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Plant Pathogenesis-Related Proteins: Molecular Mechanisms of Gene Expression and Protein Function[1]

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Higher plants accumulate several kinds of "pathogenesis-related (PR)" proteins in response to infection by pathogens such as fungi or viruses. Gene expression of one group of PR proteins is known to be mediated by phytohormone ethylene. Here we describe the signal transduction system from the ethylene receptor ETR to transcription factors, ERFs. Ethylene-inducible PR genes are expressed constitutively in roots and cultured cells even when are not infected. We discuss the mechanisms of this pathogen-independent expression of PR genes and describe recent findings in the study of molecular mechanisms of antifungal activities of the PR proteins. Genes of PR-1 and -5 proteins have now been identified in the genomes of various species of organisms, including humans and nematodes. PR proteins may contribute to the innate immunity of plants as well as to that of other organisms.

Key words: antifungal activity, ERF (EREBP), ethylene, innate immunity in plant, pathogenesis-related protein (PR protein).

Unlike animals, plants do not have the clonal-selection immune system called acquired immunity. They protect their bodies by hardening the cell wall, producing antibiotic compounds called "phytoalexins" and antibiotic proteins, and by accelerating cell death (hypersensitive reaction) to suppress the spread of infectious pathogens. Pathogenesis-related (PR) proteins have been defined as proteins encoded by the host plant but induced by various types of pathogens such as viruses, bacteria, and fungi, and those induced by the application of chemicals that mimic the effect of pathogen infection or induce similar stresses (*1*). PR proteins originally were divided into 5 groups on the basis of findings of serological and sequence analyses (*1*). Currently, another six groups of proteins induced by pathogens have been recommended for inclusion as PR proteins (*2*).

The five classic PR protein groups generally have two subclasses: an acidic subclass protein that usually is secreted to the extracellular space, and a basic subclass usually transported to the vacuole by a signal sequence located at the C-terminal end (The tobacco PR-5d, or OLP, is included because its intracellular location and gene expression is very similar to those of basic subclass proteins, although it has a neutral pI) (*3-6*).

Induction of PR gene expression during pathogen infection is mediated by various signaling molecules. Salicylic acid (the deacetylated form of aspirin) and reactive oxygen species mediate the expression of acidic PR genes (for review, Ref. *7*). Expression of basic PR genes is mediated by gaseous phytohormone ethylene (C_2H_4) and methyl jasmonate (a compound structurally related to prostaglandin, a mammalian signaling molecule for inflammation) (*8*). Protein factors, such as kinase and DNA-binding protein, that regulate the expression of these genes have been identified in recent years.

Basic PR genes also are expressed constitutively in some organs, including roots, limited parts of seedlings, and in cultured cells—independent of pathogen infection. In addition to pathogen-inducible gene expression in leaves, constitutive expression of PR protein in some organs, in particular the roots, also would be significant for plant defense. The root usually is surrounded by soil, in which microorganisms abound, and is considered to defend itself by a preexisting defense mechanism, as well as an induced one (*9*). The molecular mechanisms for the constitutive expression of PR genes are not clear.

Although all five classic PR proteins are now known to have antifungal activities (for reviews, Refs. *10* and *11*), their active molecular mechanisms are not well understood except PR-2 (β-1,3-glucanase) and PR-3 (chitinase). Molecular cloning and sequence analysis have shown that PR-1 and -5 genes also exist in other organisms, such as animals. Studies of non-plant PR proteins may help plant researchers understand the action of molecular mechanisms of plant PR proteins.

Signal transduction systems from ethylene perception to the kinases leading to various morphological and physiological changes in the plant, including basic PR gene expression, have been extensively reviewed (*e.g.*, Refs. *12* and *13*). We therefore have focused on recent information

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Abbreviations: CRISP, cysteine-rich secretory protein; ERF, ethylene-responsive element binding factor; OLP, osmotin-like protein; PR, pathogenesis-related.

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about molecular mechanisms for the expression of basic PR genes, especially nuclear factors, and the action of PR proteins.

1. Regulatory mechanism for the expression of basic PR genes

A. Signal transduction pathway from ethylene perception to transcriptional activation. Pathogen infection of the plant leaf accelerates ethylene biosynthesis in the leaf, and ethylene activates the signaling pathway leading to expression of basic PR genes (*12, 13*), but other signaling pathways may exist (*8, 14*) (Fig. 1). Studies of *Arabidopsis* mutants have identified some genes that regulate the expression of these ethylene-inducible genes (*12, 13*).

The ethylene receptor ETR1 is highly homologous to the bacterial two-component histidine kinase. It carries an N-terminal receptor domain composed of three transmembrane segments and, on the cytosolic side, a histidine kinase domain followed by a receiver domain. In many bacterial two-component systems, kinase activity results in the phosphorylation of a conserved histidine residue. The phosphate group subsequently is transferred to a second protein, the response regulator, which is phosphorylated on an aspartic acid residue of the receiver domain. Unlike ETR1, which has the receiver domain fused to the C-terminal end of the kinase domain, the other ethylene receptors, ERS of *Arabidopsis* and Nr of tomato, lack a receiver domain. The target proteins of the phospho-transfer of these two proteins have yet to be identified. A third putative ethylene receptor, ETR2, has now been cloned (*15*). Surprisingly, the histidine residue in the kinase domain (postulated to be a phosphorylation site in ETR1, ERS, and bacterial two-component proteins) is replaced by a glutamine residue. It is not clear how the signal of ethylene perception by ETR2 is transduced to downstream

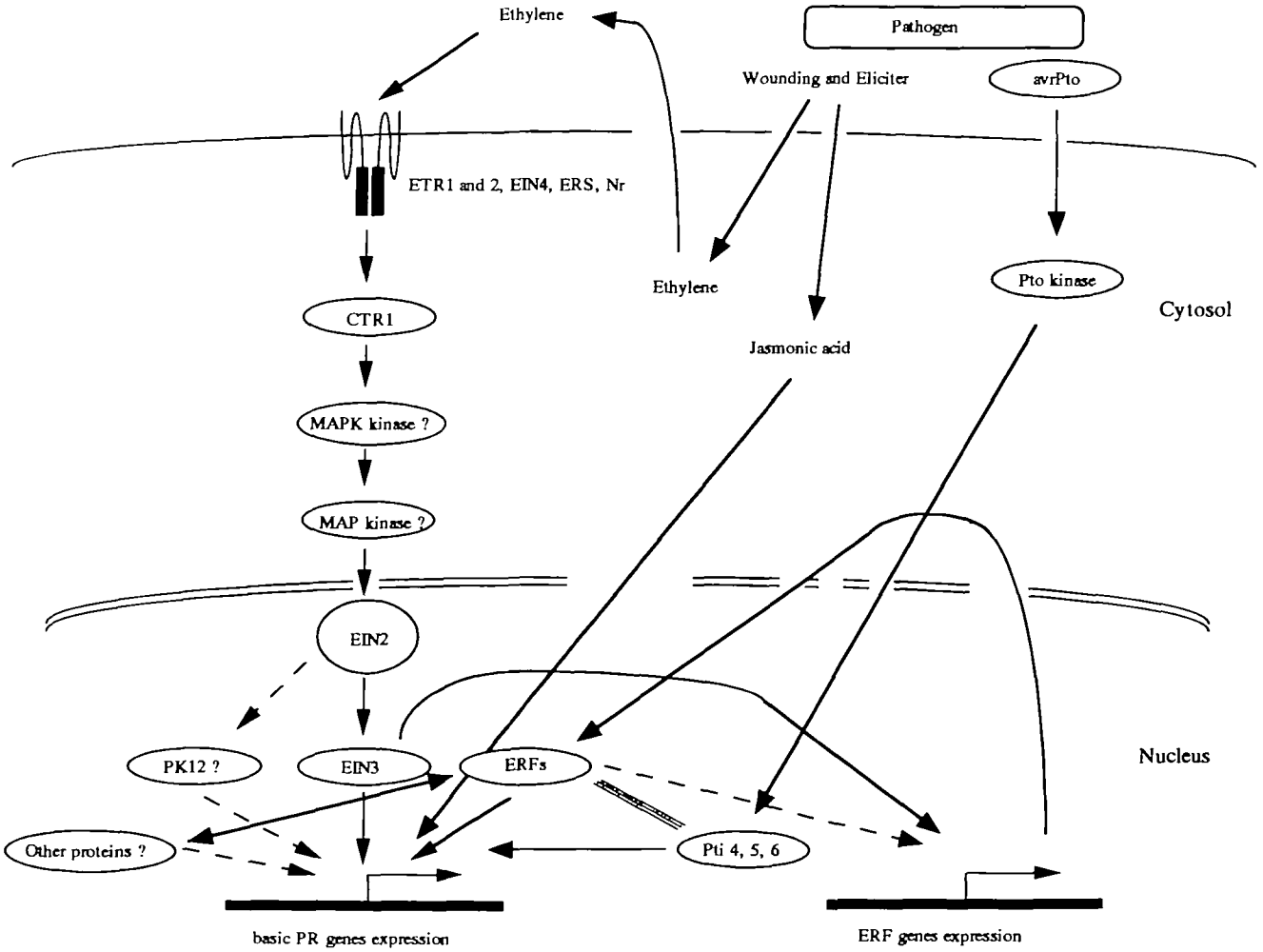

Fig. 1. **Hypothetical model of ethylene-induced PR gene expression.** CTR1, a negative regulator, is thought to be inactive when the ethylene-response occurs. The cascade of ETRs/ERS/EIN4-CTR1-EIN2-EIN3 was confirmed by genetic analysis using the *Arabidopsis* mutant. Involvement of ERF in ethylene-induced PR gene expression was confirmed by analysis using the mutated promoter of tobacco PR genes, in which ERF-recognition sequences were disrupted. The presence of MAPK kinase/MAP kinase and the involvement of PK12 and ERFs in this pathway have not been confirmed. Pti4, 5, and 6 are tomato ERF homologs which interact with Pto kinase. *Arabidopsis* ERF homolog, AtEBP, interacts with the *ocs* element-binding factor. Intracellular locations of ERFs activated by Pto kinase and of EIN2 have not been determined. The signaling pathway for the expression of ethylene-mediated ERFs and jasmonic acid-mediated PR genes has still to be determined.

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proteins.

Genetic studies showed the protein kinase CTR1 acts downstream of ETRs, ERS, and EIN4, another putative ethylene receptor (*12, 13*). The mutant *Arabidopsis ctr1*, which is thought to lack functional kinase CTR1 activity, has a constitutive ethylene-response even when not treated with ethylene. This indicates that the ethylene response is down-regulated by the kinase activity of CTR1. CTR1 is similar to mammal Raf1 protein kinase, a MAPKK kinase present in eucaryotic cells. MAPKK kinase in combination with MAPK kinase and MAP kinase, participates in phosphorylation cascades in a variety of developmental or stress signaling events.

These findings indicate that in the ethylene signaling pathway of plants, unlike most signaling pathways of eucaryotic cells, a bacterial two-component system links to the eucaryotic MAP kinase cascade, activating the ethylene response. The actual CTR1 inactivation mechanism induced by ETRs is not known, but a two-component system linked to the MAP kinase cascade operates in the osmosensing of *Saccharomyces cerevisiae* (for review, Ref. *16*), in which the phosphate group of Sln1 (a hybrid type of histidine kinase) and a receiver domain are sequentially transferred to the proteins Ypd1 and Ssk1, leading to the activation of Ssk2, a MAPKK kinase homolog, through direct interaction between Ssk1 and Ssk2 (*17*). No intermediary protein between ETRs and CTR1 has been found. Instead, ETR1 is reported to bind directly to CTR1 in yeast and *in vitro* (*18*). Although many MAPK and MAP kinases have been cloned from plants, neither type of kinase has been assigned to act downstream of the ETR1-CTR1 cascade.

Genetic analysis of the *Arabidopsis* mutant has detected proteins that act further downstream of the putative MAPK cascade (*12*). EIN2 is one such protein which has a domain similar to an ion transporter (*19*). EIN3, and probably its homologous protein, EILs, acts downstream of EIN2 (*12*). These proteins are localized in the nucleus. The involvement of EIN3 as a transcription factor in ethylene-induced gene expression of ERF, a transcription factor for basic PR genes (see below), recently was reported (*20*). A protein homologous to EIN3, TEIL, also was cloned from tobacco (*21*). A consensus DNA binding site of TEIL or its related sequences are found in the promoter regions of several ethylene-inducible genes including basic β-1,3-glucanase genes and basic chitinase genes, but it differs from the GCC box (*cis* element for ERF) described below. Although there is no evidence of its involvement in the ethylene-induced expression of the β-1,3-glucanase gene, these findings indicate that EIN3 and its tobacco homolog may directly function as transcription factors for the ethylene-induced expression of basic PR genes or indirectly *via* ERFs.

The nuclear-localized protein, kinase PK12, which belongs to the LAMMER kinase family, is expressed in leaves after ethylene-treatment (*13* and *22*). This kinase also may be a factor that mediates the ethylene-signaling pathway.

B. Structure of ERF, a transcription factor of PR genes. Characterization of the *cis* element of the promoter regions of basic PR genes provided information about a novel transcription factor, ERF, an ethylene-responsive element binding factor (formally called EREBP) that is the best studied DNA-binding protein involved in the expression of basic PR genes (*23, 24*). ERF recognizes the AGCCGCC sequence (GCC box) present in the 5′ untranscribed regions of almost all the basic PR genes of dicots (*14*), and which has been shown to be an ethylene-responsive *cis* element of the β-1,3-glucanase (*23*), PR-1 (*25*), and PR-5d (*26*) of tobacco.

Isolated ERF proteins of tobacco make up three families: ERF1/2, 3, and 4. There is no significant homology among the ERFs, except for a limited region of 59 amino acid residues located in the middle of the coding region of ERF1/2 and 4, and near the N-terminal end of ERF3. Southwestern analysis of deleted ERF2 showed that a conserved region of 59 amino acid residues confers DNA-binding activity and sequence specificity (*23*).

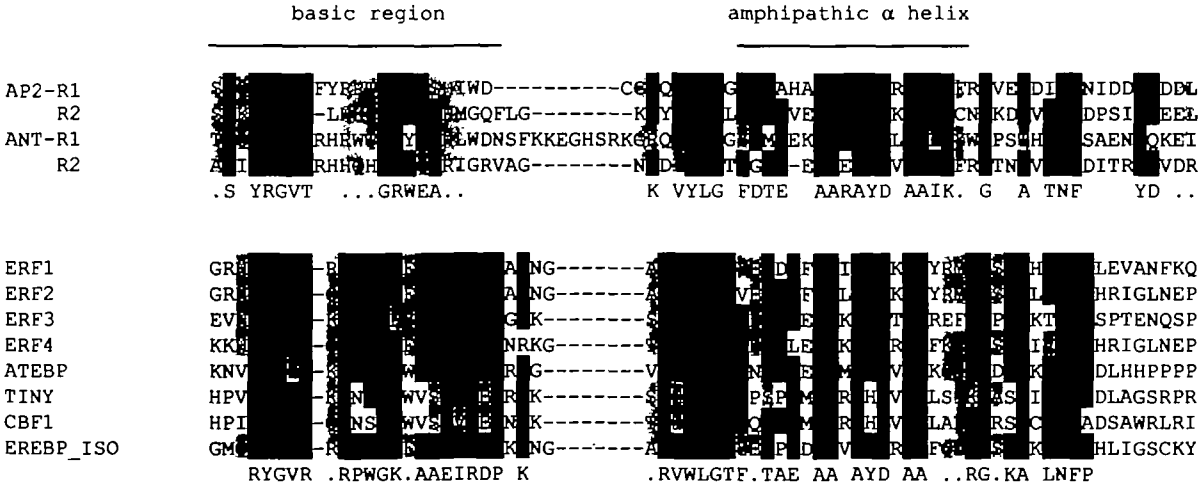

Fig. 2. **Alignment of predicted amino acid sequences of the AP2/ERF domains.** The alignment made by MacVector™ ver. 6.0 (Oxford Molecular Group) was modified manually. Identical amino acid residues are marked by black boxes and similar ones by gray boxes. Consensus amino acid residues of each subgroup are shown below the boxes. Sequences used are repeats 1 and 2 of *Arabidopsis* AP2 (DDBJ/EBML/Genbank accession no. U12546) and ANTEGUMENTA (U40256); tobacco ERF1 (D38123); ERF2 (D38126); ERF3 (D38124); ERF4 (D38125); *Arabidopsis* AtEBP (Y09942); TINY (X94698); CBF1 (U77378) and the EREBP isolog (AC002388).

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This DNA-binding domain (called the AP2/ERF domain) is now known to be widely distributed in various putative plant transcription factors, both dicot and monocot, but its functions and specificities are not the same. For instance, *Arabidopsis* APETALA2 (AP2, *27*), ANTEGUMENTA (*28* and *29*), and TINY (*30*) have this domain and function in organ development but not in ethylene signaling or PR gene expression. Actually, AP2 does not recognize the GCC box (unpublished results mentioned in Ref. *31*). It has been suggested that AP2 and ERF domain are belongs to two subdivisions, as the fewer amino acids being identical between ERF and AP2 domains in all members of the extended family (*32*).

The AP2/ERF domain is divisible into two separate small motifs (Fig. 2). The N-terminal side has about 20 amino acid residues that are rich in basic amino acids. The basic nature of this motif may contribute to the binding of the AP2/ERF domain to DNA. Recently three dimensional structure of AP2/ERF domain isolated from *Arabidopsis* was determined in complex with the DNA fragment containing GCC box by NMR. The result indicated that antiparallel β sheets were involved in DNA-binding in the major groove (*33*). In the C-terminal side motif composed of about 40 residues, 18 highly conserved amino acids were speculated to form an amphipathic α-helix structure (*31*). This amphipathic α helix may function in protein–protein interaction to control transcriptional activities or the intracellular location. This is consistent with reports that the AP2/ERF domain of the tomato homolog of ERF, Pti (Pto-interacting protein) 4, 5, and 6, has binding activity for tomato Pto kinase, a Ser/Thr protein kinase that confers resistance to the tomato bacterial speck disease caused by *Pseudomonas syringae* pv. *tomato* (*14*).

C. Role of ERFs in PR gene expression. Northern analysis showed that in general the accumulation of mRNAs in each of the tobacco ERFs, is positively regulated by ethylene in the aerial plant parts and is constitutively expressed in roots and cultured cells, as are basic PR genes (*23*). In addition to the ethylene-induced expression of basic PR genes, ERFs are thought to be essential factors in constitutive gene expression in these tissues because disruption of the GCC boxes abolished PR-5d promoter::GUS fusion gene expression in the roots and cultured cells of transgenic tobacco (*34*).

The existence of at least four isogenes of ERF suggests that they function differently in the regulation of PR gene expression; *i.e.*, each ERF having a preference for a distinct basic PR gene promoter or a role in the distinct tissue-specific expression of basic PR genes. In fact, detailed analysis has shown that individual ERF genes are expressed in slightly different manners (*23*). In untreated healthy leaves of tobacco (cv. BY4), ERF1 and 2 were expressed at low levels, and ERF4 was barely detectable. In contrast, there was a high accumulation of ERF3 mRNA in the top leaves, and expression of a basic chitinase gene was but not that of the basic β-1,3-glucanase gene. Treatment with ethephon (an ethylene-releasing compound that induces both the chitinase and β-1,3-glucanase genes) induced only ERF4 highly in both the top and lower leaves, whereas the induction of ERF1, 2, and 3 was limited to the top leaves. In the root, all the ERFs were expressed constitutively like the chitinase, β-1,3-glucanase, and other basic PR genes.

The expression system of PR genes in the root may differ from that in ethylene-treated leaves. The PR-5d (*34*) and basic β-1,3-glucanase (*35*) genes are expressed in the root tip, cortex, and vascular cylinder of the root. Although the constitutive expression of the *Arabidopsis* basic chitinase gene in the roots is, like tobacco PR-5d, thought to be GCC box/ERF-dependent, this gene is reported to be expressed in roots of an ethylene-insensitive *etr1* mutant but not in its ethylene-treated leaves (*36*).

An ERF-dependent but ethylene-independent signaling pathway may control the expression of basic PR genes in leaves as well as in roots. Direct interaction of Pti and Pto, activated by the bacterial protein AvrPto through direct interaction, suggests that such a pathway would control the expression of basic PR genes in infected leaves (*14*). Furthermore, the ethylene-insensitive tomato mutant *Never-ripe* (*Nr*) expresses the basic PR-1 gene in its leaves when infected with the virulent bacteria *Xanthomonas campestris* pv. *vesicatoria* (*37*), but whether ERFs are involved is not known.

D. Regulation of the activity of ERF proteins. The recombinant ERFs expressed in *Escherichia coli* bind to the GCC box *in vitro* (*23*). In yeast, the recombinant protein of tomato Pti5, a homolog of tobacco ERF1/2, which fuses to the GAL4 DNA-binding domain could drive the transcription of the reporter gene controlled by a promoter having the GAL4 recognition sequence (*14*). These findings and the expression patterns of the ERFs suggest that the expression of these genes is a limiting step in the regulation of the expression of basic PR genes.

Posttranscriptional or posttranslational regulation of ERFs also may control basic PR gene expression. Recent findings suggest that some intracellular proteins interact with ERFs. Interaction between Pto kinase and Pti proteins (*14*) suggests that phosphorylation of ERF protein by a specific kinase may alter the intracellular localization, DNA-binding, or transactivation activity of ERFs. Büttner and Singh (*38*) isolated an *Arabidopsis* DNA-binding protein which has similarity to ERFs in the ERF/AP2 domains and recognizes the GCC box of the tobacco basic PR-1 gene *in vitro*. This protein, named AtEBP, interacts with the *ocs* element-binding factor (*cis* element required for the expression of pathogen genes and plant defense genes) in yeast and *in vitro*, further evidence that ERFs act in coordination with other proteins to control basic PR gene expression.

During pathogenesis, complicated phenomena occur in plant cells. Signals derived from the host plant, as well as from the infecting pathogen, may cooperatively contribute to the expression of basic PR genes. Characterization of the signal transduction network is a very new and challenging area.

2. Function of PR proteins in plant defense

PR proteins are known to have antifungal activity *in vivo*, *in vitro*, or both. PR-2 and -3, respectively, are β-1,3-glucanase and chitinase, which degrade fungal cell wall components. The molecular activities of the other PR proteins, however, are not yet known. Here we focus on the action of PR-1 and -5, homologous genes recently found in animal systems.

A. PR-1. Of the PR-protein families, PR-1 proteins are the most abundantly accumulated after pathogen infection, with concomitant accumulation of the transcript occurring

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during pathogen attack. Antifungal activities of PR-1 have been shown *in vivo*, in transgenic plants that overproduce tobacco acidic PR-1 (*39*), and an *in vitro* assay (*40*). The *in vitro* assay showed that tobacco basic and acidic and tomato basic PR-1 proteins inhibit zoospore germination of *Phytophthora infestans* (*40*). The molecular mechanism of their activities, however, has yet to be determined (for review, Refs. *1*, *10*, and *11*).

Studies of proteins identified in other organisms homologous to plant PR-1 may provide information for clarifying the actual mechanism of the antifungal and unknown functions of plant PR-1. The gene family that includes mammal cysteine-rich secretary protein (CRISP) and allergen 5 (Ag5) from vespid venom belongs to a superfamily that includes plant PR-1 (*41* and *42*). Mammal CRISPs generally have a signal peptide for secretion that is absent in the mature protein. The mature protein consists of two domains, one corresponding to the entire sequence of plant PR-1, and the second in the C-terminal third where 10 of 16 conserved cysteine residues are clustered.

The function(s) of these domains is not known, but the PR-1-like domain they may be essential because most invertebrate proteins such as *Drosophila* Agr (DDBJ/EMBL/GenBank accession number L49036), hypothetical *Caenorhabditis elegans* protein (U23514 gene F48E8.1), and three yeast proteins (SWISSPROT YKZ3_YEAST, and X83502 genes J1022 and J1027) do not conserve the Cys-rich domain. Moreover, vespid venom Ag5 (M98858) and fungus *Schizophyllum commune* protein pSC7 (M81722) also lack this domain. A human cDNA clone completely lacking this domain has been isolated and, probably was generated by the alternative splicing of the CRISP1 gene (*41*).

By analogy to plant PR-1, some CRISPs are thought to be involved in the defense against pathogens (*38*, *43*, and *44*), but conclusive evidence has yet to be reported. On the other hand, involvement of mammal CRISPs in sperm maturation, the fertilization process, or both also has been suggested. The CRISP-1 gene is expressed mainly in the epididymis which holds sperm during maturation. CRISP-1 accounts for about 15% of the protein content of mouse epididymal fluid (*45*) and is loosely associated with mouse spermatozoa (*45* and *46*). Furthermore, antibodies against acidic epididymal glycoprotein (a rat homolog of CRISP-1) appear to block fertilization *in vivo* (*47*).

Inhibition of the ryanodine-sensitive Ca^{2+} channel by helothermine, a toxic peptide purified from salivary secretions of the Mexican beaded lizard, is the only well-documented molecular function of any of the PR-1-like proteins (*48*). Injection of helothermine to rodents causes lethargy, rear limb paralysis, hypothermia, and death. In a cell-free system, it reversibly inhibits the binding of ryanodine, a plant alkaloid, to the cardiac and skeletal ryanodine-sensitive Ca^{2+} channel. Furthermore, it inhibits Ca^{2+} release by this channel. Although it is not known whether its PR-1-like or Cys-rich domain is important for this action, this finding suggests that plant PR-1 proteins also may interact with the channel proteins of target cells.

B. PR-5. PR-5 proteins are sometimes called thaumatin-like proteins because their amino acid sequences are highly similar to those of thaumatin, a sweet-tasting protein isolated from the fruit of *Thaumatococcus danielli*. Basic PR-5, osmotin, accumulates in ethylene-treated leaves and in salt-adapted tobacco cells, but its function in those cells is not clear (*49*). Antifungal activities of PR-5 proteins have been reported against such fungi as *Candida albicans*, *Neurospora crassa*, *Trichoderma reesei*, *Fusarium oxysporum*, *Phytophthora infestans*, and *Asternaria solani* (*50–57*).

Detailed analyses have shown that their antifungal activities are caused by the lysis of spores, inhibition of hyphal growth and/or the reduction of spore germination (*54, 55, 57*). The precise mechanism that accounts for the antifungal activity of PR-5 proteins, however, is unknown. Zeamatin causes rapid release of cytoplasmic material from *C. albicans* and *N. crassa* at the hyphal tip or immediately behind the hyphal apical dome; places susceptible to turgor pressure. Hyphal rupture occurred in less than 15 s at 23°C (*50*). These findings suggest that permeabilization of the plasma membrane is responsible for the antifungal activity of PR-5. One hypothesis is that PR-5 protein is inserted directly into the fungal membrane forming a transmembrane pore and causing the influx of water and subsequent osmotic rupture (*50*). This is consistent with reports that the bursting of hyphal cells of *Trichoderma longibrachiatum* by osmotin (*55*) and of *Neurospora crassa*, *Trichoderma reesei*, and *Cochiliobolus miyabeanus* by PR-5d (*57*) was suppressed when a high concentration of saccharide was present in the medium. This hypothesis, however, is negated by the fact that zeamatin is active at 0°C (*50*), at which temperature pore formation is unfavorable because of the crystalline nature of the lipid bilayer. Involvement of the membrane, not the cell wall, in the sensitivity of fungi to PR-5 protein is clear because spheroplasts of *Saccharomyces cerevisiae* are sensitive to osmotin, whereas cells are insensitive (*58*). The cell wall, in fact, may act as a barrier.

Although thaumatin has amino acid sequences similar to those of other PR-5s, its antifungal activity against *C. albicans* is weak [*52* or not detectable (*57*)]. On the other hand, zeamatin (*50*) and PR-5d (Koiwa *et al.*, unpublished) do not have a sweet taste. These differences between thaumatin and PR-5s are due to their different tertiary structures. The crystal structures of zeamatin (*59*) and PR-5d (Koiwa *et al.*, in preparation) in addition to the structure of thaumatin (*60*), have recently been determined.

In general, the tertiary structures of zeamatin and PR-5d are homologous to that of thaumatin. The mature form of zeamatin is composed of 206 amino acid residues and has a total of 13 β-strands. Eleven of them form a β-sandwich, which forms the core of the protein. Residues 124–177 make up an arm composed of a short α helix which loops out from the core domain, creating a cleft. Sixteen cysteine residues, conserved among the PR-5 proteins, thaumatin, and the putative *C. elegans* PR-5-like proteins (described below) form eight pairs of disulfide bonds to stabilize the protein structure.

One of the most striking features of the zeamatin structure is its electrostatically polarized surface. The cleft on the front side is highly acidic, whereas the back side is heavily populated with arginine and lysine residues, creating a predominantly positive surface which is solvent accessible. An acidic cleft also exists in PR-5d. In contrast, thaumatin has only a few of the conserved acidic residues of the major cleft, and its cleft is basic. The back sides of

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thaumatin and PR-5d, like zeamatin, are predominantly basic.

Even if zeamatin forms a multimeric complex, it would be not sufficient to form a pore. The acidic cleft of zeamatin and PR-5d may interact electrostatically with some molecule in the fungal cell (*e.g.*, a channel or receptor protein) resulting in an influx of water or ion. This model is consistent with the finding that NaCl suppresses the inhibition of the spore germination of *Trichoderma* by osmotin (*55*) or PR-5d (*57*), probably by inhibition of their electrostatic interaction. Indeed, most of the homologous region among PR-5 proteins fall within the core domain as well as the acidic cleft, indicating these structure is involved in the antifungal activity.

The direct interaction of PR-5 and some target molecule is suggested by the recent finding of PR5K in *Arabidopsis* (*61*). The N-terminal domain of PR-5K is similar to the entire sequence of the mature PR-5 protein, and probably is arranged extracellularly. This domain is joined by a transmembrane region to a cytoplasmically oriented Ser/Thr protein kinase domain. Although its ligand has yet to be identified, this intracellular kinase domain is, by analogy to many animal receptor protein kinases involved in signal transduction pathways, thought to be activated or inactivated by the binding of a putative ligand to the extracellular PR-5 domain.

The arm structure, in contrast, may not be important, because some monocot PR-5 proteins lack this domain but have antifungal activity (*59*).

The PR-5 protein long was believed to be unique to the plant kingdom, but recent sequence analysis detected the existence of PR-5-like genes in the *C. elegans* genome. Figure 3 shows the alignment of the predicted amino acid sequences of the representative plant and putative *C. elegans* PR-5 genes. A corresponding cDNA clone (D70016) encoded by the fifth putative coding region of cosmid CEF28D1 (Z70684), has been obtained from the five putative *C. elegans* genes. Although the protein product of the PR-5 genes has not been reported in *C. elegans*, conservation of the predicted amino acid lengths (except in one gene) and the 16 cysteine residues indicate that these genes may encode functional proteins.

Whether these proteins have antibiotic activity and function in the defense system is not known, but the existence of PR-5, as well as PR-1, suggests that PR proteins contribute to the innate immunity of plants as well

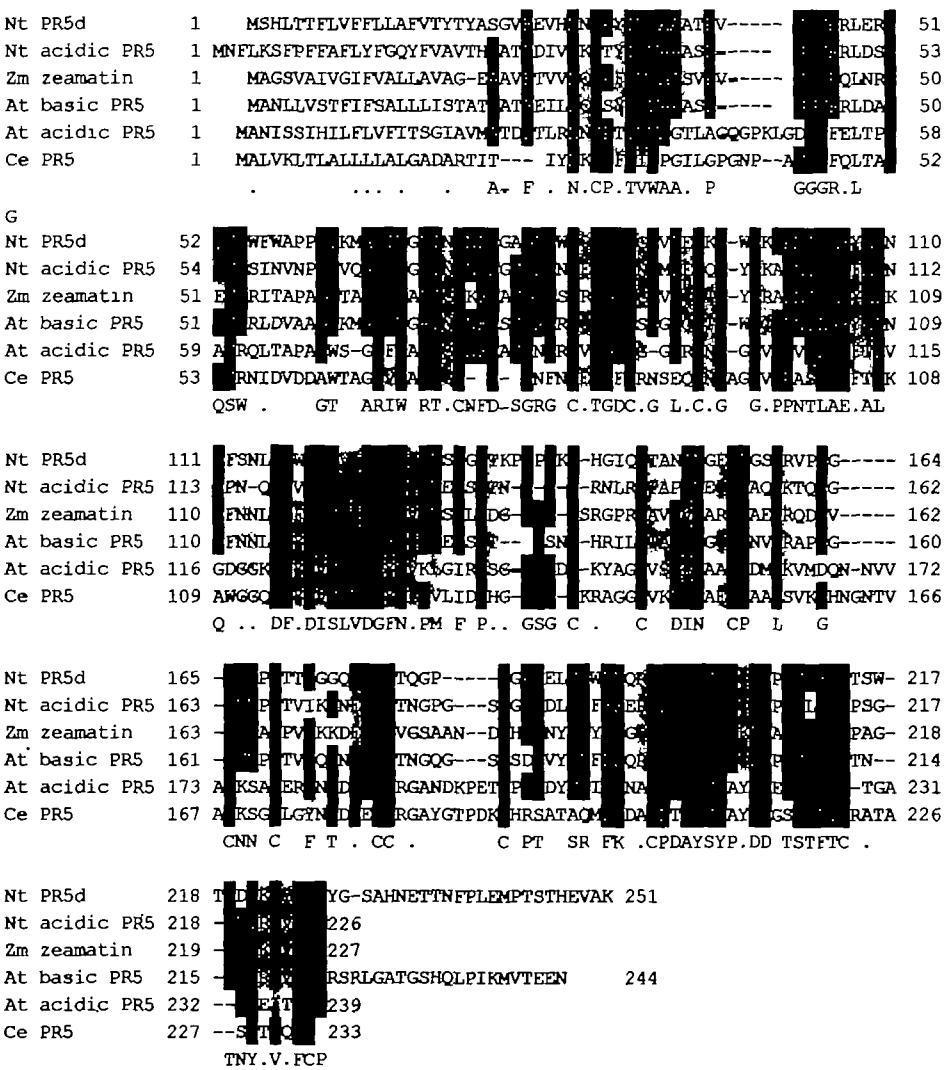

Fig. 3. **Alignment of predicted amino acid sequences of PR-5.** The alignment made by MacVector™ ver. 6.0 was modified manually. Identical amino acid residues are marked by black boxes and similar ones by gray boxes. Consensus amino acids are shown below the boxes. Sequences used are tobacco (Nt) PR-5d (P23871); acidic PR-5 (X12739); *Zea mays* (Zm) zeamatin (U06831); *Arabidopsis* (At) acidic PR-5 (M90510); basic PR-5 (X89008); and *C. elegans* (Ce) CEF28-D1.5 (Z70684).

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as in other organisms that may lack acquired immunity.

3. Conclusion

The basic plant pathogenesis-related (PR) genes are constitutively expressed in some intrinsic healthy tissues, whereas in the leaf they are induced by pathogen infection that is mediated by ethylene and other factors such as avrPto/Pto kinase. Although the signal transduction system of ethylene has been well studied, the roles of these other factors and their network have still to be determined. Transcription factor ERFs are key components in both the pathogen-induced and tissue-specific pathogen-independent expression of basic PR genes. Clarification of the regulation of ERF gene expression and activity is a very important task for understanding the regulation system of basic PR genes. The molecular mechanisms that produce the antifungal activities of some PR proteins, however, are unknown. Studies of the animal homologs of these PR proteins may help plant scientists understand the action mechanisms of plant PR proteins. Furthermore, the existence of PR proteins in animals suggests that those proteins function in the innate immunity of plants as well as of animals.

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