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Mini Review

A Large Family of Class III Plant Peroxidases

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Class III plant peroxidase (POX), a plant-specific oxidoreductase, is one of the many types of peroxidases that are widely distributed in animals, plants and microorganisms. POXs exist as isoenzymes in individual plant species, and each isoenzyme has variable amino acid sequences and shows diverse expression profiles, suggesting their involvement in various physiological processes. Indeed, studies have provided evidence that POXs participate in lignification, suberization, auxin catabolism, wound healing and defense against pathogen infection. Little, however, is known about the signal transduction for inducing expression of the *pox* genes. Recent studies have provided information on the regulatory mechanisms of wound- and pathogen-induced expression of some *pox* genes. These studies suggest that *pox* genes are induced via different signal transduction pathways from those of other known defense-related genes.

Key words: Function — Gene family — Plant peroxidase — PR protein — Signal transduction.

Abbreviations: EST, expressed sequence tag; JA, jasmonic acid; POX, class III plant peroxidase; PR, pathogenesis-related; RGP, Rice Genome Research Program; SA, salicylic acid.

Introduction

Peroxidases, a class of enzymes in animal, plant and microorganism tissues, catalyze oxidoreduction between H_2O_2 and various reductants. Class III plant peroxidase (POX, EC 1.11.1.7) is a classical enzyme whose activity was described as early as 1855 and whose purification followed a few decades later (Theorell 1951). Since POX is relatively stable at high temperature and its activity is easily measured using simple chromogenic reactions, it has been used as a model enzyme in the study of protein structure, enzyme reactions and enzyme functions, and it has been used for several practical applications.

POX is a heme-containing glycoprotein encoded by a large multigene family in plants. More than 100 expressed sequence tags (ESTs) encoding different POX isoenzymes are found in Arabidopsis. POX generally lacks strict specificity against reductants, and some POX isoenzymes have been reported to catalyze H_2O_2-independent oxidoreduction (Hinman and Lang 1965, Mäder et al. 1980, Dayan et al. 1999).

Studies have suggested that POXs play a role in lignification (Whetten et al. 1998), suberization (Espelie et al. 1986), cross-linking of cell wall structural proteins (Fry 1986), auxin catabolism (Lagrimini et al. 1997b), self-defense against pathogens (Chittoor et al. 1999), salt tolerance (Amaya et al. 1999) and senescence (Abeles et al. 1988). These functional assignments are based on the in vitro catalytic properties, expression profiles, localization and the characteristics of transgenic plants over- or under-expressing a certain *pox* gene. However, it is difficult to define the specific functions of individual POXs because of their low substrate specificity in vitro and the presence of many isoenzymes.

Among the various POX isoenzymes in plants, several are induced by pathogen infection and wounding, suggesting the importance of POX in self-defense systems in plants. The importance of POX in pathogen defense was indicated (Lovrekovich et al. 1968) earlier than the discovery of pathogenesis-related (PR) proteins as novel pathogen-inducible proteins (van Loon and van Kammen 1970), and POX was classified as PR-9 (van Loon et al. 1994). However, the signal transduction leading to the expression of pathogen- or wound-inducible genes has been more extensively studied for *PR* genes other than *pox* genes. Only recently has information been obtained on the regulated expression of pathogen- or wound-inducible *pox* genes in comparison with that of *PR* genes.

In this review, we summarize the characteristics and proposed functions of POXs and the signals controlling *pox* gene expression.

POX is one type of peroxidase

The term "peroxidase" means an enzyme catalyzing oxidoreduction between hydrogen peroxide and reductants ($H_2O_2 + AH_2 \rightarrow 2H_2O + A$). Peroxidases are found in animals, plants and microorganisms, and they are divided into three superfamilies based on their structural and catalytic properties (Welinder 1991, Table 1). The first peroxidase superfamily consists of enzymes in animals such as glutathione peroxidase

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Table 1 Classification of peroxidases

Superfamily	Class [a]	Member (EC number)	Origin	Molecular weight (kDa)
Animal peroxidase		Eosinophill peroxidase (EC 1.11.1.7)	Animal	50–75
		Lactoperoxidase (EC 1.11.1.7)	Animal	78–85
		Myeloperoxidase (EC 1.11.1.7)	Animal	79–150
		Thyroid peroxidase (EC 1.11.1.7)	Animal	90–110
		Glutathione peroxidase (EC 1.11.1.9)	Animal and plant	6–22 and 75–112 [b]
		Prostaglandin endoperoxide synthase (EC 1.14.99.1, partial) [c]	Animal	115–140
Catalase		Catalase (EC 1.11.1.6)	Animal, plant, fungus and yeast	140–530
Plant peroxidase	I	Cytochrome *c* peroxidase (EC 1.11.1.5)	Yeast and bacterium	32–63
		Catalase-peroxidase (EC 1.11.1.6)	Bacterium and fungus	150–240
		Ascorbate peroxidase (EC 1.11.1.11)	Plant	30–58
	II	Manganese-dependent peroxidase (EC 1.11.1.13)	Fungus	43–49
		Ligninase (EC 1.11.1.14)	Fungus	40–43
	III	**Peroxidase (EC 1.11.17, POX)**	**Plant**	**28–60**

[a] Established only for the plant peroxidase superfamily.
[b] Molecular weights for monomeric and tetrameric forms, respectively.
[c] Homology was observed in the central region (approximately 180 residues) with other animal peroxidases.

(EC 1.11.1.9), myeloperoxidase (EC 1.11.1.7), thyroid peroxidase (EC 1.11.1.7), eosinophill peroxidase (EC 1.11.1.7), lactoperoxidase (EC1.11.1.7) and a partial region of prostaglandin endoperoxide synthase (EC 1.14.99.1). Although glutathione peroxidase is categorized into the animal peroxidase superfamily, its activity is also detected in plants, and several cDNAs encoding its homologs have actually been isolated from plants (Churin et al. 1999). The second peroxidase superfamily consists of catalases (EC 1.11.1.6) in animals, plants, bacteria, fungi and yeast. The third superfamily consists of peroxidases in plants, fungi, bacteria and yeast. Although the active sites of myeloperoxidase and cytochrome *c* peroxidase (a member of the animal and plant peroxidase superfamilies, respectively) have similar architectures, the overall primary sequences and 3-dimensional structures of these peroxidases are quite different, implying that these superfamily genes evolved from distinct ancestral genes (Taurog 1999).

The amino acid sequences were found to be highly variable among the members of the plant peroxidase superfamily, with less than 20% identity in the most divergent cases. Based on differences in primary structure, the plant peroxidase superfamily can be divided further into three classes (Welinder 1992, classes I, II and III in Table 1). Class I plant peroxidases include the intracellular enzymes in plants, bacteria and yeast such as microbial cytochrome *c* peroxidase (EC 1.11.1.5), bacterial catalase-peroxidase (EC 1.11.1.6) and ascorbate peroxidase (EC 1.11.1.11). Ascorbate peroxidase was purified from the bovine eye, and its N-terminal sequence was found to be homologous to that of plant enzymes, implying that ascorbate peroxidase is not plant-specific (Wada et al. 1998). Class II plant peroxidases are extracellular peroxidases from fungi, including lignin peroxidase (EC 1.11.1.14) and Mn^{2+}-dependent peroxidase (EC 1.11.1.13). Class III plant peroxidases (EC 1.11.1.7), which were originally described as peroxidases and which are the focus of this article, are plant enzymes that are secreted outside the cells or transported into vacuoles. POX includes horseradish peroxidase, which is a commercially available enzyme that is frequently conjugated to an antibody for chromogenic detection.

Enzymes from these three classes of the plant peroxidase superfamily differ in their structures and catalytic properties. Class II peroxidases have an additional 40 to 60 amino acid residues in their C-termini compared to peroxidases in other classes (Welinder 1992). Members of all classes of the plant peroxidase superfamily contain 10 common α-helices. Enzymes of classes I and II have one specific helix, whereas three specific helices are present in class III peroxidases (Schuller et al. 1996, Gajhede et al. 1997). Cytochrome *c* peroxidase and ascorbate peroxidase show strong specificity against reductants (cytochrome *c* and ascorbic acid, respectively). POXs oxidize various substances, from small molecules to macromolecules. Although there is low sequence homology between the three classes, five separately positioned amino acids that are important for catalysis and structure as well as the helical folding of the entire polypeptide are strictly conserved among peroxidases in all three classes (Schuller et al. 1996, Gajhede et al. 1997).

Multiplicity of POXs

POX isoenzymes have been found in crude plant extracts by staining for activity after separation by gel electrophoresis. Twenty-five and 12 POX isoenzymes were distinguished on the

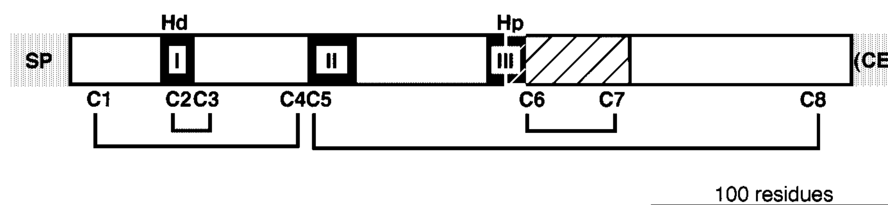

Fig. 1 Structural features of POX. The features of a representative POX, horseradish peroxidase isoenzyme C, are schematically presented according to the reported amino acid sequence (Welinder 1976) and 3-dimensional structure (Gajhede et al. 1997). N-terminal signal peptide (SP) and C-terminal extension (CE) are indicated by gray boxes. Only vacuolar POXs contain CE. Filled boxes represent a conserved catalytic and distal heme-binding domain (I), a proximal heme-binding domain (III), and a central conserved domain (II) of unknown function (Buffard et al. 1990). A region presumed to be important for determining the specific function (see text) is indicated by a hatched box. Invariable distal histidine (Hd), proximal histidine (Hp) and eight cysteine residues (C1 to C8), which form a disulfide bond network as indicated, are shown above and below the boxes, respectively.

basis of their isoelectric points in rice and tobacco, respectively (Ito et al. 1991, Lagrimini and Rothstein 1987). In rice, 42 ESTs have been identified as cDNAs encoding independent POX isoenzymes by the Rice Genome Research Program (RGP) (Yamamoto and Sasaki 1997). Since the RGP has used only tissue-specific cDNA libraries for EST preparation, the number of POX ESTs would be underestimated. Actually, prxRPA, prxRPN (Ito et al. 1994), POX22.3, POX8.1 and POX5.1 (Chittoor et al. 1997) are not found in the rice POX ESTs of the RGP, suggesting the presence of other unknown *pox* genes. Except for class III peroxidase, no peroxidase has been reported to have such a large number of isoenzymes in both plant and non-plant organisms. For example, only three distinct cDNAs have been isolated for barley glutathione peroxidase to date (Churin et al. 1999), and several variants of thyroid peroxidase mRNA appear to be transcribed from a single gene in human (Kimura et al. 1987, Barnett et al. 1990).

Comparison of POX primary sequences indicates that they are highly variable within single plant species: total amino acid sequence identity is sometimes less than 35% within the same plant species. However, nearly 90% of residues are identical among POXs from different plant species (Kjærsgård et al. 1997). These observations suggest that individual plant species possess a common set of POXs with similar characteristics among species.

The expression profiles of the *pox* genes indicate possible roles of POXs in multiple physiological processes. Lagrimini and Rothstein (1987) reported differences in the organ specificity and stress response of 12 different tobacco POXs. In rice and *Stylosanthes humilis*, *pox* genes have been found to respond differentially to pathogen infection and wounding (Harrison et al. 1995, Chittoor et al. 1997). Using gene-specific probes, the expression patterns of 21 rice *pox* genes were shown to be highly divergent with respect to their organ distribution and responses to environmental stimuli (Hiraga et al. 2000c).

Large protein families like POX are often found among plant transcription factors. In Arabidopsis, more than 100 members have been recognized in families consisting of proteins with conserved domains such as AP2/EREBP, MYB or WRKY (Riechmann and Meyerowitz 1998, Kranz et al. 1998, Meissner et al. 1999, Eulgem et al. 2000). These proteins have similar structures in only specific domains, the other regions being structurally unrelated, suggesting that the non-homologous regions define their specific functions. Actually, a correlation was found between the expression profile and sequence features of MYB-related transcription factors. These proteins have one to three repeats of homologous DNA-binding domains in the N-terminal region but no consensus structure in the C-terminal region. However, phylogenetic analysis using C-terminal non-homologous sequences of 121 plant MYB proteins (including 86 sequences from Arabidopsis) revealed that these proteins can be divided into 22 subgroups and that common sequence motifs exist in the C-terminal region of members of the same subgroup (Kranz et al. 1998). Interestingly, genes encoding the MYB proteins with common C-terminal motifs displayed similar expression patterns, suggesting that the members in the same subgroup have similar functions (Kranz et al. 1998).

Chittoor et al. (1999) showed a correlation between primary structure and the expression profile of pathogen-inducible POXs. They compared a peptide region spanning from the proximal heme-binding site to the vicinity of the 7th invariable cysteine residue (indicated by a hatched box in Fig. 1). This region is fundamentally divergent in length and sequence among POXs, and it forms a part of the substrate access channel in peanut and horseradish POXs (Schuller et al. 1996, Gajhede et al. 1997). Based on this comparison, the 62 *pox* genes could be grouped into 11 clusters. Interestingly, the majority of pathogen-inducible POXs fell into only two clusters, which shared homologous structures based on the conservation of their primary sequence. Thus, Chittoor et al. suggested that the region 40 to 50 C-terminal amino acids from the proximal heme-binding site reflected the biological function of each POX, namely the reactivity to the reductants.

POX has three highly conserved domains, the distal heme-binding domain, the central conserved domain of unknown function, and the proximal heme-binding domain (I, II and III in Fig.

1, respectively). Both the position and the arrangement of these domains is strictly conserved among POXs, whereas the position and number of conserved domains differ among the members of the AP2/EREBP and WRKY superfamilies (Riechmann and Meyerowitz 1998, Eulgem et al. 2000). Thus, the overall primary structure of POX is less divergent than that of the AP2/EREBP and WRKY transcription factors. Each POX has enzymatic activity. The substrate and resulting products are important to its specific function. Since purified POX preparations generally catalyze the oxidation of a broad range of reductants in vitro (Kvaratskhelia et al. 1997, Bernards et al. 1999, de Marco et al. 1999, Quiroga et al. 2000), it would not be reasonable to consider the functions of individual POXs on the basis of only in vitro catalytic properties. A combination of characteristics (e.g. in vitro properties, localization and gene expression profiles) defines the specific functions of respective POXs.

Proposed functions of POXs

1. Lignification, suberization and other cell wall metabolisms—POXs generally react to compounds containing a hydroxyl group(s) attached to an aromatic ring. Guaiacol (*o*-methoxy phenol) is commonly used as a substrate for the measurement of POX activity. Dehydrogenative oxidation of guaiacol by POX results in the formation of phenoxy radicals, and the subsequent coupling of unstable radicals leads to the non-enzymatic polymerization of monomers. In a similar way, hydroxycinnamic acid, hydroxycinnamyl alcohol and their derivatives are converted to phenoxy radicals by POX. Hydroxycinnamyl alcohol species are polymerized to lignin. Hydroxycinnamic acids containing aliphatic moieties are incorporated into suberin. POXs also oxidize phenolic domains of feruloylated polysaccharides (Fry 1986) and tyrosine residues of cell wall structural proteins such as hydroxyproline-rich glycoproteins (Everdeen et al. 1988, Brownleader et al. 1995). These macromolecules are cross-linked through radical coupling, resulting in the formation of more complex and larger molecules in the cell wall.

The cell wall POXs polymerize macromolecules, which are then deposited on the extracellular surface. The accumulation of these macromolecules strengthens the cell wall, thereby restricting cell expansion and pathogen invasion, and confers structural strength to the plant body, which is especially important for trees and construction of xylem vessels. Suberin deposition is thought to be important for wound healing (see below). Wall modification by a cell wall-bound tomato POX (TPX2) also seems to be associated with resistance to hyperosmotic stress (Amaya et al. 1999).

Although histochemical and in vitro biochemical studies have supported the notion of POX-catalyzed polymerization of lignin monomers, laccase (EC 1.10.3.2) remains a possible candidate for the lignin-polymerizing enzyme (reviewed by Whetten et al. 1998). Some studies have independently indicated that lignin or suberin content was either increased or unchanged in transgenic plants over- or under-expressing a certain *pox* gene (Lagrimini 1991, Sherf et al. 1993, Lagrimini et al. 1997a, Mansouri et al. 1999). Thus, there is no conclusive indication of the participation of these POXs in ligno-suberization. Recently, Quiroga et al. (2000) obtained evidence supporting the involvement of a tomato basic POX (TPX1) in ligno-suberization by a combination of kinetic and histochemical experiments. Lignin content was increased in the transgenic tomato plants overproducing TPX1 (Mansouri et al. 1999). Therefore, TPX1 seems to be a polymerizing enzyme in the formation of lignin and/or suberin.

Two basic POXs (P1 and P2) isolated from the spent medium of cultured tobacco cells were found to be localized to phloem of the root and stem and in the root epidermis of tobacco plants (de Marco et al. 1999). An in vitro enzymatic assay showed that both POXs had higher specific activities to NADH, NADPH and IAA than to monolignols, suggesting the involvement of these basic POXs in some cell wall biosynthetic processes other than polymerization of monolignols.

2. Auxin catabolism—Auxin is a classical plant hormone that affects many physiological processes, including cell division and differentiation. Several metabolic pathways of IAA, an endogenous auxin, have been proposed on the basis of results of biochemical and molecular genetic studies (Normanly and Bartel 1999). Oxidative decarboxylation by POX is thought to be one of the IAA inactivation processes, although IAA is also inactivated via conjugation with amino acids or sugars and/or via conversion to oxindole-3-acetic acid by oxidation without POX.

Since an early indication that horseradish POX oxidizes IAA (Hinman and Lang 1965), several POXs have been reported to catalyze IAA oxidation. Among IAA-oxidating POXs, a tobacco anionic POX (TOBPXDLF) has been the most intensively studied in relation to IAA degradation. This isoenzyme is a major POX in tobacco (Lagrimini and Rothstein 1987) and was shown to oxidize IAA in vitro (Gazaryan and Lagrimini 1996). In transgenic tobacco plants overexpressing TOBPXDLF, lateral root formation was suppressed, probably because of enhanced IAA degradation (Lagrimini et al. 1997b). This reduction in root growth resulted in a leaf wilting phenotype at the flowering stage due to reduced water supply from roots (Lagrimini et al. 1990). However, some studies have also indicated possible involvement of TOBPXDLF in lignification or polyphenol metabolism (Lagrimini 1991, Lagrimini et al. 1997a, Klotz et al. 1998), suggesting that this tobacco anionic POX has multiple roles during growth and development in the tobacco plant.

3. Wound healing—Wounding caused by various biotic and abiotic factors is a daily stress for plants that can lead to loss of essential organs and easy penetration of pathogens. Plants respond to wounding by activating self-defense systems to restore damaged tissues or to defend against attacks by pathogens and herbivores. Among the large number of wound-inducible proteins, POX has been shown to exhibit increases in activity or mRNA levels upon mechanical wounding in various

plants, including tobacco (Lagrimini and Rothstein 1987, Hiraga et al. 2000a), tomato (Roberts et al. 1988), potato (Roberts et al. 1988), cucumber (Svalheim and Robertsen 1990), azuki bean (Ishige et al. 1993), rice (Chittoor et al. 1997, Ito et al. 1994, Hiraga et al. 2000c), horseradish (Kawaoka et al. 1994a), *S. humilis* (Harrison et al. 1995) and sweet potato (Kanazawa et al. 1965, Huh et al. 1997).

Suberin is deposited in wounded tissues. Since suberin is a highly hydrophobic macromolecule composed of hydroxycinnamic acid and its derivatives contain conjugated aliphatic moieties, suberin deposition around the wounded tissues should aid wound healing (Kolattukudy 1980). Not only is the suberin monomer a substrate for POX but the spatial and temporal expression patterns of wound-inducible anionic POX were shown to be highly correlated with suberin deposition (Espelie and Kolattukudy 1985, Espelie et al. 1986, Bernards et al. 1999), suggesting the involvement of POX in suberization.

4. Defense against pathogen infection—Upon pathogen infection, plants newly synthesize a set of defense-related proteins such as PR proteins, which are induced in pathological or related situations (van Loon et al. 1994). POXs have been reported to be induced by infection with fungi (Thordal-Christensen et al. 1992, Harrison et al. 1995), bacteria (Reimers et al. 1992, Rasmussen et al. 1995), viruses (van Loon and Geelen 1971, Lagrimini and Rothstein 1987, Hiraga et al. 2000b) and viroids (Vera et al. 1993). Thus, specific pathogen-inducible POXs have been categorized into a family of PR proteins (PR-9, van Loon et al. 1994).

Many studies have indicated the importance of POXs in defense against pathogen infection. In tobacco, a positive correlation was found between POX activity and resistance to tobacco wildfire disease (Lovrekovich et al. 1968, Simons and Ross 1970). A cationic POX isoenzyme was induced more rapidly and at higher level when rice plants were infected with an incompatible rather than compatible strain of *Xanthomonas oryzae* pv. *oryzae* (Reimers et al. 1992). The roles of POXs in defense are considered to be as follows: (1) reinforcement of cell wall physical barriers comprising lignin, suberin, feruloylated polysaccharides and hydroxyproline-rich glycoproteins (Vance et al. 1980, Fry 1986, Bowles 1990); (2) enhanced production of reactive oxygen species as signal mediators and antimicrobial agents (Bolwell et al. 1995, Wojtaszek 1997, Kawano and Muto 2000); and (3) enhanced production of phytoalexin (Kristensen et al. 1999). Generally, multiple POXs are induced by pathogen infection (Harrison et al. 1995, Chittoor et al. 1997), suggesting that each POX is involved in a specific defense process.

5. Others—Many studies have suggested an association of POXs with production (Mäder et al. 1980, Bolwell et al. 1995) and scavenging (Mehlhorn et al. 1996, Kvaratskhelia et al. 1997) of hydrogen peroxide, porphyrin metabolism (Dayan et al. 1999), senescence (Abeles et al. 1988, Oh et al. 1997) and organogenesis (Kay and Basile 1987), indicating that POX has diverse functions.

Mechanism of induced expression of the pox gene

Increases in mRNA, protein and activity levels of POX induced by various stimuli such as pathogen infection, wounding and oxidative stress have been reported in plants. The mechanism of *pox* induction is not well understood; however, recent studies have provided information on the regulated expression of *pox* genes.

1. Wound-inducible pox genes—In wounded plant tissues, a set of defense-related genes, such as genes for basic type PR proteins and enzymes involved in phenylpropanoid synthesis (e.g. phenylalanine ammonia-lyase), are induced. Several low-molecular-weight compounds such as ABA, ethylene and jasmonic acid (JA) are postulated to be involved in the signal transduction pathways leading to the expression of these genes (Peña-Cortés et al. 1989, Farmar and Ryan 1990, Ohashi and Oshima 1992, Seo et al. 1995, O'Donnell et al. 1996).

Wound-inducible *pox* genes were induced by treatment with ABA in potato and tomato (Roberts and Kolattukudy 1989), by treatment with ethephon (an ethylene-releasing agent) in azuki bean and rice (Ishige et al. 1993, Ito et al. 1994, Hiraga et al. 2000c), and by treatment with methyl jasmonate (methyl ester of jasmonate) in *S. humilis* and rice (Curtis et al. 1997, Hiraga et al. 2000c). Two *cis*-acting elements and binding proteins for each element have been identified in the 5′ upstream region of a wound-inducible horseradish *pox* gene (Kawaoka et al. 1994b, Kawaoka et al. 2000, Kaothien et al. 2000). However, the signal compound(s) for the induced expression of the horseradish *pox* gene is not known. Recently, we isolated a tobacco *pox* gene whose transcript was accumulated rapidly and systemically after wounding. However, its expression was not affected by ethephon or salicylic acid (SA), and it was suppressed by methyl jasmonate. Since most wound-inducible genes are induced by JA and ethylene, these results suggest the presence of a novel signal transduction pathway for wound-induced expression of the *pox* gene (Hiraga et al. 2000a). Two wound-inducible rice *pox* genes were reported to be induced by ethephon treatment (Ito et al. 1994). Although an ethylene-responsive element (AGCCGCC, Ohme-Takagi and Shinshi 1995) was found in the promoter regions of each gene, neither promoter responded to ethephon treatment in transgenic tobacco plants (Ito et al. 2000), suggesting that the regulatory mechanisms for ethylene-induced gene expression in rice and tobacco plants are different. Thus, unknown signals and transcriptional regulation for wound-inducible *pox* genes should be studied further.

2. Pathogen-inducible pox genes—Pathogen infection induces the synthesis of a set of defense-related proteins. Among these, PR proteins have been well studied with regard to the regulated expression of their genes. In tobacco, genes for acidic PR proteins have been reported to be induced via SA and genes for basic PR proteins via ethylene and JA (Malamy et al. 1990, Ohashi and Oshima 1992, Seo et al. 1995, Niki et al. 1998, Ohtsubo et al. 1999).

Studies have demonstrated induction of pathogen-indu-

cible *pox* genes by defense-related signal compounds. For example, the *pox* mRNA level or activity was increased by treatment with ABA in tomato (Roberts and Kolattukudy 1989), by treatment with JA in rice and *S. humulis* (Schweizer et al. 1997, Curtis et al. 1997), and by treatment with SA in cucumber and parsley (Rasmussen et al. 1995, Thulke and Conrath 1998). In contrast, we found in a previous study that a tobacco *pox* gene, which is induced during an *N* gene-dependent hypersensitive reaction in tobacco mosaic virus-infected tobacco leaves, did not respond to well-known defense-related signal compounds such as SA, JA and ethephon (Hiraga et al. 2000b). This *pox* gene was induced at a low level only by spermine, which was found to be a novel signal compound for both acidic and basic type *PR* gene expression (Yamakawa et al. 1998, Hiraga et al. 2000b), suggesting the presence of another unknown signaling pathway for the pathogen-inducible *pox* gene expression. Although there have been several reports on the isolation and analysis of the promoter regions of pathogen-inducible *pox* genes (Mohan et al. 1993a, Mohan et al. 1993b, Curtis et al. 1997), no *cis*-acting element or transcription factor has been identified to date. Recently, neither SA, ethylene nor JA has been reported to be involved in pathogen-induced expression of a tomato *pox* gene. Interestingly, the promoter of this *pox* gene was constitutively activated in an Arabidopsis mutant that could not perceive auxin (Mayda et al. 2000).

In summary, although recent studies have provided some information on the regulation of pathogen- or wound-inducible *pox* genes, many aspects of the regulation are still not clear. Further investigations are expected to reveal novel regulatory mechanisms for the wound- or pathogen-induced *pox* gene expression as well as novel signaling pathways.

Concluding remarks

Among the numerous enzymes present in animals, plants and microorganisms, POX is characteristic in that it consists of a large number of isoenzymes in a single plant species. One possible reason for this is the importance of POX to the growth and self-defense of plants. To guarantee against accidental loss of the genes, amplification and structural modification of *pox* genes might have occurred repeatedly in plant genomes during evolution. Indeed, *pox* genes are scattered through all 12 chromosomes in rice (K. Yamamoto and T. Sasaki, unpublished), and at least four structurally related *pox* genes are located within a limited region of chromosome 7 (Chittoor et al. 1999). However, such redundancy makes it difficult to conclusively determine the functions of individual POXs. Genetic manipulation using antisense technology or screening of mutants is not a very powerful method for revealing functions of individual *pox* genes because possible interference from other *pox* genes may give complex phenotypes. Although it would be a challenge to determine all of the functions of the approximately 100 POX isoenzymes in a single plant species, accumulation of information on individual *pox* genes should lead to a better understanding of POX, an important plant enzyme.

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