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Isolation and characterization of a novel anionic peroxidase cDNA found in poplar (*Populus nigra*) suspension cultured cells

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Abstract A cDNA clone from *Populus nigra* L. var. *italica* Koehne, denoted PCY2-6, coding for an anionic peroxidase has been isolated, cloned, and characterized. PCY2-6 is 1160 bp long; and its deduced product, PnC26, contains 343 amino acid residues. The mature protein has a calculated isoelectric point of 4.09. The protein contains two motifs typical of peroxidase and 10 potential *N*-glycosylation sites. PnC26 is therefore classified as an anionic peroxidase. The mRNA of the PCY2-6 gene family was detected in immature and mature leaves and in two parts of current-year stems: the shoot tip and the older stem. The mRNA of PCY2-6 gene family was found to localize in the phloem and cortex of the current-year stems. We therefore conclude that expression of the PCY2-6 gene family is related to bark development.

Key words Anionic peroxidase · Bark · *Populus nigra* · Suspension cultured cells

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Introduction

The cell wall of higher plants is made up of roughly 90% carbohydrates and 10% proteins. The membrane-associated proteins can be divided into three groups: secretory proteins, enzymes and other proteins embedded in the cell wall, and structural proteins. Peroxidase and lectin are notable examples of secretory proteins, and the extensins and arabinogalactan proteins are well known structural proteins.

We have isolated two cDNA clones from a cDNA library constructed using poplar (*Populus nigra* L. var. *italica* Koehne) suspension cultured cells treated with *t*-zeatin, which is a kind of cytokinin. The first clone was designated PCY3-15[1] (DNA Data Base accession number D83226) and was found to encode an extensin; the other, PCY2-6 (DNA Data Base accession number D83224), encoded an anionic peroxidase. Therefore, we reasoned that these clones should encode cell wall proteins of a higher plant. We would also expect that expression of these protein-encoding genes might be dependent on the development of the plant cell wall.

It is reported that plant peroxidases contain an endoplasmic reticulum (ER)-targeting signal and some *N*-glycosylation sites in plants[2–4]; and some anionic peroxidases have been extracted from the cell wall.[5] Therefore, it is expected that anionic peroxidases are in the plant cell wall. Anionic peroxidases are considered to play the major role in the cross-linking of cell wall proteins,[6,7] making cell walls effective barriers to diffusion via suberization[3,8] and lignification.[9] It was also known that the transcription of some their genes was related to the auxin or cytokinin level (or both).[4,10,11]

In this report we describe cloning of a cDNA encoding an anionic peroxidase, termed PCY2-6, from poplar cells and study of its mRNA detection and localization in various tissues. The purpose of this study was to examine the

relation between this gene expression and poplar tissue development.

Materials and methods

Materials

Poplar (*Populus nigra* L. var. *italica* Koehne) suspension cultured cells were prepared as previously reported.[1]

cDNA sequencing

PCY2-6 was isolated as follows: A previously constructed cDNA library was screened to yield anionic peroxidase cDNAs in λgt10 with differential hybridization as a probe ^{32}P-labeled; they were subsequently subcloned into pBluescript II KS (+).[1] A series of deletion mutants of this cloned fragment were prepared with a Kilo-Sequence deletion kit (Takara shuzo, Kyoto) according to the supplier's instructions. The sequencing of the cDNA clone was performed on both strands using a cycle-sequencing dye terminator kit (ABI Prism, Perkin Elmer). Sequencing reaction mixtures were analyzed with an ABI model 373A sequencing system.

Northern blot analysis

Northern blot analysis of PCY2-6 on RNA isolated from poplar suspension cultured cells treated with *t*-zeatin was performed as previously reported.[1] Total RNA was isolated from the shoot tips (1 cm in length from the tops), old stems (5–15 cm in length from the tops) of current-year stems, and immature (first and second leaves from the shoot tip) and mature (10 cm below the shoot tip) leaves.[12] For the Northern analysis, 20 μg of total RNA was separated on 1% agarose gel containing formaldehyde and transferred to a Hybond-N+ membrane (Amersham Pharmacia, Tokyo, Japan).[13] RNA was hybridized with probes Digoxigenin (DIG)–labeled by the polymerase chain reaction (PCR). The probes were amplified from the PCY2-6 cDNA as a template by PCR using the PCY2-6 antisense specific primer (5′-AGCCAGGTTTGCATTGACTACC-3′) to label only the antisense strand of the cDNA.[14] Hybridization was performed to the manufacture's instructions (Boehringer Mannheim, Germany). Hybridization was performed in 5 × SSC (0.75 M NaCl, 75 mM Na_3 citrate), 50% formamide, 50 mM sodium phosphate buffer (pH 7.0), 7% sodium dodecyl sulfate (SDS), 2% blocking reagent (Boehringer Mannheim, Germany), 0.1% *N*-lauroylsarcosine and denatured fish sperm DNA solution (50 μg/ml) for 16 h at 50°C. The concentration of the probe was 0.2 μg/ml in the hybridization buffer. The washing steps were carried out twice for 5 min at room temperature in 2 × SSC and 0.1% SDS and twice for 15 min at 68°C in 0.1 × SSC and 0.1% SDS. Detection was performed using the DIG detection kit according to the manufacture's instructions (Boehringer Mannheim, Germany).

Southern blot analysis

The DNA was isolated from poplar suspension cultured cells.[15] After 12 h of hydrolysis by restriction enzymes, the DNA fragments were separated on 0.8% agarose gels in 90 mM Tris-borate, 2 mM Na_2-EDTA. After blotting onto a Hybond-N+ membrane (Amersham Pharmacia, Tokyo), DNA was hybridized with probes ^{32}P-labeled by random priming. Hybridization was performed in 6 × SSC, 50% formamide, 1 × Denhardt's [0.02% bovine serum albumin (BSA), 0.02% Ficoll, 0.02% polyvinyl pyrrolidone (PVP), 0.5% SDS, and denatured fish sperm DNA 100 μg/ml. The probe was the PCR-amplified DNA corresponding to PCY2-6. Hybridization was performed for 16 h at 42°C. The washings were done successively for 5 min at room temperature in 2 × SSC, for 30 min at room temperature in 2 × SSC, and twice for 1 h at 65°C in 1 × SSC.

In situ hybridization

The synthesized and labeled probes were used as described for the Northern blot analysis. Current-year stem's cuttings, which were 5.0–5.5 cm in length from the top of the stem, were fixed with FAA (50% ethanol, 5% acetic acid, 3.7% formaldehyde) and dehydrated using a graded ethanol series. Tissues were further treated with *t*-butyl alcohol and embedded in Paraprast plus (Sigma, USA).[16] Hybridization was performed in 50% formamide, 2 × SSC, 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, 1 × Denhardt's, 10% dextran sulfate, 0.25% SDS, and denatured fish sperm DNA solution 1 mg/ml for 24 h at 50°C. The concentration of the probes was 1 μg/ml in the hybridization buffer. The washing steps were carried out twice for 20 min at 50°C in 2 × SSC, twice for 20 min at 50°C in 0.2 × SSC, and twice for 20 min at 50°C in 0.1 × SSC. Detection was performed with the DIG detection kit according to the manufacture's instructions (Boehringer Mannheim, Germany). As a control, DIG-labeled DNA probe was amplified from λgt10 DNA as a template by PCR using the forward primer (5′-GCTGGGTAGTCCCCACCTTT-3′) and used for detection during subsequent hybridization.

Results and discussion

Nucleotide and deduced amino acid sequences of PCY2-6

PCY2-6 was 1160 bp long and contained a poly(A) tail. Nucleotide sequence analysis revealed a 1035-bp open reading frame (Fig. 1), which would code for a protein of 343 amino acid residues with a molecular mass of 36.5 kDa. This protein was designated PnC26 and the PnC26-encoding gene was *pnc26*. Twenty-four hydrophobic amino acid residues were found in the N-terminal region of PnC26, and might constitute a putative ER-targeting signal, as specified by von Heijine,[17] with the hydrophobic leader being cleaved off between Gly24 and Gln25. The 12-amino acid resides in the C-terminal region might be cleaved off

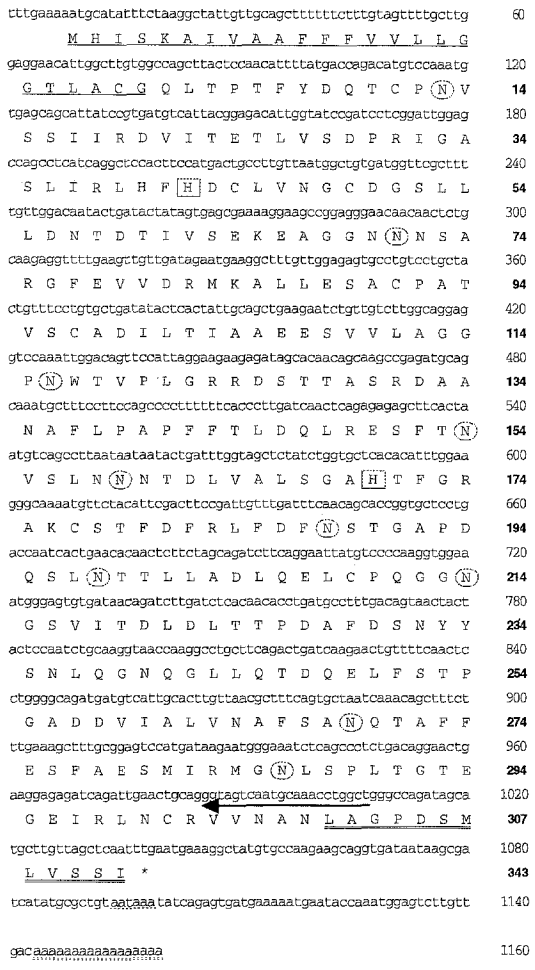

Fig. 1. Nucleotide and deduced amino acid sequences of a poplar anionic peroxidase cDNA, PCY2-6. The putative endoplasmic reticulum (ER) signal is underlined by a *single line*. The N-terminal amino acid residue of the mature protein is number 1 in the protein sequence. The distal and proximal histidines are surrounded by *squares*, and the putative *N*-glycosylation sites are surrounded by *circles*. The position of the putative C-terminal cleaved region is underlined by *double lines*. The stop codon is shown by an *asterisk*. The putative polyadenylation signal is underlined by a *dotted line* and a poly(A) tail by a *double dotted line*. The position of PCY2-6 antisense specific primer is underlined by the *arrow*

between Asn331 and Leu332. The C-termini of mature plant peroxidases indicate that they follow the default pathway out of the cell.[18] The putative mature protein (32.9kDa) contains two motifs typical of peroxidase: GASLIRLHFHDC (consensus AXXXIRIXFHXC) and DLVALSGAHTF (consensus DLXXLSGAHTV). The distal and proximal histidine residues located near the prosthetic heme group of the active center of peroxidase are present in these motifs (Fig. 1). The sequence of PnC26 also revealed eight conserved cysteines that are known to form four protein disulfide bridges in plant peroxidases[19] and 10 potential *N*-glycosylation sites. Because mature PnC26 had a calculated isoelectric point of 4.09, it was classified as an anionic peroxidase.

In a consequent homology search, PnC26 revealed significant similarities to other peroxidases. Figure 2 shows an alignment of the deduced amino acid sequence of PCY2-6 with the sequences of Pxp22 of *P. trichocarpa* (Christensen, Bauw, Boerjan and van Montagu., accession number X97349), pA2a of *P. kitakamiensis*.[20] PCY2-6 showed the highest similarity to Pxp22 from *P. trichocarpa* with 97.8% identity at the amino acid level.

Detection of the mRNA of the PCY2-6 gene family in suspension cultured cells and Southern blot analysis

Southern blot analysis of *P. nigra* genomic DNA revealed four bands hybridizing with a PCY2-6 PCR fragment as a probe (data not shown). The data suggested the presence of several homologous genes to *pnc26* in the poplar genome. Isolation of *pnc26* from the poplar genome was then carried out. An additional clone, PnAPG1 (accession number D83225), was isolated and identified as another member of the *pnc26* gene family. The deduced amino acid sequence of PnAPG1 showed considerable similarity to PnC26, with 94.2% identity (data not shown). The results supported the existence of anionic peroxidase isoenzymes in *P. nigra*. Previously, Kawai et al.[21] and Osakabe et al.[22] isolated three genes of anionic peroxidase isoenzymes from *P. kitakamiensis*, and Christensen et al. reported the existence of six anionic peroxidase isoenzymes in *P. trichocarpa*.[5]

Total RNA was prepared from suspension cultured cells at various times of *t*-zeatin treatment. The total RNA from

Fig. 2. Alignment of the deduced amino acid sequence of PCY2-6 with those of other plant peroxidases. *Asterisks* indicate identical positions. The accession numbers of the aligned peroxidase cDNAs are X97349 (*Pxp22, P. trichocarpa*) and D30652 (*pA2a, P. kitakamiensis*)

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PCY2-6                                  MHISKAIVAAFFFVVLLGGTLACGQLTPTFYDQTCPNVSSIIRDVITETLVSDPRIGASLIRLHFHDCLVNGCDGSLLLDNTDTIVSEKE   90
Pxp22                                   MHISKAIVEAFFFVVLLRGTLACGQLTPTFYDQTCPNVSSIIRDVITETLVSDPRIGASLIRLHFHDCFVNGCDGSLLLDNTDTIVSEKE   90
pA2a                                                                    FYDQTCPNVSTIIRDVITETLASDPRIGASLIRLHFHDCFVNGCDGSLLLDNSDTIVSEKE   61
                                                                        ********** ********** ********************** ************ ********

PCY2-6   AGGNNNSARGFEVVDRMKALLESACPATVSCADILTIAAEESVVLAGGPNWTVPLGRRDSTTASRDAANAFLPAPFFTLDQLRESFTNVSLNNNTDLVALSGAHTFGR   198
Pxp22    AGGNNNSARGFEVVDTMKALLESACPATVSCADILTIAAEESVVLAGGPNWTVPLGRRDSTTASRDAANAFLPAPFFTLDQLRESFTNVSLNNNSDLVALSGAHTFGR   198
pA2a     AGGNNNSARGFEVVDRMKALLESACPATVSCADILTIAAEESEVLAGGPNWTVPLGRRDSTTASRDAANAFLPAPNITLDQLRESFTNVGLNNNSDLVALSGAHTFGR   169
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PCY2-6   AKCSTFDFRLFDFNSTGAPDQSLNTTLLADLQELCPQGGNGSVITDLDLTTPDAFDSNYYSNLQGNQGLLQTDQELFSTPGADDVIALVNAFSANQTAFFESFAESMI   306
Pxp22    AKCSTFDFRLYDFNSTGAPDPSLDTTLLAALQELCPEGGNGSVITDLDLSTPDAFDSDYYSNLQGNRGLLQTDQELFSTPGADDVIALVNAFSANQTAFFESFVESMI   314
pA2a     AKCSTFDFRLYDFNSTGAPDQSLDPTLLAALQELCPQGGNGSVLTDLDLTTPDAFDSNYYSNLQGNQGLLQTDQVLFSTPGADDVIALVNAFSANQTAFFESFAESMI   277
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PCY2-6   RMGNLSPLTGTEGEIRLNCRVVNANLAGPDSMLVSSI                                                                          343
Pxp22    RMGNLSPLTGTEGEIRLNCSVVNANLAGPDSMLVSSI                                                                          343
pA2a     RMGNLRPLTGTEGEIRLNCRVVNANLAGPDSKLVSSI                                                                          314
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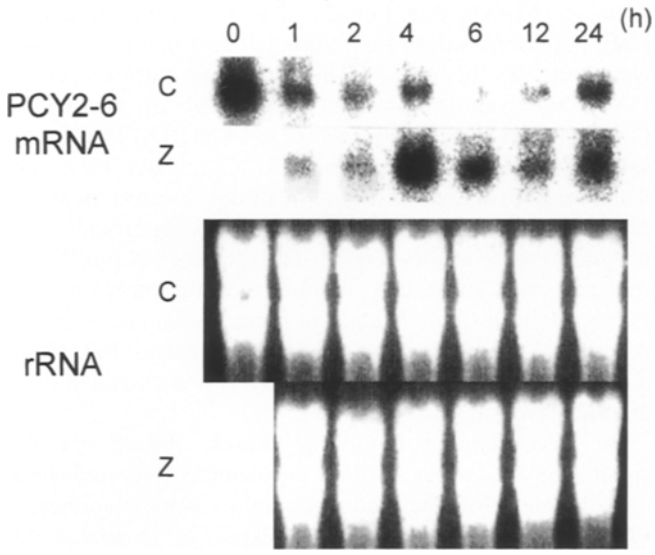

Fig. 3. Northern analysis of the expression of the PCY2-6 gene family in suspension cultured cells. Expression of the PCY2-6 gene family in suspension cultured cells with or without *t*-zeatin. *C*, total mRNA was isolated from control cells (2,4-D 1 ppm); *Z*, total RNA was isolated from *t*-zeatin-treated cells (2,4-D 1 ppm + *t*-zeatin 1 ppm). Numbers represent hours after subculture

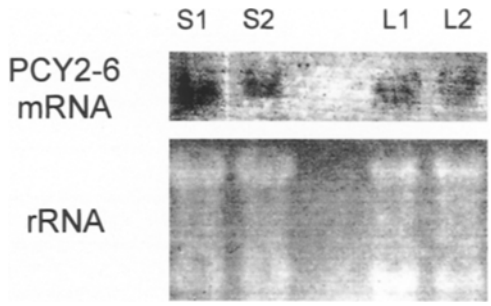

Fig. 4. Northern analysis of expression of the PCY2-6 gene family in stems and leaves. PCY2-6 mRNA expression in poplar stems and leaves. Total RNAs from shoot tip (*S1*), older stem (*S2*), immature leaf (*L1*), and mature leaf (*L2*) were analyzed by northern hybridization with digoxigenin (DIG)-labeled PCY2-6 specific polymerase chain reaction (PCR) probe

auxin-only-treated cells was used as a control. The mRNA of the PCY2-6 gene family in suspension cultured cells was detected by Northern analysis. Immediately after the treatments, the mRNA was detected with a PCY2-6-specific probe in cells treated with *t*-zeatin and the control cells (Fig. 3). Whereas the mRNA of the PCY2-6 gene family decreased to a 6-h incubation in the control cells, the mRNA decreased to a 1-h incubation and increased from a 2-h incubation in the cells treated with *t*-zeatin. Specifically, after 4 h of incubation, the transcript levels of the PCY2-6 gene family were three times higher in *t*-zeatin treated cells than in control cells. This result is likely to be due to an influence of cytokinin treatment on the transcription of the PCY2-6 gene family. Tournaire et al. also reported a variation in gene expression of an anionic peroxidase following cytokinin addition during callus growth in *Petunia*.[11]

Detection of the mRNA of the PCY2-6 gene family in poplar leaf and stem

The mRNA of the PCY2-6 gene family was detected by Northern analysis and found to be similarly expressed, albeit at low transcriptional levels, in both stems and leaves (Fig. 4). This finding is interesting because PCY2-6 shows a high degree of amino acid similarity to pA2 from *P. kitakamiensis* (95.8%). However, although the latter mRNA is also detected in the stem, it is not detected in the leaf.[20] This result demonstrates that the role of the mRNA of the PCY2-6 gene family might be physiologically different from that of pA2 mRNA despite the high level of homology between them. Similarly, Lagrimini et al. reported that the mRNA of a lignin-forming anionic peroxidase from tobacco was abundant in stem tissue but detected at only low levels in the leaf and root tissue.[23] Thus, the tissue-specific presence of mRNA of the PCY2-6 gene family differs from that of the lignin-forming anionic peroxidase mRNA as well.

The highly anionic peroxidases of the tomato appear to be related to pathogenesis. These genes are highly induced in tissues responding to fungal pathogens, wounding, or exposure to either abscisic acid (ABA) or fungal elicitor preparations. The basal levels of transcripts of these genes are low in the root, stem, leaf, and red fruit tissues of healthy tomato plants.[24–26] Detection of the tomato peroxidase transcripts in healthy tissues was similar to that of PCY2-6 in healthy poplar tissues. This might explain the low detection rate of the mRNA of the PCY2-6 gene family in the leaves and stems.

mRNA localization of the PCY2-6 gene family in poplar stem

The mRNA localization of the PCY2-6 gene family in poplar stem was determined by in situ hybridization using a matching probe. The mRNA was detected in the bark of the stem but not in the xylem (Fig. 5B), indicating tissue-specific localization. Thus, it is likely that expression of the PCY2-6 gene family has a connection with the development of the bark. Christensen et al. reported that anionic peroxidase isoenzymes were present in both bark and xylem of the stem of *P. trichocarpa* but with a different activity in each tissue.[5] It is also possible that PnC26 localizes in the bark.

In the bark, the mRNA of the PCY2-6 gene family was detected in the phloem and cortex of the poplar stem (Fig. 5B). Teichmann et al. reported the detection of anionic peroxidase (ZmAP1) mRNA in the epidermis, hypodermis, and pericycle of *Zea mays* roots,[3] and Carpin et al. detected mRNA of an anionic peroxidase (APRX) in root epidermis and some cells of the stele and in the lower and upper epidermis in zucchini.[4] The mRNA of the PCY2-6 gene family was strongly detected in the cortex of the poplar stem, near the epidermis (Fig. 5B). It is therefore possible

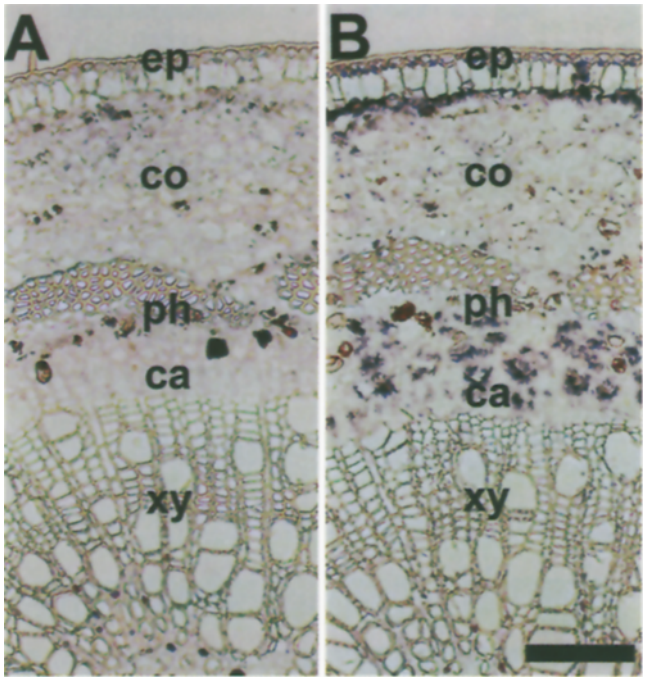

Fig. 5. In situ localization of mRNA of the PCY2-6 gene family in a poplar stem (5.0–5.5 cm in length from the top). **A** Section was hybridized with DIG-labeled λgt10 DNA. **B** Section was hybridized with DIG-labeled PCY2-6-specific PCR probe. *ep*, epidermis; *co*, cortex; *ph*, phloem; *ca*, cambium; *xy*, xylem. *Bar* 100 μm

that the mRNA of the PCY2-6 gene family is similar to ZmAP1 and APRX mRNA as far as the localization pattern surrounding the epidermis is concerned. Furthermore, Magliano and Casal reported that anionic peroxidases A_3 and A_4 as well as extensin were found in the vascular bundles and epidermis of mustard stems. These peroxidases responded to both wounding and phytochrome and were able to cross-link extensin precursors.[7] From the results showing the mRNA localization of the PCY2-6 gene family in poplar stems, we speculate that PnC26 would be detected in the vascular bundles and epidermis of poplar stems. Because PnC26 seems to be similar to A_3 and A_4 in its localization pattern and its response to wounding, it might also be able to crosslink extensin precursors.

Conclusions

We have shown that detection of the mRNA of the PCY2-6 gene family is low in healthy poplar tissues, which is also the case for peroxidases involved in pathogenesis. In addition, mRNA localization of the PCY2-6 gene family in these tissues was reminiscent of anionic peroxidases that respond to either wounding or phytochrome and crosslinking extensin precursors. Therefore, it is possible that PnC26, like other anionic peroxidases, plays a role in extensin polymerization, defense reactions against pathogen attack, and response to wounding, but these hypothetical roles of PnC26 still need to be proven. Our next step is to study the localization, extensin polymerization, and involvement of PnC26 in plant responses to wounding.

References

1. Sakuma Y, Azuma T, Kato Y, Kojima Y, Miura K (1996) Cytokinin-Induced mRNA in the suspension cultured cell of poplar (*Populus nigra*). Mokuzai Gakkaishi 42:789–794
2. Østergaard L, Abelskov AK, Mattsson O, Welinder KG (1996) Structure and organ specificity of an axnionic peroxidase from *Arabidopsis thaliana* cell suspension culture. FEBS Lett 398:243–247
3. Teichmann T, Guan C, Kristoffersen P, Muster G, Tietz O, Palme K (1997) Cloning and biochemical characterization of an anionic peroxidase from *Zea mays*. Eur J Biochem 247: 826–832
4. Carpin S, Crèvecoeur M, Greppin H, Penel C (1999) Molecular cloning and tissue-specific expression of an anionic peroxidase in zucchini. Plant Physiol 120:799–810
5. Christensen JH, Bauw G, Welinder KG, Van Montagu M, Boerjan W (1998) Purification and characterization of peroxidases correlated with lignification in poplar xylem. Plant Physiol 118: 125–135
6. Brownleader MD, Ahmed N, Trevan M, Chaplin MF, Dey PM (1995) Purification and partial characterization of tomato extensin peroxidase. Plant Physiol 109:1115–1123
7. Magliano TMA, Casal JJ (1998) In vitro cross-linking of extensin precursors by mustard extra cellular isoforms of peroxidase that respond either to phytochrome or to wounding. J Exp Bot 49:1491–1499
8. Bernards MA, Fleming WD, Llewellyn DB, Priefer R, Yang X (1999) Biochemical characterization of the suberization-associated anionic peroxidase of potato. Plant Physiol 121:135–145
9. Klotz KL, Liu T-TY, Liu L, Lagrimini M (1998) Expression of the tobacco anionic peroxidase gene is tissue-specific and developmentally regulated. Plant Mol Biol 36:509–520
10. Klotz KL, Lagrimini LM (1996) Phytohormone control of the tobacco anionic peroxidase promoter. Plant Mol Biol 31:565–573
11. Tournaire C, Kushnir S, Bauw G, Inzé D, de la Serve BT, Renaudin J-P (1996) A thiol protease and an anionic peroxidase are induced by lowering cytokinins during callus growth in *Petunia*. Plant Physiol 111:159–168
12. Mukai Y, Yamamoto N (1997) The method of RNA isolation (in Japanese). In: Shimamoto K, Sasaki T (eds) The experimental protocol of PCR in plants. Shujunsya, Tokyo, pp 60–62
13. Sugiura M (ed) (1989) Electrophoresis of RNA and Northern hybridization (in Japanese) In: Clonning and sequence. Nosonbunkasya, Tokyo, pp 50–59
14. Gyllensten UB, Erlich HA (1988) Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the *HLA-DQA* locus. Proc Natl Acad Sci USA 85:7652–7656
15. Jhingan AK (1992) A method is described to extract high molecular weight DNA from lyophilized leaf material of several plant species without employing deproteination: DNA produced is readily soluble and pure for different molecular biology applications. Methods Mol Cell Biol 3:185–187
16. Demura T (1997) In situ hybridization (in Japanese). In: Hukuda H (ed) The experimental protocol of observing plant cells. Shujunsha, Tokyo, pp 61–70
17. Von Heijine G (1988) Transcending the impenetrable: how proteins come to term with membranes. Biochim Biophys Acta 947:307–333
18. Harkin JM, Obst JR (1973) Lignification in tree: indication of exclusive peroxidase participation. Science 180:296–298
19. Welinder KG (1985) Plant peroxidases: their primary, secondary and tertiary structures, and relation to cytochrome c peroxidase. Eur J Biochem 151: 450–497
20. Osakabe K, Koyama H, Kawai S, Katayama Y, Morohoshi N (1994) Molecular cloning and the nucleotide sequences of two novel cDNAs that encode anionic peroxidases of *Populus kitakamiensis*. Plant Sci 103:167–175

21. Kawai S, Matsumoto Y, Kajita S, Yamada K, Katayama Y, Morohoshi N (1993) Nucleotide sequence for the genomic DNA encoding an anionic peroxidase gene from a hybrid poplar, *Populus kitakamiensis*. Biosci Biotech Biochem 57:131–133
22. Osakabe K, Koyama H, Kawai S, Katayama Y, Katayama Y, Morohoshi N (1995) Molecular cloning of two tandemly arranged peroxidase genes form *Populus kitakamiensis* and their differential regulation in the stem. Plant Mol Biol 28:677–689
23. Lagrimini LM, Burkhart W, Moyer M, Rothstein S (1987) Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: molecular analysis and tissue-specific expression. Proc Natl Acad Sci USA 84:7542–7546
24. Roberts E, Kolattukudy PE (1989) Molecular cloning, nucleotide sequence, and abcisic acid induction of a suberization-associated highly anionic peroxidase. Mol Gen Genet 217:223–232
25. Mohan R, Kolattukady PE (1990) Differential activation of expression of a suberization-associated anionic peroxidase gene near-isogenic resistant and susceptible tomato lines by elicitors of *Verticillium albo-atrum*. Plant Physiol 92:276–280
26. Robb J, Lee SW, Nazar RN, Mohan R, Kolattukudy PE (1991) Chemical characterization of stress-induced vascular coating. Plant Physiol 97:528–536