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Calmodulin gene family in potato: developmental and touch-induced expression of the mRNA encoding a novel isoform

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Abstract

Eight genomic clones of potato calmodulin (PCM1 to 8) were isolated and characterized. Sequence comparisons of different genes revealed that the deduced amino acid sequence of PCM1 had several unique substitutions, especially in the fourth Ca^{2+}-binding area. The expression patterns of different genes were studied by northern analysis using the 3′-untranslated regions as probes. The expression of PCM1, 5, and 8 was highest in the stolon tip and it decreased during tuber development. The expression of PCM6 did not vary much in the tissues tested, except in the leaves, where the expression was lower; whereas, the expression of PCM4 was very low in all the tissues. The expression of PCM2 and PCM3 was not detected in any of the tissues tested. Among these genes, only PCM1 showed increased expression following touch stimulation. To study the regulation of PCM1, transgenic potato plants carrying the PCM1 promoter fused to the β-glucuronidase (GUS) reporter gene were produced. GUS expression was found to be developmentally regulated and touch-responsive, indicating a positive correlation between the expression of PCM1 and GUS mRNAs. These results suggest that the 5′-flanking region of PCM1 controls developmental and touch-induced expression. X-Gluc staining patterns revealed that GUS localization is high in meristematic tissues such as the stem apex, stolon tip, and vascular regions.

Introduction

Calmodulin, a Ca^{2+}-binding, multifunctional, regulatory protein is known to play a pivotal role in Ca^{2+} signaling in eukaryotes. Calmodulin is a highly conserved protein and upon binding to Ca^{2+}, it interacts with other proteins in the cell and regulates their activity [9, 16, 21]. The activity of several enzymes such as NAD kinase, Ca^{2+}-ATPase, and protein kinase are known to be regulated by calmodulin in plants [17, 18, 22].

Multiple calmodulin genes have been reported in *Arabidopsis* and some of them are differentially expressed during development [6]. Calmodulin

isoforms have also been isolated from other plants such as petunia and rice [18]. Plant calmodulin genes have conserved features with two exons interrupted by a single intron at the 26th codon (glycine) [14]. Southern blot analysis has indicated that divergent calmodulin isoforms are coded by different genes on the genome [2, 6]. The calmodulin mRNAs are known to be developmentally regulated and signal-responsive [8, 28]. Nuclear run-on assay of calmodulin isoforms in *Arabidopsis* suggested that calmodulin gene expression is controlled at the transcriptional level [14]. The expression of calmodulin mRNA is induced by mechanical perturbations such as touching or wounding, suggesting their involvement in thigmomorphogenesis in plants [3]. However, the molecular mechanisms controlling touch-inducible expression of calmodulin genes have not been determined.

Ca^{2+} and calmodulin are implicated in the control of potato tuber development [1]. We have previously isolated the potato calmodulin cDNA clone (pPCM-1) from the stolon tip and showed that its expression is developmentally regulated [8]. Potato calmodulin mRNA is highly expressed in the stolon tip, and its expression decreases during tuber development. To study the genomic organization and expression of calmodulin genes, we have characterized different calmodulin clones from potato. Furthermore, regulation of developmental and touch-induced calmodulin gene expression was studied using transgenic plants carrying the promoter of the calmodulin isoform (PCM1) fused to the GUS reporter gene.

Materials and methods

Plant material

Potato (*Solanum tuberosum* L. cv. Russet Burbank and FL1607) plants were grown in the greenhouse around 25 °C (day) and 18 °C (night). *In vitro* grown plants were propagated by culturing single-node stem cuttings in MS medium supplemented with 2% sucrose, 100 mg/l myo-inositol, and 0.4 mg/l thiamine-HCl. Expanded leaves of about 3-week-old plants grown in the greenhouse were used to study touch response. Each leaf was gently touched between two fingers from the top and bottom for 10 s. At various times, the leaves were harvested and frozen in liquid nitrogen and stored at −70 °C until needed for DNA or RNA extraction.

Genomic Southern analysis

Potato (Russet Burbank) DNA was digested with different restriction enzymes and transferred onto a nylon membrane. The membrane was hybridized at 42 °C with ^{32}P-labeled calmodulin cDNA [8] in a solution containing 50% formamide, 6 × SSC, 2 × Denhardt's solution, 0.1% w/v SDS, and 100 μg/ml herring sperm DNA. The membrane was washed at 60 °C in 0.5 × SSC and 0.1% w/v SDS, and exposed to Kodak XAR-5 film.

Screening of potato genomic library

A potato genomic library was constructed by partial digestion of potato (Russet Burbank) DNA with *Mbo* I and ligation into λ EMBL3 vector. Plaque lifting and hybridization were performed according to standard protocols [24]. Approximately 3×10^5 plaques were screened using the ^{32}P-labeled potato calmodulin cDNA as a probe. Hybridization was performed at 60 °C in a solution containing 3 × SSC, 10 × Denhardt's solution, 0.1% w/v SDS, and 100 μg/ml herring sperm DNA. Following hybridization, the membranes were washed in 0.5 × SSC and 0.1% w/v SDS at 50 °C, and exposed to Kodak XAR-5 film. After three rounds of plaque purification, isolated clones were subjected to restriction analysis and subsequent Southern hybridization. The nucleotide sequences were determined by the dideoxynucleotide chain-termination method.

Amplification of cDNA by PCR

Total RNA was prepared from the stolon tip of 1.5-month-old Russet Burbank potato according to Verwoerd *et al.* [25], and poly(A)$^+$ RNA was

isolated by oligo (dT)-cellulose column chromato-graphy. The cDNA library was constructed in the λZAP vector (Stratagene) using a Pharmacia cDNA synthesis kit according to the manufac-turer's instructions. The cDNA was amplified by PCR using *Taq* polymerase and the reaction buffer from Promega. About 1×10^7 pfu of the amplified phage lysate was used for the PCR re-action with the bacteriophage T7 vector primer, and the gene specific primer for the 3'-untrans-lated region of the calmodulin gene. The PCR was performed using a program of 95 °C for 30 s, 42 °C for 60 s, and 72 °C for 60 s for 35 cycles.

Northern blot analysis

A 5–10 μg portion of total RNA prepared from various potato tissues [25] was denatured by gly-oxal and DMSO, and electrophoresed in an aga-rose gel. The RNAs were stained with 2 mg/l ethidium bromide and then transferred onto a nylon membrane. DNA fragments around the 3'-untranslated region of the genomic clones were subcloned into the pBluescript-KS$^+$ plasmid. The probes were created by digesting these sub-clones with the appropriate restriction enzymes (*Afl* II for PCM1, *Msc* I for PCM2-8) and using them as templates for transcription with α-[^{32}P]-UTP by T3 or T7 RNA polymerase.

Hybridization was performed at 60 °C in a so-lution containing 50% formamide, 6 × SSPE, 0.5% w/v SDS, 5 × Denhardt's solution, 100 μg/ml herring sperm DNA, and $> 10^9$ cpm/μg of ^{32}P-labeled RNA probe. Following hybridization, the membrane was washed with 0.1 × SSC and 0.1% w/v SDS at 65 °C, and exposed to Kodak XAR-5 film. To reduce non-specific binding, the membrane was treated with 2 × SSC containing 10 μg/ml RNase A at 37 °C for up to 1 h.

Construction of PCM1 promoter-GUS fusion and production of transgenic potato plants

A 6 kb *Hind* III fragment containing a putative PCM1 promoter and 49 bp of the coding region

was fused in frame to the GUS gene in the pBI101.3 binary vector (Clontech). Potato FL1607 was transformed with this construct by co-cultivation of leaf strips with *Agrobacterium tumefaciens* LBA4404 according to Wenzler *et al.* [27]. A 560 bp *Eco* RV/*Hind* III fragment con-taining the N-terminal sequence of the GUS-coding region of pBI101.3 was used for making the antisense RNA probe for northern blot analy-sis.

Histochemical localization

The histochemical assay for GUS was performed using free hand sections of different tissues. The sections were incubated overnight in a solution containing 100 mM sodium phosphate, pH 7.0, 1 mM EDTA and 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-Gluc) [7].

Results and discussion

Isolation and characterization of calmodulin genomic clones

Genomic Southern analysis of potato genomic DNA suggested the presence of a family of calm-odulin genes (Fig. 1A). DNA from diploid potato showed similar patterns as compared to the tet-raploid Russet Burbank (data not shown). Eight genomic clones (PCM1 through 8) of calmodulin genes were isolated from the potato λEMBL3 library by screening with a cDNA [8] as a probe. These clones were characterized by restriction mapping and DNA sequencing. Figure 1B shows the genomic structures of potato calmodulin genes. Nucleotide sequence analysis revealed that potato calmodulin clones PCM1, 5, 7 and 8 have two exons and one intron similar to other plant calmodulin genes [14, 26]. These exons are inter-rupted by a single intron of 1.5 to 2.7 kb in length at the conserved position of the 26th amino acid (glycine). The clones carrying PCM2, 3, 4 and 6 did not contain the first exon. The DNA sequence of the first exon of PCM6 was obtained by se-
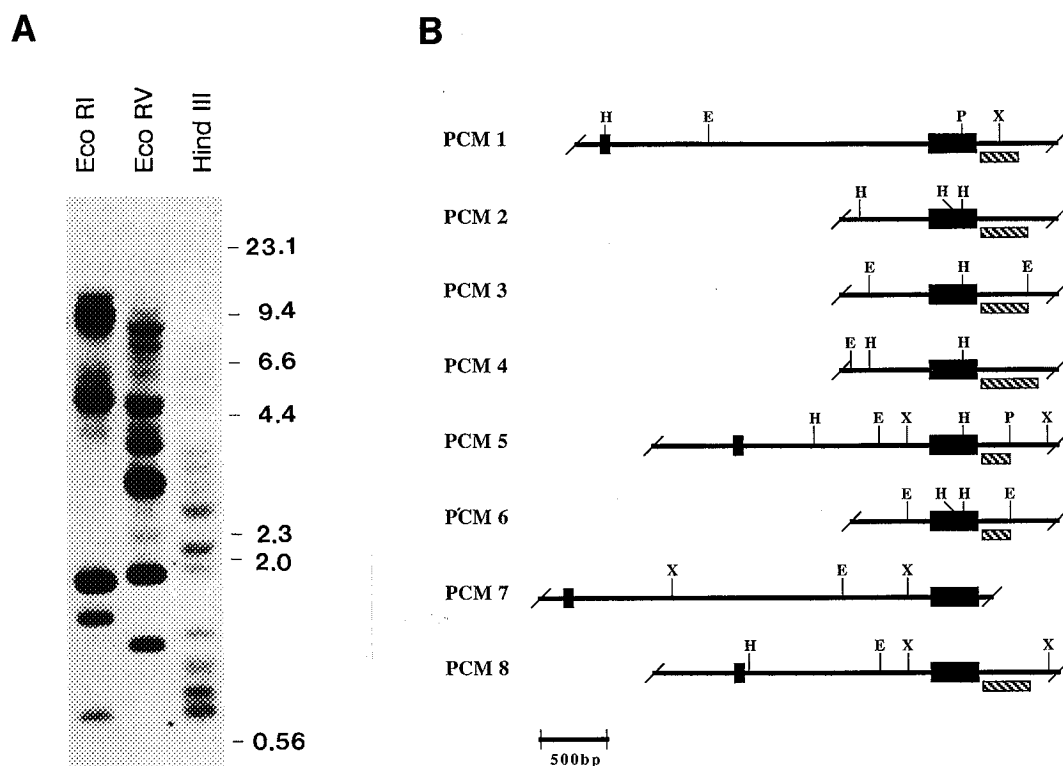
696

A

B

Fig. 1. A. Southern analysis of potato calmodulins. Five μg of potato DNA was digested with restriction enzymes and analyzed. The size of standard markers is shown in kb. B. Genomic structures of calmodulin genes (PCM1–8). Restriction sites are indicated as follows: E, *Eco* RI; H, *Hind* III; P, *Pst* I; X, *Xba* I. Coding regions are marked with dark, thick bands. Hatched areas at the 3′ end indicate the regions used for making riboprobes.

quencing cDNA which was obtained from the cDNA library by PCR. Considering the potential misincorporations during PCR amplification by *Taq* polymerase, three independent PCR products were cloned and sequenced. The nucleotide sequences of these clones were identical.

Nucleotide sequences within the coding region of these genes were compared (Fig. 2). These genes share a high degree of similarity with each other, suggesting that these genes belong to a calmodulin gene family. Despite the high nucleotide sequence identity within the coding region, the introns and the putative 5′- and 3′-untranslated regions have highly diverse sequences (data not shown). Figure 3 shows comparisons of the deduced amino acid sequences of these genes. The amino acid sequences of PCM5, 6, 7 and 8 are identical to each other and very similar to *Arabidopsis* ACaM-2, barley CaM-1; whereas,

PCM1 has unique amino acid substitutions, especially in the fourth Ca^{2+}-binding area. Interestingly, the amino acid sequences of the fourth Ca^{2+}-binding area revealed that both chick and PCM1 are identical (Fig. 3).

Expression of calmodulin isoforms

We have previously shown that calmodulin gene expression in potato is developmentally regulated using the whole PCM1 cDNA insert as a probe [8]. To study the expression of mRNA of each calmodulin isoform, northern blot analysis was carried out using total RNA from various tissues of potato plants. For northern analysis, the highly diverse 3′-untranslated regions were used as probes. Figure 4 shows that the mRNAs of calmodulin isoforms (PCM1, 5, 6 and 8) are differen-

```
PCM1 ATGGCAGAGCAGCTGACGGAGGAGCAGATCGCCGAGTTCAAGGAAGCTTTTAGCCTTTTC -60
PCM2
PCM3
PCM4
PCM5 --------T-----C--C--A--T------T-T-----------G-----C-----A---
PCM6 ----G--T-----C--C--A--T------T-T-------------C--C--T-----T
PCM7 --------T-----C--C--A--T------T-T-------------C--C--------T
PCM8 --------T-----C--C--A--C------T-T-----------G-----C-----A---

PCM1 GACAAGGATGGCGATGGCTGTATTACTACCAAGGAGTTGGGAACAGTGATGAGATCACTT -120
PCM2           --T--C--C--C--T------C-T-----C------C-T--GT-G
PCM3           --T--C--C--C--T-----AC-T-----C------C-T--GT-G
PCM4           --T--C--C--C--T------C-T-----T------C-T--GT-G
PCM5 --------C--A-----T--C-----A--T------C-T--G--T--------G--GT-G
PCM6 --------C--A-----T-----C--A--T-----AC-T-----T--A---C-G---T-G
PCM7 -----A--C--A-----T--C--C--C--T--A--C-T-----T------C-T--GT-G
PCM8 --------C--A-----T--C--C--C--T------C-T-----T------C-T--GT-G

PCM1 GGTCAGAATCCCACTGAAGCTGAACTACAGGATATGATCAGTGAAGCTGATGCTGATCAG -180
PCM2 --A--A-----A-----G-----G--T--A--C-------A-----T----------GGA
PCM3 --A--A-----A-----G-----G--T--A--C-------A-----T----------GGA
PCM4 --A--A-----A-----G-----G--T--A--C-------A-----T----------GGA
PCM5 --A-----C--A-----------G--C-----C-----A-A-----TG---------GGT
PCM6 --G-----C--A-----G-----G--T--A--C-------A-----T----------GGA
PCM7 --A--A-----A-----G-----G--T--A--C-------A-----T----------GGA
PCM8 --A--A-----A-----G-----G--T--A--C-------A-----T----------GGA

PCM1 AATGGAACCATTGATTTTCCAGAGTTCTTGAATCTGATGGCACGTAAGATGAAGGACACT -240
PCM2 ---------------C--C--C------C-T--CT------T--G---------A------
PCM3 ---------------C--C--C------C-T--CT-------T--G----A--A------
PCM4 ---------------C--C--C------C-T----------T--G--------A------
PCM5 -----------C--C--C--------T-----C--C-----GA-G----------T--A
PCM6 -----G--------------------A--C--------T--C---------------
PCM7 ---------------C--C--C------C----CT------T--G--------A------
PCM8 ---------------C--C--C------C-T--CT------T--G--------A------

PCM1 GATTCTGAGGAGGAACTCAAAGAGGCTTTCAAGGTTTTCGATAAAGATCAGAATGGCTTT -300
PCM2 ---------------G-----G--A-------GA--G--T--C--G-----------A--C
PCM3 ----T----------G-----G--A-------GA--G--T--C--G------G------A--C
PCM4 --C-----A-----G-----G--A-------GA--G--T--C--G-----------A--C
PCM5 --C--A--------G--G--G-----A----GA--G--T--C--G--C--------G--C
PCM6 ---------------G-----G--A-------GA--G--T--C--G-----------A--C
PCM7 ---------------G-----G--A-------GA--G--T--C--G-----------A--C
PCM8 ---------------G-----G--A-------GA--G--T--C--G-----------A--C

PCM1 ATTTCTGCAGCTGAGCTTCGTCATGTAATGACAAACCTTGGAGAGAAGCTGACTGATGAA -360
PCM2 ----------------A--C-----C-----T-----A--C--------T--A------
PCM3 ----------------A-CC----C----------A--C--------T--A------
PCM4 ----------------A--C-----C----------A--C-----A--T--A------
PCM5 --C-----T--------C---------------T--------T--------T---------
PCM6 -----C----------------------C----------A--C--A-----T---------
PCM7 ---------------A--A--C-----C----------A--C--------T--A------
PCM8 ----------------A--C-----C-----T--------T--------T---------

PCM1 GAGGTGGATGAGATGATCCGAGAGGCAGATATTGATGGTGATGGGCAAGTTAATTATGAG -420
PCM2 -----T--C--------T--T--A--T--CG-G-----------A--GA-C--C-----C
PCM3 -----T--C-----A--T--T--A--T--CG-G---T-------A--GA-C--C-----C
PCM4 -----T--C--------T--T--A--T--CG-G-----------A--GA-C--C-----C
PCM5 --A--T-----A-------A-G-----T---G-------C-----A---A----C--C--T
PCM6 -----T------------T--T--A--T---G-G------C--------GA-C--C--C--C
PCM7 -----T--C--------T--T--A--T---G-G-----------A--GA-C--C-----C
PCM8 --A--T-----A------A-G-----T---G-C-----C-----A---A----C--C--T

PCM1 GAGTTTGTCCGTATGATGCTTGCCAAGTGA -450
PCM2 -----C---AAGG-C---A-G---------
PCM3 -----C---AAGG-C---A-G---------
PCM4 ---------AAGG-C---A-G---------
PCM5 --------TAAGG-C---A-G---------
PCM6 -----C---AAGG-T---A-G---------
PCM7 -----C---AAGG-C---A-G---------
PCM8 --------TAAGG-C---A-G---------
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Fig. 2. Nucleotide sequence comparison of potato calmodulin genes (PCM1–8). Arrow indicates the position of intron.

tially expressed in various organs during development. The expression of PCM1 mRNA was highest in the stolon tip, high in stems, moderate in roots, and very low in leaves. During tuber development, the expression of PCM1 mRNA

was significantly reduced. The expression patterns of PCM5 and 8 show some similarity to PCM1. In contrast to these isoforms, PCM6 showed nearly steady-state expression in all of the tissues tested, except in the leaves where the

```
                                * * * * *  *
PCM1          AEQLTEEQIAEFKEAFSLFDKDGDGCITTKELGTVMRSL
PCM5          -D----D--S-----------------------------
PCM6          -D----D--S-----------------------------
PCM7          -D----D--S-----------------------------
PCM8          -D----D--S-----------------------------
Arabidopsis   -D----D--S-----------------------------
Barley        -D----D--------------------------------
Chick         -D----------------------T--------------


                                * * * * *  *
PCM1          GQNPTEAELQDMISEADADQNGTIDFPEFLNLMARK
PCM5          --------------N-V---G---------------
PCM6          --------------N-V---G---------------
PCM7          --------------N-V---G---------------
PCM8          --------------N-V---G---------------
Arabidopsis   --------------N-V---G---------------
Barley        --------------N-V---G---------------
Chick         --------------N-V---G---------TM----


                                * * * * *  *
PCM1          MKDTDSEEELKEAFKVFDKDQNGFISAAELRHVMTNL
PCM5          ---------------R--------------------
PCM6          ---------------R--------------------
PCM7          ---------------R--------------------
PCM8          ---------------R--------------------
Arabidopsis   ---------------R--------------------
Barley        ---------------R--------------------
Chick         ---------IR---R-----G--Y------------


                                * * * * *  *
PCM1          GEKLTDEEVDEMIREADIDGDGQVNYEEFVRMMLAK
PCM5          ------------------V-----I--D---KV-M--
PCM6          ------------------V-----I--D---KV-M--
PCM7          ------------------V-----I--D---KV-M--
PCM8          ------------------V-----I--D---KV-M--
Arabidopsis   ---------------K---V-----I--D---KV-M--
Barley        ------------------V-----I--D---KV-M--
Chick         ---------------------------------Q--T--
```

Fig. 3. A comparison of the deduced amino acid sequences of potato calmodulin genes with calmodulins from other sources. PCM1, 5, 6, 7, 8, *Arabidopsis* ACaM-2 [13], barley [12], and chicken [20] calmodulins are shown. Asterisks indicate the position of amino acids involved in Ca^{2+} binding.

Fig. 4. Northern blot analysis of total RNA from different tissues of potato plants using 3′-untranslated region of calmodulin isoforms (PCM1, 5, 6, and 8) as probes. Each lane contain 5 μg of RNA isolated from different tissues indicated as follows. L (leaf), S (stem), R (root), St (stolon tip), Dt (developing tuber), T (tuber). The autoradiographs of PCM5 and 8 were exposed longer than the autoradiographs of PCM1 and 6. The ethidium bromide-stained gel shows two ribosomal RNA bands (25S and 18S).

expression was lower. The ethidium bromide-stained gels verified that similar amounts of RNA were loaded per lane. To confirm that the probe used for northern blot analysis was gene specific, the 3′-untranslated region of each clone was tested for cross-hybridization. Among all the PCM clones tested, only the 3′ region of PCM5 and 8 cross-hybridized. The restriction pattern has some similarity as well (Fig. 1B). However, the amount of cross-hybridization was low after the blot was treated with RNase A. Because the level of PCM4 mRNA was very low in all the tissues, it was not studied further. PCM1, 4, 5, 6 and 8 have similar transcript sizes as previously shown when the calmodulin coding region was used as a probe [8]. The expression of PCM2 and PCM3 mRNA was undetectable in all of the organs tested. However, PCM2 and PCM3 could
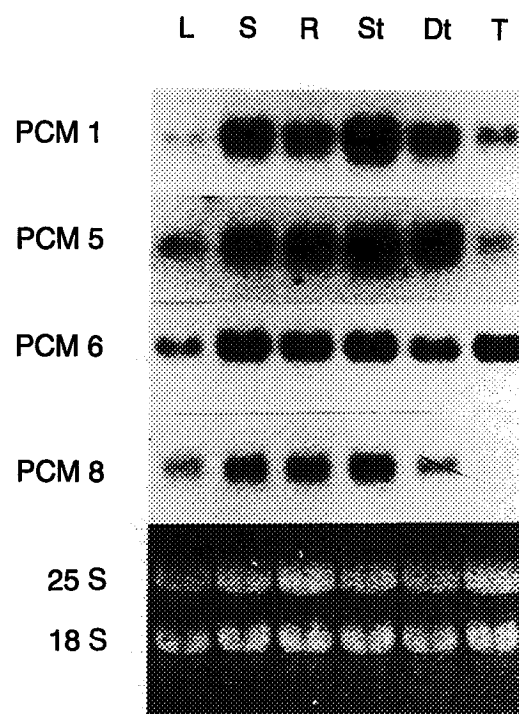
be expressed in the specialized tissues or organs such as the germinating pollen or flower. These organs were not tested in this investigation. It is possible that these isoforms may respond to other signals. The possibility that PCM2 and PCM3 are pseudogenes cannot be ruled out. The expression of PCM7 was not investigated since it contained a very short 3′ region (Fig. 1B). Therefore, the expression of PCM1, 5, 6, and 8 were further studied.

Touching is known to increase the mRNA expression of at least three different calmodulin isoforms in *Arabidopsis* [14]. We also studied touch-induced changes in mRNA accumulation of potato calmodulin isoforms by northern analysis. Figure 5 shows that touching of potato leaves re-
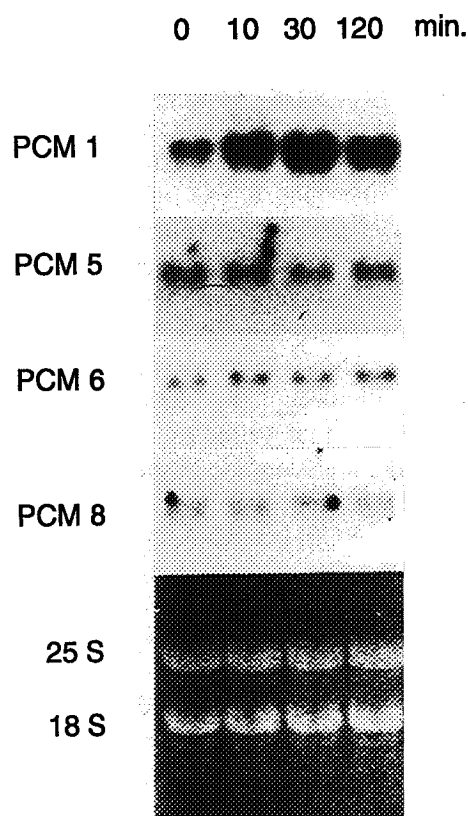
0 10 30 120 min.

PCM 1

PCM 5

PCM 6

PCM 8

25 S

18 S

Fig. 5. Northern analysis showing touch-induced expression of calmodulin isoforms (PCM1, 5, 6, and 8). The leaves were stimulated by repeated touching for 10 s and harvested at the indicated times. The ethidium bromide-stained gel shows two ribosomal RNA bands (25S and 18S).

A
```
  1   TTAGCGATGCTTTCAATGTGGTATTGATTATTGAAAAAAAGAAGAAGAGG
 51   GAAACATTGAAAGCACCCCTAAACTCGGTGCACACTATTAATTTCATCTC
101   TGAATTATTGATAGCCTTGAAAGACACTCTTTACTGACTAACTGAACTTA
151   TATACACCTTCGATCTTAAAACATAATTGAAATACACCCCTAAATTATTG
201   TCGCCAATTGAGGGTGTTTTCAACACTTTTCTCGAGTTTTTATTAAGAGT
251   TTCATGCTCCTACTAAAATTTGAGATAACTTGTTATGTCATGTGACAAAC
301   TGAGTGTATATCTAAGTTTAGTTAGTCTAACATAATAGTATTTTTAAAGT
351   TGTCAATAGTTCAGACACTAACCTAATAATTTGCGTCAAATTTAAAAGTG
401   TTTCACTATTTCTCTTATATTTCTTCGTATTATTTATTTTAGTAAATTTT
451   CAAATTTAAAAGACAAAAAGAAAAAAGTAAGCGCTCCTCGAATTAAGGGC
501   CCATTTATGTGTGGATATTTACCGTACGATATTTCCACATTATTTCGAGA
551   AGTCCTCTTTTATCGGCGAGTCAAGATATTTCTACTCGTTTTCCCCTTCT
601   TTAAATACCGTCTTATGTGTCTAAACGAAATCAAAAGCTAAAACTCGGGC
651   ATAGGGTTCTTACAGTTCGATCGAAGAAATTGTTAAAAAAAAAGAGAGGA
701   AAAAAA ATG GCA GAG CAG CTG ACG GAG GAG CAG ATC GCC
751   GAG TTC AAG GAA GCT T
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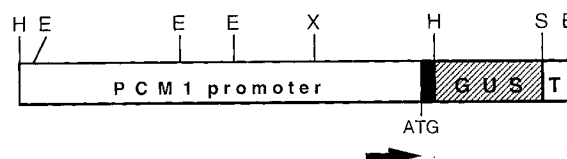
B

Fig. 6. A. Nucleotide sequence of the PCM1 promoter and the N-terminal coding region. The putative TATA box is double underlined and the start codon is boxed. The *Hin*d III site (AAGCTT) that was used for making the translational fusion to GUS is bolded and underlined. B. Construct used in the production of transgenic plants carrying the GUS reporter gene fused to the PCM1 promoter. The black box indicates the 49 bp coding region of PCM1 including the start codon (ATG). The terminator of nopaline synthase is designated as T. The arrow indicates the region around the nucleotide sequence shown in A. Restriction sites are indicated as follows: E, *Eco* RI; H, *Hin*d III; S, *Sst* I; X, *Xba* I.

sulted in a rapid increase in PCM1 expression. The amount of PCM1 mRNA increased about 5-fold 30 min after touching. After 120 min, there was an obvious decline in PCM1 mRNA. In contrast to PCM1, the expression of PCM5, 6 and 8 were unaffected by touch.

Expression of PCM1-GUS fusion in transgenic plants

To study the regulation of developmental and touch-induced expression of PCM1, the 5′-flanking region of PCM1 was isolated and sequenced. Figure 6A shows part of the nucleotide sequence of the 5′-flanking region of PCM1, which contains the putative promoter elements.

Sequence analysis of the PCM1 promoter revealed that the putative TATA box (TAAATA), similar to the sequence found in *Arabidopsis* ACaM-3 [14] promoter, is present at the −105 position from the translation start site.

To test whether the PCM1 promoter is responsible for its expression during plant development and signal response, a chimeric PCM1 promoter-GUS gene was constructed and its expression in transgenic plants was analyzed. A 6 kb *Hin*d III fragment containing a putative promoter and a

C   L   S   R   Dt   T
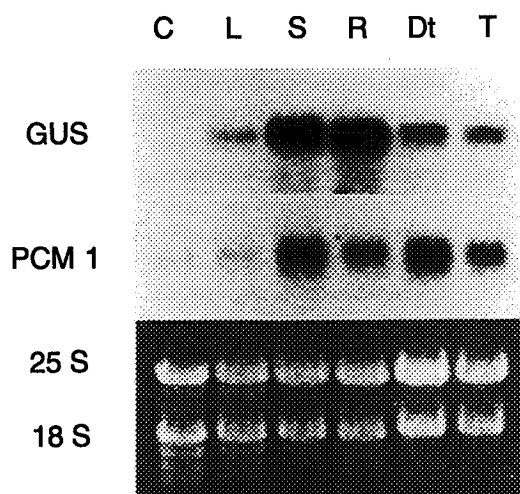
GUS

PCM 1

25 S

18 S

Fig. 7. Northern analysis showing the expression of the GUS reporter gene and PCM1 in different parts of transgenic potato plants. C (leaf tissues from untransformed control), L (leaf), S (stem), R (root), Dt (developing tuber) and T (tuber). The ethidium bromide-stained gel shows two ribosomal RNA bands (25S and 18S).

49 bp coding region of PCM1 was fused in frame to the GUS reporter gene (Fig. 6B) and transgenic potato plants carrying this construct were produced. To study GUS gene expression in transgenic plants, northern blot analysis was carried out using total RNA from different tissues. Figure 7 shows the expression of the GUS reporter and PCM1 mRNA in transgenic plants. The mRNA expression of GUS driven by the PCM1 promoter and endogenous PCM1 was positively correlated, except for the expression of GUS mRNA which was higher in the root and slightly lower in developing tuber. Although the reason has not been addressed yet, the higher accumulation of GUS mRNA could be because GUS mRNA has different stability from PCM1 in roots and the developing tuber. The general positive correlation between GUS and PCM1 expression in transgenic plants implies that the differential accumulation of PCM1 mRNA in different organs is a consequence of differential transcription of the PCM1 gene. This suggests that the main transcriptional determinants for PCM1 mRNA expression lies within the 6 kb *Hin*d III fragment tested in those experiments.

Localization of GUS was studied by X-gluc staining using various cross and longitudinal sections of potato organs (Fig. 8). X-gluc staining was localized primarily in the meristematic region of the shoot tip (Fig. 8, top left), the tip of the developing tuber (Fig. 8, top right), and the vascular zones of petiole and tuber (Fig. 8, bottom left and right). We did not detect a significant amount of GUS staining in mesophyll cells and young leaves (Fig. 8, top left). The expression of calmodulin mRNA is known to be correlated with active cell division [15]. Immunolocalization studies indicate that calmodulins are highly expressed in the meristematic tissues of the root tip [5], implying their involvement in cell division. Furthermore, PCM1 mRNA is also highly expressed in the tips of dark-grown sprouts (data not shown). However, it should be noted that the relative degrees of vacuolation of the different cell types affects GUS staining.

Touch-induced expression of GUS mRNA was also studied using these transgenic plants. Figure 9 shows that both GUS and PCM1 mRNA increased 10 min after touch stimulation, peaked around 30 min, declined after 60 min, and reached a steady state thereafter. Similar kinetics of GUS and PCM1 mRNA accumulation suggest an important role for the PCM1 promoter in the regulation of touch-induced gene expression. It is necessary to perform nuclear run-on assay and RNA analysis using the inhibitor for transcription to determine the transcription rate of the PCM1 during touch induction. Touch-induced changes in calmodulin gene expression suggest that calmodulin could play an important role in transducing external signals in plants. Touch stimulus is known to induce a transient increase in cytosolic Ca^{2+} concentration [10, 11]. The elevated Ca^{2+} concentration in turn alters the expression of calmodulin-like TCH genes in *Arabidopsis* [4]. However, it is not clear how cytosolic Ca^{2+} modulates the expression of TCH genes.

It is likely that the isoforms that showed different mRNA expression patterns may have altered promoter elements. For instance, PCM5 and 8 are not touch inducible, but they show

Fig. 8. Histochemical localization of PCM1 promoter activity. GUS activity in transgenic potato plants (FL1607) carrying the calmodulin promoter and GUS-fusion. Longitudinal sections through the stem apex and a developing tuber from a transgenic plant were assayed for GUS activity. Stem apex, 11 × (top left); stolon tip initiating tuber formation, 26 × (top right); cross section of young petiole, 26 × (bottom left); apical end of developing tuber, 26 × (bottom right).

expression patterns similar to PCM1 during development. Differential expression, but not touch-

inducible expression of PCM5 and 8 implies the presence of two independent mechanisms con-

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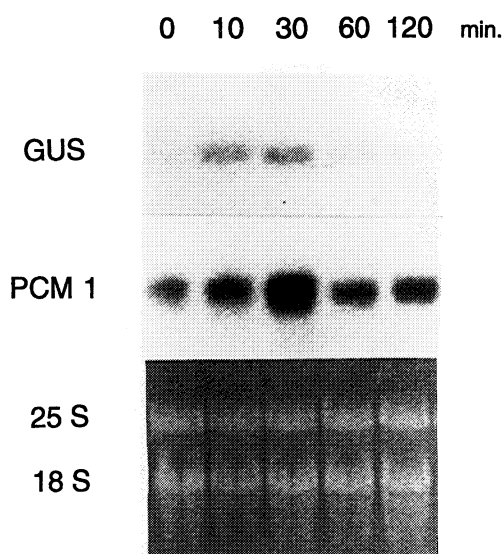
Fig. 9. Northern analysis showing touch-induced expression of the GUS reporter gene and PCM1 in leaves of transgenic plants. Samples were collected at 0 to 120 min after touch stimulation. The ethidium bromide-stained gel shows two ribosomal RNA bands (25S and 18S).

trolling calmodulin gene expression. Promoters of these calmodulins may be differentially regulated by a similar mechanism to PCM1, but they may lack the cis-elements required for touch induction. The identification of cis-elements and trans-acting factors is critical to the understanding of the molecular mechanisms that control calmodulin gene expression.

PCM1, which has unique amino acid substitutions as compared to other isoforms, may play an important role in plant growth and development and signal transduction. To study the role of PCM1 in growth and development, we produced transgenic potato plants expressing varying levels of PCM1 [19]. These plants show striking differences in growth and development, which further suggests that PCM1 plays an important role in plant growth and development.

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