PROGRAMMED CELL DEATH IN
PLANT-PATHOGEN INTERACTIONS

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ABSTRACT
Plants cope with pathogen attacks by using mechanisms of resistance that rely both on preformed protective defenses and on inducible defenses. The latter are the most well studied, and progress is being made in determining which induced responses are responsible for limiting pathogen growth. Many plant-pathogen interactions are accompanied by plant cell death. Recent evidence suggests that this cell death is often programmed and results from an active process on the part of the host. The review considers the roles and possible mechanisms of plant cell death in response to pathogens.

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INTRODUCTION

Plants’ defense mechanisms against pathogens divide into two classes: those that are present constitutively [so-called preformed defenses (58 and references therein)] and those that are induced upon exposure to a pathogen. The latter class of defense mechanisms has been the subject of intense interest because of the possibility of exploiting these natural inducible defenses to engineer broad-spectrum pathogen resistance. Although some pathogens do not appear to elicit a robust defense response on their hosts (e.g. 13), some pathogens do elicit plant defenses but can still parasitize the host, possibly because some pathogens can grow or develop faster than the host can elaborate its defenses or because the pathogen can tolerate the induced defenses or detoxify them (e.g. 87). In this context, it is useful to consider that plant-pathogen interactions are dynamic: There may be much communication between the host and the parasite, and the outcome of the interaction may reflect the attempts of the host and the parasite to adapt to defenses and virulence factors, respectively. The most robust resistance to pathogens often occurs when a plant can specifically recognize the pathogen and rapidly induce a variety of potential defenses that limit its growth and/or development.

Often the interaction between a plant and a pathogen results in plant cell death. Plant cell death can occur when the pathogen unsuccessfully parasitizes the host as well as when the pathogen successfully causes disease. The effects of host cell death on a pathogen may vary greatly depending on the lifestyle of the parasite. For example, some pathogens are obligate parasites that depend on living cells to grow and develop. Other pathogens may benefit from the release of nutrients from dead cells. The purpose of this review is to reevaluate the role and regulation of plant cell death during plant-pathogen interactions and to examine the relationship between cell death and the activation of other defenses.

THE ROLE OF CELL DEATH DURING PATHOGENESIS

When a plant is infected by a pathogen that can grow extensively, the interaction is called “susceptible.” Cell death during such a susceptible interaction may benefit pathogens that do not rely on living tissue to grow and develop. Cell death can be useful for obtaining nutrients and providing a reservoir for pathogen dissemination if the pathogen is released onto the surface of the plant. However, is cell death really beneficial to the pathogen? If, for example, the cell death is accompanied by dehydration in a dry environment, it may be deleterious to the pathogen. To answer this question, it is important to look at
the whole life cycle of a pathogen as well as the environmental conditions in which it grows. For example, many foliar bacterial pathogens that cause the death of the host leaf cells are thought to gain access to plant tissue through wound sites. These pathogens, such as Xanthomonads and Pseudomonads, then multiply between the plant cells in the apoplasm. However, it is unlikely that cell death per se is required for the multiplication of these bacteria. This is because there are natural plant isolates (84) and mutants (8) that are “tolerant” to bacteria; that is, they show little or no symptoms after pathogen attack and yet they support growth of the pathogen that is equal to that seen on plants where symptoms occur. These natural and induced mutations benefit the plant because less cell death occurs during the infection. However, note that bacteria may persist longer in such tolerant tissue, and when the leaves senesce and fall into the soil they may provide a higher reservoir of bacteria than leaves that show cell death. Release of pathogens onto the surface of these natural variants and mutants and long-term survival of pathogens within these tissues need to be tested to clarify the role of cell death in susceptible interactions.

CELL DEATH MECHANISMS DURING SUSCEPTIBLE INTERACTIONS

How cell death occurs during susceptible interactions is not well understood for most plant-pathogen combinations. Tolerant mutants and natural variants that show decreased cell death during susceptible interactions but normal pathogen growth may provide clues about the mechanisms of cell death. One of three possibilities seems likely to explain the basis of pathogen-induced cell death: A toxin from the microbe might directly kill the plant cells, a secreted virulence factor might cause the plant to kill itself by causing a metabolic catastrophe (such as the disruption of membrane integrity), or an endogenous cell death program might be triggered. In one case, a mutant of Arabidopsis that was tolerant of both Pseudomonas syringae (the causal agent of leaf spot disease) and Xanthomonas campestris (the causal agent of black rot), ein2, was identified among mutants that were isolated because of their insensitivity to the hormone ethylene (8). Mutations in other loci that also caused ethylene insensitivity showed normal symptom development. These observations suggest that either the ein2 mutation is less leaky than the other ethylene-insensitive mutations or that the ein2 mutations are pleiotropic and affect both the perception of ethylene and the perception of an additional pathogen-derived signal. The possibility that ethylene has a role in symptom development is intriguing because this hormone is required for the timing of the onset of senescence in Arabidopsis (34) and fruit ripening in tomato (57, 70), two
processes that result in the death of cells in processes that require de novo protein synthesis. The possible involvement of hormones and other host factors in modulating cell death responses to pathogens suggests that symptom development is not necessarily due to toxic microbial products or toxic plant products. Additional evidence that cell death during susceptible interactions is genetically programmed comes from the observation that many mutants of maize mimic pathogenic diseases at the level of the visible symptoms (45, 88, 89). However, these mutants have not been characterized extensively to determine whether they exhibit the expected biochemical markers that appear during a pathogen infection. The recent isolation of mutants of Arabidopsis that mimic leaf spot disease and do express typical biochemical defense markers seen during this disease suggests that the cell death associated with this disease is genetically determined (JT Greenberg, unpublished information).

Susceptibility to pathogens is often genetically conditioned. In the case of the tomato fungal pathogen *Alternaria alternata*, susceptibility of tomato to stem canker disease is conditioned by the *Asc* gene (17). This same gene is required for susceptibility to the *Alternaria* AAL-toxin, which causes cell death (17). Experiments with purified toxin on susceptible plants have implicated ethylene in symptom development. First, toxin-induced cell death and leaf epinasty. The latter symptom is a typical ethylene response of tomato plants. Measurement of ethylene levels of toxin-treated plants were increased, and application of ethylene inhibitors partially blocked symptom production (67). Because ethylene on its own does not induce cell death, it should be considered a modulator of cell death processes. The metabolic state of the plant also conditions susceptibility to the toxin. Thus, elevated pyrimidine biosynthetic intermediates blocked symptom development, whereas inhibiting pyrimidine biosynthesis alone gave symptoms similar to those induced by the toxin (67). Although the molecular basis of the cell death induced by toxin has not been determined, recently it was shown that the induced cell death is an example of programmed cell death (pcd) in plants (91). This is one of the first examples of pcd in plants that has many of the same characteristics as those seen in animal cells undergoing a common form of pcd called apoptosis. Thus, tomato protoplasts treated with toxin exhibit internucleosomal cleavage, DNA breaks with 3'OH ends indicative of endonucleolytic cleavage, membrane blebbing, and apoptotic bodies. As would be expected for cell death that is an active process, the induction of internucleosomal DNA fragments by toxin was dependent on protein synthesis (91). Intact tomato leaves treated with toxin also exhibited DNA cleavage (91). The same toxin also induced pcd in African green monkey kidney cells, suggesting that the mechanism of activation of pcd in plants and animal cells may be conserved (90).
Cell death that appears in many different susceptible interactions may be caused by pathogen-derived factors taking advantage of endogenous plant cell death programs to effect their functions, but this remains to be determined. In the case of AAL-toxin, the pathogen may have evolved to take advantage of pcd caused by low pyrimidine levels, which on its own caused cell death (see above). However, cells with inhibited pyrimidine synthesis have not been examined for apoptotic features. Pyrimidine levels may be limiting in senescing tissue and may serve as an endogenous trigger for cell death. Senescence itself is likely a form of pcd where nutrients are reapportioned from older tissues to reproductive ones and the older cells die under the control of newly synthesized protein (35). The strategy of a pathogen causing the host to commit suicide is common in animal-pathogen interactions (94), particularly with viruses (79). It will be interesting to determine if many plant pathogens harbor virulence factors that act by regulating host pcd or if cell death arises by many different mechanisms.

CELL DEATH DURING A RESISTANT RESPONSE

Overview of the Resistant Response

Sometimes when a plant is infected with a pathogen, it can mount a rapid defense response and effectively prevent the pathogen from growing and/or developing. Such an outcome of an interaction between a pathogen that normally causes a susceptible interaction and its host plant can occur if two conditions are met: First, the pathogen must harbor a gene called an avirulence (avr) gene, and second, the plant must harbor a single gene called a resistance (R) gene that allows the plant to specifically recognize a pathogen carrying the avirulence gene. Both functions must be present to limit pathogen growth and/or development, a phenomenon termed “gene-for-gene” resistance [reviewed by Keen (47)]. The mode of recognition of avr functions is not known in many cases. However, in some cases avr gene products are proteins that are either known to be directly secreted into the apoplasm (e.g. 38) or thought to be secreted directly into the plant cell using a specialized bacterial secretion apparatus (type III) common to both animal and plant pathogens (32). Not all avr products are proteins; for example, the avrD operon encodes proteins that synthesize a c-glycosyl lipids called a syringolides (81). Several R genes have been cloned that are each specific for a variety of different pathogens including *Pseudomonas syringae, Tobacco Mosaic Virus (TMV), Cladosporium fulvum*, and *Melampsora lini*. The remarkable finding about these R genes is that they are all similar in structure even though they are specific for pathogens with
radically different modes of pathogenicity, from an intracellular virus to an extracellular bacteria to fungi that are both intra- and extracellular. The notable features of several cloned \( R \) genes are that they have leucine-rich repeats that are suggestive of a domain that interacts with other proteins and a sequence that may encode a nucleotide-binding domain (82). Other plant genes involved in resistance include typical signal transduction molecules such as the Pto kinase and the Pti kinase of tomato (61, 97).

As alluded to above, resistance to pathogens that is conditioned by a gene-for-gene interaction is an active process. Attempts to understand the basis of resistance in many different plant-pathogen systems have revealed that many potential defense functions are induced during a resistance response. These include the de novo induction of many mRNA transcripts and proteins termed defense-related proteins. Often these transcripts and proteins are induced during a susceptible interaction as well as a resistant interaction, but the timing and abundance of the transcript or protein is either faster or higher, respectively, when resistance occurs. The same phenomenon has been observed for the production of plant antibiotics (phytoalexins). For example, in Arabidopsis plants undergoing a resistance response, phytoalexins accumulate to a higher level than in a susceptible interaction (30). Defense-related proteins and phytoalexins are proposed to be important for directly limiting pathogen growth. In some cases, there is direct evidence for antimicrobial activity of inducible defense-related genes and phytoalexins. For example, overproduction of the defense-related gene \( PR1a \) confers resistance to oomycete fungi (1), and loss of phytoalexin synthesis in Arabidopsis causes plants to become more susceptible to \( P. syringae \) pathogens (30). Some defense-related proteins require the production of the signal molecule salicylic acid (SA) to be induced during a resistance response. For example, this is the case for the TMV-induced resistance response in tobacco (19). Plants that cannot accumulate SA because of the presence of a transgene called \( nahG \) from \( Pseudomonas putida \), whose product converts SA into the inactive catechol, have a reduced capacity to limit pathogen growth during a resistance response (29).

Other potential defenses that are induced during a resistance response are alterations of plant cell walls: At least one protein is crosslinked in the cell walls possibly by dityrosine bridges in a process that is likely to depend on \( H_2O_2 \) (11, 12). Phenolic compounds also become crosslinked in the cell walls, which become lignified during the resistant response. Modifications of the cell wall induced during the resistant response protect the cell wall from digestion by pathogens (12). The \( H_2O_2 \) required for cell wall modifications may be supplied by the activation of a membrane-localized NADPH-dependent oxidase in a process termed the oxidative burst [reviewed by Mehdy (63)]. In
some cases, it appears that the H$_2$O$_2$ comes from the dismutation of superoxide in the apoplasm (3) generated from the oxidase. It is unclear whether this superoxide is dismutated spontaneously or enzymatically because no extracellular superoxide dismutase has yet been identified.

Additional changes that occur during the resistant response include the transient accumulation of Ca$^{2+}$ into cells (55) and the activation of H$^+/K^+$ exchange (2, 5), processes that may be involved in signaling (see below). Finally, most characterized resistance responses are accompanied by rapid cell death of the infected cells and to a limited degree the neighboring cells. This rapid cell death is termed the hypersensitive response (HR). Sometimes the HR is referred to interchangeably with a resistance response, but for the purpose of this review the HR refers only to cell death that is associated with the resistant response. Plants that have undergone a resistance response that includes the HR on one or a few leaves develop immunity to many other pathogens in the leaves that have not previously been exposed to pathogens. This immunity is called systemic acquired resistance, and it requires the signal SA for its induction (29).

Many of the diverse biochemical and cell biological changes that occur during the resistant response do not require the continuous exchange of information between the pathogen and the host, but rather are the result of an initial stimulus that sets in motion a cascade of plant responses. Evidence for this comes from two types of observations: A number of pathogen-derived molecules when applied to plants as purified products (called elicitors) appear to induce all aspects of the resistant response (4, 83). In addition, there are plant mutants that also bypass pathogen exposure to activate an apparent resistant response spontaneously (21, 37). The cascade of responses likely involves the activation of multiple signal transduction pathways because in the case of gene expression, many defense-related genes are activated with different kinetics in response to the same stimulus (e.g. see references 26, 37, 72). One of these transduction pathways is regulated by SA because this molecule is required for full resistance during avirulent Pseudomonas infection of Arabidopsis (19). However, SA is not responsible for regulating phytoalexin production (JT Greenberg, unpublished information) or LOX1 gene expression (64), two defense-related responses that are differentially regulated during the resistant response (30, 64).

**Hypersensitive Cell Death Function**

Because the resistance response is correlated with diverse biochemical and cell biological changes, it is often unclear whether all these changes are necessary
for the limitation of pathogen growth and/or development. It is possible that
different pathogens are affected by distinct aspects of the resistant response or
that some potential defenses act in a redundant fashion. In the case of the HR,
it has long been speculated that the cell death is directly responsible for
limiting pathogen growth and development. Evaluation of this hypothesis in
the absence of a way to block only the HR during a resistant response is
difficult. In the case of fungal organisms that must develop within live plant
cells to grow, it is possible to make limited inferences about the role of the HR
in the resistance response. This is because it is possible to detect the stage at
which a fungus is arrested and determine if it occurs before, concomitant with,
or after the onset of cell death. In many cases this level of detail has not been
characterized. However, one elegant study by Gorg et al (33) examined the
timing of cell death in the interaction between powdery mildew and barley.
Different R genes cause barley to arrest powdery mildew at distinct steps in
development. In the case of the Mlg resistance locus, the fungus is arrested
before the establishment of the haustorium, and the percentage of germinated
spores that are arrested is dependent on the gene dosage of Mlg. Thus, one
copy of Mlg causes 80% arrest, and two copies causes 100% arrest. However,
upon close examination, infections of heterozygous plants were not accompa-
nied by cell death at the time of the arrest of fungal development. Homozygous
plants showed cell death concomitant with the developmental arrest. The
authors suggested that because arrest could occur before cell death in heterozy-
gous plants that cell death was not an obligatory step in achieving resistance.
However, they suggested that cell death might be a back-up defense that
ensures quantitative resistance to the fungus by the creation of a highly antimi-
crobial environment. It is worth noting in this context that powdery mildew is
an obligate biotroph that causes disease by establishing a haustorium in a
living plant cell and then undergoes further development and reproduction.
This pathogen cannot establish an infection if cell death occurs. In contrast to
the Mlg mechanism, resistance to powdery mildew conditioned by the Mla
locus causes arrest later in the development of the fungus at the haustorium
stage and is always accompanied by cell death. However, a gene dosage
experiment similar to that done with Mlg plants has not been performed.

Because fungal arrest can occur at different stages of development, it is
difficult to generalize about the role of cell death in directly affecting fungal
growth. Cell death patterns can also vary greatly during a resistance response
triggered by obligate biotrophic fungi. For example, *Peronospora parasitica*
can cause at least six distinct cell death patterns depending on which R gene
the host carries (42). In some cases cell death occurs only in the infected cell,
and in some cases the cell death occurs distal to the site of infection. These
different patterns of cell death may reflect different roles that cell death plays in pathogen limitation. Implicit in this discussion is the idea that \( R \) genes may not all activate exactly the same distribution of potential defenses judging from the cell death pattern as well as the developmental stage at which the pathogen is arrested. This is further supported by the observation that some defense-related genes are induced during a resistant response only when a specific \( R \) gene is present (72, 73), and different physiological changes can be mediated by different \( R \) genes (39). It is likely that there are a core set of events that occur during a resistant response and that these are added upon by the particular \( R \) gene that has evolved to recognize the appropriate \( avr \) product. For example, even though the Arabidopsis \( R \) genes \( Rpm1 \) and \( Rpt2 \) recognize \( avrRpm1 \) and \( avrRpt2 \) genes, respectively, and activate some defense-related genes in an \( Rpm1 \)- or \( Rpt2 \)-specific manner, both \( R \) genes share at least one signal transduction step. This is evidenced by the phenotype of Arabidopsis \( ndr \) mutants, which are unable to mount effective resistance against bacteria carrying either \( avrRpt2 \) or \( avrRpm1 \) (15).

It is straightforward to imagine that cell death may play a role in quantitative resistance to biotrophic fungi because they need living host cells to achieve a successful level of parasitism. How might cell death influence growth of pathogens other than obligate biotrophic fungi? In the case of intracellular pathogens such as viruses, cell death might affect the plasmodesmata through which the virus travels from one cell to another (20). In the case of a bacterial pathogen, dehydration, which often accompanies the HR, might cause a decrease in growth or long-term survival within the plant tissue. Cell death may also cause the release of defense-related proteins and metabolites into the apoplasm where bacterial and some fungal pathogens reside.

**The HR: An Example of Programmed Cell Death**

Pcd is the formal term used to describe cell death that is genetically programmed and thus requires active participation by the host. The term pcd implies nothing about the mechanism by which the cell death occurs. That the HR is likely a form of pcd and not due to the action of pathogen-secreted toxins that directly kill the host is bolstered by several observations. First, plants must have active protein synthesis to show an HR induced by bacteria (18, 48). Second, purified bacterial elicitors of the HR require plants to have active metabolism to show cell death (40). These same elicitors induce the expected array of defense-related biochemical markers (83). Third, overproduction of a component of the \( R \) gene \( Pto \) signal transduction pathway of tomato expressed in tobacco caused an amplification of the HR. Finally,
Arabidopsis mutants that show an apparent HR spontaneously or in response to aberrant stimuli argue that the HR is genetically controlled and coordinately regulated with other defense-related biochemical events typically seen during the resistant response (21, 37). Some maize mutations at the complex resistance locus rpl also result in spontaneous cell death lesions, indicating that cell death is genetically controlled and can be caused by mutations in a signal transduction component of the resistance response (43). These observations prompted a number of groups to examine whether the pcd observed in plants might be similar to pcd observed in animals when they undergo a common type of pcd called apoptosis. Typical apoptotic events include the fragmentation of DNA into ~50-kDa pieces and/or internucleosomal-sized fragments with 3’OH ends indicative of endonucleolytic cleavage (so-called DNA ladders), membrane blebbing, nuclear condensation, and fragmentation with nucleic acids found in membrane-bound vessels (apoptotic bodies) and cytoplasmic condensation. One or more of these parameters has been examined in a few plant-pathogen systems when the HR is occurring. Tomato protoplasts treated with the HR elicitor arachidonic acid showed DNA ladders and 3’OH DNA ends (91). Soybean culture cells, intact soybean, and Arabidopsis tissue all showed DNA fragmented into large pieces and a morphology suggestive of apoptotic bodies (55). In this case, the authors found that the apparent apoptosis was only elicited by bacteria harboring an avr gene that could be recognized by plant cells with the corresponding R gene. In soybean suspension cultures, induction of the HR by bacteria was an active process that required Ca\(^{2+}\) transport and kinase activity (55).

Ryerson & Heath (74) found that cowpea infected with the obligate fungal parasite Uromyces vignae showed in situ evidence of 3’OH DNA ends as well as DNA laddering. Killing the cells by abiotic treatment generally did not elicit DNA laddering (74), except for KCN (74, 91). Tomato protoplasts treated with the kinase inhibitor staurosporine showed DNA fragmentation, whereas soybean suspension cells treated with this drug blocked elicitor-induced oxidative burst (56). Finally, tobacco infected with TMV showed in situ evidence of 3’OH ends in the DNA. Although diverse pathogens exhibited one or more markers of apoptosis on a variety of different plants, the HR of lettuce after bacterial infection did not show apoptotic morphological features (9). This may indicate that there are two or more mechanisms of effecting pcd during the HR. Alternatively, pcd in lettuce may share some features with the other systems listed above but then diverges at a step that determines cell morphology. For example, DNA fragmentation mediated by endonucleases may occur in lettuce, although it has not yet been examined. In animals, though apoptosis
is a common way for pcd to occur, there is at least one other alternative mechanism of pcd that has been observed during development (77).

**Regulation and Execution of the HR**

The HR is correlated with the oxidative burst, membrane damage, ion fluxes, endonuclease activation, DNA cleavage, and gene expression. It is not clear a priori which of these events may be involved specifically in the regulation or execution of the HR and which may be involved in the other aspects of the resistance response. To sort out these questions, a number of laboratories have tried to determine the role of individual changes that occur during the resistant response that might be specifically related to the regulation or execution of the HR. In particular, much emphasis has been placed on understanding the role and regulation of the oxidative burst and its relationship to the HR. In some systems, it is possible to detect superoxide in the apoplast of cells undergoing an HR (3, 22), whereas in other systems only H$_2$O$_2$ accumulates to detectable degrees (e.g. 56). The oxidative burst (superoxide and/or hydrogen peroxide) proceeds cell death, which also makes it a candidate for a source of signaling molecules. In animal cells, oxidative stress activates apoptosis by two independent signaling mechanisms (76), making the oxidative burst in plant cells a prime candidate for the HR trigger in plants.

To determine whether the oxidative burst is responsible for triggering the HR, Glazener et al (31) used bacterial mutants, which are defective in eliciting the HR because of a mutation in the hrp locus (a locus important for the secretion of virulence and avirulence factors), to infect tobacco cells in culture and monitored plant responses. They found the production of active oxygen, detected as H$_2$O$_2$, using this hrp$^-$ strain was indistinguishable from that elicited by a wild-type strain. However, plant cell death did not occur with the hrp$^-$ strain. Thus, using the hrp$^-$ strain, the oxidative burst was uncoupled from the cell death response (the HR). However, Levine et al (56) have found that the oxidative burst, manifest as H$_2$O$_2$ production in their soybean suspension culture system, might play a role in triggering the HR. The oxidative burst induced by the elicitor Pmg, a glucan from the mycelial walls of the fungal pathogen Phytophthora megasperma f. sp. glycinea, is completely blocked by the kinase inhibitors K252A and staurosporine. When H$_2$O$_2$ production was enhanced by inhibiting catalase during an HR elicited by avirulent bacteria, the amount of cell death was greatly increased (56). In addition, if the oxidative burst was blocked by an inhibitor of NADPH oxidase (which generates the oxidative burst) or the kinase activity was blocked by K252A, the amount of cell death of the plant cells induced by avirulent *Pseudomonas* was decreased.
by a factor of two. The fact that K252A completely blocked the oxidative burst by Pmg elicitor but did not completely inhibit cell death induced by avirulent bacteria suggests that H₂O₂ may not act alone to trigger cell death. However, since the oxidative burst was not monitored after avirulent bacteria inoculation in the presence of K252A, it is also possible that K252A completely inhibits the oxidative burst by Pmg but not by avirulent bacteria. Thus, the residual active oxygen may remain when soybean cells are treated with K252A and avirulent bacteria. The results of Levine et al (56) and Glazener et al (31) indicate that H₂O₂ may not be sufficient to account for all the cell death that is observed during the resistant response. However, it is not clear whether activation of all resistance responses results in the same amount of H₂O₂ production. One possibility is that if the concentration of H₂O₂ is high enough, additional signals are not necessary. This might account for the observation that 8 mM H₂O₂ was required to induce apoptosis in suspension cultures of soybean when it was estimated that the amount of H₂O₂ that is generated during the oxidative burst of tobacco cells in response to bacteria is much lower, about 12 µmol • min⁻¹ (31 and references therein). It has been suggested that because catalase may be inhibited by SA (16), a rise in H₂O₂ at the site of lesion formation might contribute to the coordinated activation of cell death (54).

It is also possible that superoxide is involved in triggering cell death. Infiltration of superoxide dismutase into tobacco leaves infected with TMV compromised the development of the HR (25). Recently it was shown that the lsd1 mutant of Arabidopsis, which shows an apparent HR after shifting uninfected plants from short-day to long-day growth conditions, accumulates superoxide in the apoplasm of leaf tissue (44). These observations point to a possible function for superoxide in regulating the initiation and/or extent of cell death during the HR. Because both plants and animals show apoptosis in response to oxidative signals, it will be interesting to determine whether there is any similarity to the mechanism of apoptotic activation in these highly diverged systems.

Other potential signals for HR induction are the flux and exchange of ions. Early during the resistance response, there is an efflux of K⁺. A hint that ion fluxes do play an important role in regulating the HR comes from tobacco plants that constitutively express the bacterio-opsin (bO) protein, a bacterial proton pump from Halobacterium halobium that requires rhodopsin for active proton pumping in bacteria. Such transgenic plants showed an apparent spontaneous HR accompanied by 3′OH DNA ends indicative of endonucleolytic processing and expressed many defenses normally seen during a resistance response (66). In addition, bO-expressing tobacco showed elevated levels of DNA endonucleases that were also activated during a TMV-induced resistance
response on tobacco (66). Tobacco plants expressing a mutant form of the bO that should not form a functional ion channel did not show any features of the defense response. A key question in interpreting these experiments is whether the bO protein is acting by promoting passive or active translocation of protons. To do the latter, it would need to bind rhodopsin, which is not known to be present in higher plants. To translocate protons even passively, the bO protein would need to be localized to the plasma membrane to function as an ion channel and activate the resistance response downstream of pathogen recognition.

Ca\(^{2+}\) fluxes may also play a role in the execution of the HR. Blocking Ca\(^{2+}\) ion channels reduces cell death of soybean suspension cells in response to avirulent bacteria or H\(_2\)O\(_2\) (55). Treatment of such plant cells with a Ca\(^{2+}\) ionophore also induced pcd. In the case of soybean suspension cells, Ca\(^{2+}\) fluxes were not associated with the expression of defense related genes that were induced by H\(_2\)O\(_2\) (55). Because Ca\(^{2+}\) was also required for activation of DNA endonucleases that are associated with the resistance response (65), it is possible that DNA cleavage is a necessary step in the suicide process. Conversely, DNA breakdown may occur after the cell has committed to a cell death program and may facilitate recycling of cellular constituents.

Because the membrane changes its properties during the resistant response, it is possible that lipid-based signals are responsible for regulating the HR. Because of the differential activation and/or localization of lipoxygenase and phospholipase D, respectively, during the resistance response, it has been suggested that the products of these particular enzymatic reactions might be active as signal molecules in the activation of the HR (18, 96). Interestingly, lipoxygenase enzymes generate hydroperoxidized lipids that have been seen to induce apoptosis in animals (75).

Using pharmacological, genetic, and transgenic approaches, it seems clear that ion channels are involved in signaling the onset of cell death. Other steps in the regulation of the HR include phosphorylation and dephosphorylation (27, 55, 97) and protease activity (56). In most cases, the particular molecules involved in these activities have not been identified, but their requirement for HR regulation has been inferred using pharmacological agents that may not be specific for only one target. Many questions remain open about the regulation and execution of the HR. Are H\(_2\)O\(_2\) and O\(_2^-\) obligatory signals in the induction of the HR? If other signals exist, what are they? Could they be lipid peroxides? What is the role of DNA cleavage? Are there HR-specific proteases as there are apoptosis-specific proteases in animals? What are the targets that become phosphorylated, dephosphorylated, or proteolyzed?
Answering these questions should contribute to a full understanding of the regulation and function of pcd resulting from plant-pathogen interactions.

**CELL DEATH DURING SYSTEMIC ACQUIRED RESISTANCE**

Plants that have undergone a resistance response on one part of a plant become more resistant at distal sites to subsequent attack by pathogens that would normally cause a susceptible interaction. As mentioned above, this type of resistance is called systemic acquired resistance (SAR) and requires the signal molecule SA (29). In addition, some pathogens that normally cause a resistance response on a particular host will apparently be limited to an even greater extent if these plants have SAR. Direct application of SA, a synthetic analog of SA, 2,6-dichloroisonicotinic acid (INA), or benzothiadiazole (BTH) to plants triggers the synthesis of the same biochemical markers that appear during induction of SAR by a biological agent (28, 52, 85, 92). It is possible that SA, INA, and BTH activate SAR at the same target (52, see 93 and references therein). These chemicals have been used to study basic aspects of the regulation of SAR. The observation that pathogens that normally can cause disease on a host can be prevented from doing so because of SAR has prompted several laboratories to investigate the possible mechanism of pathogen growth and/or developmental arrest. Remarkably, susceptible barley plants that are treated with INA and then are infected with powdery mildew show developmental arrest of the fungi accompanied by rapid plant cell death. These arrested fungi apparently look identical to those that are arrested because of infection of plants carrying the Mlg resistance gene (49). This type of observation is not unique to barley: Arabidopsis plants treated with INA or a pathogen that induces SAR show rapid cell death when infected by a normally pathogenic downy mildew isolate that does not cause cell death on control plants (62, 86). Furthermore, the induction of rapid cell death is not limited to infections by biotrophic pathogens because Arabidopsis with SAR induced by avirulent *P. syringae* also shows rapid cell death when infected with virulent *P. syringae* (14). Tobacco plants treated with SA also show a rapid cell death response when infected with the soft-rot pathogen *Erwinia carotovora* subsp. *carotovora* (71). Other cases of rapid cell death induced by normally virulent pathogens on immunized plants were noted by Kuc (50).

What might be the basis of this rapid cell death? One possibility is that induction of SAR causes either the induction or the modification of latent plant receptors that can recognize products secreted by normally virulent pathogens. If this is the case, then there may be a range of such receptors that can
recognize products from all kinds of pathogens such as one finds for \( R \) genes. An alternative model is that some plant product that is generated during virulent pathogen attack is perceived by the plant differently if the plant has elevated levels of SA. In this view, SA induces a “receptor” to a plant product instead of a pathogen-derived product. A role for SA in modifying the regulation of a defense response other than cell death has been reported. For example, SA treatment or bacterial infection of tobacco induces the defense-related transgene fusion \( AoPR1-GUS \) only modestly, but pretreatment of the plants with SA before bacterial infection caused a 10-fold increase in the induction of the fusion (68). SA itself is also subject to the potentiating stimulus ethylene. Thus, treatment of plants with low levels of SA that did not cause defense-related gene activation caused the defense-related gene \( PRI \) to become inducible by ethylene. Ethylene alone did not induce \( PRI \) (53).

The cell death that occurs on plants with activated SAR may be triggered in a similar way as the HR. In this regard, it is intriguing that treating parsley suspension cells with SA causes them to undergo an amplified oxidative burst when exposed to fungal elicitor (46). This amplified oxidative burst is blocked by the same kinase inhibitor, K252A, that blocks the oxidative burst that occurs because of a resistant response (56). An alternative hypothesis is that cell death induced by pathogens is a default response of plants that pathogens have evolved to suppress except in special cases, such as when \( \text{avr} \) genes are present. Suppressors of the HR derived from virulent fungi have been documented (23, 24). Cell death in immune plants could be caused by an ability of plants to inhibit cell death suppressors.

Although SAR causes plants to undergo a rapid cell death response with normally virulent bacterial and fungal pathogens, it causes a suppression of cell death when plants are infected with avirulent viruses. These viruses normally cause localized cell death during a resistance response. When plants with a functional \( R \) gene that conditions resistance to an avirulent virus have also undergone SAR, the cell death response of the plants is decreased, as shown by a decrease in lesion size. This observation has been interpreted to mean that plants become even more resistant to the virus. Plants that cannot accumulate SA show an increase in lesion size upon viral infection (29). How should a change in lesion size be interpreted? First it is important to establish the underlying assumptions about what regulates lesion size. Lesion size may reflect at least two things: the rate of viral movement relative to the onset of cell death and/or the amount of cell death after viral recognition. Cell death during a resistant response is not entirely cell autonomous: Maize plants mosaic for the \( RP1 \) resistance gene showed some cell death in the neighboring \( rp1 \) tissue after infection with \( Puccinia sorgum \), if the recognition of pathogen
occurred in the RP1 tissue sectors (7). Thus, lesion size may not be a reflection of pathogen replication as much as it is one of the activation of plant cell-cell communication that results in cell death. Arabidopsis plants that cannot accumulate SA exhibit more cell death when exposed to ozone (78). Sharma et al (78) suggested that this may be because of the absence of antioxidants that are regulated by SA. At least one catalase gene was activated by SA in potato plants (69). Arabidopsis also showed increased catalase in SA-treated tissue (A Castillo, P Hradecky & JT Greenberg, unpublished information). SA also induces Mn-superoxide dismutase in tobacco (10). It appears that SA may serve to both condition the activation of a cell death response and regulate the extent of cell death at the same time. These roles for SA are also consistent with the observation that some mutants of Arabidopsis (lsd6 and lsd7, lesions simulating disease) that spontaneously accumulate cell death patches show suppressed cell death if SA levels are decreased (93).

CELL DEATH AND THE INDUCTION OF DEFENSES

Because SAR occurs at or after the time of HR development and occasionally after susceptible interactions that involve cell death, it has been widely assumed that there is a causal relationship between cell death and SAR establishment. It has been found, for example, that more numerous TMV lesions are correlated with more SA production (95). However, in some cases, the amount of cell death did not correlate with the amount of resistance observed in plants (14). Excision of a cucumber leaf undergoing a resistant response 6 h after treatment with *P. syringae* still allowed development of SAR even though cell death would not occur on the primary infected leaf until 24 h after inoculation. Thus, SAR can be triggered before any cell death (80). It is also unlikely that cell death per se causes SAR, because many situations can arise where plant cells die yet no SA or SAR ensues. Senescent leaves did not trigger SAR in young tissues, even though senescence is a form of pcd (see reference 35; JT Greenberg, unpublished information). Cell death occurs throughout plant development without triggering SAR (35). Plant cells killed by wounding (51, 60), freezing (59), or leaf spot disease (14) do not elicit SAR. Several Arabidopsis mutants that showed spontaneous cell death (21; JT Greenberg, unpublished information) did exhibit SAR. The connection between cell death and SAR may simply be that cell death occurs fortuitously at the same time as the development of SAR. One possibility is that when a resistance response is triggered, a number of pathways are coordinately regulated, including one for cell death and one for SAR, and one or more for other local defenses. Thus, one event in the infected leaf might lead to SAR development at distal sites...
and also result in a signal to generate localized cell death. In this scenario, cell death and SAR are coupled because of an early event, but cell death does not cause SAR.

CONCLUDING REMARKS

Cell death occurs in response to diverse pathogens and appears to occur by at least two pathways: one that resembles apoptosis that is seen in animal systems and one that resembles necrotic cell death morphologically, but that may also be an example of programmed cell death. Recognition of pathogens in a gene-for-gene interaction is only one of several ways that programmed cell death is triggered. It is likely that many factors influence the control of cell death during pathogenesis as exemplified by the diversity of cell death patterns seen after pathogen attack and by the numerous cell death defense mutants in maize and Arabidopsis (21, 36, 37, 45, 93). An emerging area of interest is how the metabolic state of the plant influences the cell death rate and patterns during plant-pathogen interactions. Indeed, alterations in sugar levels or modification of the ubiquitin pathway appear to trigger the HR spontaneously and alter cell death responses to pathogens (6, 41). Herbers et al (41) suggested that this may reflect a role for cell wall invertases in controlling pathogen-triggered cell death. What is clear is that cell death during plant-pathogen interactions is a common event that may be important for robust resistance as well as susceptibility to pathogens depending on the lifestyle of the pathogen in question.

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