

## Review

# The family 10 of plant pathogenesis-related proteins: Their structure, regulation, and function in response to biotic and abiotic stresses

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**Abstract**

The aim of this review is to present the current state of our understanding on the structure, regulation, and function of plant pathogenesis-related protein family 10 (PR-10). This protein family consists of relatively diverse members subgrouped into classes that suggest different functions. It is believed that PR-10 proteins are involved in plant defense because their genes are usually induced upon the attack of various pathogens and by environmental stresses. However, updated evidence shows that PR-10 proteins display several additional functions, including a role in developmental processes and enzymatic activities in secondary metabolism. Because of the complexity of the PR-10 gene family and its potential multiple functions, it is important to summarize current knowledge as basis for a further dissection of the functions of PR-10 proteins and a better understanding of their structural adaptation. This paper provides the first review of existing knowledge of plant PR-10 proteins and examines their structural and functional adaptations.

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**Keywords:** Pathogenesis-related protein family 10; Plant defense response; Biotic and abiotic stresses

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**1. Introduction**

In response to infection by a variety of pathogens, plants are induced to express a set of genes at increased levels. Some of these genes associated with plant defense responses, which including pathogenesis-related (PR) and antimicrobial genes, have shown biological activities related to disease resistance. PR proteins are well-known proteins proven to be induced by pathogens and related abiotic stresses [1,2]. Based on their structural and functional properties, up to 17 different families of PR proteins have been identified thus far in mono- and dicotyledonous plants. These PR families function in a wide range from cell wall rigidification to signal transduction and antimicrobial activity [3,4].

Among PR proteins, the parsley “PR-1” protein [5,6] is a typical member of the PR-10 family. The PR-10 proteins are regulated developmentally and environmentally and they are widely distributed in seed plants. Based on

similarities in their amino acid sequences, subcellular location, and putative function, the PR-10 family comprises two distinct groups: intracellular pathogenesis-related proteins (IPR) with homology to ribonucleases [1], and (S)-norcochlorine synthases (NCS, EC 4.2.1.78) [7]. For the first time, here we review recent data on the PR-10 protein structure, expression, and their involvement in plant defense and other biological processes.

**2. Characterization of plant PR-10 proteins***2.1. Occurrence of PR-10 proteins*

More than 100 PR-10 or PR-10-related sequences have been identified from various flowering plants. PR-10 proteins are identified primarily as IPR proteins, including tree pollen allergens and major food allergens [8]. They have been reported in a variety of higher plant species of both angiosperms and gymnosperms. Among them, PR-10 proteins have been found in numerous dicots, including parsley (*Petroselinum crispum*) [5], pea (*Pisum sativum*) [9], potato (*Solanum tuberosum*) [10], white birch (*Betula*

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*verrucosa*) [11,12], bean (*Phaseolus vulgaris* L.) [13], soybean (*Glycine max* cv Mandarin) [14], alder (*Alnus glutinosa*) [15], apple (*Malus domestica*) [16], celery (*Apium graveolens*) [17], and alfalfa (*Medicago sativa*) [18,19]. Among the monocots, PR-10 proteins are known to occur in asparagus (*Asparagus officinalis*) [20], rice (*Oryza sativa*) [21], lily (*Lilium longiflorum*) [22], and sorghum (*Sorghum bicolor*) [23]. Among gymnosperms, PR-10 proteins have been found in sugar pine (*Pinus lambertiana*), eastern white pine (*Pinus strobus*), western white pine (*Pinus monticola*) [24,25], Douglas-fir (*Pseudotsuga menziesii*) [26], maritime pine (*Pinus pinaster*) [27], and white spruce (*Picea glauca*) [28].

## 2.2. Sequence and structural properties of PR-10 protein family

Most genes of the PR 10 IPR group have an open reading frame (ORF) from 456 to 489 bp encoding a polypeptide of 151–162 amino acids with molecular masses of 15–18 kDa. PR-10 ORF is usually interrupted by an intron of 76–359 bp at a position highly conserved in seed plants [29]. However, one subclass of the *Malus* PR 10 family is intronless [30]. Members of the PR-10 NCS group contain longer ORFs from 633 to 696 bp with predicted molecular masses around 26 kDa, and they share 28–38% identity as well as putative structural homology with IPR proteins [7,31]. Multiple members of the PR-10 family have been identified in plant genomes. Most PR-10 genes are clustered in the chromosomes [30,32,33].

The amino acid sequences of PR-10 proteins diverge considerably (Fig. 1). Members of PR-10 IPR group share several conserved features, including small molecular masses, acidic pI, cytosolic localization, and similar three-dimensional (3D) structure [1,34]. However, members of NCS group are distinctive in the PR-10 family due to oligopeptide extensions at both their C- and N-terminals (Fig. 1). The presence of putative N-terminal signal peptides in NCS proteins suggests that they have an association with a subcellular compartment other than the cytosol [7,31]. Amino acid sequence alignment of representative members shows that there are only four amino acids invariable across the PR-10 family (Fig. 1). The only highly conserved region, P-loop motif (GxGGxGxxK), is presumed to function as a binding site for nucleotides involved in RNase activity for some PR-10 proteins.

Crystal structures of a few PR-10 proteins have been determined by X-ray diffraction and NMR spectroscopy. They include Bet v 11, a major allergen from birch pollen [35]; Bet v 11, a low-allergenic isoform of Bet v 1 [34]; Pru av 1, a major allergen from cherry [36]; and LIPR10-1A and LIPR10-1B from yellow lupine [37]. The 3D structures of these PR-10 proteins are very similar (Fig. 2). In general, each consists of a 25-amino-acid long C-terminal  $\alpha$ -helix ( $\alpha 3$ ) surrounded by a seven-stranded antiparallel  $\beta$ -sheet (from  $\beta 1$  to  $\beta 7$ ) and two N-terminal short  $\alpha$ -helices ( $\alpha 1$  and  $\alpha 2$ ) between  $\beta 1$  and  $\beta 2$  strands. The connection sequences

between  $\alpha$ -helix or/and  $\beta$ -strand are short loop structures (from L1 to L9). The conserved P-loop motif (GxGGxGxxK) is localized in the L4 region between  $\beta 2$  and  $\beta 3$  (Fig. 2). A 30-Å-deep, Y-shaped, hydrophobic pocket has been identified in the crystal structure of LIPR10-1A, LIPR10-1B, and Bet v 11. It occurs between residues lining the interior face of the  $\beta$ -sheet and the three  $\alpha$ -helices, and functions as the ligand-binding site [34,37].

## 2.3. Phylogenetic clustering of PR-10 proteins

Exploring the evolutionary relationships in the PR 10 family provides insight into the origin and functional adoption of this universal family. A neighbor-joining (NJ) phylogram was used to construct a phylogenetic tree based on the amino acid sequence alignment analysis of 95 PR-10 proteins from a variety of seed plants including both angiosperm and gymnosperm species. Because major latex proteins (MLPs) show low homologies with PR-10 family members [38], an *Arabidopsis* MLP (At-MLP) was included as the out-group in this analysis. As shown in Fig. 3, this representative tree is highly divergent and contains two major clades or branches. Most PR-10 proteins are grouped into one large clade, while NCS proteins are clearly grouped with *Papaver somniferum* PR-10 proteins (PsPR10-1 and -2) in another small clade at the basal positions of this family tree. This topological pattern suggests that the NCS clade might have originated prior to the divergence of seed plants. In the big major clade, the highly divergent IPR members are divided into four subclades. Most PR-10 proteins from dicots form subclade-I, while those members from monocots form subclades-II and -IV, and those from conifers form subclade-III. In this phylogenetic tree, most sequences from the same species are clustered together, consistent with previous studies [8,39]. Early in the evolution of flowering plants, an ancestral phylogenetic burst might have happened, resulting in duplication or alleles of PR-10 genes. PR-10 proteins occurring in both conifers and angiosperms may have evolved from the same ancestral gene; they adopted different functions as they underwent structural divergences during their evolutionary history.

## 3. Expression and induction following abiotic and biotic stresses

### 3.1. Constitutive expression of PR-10 proteins

Some PR-10 proteins display constitutive expression patterns unrelated to the pathogenic response. It has been shown that PR-10 proteins are developmentally regulated in different plant tissues and organs [40,41]. The constitutive accumulation of PR-10 proteins has been detected during plant growth and development in plant flower organs [11,22,42–45], pollen grain [11,46], fruits [16,47,48], seeds [44,49], vegetative organs of roots [14,40,50–53], stems [44,54], and leaves [14,39,54]. As pollen or food

|            |                                                                  |     |
|------------|------------------------------------------------------------------|-----|
| PmPR10-2.1 | -----MVAGTVTTEYVSQVETKRLW                                        | 20  |
| PmPR10-3.1 | -----MVSGTATSEHFSEVEARRIW                                        | 20  |
| PmPR10-1.1 | -----MVSGTSSTEEVVQVEARRLW                                        | 20  |
| CaPR-10    | -----MGAYTFTDKSTASVAPSRLF                                        | 20  |
| HpHyp-1    | -----MAAYTIVKEEESPTAPHRLF                                        | 20  |
| LaPR10     | -----MGIFTFEDESTSTVAPAKLY                                        | 20  |
| SPE16      | -----MGVVFVRDETSSSVAPAKLY                                        | 20  |
| Bet V1     | -----MGVFNYTEATSVIPAARLF                                         | 20  |
| GaPR10     | -----MGVVSYEFEVTSPIAPARLF                                        | 20  |
| Ot-Oca     | -----MGVVFVEDEITTTTISPTRVF                                       | 20  |
| Vr-CSBP    | -----MVKEFNTQTELSVRLEALW                                         | 19  |
| TfNCS      | MMKMEVVFVFLMLLGTINCQKLILTGRPFLHHQGI INQVSTVTKVIHHELEVAASADDIW    | 60  |
| *****      |                                                                  |     |
| PmPR10-2.1 | NAMVKDGHNLYPKALHEFHISVSLHGDGGVGTVRQLNFTSANKDFSHIKERLDVIDEE       | 80  |
| PmPR10-3.1 | NALVKDSHNLLPKILPDF-FSSATFLOGDGGVGTIKETNFTPANKDFSYPKERIDEIDEE     | 79  |
| PmPR10-1.1 | NATTKDSHNFLPKVLPGV-FTSVTLLOGDGGVGTVKQLNFTP GKKDFSFIKERVDELDEE    | 79  |
| CaPR-10    | KALVIDFNNLVS-KLAPDV-KSIENVEGDGGAGTIKKMTFV-EGGPIKYMKKHIVIDEK      | 77  |
| HpHyp-1    | KALVLERHQVLV-KAQP HVFKSGEII ECDGGVGT VTKITFV-DGHPLTYMLHKFDEIDAA  | 78  |
| LaPR10     | KALVADANI IIP-KAV-EAIQSVENVEGNGGPGTIKKLTFI-EDGETKYVLHKIEIDEA     | 77  |
| SPE16      | KALTKDSDTIAQ-KID-GPIQSI ELVEGNGGPGTIKKITAN-EGDKTSFVLQKVDAIDEA    | 77  |
| Bet V1     | KAFILDGDNLFV-KVAPQAISVENIEGNGGPGTIKKISFP-EGFPFKYVKDRVDEVDHT      | 78  |
| GaPR10     | KAFVLEAAKIWP-TAAPHAVKSVE-LEGDASPGSIVKITFV-EGLPYQYMKHQIGHDEN      | 77  |
| Ot-Oca     | DSFVN-ADV VFL-KVAAEHKSVETLEGDGGVGTIRKIVFH-EGHG-GYIKQKIEVVDKE     | 76  |
| Vr-CSBP    | AVLSKDFITVVP-KVLPHIVKDVQLIEGDGGVGTILIFNFLPEVSP-SYQREEITEFDES     | 77  |
| TfNCS      | TVYSWPGLAKHLPLDLLPGAFAKLE-I I ECDGGVGTILDMTFVPGEFPHEY-KEKFILVDNE | 118 |
| *          |                                                                  |     |
| PmPR10-2.1 | KMVHKYAAIEGGS LGKKLSALNFE LKFVRREEGGCVLTWICNYETLPGAPDGEARVDEIK   | 140 |
| PmPR10-3.1 | KMVIKYTTIEGGLGKKLSASNFEIKIVPKEGGC VSWICNYETLSGAPFDEGKAQEMK       | 139 |
| PmPR10-1.1 | NFVYKYTAIEGGPLGKKLSACFEV KLVPRKEGGCAKWT CNYETLPGVQPDGKFKKEIK     | 139 |
| CaPR-10    | NLVTKYSLIESDV TENKAESVDYDGKFEASADGGSVCTT VTVYNTKGDYVVTEEEHNVHK   | 137 |
| HpHyp-1    | NFYCKYTI FEGDVL RDNIEKV VYEVKLEAVG-GGSKGITVSYHPKPGCTVNEEEVKIGE   | 137 |
| LaPR10     | NLGYNYSIVGGVGLPDTVEKITFETKLVEGVNGGSIGKVTIKIETKGDAPNEQEGKAAK      | 137 |
| SPE16      | NLGVDYSIVGGTGLPESLEKLSFETKVVAGSGGGSISKVTLKFHTKGDAPLSDAVRDDAL     | 137 |
| Bet V1     | NFKYNYSVIEGGPIGDTLEKISNEIKIVATPDGGSILKISNKYHTKGDHEVKAEQVKASK     | 138 |
| GaPR10     | NFSYSYSMIEGGPLGDKLEKISYENQFVAADGGSICKSSIKYYTVGDYVITEDEIKTLI      | 137 |
| Ot-Oca     | NLQYNDSVIEGDAIVGSIEKILNENKIIPNADGGCTVKSSTFYTSDESEI PAQILAEGK     | 136 |
| Vr-CSBP    | SHEIGLQVIEGGYLNQGLSYYKTTFKLSEIEEDKTLNVNKISYDHDSDIEEKVTPTKTSQ     | 137 |
| TfNCS      | HRLKKVQMIEGGYLDLGVTYYMDTIHV VPTGKDCSVIKSSTEYHVKPEFVKIVEPLITTG    | 178 |
| * *        |                                                                  |     |
| PmPR10-2.1 | KMDAMFRKIEQYLLISNPDLYC-----                                      | 162 |
| PmPR10-3.1 | EQSNHMFKKIEQYLLSNFSLYC-----                                      | 161 |
| PmPR10-1.1 | EDSFGMLKKVEQYLLSNPNLYC-----                                      | 161 |
| CaPR-10    | EKANDLLKAI EAYLLANPSVYV-----                                     | 159 |
| HpHyp-1    | KKAYEFYKQVEEYLAANPEVF A-----                                     | 159 |
| LaPR10     | ARGDAFFKAIETYLSAHPDYN-----                                       | 158 |
| SPE16      | AKGAGFFKAIETYL-----                                              | 151 |
| Bet V1     | EMGETLLRAVESYLLAHSDAYN-----                                      | 160 |
| GaPR10     | KGSEVVYKAI EAYLLANPDACN-----                                     | 159 |
| Ot-Oca     | EKRLGMFKATEAYLLANPEAFN-----                                      | 158 |
| Vr-CSBP    | S-TLMYLRRLERYLSNGSA-----                                         | 155 |
| TfNCS      | PLAAMADAISKLVLEHKS KSN SDEIEAAIITV                               | 210 |

Fig. 1. Alignment of deduced amino acid sequences of three *Pinus monticola* PR-10 proteins and representative PR-10 members with identified biological functions. Dashes indicate gaps that were introduced to optimize alignment. Highlighted portions show the residues identical to the column consensus. Non-highlighted portions indicate the residues neither similar nor identical to the column consensus. Four residues that are strictly conserved are highlighted in black and highly conserved residues that do not vary in at least half of the members are in gray. Numbers to the right of the sequences indicate the amino acid residue locations. The P-loop motif (G<sub>47</sub>–T<sub>53</sub>, E<sub>103</sub>, E<sub>150</sub>, and Y<sub>152</sub> (as positioned in PmPR10) involved in ribonuclease activity are indicated with asterisks (\*). Amino acid sequences are deduced from cDNA or genomic DNA sequences from *Pinus monticola* PmPR10-1.1, -2.1, -3.1 (GenBank accession numbers AY064193, AY596271, and AY596273), *Betula verrucosa* Bet V1 (Z72429), *Capsicum annuum* CaPR-10 (AF244121), *Gossypium arboreum* GaPR10 (AF416652), *Hypericum perforatum* HpHyp-1 (AAN65449), *Lupinus albus* LaPR10 (AJ000108), *Oxalis tuberosa* Ot-Oca (AF333436), *Pachyrhizus erosus* SPE16 (AY433943), *Thalictrum flavum* TfNCS (AAR22502), and *Vigna radiata* Vr-CSBP (AB012218).

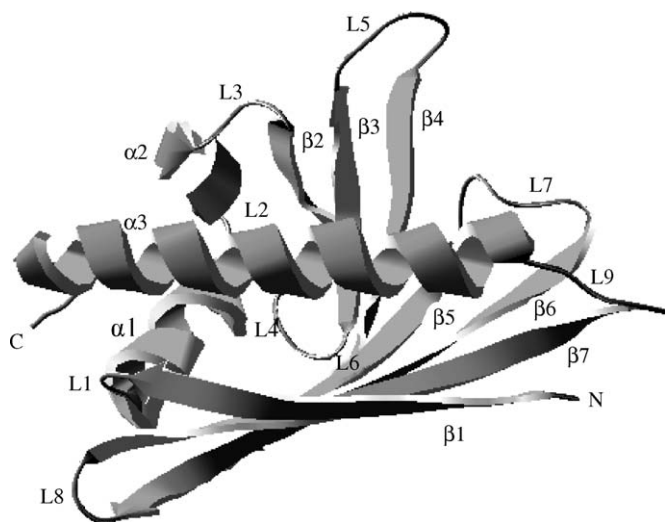


Fig. 2. Three-dimensional structure of the *Pinus monticola* PR-10 protein PmPR10-3.1 modulated and represented by a ribbon diagram. The 3D structure of PmPR10-3.1 protein was simulated using the SWISS-MODEL software package (Swiss Institute of Bioinformatics, <http://swissmodel.expasy.org/>). To manipulate the 3D structure and model of the PmPR10-3.1 protein, the Deep View Swiss-PdbViewer software package was used. The 3D structure of PmPR10-3.1 is composed of three  $\alpha$ -helices ( $\alpha 1$  to  $\alpha 3$ ), a seven-stranded  $\beta$ -sheet ( $\beta 1$  to  $\beta 7$ ), and nine junction loops (L1–L9).

allergens, high levels of PR-10 proteins are detected in birch pollen [11], hornbeam (*Carpinus betulus*) [55], apple [16], celery [17], pear [56], and soybean [33].

### 3.2. Induced expression by biotic and abiotic stresses

The inducible expression of PR-10 genes in response to attacks by pathogens has been widely investigated in a number of plant species. Pathogens triggering a PR-10 response include viruses [48,57–59], bacteria [18,19,60], and fungus [12,13,54,58,61,62,63,64]. Immunocytochemical localization has shown that the PR-10 proteins bind to the cell wall of blister rust fungus (*Cronartium ribicola*) both

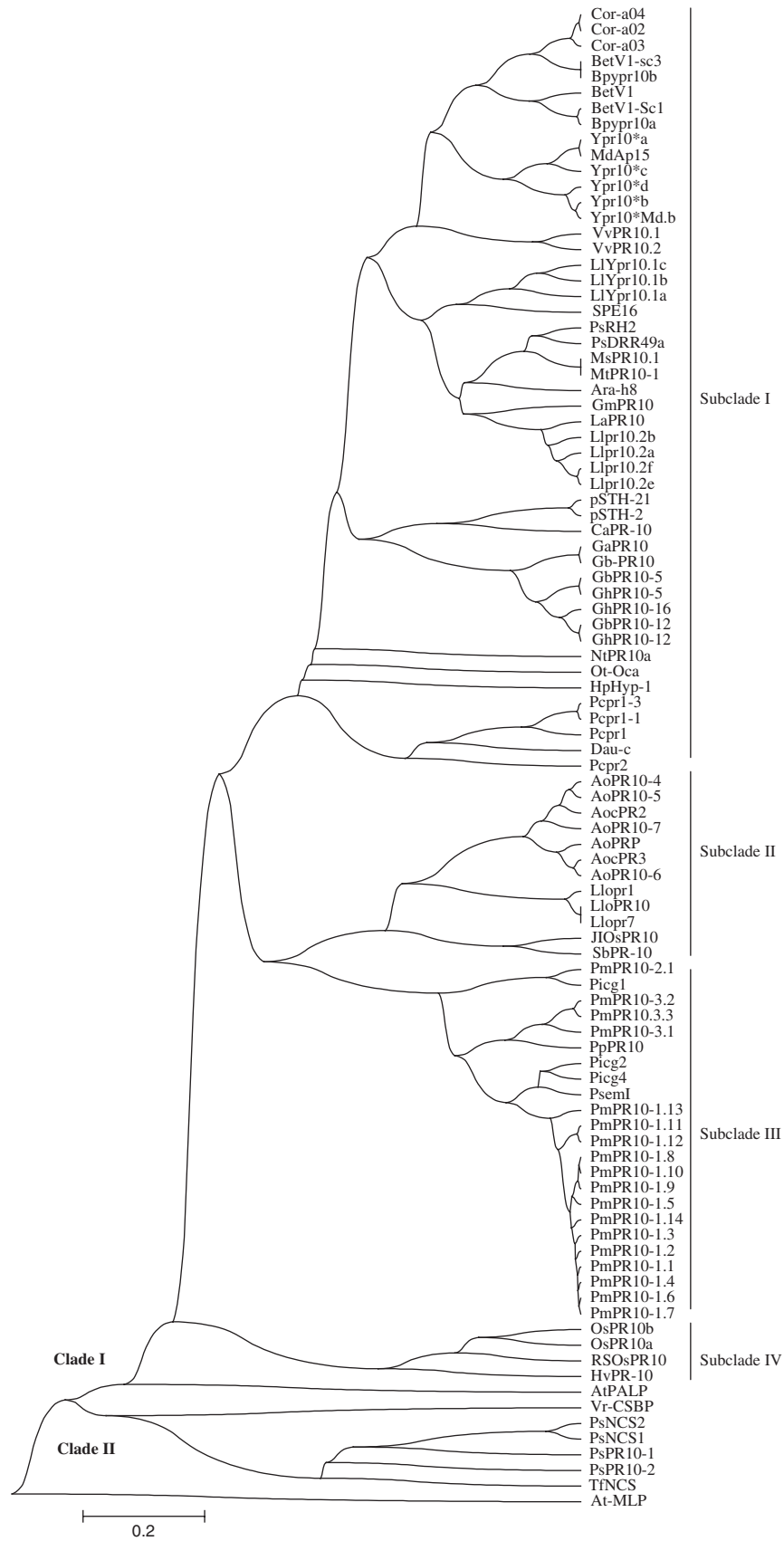
intracellularly and extracellularly in infected sugar pine needles [65]. Immunofluorescence analysis has revealed that a Douglas-fir PR-10 protein is induced in cortical tissues of roots infected with the fungal pathogen, *Phellinus sulphurascens* Pilat (Islam et al. unpublished data). GUS activity regulated by the *Ypr10\*a* promoter from apple (*Malus domestica*) is induced in young leaves by multiple stress factors, including pathogen attack and a fungal elicitor [48].

Wound treatment usually induces PR-10 expression. As quantified by Northern blot and PR-10 promoter- $\beta$ -glucuronidase fusion analysis, an asparagus PR-10 gene (AoPR1) transcript is accumulated after wounding [20,45]. Potato PR-10a is rapidly induced by wounding or by an elicitor, and becomes detectable 6 h after treatment, but the accumulation of PR-10c in potato is unaffected by wounding or by an elicitor [42,66]. Differential expression profiles have been reported for birch PR-10a and PR-10c in response upon wounding [67]. In western white pine, both PR-10 mRNA transcripts and proteins are rapidly induced upon wounding [54,63]. Furthermore, wounding can mimic *Cronartium ribicola* disease-activated PR-10 accumulation pattern in western white pine needles. *Cronartium ribicola* infection leads to expression of 10 PR-10 isoforms in needles, while nine of them accumulate after wounding [63].

As an important environmental factor, cold hardiness affects the PR-10 expression. PR-10 protein accumulates to the highest level in the roots of sugar pine and western white pine in winter [24]. The accumulation of PR-10 proteins by cold acclimation has also been observed in peach [68] and mulberry [41].

The expression of PR-10 genes is also induced by other abiotic stresses, for example, by salinity in the roots of rice [69], by drought stress in maritime pine [27] and hot pepper [57], by dormancy in a desert legume (*Retama raetam*) [70], by copper stress and other related oxidative stress in roots and leaves of birch (*Betula pendula*) [71,72], and by ultraviolet radiation in *Lupinus albus* L. [58].

Fig. 3. Phylogenetic tree based on the alignment of the deduced amino acid sequences of PR10 proteins from western white pine, other conifers, and angiosperms. Protein sequences were aligned with the Clustal W network service at the European Bioinformatics Institute (Cambridge, UK). Based on the alignment of deduced amino acid sequences, a phylogenetic tree was constructed by the NJ method, using the MEGA2 software package (<http://www.megasoftware.net/>). *Arabidopsis thaliana* MLP-related protein (At-MLP, NM\_128163) is included as an outgroup. Sequence abbreviations of PR10 proteins are given with GenBank accession numbers in parentheses as below: *Arabidopsis thaliana* AtPALP (AAM65899), *Arachis hypogaea* Ara-h8 (AY328088); *Asparagus officinalis* AoPRP, AocPR2–AocPR7 (X62103, X69562, X69563, AJ132610 to AJ132613); *Betula pendula* Betv1, Betv1-sc1, -sc3, Bpypr10a-b (Z72429, X77599, X77601, AJ289770, AJ289771); *Capsicum annuum* CaPR-10 (AF244121); *Corylus avellana* Cor-a02–Cor-a04 (AF323973 to AY323975); *Daucus carota* Dau-c (AF456481); *Gossypium arboreum* GaPR10 (AF416652); *Gossypium barbadense* Gb-PR10, GbPR10-5, -12 (AY588276, AY560553, AY560552); *Glycine max* GmPR10 (AF529303); *Gossypium hirsutum* GhPR10-5, -12, -16 (AF305064, AF305066, AF305067); *Hordeum vulgare* HvPR-10 (AY220734); *Hypericum perforatum* HpHyp-1 (AAN65449); *Lilium longiflorum* LloPR10, Llopr10-1, -7 (AAD17336, AF021848, AF021853); *Lupinus luteus* LiYpr10.1a-c, Llopr10.2a, b, e, f (AF002277, AF002278, AF180941, AF170091, AF170092, AY288355, AY303549); *Lupinus albus* LaPR10 (AJ000108); *Malus x domestica* Ypr10\*a–Ypr10\*d, Ypr10\*Md.b, and MdAp15 (AY026908 to AY026911, AY186248, L42952); *Medicago sativa* MsPR10.1 (AJ311049); *Medicago truncatula* MtPR10-1 (Y08641); *Nicotiana tabacum* NtPR10a (AY055111); *Oryza sativa* JIOsPR10, OsPR10a, b, RSOsPR10 (AF395880, AF274850, AF274851, AB127580); *Oxalis tuberosa* Ot-Oca (AF333436); *Pachyrhizus erosus* SPE16 (AY433943); *Papaver somniferum* PsNCS1, 2, PsPR10-1, -2 (AAX56303, AAX56304, AAX56075, AAX56076); *Petroselinum crispum* Pcp1, 2, Pcp1-1, -3 (X98688, X58698, U48862, X12573); *Picea glauca* Picg1,2,4 (AAF12810–AAF12812); *Pinus montica* PmPR10 (AY064193–AY064206, AY596271, AY596273–AY596275); *Pinus pinaster* PpPR10 (CAC83079); *Pisum sativum* PsDRR49a and PsRH2 (vU31669, S74512); *Pseudotsuga menziesii* Psem1 (AAF60972); *Solanum tuberosum* pSTH2 and pSTH21 (M25155, M25156); *Sorghum bicolor* SbPR-10 (U60764); *Thalictrum flavum* TfNCS (AAR22502), *Vigna radiata* Vr-CSBP (AB012218), *Vitis vinifera* VvPR10.1 and VvPR10.2 (AJ291705, AJ291704).



PR-10 gene expression is also regulated by plant hormones and defense-related signaling molecules, including jasmonic acid [63,64,69,73,74], abscisic acid (ABA) [74], and salicylic acid [64]. Okadaic acid, a specific inhibitor of type 1 or type 2A serine/threonine protein phosphatase, enhances wound-induced PR-10 protein synthesis [63]. These data suggest that defense-related signal chemicals and protein phosphorylation are involved in the signal transduction pathway leading to PR-10 activation. Based on investigation using lily anthers, Wang et al. [74] have proposed that two separate signal transduction pathways are involved in the induction of PR-10 genes by ABA and methyl jasmonate (MeJA), respectively.

#### 4. Promoter analysis and regulations of PR-10 genes

PR-10 promoters have been cloned and functionally analyzed in several angiosperm species, including parsley [75–77], asparagus [45], bean [52], apple [48], potato [66,78], and alfalfa [79] and in the gymnosperm western white pine [40,54]. Walter et al. [52] have shown that the bean *Ypr10c* promoter directs GUS expression in an organ-specific, dark-dependent, and glutathione- or SA-inducible manner. However, no specific *cis*-element has been characterized in the *Ypr10c* promoter sequence. Several putative motifs, including G-box, H-box, and a 9-bp sequence (ATTTGACCG), have been found in a wounding-responsive *Asparagus* PR-10 (*AoPR1*) promoter fragment (from –982 to 1) [44,45]. Some PR-10 gene promoters, including parsley (*Petroselinum crispum*) *PcPR1* genes [76,80] and *PcPR2* gene [75] and potato (*Solanum tuberosum*) *PR-10a* gene [21,22,61], have been characterized for the presence of *cis*-elements and their *trans*-acting protein factors. A detailed study on the promoter of the parsley *PcPR1* gene has shown that the promoter sequence from –240 to –130 is essential for fungal elicitation [76]. Further work has revealed that the interaction of WRKY proteins (WRKY1, 2, and 3) and W-boxes with the core sequence (T)TGAC(C) is responsible for elicitor-responsive expression of the parsley *PcPR1* gene [80,81]. In another parsley PR-10 promoter of the *PcPR2* gene, a 11-bp motif (CTAATTGTTTA) is necessary for elicitor-mediated expression [75].

In western white pine different regions of the *PmPR10-1-14* promoter are responsible for its root-specific expression in transgenic tobacco, including a long repeated sequence [40]. In another western white pine PR-10 promoter (*PmPR10-1-13*), two regions (–1316 to –930 and –309 to –100) are responsible for the disease and wound-inducible activities [54]. A few *cis*-regulatory elements, responsible for inducible expression in angiosperms, have also been identified in the *PmPR10-1.13* promoter [54], such as the typical box-W1 (TTGACC), elicitor-responsive-element (EIRE)-like sequence (TTCGTCC) [82], MeJA-responsive motifs (CGTCA/TGACG) [83], and ethylene-responsive enhancer element (ERE)-like motif (ATTTCAAG) [84]. Promoter 5'-deletion and promoter

activity comparison analyses in transgenic *Arabidopsis* have revealed that the EIRE-box, G-box, and a putative palindromic element (ATGAAGTTCAT) are potentially involved in disease- and wound-induced PR-10 gene expression [54]. Further investigation of *cis*-acting elements that regulate the pathogen-inducible expression of PR-10 genes will shed light on the mechanisms of the defense response against pathogen attack.

Another detailed investigation of PR-10 *cis*-elements has been conducted on the potato PR-10 family. Examination of the 1015-bp 5'-flanking sequence of the potato *PR-10a* gene (*STH-2*) in transgenic potato plants has identified a positive *cis*-regulatory region from –155 to –52 with fungal or wounding induction, and a possible negative regulatory region between –52 and –28 [78]. A more detailed study has shown that a 50-bp region (–155 to –105) in the potato *PR-10a* promoter is responsible for complete elicitor responsiveness. The recognition and binding at the region from –135 to –105 by the phosphorylated nuclear factor PBF1 is necessary for the PR-10a activation [66]. The binding of an elicitor response element (ERE) (TGACAnnnnTGTC) with the nuclear factor PBF-2 is also crucial for PR-10a activation [85]. Boyle and Brisson [86] have further identified a silencing element-binding factor that shows sequence-specific binding to the sequence PyTGTCNC and represses the activity of a PR-10a reporter construct by over-expression. These findings suggest that there is a complex network to modulate PR-10 gene expression. Whirly (StWhy1), a novel transcriptional factor binding to a single-stranded DNA element (GTCAAAA) of the PR-10a promoter, has been implicated in the regulation of potato PR-10a gene, and its *Arabidopsis* ortholog (AtWHY1) is necessary for salicylic acid-dependent disease resistance [87].

#### 5. Biological functions of PR-10 proteins

##### 5.1. Antimicrobial activity and in vitro ribonuclease activity

Direct evidence of antimicrobial activity conferred by PR-10 proteins comes from in vitro microbe inhibition experiments. Ocatin, a PR-10 protein from the Andean tuber oca (*Oxalis tuberosa*), has been shown to have antibacterial and antifungal activities [88]. The recombinant CaPR-10 protein from hot pepper (*Capsicum annuum*) inhibits the growth of the oomycete pathogen *P. capsici*; it also exhibits ribonucleolytic activity against tobacco mosaic virus RNA [57]. SsPR10 from *Solanum surattense* shows both ribonucleolytic and antimicrobial activity [89]. Recently, it has been found that one recombinant protein of western white pine PmPR-10 inhibits the growth of Douglas-fir root rot fungus *Phellinus sulphurascens* (Williams et al. unpublished data).

The results of over-expressing PR-10 genes in transgenic plants are not consistent. Over-expression of *STH-2*, a member of the *Ypr10* family, has not resulted in enhanced

resistance of potato to pathogens [90]. Similar negative results have also been observed in the studies of pea PR-10-1 [91]. Unlike many defense-related genes described in similar systems, expression of PR-10-homologous SRG1-like genes does not correlate with resistance to *Colletotrichum trifolii* [92]. However, expression of the pea PR-10.1 gene in potato confers resistance to early dying disease [93]. Leaf extracts from transgenic tobacco expressing the maize ZmPR-10 demonstrate RNase activity and inhibit the growth of maize pathogen *Aspergillus flavus* [94].

One explanation linking the biological role of PR-10 genes with plant defense responses is that some PR-10 proteins have shown in vitro ribonuclease activity. PR-10 proteins with RNase activity may help to protect plants during programmed cell death around infection sites or to act directly on the pathogens. Two ribonucleases (RNase 1 and 2) from ginseng share high structural homology with PR-10 proteins [95,96]. Recently, ribonuclease activity has been confirmed for some recombinant and natural PR-10 proteins: Bet v 1 from birch (*Betula verrucosa*) pollens [43,97], LaPR-10 from lupine (*Lupinus albus*) roots [98], PR-10c from birch (*Betula pendula*) [99], GaPR-10 from *Gossypium arboreum* [100], SPE-16, a PR-10 protein from *Pachyrrhizus erosus* [49], and CaPR-10 from hot pepper (*Capsicum annuum*) [57]. Increased ribonuclease activities have been observed in tobacco leaves during the hypersensitive response to tobacco mosaic virus [101]. All evidence to date suggests that the PR-10 proteins may be synthesized and released from host cells damaged by the fungus penetration, which implies a role in defense against pathogen attack.

The 3D structures of the birch Bet v1 [35] and the yellow lupine PR-10 protein [37] show that E96, E148, and Y150 (as positioned in Bet v 1) are close together, and thus are predicted as an activity site for the catalytic reaction of ribonuclease. Associated with ribonuclease-like activity, the P-loop (GxGGxGxxK), a motif believed to be involved in ATP or GTP binding, has been shown to be critical for the RNase activity in SPE-16 [49]. Phosphorylated CaPR-10 has shown a 12.4-fold higher RNase activity than the non-phosphorylated form in a yeast tRNA-degradation test [57]. The presence of putative phosphorylation sites in CaPR-10 and other PR-10 proteins suggests that phosphorylation may be involved in the RNase activity of PR-10 proteins.

However, ribonucleolytic activity has been detected only in a few PR-10 proteins. Recent data indicate that not all PR10 proteins possess this catalytic property. For example, LIPR10.1B has displayed RNase activity at some level, but its homologous protein from the same species, LIPR10.1A, has shown none at all [37]. The recombinant protein of PmPR10-1.10 from western white pine also does not demonstrate any RNase activity [39]. Therefore, it has been proposed that RNase activity of PR-10 proteins is possibly incidental and of no biological importance [37].

## 5.2. Ligand-binding ability

Cytokinins are plant-growth hormones that control the differentiation and proliferation of plant cells. The first indication of cytokinin-specific binding activity for a PR-10 protein has come from a study on VrCSBP, a PR-10 protein purified from mung bean (*Vigna radiata*) [102]. A 34-kD protein from moss (*Physcomitrella patens*), significantly homologous to classic PR-10 proteins, also shows cytokinin-binding ability [103]. Recently, the major birch allergen, Bet v 1, has been found to have the capacity to bind a range of physiological ligands, including cytokinins, fatty acids, and flavonoids [104]. Pru av 1, a cherry allergen PR-10 protein, is able to bind the phytosteroid homocastasteron [36]. Bet v 11 can bind deoxycholate, brassinolide, and 24-epicastasterone by a specific non-covalent interaction [34]. Protein–ligand interaction studies with saturation transfer difference NMR have revealed that birch PR-10c interacts with several biologically important molecules, including cytokinins, flavonoid glycosides, sterols, and emodin [105]. Brassinolide, one of the most important brassinosteroids in the hormonal regulation of plant growth and development, has been found to induce disease resistance in plants [106]. Therefore, PR-10 proteins may take part in steroid hormone-mediated disease resistance in plant defense response or in developmental regulation.

AtBRI1, an *Arabidopsis* receptor kinase, has an extracellular leucine-rich repeat and a transmembrane domain that are involved in the binding of brassinosteroids [107]. PR-10 proteins may act in plant cells as a carrier to move ligands to their soluble receptors, both increasing ligand concentration and providing the means for specific interaction [34]. Apple PR-10 protein (Mal d 1) has the ability to bind to another protein MdAP [108], suggesting its potential role in signaling. The interaction of PR-10 protein with other proteins may be a transport or storage mechanism, thus may be involved in signaling of phytohormones [104].

The Y-shaped, hydrophobic pocket in the 3D structure of PR-10 proteins [34] is responsible for the specific non-covalent interaction with apolar ligands, and perhaps with other substrates for enzymatic activities. A study of the western white pine PR-10 family based on  $K_a/K_s$  estimation and computer modeling indicates that the amino acid residues constituting this type of Y-shaped pocket might have been under positive, selective pressure during evolution. The selective pressure on the putative ligand/substrate-binding site allows a homologous PR-10 protein to obtain a new function with little change in 3D structure [39]. This kind of evolutionary process would permit proteins to distinguish ligands/substrates that meet specific physiological requirements for each of the various functional roles of PR-10 proteins. LIPR-10.2A, a member of a new PR-10 group from lupin, has different structural properties from LIPR-10.1A and LIPR-10.1B. The hydrophobic ligand-binding cavity of LIPR-10.2A has been shown to have a different shape and volume [109]. This

suggests that there are different binding affinities among PR-10 isoforms for plant functional ligands or enzymatic substrates. The interaction of birch PR-10c with several biologically important ligands reveals the potential presence of three different binding sites, suggesting that it is a multifunctional protein [105]. It is believed that the role of ligand binding and transport in PR-10 proteins may be crucially important for plant defense response to pathogens, as well as for plant growth and development [34]. It appears that the PR-10 family members may serve different activities and biological functions in specific tissues. To elucidate the mechanism underlying evolution and functional adaptation of plant PR-10 proteins, much more experimental work is required to investigate ligand/substrate-binding affinity and specificity, as well as genetic mutants at the putative binding sites.

### 5.3. Enzymatic activities in plant secondary metabolisms

The novel catalytic functions of NCS and HpHyp-1 proteins define a new class of plant secondary metabolic enzymes within the PR-10 family [7]. The biological activity of some PR-10 proteins in plant secondary metabolism has been first reported from a *Hypericum perforatum* gene (*HpHyp-1*) that encodes an enzyme (HYP1) responsible for the biosynthesis of medicinal compound hypericin, which involves the condensation of emodine and emodine anthrone followed by dehydration and successive phenolic oxidations to produce naphthodianthrone [110]. Recently, Koistinen et al. [105] have reported that birch PR-10c-His interacts with emodin on the basis of ligand docking by the binding of two molecules at the same time. NCS proteins catalyze the formation of (S)-norcoclaurine from the condensation of 3,4-dihydroxyphenylethylamine (dopamine) and 4-hydroxyphenylacetaldehyde in the biosynthesis of benzyloquinoline alkaloids. However, homologous PR-10 proteins, including PsPR10-1, PsPR10-2, OsPR10, VrCSBP, HpHYP1, BbMAP (Bet v 1a), and PmPR10 do not have NCS activity at detectable levels [31].

### 5.4. Role in abiotic stress

Constitutive expression of pea *PR-10.1* enhances the germination of *Brassica napus* under saline conditions [111], suggesting a crucial role for PR-10 protein in plant stress tolerance. The WAP18, a PR-10 from mulberry (*Morus bombycis* Koidz.), exhibit in vitro cryoprotective activity for a freeze-labile model enzyme (L-lactate dehydrogenase-LDH), suggesting that some PR-10 proteins might function in the freezing-tolerance mechanism during winter [41]. An electrolyte-leakage analysis has revealed a significant correlation between PR-10 protein level and frost hardiness during seasonal cold acclimation [24]. PR-10 proteins are induced by dormancy and possess high numbers of polar residues (~40%), comparable to dehydrin (~50%) [70]. Thus, it is possible that PR-10

protein might function as a LEA-like protein to protect other proteins and cellular structures during dormancy, cold hardiness or salinity. Meanwhile, because of their high accumulation levels in winter, it is speculated that PR-10 proteins may be nitrogen-storage proteins that support subsequent shoot growth in the following spring [41,112].

## 6. Conclusion

As a diverse family in higher plants, PR-10 proteins show a complex expression pattern and display various biological activities in different species. A highly complex PR-10 gene family has been identified in birch [12], apple [30,47,113], and western white pine [39]. Multiple PR-10 members are differentially expressed under different environmental stresses or at different stages during plant growth and reproduction. Therefore, it is not reasonable to assign a unique function to PR-10 protein groups throughout the plant kingdom. It is possible that some PR-10 proteins might have evolved the capacity for catalytic activities or/and ligand-binding activities due to selective pressure.

Inducible expression, RNase and ligand-binding activities have linked PR-10 proteins to plant defense as well as to plant growth and development. However, our understanding of PR-10 protein biochemical activities in plant defense response is still limited by investigation of transgenic plants with PR-10 over-expression or by in the vitro test of inhibition activity against pathogens. The in vitro ligand-binding activities possessed by some PR-10 proteins could not tell us what role PR-10 proteins play in plant hormone-mediated signal transduction pathway in living tissues. One obstacle confounding the functional study of PR-10 proteins is the presence of many members of this divergent family in a single species. The development of functional genomics technology will increase our ability to evaluate the contribution of each specific member of PR-10 family in plant defense and development.

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