additionally, transcripts associated with senescence were reduced during drying compared with hydrated controls (181). These results are consistent with studies that demonstrated increased expression of cysteine protease inhibitors (phytocystatins) in the resurrection plant *Sporobolus stapfianus* during dehydration and desiccation (10). It is plausible that at least some resurrection plants manipulate autophagy to suppress drought-induced senescence and apoptotic-like cell death to survive desiccation.

Once Activated, How Do Extremophiles Regulate Autophagy Pathways?

Autophagy pathways are triggered in both desiccation-sensitive and desiccation-tolerant species, which suggests that the induction of autophagy alone is insufficient to provide desiccation tolerance. Resurrection plants must therefore utilize strategies to regulate autophagy during desiccation. Trehalose is a nonreducing disaccharide and chemical chaperone that prevents denaturation and aggregation of proteins in bacteria, yeast, and insects (46). In addition to its role as a chemical chaperone, trehalose can induce autophagy, suppress apoptosis, and protect against Bax-induced cell death in human neuroblastoma cells (140). Trehalose does not accumulate in most land plants but has been detected in several resurrection plants, albeit not to levels sufficient to function as an osmoprotectant or energy source (80). Notably, the addition of trehalose prolongs the vase life of gladiolus flowers, and treated flowers exhibit decreased protein degradation, higher membrane integrity, and higher relative water content (185). Despite these observations, the precise molecular mechanisms of trehalose in desiccation tolerance were unknown until recently, when Williams et al. (181) showed that it triggers autophagy in *T. loliiformis*. Additionally, resurrection plants encode an effective suite of ROS scavenging systems that remain active even in desiccated tissue. ROS molecules are potent inducers of PCD, including autophagy, and the increased ability of resurrection plants to mop up erroneous ROS could enable the plants to survive in stressful conditions.

It is apparent that resurrection plants, and possibly other extremophiles, use diverse strategies to tolerate desiccation, including the induction of autophagy. Thus, the investigation of cell death control in extremophiles provides significant opportunities for molecular dissection of the players that regulate cell death programs in plants and should provide interesting information in the future. Future work should focus on whether resurrection plants tolerate stress indirectly by regulating PCD pathways via efficient management of energy homeostasis or directly through unknown mechanisms.

PERSPECTIVE

Counterintuitively, PCD is of paramount importance to the well-being of all multicellular organisms; it is used to regulate development and aging, mitigate abiotic and biotic stresses, and maintain homeostasis (182). As is the case with innumerable fundamental cellular processes, PCD spans broad evolutionary distances, but in the absence of sequence similarity for canonical PCD players—particularly those involved in apoptosis—PCD in plants remains controversial (170, 171).

In particular, a failure to detect key players of mammalian apoptosis pathways such as caspases and Bcl-2 family members at the sequence level and the absence of phagocytic removal of apoptotic cells in plants have raised doubts as to whether PCD conservation extends to apoptotic-like cell death in plants (170, 171). Much of the controversy stems from the original classifications of cell death, the biochemical players identified in animals, and an inability to identify core inducers and inhibitors of PCD in plants.

That said, strictly speaking, apoptosis is defined by the detection of a suite of morphological traits that cells undergo during death. As discussed throughout this review, many of these hallmark traits (including plasma membrane retraction and blebbing and nuclear pyknosis) are also present in plant cells that are experiencing attrition induced by developmental or pathobiological demands or constraints, but which, it should be noted, are held within the confines of a rigid cell wall in a system that is not patrolled by phagocytic cells. Additionally, exogenous expression of animal apoptotic players such as *Bcl-2* and *IAP* genes regulates plant PCD outcomes. Metacaspases, VPEs, and subtilases are promising candidates for proteases that are the ideological equivalents of caspases. At the very least, there may be a case to relax the requirements that designate apoptosis in plants. We agree that, as proposed by Reape & McCabe (131), the term apoptotic-like PCD is appropriate when describing plant cell death events until the biochemical context of plant PCD has been sufficiently addressed. The identification of clear regulatory and protease elements involved in plant PCD presents unique and exciting opportunities for plant biologists to understand how plant cells achieve or are forced into cellular demise.

SUMMARY POINTS

1. As it is in all eukaryotes, the appropriate control of programmed cell death (PCD) is important for proper plant development and responses to abiotic and biotic stresses. Despite broad transkingdom conservation of PCD pathways, it is apparent that plants and animals execute PCD differently. Despite the absence of core mammalian PCD regulators, plants display many of the morphological traits associated with PCD in animals.
2. Although obvious caspase homologs are not encoded in plant genomes, plants undergoing PCD require proteases such as metacaspases, vacuolar processing enzymes (VPEs), and subtilases. These proteases share varying degrees of similarity with caspases, such as their domain architecture and cleavage-site recognition, but look very different at the primary amino acid level. Future research efforts focused on identifying the substrates of these proteases will reveal more about the molecular regulators of PCD in plants.
3. Inhibitor of apoptosis (IAP) proteins are negative regulators of caspase activity that are also not encoded in plant genomes. Interestingly, the ectopic expression of IAPs in tomato and tobacco suppresses apoptotic-like PCD in these plants in response to several abiotic and biotic stresses. The identification of IAP partners in plants could reveal novel regulators of plant PCD.
4. The discovery of the Bcl-2-associated athanogene (BAG) family of co-chaperones in plants suggests that, despite the absence of core apoptotic regulators, some aspects of cell death regulation are conserved between animals and plants. Plant BAGs play cytoprotective roles, particularly in response to stress and developmental cues. The varied subcellular localization of the plant BAGs is not observed in animals and may indicate the evolution of distinct plant BAG functions.

5. PCD pathways are tightly aligned with cellular energy status and the cell cycle. The manner in which organisms control their energy metabolism and the stage of the cell cycle when stress occurs may dictate PCD outcomes.
6. We propose that the term apoptotic-like PCD is appropriate when describing plant cell death that displays animal apoptotic features until the biochemical context of plant PCD has been sufficiently addressed.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGEMENTS

We thank Aislinn Bartholomay for generating the graphics in this review and Professors Caitilyn Allen and Andrew Bent for stimulating discussions and valuable comments. M.K. is supported by the Wisconsin Soybean Marketing Board (grant 193446) and the North Central Soybean Research Program (grant 187452). B.W. is supported by a Queensland University of Technology Vice Chancellor's Research Fellowship. R.K. is supported by National Institutes of Health National Research Service Award T32 GM007215.

LITERATURE CITED

1. Alberti S, Demand J, Esser C, Emmerich N, Schild H, Hohfeld J. 2002. Ubiquitylation of BAG-1 suggests a novel regulatory mechanism during the sorting of chaperone substrates to the proteasome. *J. Biol. Chem.* 277:45920–27
2. Alpert P. 2005. The limits and frontiers of desiccation-tolerant life. *Integr. Comp. Biol.* 45:685–95
3. Arndt V, Daniel C, Nastainczyk W, Alberti S, Hohfeld J. 2005. BAG-2 acts as an inhibitor of the chaperone-associated ubiquitin ligase CHIP. *Mol. Biol. Cell* 16:5891–900
4. Baena-González E, Sheen J. 2008. Convergent energy and stress signaling. *Trends Plant Sci.* 13:474–82
5. Bao Q, Shi Y. 2007. Apoptosome: a platform for the activation of initiator caspases. *Cell Death Differ.* 14:56–65
6. Bashir Z, Ahmad A, Shafique S, Anjum T, Shafique S, Akram W. 2013. Hypersensitive response—a biophysical phenomenon of producers. *Eur. J. Microbiol. Immunol.* 3:105–10
7. Bassham DC, Laporte M, Marty F, Moriyasu Y, Ohsumi Y, et al. 2006. Autophagy in development and stress responses of plants. *Autophagy* 2:2–11
8. Behl C. 2016. Breaking BAG: the co-chaperone BAG3 in health and disease. *Trends Pharmacol. Sci.* 37:672–88
9. Beilina A, Rudenko IN, Kaganovich A, Civiero L, Chau H, et al. 2014. Unbiased screen for interactors of leucine-rich repeat kinase 2 supports a common pathway for sporadic and familial Parkinson disease. *PNAS* 111:2626–31
10. Blomstedt CK, Griffiths CA, Fredericks DP, Hamill JD, Gaff DF, Neale AD. 2010. The resurrection plant *Sporobolus stapfianus*: an unlikely model for engineering enhanced plant biomass? *Plant Growth Regul.* 62:217–32
11. Bögre L, Henriques R, Magyar Z. 2013. TOR tour to auxin. *EMBO J.* 32:1069–71
12. Bolouri-Moghaddam MR, Le Roy K, Xiang L, Rolland F, Van den Ende W. 2010. Sugar signalling and antioxidant network connections in plant cells. *FEBS J.* 277:2022–37

13. Brive L, Takayama S, Briknarova K, Homma S, Ishida SK, et al. 2001. The carboxyl-terminal lobe of Hsc70 ATPase domain is sufficient for binding to BAG1. *Biochem. Biophys. Res. Commun.* 289:1099–105
14. Burstein E, Ganesh L, Dick RD, van De Sluis B, Wilkinson JC, et al. 2004. A novel role for XIAP in copper homeostasis through regulation of MURR1. *EMBO J.* 23:244–54
15. Caldana C, Li Y, Leisse A, Zhang Y, Bartholomaeus L, et al. 2013. Systemic analysis of inducible target of rapamycin mutants reveal a general metabolic switch controlling growth in *Arabidopsis thaliana*. *Plant J.* 73:897–909
16. Cameron P, Limjuco G, Rodkey J, Bennett C, Schmidt JA. 1985. Amino acid sequence analysis of human interleukin 1 (IL-1). Evidence for biochemically distinct forms of IL-1. *J. Exp. Med.* 162:790–801
17. Cerio RJ, Vandergaast R, Friesen PD. 2010. Host insect inhibitor-of-apoptosis SfIAP functionally replaces baculovirus IAP but is differentially regulated by its N-terminal leader. *J. Virol.* 84:11448–60
18. Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Ness KV, et al. 1992. Molecular cloning of the interleukin-1β converting enzyme. *Science* 256:97–100
19. Chaves MM, Maroco JP, Pereira JS. 2003. Understanding plant responses to drought—from genes to the whole plant. *Funct. Plant Biol.* 30:239–64
20. Chen S, Dickman MB. 2004. Bcl-2 family members localize to tobacco chloroplasts and inhibit programmed cell death induced by chloroplast-targeted herbicides. *J. Exp. Bot.* 55:2617–23
21. Chichkova NV, Shaw J, Galiullina RA, Drury GE, Tuzhikov AI, et al. 2010. Phytaspase, a relocalisable cell death promoting plant protease with caspase specificity. *EMBO J.* 29:1149–61
22. Coffeen WC, Wolpert TJ. 2004. Purification and characterization of serine proteases that exhibit caspase-like activity and are associated with programmed cell death in Avena sativa. *Plant Cell* 16:857–73
23. Coll NS, Vercammen D, Smidler A, Clover C, Van Breusegem F, et al. 2010. *Arabidopsis* type I metacaspases control cell death. *Science* 330:1393–97
24. Crook NE, Clem RJ, Miller LK. 1993. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. *J. Virol.* 67:2168–74
25. Cui H, Tsuda K, Parker JE. 2015. Effector-triggered immunity: from pathogen perception to robust defense. *Annu. Rev. Plant Biol.* 66:487–511
26. Curtis MJ, Wolpert TJ. 2004. The victorin-induced mitochondrial permeability transition precedes cell shrinkage and biochemical markers of cell death, and shrinkage occurs without loss of membrane integrity. *Plant J.* 38:244–59
27. Cushman JC, Oliver MJ. 2011. Understanding vegetative desiccation tolerance using integrated functional genomics approaches within a comparative evolutionary framework. In *Plant Desiccation Tolerance*, ed. U Lüttge, E Beck, D Bartels, pp. 307–38. Ecol. Stud. 215. Berlin: Springer
28. Dai Q, Qian SB, Li HH, McDonough H, Borchers C, et al. 2005. Regulation of the cytoplasmic quality control protein degradation pathway by BAG2. *J. Biol. Chem.* 280:38673–81
29. Das G, Shravage BV, Baehrecke EH. 2012. Regulation and function of autophagy during cell survival and cell death. *Cold Spring Harb. Perspect. Biol.* 4:a008813
30. del Pozo O, Lam E. 1998. Caspases and programmed cell death in the hypersensitive response of plants to pathogens. *Curr. Biol.* 8:1129–32
31. Demand J, Alberti S, Patterson C, Hohfeld J. 2001. Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. *Curr. Biol.* 11:1569–77
32. Deprost D, Truong HN, Robaglia C, Meyer C. 2005. An *Arabidopsis* homolog of RAPTOR/KOG1 is essential for early embryo development. *Biochem. Biophys. Res. Commun.* 326:844–50
33. Deprost D, Yao L, Sormani R, Moreau M, Leterreux G, et al. 2007. The *Arabidopsis* TOR kinase links plant growth, yield, stress resistance and mRNA translation. *EMBO Rep.* 8:864–70
34. Desmots F, Russell HR, Lee Y, Boyd K, McKinnon PJ. 2005. The reaper-binding protein scythe modulates apoptosis and proliferation during mammalian development. *Mol. Cell. Biol.* 25:10329–37
35. Desmots F, Russell HR, Michel D, McKinnon PJ. 2008. Scythe regulates apoptosis-inducing factor stability during endoplasmic reticulum stress-induced apoptosis. *J. Biol. Chem.* 283:3264–71
36. Dickman MB, Fluhr R. 2013. Centrality of host cell death in plant-microbe interactions. *Annu. Rev. Phytopathol.* 51:543–70
37. Dickman MB, Park YK, Oltersdorf T, Li W, Clemente T, French R. 2001. Abrogation of disease development in plants expressing animal antiapoptotic genes. *PNAS* 98:6957–62

38. Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincentz M, et al. 2016. TOR signaling and nutrient sensing. *Annu. Rev. Plant Biol.* 67:261–85
39. Dobrenel T, Marchive C, Azzopardi M, Clement G, Moreau M, et al. 2013. Sugar metabolism and the plant target of rapamycin kinase: a sweet operaTOR? *Front. Plant Sci.* 4:93
40. Doukhanina EV, Chen S, van der Zalm E, Godzik A, Reed J, Dickman MB. 2006. Identification and functional characterization of the BAG protein family in *Arabidopsis thaliana*. *J. Biol. Chem.* 281:18793–801
41. Du C, Fang M, Li Y, Li L, Wang X. 2000. Smac, a mitochondrial protein that promotes cytochrome *c*–dependent caspase activation by eliminating IAP inhibition. *Cell* 102:33–42
42. Duckett CS, Nava VE, Gedrich RW, Clem RJ, Van Dongen JL, et al. 1996. A conserved family of cellular genes related to the baculovirus *iap* gene and encoding apoptosis inhibitors. *EMBO J.* 15:2685–94
43. Dunlop EA, Hunt DK, Acosta-Jaquez HA, Fingar DC, Tee AR. 2011. ULK1 inhibits mTORC1 signaling, promotes multisite Raptor phosphorylation and hinders substrate binding. *Autophagy* 7:737–47
44. Dunlop EA, Tee AR. 2014. mTOR and autophagy: a dynamic relationship governed by nutrients and energy. *Semin. Cell Dev. Biol.* 36:121–29
45. Egan D, Kim J, Shaw RJ, Guan KL. 2011. The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. *Autophagy* 7:643–44
46. Elbein AD, Pan YT, Pastuszak I, Carroll D. 2003. New insights on trehalose: a multifunctional molecule. *Glycobiology* 13:17R–27R
47. Elmore S. 2007. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35:495–516
48. Farrant PB, Emerson R. 2007. Hyfrecation and curettage as a treatment for fibrofolliculomas in Birt-Hogg-Dube syndrome. *Dermatol. Surg.* 33:1287–88
49. Favaloro B, Allocati N, Graziano V, Di Ilio C, De Laurenzi V. 2012. Role of apoptosis in disease. *Aging* 4:330–49
50. Fernández MB, Daleo GR, Guevara MG. 2015. Isolation and characterization of a Solanum tuberosum subtilisin-like protein with caspase-3 activity (StSBTc-3). *Plant Physiol. Biochem.* 86:137–46
51. Friesen TL, Zhang Z, Solomon PS, Oliver RP, Faris JD. 2008. Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. *Plant Physiol.* 146:682–93
52. Froesch BA, Takayama S, Reed JC. 1998. BAG-1L protein enhances androgen receptor function. *J. Biol. Chem.* 273:11660–66
53. Gaff DF. 1971. Desiccation-tolerant flowering plants in southern Africa. *Science* 174:1033–34
54. Gaff DF. 1999. Resurrection plants. In *Plants in Action*, ed. BJ Atwell, PE Kriedemann, CGN Turnbull, feature essay 15.1. Melbourne: Macmillan Educ. Aust. **http://plantsinaction.science.uq.edu.au/edition1/?q=content/feature-essay-15-1-resurrection-plants**
55. Gaff DF, Bartels D, Gaff JL. 1997. Changes in gene expression during drying in a desiccation-tolerant grass *Sporobolus stapfianus* and a desiccation-sensitive grass *Sporobolus pyramidalis*. *Aust. J. Plant Physiol.* 24:617–22
56. Gaff DF, Latz PK. 1978. The occurrence of resurrection plants in the Australian flora. *Aust. J. Bot.* 26:485–92
57. Gamerdinger M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, Behl C. 2009. Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *EMBO J.* 28:889–901
58. Gechev TS, Dinakar C, Benina M, Toneva V, Bartels D. 2012. Molecular mechanisms of desiccation tolerance in resurrection plants. *Cell. Mol. Life Sci.* 69:3175–86
59. Gehrmann M, Marienhagen J, Eichholtz-Wirth H, Fritz E, Ellwart J, et al. 2005. Dual function of membrane-bound heat shock protein 70 (Hsp70), Bag-4, and Hsp40: protection against radiation-induced effects and target structure for natural killer cells. *Cell Death Differ.* 12:38–51
60. Gilbert BM, Wolpert TJ. 2013. Characterization of the *LOV1*-mediated, victorin-induced, cell-death response with virus-induced gene silencing. *Mol. Plant-Microbe Interact.* 26:903–17
61. Gilchrist DG. 1997. Mycotoxins reveal connections between plants and animals in apoptosis and ceramide signaling. *Cell Death Differ.* 4:689–98
62. Gohre V, Robatzek S. 2008. Breaking the barriers: microbial effector molecules subvert plant immunity. *Annu. Rev. Phytopathol.* 46:189–215

63. Griffiths CA, Gaff DF, Neale AD. 2014. Drying without senescence in resurrection plants. *Front. Plant Sci.* 5:36
64. Han S, Wang Y, Zheng X, Jia Q, Zhao J, et al. 2015. Cytoplastic glyceraldehyde-3-phosphate dehydrogenases interact with ATG3 to negatively regulate autophagy and immunity in *Nicotiana benthamiana*. 27:1316–31
65. Hanaoka H, Noda T, Shirano Y, Kato T, Hayashi H, et al. 2002. Leaf senescence and starvation-induced chlorosis are accelerated by the disruption of an Arabidopsis autophagy gene. *Plant Physiol.* 129:1181–93
66. Hardie DG. 2007. AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy. *Nat. Rev. Mol. Cell Biol.* 8:774–85
67. Hatsugai N, Iwasaki S, Tamura K, Kondo M, Fuji K, et al. 2009. A novel membrane fusion-mediated plant immunity against bacterial pathogens. *Genes Dev.* 23:2496–506
68. Hatsugai N, Kuroyanagi M, Yamada K, Meshi T, Tsuda S, et al. 2004. A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. *Science* 305:855–58
69. He C, Klionsky DJ. 2009. Regulation mechanisms and signaling pathways of autophagy. *Annu. Rev. Genet.* 43:67–93
70. Heath MC. 2000. Hypersensitive response-related death. *Plant Mol. Biol.* 44:321–34
71. Hedbacker K, Carlson M. 2008. SNF1/AMPK pathways in yeast. *Front. Biosci.* 13:2408–20
72. Henry E, Fung N, Liu J, Drakakaki G, Coaker G. 2015. Beyond glycolysis: GAPDHs are multifunctional enzymes involved in regulation of ROS, autophagy, and plant immune responses. *PLOS Genet.* 11:e1005199
73. Hiraiwa N, Nishimura M, Hara-Nishimura I. 1999. Vacuolar processing enzyme is self-catalytically activated by sequential removal of the C-terminal and N-terminal propeptides. *FEBS Lett.* 447:213–16
74. Hoang TML, Moghaddam L, Williams B, Khanna H, Dale J, Mundree SG. 2015. Development of salinity tolerance in rice by constitutive-overexpression of genes involved in the regulation of programmed cell death. *Front. Plant Sci.* 6:175
75. Hoang TML, Williams B, Khanna H, Dale J, Mundree SG. 2014. Physiological basis of salt stress tolerance in rice expressing the antiapoptotic gene *SfIAP*. *Funct. Plant Biol.* 41:1168–77
76. Hofius D, Munch D, Bressendorff S, Mundy J, Petersen M. 2011. Role of autophagy in disease resistance and hypersensitive response-associated cell death. *Cell Death Differ.* 18:1257–62
77. Ingram J, Bartels D. 1996. The molecular basis of dehydration tolerance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:377–403
78. Ishida H, Yoshimoto K, Izumi M, Reisen D, Yano Y, et al. 2008. Mobilization of Rubisco and stroma-localized fluorescent proteins of chloroplasts to the vacuole by an *ATG* gene-dependent autophagic process. *Plant Physiol.* 148:142–55
79. Itakura E, Kishi C, Inoue K, Mizushima N. 2008. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol. Biol. Cell* 19:5360–72
80. Iwaya-Inoue M, Nonami H. 2003. Effects of trehalose on flower senescence from the view point of physical states of water. *Environ. Control Biol.* 41:3–15
81. Jiang Y, Woronicz JD, Liu W, Goeddel DV. 1999. Prevention of constitutive TNF receptor 1 signaling by silencer of death domains. *Science* 283:543–46
82. Kabbage M, Dickman MB. 2008. The BAG proteins: a ubiquitous family of chaperone regulators. *Cell. Mol. Life Sci.* 65:1390–402
83. Kabbage M, Li W, Chen S, Dickman MB. 2010. The E3 ubiquitin ligase activity of an insect anti-apoptotic gene (SfIAP) is required for plant stress tolerance. *Physiol. Mol. Plant Pathol.* 74:351–62
84. Kabbage M, Williams B, Dickman MB. 2013. Cell death control: the interplay of apoptosis and autophagy in the pathogenicity of *Sclerotinia sclerotiorum*. *PLOS Pathog.* 9:e1003287
85. Kacprzyk J, Daly CT, McCabe PF. 2011. The botanical dance of death: programmed cell death in plants. In *Advances in Botanical Research*, Vol. 60, ed. J-C Kader, M Delsney, pp. 168–261. London: Academic
86. Kadota Y, Watanabe T, Fujii S, Higashi K, Sano T, et al. 2004. Crosstalk between elicitor-induced cell death and cell cycle regulation in tobacco BY-2 cells. *Plant J.* 40:131–42
87. Kalia SK, Lee S, Smith PD, Liu L, Crocker SJ, et al. 2004. BAG5 inhibits parkin and enhances dopaminergic neuron degeneration. *Neuron* 44:931–45

88. Kang CH, Jung WY, Kang YH, Kim JY, Kim DG, et al. 2006. AtBAG6, a novel calmodulin-binding protein, induces programmed cell death in yeast and plants. *Cell Death Differ*. 13:84–95
89. Karbaschi MR, Williams B, Taji A, Mundree SG. 2016. *Tripogon loliiformis* elicits a rapid physiological and structural response to dehydration for desiccation tolerance. *Funct. Plant Biol.* 43:643–55
90. Kim J, Kundu M, Viollet B, Guan KL. 2011. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* 13:132–41
91. Kim J, Lee H, Lee HN, Kim SH, Shin KD, Chung T. 2013. Autophagy-related proteins are required for degradation of peroxisomes in *Arabidopsis* hypocotyls during seedling growth. *Plant Cell* 25:4956–66
92. Kim KS, Min JY, Dickman MB. 2008. Oxalic acid is an elicitor of plant programmed cell death during *Sclerotinia sclerotiorum* disease development. *Mol. Plant-Microbe Interact.* 21:605–12
93. Kim SM, Bae C, Oh SK, Choi D. 2013. A pepper (*Capsicum annuum* L.) metacaspase 9 (*Camc9*) plays a role in pathogen-induced cell death in plants. *Mol. Plant Pathol.* 14:557–66
94. Kim WY, Lee SY, Jung YJ, Chae HB, Nawkar GM, et al. 2011. Inhibitor of apoptosis (IAP)-like protein lacks a baculovirus IAP repeat (BIR) domain and attenuates cell death in plant and animal systems. *J. Biol. Chem.* 286:42670–78
95. Knapp RT, Wong MJ, Kollmannsberger LK, Gassen NC, Kretzschmar A, et al. 2014. Hsp70 cochaperones HspBP1 and BAG-1M differentially regulate steroid hormone receptor function. *PLOS ONE* 9:e85415
96. Kullmann M, Schneikert J, Moll J, Heck S, Zeiner M, et al. 1998. RAP46 is a negative regulator of glucocorticoid receptor action and hormone-induced apoptosis. *J. Biol. Chem.* 273:14620–25
97. Kumar S. 2007. Caspase function in programmed cell death. *Cell Death Differ.* 14:32–43
98. Kuroyanagi M, Yamada K, Hatsugai N, Kondo M, Nishimura M, Hara-Nishimura I. 2005. Vacuolar processing enzyme is essential for mycotoxin-induced cell death in *Arabidopsis thaliana*. *J. Biol. Chem.* 280:32914–20
99. La Farge C, Williams KH, England JH. 2013. Regeneration of Little Ice Age bryophytes emerging from a polar glacier with implications of totipotency in extreme environments. *PNAS* 110:9839–44
100. Li F, Vierstra RD. 2012. Autophagy: a multifaceted intracellular system for bulk and selective recycling. *Trends Plant Sci.* 17:526–37
101. Li L, Xing Y, Chang D, Fang S, Cui B, et al. 2016. CaM/BAG5/Hsc70 signaling complex dynamically regulates leaf senescence. *Sci. Rep.* 6:31889
102. Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, et al. 1997. Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91:479–89
103. Li W, Kabbage M, Dickman MB. 2010. Transgenic expression of an insect inhibitor of apoptosis gene, SfIAP, confers abiotic and biotic stress tolerance and delays tomato fruit ripening. *Physiol. Mol. Plant Pathol.* 74:363–75
104. Li Y, Kabbage M, Liu W, Dickman MB. 2016. Aspartyl protease-mediated cleavage of BAG6 is necessary for autophagy and fungal resistance in plants. *Plant Cell* 28:233–47
105. Liu R, Takayama S, Zheng Y, Froesch B, Chen GQ, et al. 1998. Interaction of BAG-1 with retinoic acid receptor and its inhibition of retinoic acid-induced apoptosis in cancer cells. *J. Biol. Chem.* 273:16985–92
106. Liu Y, Bassham DC. 2012. Autophagy: pathways for self-eating in plant cells. *Annu. Rev. Plant Biol.* 63:215–37
107. Lu CA, Lin CC, Lee KW, Chen JL, Huang LF, et al. 2007. The SnRK1A protein kinase plays a key role in sugar signaling during germination and seedling growth of rice. *Plant Cell* 19:2484–99
108. Lu H, Chandrasekar B, Oeljeklaus J, Misas-Villamil JC, Wang Z, et al. 2015. Subfamily-specific fluorescent probes for cysteine proteases display dynamic protease activities during seed germination. *Plant Physiol.* 168:1462–75
109. Marshall RS, Li F, Gemperline DC, Book AJ, Vierstra RD. 2015. Autophagic degradation of the 26S proteasome is mediated by the dual ATG8/ubiquitin receptor RPN10 in *Arabidopsis*. *Mol. Cell* 58:1053–66
110. Matsuzawa S, Takayama S, Froesch BA, Zapata JM, Reed JC. 1998. p53-inducible human homologue of *Drosophila seven in absentia* (Siah) inhibits cell growth: suppression by BAG-1. *EMBO J.* 17:2736–47
111. McGee SL, Hargreaves M. 2008. AMPK and transcriptional regulation. *Front. Biosci.* 13:3022–33
112. McKersie BD, Lesheim Y. 2013. *Stress and Stress Coping in Cultivated Plants*. Dordrecht, Neth.: Springer

113. Meikrantz W, Schlegel R. 1995. Apoptosis and the cell cycle. *J. Cell Biochem.* 58:160–74
114. Menand B, Meyer C, Robaglia C. 2004. Plant growth and the TOR pathway. *Curr. Top. Microbiol. Immunol.* 279:97–113
115. Michaeli S, Galili G, Genschik P, Fernie AR, Avin-Wittenberg T. 2016. Autophagy in plants—what's new on the menu? *Trends Plant Sci.* 21:134–44
116. Michaeli S, Honig A, Levanony H, Peled-Zehavi H, Galili G. 2014. *Arabidopsis* ATG8-INTERACTING PROTEIN1 is involved in autophagy-dependent vesicular trafficking of plastid proteins to the vacuole. *Plant Cell* 26:4084–101
117. Mills K, Daish T, Harvey KF, Pfleger CM, Hariharan IK, Kumar S. 2006. The *Drosophila melanogaster* Apaf-1 homologue ARK is required for most, but not all, programmed cell death. *J. Cell Biol.* 172:809–15
118. Minina EA, Sanchez-Vera V, Moschou PN, Suarez MF, Sundberg E, et al. 2013. Autophagy mediates caloric restriction-induced lifespan extension in *Arabidopsis*. *Aging Cell* 12:327–29
119. Mock JY, Chartron JW, Zaslaver M, Xu Y, Ye Y, Clemons WM Jr. 2015. Bag6 complex contains a minimal tail-anchor-targeting module and a mock BAG domain. *PNAS* 112:106–11
120. Mufti AR, Burstein E, Csomos RA, Graf PC, Wilkinson JC, et al. 2006. XIAP is a copper binding protein deregulated in Wilson's disease and other copper toxicosis disorders. *Mol. Cell* 21:775–85
121. Munné-Bosch S, Alegre L. 2004. Die and let live: leaf senescence contributes to plant survival under drought stress. *Funct. Plant Biol.* 31:203–16
122. Nakaune S, Yamada K, Kondo M, Kato T, Tabata S, et al. 2005. A vacuolar processing enzyme, δVPE, is involved in seed coat formation at the early stage of seed development. *Plant Cell* 17:876–87
123. Oliver MJ, Guo L, Alexander DC, Ryals JA, Wone BW, Cushman JC. 2011. A sister group contrast using untargeted global metabolomic analysis delineates the biochemical regulation underlying desiccation tolerance in *Sporobolus stapfianus*. *Plant Cell* 23:1231–48
124. Oliver MJ, Woods AJ, O'Mahoney P. 1998. "To dryness and beyond"—preparation for the dried state and rehydration in vegetative desiccation-tolerant plants. *Plant Growth Regul.* 24:193–201
125. Osuna D, Usadel B, Morcuende R, Gibon Y, Blasing OE, et al. 2007. Temporal responses of transcripts, enzyme activities and metabolites after adding sucrose to carbon-deprived Arabidopsis seedlings. *Plant J.* 49:463–91
126. Padmanabhan MS, Ma S, Burch-Smith TM, Czymmek K, Huijser P, Dinesh-Kumar SP. 2013. Novel positive regulatory role for the SPL6 transcription factor in the N TIR-NB-LRR receptor-mediated plant innate immunity. *PLOS Pathog.* 9:e1003235
127. Parrish AB, Freel CD, Kornbluth S. 2013. Cellular mechanisms controlling caspase activation and function. *Cold Spring Harb. Perspect. Biol.* 5:a008672
128. Peters S, Mundree SG, Thomson JA, Farrant JM, Keller F. 2007. Protection mechanisms in the resurrection plant *Xerophyta viscosa* (Baker): both sucrose and raffinose family oligosaccharides (RFOs) accumulate in leaves in response to water deficit. *J. Exp. Bot.* 58:1947–56
129. Preston JC, Hileman LC. 2013. Functional evolution in the plant *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE* (*SPL*) gene family. *Front. Plant Sci.* 4:80
130. Reape TJ, McCabe PF. 2008. Apoptotic-like programmed cell death in plants. *New Phytol.* 180:13–26
131. Reape TJ, McCabe PF. 2013. Commentary: the cellular condensation of dying plant cells: programmed retraction or necrotic collapse? *Plant Sci.* 207:135–39
132. Ren M, Venglat P, Qiu S, Feng L, Cao Y, et al. 2012. Target of rapamycin signaling regulates metabolism, growth, and life span in *Arabidopsis*. *Plant Cell* 24:4850–74
133. Riedl SJ, Shi Y. 2004. Molecular mechanisms of caspase regulation during apoptosis. *Nat. Rev. Mol. Cell Biol.* 5:897–907
134. Rodriguez MC, Edsgard D, Hussain SS, Alquezar D, Rasmussen M, et al. 2010. Transcriptomes of the desiccation-tolerant resurrection plant *Craterostigma plantagineum*. *Plant J.* 63:212–28
135. Rojo E, Martín R, Carter C, Zouhar J, Pan S, et al. 2004. VPEγ exhibits a caspase-like activity that contributes to defense against pathogens. *Curr. Biol.* 14:1897–906
136. Rosati A, Graziano V, De Laurenzi V, Pascale M, Turco MC. 2011. BAG3: a multifaceted protein that regulates major cell pathways. *Cell Death Disease* 2:e141
137. Rose TL, Bonneau L, Der C, Marty-Mazars D, Marty F. 2006. Starvation-induced expression of autophagy-related genes in Arabidopsis. *Biol. Cell* 98:53–67

138. Rothe M, Pan MG, Henzel WJ, Ayres TM, Goeddel DV. 1995. The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. *Cell* 83:1243–52
139. Ruckenstuhl C, Netzberger C, Entfellner I, Carmona-Gutierrez D, Kickenweiz T, et al. 2014. Lifespan extension by methionine restriction requires autophagy-dependent vacuolar acidification. *PLOS Genet*. 10:e1004347
140. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. 2007. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and α-synuclein. *J. Biol. Chem.* 282:5641–52
141. Schepetilnikov M, Dimitrova M, Mancera-Martinez E, Geldreich A, Keller M, Ryabova LA. 2013. TOR and S6K1 promote translation reinitiation of uORF-containing mRNAs via phosphorylation of eIF3h. *EMBO J.* 32:1087–102
142. Schile AJ, García-Fernández M, Steller H. 2008. Regulation of apoptosis by XIAP ubiquitin-ligase activity. *Genes Dev.* 22:2256–66
143. Shalini S, Dorstyn L, Dawar S, Kumar S. 2015. Old, new and emerging functions of caspases. *Cell Death Differ*. 22:526–39
144. Shlezinger N, Minz A, Gur Y, Hatam I, Dagdas YF, et al. 2011. Anti-apoptotic machinery protects the necrotrophic fungus *Botrytis cinerea* from host-induced apoptotic-like cell death during plant infection. *PLOS Pathog*. 7:e1002185
145. Singh S, Ambastha V, Levine A, Sopory SK, Yadava PK, et al. 2015. Anhydrobiosis and programmed cell death in plants: commonalities and differences. *Curr. Plant Biol.* 2:12–20
146. Smith AM, Stitt M. 2007. Coordination of carbon supply and plant growth. *Plant Cell Environ*. 30:1126–49
147. Song J, Takeda M, Morimoto RI. 2001. Bag1-Hsp70 mediates a physiological stress signalling pathway that regulates Raf-1/ERK and cell growth. *Nat. Cell Biol.* 3:276–82
148. Srinivasula SM, Ashwell JD. 2008. IAPs: What's in a name? *Mol. Cell* 30:123–35
149. Stone JM, Liang X, Nekl ER, Stiers JJ. 2005. Arabidopsis *AtSPL14*, a plant-specific SBP-domain transcription factor, participates in plant development and sensitivity to fumonisin B1. *Plant J.* 41:744–54
150. Stone SL, Hauksdottir H, Troy A, Herschleb J, Kraft E, Callis J. 2005. Functional analysis of the RING-type ubiquitin ligase family of Arabidopsis. *Plant Physiol.* 137:13–30
151. Sundstrom JF, Vaculova A, Smertenko AP, Savenkov EI, Golovko A, et al. 2009. Tudor staphylococcal nuclease is an evolutionarily conserved component of the programmed cell death degradome. *Nat. Cell Biol.* 11:1347–54
152. Suttangkakul A, Li F, Chung T, Vierstra RD. 2011. The ATG1/ATG13 protein kinase complex is both a regulator and a target of autophagic recycling in *Arabidopsis*. *Plant Cell* 23:3761–79
153. Takahashi R, Deveraux Q, Tamm I, Welsh K, Assa-Munt N, et al. 1998. A single BIR domain of XIAP sufficient for inhibiting caspases. *J. Biol. Chem.* 273:7787–90
154. Takayama S, Reed JC. 2001. Molecular chaperone targeting and regulation by BAG family proteins. *Nat. Cell Biol.* 3:E237–41
155. Takayama S, Sato T, Krajewski S, Kochel K, Irie S, et al. 1995. Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. *Cell* 80:279–84
156. Takayama S, Xie Z, Reed JC. 1999. An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. *J. Biol. Chem.* 274:781–86
157. Tardieu F. 2005. Plant tolerance to water deficit: physical limits and possibilities for progress. *C. R. Geosci.* 337:57–67
158. Tenev T, Zachariou A, Wilson R, Paul A, Meier P. 2002. Jafrac2 is an IAP antagonist that promotes cell death by liberating Dronc from DIAP1. *EMBO J.* 21:5118–29
159. Thomma BP, Nurnberger T, Joosten MH. 2011. Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *Plant Cell* 23:4–15
160. Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, et al. 1992. A novel heterodimeric cysteine protease is required for interleukin-1β processing in monocytes. *Nature* 356:768–74
161. Thress K, Evans EK, Kornbluth S. 1999. Reaper-induced dissociation of a Scythe-sequestered cytochrome *c*-releasing activity. *EMBO J.* 18:5486–93

162. Thress K, Henzel W, Shillinglaw W, Kornbluth S. 1998. Scythe: a novel reaper-binding apoptotic regulator. *EMBO J.* 17:6135–43
163. Tran V, Weier D, Radchuk R, Thiel J, Radchuk V. 2014. Caspase-like activities accompany programmed cell death events in developing barley grains. *PLOS ONE* 9:e109426
164. Tschopp J, Martinon F, Hofmann K. 1999. Apoptosis: silencing the death receptors. *Curr. Biol.* 9:R381–84
165. Tsiatsiani L, Timmerman E, De Bock PJ, Vercammen D, Stael S, et al. 2013. The *Arabidopsis* METACASPASE9 degradome. *Plant Cell* 25:2831–47
166. Tsiatsiani L, Van Breusegem F, Gallois P, Zavialov A, Lam E, Bozhkov PV. 2011. Metacaspases. *Cell Death Differ.* 18:1279–88
167. Twiddy D, Cohen GM, Macfarlane M, Cain K. 2006. Caspase-7 is directly activated by the approximately 700-kDa apoptosome complex and is released as a stable XIAP-caspase-7 approximately 200-kDa complex. *J. Biol. Chem.* 281:3876–88
168. Uren AG, O'Rourke K, Aravind LA, Pisabarro MT, Seshagiri S, et al. 2000. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol. Cell* 6:961–67
169. Usadel B, Blasing OE, Gibon Y, Retzlaff K, Hohne M, et al. 2008. Global transcript levels respond to small changes of the carbon status during progressive exhaustion of carbohydrates in Arabidopsis rosettes. *Plant Physiol.* 146:1834–61
170. van Doorn WG. 2011. Classes of programmed cell death in plants, compared to those in animals. *J. Exp. Bot.* 62:4749–61
171. van Doorn WG, Beers EP, Dangl JL, Franklin-Tong VE, Gallois P, et al. 2011. Morphological classification of plant cell deaths. *Cell Death Differ.* 18:1241–46
172. Van Hautegem T, Waters AJ, Goodrich J, Nowack MK. 2015. Only in dying, life: programmed cell death during plant development. *Trends Plant Sci.* 20:102–13
173. Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, et al. 2000. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* 102:43–53
174. Wang HG, Takayama S, Rapp UR, Reed JC. 1996. Bcl-2 interacting protein, BAG-1, binds to and activates the kinase Raf-1. *PNAS* 93:7063–68
175. Wang X, Guo J, Fei E, Mu Y, He S, et al. 2014. BAG5 protects against mitochondrial oxidative damage through regulating PINK1 degradation. *PLOS ONE* 9:e86276
176. Watanabe N, Lam E. 2011. Arabidopsis metacaspase 2d is a positive mediator of cell death induced during biotic and abiotic stresses. *Plant J.* 66:969–82
177. Whittaker A, Martinelli T, Bochicchio A, Vazzana C, Farrant J. 2004. Comparison of sucrose metabolism during the rehydration of desiccation-tolerant and desiccation-sensitive leaf material of *Sporobolus stapfianus*. *Physiol. Plant.* 122:11–20
178. Whittaker A, Martinelli T, Farrant JM, Bochicchio A, Vazzana C. 2007. Sucrose phosphate synthase activity and the co-ordination of carbon partitioning during sucrose and amino acid accumulation in desiccation-tolerant leaf material of the C_4 resurrection plant *Sporobolus stapfianus* during dehydration. *J. Exp. Bot.* 58:3775–87
179. Williams B, Kabbage M, Britt R, Dickman MB. 2010. *AtBAG7*, an *Arabidopsis* Bcl-2-associated athanogene, resides in the endoplasmic reticulum and is involved in the unfolded protein response. *PNAS* 107:6088–93
180. Williams B, Kabbage M, Kim HJ, Britt R, Dickman MB. 2011. Tipping the balance: *Sclerotinia sclerotiorum* secreted oxalic acid suppresses host defenses by manipulating the host redox environment. *PLOS Pathog.* 7:e1002107
181. Williams B, Njaci I, Moghaddam L, Long H, Dickman MB, et al. 2015. Trehalose accumulation triggers autophagy during plant desiccation. *PLOS Genet.* 11:e1005705
182. Williams B, Verchot J, Dickman MB. 2014. When supply does not meet demand-ER stress and plant programmed cell death. *Front. Plant Sci.* 5:211
183. Wrzaczek M, Brosche M, Kollist H, Kangasjarvi J. 2009. *Arabidopsis* GRI is involved in the regulation of cell death induced by extracellular ROS. *PNAS* 106:5412–17

184. Wu G, Chai J, Suber TL, Wu J-W, Du C, et al. 2000. Structural basis of IAP recognition by Smac/DIABLO. *Nature* 408:1008–12
185. Yamada T, Takatsu Y, Manabe T, Kasumi M, Marubashi W. 2003. Suppressive effect of trehalose on apoptotic cell death leading to petal senescence in ethylene-insensitive flowers of gladiolus. *Plant Sci*. 164:213–21
186. Yang YL, Li XM. 2000. The IAP family: endogenous caspase inhibitors with multiple biological activities. *Cell Res*. 10:169–77
187. Yang Z, Klionsky DJ. 2010. Eaten alive: a history of macroautophagy. *Nat. Cell Biol.* 12:814–22
188. Ye CM, Chen S, Payton M, Dickman MB, Verchot J. 2013. TGBp3 triggers the unfolded protein response and SKP1-dependent programmed cell death. *Mol. Plant Pathol.* 14:241–55
189. Yobi A, Wone BW, Xu W, Alexander DC, Guo L, et al. 2012. Comparative metabolic profiling between desiccation-sensitive and desiccation-tolerant species of *Selaginella* reveals insights into the resurrection trait. *Plant J*. 72:983–99
190. Yobi A, Wone BW, Xu W, Alexander DC, Guo L, et al. 2013. Metabolomic profiling in *Selaginella lepidophylla* at various hydration states provides new insights into the mechanistic basis of desiccation tolerance. *Mol. Plant* 6:369–85
191. Young AR, Chan EY, Hu XW, Kochl R, Crawshaw SG, et al. 2006. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J. Cell Sci.* 119:3888–900
192. Yu X, Wang L, Acehan D, Wang X, Akey CW. 2006. Three-dimensional structure of a double apoptosome formed by the *Drosophila* Apaf-1 related killer. *J. Mol. Biol.* 355:577–89
193. Yuan J, Shaham S, Ledoux S, Ellis HM, Horvitz HR. 1993. The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1β-converting enzyme. *Cell* 75:641–52
194. Zhang H, Dong S, Wang M, Wang W, Song W, et al. 2010. The role of vacuolar processing enzyme (VPE) from *Nicotiana benthamiana* in the elicitor-triggered hypersensitive response and stomatal closure. *J. Exp. Bot.* 61:3799–812
195. Zimmermann D, Gomez-Barrera JA, Pasule C, Brack-Frick UB, Sieferer E, et al. 2016. Cell death control by matrix metalloproteinases. *Plant Physiol.* 171:1456–69
196. Zipfel C, Robatzek S. 2010. Pathogen-associated molecular pattern-triggered immunity: veni, vidi. . .? *Plant Physiol.* 154:551–54
197. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. 1997. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome *c*-dependent activation of caspase-3. *Cell* 90:405–13
198. Zou H, Li Y, Liu X, Wang X. 1999. An APAF-1·cytochrome *c* multimeric complex is a functional apoptosome that activates procaspase-9. *J. Biol. Chem.* 274:11549–56