The 12th Symposium on Plant Biotechnology
Vol. 12 : 3~9(1998)

PLANT DEFENSE SIGNALLING

Moo Je CHO

Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Chinju, 660-701, Korea

INTRODUCTION

Plants are hosts to thousands of diseases caused by a variety of phytopathogenic fungi, bacteria and virus. However a relatively limited numbers of pathogens invade the plant successfully and cause disease because of self-defense mechanisms.

Plants recognize and resist many invading phytopathogens by inducing a rapid defense response, termed the hypersensitive response (HR). The HR results in localized cell death at the site of infection, which constrains further spread of the infection (1). This local response triggers nonspecific resistance throughout the plant, a phenomenon known as systemic acquired resistance (SAR) (2). An understanding of the defense signal transduction pathway associated SAR is interesting as a paradigm for signal transduction, and it may provide practical application to either genetically engineered plants with enhanced disease resistance or novel mode of action plant protection chemicals that act by stimulating the plant's disease resistance mechanisms.

Hypersensitive Response

The defense response of HR include an oxidative burst leading to production of reactive oxygen intermediates (ROIs), expression of defense related genes, alteration of membrane potentials, an increase in lipoxygenase activity, cell wall modification, lignin decomposition, and production of antimicrobial compounds such as phytoalexins. The HR is the outcome of recognition by ligand/receptor interactions specified by plant resistance (R) and pathogen avirulence (avr) genes.

One of the earliest events in the HR is a burst of oxidative metabolism leading to the generation of superoxide (O_2^-) and subsequent accumulation of hydrogen peroxide (H_2O_2) (3). These ROIs are directly protective and also drive oxidative cross-linking of the cell wall (4). Moreover, ROIs may directly trigger the HR or cell death and the subsequent induction of defense related genes as a key signaling molecules (Fig. 1). Recently several evidence suggest potential involvement of nitric oxide as plant defense signaling molecule (5).

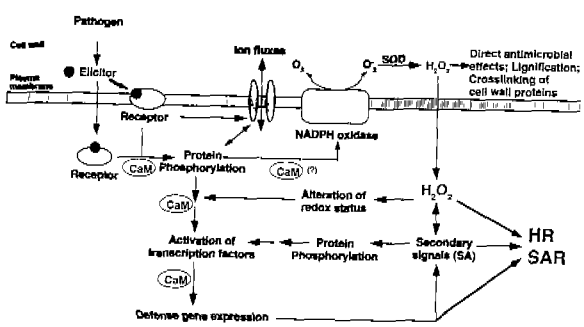

Fig. 1. A model for signal transduction in plant defense response.

In the *Arabidopsis*, *lsd*1 mutant, which form HR-like lesions spontaneously, causes runaway cell death and such lesion mimic phenotypes suggest that the host cell death response is under genetic control (6, 7).

Systemic aquired resistance

SAR refers to a distinct signal transduction pathway that play an important role in the ability of plants to defend themselves against pathogens. SAR activation results in the development of a broad-spectrum resistance. An understanding of the basic molecular mechanisms leading to the resistance could enable the development of either genetically engineered plants with enhanced disease resistance.

Salicylate including salicylic acid (SA), methyl salicylate and SA glucoside are natural products of plant metabolism and has been known to play a key role in SAR signaling (8, 9, 10). Acetyl salicylate, aspirin, has been widely used to relieve inflammation, fever and pain. The primary action of salicylates in mammals has been attributed to the disruption of eicosanoic acid metabolism thereby altering the levels of prostaglandins and leukotrienes.

SA was found to increase by several hundred-folds in tobacco and cucumber after pathogen infection, and this increase was shown to correlate with SAR, however the exact mechanism by which SA induces SAR is not certain.

Several laboratories put their efforts to identify SA-effector proteins and have identified several proteins that interact with SA (11, 12). Most of these are either ion sulfur- or heme-containing proteins and a catalase has been identified as the first SA binding protein in plant (13). SA inhibited catalase's H_2O_2-degrading activity and that H_2O_2 induced PR gene expression led to the proposal that one mechanism of SA's action was to elevate the level of H_2O_2 or H_2O_2-derived ROIs, which then serve as intermediates in the SA signaling pathway (3). The observation that SA also inhibited the activity of ascorbate peroxidase, the other major H_2O_2-scavenging enzyme support this model. Furthermore, prooxidants induced the expression of PR-1 genes while several antioxidants suppressed SA-mediated expression of these genes (13, 14).

The role of SA-mediated catalase inhibition and elevated H_2O_2 level in plant defense responses is an area of active debate. While H_2O_2 and H_2O_2-inducing chemicals activated PR-1 gene in wild type tobacco, PR-1 induction by these compounds was strongly suppressed in *NahG* transgenic plants (15, 16). In addition, no detectable increase of H_2O_2 levels was found during the onset of SAR (15, 16). Neuenschwander et al., (16) was able to detect that H_2O_2 at very high concentrations (150mM-1000mM) can stimulate accumulation of SA and activate the SA-forming enzyme, benzoic acid-2 hydroxylase. Moreover, transgenic tobacco plants exhibiting drastically reduced catalase expression, due to cosuppression or synthesis of antisense RNA, fail to constitutively accumulate elevated levels of PR-1 mRNA or protein (17, 18). Taken together, these results argue that H_2O_2 acts upstream of SA in the signal transduction pathway rather than, or in addition to, acting downstream of SA.

Another stream of evidence suggest that SA is not the translocated signal that triggers SAR in distal plant organs. Recently, we have identified the presence of new SA-independent SAR pathway mediated by specific calmodulin (CaM) isoforms. We will briefly review the role of CaM isoforms in plant defense signaling in the next chapter.

Specific-calmodulin isoform-mediated plant defense signalling

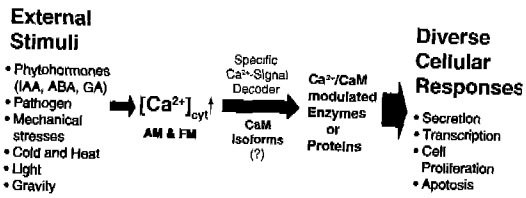

Fig. 2. Ca^{2+}/CaM-dependent signaling in plants.

Ca^{2+} acts as a second messenger in many of the diverse range of signal-transduction pathways of animals and plants. A variety of external stimuli induce increase in cytosolic Ca^{2+} concentration with different amplitude and frequency (Fig. 2). This phenomenon raises fundermental questions regarding how the variety of stimulus specific Ca^{2+}-signals can be decoded to transduce to the downstream elements in the signaling cascade. Recent work in our lab has pointed to potential role for CaM isoforms in the control of Ca^{2+}-signal specificity which exhibit different Ca^{2+}-binding affinity, and Ca^{2+} dose effect on target enzyme activation. In animals, it has also been proposed that protein phosphorylation and dephosphorylation may provide a mechanism by which the signaling information encrypted in Ca^{2+} spike and oscillation in cytosolic calcium concentration (19). The presence of protein kinases and phosphatases with different Ca^{2+} activation kinetics could allow differential decoding of stimulus-specific pattern of oscillation in cytosolic Ca^{2+} concentration into a range of physiological responses.

Many protein kinases and phosphatases show Ca^{2+}/CaM-dependent activation. Moreover, several CaM isoforms regulate target enzymes reciprocally (22). A CaM isoform isolated from soybean (SCaM1) activate a Ca^{2+}/CaM-dependent protein phosphatase, calcineurin, and NAD kinase while another soybean CaM isoform (SCaM4) serves as a competitive antagonist of these activation. The reciprocal was true for nitric oxide synthetase (NOS)(22).

The reciprocal regulation exhibited by these isoforms suggest that their differential expression may allow for a bifurcation in a Ca^{2+}/CaM signal transduction pathway, allowing selective activation and inhibition of particular sets of CaM target enzymes and producing alteration in cellular function.

CaM has been known to be highly conserved and ubiquitously distributed protein in higher eukaryotes. The essential role of CaM in a variety of cellular processes may be the reason for strict conservation of the primary structure of CaM during evolution. However, recent studies in plant systems revealed the presence of multiple CaM isoforms in a single organism (20, 23, 24, 25). This is very interesting because there exists only a single form of CaM in animal systems although a diverse CaM isoform called CaM like protein (hCLP) has been isolated from human mammary epithelial cell (26, 27).

Plant CaM isoforms found in *Arabidopsis* (25), wheat (23) and potato (24) have minor amino acid sequence divergency which show one to six amino acid difference. However CaM isoforms (SCaM4 and 5) isolated from soybean show very divergent amino acid sequence (20). Furthermore all the plants tested thus far have SCaM4 and 5 homolog although they have not isolated yet. This implicates that plant system may have a unique feature although the overall Ca^{2+}/CaM-mediated signal transduction mechanism is similar to that of animal system.

We have recently cloned 5 CaM isoforms from soybean (SCaM-1 through 5). While some of these isoforms (SCaM-1, 2 and 3) are >90% identical with mammalian CaM, two isoforms (SCaM-4 and SCaM-5) exhibit only ~78% identity and are the most divergent CaM isoforms reported thus far in the plant or animal kindom (Fig. 3).

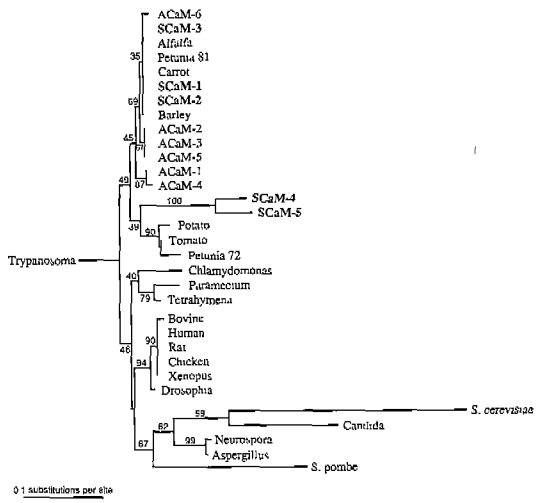

Fig. 3. Relationship of soybean calmodulin isoforms to other calmodulins.

ACaM indicates six calmodulin cDNAs isolated from *Arabidopsis thaliana*.

CaM modulates activities of a variety of enzymes upon activation by Ca^{2+}. We have identified that plant possess divergent CaM isoforms that have different ability to activate CaM target enzymes, NAD kinase and phosphodiesterase (20). Subsequent studies on the failure of SCaM-4 in the NAD kinase activation among CaM isoforms relied on the differences in their primary structures (21). The differential abilities of CaM isoforms in the activation of target enzymes suggest that CaM isoforms may have their own targets, i.e., CaM isoform-specific target. Also, in the activation of NAD kinase, SCaM-4 was shown to be able to bind the enzyme but cannot activate it, which suggest that SCaM-4 may act as a competitive antagonist in the activation of NAD kinase by SCaM-1. Indeed, in the case of animal system, hCLP was shown to selectively activate or inhibit CaM-dependent enzymes (26, 27).

Recently we have determined the effect of SCaM-1 and -4 on the activation of 10 different CaM dependent enzymes, 7 from animal sources and 3 from plant sources. As summarized in Table 1, SCaM-1, in general, is a good activator for most of target enzymes except NOS nearly

Table 1. Differential activation of calmodulin-dependent enzymes by SCaM isoforms.

	SCaM-1		SCaM-4		Brain CaM	
Animal	% miximal activation	K_{act}(nM)/Relative K_{act}	% miximal activation	K_{act}(nM)/ Relative K_{act}	% miximal activation	K_{act}(nM)/ Relative K_{act}
Phosphodiesterase	100	7.6(6.3)	104	6.2(5.2)	100	1.2(1.0)
Calcineurin	100	19.0(1.3)	50	17.0(1.2)	100	14.0(1.0)
Nitric oxide synthase	20	270.0(9.0)	80	680.0(22.0)	100	30.0(1.0)
smMLCK	100	N.D.	No activation		100	N.D.
Ca^{2+}-ATPase (RBC)	80	N.D.	No activation		100	N.D.
CaM Kinase II d (SR)	85	55.0(1.1)	81	144.6(2.9)	100	~50(1.0)
Plant						
NAD kinase	100	7.5(1.0)	No activation		100	~7.0(1.0)
Glutamate decarboxylase	100	24.3(1.0)	75	98.8(4.1)	100	15.0(0.6)
Ca^{2+}-ATPase (Radish)	100	8.8(1.0)	111	154.0(17.5)	107	12.2.(1.4)

N.D, not determined; smMLCK, skeletal muscle myosine light chain kinase; SR, sarcoplasmic reticulum

as effective as bovine CaM. In contrast, SCaM-4 was a poor activator of CaM-dependent enzymes as compared to SCaM-1 and bovine CaM, which is determined by increased K_{act} values and/or decreased % maximal activations of SCaM-4. However, in the case of NOS, SCaM-1 cannot activate the enzyme whereas SCaM-4 can near maximally activate the enzyme. Furthermore SCaM-1 competitively inhibit the activation of NOS by SCaM-4 with Ki 159 nM. This is very interesting because SCaM-4 acts as a competitive inhibitor of calcineurin (CaN) activation by SCaM-1. Therefore, each isoform has a selective sets of target enzymes where it activates or competitively antagonizes the activation of them by other CaM isoforms. Thus multiple CaM isoforms play a double reciprocal regulatory role in target enzyme activation/ inhibition. These results suggest the potential application of SCaM-1 as a selective inhibitor of NOS activity because SCaM-1 did not inhibit other CaM target enzymes tested so far except NOS. NOS is an important enzyme for a variety of cellular activity such as proliferation and neuronal function. But NO overproduction kills cells, thus selective inhibitor of the enzyme has been eagerly searched for. However, the blocking of the enzyme activity at the CaM activation step was not possible yet.

Another interesting feature observed is that Ca^{2+} pump isolated from two different plasma membrane sources, one from radish seedling and another from red blood cell, exhibited different sensitivity to SCaM isoforms. Therefore this suggest that the same enzyme from different organisms may differently regulated by SCaM isoforms.

The resistance of plants to invading pathogens is accompanied by the deployment of a complex array of defense responses (30). These include rapid death of challenged cells leading to the formation of local lesions (termed the hypersensitive response; HR) (31) and non-specific immunity to subsequent infection by a variety of pathogens known as systemic acquired resistance (SAR) (2). Accumulating evidence implicates the involvement of a Ca^{2+} signal in certain plant defense responses. A Ca^{2+} ion influx is one of the earliest events in challenged cells (1) and has been shown to be essential for the activation of defense responses such as phytoalexin biosynthesis, induction of defense-related genes, and hypersensitive cell death (32). However, the molecular target(s) of this Ca^{2+} signal and how it regulates downstream events in the defense signaling pathway is not well understood. In particular, little is known about the Ca^{2+} signal receptors and the mechanism by which these Ca^{2+}-modulated proteins transduce the Ca^{2+} signal into defense responses. Based on pharmacological studies with CaM antagonists (32), it has been proposed that CaM, a major Ca^{2+} signal transducer in both animals and plants (33), is involved. However, CaM antagonists can also influence other cellular processes not related to the Ca^{2+}/CaM signaling (33). Thus, whether CaM is an actual component of plant defense signaling and, if so, what is the identity of the CaM-modulated enzymes and/or proteins invovled remains to be determined.

Recently we demonstrate a central role for the major Ca^{2+} signal transducer, CaM, in plant defense signaling. Our results argue that the divergent CaM isoforms act as both signal receptor and transmitter of the pathogen-induced Ca^{2+} signal. Divergent CaM isoforms (SCaM-4, 5) resemble immediate early genes such as *fos* and *jun* in animal system in that certain external stimuli immediately activate their expression which then leads to cellular responses (34). Thus, the divergent CaM isoforms represent novel inducible components of the plant defense responses.

Transgenic plants that constitutively expressed these divergent CaM isoforms had

phenotypes similar to those of spontaneous lesion-mimic mutants; however, there are several notable differences. The first concerns the causal relationship between cell death and PR gene expression. While PR gene expression is tightly linked to cell death in *lsd* and *acd* mutants, it is independent of cell death in the SCaM-4 and SCaM-5 transgenic plants. The second major difference is SA dependence. SA levels in most lesion-mimic mutants are substantially higher than those in normal plants (36, 37). In contrast, in the SCaM-4 and SCaM-5 transgenic plants disease resistance responses were activated without concurrent elevation of endogenous SA level. Furthermore, removal of SA in these transgenic plants by co-expression of the *nahG* gene did not block the constitutive expression of PR genes (35). These observations strongly suggest that the divergent CaM isoforms activate plant disease resistance responses via a SA-independent pathway(s) as suggested in Fig. 4.

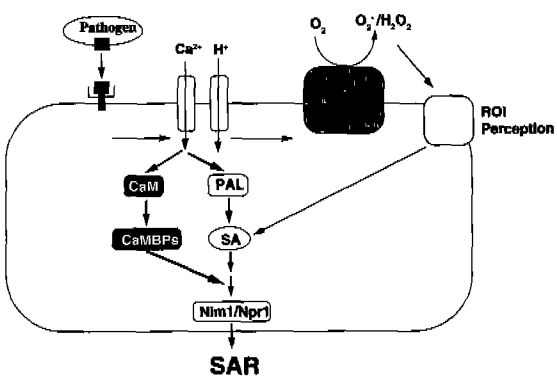

Fig. 4. A simplified proposed model for plant defense signaling pathway.

Our results provide the first *in vivo* evidence for functional differences among plant CaM isoforms. Only divergent CaM isoforms are induced by pathogens and could trigger defense responses in transgenic plants, whereas the other, highly conserved CaM isoforms such as SCaM-1 and SCaM-2 did not have these properties (35). Harding et al., (39) propose that, Ca^{2+} activation of CaM stimulates NAD kinase and the resulting increase of cellular $NADP^+$ levels then activates $NADP^+$ oxidase, which produces ROS. This Ca^{2+}/CaM pathway in ROS production is thought to be mediated by the highly conserved CaM isoforms since the divergent CaM isoforms are unable to activate NAD kinase (39). These observations support a model for concerted roles of CaM isoforms in plant defense response against pathogens, in which the highly conserved CaM isoforms mediate ROS increase, while the divergent CaM isoforms activate programmed cell death and defense gene expression.

Transgenic plants constitutively expressing several other transgenes also have been shown to have altered disease resistance. However, it is not clear whether these genes are *bona fide* components of the plant defense response pathway(s). Thus, the divergent CaM isoforms represent one of the first "natural" pathogen-inducible components in plant defense signaling whose constitutive expression leads to enhanced disease resistance. The results presented here not only enhance our understanding of the pathway(s) leading to plant disease resistance but may also provide new opportunities to genetically engineer plants with resistant to a wide spectrum of pathogens.

REFERENCE

1. Dixon, R.A., M.J. Harrison and C.J. Lamb (1994) Ann. Rev. Phytopathol. 32, 479-501
2. Ryals, J.A., U.H. Neuenschwander, M.G. Willits, A. Molina, H.Y. Steiner and M.D. Hunt (1996) Plant Cell 8, 1809
3. Chen, Z.X., H. Silva and D.F. Klessig (1993) Science 262, 1883-1886
4. Lamb, C. and R.A. Dixon (1997) Ann. Rev. Plant Physiol. Plant Mol. Biol. 48, 251-257
5. Durner, J., D. Wendehenne and D.F. Klessig (1998) Proc. Natl. Acad. Sci. (submitted)

6. Dietrich, R.A., T.P. Delany, S.J. Uknes, E.R. Ward, J.A. Ryals and J.L. Dangl (1994) Cell 77, 565-577
7. Greenberg, J.T., A, Guo, D.F. Klessig and F.M. Ausubel (1994) Cell 77, 551-563
8. Malamy, J., J.P. Carr, D.F. Klessig and I. Raskin (1990) Science 250, 1004-1005
9. Yang, Y., J. Shah and D.F. Klessig (1997) Genes and Dev. 11, 1621-1639
10. Rasmussen, J.B., R. Hammerschmidt and M.N. Zook (1991) Plant Physiol. 97, 1342-1347
11. Durner, J., J. Shah and D.F. Klessig (1997) Trends Plant Sci. 2, 266-274
12. Shah, J. and D.F. Klessig (1998) In: Biochemistry and Molecular Biology of Plant Hormones, Elsevier. In press
13. Conrath, U., Z. Chen, J.R. Ricigliano and D.F. Klessig (1995) Proc. Natl. Acad. Sci. (USA) 92, 7143-7147
14. Wendehenne, D., J. Durner, Z. Chen and D.F. Klessig (1997) Phytochemistry 47, 651-657
15. Bi, Y.M., P. Kenton, L. Mur, R. Darby and J. Draper (1995) Plant J. 8, 235-345
16. . Neuenschwander, U., B. Vernooij, L. Friedrich, S. Hknes, H. Kessmann and J. Ryals (1995) Plant J. 8, 227-233
17. Chamnongpol, S., H. Willekens, C. Langebartels, M. Van Montagu, D. Inze, W. Van Camp (1996) Plant J. 10, 491-503
18. Takahashi, H., Z. Chen, H. Du, Y. Lki and D.F. Klessig (1997) Plant J. 11, 993-1005
19. Goldbeter, A., G. Dupont and M.J. Barridge (1990) Proc. Natl. Acad. Sci. 87, 1461
20. Lee, S.H., J.C. Kim, M.S. Lee, W.D. Heo, H.Y Seo, H.W. Yoon, J.C. Hong, S.Y. Lee, J.D. Bahk, I. Hwang and M.J. Cho (1995) J. Biol. Chem. 270, 21806
21. Lee, S.H., H.Y. Seo, J.C. Kim, W.D. Heo, W.S. Chung, K.J. Lee, M.C. Kim, Y.H. Cheong, J.Y. Choi, C.O. Lim and M.J. Cho (1997) J. Biol. Chem. 272, 9252
22. Cho, M.J., P.L. Vaghy, R. Kondo, S.H. Lee, J. Davis, R. Rehl, W.D. Heo and J.D. Johnson, J. Biol. Chem. (submitted)
23. Yang, T., G. Segal, S. Abbo, M. Feldman and H. Fromm (1996) Mol. Gen. Genet. 252, 684
24. Poovaiah, B.W., D. Takezawa, G. An and T.J. Han (1996) Plant Physiol. 149, 553
25. Gawienwski, M.C., D. Szymanski, I.Y. Perera and R.E. Zielinski (1993) Plant Mol. Biol. 22, 215
26. Yaswen, P., A. Smott, J. Hosoda, G. Parrydnd, and M.R. Stampfer (1992) Cell growth & Differentiation 3, 335
27. Edman, C.F., S.E. George, A.R. Means, H. Schulman and P. Yaswen (1994) Eur. J. Biochem. 226, 725
28. Barbato, G. *et al.* (1992) Biochemistry 31, 5269
29. Cho, M.J. *et al.* unpublished data
30. Jackson, A.O. and C.B. Tayler (1996) Plant Cell 8, 1651
31. Goodman, R.N. and A.J. Novacky The Hypersensitive Reaction in Plants to Pathogens: A Resistance Phenomenon. (APS Press, St. Paul, 1994)
32. Sacks, .W.R. *et al.*, in Advances in Molecualr Genetics of Plant-Microbe Interactions, E.W. Nester and D.P.S. Verma, Eds. (Kluwer Academic Publishers, Dordrecht, 1993) Vol. 2, pp. 485-495
33. Roberts, D.M. and A.C. Harmon (1992) Annu. Rev. Plant Physiol. Plant Mol. Biol. 43, 375
34. Roberts, D.M. and A.C. Harmon (1986) Annu. Rev. Plant Sci. 4, 311
35. Heo, W.D., S.H. Lee, and M.J. Cho, unpublished Data
36. Dangl, J.L., R.A. Dietrich, and M.H. Richberg (1996) Plant Cell 8, 1793
37. Durner, J., J. Shan, and D.F. Klessig (1997) Trends Plant Sci. 2, 266
38. Morgan, J.I., and T. Curran (1989) Trends Neurosci. 12, 459
39. Harding, S.A., S.-H. Oh, and D.M. Roberts (1997) EMBO J. 16, 1137