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Review

The hypersensitive response and the induction of cell death in plants

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Abstract

The hypersensitive response, or HR, is a form of cell death often associated with plant resistance to pathogen infection. Reactive oxygen intermediates and ion fluxes are proximal responses probably required for the HR. Apoptosis as defined in animal systems is, thus far, not a strict paradigm for the HR. The diversity observed in plant cell death morphologies suggests that there may be multiple pathways through which the HR can be triggered. Signals from pathogens appear to interfere with these pathways. HR may play in plants the same role as certain programmed cell deaths in animals with respect to restricting pathogen growth. In addition, the HR could regulate the defense responses of the plant in both local and distant tissues.

Keywords: HR; oxidative burst; resistance; biotrophic and necrotrophic pathogens; suppression and negation of plant defenses

Abbreviations: HR, Hypersensitive Response; XR, ion fluxes; ROIs, Reactive Oxygen Intermediates; SA, Salicyclic Acid; SAR, Systemic Acquired Resistance

Introduction

Plants have evolved sophisticated and efficient mechanisms to prevent the invasion of their tissues by pathogens, and disease rarely occurs. One common feature of disease resistance is the rapid development of cell death at and immediately surrounding infection sites, called the Hypersensitive Response, or HR (Agrios, 1988; Goodman and Novacky, 1994). The HR can be triggered by a wide variety of pathogens and occurs within a few hours following pathogen contact. It is important to note that what plant pathologists traditionally call necrosis is not equivalent to necrosis in animal systems (by opposition to apoptosis). Rather, necrosis historically denoted a macroscopic phenomenon without mechanistic connotations. Cell death is also visible in the development of disease symptoms, but occurs temporally much later accompanying pathogen ingress. In this review, we will refer to HR as the development of cell death as a consequence of disease resistance, and to necrosis as the development of cell death during the process of disease. Note that this use of terms is still not intended to necessarily connote mechanistic differences.

The HR is often conditioned by the presence in the pathogen of an avirulence (*avr*) gene, the direct or indirect product of which is recognized by a plant possessing the corresponding resistance (*R*) gene. An interaction leading to disease is termed compatible and, when resistance is effective, the interaction is called incompatible. This specific pathogen recognition accounts for many, but not all, plant disease resistances (Dangl, 1995; Staskawicz *et al*, 1995). The simplest mechanistic model is that the *avr* gene encodes a ligand that is recognized by the product of the matching *R* gene which then triggers the HR and disease resistance (Bent, 1996). In addition, molecules from the pathogen called elicitors are able to trigger HR (Ebel and Cosio, 1994). Plant receptors are also thought to be involved in recognition of these elicitors (Nürnberger *et al*, 1994; Umemoto *et al*, 1997).

Subsequent to recognition, biochemical and metabolic plant modifications are well conserved among different plant-microbe interactions (Hammond-Kosack and Jones, 1996). Following pathogen recognition, constitutively expressed signal transduction pathways are engaged. A large set of inducible genes, commonly known as defense related genes, are expressed as resistance develops. They include enzymes involved in the synthesis of anti-microbial compounds called phytoalexins, structural proteins incorporated into the cell wall (Bradley *et al*, 1992), and the pathogenesis related (PR) proteins, some of which have known anti microbial activities (Schlumbaum *et al*, 1986). The induction of these defense genes is not specific to plant-pathogen interactions. Abiotic treatments and physical stresses have been shown to activate them (Brederode *et al*, 1990), and they often are expressed during normal development (Samac and Shah, 1991; Dangl, 1992).

While the mechanisms of cell death in animals have been studied in great detail, our understanding of the mechanism of cell death in plants is still poor. In plants, cell death is also invoked developmentally during xylogenesis, senescence, and reproduction (Hatfield and Bennett, 1997; Fukuda, 1997; Greenberg, 1996; Jones and Dangl, 1996). Here we will address the following key questions:

- Is the HR programmed?
- What is the cytological morphology of HR?
- How is the HR induced?

- Has the HR a causal role in disease resistance?
- Are there mechanistic differences between the cell death associated with the HR and that associated with disease?

As the HR may be driven by signals from both the host and the pathogen, a particular emphasis is given to the *context* of plant-microbe interaction in which this phenomenon occurs.

Is the HR programmed?

Several lines of evidence suggest that the HR results from the activation of an intrinsic program:

(1) A large class of plant mutants, called disease lesion mimics, show spontaneous cell death resembling HR or disease symptoms (Dangl *et al*, 1996). In a subset of disease lesion mimics (*lsd* and *acd* mutants), the development of cell death is associated with the induction of defense-related markers such as callose deposition, PR gene expression and heightened resistance to otherwise virulent pathogens (Dietrich *et al*, 1994; Greenberg *et al*, 1994). Therefore these mutants are likely to represent defects in the pathway leading to the HR and disease resistance and not simply metabolic perturbations triggering cell death. Two subclasses of *lsd* mutants were established based on the phenotypes observed. First, initiation mutants display lesions which are limited in size (e.g. the *lsd5* Arabidopsis mutant; Dietrich *et al*, 1994), and probably represent defects in the triggering of cell death. Second, propagation class mutants express lesions which, once initiated, spread and usually engulf the entire leaf (see *lsd1* and *lls1* below). These propagation mutants have been hypothesized to represent defects in mechanisms that negatively control HR (Walbot *et al*, 1983; Dietrich *et al*, 1994). Recently, Hu *et al* (1996) demonstrated that some maize lesion mimics are caused by mutations in the rust disease resistance gene *Rp1*, indicating that a mutant form of an *R* gene can also trigger pathogen-independent cell death.

(2) The HR requires active plant gene transcription and translation (He *et al*, 1994). Therefore it appears that the HR is an active process, genetically controlled, and does not necessarily or only result from damage caused by the pathogen.

(3) The expression of various transgenes in the plant sometimes results in the development of cell death reminiscent of HR (Dangl *et al*, 1996; Mittler and Lam, 1996). Despite the fact that these phenotypes could be due to perturbation of plant cellular homeostasis, it is interesting to note that in some cases the overexpressed transgene was previously implicated in plant-pathogen interactions. For instance, proton pump ATPases are active in the early steps of many defense responses (Atkinson and Baker, 1989; see below), and overexpression of a bacterial light-driven proton pump gene in tobacco results in the formation of lesions (Mittler *et al*, 1995).

(4) There is no requirement for the presence of a living pathogen to trigger the HR. For example, certain purified elicitors can induce many of the physiological changes occurring during disease resistance (Nürnberger *et al*, 1994) and lesions resembling the HR (He *et al*, 1993; May *et al*, 1996). Therefore the destructive potential of an active pathogen is not necessary. Purified pathogen phytotoxins can have similar effects (Gilchrist, 1997; Levine *et al*, 1996).

Thus, there are plant genes and signaling programs controlling the HR. The analysis of model systems, such as cell death control mutants and transgenic plants showing spontaneous lesions, is likely to provide useful information regarding the plant components involved during the HR, in absence of pathogen interference.

Morphologies of HR

In most studied pathosystems, pathogen infection is non-synchronous. This renders the chronological ordering of the cytological events leading to HR difficult. Several systems are utilized to describe the development of HR in living plant tissues where individual infection events can be followed. One well characterized system is the interaction between the biotrophic fungus *Uromyces vignae* and cowpea. At 15 h after inoculation during an incompatible interaction, Chen and Heath (1991) observed the following sequence of cytological events: (i) migration of the nucleus to the site of fungal penetration and intense cytoplasmic streaming, (ii) cessation of cytoplasmic streaming, Brownian motion of the organelles, condensation of the nucleus, accumulation of granules at the periphery of the cytoplasm, shrinkage of the protoplast and (iii) collapse of the cytoplasm and death of the infected cell. Similar cytological changes were observed in the interaction between *Erysiphe graminis* f.sp *hordei* and barley plants carrying the Mla_{12} resistance gene (Bushnell, 1981). These changes were not observed in an isogenic susceptible plant, indicating that they are under the control of the Mla_{12} resistance gene. The timing of these events has been precisely established using video microscopy during an incompatible interaction between *Phytophthora infestans* and potato. Only 26 s are necessary for plant cell collapse and death, and death of the fungus follows 20 s later (Freytag *et al*, 1994). Such rapid responses could make detection of intermediate steps almost impossible using fixed tissues.

As yet there is no specific molecular or cytological marker in plants which would allow clear discrimination between necrosis and the HR. Therefore recent investigations have often applied criteria established in animal systems. Some characteristics of animal apoptosis have been shown to occur in plants during interactions with pathogens or purified elicitors. Levine *et al* (1996) detected plasma membrane blebbing, cell shrinkage, condensation of both the cytoplasm and nucleus, and structures that might be interpreted as apoptotic bodies during the HR triggered by bacterial pathogens, but not in susceptible tissues. However, they did not detect DNA laddering. Fragmentation of nuclear DNA (but no DNA laddering) was observed in resistant tobacco plants infected with TMV

(Mittler *et al*, 1996). In contrast Ryerson and Heath (1996) demonstrated the presence of oligonucleosomal fragments during an incompatible interaction between *Uromyces vignae* and cowpea. HR-induced endonucleases may play a role in this process (Mittler and Lam, 1995). Finally, apoptotic bodies were also detected in isolated protoplasts from susceptible plants treated with the AAL-toxin (Wang *et al*, 1996). Because there is no known system in plants capable of scavenging such corpses, this finding begs the question of how the plant disposes of dead cell debris.

Thus there is so far no clear correlation between one particular morphology of cell death and either the HR or disease symptoms. There are only a few examples correlating disease symptoms with cytologically defined necrosis (as defined in animal systems) and resistance with apoptosis-like cell death (Levine *et al*, 1996). In other cases, resistance is associated with cytological changes reminiscent of animal necrosis (Bestwick *et al*, 1995). Although cell death in plants could functionally play the same role as in animals, it may be that the mechanisms underlying this process evolved differently (Mittler and Lam, 1996). Moreover, signals from both the plant and the pathogen can intervene to affect progression to cell death. Thus assessing cell death in the context of the interaction in which it occurs may facilitate our understanding.

Inducers, effectors and regulators of the HR

Some recent reviews provide detailed information concerning the induction and signal transduction leading to disease resistance (Bent, 1996; Hammond-Kosack and Jones, 1996) and here we will only review recent findings relevant to our understanding of the HR. At least two steps are necessary to induce the HR: recognition of the pathogen and transduction of the perceived signal(s) to the effector(s) of cell death (Figure 1).

How is the HR induced?

The specific pathogen recognition model suggests that the first event in triggering the HR could be the direct recognition of the pathogen *avr* gene product by the corresponding plant *R* gene product. Recent evidence indicates that there is such a direct interaction between the tomato *Pto* resistance gene product and the product of the avirulence gene *avrPto* from *Pseudomonas syringae* pv *tomato* (Scofield *et al*, 1997; Tang *et al*, 1997). To date, the analysis of the sequences of the different cloned resistance genes suggests that this possible type of direct interaction may not only happen in the plasma membrane but also in the cytoplasm (Jones, 1997) and in the nucleus (Leister *et al*, 1996; Van den Ackerveken *et al*, 1996).

The earliest changes observed following pathogen recognition are an oxidative burst, resulting in production of Reactive Oxygen Intermediates (ROIs) (Baker and Orlandi, 1995; Levine *et al*, 1994; May *et al*, 1996) and rapid ion fluxes across the plasma membrane, termed the XR (Atkinson and Baker, 1989). There is some debate concerning the nature of the ROIs involved. While recent studies suggest that H_2O_2 is sufficient to induce soybean cell death (Levine *et al*, 1994, 1996), compelling evidence indicates that superoxide radical ($O_2^{\cdot-}$) is the key ROI in triggering cell death in the Arabidopsis *lsd1* mutant (Jabs *et al*, 1996). Superoxide is weakly diffusable and could be dismutated to H_2O_2 or other diffusable, toxic ROIs, which then can cross or damage the plasma membrane. A membrane NADPH oxidase analogous to that found in mammalian neutrophils may be involved in this process (Groom *et al*, 1996). ROIs could also act as a signal via the production of lipid peroxides (May *et al*, 1996; Mehdy, 1994).

The XR is characterized by an uptake of Ca^{2+} (Levine *et al*, 1996) and export of Cl^- and K^+ driven by H^+-ATPases, resulting in alkalinization of the cytoplasm (Atkinson and Baker, 1989).

There is still some debate concerning the order in which these responses occur. In a study using cultured parsley cells and a glycoprotein elicitor (which does not trigger cell death under these conditions), Jabs *et al* (1997) established that the XR precedes the oxidative burst. In contrast, using a different system (soybean cultured cells treated with H_2O_2), Levine *et al* (1996) demonstrated that the oxidative burst precedes and stimulates a rapid influx of Ca^{2+}, leading to cell death. Glazener *et al* (1996) showed that a mutant of *Pseudomonas syringae* pv *syringae* unable to trigger either an HR on tobacco leaves or cell death in cell suspensions was still able to elicit a normal XR and oxidative burst in cell suspensions. Thus the oxidative burst and the XR are probably necessary but may not be generally sufficient in each system to initiate the cell death process.

Little is known about the molecular events following the initial recognition of the avirulence signal and the earliest cellular responses described above (reviewed by Hammond-Kosack and Jones, 1996; Low and Merida, 1995). Large surveys for mutants in loci necessary for normal *R* gene function have been undertaken (Freialdenhoven *et al*, 1994, 1996; Hammond-Kosack *et al*, 1994). Using such a genetic approach, Salmeron *et al* (1996) identified a gene, *Prf*, necessary for *Pto* function. *Prf* encodes a protein with leucine-zipper, nucleotide binding and leucine-rich repeat motifs, as are found in a number of disease resistance genes (Bent, 1996). *Pto* was previously found to encode a cytoplasmic serine-threonine kinase (Loh and Martin, 1995; Martin *et al*, 1993), suggesting that kinase cascades may be involved in triggering HR. By using the yeast two-hybrid system, Zhou *et al* (1995) have shown that a serine-threonine kinase, Pti, is phosphorylated by Pto and that overexpression of Pti is sufficient for further signaling of the HR in transgenic tobacco.

Genetic approaches have proven useful to further analyze the signal transduction pathways leading to the HR. In barley, gene interaction studies have shown that a mutant allele of a gene required for *Mla*-based resistance (*rar1*; Freialdenhoven *et al*, 1994) does not abolish *Mlg*-based resistance at the macroscopic level. Interestingly, at the microscopic level, the *rar1* mutation modifies the *Mlg* resistance phenotype. This is manifested by an increase of fungal penetration events in *rar1/Mlg* plants compared to *Rar1/Mlg* plants. However, although *rar1* suppresses the

HR phenotype of the *Mla*-mediated resistance, it does not suppress the cell death response of the *Mlg*-mediated resistance (Peterhänsel *et al*, 1997). This result suggests that, in barley, alternative routes leading to HR exist and may or may not converge (Figure 2A). Similarly, Parker *et al* (1996) isolated an Arabidopsis mutant, *eds-1*, suppressing the action of several different *R* genes directed against isolates of the biotrophic pathogen *Peronospora parasitica*, but not to an incompatible bacterial pathogen. Thus *eds-1* defines a function upstream from a possible convergence of bacterial and fungal resistance gene signaling pathways. In contrast, the Arabidopsis *ndr-1* mutant is impaired in resistance against both bacteria and *Peronospora* (Figure 2B; Century *et al*, 1995). *Ndr* could thus represent a downsteam step in signaling, after the convergence of pathways specific to fungal and bacterial pathogens.

Effectors of cell death

The nature of the effectors of HR remains elusive. Some components of the defense response are potentially toxic for the plant cell (e.g. ROIs, phytoalexins, SA) (see Ward *et al*, 1991 concerning SA) and they could participate directly in cell death (Figure 3A). ROIs can cause loss of cell integrity and viability because of their elevated reactivity towards membrane lipids, proteins and nucleic acids (Baker and

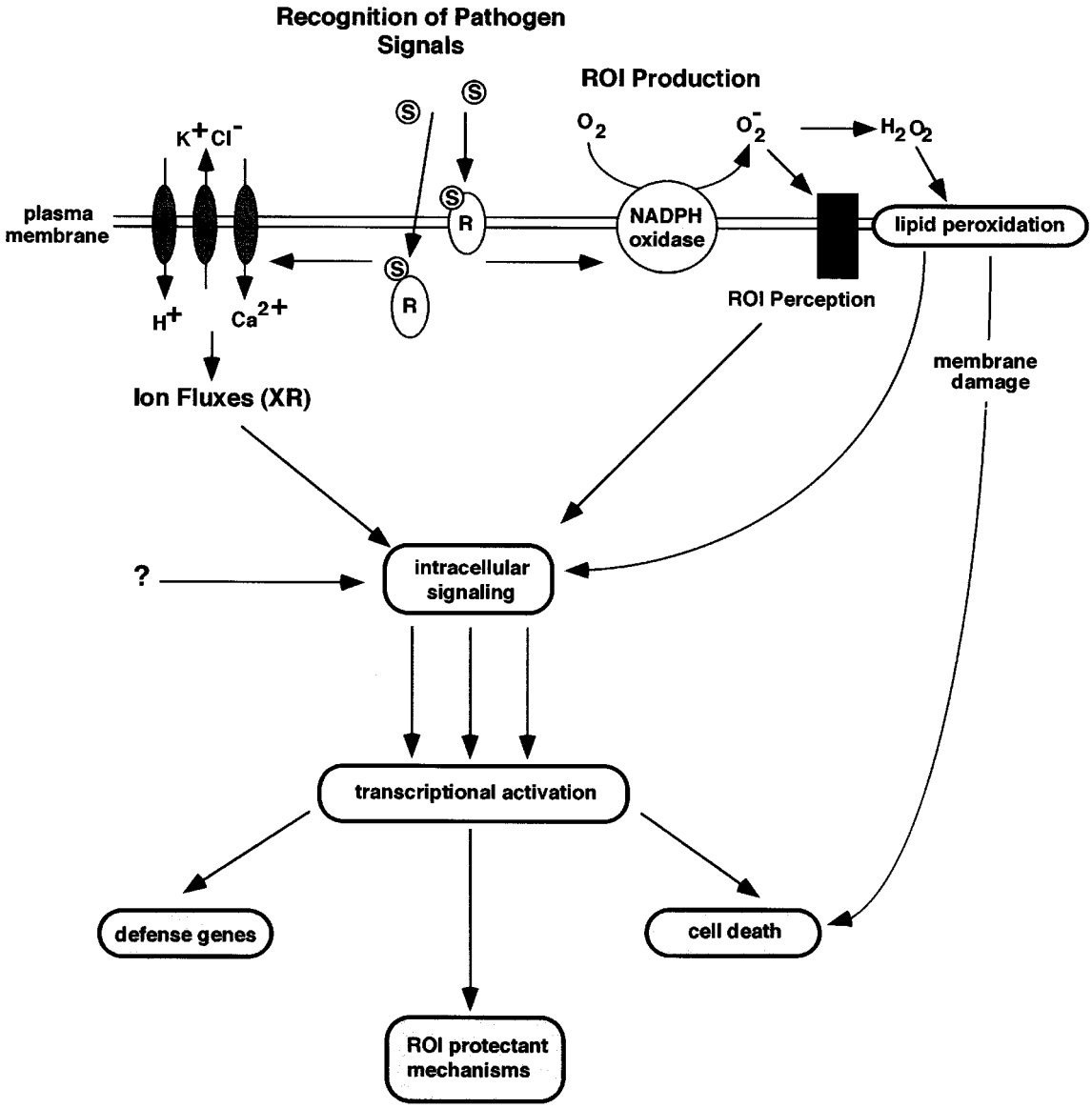

Figure 1 A simplistic picture of the transduction pathways leading to the HR. After initial recognition of the pathogen signal (S) via an extra- or intracellular receptor (R; the product of a plant *R* gene or an elicitor receptor), an oxidative burst and ion fluxes (XR) trigger intracellular signaling (mediated by ROI perception, kinase/phosphatase cascades, lipid peroxidation), which in turn results in the activation of defense responses. These defense responses are composed of defense gene activation (structural proteins, phytoalexins biosynthesis genes, anti-fungal proteins) and cell death (endonucleases, proteases). Cellular protectant mechanisms are also induced in order to control the extent of the cell death (superoxide dismutases, catalases, glutathione peroxidases and S-transferase)

Orlandi, 1995). Serine and cysteine proteinases and endonucleases may also be part of a complex machinery set in motion during the HR (Levine *et al*, 1996; Mittler and Lam, 1995). To date, plant homologues to animal caspases have not been described.

However, as some of the induced defense molecules appear well after the first signs of cell death, they are probably not determinants of HR (Goodman and Novacky, 1994; Schröder *et al*, 1992). An Arabidopsis mutant (*pad3*) deficient in the synthesis of the major phytoalexin

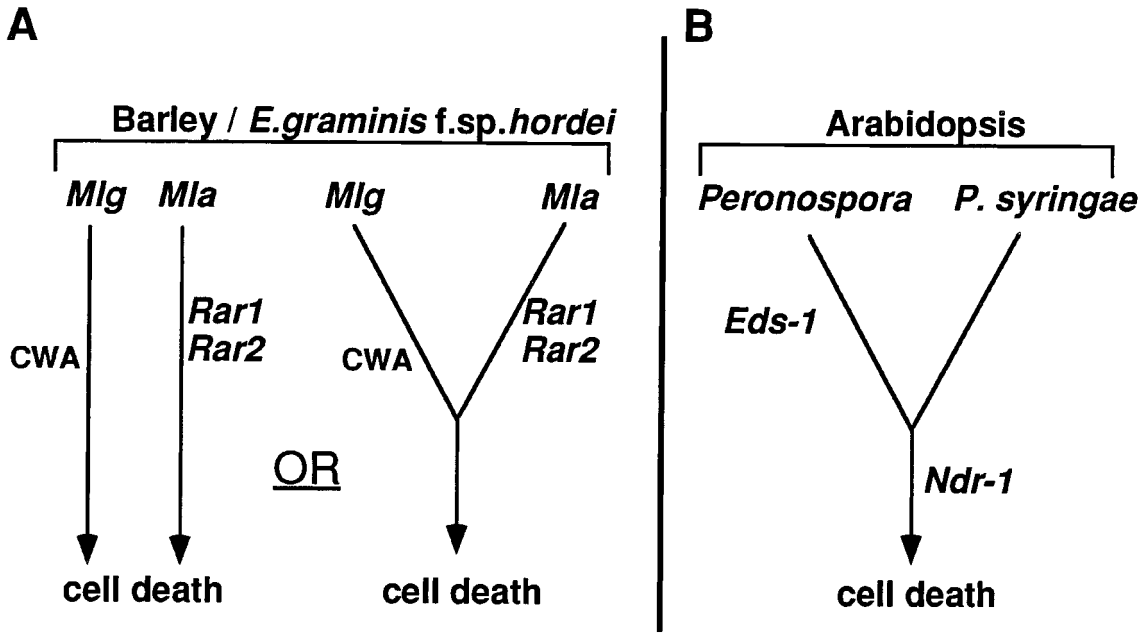

Figure 2 Genetic dissection of the pathways leading to the HR. (**A**) The *Mlg* and *Mla* based resistances of barley could function independently and lead to plant cell death. Alternatively, the *Mlg* and *Mla* pathways could converge. A requirement for the *Rar1* and *Rar2* products for *Mla*-mediated cell death, but not *Mlg*-mediated cell death, and the occurrence of cell wall appositions (CWA) only in *Mlg* responses is detailed in the text. (**B**) Definition of the *Ndr-1* locus suggests that resistance to both bacterial and oomycete pathogens can share common steps. Note that wild-type *Eds1* function is required for resistance to some, but not all, isolates of *Peronospora parasitica*, and one of several *Pseudomonas Syringae aur* genes

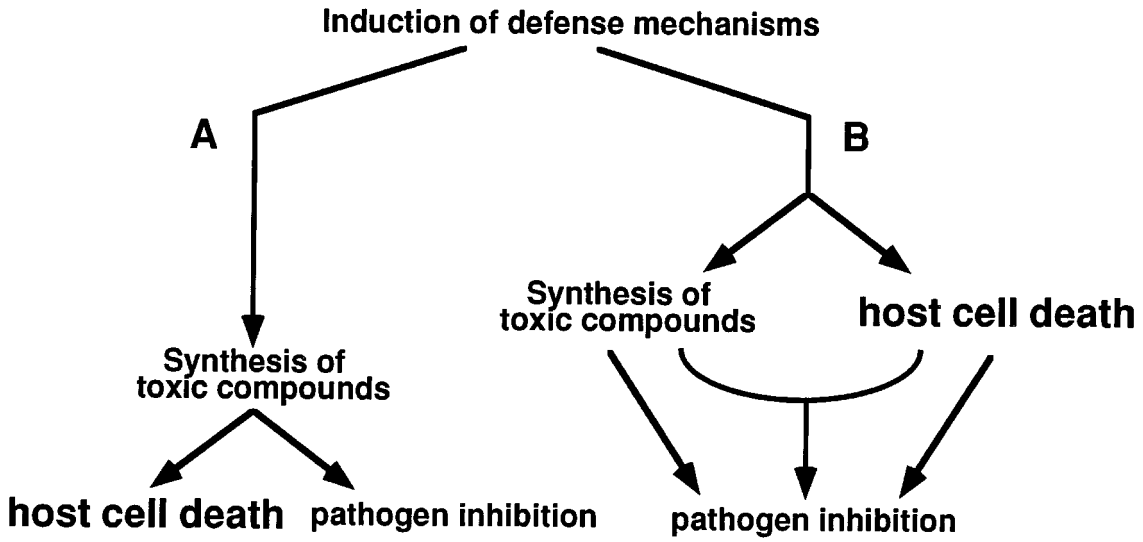

Figure 3 Possible mechanisms leading to the HR. Because many of the plant defense products are also toxic for the plant cell (ROIs, phytoalexins, SA), they could be directly responsible for the death of the host cells (**A**). However, the possible uncoupling of cell death and other defense responses suggests that they act in parallel pathways, with possible cross-talk (**B**)

camalexin, is still able to mount an HR to bacterial pathogens (Glazebrook and Ausubel, 1994). This suggests that camalexin is not necessary for the induction of the HR. Moreover, defense gene mRNA accumulation can be found in the absence of HR, with kinetics and amplitude similar to those observed during the HR (Jakobek and Lindgren, 1993; Schröder *et al*, 1992). These results further suggest that the initial signaling pathway can fork and give rise to at least 2 branches: one activates the synthesis of phytoalexins and defense proteins while the other one specifically results in the cell death (Figure 3B). Accordingly, Rustérucci *et al* (1996) used a purified fungal elicitin and cultured tobacco cells to show that the induction of lipid peroxidation and cell death were dependent on the generation of ROIs while phytoalexin synthesis was not.

Protectant mechanisms and anti-cell death pathways

Potential ROI protectant mechanisms include anti-oxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase and polyubiquitin. Expression of these genes occurs concomitantly with cell death and H_2O_2 may play a role in their induction (Levine *et al*, 1994, 1996; see below). The induction of these protectant mechanisms, in contrast to the induction of defense genes and cell death, can be independent of Ca^{2+} signaling (Levine *et al*, 1996). This further suggests that the induction of defense genes, cell death and anti-oxidant protectant mechanisms are probably controlled by divergent pathways.

Because uncontrolled cell death would lead to deleterious damage at the tissue level, plants apparently have evolved anti-cell death pathways. These pathways may be different than the ones existing in animals, since transgenic tobacco plants carrying the Bcl-X_L gene do not show altered response to the TMV virus or to *P. syringae* (Mittler *et al*, 1996). Functional homologues of the animal anti-cell death *dad-1* gene (Gallois *et al*, 1997; Sugimoto *et al*, 1995) have recently been found in maize, rice and Arabidopsis, but their involvement in the HR remains to be established. Additionally, plant proteins with conserved domains of the animal ced-9/Bcl-2 protein family have yet to be described.

The best evidence that anti-cell death pathways exist in plants comes from the existence of propagation class cell death control mutants (*lsd1* and *lls1*). The recent finding that the *lls1* mutant from maize encodes a probable dioxygenase (Gray *et al*, 1997) raises the possibility that this gene is involved in detoxification of oxidized phenolic compounds such as salicyclic acid (SA). SA can promote cell death (Naton *et al*, 1996; Shirasu *et al*, 1997) and considerable levels of SA accumulate during the HR (Eneydi *et al*, 1992). Therefore *Lls1* could act as a suppressor of cell death by scavenging SA or a related phenolic compound. Another good candidate for a negative regulator of both cell death and damping of basal level expression of disease resistance pathways is the *lsd1* gene from *Arabidopsis*. *lsd1* mutants exhibit a lowered threshold to trigger HR, and an inability to control HR once it is initiated. The *lsd1* mutation defines a gene encoding a novel zinc-finger protein (Dietrich *et al*, 1997). Thus LSD1 could act as a transcriptional regulator of cell death effectors.

In sum, the induction of HR involves several plant signals generated in the plant plasma membrane (ROIs, ion fluxes). These signals seem to converge into a few genetically and pharmacologically separable pathways. Subsequently, defense genes, ROI protectant mechanisms and cell death can be induced via divergent pathways (Figure 1).

Is HR the ultimate response triggered by the plant?

Although the HR is often associated with disease resistance, there are also examples where HR is not causal to resistance. In this respect, barley *mlo*-, *Mlg*- and *Mla*-mediated resistances to *Erysiphe graminis* f.sp. *hordei* have proven to be an incomparable model. This obligate biotrophic fungus causes powdery mildew. Recessive *mlo* alleles confer resistance to nearly all races of *E. graminis* f.sp. *hordei* without development of an HR. Despite the absence of cell death during this resistance response, there may be a link between resistance and deregulated cell death in *mlo* plants. One pleitropic effect of *mlo* alleles is to trigger spontaneous cell death in the absence of pathogen. The development of foliar lesions in *mlo* plants under certain conditions suggests that one stimulus (fungus) does not trigger cell death while others (e.g. low temperatures; Wolter *et al*, 1993) can. Moreover, the analysis of a large number of *mlo* alleles established a correlation between the frequency of necrosis under pathogen-free conditions and the effectiveness of resistance (cited in Büschges *et al*, 1997). Thus HR may represent the final step in a chain of increasingly severe cellular defense reactions, and this step is simply not reached during *mlo*-dependent resistance reactions. The *mlo* phenotype may further indicate a threshold necessary to engage the HR pathway, as was mentioned above for *lsd1*. In this scenario, the wild-type *Mlo* gene could function to down regulate a low level, constitutive defense response. *Mlo* encodes a probable transmembrane protein with no homologues in animal gene databases (Büschges *et al*, 1997).

Further evidence for differential thresholds activating different *R* genes is provided by comparison of barley *Mlg* and *Mla* function. HR is observed during *Mlg*-directed response of barley to an incompatible fungal isolate, but is probably not causal for resistance. The interaction between tomato and the fungus *Cladosporium fullvum* could be another example in which HR may not be causal to resistance (Hammond-Kosack and Jones, 1994). In contrast, HR is a key component of *Mla*-mediated resistance (Bushnell, 1981). In *Mlg* barley plants HR appears after the induction of a first set of defense responses, namely cell wall apposition which stops fungal penetration (CWA; Görg *et al*, 1993). CWA do not form during *Mla*-mediated responses. It is thus interesting to note that the *rar1* mutation, which abolishes *Mla*-mediated HR, does not suppress *Mlg*-mediated HR (Peterhänsel *et al*, 1997). Thus, in *Mlg*-based resistance, the HR may result

from overall signal levels passing a threshold. By contrast, in *Mla*-based resistance this threshold requires *Rar1* function for commitment to HR and is reached before, or independent of, CWA formation. Thus the pathways triggering HR in *Mlg* and *Mla* plants may be independent (see above; Figure 2A). In a case similar to *Mlg* resistance, cell death only occurs after what appeared to be an unsuccessful penetration attempt that induced a first set of defense reactions (Meyer and Heath, 1988) during incompatible interactions between cowpea and the fungus *Erysiphe cichoracearum*.

Thus, although HR is sometimes not causal for disease resistance, it appears that HR represents the final stage invoked by plants to resist infection. A threshold seems to be necessary for irrevocable commitment to HR, while the transcriptional activation of defense responses can be activated below this threshold.

Pathogen lifestyles and cell death

Because pathogens have developed various strategies for growth and reproduction, the requirements for cell death may depend on the nature of the plant-pathogen interaction. The impact of the HR may also vary depending on the lifestyle of the pathogen. In this respect, two parameters should be considered: whether the parasite is intra- or extra-cellular, and whether it is a biotrophic, hemibiotrophic or necrotrophic pathogen. In addition, pathogens use at least three strategies in order to efficiently colonize their hosts. First, if they do not produce any signal molecule recognized by the host, they can evade detection. Second, in the presence of such molecules, they can actively attempt to avoid further triggering of the defense responses (suppression). Third, they can co-opt the plant defense responses by purposely killing the plant cell (negation). Only the last two strategies are presented here because of their consequences for the host cell.

Biotrophic and obligate pathogens

Viruses are intracellular obligate parasites and need the host cell machinery in order to replicate. Thus cell death of the invaded cell appears to be a good means of blocking multiplication of the pathogen. Because it also results in the mechanical isolation of the infected cell from the neighboring cells, the HR could prevent further viral spread. Yet, by taking a random sample from the literature, Fraser (1990) could find that more than 65% of the viral resistance genes were not associated with an HR, but rather with reduced multiplication of the virus or total immunity (e.g. the potato *Ry* gene against PVY; Baker and Harrison, 1984). Thus, HR is not the major resistance mechanism used by plants to protect themselves against viruses.

Biotrophic and hemibiotrophic fungal pathogens develop specialized structures called haustorium which invade the tissue and salvage nutrients. This haustorium penetrates the cell wall and establishes an active interface consisting of the plasma membranes of both the fungus and a living host cell where the uptake of nutrients takes place. In this type of parasitism, the pathogen needs living host cells for its development. Therefore death of the invaded cells could deprive the pathogen of nutrients. Accordingly, in many cases HR precedes growth arrest and death of the incoming pathogen at the haustorial developmental stage of the fungus (Bennett *et al*, 1996; Bushnell, 1981; Chen and Heath, 1991; Naton *et al*, 1996). In such cases, HR could cause pathogen death, or the mechanism which kills the plant cells could also kill the pathogen. However, there are also situations where HR is thought to occur too late to be the causal event for resistance (e.g. Barley *Mlg* resistance gene discussed above; Görg *et al*, 1993; Koga *et al*, 1990).

The overall plant defense response is easily inducible (Brederode *et al*, 1990; Hammond-Kosack and Jones, 1996), and it is surprising that in many cases they are not activated by fungal infection. Fungal pathogens may avoid detection by not producing any effective signal molecule recognized by the plant. Alternatively, they may not produce enough of the signals critical for efficient triggering of the defense responses (Kamoun *et al*, 1997). This seems unlikely given the mechanical and physiological stresses that a fungus causes in order to colonize its host. In addition, biotrophic fungal pathogens seem to actively inhibit host cell death to prevent the infected cells from dying (e.g. green islands induced by virulent pathogens in otherwise senescing leaves; Johal *et al*, 1995b). Several reports have demonstrated that some hemibiotrophic pathogens can also suppress defense response activation. This phenomenon parallels well characterized cases in animal diseases caused by viruses, where virus gene products suppress cell death pathways during the multiplication phase (Shen and Shenk, 1995). A large number of fungal-derived molecules suppressing plant defense responses are known (Kunoh, 1995; Yamamoto, 1995). Here we will only mention two of them where the potential plant targets have been identified. The pea pathogen *Mycosphaerella pinodes* produce glycopeptides, supprescins A and B, able to partially suppress defense responses (Shiraishi *et al*, 1991). Supprescin B may affect the signaling pathway leading to resistance by inhibiting plasma membrane H^+-ATPase (Kato *et al*, 1993). In the interaction between potato and *Phythopthora infestans*, an Hypersensitivity Inhibiting Factor (HIF) was identified. This HIF, a β, 1-3 glucan, suppresses cell death and HIF from virulent isolates is more active than HIF from avirulent ones (Doke and Tomiyama, 1980; Maniara *et al*, 1984). Doke (1985) showed that the HIF suppresses NADPH-dependent ROI production. Thus biotrophic pathogens can suppress the host defense response to successfully invade their host. Figure 4A provides a possible model for the mechanisms of action of such suppressors.

Necrotrophic pathogens

Necrotrophic pathogens are largely extracellular parasites or may also develop intracellular structures. They usually possess all the enzymatic activities required to utilize the extracellular matrix of the plant cells as a nutrient source. Moreover, they often trigger nutrient leakage from the host cells and are able to live from dead tissues. Thus, the role of host cell death, and which partner it ultimately benefits,

is questionable in this type of interaction. Nevertheless, development of an HR in these interactions is associated with *R* gene action. This might reflect the fact that the pathways leading to resistance are linked to those resulting in HR (see Figure 1 and above). In this scenario, HR may simply be the consequence of simultaneous activation of cell death and defense response pathways. Alternatively, cell death and the associated cellular decompartmentalization could provoke the release of toxic compounds (phytoalexins) accumulated in the vacuoles and precede the arrest of the pathogen. Finally, the desiccation process accompanying the HR may generate an anti-microbial environment (Bestwick *et al*, 1995). Because cell death *per se* may be insufficient to

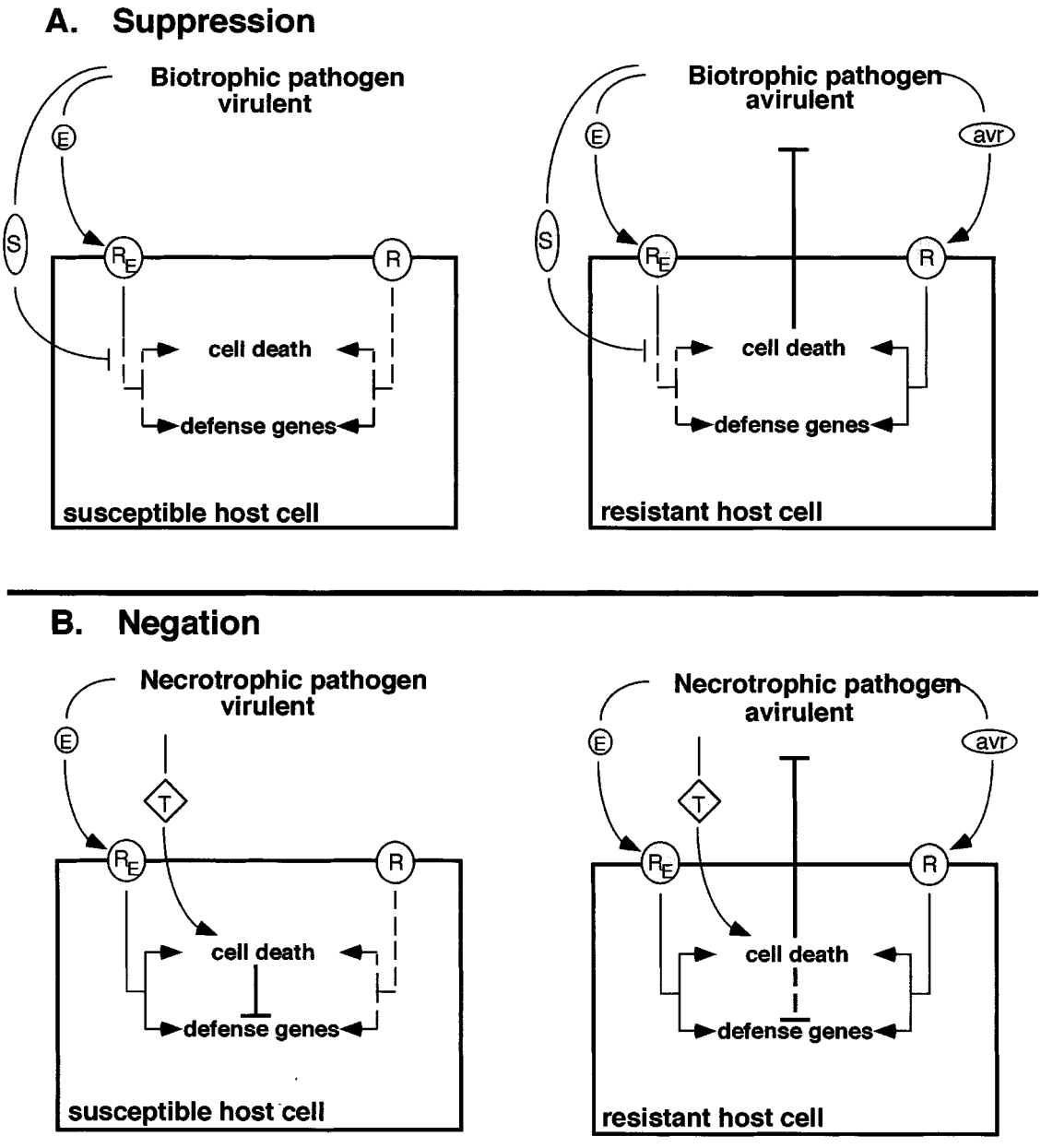

Figure 4 Suppression and negation of the plant defenses. The gene-for-gene specific and elicitor-mediated pathways have been indicated separately but could be identical. Solid lines and dashed lines respectively indicate that the pathway is on or off. E: elicitor; RE: elicitor receptor; avr: avirulence signal; R: resistance gene product; S: suppressor; T: toxin. Arrows indicate positive regulation, flat arrowheads indicate negative regulation. Thick bar with flat arrowhead indicates a successful disease resistance response. (**A**) Suppression. In the absence of avirulence signals (susceptible host cell), the production of a suppressor molecule can inactivate the induction of the plant defenses by non-specific elicitors. Then in the presence of specific recognition of avirulence signals by *R* products (resistant host cell), the host cell triggers defense mechanisms despite the inhibiting effect of the suppressor. (**B**) Negation. In the absence of avirulence signals (susceptible host cell), the production of a molecule (e.g. toxin) impairs the host's ability to respond to elicitors that trigger the defense response. Then in the presence of specific recognition of avirulence signals by *R* products (resistant host cell), the host cell coordinates an appropriate defense response which overcomes toxin-mediated negation

halt necrotrophic growth, overall coordination of defense responses and HR is important.

It is also conceivable that a necrotrophic pathogen may utilize a plant cell death pathway (in order to negate the defense responses by purposely killing the cells) to its own advantage, since dead cells are a good growth substrate for such a parasite (Figure 4B; Johal *et al*, 1995a). Again, animal viruses use similar strategies in order to spread in the infected organism (Shen and Shenk, 1995). A good example of such a strategy could be illustrated by the AAL-toxin which, although leading to disease when secreted onto a susceptible host, triggers cell death morphologically reminiscent of apoptosis (Wang *et al*, 1996; Gilchrist, 1997).

Bacterial pathogens can also co-opt the plant defense responses. *Pseudomonas syringae* pv *syringae* produces a cell death-inducing toxin, syringomycin. The target of this small lipopeptide is the plant host plasma membrane where it forms pores (Hutchison *et al*, 1995). At low concentrations, syringomycin promotes passive membrane ion fluxes reminiscent of the ion fluxes triggered during the HR (Takemoto, 1992). Moreover, some but not all defense responses, are induced by syringomycin (e.g. callose deposition), suggesting that partial induction of host response pathways may be used by *P. syringae* pv *syringae* in order to negate the full set of resistance responses of the plant. Alternatively, that portion of the defense response triggered by syringomycin may be involved in both HR and disease. The finding by Levine *et al* (1996) that syringomycin triggers cytologically defined necrosis is not contradictory with this interpretation since, at high concentrations, syringomycin acts as a surfactant and rapidly disrupts plasma membranes (Hutchison *et al*, 1995).

Necrotrophic pathogens can also suppress plant defenses (in contrast to negate by killing). Coronatine is a chlorosis-inducing toxin produced by *P. syringae* pathovars. It does not induce death of the host cells but shrinkage of the chloroplasts, the consequence of which could be to slow down the metabolism of the attached cells (Palmer and Bender, 1995). It has been observed that mutant strains of *Pseudomonas syringae* pv *tomato* which do not produce coronatine induce higher levels of several host defense genes than does the wild-type strain. Thus the function of coronatine could be to suppress the induction of plant defense responses until the bacteria population increases to a level at which it is no longer possible for the plant to limit the infection (Mittal and Davis, 1995). As an additional example, the BZR-toxin produced by *Bipolaris zeicola* race 3 has a dual mode of action. On rice, it induces cell death and therefore may participate in the negation of the plant defenses. In contrast, the BZR-toxin is not toxic (as measured by the absence of root growth inhibition) and does not trigger cell death on maize or wheat. However, on these plants BZR-toxin induces susceptibility to subsequent infection by normally non-pathogenic fungi (Xiao *et al*, 1991) and therefore could act by suppressing the host defense responses (Xiao *et al*, 1992). The mechanisms of action of the BZR-toxin are unknown and it would be interesting to determine how the same molecular component can trigger cell death on one plant (rice) and suppress resistance on others (maize and wheat).

Although these examples do not provide direct evidence that cell death is necessary for resistance, they suggest at least that pathogens try to inhibit it in order to more effectively colonize their host. This inhibition can be performed either by suppression (Figure 4A, biotrophic and necrotrophic pathogens) or by negation (Figure 4B, necrotrophic pathogens). These examples also emphasize that a complex network of signals is exchanged during plant-pathogen interactions: the respective interests of each protagonist must be considered in order to assign the role of cell death in disease resistance and disease symptom development. The cell death morphology is the result of the juxtaposition and summing of these different signals. This may explain the diversity of morphologies so far observed during the HR and during the development of disease symptoms.

Is there a social role for the HR?

Several reports show that expression of defense responses occur in cells surrounding necrotic infection sites (Heitz *et al*, 1994; reviewed by Kombrick and Somssich, 1995). Using the GUS reporter gene fused to the promoter region of the defense gene chitinase, Samac and Shah (1991) could monitor the induction of this gene after infection. When infected with *P. infestans*, GUS activity was detected in a sharp zone surrounding the necrotic lesions but not around prenecrotic spots. This induction of defense responses in the sharp zone surrounding the HR cells might be essential to restrict pathogen spread. It is known for example that in Tobacco Mosaic Virus-induced HR, the cells immediately beyond the necrotic region contain virus particles (Konate *et al*, 1982). It was suggested that cells undergoing HR might release signals regulating defense responses in the tissue next to the infection site (Kombrick and Somssich, 1995). In this model, the cells directly in contact with the pathogen overreact to the pathogen, and amplify signals until they commit suicide. This amplification may reflect a runaway cycle where SA and ROIs are involved. Indeed H_2O_2 can induce SA synthesis (León *et al*, 1995; Neuenschwander *et al*, 1995) which, at very high concentrations, can inhibit catalase and other scavenging enzymes (Chen *et al*, 1993), potentiating further accumulation of H_2O_2 (Shirasu *et al*, 1997).

Cell death may shut down further amplification and trigger signals to neighboring cells. Levine *et al* (1994) used an experimental design where infected cells were separated from uninfected ones by a dialysis membrane, and showed that H_2O_2 can function as a short distance signal. The local oxidative burst in response to infection triggered induction of Glutathione S-transferase but not cell death in the uninfected cells. Thus, although the authors did not examine the induction of other defense genes in this experiment, they could demonstrate the existence of a communication system between uninfected and infected cells. Recently, a diffusible signal able to induce several defense genes (e.g. sesquiterpene cyclase, chitinases, but not PR-1) has been identified after treatment of tobacco

cells with the cell death inducing elictin cryptogein (Chappell *et al*, 1997). The detection of superoxide production at the lesion margin of the Arabidopsis *lsd1* mutant is another indication that dying cells are triggering cell non-autonomous signals to the neighboring cells (Jabs *et al*, 1996). Therefore, besides its role in the infected cells, the HR may be used to coordinate the defense responses in neighboring cells (local resistance).

Plants have developed a broad-range secondary resistance known as Systemic Acquired Resistance (SAR). The SAR pathway confers non-specific heightened and prolonged levels of resistance in uninoculated tissues to secondary infections by a broad range of pathogens (reviewed by Ryals *et al*, 1996). Salicyclic Acid has been shown to play a central role in the establishment of the SAR in at least Arabidopsis and tobacco (Gaffney *et al*, 1993; Delaney *et al*, 1994). SAR is biologically triggered by both avirulent and virulent pathogens that cause cell death (as a result of the HR or disease symptoms). Therefore there might be a correlation between cell death and the establishment of SAR. However, if the tissue inoculated by a necrotizing pathogen is removed before the onset of macroscopic cell death, SAR is still observed in systemic inoculated tissues (Smith *et al*, 1991). In this experiment, the presence of macroscopic lesions was the criteria used for the presence of cell death and it is possible that microscopic cell death had occurred without any visible symptoms (as initiation of the HR and its magnitude may be separable; Hammond-Kosack *et al*, 1996). Thus, cell death appears to be necessary to trigger the SAR.

Concluding remarks

The HR is an intrinsically programmed process. However, because of the great diversity of triggers (Dangl *et al*, 1996; Jones and Dangl, 1996) and morphologies of the cell deaths (Heath, 1980), there are probably several ways in which a cell may die. It does not seem that apoptosis as traditionally defined is a strict paradigm for the HR. The attacked cell and its neighbors are probably not receiving the same signals in both quantity and quality (Levine *et al*, 1994). In animals, the severity of the initial signal can determine whether the cells undergo necrosis or apoptosis (Bonfoco *et al*, 1995) and the same could be true in the case of the HR. Thus both apoptosis and necrosis could occur within a single HR region (Kosslack *et al*, 1996). Plant pathologists still need to establish criteria and find strict markers (if such exist) to differentiate between cell death resulting from environmental or metabolic perturbation and cell death resulting from the activation of the internal HR program. However, the morphological characterization of the HR may be difficult due to the rapidity at which the cellular modifications occur (Freytag *et al*, 1994). Genetic approaches and cloning of plant genes (such as the genes responsible for the disease lesion mimic phenotypes and *R* gene suppressors) will shed new light on the mechanisms involved in regulating and executing the HR. The model depicted in Figure 1 suggests that it should be possible to isolate mutants specifically impaired in their induction of the HR, but not in the induction of the other defense responses.

Genetic dissection of the signal transduction leading to HR is underway and has already suggested that various signal pathways exist. These may or may not converge (Parker *et al*, 1996; Century *et al*, 1995; Peterhänsel *et al*, 1997). The HR also results from a complex interplay of signals from both the plant and the pathogen. The latter can sometimes interfere with these processes in order to successfully colonize the plant (Figure 4). A better understanding of pathogenicity factors and their targets in the host are necessary to interpret the phenomena observed in the challenged plants.

It does not seem that HR is always necessary for resistance (Fraser, 1990; Wolter *et al*, 1993). Rather coordination between the different induced mechanisms is required for successful resistance. Cell death during the HR appears to be part of a continuous process where different pathways cross talk. This is also suggested by the fact that in their attempt to isolate mutants with enhanced disease susceptibility, Glazebrook *et al* (1996) isolated several mutants affected in their resistance to normally avirulent pathogens (*nim1/ndr1*: Cao *et al*, 1994; Delaney *et al*, 1995). Moreover, many of the maize lesions mimics mutants display lesions resembling disease symptoms rather than HR, suggesting that there is also host genetic control of disease symptom development (Johal *et al*, 1995b). Hence cell death associated with disease symptoms and HR probably share common mechanisms and study of susceptibility will probably give us new insights into resistance mechanisms.

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