Reactive Oxygen Species in Plant Cell Death[1]

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CELL DEATH IN PLANTS

Paradoxically, death is an integral part of life. Cell death is essential for growth and development of eukaryotes, by maintaining tissue and organ homeostasis in concert with cell proliferation, growth, and differentiation. Until recently, the wide variety of cell death types reported in the literature was mostly caged in two semantic categories: apoptosis and necrosis. Discrimination between these two forms was based on the presence or absence of specific biochemical and molecular hallmarks, such as DNA laddering, cytochrome c release, caspase involvement, ATP depletion, cytoplasmic swelling, and loss of membrane integrity (Pennell and Lamb, 1997). However, over the last decade this arbitrary division had clearly become too simplistic and a more accurate description of plant cell death needed to be established (van Doorn and Woltering, 2005). To avoid a Babel confusion of languages, we propose necrosis to depict accidental cell death caused by extrinsic factors, such as phytotoxic accumulation of specific molecules after a traumatic stress event. Thus, plant cell death through necrosis is passive, indiscriminate, and often follows irreversible injury. It is characterized by a progressive loss of membrane integrity that results in swelling of the cytoplasm and release of cellular constituents. Accordingly, the terms active or programmed cell death (PCD) define any form of cell death involving a single or a series of molecular and cellular orderly processes mediated by intracellular death programs, regardless of the (external) trigger or the hallmarks it exhibits (Jacobson et al., 1997; Dangl et al., 2000). Although both cell death events are quite well defined in animals, in plants there seems to be much more overlap between the phenotypic and molecular hallmarks of necrosis and PCD, thus making it harder to discriminate between the two events. In plants, PCD can occur during many developmental processes and abiotic stress conditions. For instance, PCD has been described during hypoxia stress (Drew et al., 2000), extremes in temperature (Vacca et al., 2004), and ozone (Langebartels et al., 2002). Developmental PCD has been reported during tracheary element formation (Kuriyama and Fukuda, 2002), seed development, germination (Souter and Lindsey, 2000; Young and Gallie, 2000), and senescence processes (Gunawardena et al., 2004). However, cell death events remain best described during incompatible plant-pathogen interactions that form the basis for the hypersensitive response (HR; Pennell and Lamb, 1997). This multitude of plant PCD events clearly illustrates a functional analogy between cell death across kingdoms: sculpting and deleting structures, eliminating cells to control cell quality and quantity after trauma, and producing differentiated cells without organelles (Jacobson et al., 1997). But, is there more than just a functional similarity between PCD in plants and animals? In animals, reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide ion, and nitric oxide (NO) are well-recognized triggers of cell death (Jabs, 1999). In contrast, involvement of these molecules during plant PCD was, for a long time, rather hypothetical. In addition to the lack of accurate and quantifiable detection methods, the inherent toxic nature of ROS masked their underlying function in various signaling networks. Only recently, the prominent role of ROS has been revealed in the induction, signaling, and execution of plant cell death. The initial phenotypic and pharmacological evidence for ROS signaling during plant PCD has been confirmed genetically with the identification of Arabidopsis (*Arabidopsis thaliana*) mutants incapable either of arresting ROS-driven PCD or of undergoing a PCD despite high ROS levels. Recently, the first genes involved in ROS perception and signal transduction have been identified and now we are faced with the challenge of uncovering the other players in the gene regulatory network of ROS-dependent cell death. In this update, we will describe the current knowledge on ROS homeostasis and cell death events in plants.

ROS HOMEOSTASIS, A DEDICATED BALANCE BETWEEN DEATH AND SIGNALING

Life under aerobic conditions is intimately linked with ROS production. Indeed, ROS are generated as

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by-products of the most essential energy-generating processes, photosynthesis and respiration. Together with an extensive battery of oxidases, chloroplasts, peroxisomes, and mitochondria are the main organellar ROS producers (Vranová et al., 2002; Apel and Hirt, 2004). Despite an efficient antioxidant machinery in these organelles, subtle changes in ROS homeostasis are inevitable (Foyer and Noctor, 2005). When the increase in ROS is relatively small, the housekeeping antioxidant capacity is sufficient to reset the original balance between ROS production and scavenging, thus reestablishing redox homeostasis. Under growth-limiting environmental conditions, this delicate redox balance is easily disturbed, potentially leading to a significant ROS accumulation. In fact, a 3- to 10-fold increase in ROS levels has been calculated under stress conditions (Polle, 2001). Thus, it is not surprising that a transient oxidative burst and a subsequent temporary shift in the intracellular redox state are common features of both biotic and abiotic stress responses (Dat et al., 2000; Mittler et al., 2004). Under unfavorable environmental conditions, such as temperature extremes, drought, or salt stress, the rate of carbon fixation is limited, causing an increase in photoinhibition potentially steering the photosystem toward overproduction of superoxide radicals and H_2O_2 (Foyer and Noctor, 2005). Similarly during ozone exposure, ROS are generated following the entry of ozone through the stomata and its conversion in the leaf apoplast, eventually leading to the formation of HR-like lesions (Pellinen et al., 1999; Rao and Davis, 2001). Furthermore, most incompatible plant-pathogen interactions are typified by a biphasic oxidative burst during which both infected and adjacent cells are sacrificed to limit pathogen spreading (Doke, 1997; Draper, 1997). The accumulation of ROS toward the apoplast is considered to originate mainly from an increased activity of apoplastic peroxidases, amine oxidases, and an NADPH-oxidase complex coupled to a decrease in cellular ROS-scavenging capacity (Bolwell, 1999; Mittler et al., 1999; Torres and Dangl, 2005).

At high concentrations (oxidative stress), the various ROS can certainly behave as extremely reactive molecules. Their ability to react indiscriminately with almost all cellular components provokes, among others, destructive protein modifications as well as mutagenic DNA strand breaks, purine oxidations, and protein-DNA cross links (Beckman and Ames, 1997; Berlett and Stadtman, 1997). Lipid peroxidation, in both cellular and organellar membranes, takes place when above-threshold ROS levels are reached, thereby not only directly affecting normal cellular functioning, but also aggravating the oxidative stress through production of lipid-derived radicals (Montillet et al., 2005). Consequently, the cellular damage resulting from high ROS levels shows some of the typical hallmarks of necrosis, because phytotoxic levels of oxidants indiscriminately attack cellular constituents, leading to membrane leakage and cell lysis. To avoid such collateral damage, a tight regulation of ROS homeostasis is necessary, which is provided by a complex gene network that operates in all subcellular compartments and through complex feed-forward/feed-back loops between oxidants and scavengers. As an illustration of this complexity, at least 158 genes in the Arabidopsis genome specifically master the spatio-temporal network of ROS production and scavenging (Mittler et al., 2004). Simultaneously, the tight ROS homeostasis regulation also creates a baseline on which spikes of ROS can signal in different cellular processes. In addition, depending on their type and their subcellular production sites, ROS may determine the specificity of ensuing cellular responses. Although the underlying signaling function of ROS has long been masked by the inherent toxic nature of oxidants, their triggering ability during various developmental processes and environmental stress responses is now widely recognized (Foyer and Noctor, 2005).

ROS SIGNALS INVOLVED IN PLANT CELL DEATH

The first indications for an involvement of ROS in PCD were based mainly on spatio-temporal correlations between increased ROS levels and cell death. The initial experimental evidence that ROS also acted as a signal in plant PCD was obtained in cell suspensions by demonstrating that H_2O_2-induced cell death could be blocked by cycloheximide and protease inhibitors (Levine et al., 1994). Since then, there has been ever growing support for a key role for ROS as triggers of PCD. Whereas initial results were generally obtained by exogenous application of oxidants, more recently, transgenic plants with perturbed levels of cellular antioxidants and mutants unable to stop the initiation or propagation of ROS-driven cell death have elegantly demonstrated the important role of ROS in the initiation phase of different plant PCD events (Mittler and Rizhsky, 2000; Lorrain et al., 2003).

The best studied examples of ROS-derived PCD are those following the typical biphasic oxidative burst during both the HR and ozone stress. The ensuing cell death is characterized by discrete cellular lesions that are generally preceded by the appearance of several hallmarks of PCD, such as chromatin condensation, DNA laddering, and cytochrome c release (Lam, 2004). Moreover, these features can be halted by administrating either high concentrations of antioxidants or inhibitors of both translation and transcription and of known signal transducing components, such as kinases or phytohormones. Accordingly, transgenic plants with low or high levels of several antioxidants (superoxide dismutase, catalase, and ascorbate peroxidase) also exhibit an altered response to both pathogen- and ozone-driven PCD, again demonstrating the crucial importance of a tightly orchestrated redox balance (Van Camp et al., 1994; Örvar et al., 1997; Mittler et al., 1999). Further evidence for a ROS-dependent PCD

pathway in plants has been provided by transgenic plants deficient in catalase (Cat1AS), the major H_2O_2-scavenging enzyme. In Cat1AS leaves of tobacco (*Nicotiana tabacum*), accumulation of photorespiratory H_2O_2 induced an active cell death of leaf palisade parenchyma cells. In addition to the presence of several PCD hallmarks, this high light-driven cell death could be blocked by infiltration of various antagonists of HR-like PCD (Dat et al., 2003). Interestingly, we have also been able to uncouple a necrosis-like response, provoked by continuous high light-dependent H_2O_2 accumulation, from PCD triggered by a transient, nonlethal, in planta ROS accumulation. The short H_2O_2 pulse sufficient to induce PCD in leaves had a lipoxygenase-dependent oxylipin signature similar to that induced by a pathogenic elicitor (cryptogein). In contrast, the continuous H_2O_2 accumulation generated by long-term high light exposure or H_2O_2 feeding led to necrosis and ROS-mediated lipid peroxidation (Montillet et al., 2005). The existence of a dose-dependent ROS threshold below which PCD will be triggered and above which necrotic cell death will prevail might be the reason for the overwhelming effect that phytotoxic ROS levels have on PCD hallmarks. Alternatively, similarly to what is reported in animals, high doses of oxidants might inhibit components of the PCD pathway (Kazzaz et al., 1996). Complementary to the data provided by transgenic plants, a number of Arabidopsis mutants have demonstrated the specificity in the signaling capacity of different ROS in initiating PCD. In two mutants of Arabidopsis, *lesion-stimulating disease1* (*lsd1*) and *radical-induced cell death1* (*rcd1*), elevated ROS levels are necessary and sufficient to induce spreading of cell death (Jabs et al., 1996; Overmyer et al., 2000; Mateo et al., 2004). The conditional *fluorescent* (*flu*) mutant of Arabidopsis that generates singlet oxygen upon a dark-to-light shift initiates a cell death response immediately after the release of singlet oxygen (op den Camp et al., 2003). Singlet oxygen is interpreted differently at the molecular level than H_2O_2 and superoxide, thus providing further evidence for a selective signaling effect of various ROS in plants (Danon et al., 2005). The ozone-sensitive mutant *vitamin c-1* (*vtc-1*), deficient in L-ascorbic acid, shows small patches of spontaneous lesions on its leaves (Pavet et al., 2005). These lesions were attributed to changes in redox homeostasis rather than to H_2O_2, as evidenced by lower ascorbate levels in *vtc* mutants than in the wild-type plants (Pavet et al., 2005). Finally, the localized expression pattern of the copper amine oxidase-encoding gene *ATAO1*, which produces H_2O_2 associated with PCD in lateral root cap cells and developing tracheary elements, also hints to H_2O_2 as a trigger for certain developmental cell death processes (Møller and McPherson, 1998). In summary, various ROS species and cellular redox changes can trigger different signaling cascades leading to PCD. However, the understanding of how ROS signals are perceived and transduced is still in its infancy.

ROS SIGNAL RELAY DURING PLANT CELL DEATH

In animal cells, mitochondria have long been recognized as central players in ROS-dependent apoptotic cell death. One key event is the release of cytochrome c necessary for caspase activation that precedes mitochondrial membrane depolarization and nuclear condensation as well as other hallmarks of apoptosis (Fleury et al., 2002). In plants, mitochondria may also serve as first relay stations where the initial alteration in ROS homeostasis is amplified, triggering cytochrome c release through mitochondrial transition pore opening and morphological changes (Tiwari et al., 2002; Dat et al., 2003; Casolo et al., 2005; Yoshinaga et al., 2005). The role of mitochondria is further corroborated by the up-regulation of both manganese superoxide dismutase and the alternative oxidase mitochondrial antioxidant genes early during ROS-driven PCD (Robson and Vanlerberghe, 2002; Dat et al., 2003). In plants, besides mitochondria, chloroplasts are also important ROS suppliers, and they may generate intermediate signals involved in PCD. For example, during cryptogein-induced PCD, an H_2O_2-dependent activation of lipoxygenases targets chloroplastic polyunsaturated fatty acids, releasing oxylipins that are sufficient for inducing PCD (Rustérucci et al., 1999; Maccarrone et al., 2000; Montillet et al., 2005). A plastid involvement in PCD is further substantiated by the ectopic expression in the chloroplasts of mammalian antiapoptotic B-cell leukemia/lymphoma (BCL2) members, which protect transgenic tobacco plants from herbicide-induced PCD (Chen and Dickman, 2004). Finally, recent data on the role of phytochrome signaling during the establishment of the HR clearly implicate the necessity for a chloroplastic factor in the pathway leading to the HR (Genoud et al., 2002; Karpinski et al., 2003). Thus, in addition to the recognized participation of mitochondria in PCD, an active involvement of chloroplast-derived signals is also primordial during plant PCD.

A strong interplay between ROS and other signaling molecules (phytohormones) exists during plant PCD (Overmyer et al., 2005). The fact that ROS-dependent PCD is associated with increased levels of both salicylic acid (SA) and ethylene and that the overproduction of an SA-degrading enzyme (catechol) in ethylene mutants tempers PCD, strongly positions both ethylene and SA within a positive feed-back cycle that promotes ROS-dependent cell death (de Jong et al., 2002; Moeder et al., 2002; Danon et al., 2005). On the other hand, jasmonic acid can either induce or act antagonistically in this oxidative-dependent cell death cycle depending on whether the initial ROS signal is singlet oxygen or superoxide. This different interaction with oxylipins adds another level of specificity and complexity to ROS signaling during PCD (Overmyer et al., 2000; Danon et al., 2005). An additional player in ROS-dependent PCD is NO that operates in a dose-dependent manner synergistically with H_2O_2 in HR cell death (Delledonne et al., 2001; Zago et al., 2006).

Moreover, NO may directly interact with SA, because infiltration of tobacco leaves with NO results in a significant accumulation of SA (Durner et al., 1998). Thus, redox-dependent plant PCD involves a range of signaling molecules, but this interactive regulatory network is just starting to be elucidated (Fig. 1).

MOLECULAR TARGETS OF ROS SIGNALING DURING PLANT CELL DEATH

One of the first genes identified in ROS-dependent cell death was isolated through a genetic screen. The Arabidopsis *lsd1* mutant is unable to cope with increased superoxide levels and develops spontaneous superoxide- and SA-dependent runaway lesions (Jabs et al., 1996). LSD1 encodes a zinc-finger protein that, together with two other zinc-finger proteins (LOL1 and LOL2), could act as a molecular rheostat, sensing changes in ROS homeostasis, thereby repressing a default death pathway or controlling an antideath mechanism through the regulation of cell death genes (Dietrich et al., 1997; Epple et al., 2003). The ozone-sensitive lesion mimic mutant, *rcd1*, also displays PCD lesions. RCD1 has been proposed to modulate the activity of target proteins through ADP ribosylation (Ahlfors et al., 2004; Overmyer et al., 2005). In a landmark paper, ROS-induced PCD has recently been shown not to be mediated by photooxidative damage but through a genetically determined PCD pathway (Wagner et al., 2004). In fact, inactivation of the Arabidopsis *Executer1* gene completely abolished singlet oxygen-induced cell death (Wagner et al., 2004). This hitherto unknown nucleus-encoded chloroplastic protein might perceive nonscavenged singlet oxygen species within the chloroplast. Whether *Executer1* is specifically involved in singlet oxygen perception or also in sensing other ROS species remains to be tested.

Other important stress signal transducers include mitogen-activated protein kinases (MAPKs) that act upstream of the oxidative burst during ozone treatment and the HR (Ren et al., 2002; Samuel and Ellis, 2002).

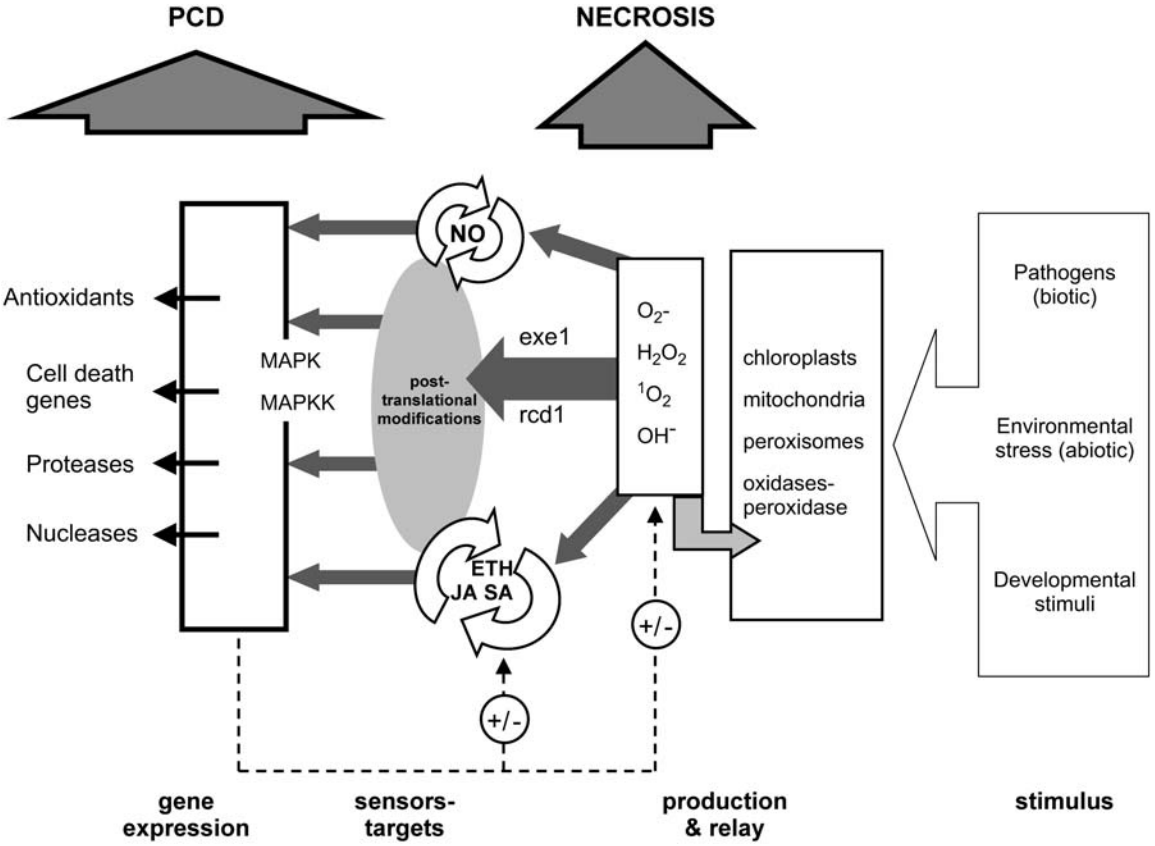

Figure 1. Schematic representation of ROS-dependent cell death pathways. Environmental and developmental changes stimulate the production of ROS through various organelles and enzymes. This initial increase in ROS levels may be further enhanced by various relay and production centers. Sustained ROS production provokes the accumulation of phytotoxic levels of ROS leading to necrotic cell death. Transient increases may initiate signal transduction cascades involving cross talk with other phytohormones, SA, jasmonate, ethylene, and NO. This cross talk can amplify or diminish the ensuing response or channel the cascade through more specific transducer sensors and targets toward ROS-dependent and cell death-related gene expression. Besides MAPK-driven phosphorylation cascades, other regulatory posttranslational modifications, such as protein oxidation and nitrosylation might be involved in ROS-dependent cell death pathways. ETH, Ethylene; exe1, executer1; JA, jasmonic acid.

However, MAPKs might also work downstream in ROS-dependent cell death events. The primary ROS-activated tobacco MAPK is the SA-induced protein kinase, which is required during harpin-dependent PCD (Samuel et al., 2005). A MAPKKK of alfalfa (*Medicago sativa*) activates cell death induced by H_2O_2 through a specific MAPK-scaffolding action (Nakagami et al., 2004). Overlapping features of animal and plant PCD have also inspired different functional approaches by introducing animal genes into plants to gather mechanistic insight into oxidative stress-dependent cell death events. No obvious homologs of the BCL-like animal cell death suppressors have been identified in plant genomes to date; nevertheless, protection against mitochondrial and chloroplast-derived ROS-dependent cell death is conferred by their overproduction in tobacco. Although functional plant homologs have been successful in protecting against ROS-mediated PCD in the transgenic lines, their existence remains unclear (Mitsuhara et al., 1999; Chen and Dickman, 2004). However, recently an evolutionarily conserved Arabidopsis BCL2-associated athanogene protein has been shown to be induced by H_2O_2 and capable of provoking PCD in both yeast (*Saccharomyces cerevisiae*) and plants (Kang et al., 2006).

CONCLUSION AND PERSPECTIVES

Research on redox-dependent PCD has demonstrated that when ROS production exceeds the cellular scavenging capacity, ROS levels may rise above two different threshold levels both leading to cell death. Unleashed ROS accumulation will build up to phytotoxic levels hereby indiscriminately attacking and damaging proteins, lipids, and DNA. This type of ROS-dependent cell death leads to a necrotic phenotype and is commonly encountered under conditions favoring leakage from electron transport chains together with reduced antioxidant potential. Under many abiotic stress conditions, cell death execution is steered through this ROS overaccumulation. When the accumulation of ROS is insufficient to kill the cell directly, changes in cellular redox homeostasis appear to switch on a signaling cascade, leading to PCD, as typically encountered during the HR.

The recent identification of plant genes involved in ROS-dependent PCD is merely the start of a new era during which unearthing the remainder of the gene regulatory network that controls this mechanism will be crucial. Initial optimism that functional equivalents of animal cell death genes could be identified in plants on the basis of sequence homologies has gradually declined. Until today, no homologs of recognized apoptotic regulators, such as caspases or BCL2-associated X protein, have been detected in plants. Thus, current stakes are on the outcome of ongoing mutant and functional screens. Additional suppressor screens for ROS-induced cell death and cloning of the remaining alleles already reported to revert or induce PCD in Arabidopsis mutants will increase the number of genes in the plant PCD pathway schemes. Finalized genome-wide transcriptome studies have identified hundreds of genes that are rapidly induced by ROS signals (Desikan et al., 2001; op den Camp et al., 2003; Vandenabeele et al., 2003; Vanderauwera et al., 2005; Gadjev et al., 2006). Within these inventories, a cohort of novel genes involved in early ROS perception and transduction will undoubtedly be discovered. Functional screens, in which these genes are either up- or down-regulated and scored for their potential to modulate ROS-dependent cell death, will assist in their identification. This approach, together with the characterization of target proteins that undergo regulatory posttranslational modifications, such as protein oxidation and nitrosylation during cell death, will lead to a better understanding of cell death in plants.

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LITERATURE CITED

Ahlfors R, Lång S, Overmyer K, Jaspers P, Brosché M, Tauriainen A, Kollist H, Tuominen H, Belles-Boix E, Piippo M, et al (2004) Arabidopsis RADICAL-INDUCED CELL DEATH1 belongs to the WWE protein-protein interaction domain protein family and modulates abscisic acid, ethylene, and methyl jasmonate responses. Plant Cell **16:** 1925–1937

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol **55:** 373–399

Beckman KB, Ames BN (1997) Oxidative decay of DNA. J Biol Chem **272:** 19633–19636

Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem **272:** 20313–20316

Bolwell GP (1999) Role of active oxygen species and NO in plant defence responses. Curr Opin Plant Biol **2:** 287–294

Casolo V, Petrussa E, Krajňáková J, Macrì F, Vianello A (2005) Involvement of the mitochondrial K_{TP} channel in H_2O_2- or NO-induced programmed death of soybean suspension cell cultures. J Exp Bot **56:** 997–1006

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:** 2617–2623

Dangl JL, Dietrich RA, Thomas H (2000) Senescence and programmed cell death. *In* B Buchanan, W Gruissem, R Jones, eds, Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists Press, Rockville, MD, pp 1044–1100

Danon A, Miersch O, Felix G, op den Camp RGL, Apel K (2005) Concurrent activation of cell death-regulating signaling pathways by singlet oxygen in *Arabidopsis thaliana*. Plant J **41:** 68–80

Dat J, Vandenabeele S, Vranová E, Van Montagu M, Inzé D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci **57:** 779–795

Dat JF, Pellinen R, Beeckman T, Van De Cotte B, Langebartels C, Kangasjärvi J, Inzé D, Van Breusegem F (2003) Changes in hydrogen peroxide homeostasis trigger an active cell death process in tobacco. Plant J **33:** 621–632

de Jong AJ, Yakimova ET, Kapchina VM, Woltering EJ (2002) A critical role for ethylene in hydrogen peroxide release during programmed cell death in tomato suspension cells. Planta **214:** 537–545

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci USA **98:** 13454–13459

Desikan R, Mackerness SA-H, Hancock JT, Neill SJ (2001) Regulation of the Arabidopsis transcriptome by oxidative stress. Plant Physiol **127:** 159–172

Dietrich RA, Richberg MH, Schmidt R, Dean C, Dangl JL (1997) A novel zinc finger protein is encoded by the Arabidopsis *LSD1* gene and functions as a negative regulator of plant cell death. Cell **88:** 685–694

Doke N (1997) The oxidative burst: roles in signal transduction and plant stress. *In* JG Scandalios, ed, Oxidative Stress and the Molecular Biology of Antioxidant Defenses, Monograph Series, Vol 34. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 785–813

Draper J (1997) Salicylate, superoxide synthesis and cell suicide in plant defence. Trends Plant Sci **2:** 162–165

Drew MC, He C-J, Morgan PW (2000) Programmed cell death and aerenchyma formation in roots. Trends Plant Sci **5:** 123–127

Durner J, Wendehenne D, Klessig DF (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci USA **95:** 10328–10333

Epple P, Mack AA, Morris VRF, Dangl JL (2003) Antagonistic control of oxidative stress-induced cell death in *Arabidopsis* by two related, plant-specific zinc finger proteins. Proc Natl Acad Sci USA **100:** 6831–6836

Fleury C, Mignotte B, Vayssière J-L (2002) Mitochondrial reactive oxygen species in cell death signaling. Biochimie **84:** 131–141

Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell **17:** 1866–1875

Gadjev I, Vanderauwera S, Gechev TS, Laloi C, Minkov IN, Shulaev V, Apel K, Inzé D, Mittler R, Van Breusegem F (2006) Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis. Plant Physiol **141:** 436–445

Genoud T, Buchala AJ, Chua N-H, Métraux J-P (2002) Phytochrome signalling modulates the SA-perceptive pathway in *Arabidopsis*. Plant J **31:** 87–95

Gunawardena AHLAN, Greenwood JS, Dengler NG (2004) Programmed cell death remodels lace plant leaf shape during development. Plant Cell **16:** 60–73

Jabs T (1999) Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. Biochem Pharmacol **57:** 231–245

Jabs T, Dietrich RA, Dangl JL (1996) Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. Science **273:** 1853–1856

Jacobson MD, Weil M, Raff MC (1997) Programmed cell death in animal development. Cell **88:** 347–354

Kang CH, Jung WY, Kang YH, Kim JY, Kim DG, Jeong JC, Baek DW, Jin JB, Lee JY, Kim MO, et al (2006) AtBAG6, a novel calmodulin-binding protein, induces programmed cell death in yeast and plants. Cell Death Differ **13:** 84–95

Karpinski S, Gabrys H, Mateo A, Karpinska B, Mullineaux PM (2003) Light perception in plant disease defence signalling. Curr Opin Plant Biol **6:** 390–396

Kazzaz JA, Xu J, Palaia TA, Mantell L, Fein AM, Horowitz S (1996) Cellular oxygen toxicity: oxidant injury without apoptosis. J Biol Chem **271:** 15182–15186

Kuriyama H, Fukuda H (2002) Developmental programmed cell death in plants. Curr Opin Plant Biol **5:** 568–573

Lam E (2004) Controlled cell death, plant survival and development. Nat Rev Mol Cell Biol **5:** 305–315

Langebartels C, Wohlgemuth H, Kschieschan S, Grün S, Sandermann H (2002) Oxidative burst and cell death in ozone-exposed plants. Plant Physiol Biochem **40:** 567–575

Levine A, Tenhaken R, Dixon R, Lamb C (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell **79:** 583–593

Lorrain S, Vailleau F, Balagué C, Roby D (2003) Lesion mimic mutants: keys for deciphering cell death and defense pathways in plants? Trends Plant Sci **8:** 263–271

Maccarrone M, Van Zadelhoff G, Veldink GA, Vliegenthart JFG, Finazzi-Agrò A (2000) Early activation of lipoxygenase in lentil (*Lens culinaris*) root protoplasts by oxidative stress induces programmed cell death. Eur J Biochem **267:** 5078–5084

Mateo A, Mühlenbock P, Rustérucci C, Chi-Chen Chang C, Miszalski Z, Karpinska B, Parker JE, Mullineaux PM, Karpinski S (2004) *LESION SIMULATING DISEASE 1* is required for acclimation to conditions that promote excess excitation energy. Plant Physiol **136:** 2818–2830

Mitsuhara I, Malik KA, Miura M, Ohashi Y (1999) Animal cell-death suppressors Bcl-x_L and Ced-9 inhibit cell death in tobacco cells. Curr Biol **9:** 775–778

Mittler R, Hallak Herr E, Orvar BL, Van Camp W, Willekens H, Inzé D, Ellis BE (1999) Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. Proc Natl Acad Sci USA **96:** 14165–14170

Mittler R, Rizhsky L (2000) Transgene-induced lesion mimic. Plant Mol Biol **44:** 335–344

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) The reactive oxygen gene network in plants. Trends Plant Sci **9:** 490–498

Moeder W, Barry CS, Tauriainen AA, Betz C, Tuomainen J, Utriainen M, Grierson D, Sandermann H, Langebartels C, Kangasjärvi J (2002) Ethylene synthesis regulated by biphasic induction of 1-aminocyclopropane-1-carboxylic acid synthase and 1-aminocyclopropane-1-carboxylic acid oxidase genes is required for hydrogen peroxide accumulation and cell death in ozone-exposed tomato. Plant Physiol **130:** 1918–1926

Møller SG, McPherson MJ (1998) Developmental expression and biochemical analysis of the *Arabidopsis atao1* gene encoding an H_2O_2-generating diamine oxidase. Plant J **13:** 781–791

Montillet J-L, Chamnongpol S, Rustérucci C, Dat J, Van de Cotte B, Agnel J-P, Battesti C, Inzé D, Van Breusegem F, Triantaphylidès C (2005) Fatty acid hydroperoxides and H_2O_2 in the execution of hypersensitive cell death in tobacco leaves. Plant Physiol **138:** 1516–1526

Nakagami H, Kiegerl S, Hirt H (2004) OMTK1, a novel MAPKKK, channels oxidative stress signaling through direct MAPK interaction. J Biol Chem **279:** 26959–26966

op den Camp RGL, Przybyla D, Ochsenbein C, Laloi C, Kim C, Danon A, Wagner D, Hideg E, Göbel C, Feussner I, et al (2003) Rapid induction of distinct stress responses after the release of singlet oxygen in Arabidopsis. Plant Cell **15:** 2320–2332

Örvar BL, McPherson J, Ellis BE (1997) Pre-activating wounding response in tobacco prior to high-level ozone exposure prevents necrotic injury. Plant J **11:** 203–212

Overmyer K, Brosché M, Pellinen R, Kuittinen T, Tuominen H, Ahlfors R, Keinänen M, Saarma M, Scheel D, Kangasjärvi J (2005) Ozone-induced programmed cell death in the Arabidopsis *radical-induced cell death1* mutant. Plant Physiol **137:** 1092–1104

Overmyer K, Tuominen H, Kettunen R, Betz C, Langebartels C, Sandermann H Jr, Kangasjärvi J (2000) Ozone-sensitive Arabidopsis *rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell **12:** 1849–1862

Pavet V, Olmos E, Kiddle G, Mowla S, Kumar S, Antoniw J, Alvarez ME, Foyer CH (2005) Ascorbic acid deficiency activates cell death and disease resistance responses in Arabidopsis. Plant Physiol **139:** 1291–1303

Pellinen R, Palva T, Kangasjärvi J (1999) Subcellular localization of ozone-induced hydrogen peroxide production in birch (*Betula pendula*) leaf cells. Plant J **20:** 349–356

Pennell RI, Lamb C (1997) Programmed cell death in plants. Plant Cell **9:** 1157–1168

Polle A (2001) Dissecting the superoxide dismutase-ascorbate-glutathione-pathway in chloroplasts by metabolic modeling: computer simulations as a step towards flux analysis. Plant Physiol **126:** 445–462

Rao MV, Davis KR (2001) The physiology of ozone induced cell death. Planta **213:** 682–690

Ren D, Yang H, Zhang S (2002) Cell death mediated by MAPK is associated with hydrogen peroxide production in *Arabidopsis*. J Biol Chem **277:** 559–565

Robson CA, Vanlerberghe GC (2002) Transgenic plant cells lacking mitochondrial alternative oxidase have increased susceptibility to mitochondria-dependent and -independent pathways of programmed cell death. Plant Physiol **129:** 1908–1920

Rustérucci C, Montillet J-L, Agnel J-P, Battesti C, Alonso B, Knoll A, Bessoule J-J, Etienne P, Suty L, Blein J-P, et al (1999) Involvement of lipoxygenase-dependent production of fatty acid hydroperoxides in the development of the hypersensitive cell death induced by cryptogein on tobacco leaves. J Biol Chem **274:** 36446–36455

Samuel MA, Ellis BE (2002) Double jeopardy: both overexpression and suppression of a redox-activated plant-mitogen-activated protein kinase render tobacco plants ozone sensitive. Plant Cell **14:** 2059–2069

Samuel MA, Hall H, Krzymowska M, Drzewiecka K, Hennig J, Ellis BE (2005) SIPK signaling controls multiple components of harpin-induced cell death in tobacco. Plant J **42:** 406–416

Souter M, Lindsey K (2000) Polarity and signalling in plant embryogenesis. J Exp Bot **51:** 971–983

Tiwari BS, Belenghi B, Levine A (2002) Oxidative stress increased respiration and generation of reactive oxygen species, resulting in ATP depletion, opening of mitochondrial permeability transition, and programmed cell death. Plant Physiol **128:** 1271–1281

Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr Opin Plant Biol **8:** 397–403

Vacca RA, de Pinto MC, Valenti D, Passarella S, Marra E, De Gara L (2004) Production of reactive oxygen species, alteration of cytosolic ascorbate peroxidase, and impairment of mitochondrial metabolism are early events in heat shock-induced programmed cell death in tobacco Bright-Yellow 2 cells. Plant Physiol **134:** 1100–1112

Van Camp W, Willekens H, Bowler C, Van Montagu M, Inzé D, Reupold-Popp P, Sandermann H Jr, Langebartels C (1994) Elevated levels of superoxide dismutase protect transgenic plants against ozone damage. Biotechnology **12:** 165–168

Vandenabeele S, Van Der Kelen K, Dat J, Gadjev I, Boonefaes T, Morsa S, Rottiers P, Slooten L, Van Montagu M, Zabeau M, et al (2003) A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. Proc Natl Acad Sci USA **100:** 16113–16118

Vanderauwera S, Zimmermann P, Rombauts S, Vandenabeele S, Langebartels C, Gruissem W, Inzé D, Van Breusegem F (2005) Genome-wide analysis of hydrogen peroxide-regulated gene expression in Arabidopsis reveals a high light-induced transcriptional cluster involved in anthocyanin biosynthesis. Plant Physiol **139:** 806–821

van Doorn WG, Woltering EJ (2005) Many ways to exit? Cell death categories in plants. Trends Plant Sci **10:** 117–122

Vranová E, Inzé D, Van Breusegem F (2002) Signal transduction during oxidative stress. J Exp Bot **53:** 1227–1236

Wagner D, Przybyla D, op den Camp R, Kim C, Landgraf F, Lee KP, Würsch M, Laloi C, Nater M, Hideg E, et al (2004) The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. Science **306:** 1183–1185

Yoshinaga K, Arimura S-I, Niwa Y, Tsutsumi N, Uchimiya H, Kawai-Yamada M (2005) Mitochondrial behaviour in the early stages of ROS stress leading to cell death in *Arabidopsis thaliana*. Ann Bot (Lond) **96:** 337–342

Young TE, Gallie DR (2000) Programmed cell death during endosperm development. Plant Mol Biol **44:** 283–301

Zago E, Morsa S, Dat JF, Alard P, Ferrarini A, Inzé D, Delledonne M, Van Breusegem F (2006) Nitric oxide- and hydrogen peroxide-responsive gene regulation during cell death induction in tobacco. Plant Physiol **141:** 404–411