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Priming as a mechanism in induced systemic resistance of plants

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Abstract

Induced systemic resistance is a plant defence state that is associated with an enhanced ability – the so-called priming – to resist pathogen attack by stronger activation of cellular defence responses. So far, however, priming has not been widely appreciated when studying induced plant disease resistance. During the past several years, it has been demonstrated that pre-treatment of cultured parsley cells with inducers of systemic resistance, salicylic acid or a benzothiadiazole, leads to the direct activation of a set of defence-related genes and also primes the cells for stronger elicitation of another set of defence genes including those encoding phenylalanine ammonia-lyase. From these results, it was concluded that the resistance inducers have at least a dual role in plant defence-gene activation. When elucidating whether priming plays a role in induced systemic resistance of Arabidopsis, pre-treating plants with benzothiadiazole was found to augment the subsequent activation of phenylalanine ammonia-lyase genes by *Pseudomonas* infection, wounding and osmotic stress and also to enhance wound/osmotic stress-induced callose production. The augmentation of phenylalanine ammonia-lyase gene activation or/and callose deposition was not seen in the Arabidopsis non-expresser of pathogenesis-related genes1 mutant which is compromised in induced resistance, while it was present, without benzothiadiazole pre-treatment, in the constitutive expresser of pr genes1 and 5 mutants in which induced resistance is constitutive. Together these studies point to priming as an important cellular mechanism in induced systemic resistance of plants which requires the intact non-expresser of pathogenesis-related genes1 gene.

Abbreviations: BTH – benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester (acibenzolar-S-methyl); cpr – constitutive expresser of pr genes; INA – 2,6-dichloroisonicotinic acid; ISR – induced systemic resistance; npr – non-expresser of pr genes; PAL – phenylalanine ammonia-lyase; PR – pathogenesis-related; SA – salicylic acid.

Introduction

As early as the beginning of the past century, Beauverie (1901) and Ray (1901) realised that upon infection by a pathogen plants may develop an enhanced resistance to further pathogen attack. About thirty years later, Chester (1933) provided a descriptive study in which he summarised various phenomena of pathogen-induced disease resistance of plants. One of these phenomena is nowadays known as induced systemic resistance (ISR; Kuć, 1995) or, synonymously, systemic acquired resistance (SAR; reviewed by Ryals et al., 1996, Sticher et al., 1997). This plant defence state is induced by most pathogens that cause tissue necrosis. The phenotypic

hallmarks of ISR are an enhanced defence capacity of the plant against many different, though not all, pathogens and the development of resistance not only in the area of primary infection but also in distal, non-inoculated organs (Ryals et al., 1996).

In tobacco and Arabidopsis, development of ISR is associated with the co-ordinate expression of a complex set of so-called 'sar genes' (Ryals et al., 1996; Ward et al., 1991) which include genes for some of the pathogenesis-related (PR) proteins (Cutt and Klessig, 1992; Stintzi et al., 1993, Van Loon and Van Strien, 1999). The enzymatic activities of several PR proteins have been identified and include β-1,3-glucanases (PR-2) and chitinases (PR-3), which can hydrolyse microbial cell wall components (Stintzi et al., 1993). Furthermore, over-expression of several pr genes in transgenic plants has been shown to enhance their resistance to certain fungal pathogens (see, for example Alexander et al., 1993; Liu et al., 1994; Zhu et al., 1994). Therefore, the expression of pr genes and the associated accumulation of the encoded PR proteins has often been considered as the molecular basis of ISR. However, this assumption neglects the fact that ISR is often effective against a variety of bacterial and viral pathogens as well as against fungi (Kuć, 1995; Ryals et al., 1996; Sticher et al., 1997). Antibacterial and antiviral activity have not yet been shown for any PR protein, nor has enhanced resistance to any of these two types of pathogens been reported from transgenic plants over-expressing pr genes. Thus, although the accumulation of PR proteins may contribute to ISR, additional mechanisms are likely to be involved in its development.

In this context, it is interesting to note that, upon pathogen infection, there is activation of various cellular defence responses in attacked cells of both susceptible and resistant plants. However, in the case of resistance, cellular defence responses are induced more rapidly and more strongly than they are in susceptible interactions. Thus, an enhanced ability for the rapid and effective activation of cellular defence responses which are not induced until pathogen attack is another hallmark of plant tissue with ISR. According to the terminology for mammalian monocytes and macrophages (see 'Similarity between the administration of defence responses in primed cells of plants and humans' below) we have called this state of enhanced ability to activate cellular defence responses the 'primed' state of the plant (Katz et al., 1998). In fact, Hammerschmidt and Kuć (1982) associated a sensitised state for stronger cell wall lignification with ISR of cucumber plants eighteen years ago. For unknown reasons, these and later studies on the same phenomenon (for examples, see Schmele and Kauss, 1990; Siegrist et al., 1994) have not been widely appreciated by the ISR community and, thus, basic ISR research has mainly focussed on the PR proteins and their assumed role in ISR. This may, at least in part, be due to the fact that the enhanced ability of the ISR tissue to better activate cellular defence responses does not become obvious until pathogen attack of that tissue. Equally, the enhanced cellular defence responses are only induced in the few cells under attack thus making their investigation difficult. Therefore, although the priming phenomenon has been known for many years, little is known about the molecular and biochemical mechanism(s) that mediate tissue priming.

Salicylic acid is an endogenous signal that mediates the ISR response

Though there is still controversy about the identity of the long-distance signal that is released upon primary pathogen attack and is distributed throughout the plant to trigger ISR, it is generally appreciated that salicylic acid (SA) is needed in the signal transduction mechanism that leads to ISR (reviewed by Dempsey et al., 1999; Shah and Klessig, 1999). The most compelling evidence for the important role of SA in ISR development comes from studies with transgenic tobacco and Arabidopsis plants that express the nahG gene encoding a salicylate hydroxylase of *Pseudomonas* origin. The active enzyme destroys the SA signal by converting it to catechol. Upon pathogen attack, nahG transgenic tobacco and Arabidopsis plants do not accumulate enhanced levels of SA nor do they establish ISR, presumably due to the destruction of the SA signal (Delaney et al., 1994; Gaffney et al., 1993).

Chemical activators of ISR

Since it was clear that SA is an endogenous signal for the activation of ISR, there was increased characterisation of synthetic chemicals that are able to mimic SA in ISR induction. 2,6-dichloroisonicotinic acid and its methyl ester (both referred to as INA) were the first synthetic compounds shown to activate ISR, thus providing broad-spectrum disease resistance (Métraux et al., 1991; Ryals et al., 1996). As INA is insufficiently tolerated by some crops, a novel benzothiadiazole (BTH) recently became a very attractive, commercial ISR

activator (Friedrich et al., 1996; Görlach et al., 1996; Lawton et al., 1996). SA, INA and BTH are assumed to activate ISR through a similar signalling mechanism (Ryals et al., 1996), but little is known about how these compounds induce the ISR state.

Priming can be studied in a parsley cell culture/fungal elicitor model system

Over the past two decades, the interaction of cultured parsley (*Petroselinum crispum)* cells with a crude cell wall elicitor from *Phytophthora sojae* has been useful to investigate the activation of various cellular defence responses (Nürnberger et al., 1994). These include the so-called early oxidative burst (Kauss and Jeblick, 1995), rapid alterations in ion transport across the plasma membrane (Conrath et al., 1991), the activation of a mitogen-activated protein kinase activity (Ligterink et al., 1997), the synthesis and secretion of furanocoumarin phytoalexins and various cell wall phenolics (Hahlbrock and Scheel, 1989; Kauss et al., 1993) and the transcriptional activation of various defence-related genes (Somssich et al., 1989; Thulke and Conrath, 1998).

In the early 1990s, our laboratory reported that parsley cell cultures may be an ideal system to investigate cell priming and the resulting augmentation of cellular defence responses. Pre-incubation of parsley suspension cells with SA or INA did not directly induce coumarin secretion but greatly enhanced the subsequent low-dose elicitation of this defence response (Kauss et al., 1992a). The augmented phytoalexin production was associated with enhanced activity of coumarin biosynthetic enzymes such as phenyalanine ammonia-lyase (PAL) and xanthotoxol O-methyl transferase (Kauss et al., 1992a). Based on these findings Kauss et al. (1992a) speculated that 'among the PR proteins (that are accumulated upon treatment with inducers of ISR) there may be components of the elicitor signal transduction pathway'. In an extension of their studies, Kauss and co-workers then reported that pre-incubation of parsley cells with INA or SA also augmented the elicitor-induced incorporation of various cell wall phenolics and a lignin-like polymer (Kauss et al., 1993) as well as the elicitor induction of the early oxidative burst (Kauss and Jeblick, 1995). In addition, we have recently found that primed parsley suspension cells also display augmented alterations in ion transport across the plasma membrane (V. Katz, A. Fuchs and U. Conrath, unpubl.) and enhanced activation of a mitogen-activated protein kinase activity (S. Simonis and U. Conrath, unpubl.) upon treatment with *Phytophthora sojae* cell wall elicitor.

Inducers of ISR have a dual role in defence gene activation in parsley

While the above studies provided a detailed analysis of the priming phenomena on the enzyme activity and product levels, little was known about the influence of pre-treatment with ISR inducers on the elicitor activation of defence genes in parsley cell cultures. This led us to investigate, in detail, the influence of pre-treatment with SA and BTH on the activation of various defence-related genes in parsley suspension cells as a next step toward understanding the molecular basis of defence response augmentation.

By doing so, we found that the effect of SA and BTH on the activation of defence genes in cultured parsley cells depended on the gene that was being monitored. One group of genes, such as those encoding anionic peroxidase and mannitol dehydrogenase, was found directly responsive to treatment with either of the two inducer compounds tested and, thus, they behaved as classical pr genes of tobacco, cucumber and Arabidopsis. A second group of parsley defence genes, represented by those for PAL, 4-coumarate : CoA ligase, PR-10 proteins and a hydroxyproline-rich glycoprotein, was found only scarcely responsive to treatment with exogenous SA or BTH. Yet, these genes displayed SA/BTH-dependent augmentation of their expression upon low-dose elicitation (Katz et al., 1998; Thulke and Conrath, 1998). The enhancement by SA and BTH of pal gene activation, which was induced by elicitor treatment, strongly depended on a pre-incubation period but was not due to increased accumulation of free SA or BTH within the cells (Kohler and Conrath, unpubl.; Thulke and Conrath, 1998). Thus, the ISR inducers are mediating a time-dependent response that shifts the cells on the alert, probably by continuous synthesis of one or more cellular factor(s) with important functions in the administration of various defence responses. We suppose that some of these cellular components may directly activate the mechanism that leads to the induction of ISR inducer-responsive genes, such as the anionic peroxidase and mannitol dehydrogenase genes of parsley. Other factors may function in cooperation with elicitor-inducible signalling components resulting in augmentation of defence response induction, including activation of certain defence-related genes such as

the parsley pal, 4-coumarate : CoA ligase, pr-10 and hydroxyproline-rich glycoprotein genes.

Similarity between the administration of defence responses in primed cells of plants and humans

The proposed mode of regulation of defence responses in parsley cells has striking similarity to the administration of defence responses in humans (Figure 1). Pre-treatment with granulocyte-macrophage colony-stimulating factor or interferon-γ has been reported to prime human monocytes/macrophages for augmented lipopolysaccharide-induced production of various defence-related cytokines such as interferon-α, tumour necrosis factor and interleukin-12 (Hayes and Zoon, 1993; Hayes et al., 1991; 1995a). Using tumour necrosis factor induction as a model, Hayes et al. (1995b) demonstrated that monocyte priming by interferon-γ required a several hour pre-incubation period. In addition, interferon-γ-induced monocyte priming was primarily manifested at the level of tumour necrosis factor transcript accumulation: a fact that emphasizes the proposed similarity to defence response administration in parsley culture cells.

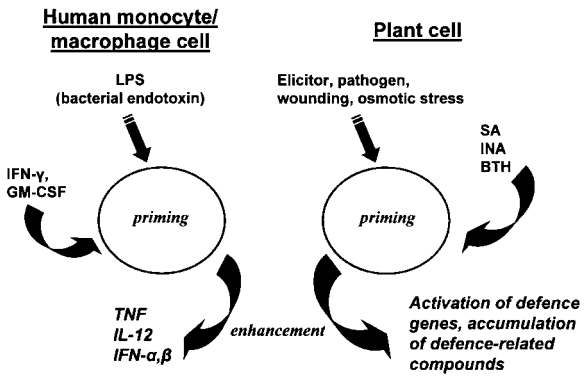

Figure 1. Similarity between administration of defence responses in human monocytes/macrophages and plant cells. Pre-treatment of human monocytes/macrophages with interferon (IFN)-γ or granulocyte-macrophage colony-stimulating factor (GM-CSF) augments the subsequent induction by lipopolysaccharides (LPS) of defense-related cytokines such as tumour necrosis factor (TNF), interleukin (IL)-12 and interferon-α/β. In analogy, pre-incubation of cultured parsley cells or Arabidopsis plants with inducers of ISR such as salicylic acid (SA), 2,6-dichloroisonicotinic acid (INA) or a benzothiadiazole (BTH) enhances the subsequent induction by elicitor (parsley), pathogen infection, wounding or osmotic stress (Arabidopsis) of a variety of defense responses. These include the activation of defence genes and the accumulation of defense-related compounds (for details, see text).

In Arabidopsis, priming causes augmentation of cellular responses induced by pathogen attack, wounding and osmotic stress

In order to investigate the priming phenomenon in association with ISR, that is at the level of whole plants, and to further elucidate the genetic basics of priming the model plant Arabidopsis was recently included in our priming experiments. By doing so we found that, in addition to directly activating pr-1 genes, the resistance inducer BTH primes Arabidopsis plants for stronger pal gene expression following infection with *Pseudomonas syringae* pv. *tomato*. The pre-treatment with BTH was found to also prime Arabidopsis plants for enhanced pal gene activation and callose deposition following mechanical wounding of leaves with forceps or after infiltrating them with water (A. Kohler, S. Schwindling and U. Conrath, unpubl.). Together, these findings confirmed the proposed dual role for BTH in the activation of defence responses in Arabidopsis. In contrast to pr gene activation, priming Arabidopsis plants for stronger pal gene expression or/and augmented callose deposition was not seen until further stimulation by pathogen attack, wounding or osmotic stress. This might explain why, over the past two decades, ISR research almost exclusively focussed on the exploration of pr gene expression rather than on the augmentation of the plants' cellular defence mechanisms (see the Introduction).

From the above results with Arabidopsis, it becomes obvious that systemic resistant plants are in an alerted state that positively affects the induction of responses to pathogen attack as well as to wounding and osmotic stress. Until recently these responses have been considered as more or less independent mechanisms. At least from the physiological point of view, priming of the ISR tissue for pathogen, wound and osmotic stress responses makes sense as pathogen attack is generally associated with tissue damage and changes in turgor pressure.

Priming for enhanced responses to pathogen infection, wounding and osmotic stress is closely associated with ISR

From previous studies with parsley cell cultures (Katz et al., 1998; Thulke and Conrath, 1998) and Arabidopsis, it was concluded that priming and the resulting augmentation of various cellular defence responses is a major mechanism of ISR in plants. If

this assumption holds true, then it should be expected that priming would be absent in ISR-deficient plants. To address this issue, we included the Arabidopsis non-expresser of pr genes (npr)1 mutant (Cao et al., 1994) which has also been called non-inducible immunity1 (Delaney et al., 1995) or salicylic acid-insensitive1 (Shah et al., 1997) in our priming experiments. Although this mutant is able to accumulate wild type levels of SA upon infection by incompatible pathogens it does not express ISR (Cao et al., 1994; Delaney et al., 1995; Shah et al., 1997). Intriguingly, the augmentation by BTH-pre-treatment of *Pseudomonas*-induced pal gene activation as well as the enhancement of wound-induced and osmotic stress-elicited pal gene activation and callose production were absent in leaves of the ISR-deficient npr1 mutant (A. Kohler, S. Schwindling and U. Conrath, unpubl.).

The Arabidopsis constitutive expresser of pr genes (cpr)1 (Bowling et al., 1994) and cpr5 (Bowling et al., 1997) mutants have been shown to express ISR against fungal and bacterial pathogens in the absence of pre-treatment with ISR inducers (Bowling et al., 1994; 1997; Cao et al., 1998). In leaves of these two mutants there was no need for BTH-pre-treatment to strongly induce pal gene expression upon *Pseudomonas* infection or both pal gene activation and callose deposition upon wounding the leaves or infiltrating them with water. Probably due to the enhanced endogenous levels of SA (Bowling et al., 1994; 1997) the Arabidopsis cpr1 and cpr5 mutants are permanently primed for strong induction of certain cellular defence responses such as pal gene activation and callose production once wound/stress-stimulated or attacked by a pathogen.

It is interesting that, in contrast to the two cpr mutants, npr1-overexpressing Arabidopsis plants obviously are not constitutively primed for wound/stress-induced pal gene expression (A. Kohler, S. Schwindling and U. Conrath, unpubl.). However, upon pathogen infection of the npr1-overexpresser, accumulation of molecular ISR markers is stronger than in the non-transformed controls (Cao et al., 1998). Therefore, while the intact npr1 gene is needed for and positively affects the priming/augmentation mechanism it is not sufficient to permanently prime Arabidopsis plants.

Dong and co-workers (Zhang et al., 1999), the Klessig laboratory (Zhou et al., 2000) and Després et al. (2000) recently reported that the Arabidopsis NPR1 protein may interact with transcription factors of the TGA/OBF basic leucine zipper protein family to activate pr-1 genes in Arabidopsis. As concluded from the experiments with the parsley cell culture (see 'Inducers of ISR have a dual role in defence gene activation in parsley' above) and our studies with Arabidopsis, we assume that during pre-treatment with ISR inducers there is synthesis of cellular factors with an important role in the direct activation of a certain set of defence-related genes, such as the Arabidopsis pr-1 genes. By binding to the promoter of yet another set of defence genes, such as the pal genes of Arabidopsis, these cellular factors might not directly cause gene activation, rather they may prepare their target gene(s) for better expression once stimulated by pathogen attack, wounding or osmotic stress. Interestingly, a binding sequence for NPR1-interacting TGA/OBF transcription factors is also present in the promoter of the Arabidopsis pal gene.

Biological activity to induce ISR correlates with ability to prime for augmented cellular responses to pathogen infection, wounding and osmotic stress

By assaying a variety of compounds, which have been reported to induce, or not induce, ISR against tobacco mosaic virus in tobacco (Conrath et al., 1995), a strong correlation was found between the ability to trigger ISR and the capability to augment pal gene activation induced by elicitor treatment in parsley culture cells (Katz et al., 1998; Thulke and Conrath, 1998) or by wounding or osmotic stress in Arabidopsis plants (A. Kohler, S. Schwindling and U. Conrath, unpubl.). This correlation suggests that the ability of ISR inducers to cause systemic resistance is mediated, at least in part, by their ability to augment defence gene activation. The important role of defence response augmentation in ISR has been further supported by Siegrist et al. (1994), who demonstrated that growing cucumber seedlings in SA or INA led to increased deposition of cell wall phenolics and enhanced enzyme activity of both chitinase and peroxidase upon *Colletotrichum lagenarium* infection of the systemically protected tissue. In addition, Mur et al. (1996) reported augmented pr-10 : uidA (Gus) and pal-3 : uidA (Gus) gene activation upon challenge infection of systemically protected transgenic tobacco plants.

It is interesting to note that, in parsley culture cells, priming for enhanced induction of phenylpropanoid defence responses and for augmented elicitation of the early oxidative burst can also be induced by pre-treating the cell cultures with methyl jasmonate (Kauss et al.,

1992b; 1994). This signalling compound was recently found to also prime Arabidopsis plants for augmented pal gene activation induced by mechanical wounding (A. Kohler and U. Conrath, unpubl.). Furthermore, Low and co-workers (Stennis et al., 1998) reported that pre-treatment with the wound-generated peptide messenger, systemin, enhanced the activation of the oxidative burst induced by oligogalacturonide elicitors or osmotic stress in cultured tomato cells. Together, these observations indicate a complex, multi-entrance nature for plant cell priming.

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