Plant Molecular Biology **44:** 335–344, 2000.
E. Lam, H. Fukuda and J. Greenberg (Eds.), Programmed Cell Death in Higher Plants.

Transgene-induced lesion mimic

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Key words: biotechnology, lesion mimic, plant-pathogen interactions, programmed cell death

Abstract

Lesion mimic, i.e., the spontaneous formation of lesions resembling hypersensitive response (HR) lesions in the absence of a pathogen, is a dramatic phenotype occasionally found to accompany the expression of different, mostly unrelated, transgenes in plants. Recent studies indicated that transgene-induced lesion formation is not a simple case of necrosis, i.e., direct killing of cells by the transgene product, but results from the activation of a programmed cell death (PCD) pathway. Moreover, activation of HR-like cell death by transgene expression is viewed as an important evidence for the existence of a PCD pathway in plants. The study of lesion mimic transgenes is important to our understanding of PCD and the signals that control it in plants. PCD-inducing transgenes may provide clues regarding the different entry points into the cell death pathway, the relationships between the different branches of the pathway (e.g., developmental or environmental), or the different mechanisms involved in its induction or execution. Cell death-inducing transgenes may also be useful in biotechnology. Some lesion mimic transgenes were found to be induced in plants a state of systemic acquired resistance (SAR). These genes can be used in the development of pathogen-resistant crops. Other cell death-inducing transgenes may be used as specific cell ablation tools. Although mainly revealed unintentionally, and at times considered 'an adverse phenotype', lesion mimic transgenes should not be ignored because they may prove valuable for studying PCD as well as developing useful traits in different plants and crops.

Introduction

Programmed cell death and plant defense

The recognition of an invading pathogen by plant cells results in the induction of different antimicrobial defenses. These include production of reactive oxygen intermediates (ROI), strengthening of cell walls, synthesis of phytoalexins, and induction of pathogenesis-related (PR) proteins (Hammond-Kosack and Jones, 1996; Yang *et al.* 1997). Occasionally these responses are accompanied by a rapid death of cells at and around the site of infection (Dangl *et al.*, 1996). This response results in the formation of a zone of dead cells referred to as a 'lesion'. Since lesions may also form in plants during late stages of infection (i.e., as disease symptoms), the appearance of lesions at an early stage of infection makes it seem as if the plant hyper-reacts to the pathogen. This apparent phenomenology led to the coining of the term 'hypersensitive response' (HR), which is used to describe the early and rapid cell death response that accompanies pathogen resistance (Goodman and Novacky , 1994).

The exact cause of HR cell death is not clear. However, it is now established that this death is not directly caused by the pathogen. Thus, as opposed to cell death that occurs in a susceptible plant, late during infection, usually caused by pathogen proliferation or by different toxins produced and secreted by the pathogen, the early and rapid cell death response that occurs during the HR appears to be the outcome of a plant encoded mechanism for programmed cell death (PCD; Dangl *et al.,* 1996; Greenberg, 1996; Mittler and Lam, 1996; Pennel and Lamb, 1997). The term PCD is used to describe cell death which results from the activation of a cell suicide pathway encoded by the

genome of the dying cell (Raff, 1992; Schwartzman and Cidlowski, 1993). This type of cell death is different from that which occurs during necrosis, i.e., a death not controlled or mediated by the cell (Mittler and Lam, 1996). Plants appear to activate a PCD pathway as part of their defense response against invading pathogens (i.e., the HR). It is thought that by killing of cells at and around the site of infection the plant generates a physical barrier composed of dead cells and limits the availability of nutrients to the pathogen due to the rapid dehydration that accompanies tissue death (Goodman and Novacky, 1994; Dangl *et al*., 1996). As indicated above, it is not entirely clear whether the death of plant cells during the HR is a 'side-effect' caused by the activation of other defense responses such as increased ROI production and synthesis of phytoalexins, or is due to a specific cell death signal that may be similar to the signal(s) that activate PCD during developmental processes such as formation of tracheary elements (Dangl *et al.*, 1996; Fukuda, 1997).

Evidence supporting the assumption that HR cell death is a PCD includes: (1) the activation of the HR in a gene-dependent manner by different elicitors which are compounds produced or secreted by pathogens; (2) the inhibition of pathogen-induced HR cell death by different metabolic inhibitors such as cycloheximide or amanitin; (3) the spontaneous formation of HR lesions in the absence of pathogens in different mutants; and (4) the activation of HR cell death by expression of specific transgenes (Dangl *et al.*, 1996; Mittler and Lam, 1996). Of these the activation of HR cell death in transgenic plants or mutants in the absence of a pathogen (i.e., lesion mimics), is perhaps the strongest evidence for the existence of a cell suicide pathway that is activated by the plant upon pathogen recognition. In this review we will discuss the different examples of transgene-induced lesion mimics, their mode of action, and their possible applications.

Naturally occurring lesion mimic mutants

Key to our understanding of transgene-induced lesion mimics are studies of naturally occurring lesion mimic mutants. Reports of lesion mimics date as early as 1923 and 1948 (Emerson, 1923; Langford, 1948), and include different plants such as maize, rice, barley, tomato, and *Arabidopsis* (Walbot *et al.*, 1983; Greenberg and Ausubel, 1993; Wolter *et al.*, 1993). Mutants similar to the naturally occurring lesion mimics can also be isolated after mutagenesis (i.e., chemical-, radiation-, or transposon-induced; Greenberg and Ausubel, 1993; Dietrich *et al.*, 1994; Greenberg *et al.*, 1994). Lesion mimics were classified according to their appearance into two groups: initiation, and feedback or propagation mutants (Walbot *et al.*, 1983; Dietrich *et al.*, 1994). This classification is based upon the assumption that two different mechanisms are involved in coordinating the HR: (1) a pathway for the initiation of PCD, and (2) a mechanism for the suppression of PCD. Initiation mutants develop spontaneous lesions with a defined border. They are thought to be defective in regulating the activation of PCD, but not its inhibition at the boundary of the lesion. The abnormal activation of cell death in these mutants may result from the lack of a negative regulator of cell death initiation (a recessive initiation mutant) or from the constitutive activation of a cell death signal (a dominant initiation mutant). Propagation or feedback mutants form spontaneous or induced lesions that spread indeterminately. They are presumed to be defective in down-regulating PCD in cells surrounding a developing lesion (recessive mutations). In these mutants cell death which is initiated randomly, upon infection, or after a mechanical injury will propagate uncontrollably, eventually resulting in the complete death of the leaf. The majority of naturally occurring cell death mutants appear to belong to the dominant initiation class (e.g., 23 of 32 different lesion mimic mutants in maize are dominant gain-of-function mutations (Walbot *et al.*, 1983; Dangl *et al.*, 1996). This observation suggests that a variety of cellular signals, some of which may not be directly related to pathogens, can activate the HR-PCD pathway (explained below; Dangl *et al.*, 1996; Mittler and Lam, 1996).

Several cell death mutants express molecular and biochemical markers associated with the antimicrobial defense response of plants. These include enhanced expression of PR proteins, accumulation of salicylic acid (SA), deposition of callose or other cell wall-strengthening compounds, and synthesis of phytoalexins (Dietrich *et al.*, 1994; Greenberg *et al.*, 1994). The activation of these antimicrobial defenses in the absence of a pathogen further suggests that the PCD pathway activated in these mutants may be similar to that activated during the response of plants to incompatible pathogens (Dangl *et al.*, 1996).

Cell death mutants are powerful tools for the study of PCD in plants. The cloning of several lesion mimic genes was recently reported and it is believed that their analysis will unravel the molecular mechanisms involved in the regulation of PCD in plants (Shirasu and

Schulze-Lefert, this issue). In addition, by crossing of different mutants for complementation studies the order of cell death genes along the PCD pathway may be determined (Dangl *et al.*, 1996).

Transgene-induced lesion mimics

Spontaneous formation of HR-like lesions in the absence of a pathogen has been reported in a number of transgenic plants that express foreign or modified genes (Table 1). In some cases, the activation of cell death was accompanied by the induction of multiple defense mechanisms and the induction of enhanced resistance, similar to systemic acquired resistance (SAR; Dangl *et al.*, 1996; Mittler and Lam, 1996). Most of these examples resemble the dominant initiation class of lesion-mimic mutants. Transgene-induced lesion mimics may be classified into four different groups: pathogen-derived genes, signal transduction-inducing genes, general metabolism-perturbing genes, and killer genes (Figure 1, Table 1). These genes can be expressed in a constitutive manner or may be placed under the control of an inducible promoter. As their classification implies, they may have different modes of action.

Pathogen-derived transgenes such as AvrRpt2, elicitin, and Avr9 (Culver and Dawson, 1991; Hammond-Kosack *et al.*, 1994; Keller *et al.*, 1999) appear to function as elicitors, thereby possibly directly interacting with a plant receptor, or a resistant (R) gene product, and activating the HR in the same manner a pathogen would. Their action therefore depends on the genetic make-up of the plant, and requires the presence of an R gene for a proper 'gene-for-gene' interaction (Flor, 1956; Bent, 1996). They are usually expressed from an inducible promoter since they induce a massive cell death response that completely kills the transgenic plant. At least in one instance the activation of the HR response by such a gene (Avr9) was found to depend on the developmental stage of the plant since cell death was initiated only at day 13 of seed germination (using a constitutive promoter; Hammond-Kosack *et al.*, 1994). The action of pathogen-derived genes seems to be somewhat different from that of the dominant lesion mimic genes since instead of causing the sporadic appearance of lesions throughout the leaf, the pathogen-derived genes appear to cause complete death of all cells in the leaf (with the exception of the TMVcp gene in the N′ background; Culver and Dawson, 1991). Pathogen-derived genes are shown in Figure 1 (class A) as genes that mimic the presence of the pathogen and interact with the plant receptors for pathogen recognition.

Signal transduction-inducing genes are transgenes that may activate or effect different components of the signal transduction pathway involved in pathogen recognition or defense response activation (Figure 1). They may mimic the flux of protons across the plasma membrane during the early stages of the HR (i.e., the XR; Yang *et al.*, 1997), such as the bO gene (Mittler *et al.*, 1995), or directly effect different signaling events that take place during the HR, as may be the case with the expression of a small GTP-binding protein, or the cholera toxin (i.e., G-protein signaling; Sano *et al.*, 1994; Beffa *et al.*, 1995). Antisense constructs for the peroxide detoxifying enzymes catalase (CAT; Chamnongpol *et al.*, 1996, 1998; Takahashi *et al.*, 1997) and ascorbate peroxidase (APX; Orvar and Elli, 1997), and an antisense gene for protoporphyrinogen oxidase (PPO; Molina *et al.*, 1999) were also found to induce the formation of lesions, either spontaneously (PPO) or in response to different environmental conditions (CAT or APX), in a manner that resembles lesion mimic mutations. These transgenes may mimic the enhanced production of ROI that accompanies the HR (i.e., the oxidative burst; AOII; Levine *et al.*, 1994; Hammond-Kosack and Jones, 1996). Although they appear to directly effect the signal transduction pathway that is activated during a pathogen-induced HR, signal transduction-inducing transgenes may also activate the HR response via pathways that are not directly related to the HR, much like the metabolic perturbing genes (explained below). The majority of signal transduction-related transgenes were found to induce a lesion mimic phenotype similar to that induced in the dominant initiation mutants. Signal transduction-inducing genes are shown in Figure 1 (class B) as genes that mimic different signal transduction events that occur during the HR.

Expression of metabolism-perturbing transgenes in plants is thought to result in the alteration of cellular homeostasis and the generation of a signal which activates the PCD response. Uncontrolled expression of genes such as invertase or hexokinase may drastically alter the metabolic balance of cells due to changes in hexose transport or metabolism (Herbers *et al.*, 1996; D. Granot, personal communication). A different effect on cellular metabolism may be caused by expression of genes such as rPS14 and CaMV gVI that may affect protein translation in transgenic plants (Takahashi *et al.*, 1989; Karrer *et al.*, 1998). It is possible that metabolism-perturbing transgenes activate PCD

Table 1. Lesion mimic transgenes.

Transgene	Source	Function	Defense	Reference
Class A. Pathogen-derived genes				
TMVcp	TMV (N′)	Avr elicitor	NT	Culver and Dawson, 1991
Avr9	*C. fulvum*	Avr elicitor	Yes	Hammond-Kosack *et al.*, 1994
Elicitin	*P. cryptogea*	Avr elicitor	Yes	Keller *et al.*, 1999
AvrRpt2	*P. syringeae*	Avr elicitor	Yes	McNellis *et al.*, 1998
Class B. Signal transduction-inducing genes				
bO	*H. halobium*	proton pump	Yes	Mittler *et al.*, 1995
Cholera toxin	*V. cholerae*	Inhibit GTPase	Yes	Beffa *et al.*, 1995
sGTP-BP	plant	GTP-binding protein	Yes	Sano *et al.*, 1994
Antisense CAT	plant	removal of ROI	Yes	Chamnongpol *et al.*, 1996; Takahashi *et al.*, 1997
Antisense APX	plant	removal of ROI	NT	Orvar and Ellis, 1997
Antisense PPO	plant	heme biosynthesis	Yes	Molina *et al.*, 1999
Class C. General metabolism-perturbing genes				
Invertase	yeast	hexose transport	Yes	Herbers *et al.*, 1996
Hexokinase	plant	hexose metabolism	Yes	D. Granot, pers. commun.
CaMV gVI	CaMV	inclusion body protein	Yes	Takahashi *et al.*, 1989
rPS14	plant	ribosomal protein	NT	Karrer *et al.*, 1998
Class D. Killer genes				
Barnase	*B. amyloliquefaciens*	RNase	Yes	Strittmatter *et al.*, 1995
DTA	*D. pertussis*	inhibits translation	NT	Nilsson *et al.*, 1998
Protease-related (Class B/C/D)				
Ubiquitin	plant	protein degradation	Yes	Bachmair *et al.*, 1990
Kunitz-type trypsin inhibitor	plant	protein degradation	NT	Karrer *et al.*, 1998

Abbreviations: APX, ascorbate peroxidase; bO, bacterio-opsin; CaMV, cauliflower mosaic virus; CAT, catalase; DTA, diphtheria toxin A subunit; NT, not tested; PPO, protoporphyrinogen oxidase; ROI, reactive oxygen intermediates; sGTP-BP, small GTP-binding protein; TMVcp, tobacco mosaic virus coat protein.

via a pathway that is unrelated to pathogen attack. However, infection of plants with some pathogens such as viruses or bacteria may cause general alterations in the metabolic balance of cells, similar to the changes induced by hexokinase or the CaMV gVI gene. Such pathogen-dependent perturbation in cellular metabolism may in turn activate PCD (Dangl *et al.*, 1996; Mittler and Lam, 1996). Therefore, the activation of PCD by some of the metabolic-perturbing genes may occur via the same pathway that is activated during a 'bona fide' HR. In animals many perturbations in cellular metabolism were shown to activate PCD and the PCD pathway was suggested to act as a 'funnel' that 'channels in' many different signals (Raff, 1992; Schwartzman and Cidlowski, 1993). Since, at least in animals, PCD appears to be activated as part of a general defense mechanism that prevents the growth and proliferation of damaged, infected, or mutated cells (including cells with general metabolic alterations), we included these genes as a separate group. The existence of metabolism-perturbing transgenes may explain the large number of dominant lesion mimic mutants. Many of these may be mutations in general housekeeping genes that cause alterations in cellular metabolism and activation of PCD. Some alterations in cellular homeostasis are thought to result in the excess production of ROI. As shown in Figure 1, ROI produced by these alterations may act as triggers for the induction of PCD. Although of pathogen origin, we classified the CaMV gVI gene as a metabolism-perturbing transgene. The CaMV gVI protein was suggested to affect the translational apparatus of plants and may therefore change the cellular homeostasis of cells (De Tapia *et al.*, 1993). Metabolism-perturbing transgenes are shown in Figure 1 (class C) as genes that activate the HR via the generation of different cellular signals that may be channeled into the HR-PCD pathway.

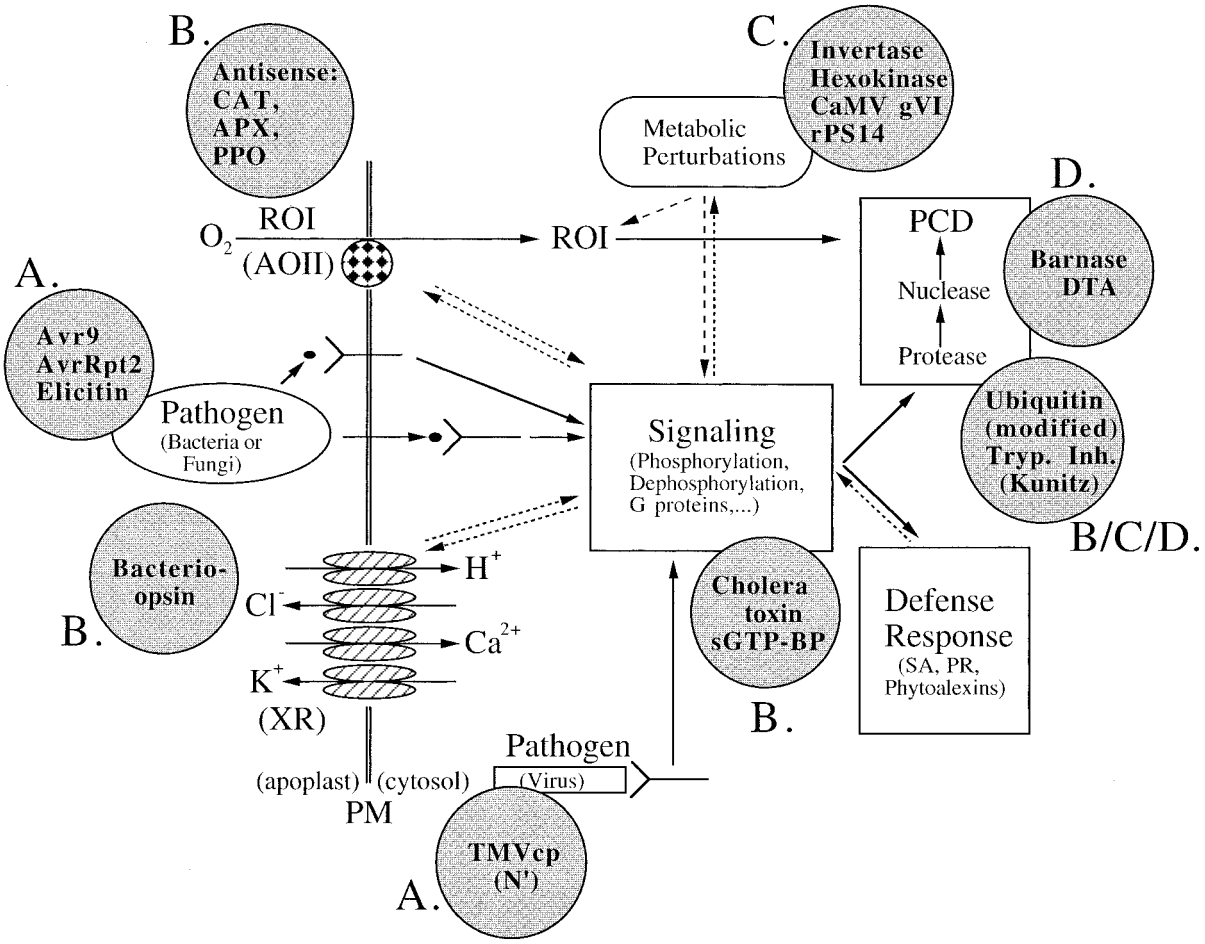

Figure 1. A model showing the different modes of PCD activation by lesion mimic transgenes, in relation to the HR pathway. HR-inducing transgenes (shaded circles; see Table 1 for abbreviations, description, and classification of the different transgenes) were classified into four groups (A to D) and placed at their possible sites of action along the pathogen-induced HR pathway (after Mittler *et al.*; Mittler and Lam, 1996). Abbreviations and symbols: AOII, oxidative burst; PCD, programmed cell death; PM, plasma membrane; PR, pathogen-related; ROI, reactive oxygen intermediates; SA, salicylic acid; XR, ion translocation response, >–, receptor.

Killer genes are genes thought to directly cause cell death. The expression of barnase, an RNase gene, in plants is one such example (Strittmatter *et al.*, 1995). Other genes that may function as killer genes are DNases, specific proteases, or subunit A of the diphtheria toxin (Nilsson *et al.*, 1998). Killer genes may mimic the action of different components of the PCD pathway, in particular cell death-executing genes. However, it should be noted that the expression of such genes may not be the direct cause of cell death and that they may simply be triggering a pre-existing PCD pathway by generating a cell death signal, similar to some examples in animals (Schwartzman and Cidlowski, 1993). It was suggested that in animals the PCD pathway is already present in cells in a 'ready-for-execution' mode which is not dependent on additional reactions of RNA or protein synthesis (Raff, 1992). If such a mechanism also functions in plant cells then a killer gene such as barnase or DTA may activate it and cause the execution of cell death in a manner that is independent of RNA metabolism or translation. Evidence for and against the existence of a 'ready-for-execution' cell death machinery in plants was reported (He *et al.*, 1994; Pennel and Lamb, 1997). Killer transgenes are shown in Figure 1 (class D) as genes that may mimic the action of plant genes involved in the final execution phase of the HR.

Proteases were recently suggested to be involved in the signal transduction pathway that leads to the activation and execution of PCD in plants and an-

imals (Fraser and Evan, 1996; del Pozo and Lam, 1998). A cascade of proteases is thought to be activated during PCD and directly cause the death of cells by proteolytic cleavage (Fraser and Evan, 1996). At least two examples of cell death inducing transgenes involving proteases were reported. A modified ubiquitin gene unable to polymerize (an essential step in the protein degradation pathway) was found to induced a lesion mimic phenotype (Bachmair *et al.*, 1990; Becker *et al.*, 1993), and a 'Kunitz'-type trypsin inhibitor was found to induce cell death upon expression in plant cells (Karrer *et al.*, 1998). The action of these genes may be directly related to the protease execution machinery (i.e., Killer, D class genes), may effect the signal transduction pathway involved in the transmitting of pathogen-derived signals (i.e., signal transduction, B class gene), or may act through causing alterations in cellular metabolism (i.e., metabolism-perturbing, C class genes). These genes are marked as class B/C/D. in Figure 1.

Inducible expression of lesion mimic transgenes in plants

At least three different lesion mimic transgenes were expressed under the control of an inducible promoter in transgenic plants. The barnase gene was expressed in plants under the control of a PR promoter (prp1-1; Strittmatter *et al.*, 1995). It was found that upon infection of plants with a fungal pathogen the barnase gene was expressed and caused the induction of PCD. This induction enhanced the resistance of transgenic plants to pathogen attack, supporting the hypothesis that death of cells at and around the site of infection plays an important role in preventing pathogen proliferation. Ethylene was also found to induce the expression of the barnase gene and the activation of PCD (in the absence of a pathogen; Strittmatter *et al.*, 1995). Another pathogen-responsive promoter used to drive the expression of a lesion-inducing transgene is the *hrs203J* promoter (Keller *et al.*, 1999). This promoter was used to express the elicitor protein cryptogein (elicitin) in transgenic tobacco plants. Transgenic plants in which the elicitin gene was induced by a pathogen attack-activated PCD and enhanced the synthesis of PR proteins. These plants also displayed enhanced resistance to attack by different pathogens (Keller *et al.*, 1999).

McNellis *et al.* (1998) used a glucocorticoid-inducible promoter to drive the expression of avrRpt2 in the RPS2 genetic background. In this transgenic system the expression of avrRpt2 is controlled artificially by the addition of dexamethasone (DEX). Application of DEX caused the induction of PCD and the activation of defense mechanisms such as PR-1 gene expression. As opposed to the two inducible systems described above (Keller *et al.*, 1999; Strittmatter *et al.*, 1995), this system was not designed to enhance the tolerance of plants to pathogens. In place, it supplies an excellent platform for studying the PCD response in plants. Thus, PCD can be activated in a synchronous manner and the different events that take place during this response, such as activation of proteases or nucleases, can be followed in the absence of the pathogen. The DEX-inducible cell death phenotype may also be used in screening for *Arabidopsis* mutants that have a defective PCD pathway. Such mutants may be isolated by selection for mutated transgenic seedlings that survive the application of DEX (McNellis *et al.*, 1998).

An interesting system for identifying novel lesion mimic genes via the use of a virus-based expression vector was reported by Karrer *et al.* (1998). A plant cDNA library was constructed in tobacco mosaic virus (TMV) and used to infect tobacco plants incapable of inducing the HR in response to TMV infection. cDNAs that induced PCD were identified by the formation of lesions that resembled HR lesions after infection of plants with the TMV vector. cDNA inserts which encoded for cell death-inducing transgenes were cloned from the lesions by RT-PCR. In addition to a large number of unidentified novel genes, Karrer *et al.* isolated a ribosomal protein (rPS14), a 'Kunitz'-type protease inhibitor, with homology to the tobacco tumor-related protein miraculin, a glycine-rich protein, and a ubiquitin gene. The ubiquitin gene appeared to function in a manner similar to that of the ubiquitin mutant, which induced a lesion mimic phenotype in transgenic tobacco plants (Karrer *et al.*, 1998).

Use of transgene-induced lesion mimics in biotechnology

Two of the main uses for lesion mimic genes in biotechnology are enhancement of pathogen resistance (Strittmatter *et al.*, 1995; Mittler and Lam, 1996; Keller *et al.*, 1999) and tools for specific cell ablation (Nilsson *et al.*, 1998). Lesion mimic genes can be used to enhance pathogen resistance in two ways.

They can be expressed in a constitutive manner causing the activation of defense responses, appearance of lesions, and induction of a systemic state of resistance. This mode of expression results in a phenotype similar to the naturally occurring lesion mimic mutants that were used for many years by breeders to introduce resistance from wild-type cultivars into commercial plants (Langford, 1948). Alternatively, lesion mimic genes may be expressed from an inducible promoter (as described above; Keller *et al.*, 1999; Strittmatter *et al.*, 1995) that will limit their action to the site and time of infection. This method will not cause the constitutive induction of various defense mechanisms that may have adverse effects on the growth and yield of the plant due to high energetic requirements, the constitutive synthesis of various compounds such as phytoalexins and SA, and the presence of areas of dead cells (i.e., lesions). The choice between the two expression strategies may depend on the cultivar, its main disease agent, and the availability of a suitable promoter.

The productivity of some cultivars may not be drastically affected by the constitutive expression of a lesion mimic transgene. Thus, a constitutive expression system may be used in these plants to induce a high level of resistance without paying a high price in yield. The level of constitutive expression may also be controlled by the strength of the promoter; therefore, cultivars can be produced in which the lesion mimic gene is expressed at a low level that causes minimal adverse effects, but provides sufficient resistance against different pathogens (E. Lam, personal communication). Another crop-related consideration is that some crops, especially those grown in developing countries, may be under a continuous attack by a number of pathogens. In these cases an inducible promoter that will always stay active may be of no advantage over a strong constitutive promoter. The type of crop used, as well as its response to different levels of constitutive expression, and the area of the world in which it is grown may therefore greatly affect the choice of a promoter.

The disease agent(s) causing the most extensive damage to the crop of interest is also a key determinant in the choice of an expression strategy. Since the successful use of an inducible promoter is mainly dependent upon the timing of its induction, it may not be possible to use some of the existing inducible promoters to block the spread of a very aggressive pathogen. Developing resistance to these pathogens may require the use of a constitutive promoter. In addition, different pathogens may activate a specific promoter at different rates, so one inducible promoter may not be sufficient to combat a number of possible disease agents. Therefore, the choice of an expression system is also determined by the aggressiveness of the pathogen and its specific interactions with the plant.

The availability of suitable promoters appears to be the most limiting factor in developing lesion mimic-expressing crops that are pathogen-resistant. Especially critical is the availability of inducible promoters. An ideal inducible promoter is one that will have no basal level of expression and will be specifically and rapidly induced upon pathogen infection. The main problem with the currently available promoters is specificity. Some pathogen-responding promoters are also induced by factors that are not solely related to pathogen attack. For example, the prp1-1 promoter is induced by ethylene that may be produced in plants in the absence of a pathogen attack (Strittmatter *et al.*, 1995). In addition, some promoters of PR proteins are expressed in a development-dependent manner in the absence of pathogens, such as expression in flowers (Lotan *et al.*, 1989). In addition, the rapid induction of a PR promoter depends upon the recognition of the pathogen by the plant (i.e., the 'gene-for-gene' interaction; Flor, 1956; Bent, 1996). Therefore, some pathogens that may not be recognized by the plant may not cause a rapid enough induction of the PR promoter. In these specific cases the induction of the PR promoter will be a secondary event that occurs only late during infection. The 'gene-for-gene' recognition event may also be critical for choosing a lesion mimic gene. Some lesion mimic genes such as those belonging to class B, C or D (Figure 1) do not depend upon recognition and may function to induce PCD in many different plants; however, elicitor genes that belong to the class A genes (Figure 1) will require the presence of an R gene. These lesion mimic transgenes will depend on the genetic background of the plant and may not function in all plants. One solution to this problem is to introduce the R gene into the plant as well. This approach has successfully been tested (Hammond-Kosack *et al.*, 1998). At present it appears as if the successful use of lesion mimic genes in an inducible system will require the isolation of more inducible promoters, the molecular modification of currently available promoters, or the use of a combined strategy in which different constructs with different promoters will be used to transform the same plant.

Chemically inducible promoters such as DEX- or tetracycline-inducible promoters (Gatz, 1995) may also be used to drive the expression of lesion mimic transgenes. These promoters will be activated by the application of the chemical inducer only when disease symptoms are detected in the field or as part of a preventive maintenance program. As opposed to the other strategies described above the use of chemically inducible promoters will require continuous monitoring of fields, combined with chemical application.

Perhaps the most critical consideration with respect to the biotechnological use of lesion-inducing transgenes as resistance-enhancing tools is that they may not provide resistance against all pathogens. Since lesion mimic genes activate the plant's own defense mechanisms and induce PCD they may not be efficient against pathogens that the plant is incapable of resisting against even with all its defenses activated. Thus, against some pathogens a combined approach may be needed, that is, one that makes use of additional genes that encode for defenses which may not naturally occur in the plant, such as small lytic peptides. In addition to this consideration, there is always the consideration of evolutionary pressure on pathogens. Persistent use of a transgenic plant that activates all of its defense mechanisms continuously or in response to pathogen attack, for example by constitutive or inducible expression of a lesion mimic gene such as the bO gene, may exert an evolutionary pressure that will cause the development of resistance-breaking strains of pathogens. These are likely to be problematic since the plant will have no defenses left to use against them. In order to combat this problem refuge plots will have to be used.

Lesion-inducing transgenes can also be used as cell ablation tools. They can be expressed under the control of a tissue-specific promoter and used for the production of male-sterile plants, or seedless fruits. However, it was found that the action of some of these genes, such as bO or Avr9, is dependent on the developmental stage of the tissue. Therefore, in some tissues the activation of PCD will be prevented or suppressed by a particular developmental signal. In these examples other cell death-inducing genes may be used.

Conclusions and perspectives

The activation of HR cell death by transgene expression provides scientists with an excellent research tool to address different questions regarding the HR. For example, the various relationships between the pathogen and the plant, as well as the effect the pathogen may have on the activation and coordination of defense responses by the plant, may be addressed by comparing a transgene-induced HR that occurs in the absence of the pathogen to a pathogen-induced HR. This point may be specifically important for the understanding of complex plant-pathogen interactions such as those that occur between hemitrophic fungi and plants. In addition, transgenic plants that contain an inducible expression system for a cell death transgene may be used to study different biochemical, physiological, and molecular aspects of the PCD response, as well as for the isolation of mutants deficient in different steps along the PCD pathway (i.e., mutants that fail to die upon activation of the transgene; McNellis *et al.*, 1998).

HR cell death appears to be controlled in part by different developmental signals (Hammond-Kosack *et al.*, 1994; Mittler *et al.*, 1995). Lesion mimic transgenes expressed from different developmentally controlled promoters may unravel some of the basic relationships between development and the HR. Thus, we may find that plant cells undergoing a particular developmental program cannot enter the PCD pathway even though the HR-inducing transgene is expressed. Since we know of different lesion mimic transgenes that may function via different routes to induce PCD (i.e., classes A to D in Figure 1) we may test whether the developmental signal that inhibits, for example, class A or B of PCD-initiating genes will also inhibit class C or even D.

One interesting question that may be related to developmental signals is why does the activation of cell death in some of the lesion mimic transgenes or mutants occur sporadically throughout the leaf. What is the cause of cell death initiation in these particular areas of the leaf? This question is puzzling since the transgene or the mutated gene is present in all of the leaf cells. Is there a particular cellular parameter that may distinguish certain cells from others and make them more prone to the activation of PCD? This parameter/factor may be the level of a particular hormone, the cell cycle stage, or the presence or absence of other cellular or developmental signals. A dependence of PCD activation on a cellular parameter such as the cell cycle is known to occur in animals (Raff, 1992). An alternative explanation is that the cell death response may be randomly initiated in different cells of the leaf. The activation of PCD in these cells may cause the induction of a cell death repressor

mechanism in neighboring cells thus resulting in the appearance of lesions only at and around the cells that randomly initiated the cell death response.

In addition to playing an important role in the study of PCD in plants, lesion mimic transgenes may be valuable for the biotechnological development of crops with enhanced resistance to pathogens. Transgenes that induce the lesion mimic phenotype may be incorporated into our arsenal of genes used to combat plant pathogens. They may be used alone or in combination with other defense strategies to create a 'super plant' with an enhanced resistance to attack by many different pathogens.

In the future, isolation and characterization of new lesion mimic transgenes will considerably advance our understanding of the PCD response and provide us with new tools to study and control this response as well as harness it to the development of crops with enhanced resistance traits.

Acknowledgements

We wish to thank Drs Leonora Reinhold, Robert Fluhr, and Alex Levine for critical comments. We gratefully acknowledge Drs David Granot and Eric Lam for sharing unpublished results. This work was supported by funding provided by the Israel Ministry of Agriculture, the Yigal Alon Fellowship, and the Hebrew University Intramural Research Fund Basic Project Awards.

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