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Molecular cloning and sequencing of a cDNA for plant calmodulin: Signal-induced changes in the expression of calmodulin

(auxin/calcium/light/signal transduction/tuberization)

P. K. JENA, A. S. N. REDDY, AND B. W. POOVAIAH

Laboratory of Plant Molecular Biology and Physiology, Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164-6414

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ABSTRACT **A cDNA clone (pPCM-1) for plant calmodulin was isolated by screening a potato stolon tip cDNA library with a chicken calmodulin cDNA. Nucleotide sequence analysis of pPCM-1 revealed that it contained 80 base pairs of 5′ untranslated region, the entire coding region, and 376 base pairs of 3′ untranslated region. Comparison of the nucleotide sequence of coding regions of potato and chicken calmodulin mRNA showed 78% homology. Comparison of the predicted amino acid sequence of potato calmodulin with other known calmodulin sequences indicated a high degree of homology with a few exceptions. Three changes in the amino acid sequence were found to be unique to the potato calmodulin sequence. In our earlier studies we showed the involvement of calcium and calmodulin in potato tuberization. The pPCM-1 clone was used as a probe to study the expression of calmodulin mRNA during tuberization and to monitor calmodulin mRNA level in various parts of the potato plant. Stolon tips showed the highest levels of calmodulin mRNA, suggesting a role for calmodulin in the tuberization process. In addition, pPCM-1 was used to investigate the effect of auxin and light on calmodulin gene expression in auxin-responsive strawberry fruit and light-responsive Merit corn roots, respectively. Both auxin and light signals were found to increase the level of mRNA for calmodulin. These results suggest that the altered calmodulin gene expression could be one of the molecular events involved in the signal transduction process in plants.**

Signals such as hormones, light, and gravity control diverse physiological processes in plants (1, 2). However, the biochemical and molecular events involved in the transduction of these signals are not clearly understood. Investigations from several laboratories suggest that calcium acts as a messenger in signal transduction (3–6). As in animals, many of the effects of calcium ions in plant cells are mediated by a calcium-binding regulatory protein, calmodulin (7). Calmodulin is a highly conserved, heat-stable, acidic protein with four calcium-binding domains (4, 7). Calmodulin binds to calcium, undergoes a conformational change, and then interacts with enzyme molecules. The Ca^{2+}–calmodulin complex can regulate enzyme activities either directly or indirectly through protein phosphorylation by activating protein kinases (4, 7–9). In plants, enzymes such as NAD kinase, Ca^{2+}-ATPase, H^+-ATPase, quinate:NAD^+ oxidoreductase, and protein kinases have been shown to be regulated by calcium and calmodulin (4, 7).

Calmodulin gene structure and its expression have been well studied in animals (10–13). In animal cells, intracellular levels of calmodulin were shown to be unaffected by a number of steroid and peptide hormones (14). However, calmodulin levels were found to be elevated in transformed cells and also at the G_1/S boundary of the growth cycle of mammalian cells (15–18). In these cases, the changes in calmodulin were found to be accompanied or preceded by changes in the level of its mRNA (16, 18). Although calmodulin protein has been isolated and characterized in plants (4, 7), the gene sequence, structure, and regulation of its expression are unknown. To understand calmodulin gene expression in plants, we isolated and sequenced a cDNA for plant calmodulin.* Potato calmodulin cDNA was used as a probe to study the expression of calmodulin mRNA during tuberization and to monitor calmodulin mRNA level in various parts of a potato plant. Since calmodulin is known to play a vital role in the signal transduction process, we investigated the effect of auxin and light signals on the expression of calmodulin. Strawberry fruit, which requires auxin for its growth, and Merit corn roots, which show light-dependent gravitropism, were used to study whether or not calmodulin gene expression is affected by auxin and light signals.

MATERIALS AND METHODS

Materials. Potato plants (*Solanum tuberosum* L. var. Russet Burbank) were grown in the greenhouse at 24°C (day) and 18°C (night) as described (19). Different parts of the potato plant (roots, stem, stolon tips, small developing tubers, and stolons) were harvested from tuberizing plants, frozen in liquid nitrogen, and stored at −70°C until use. Strawberry plants (*Fragaria ananassa* Duch. cv. Ozark beauty) were grown in the greenhouse as described (20). Fully opened flowers were pollinated and the age of the fruit was determined from the day of pollination. Corn (*Zea mays* L., cv. Merit) seeds were germinated in the dark for 48 hr (21). Chicken calmodulin cDNA clone, pGM-CaMC, was kindly provided by Anthony Means (Baylor College of Medicine). The cDNA synthesis kit was purchased from Boehringer Mannheim. The oligolabeling kit was obtained from Pharmacia.

Isolation of Poly(A)$^+$ RNA. Total nucleic acids were isolated according to Murray and Thompson (22) with modifications. The tissue was ground to fine powder in the presence of liquid nitrogen. Then 2% (wt/vol) 2-mercaptoethanol and boiling 2× extraction buffer [2% (wt/vol) hexadecyltrimethylammonium bromide/100 mM Tris·HCl, pH 8.0/20 mM EDTA/1.4 M NaCl/1% polyvinylpyrrolidone] were added and incubated in a water bath at 55°C. An equal volume of chloroform/isoamyl alcohol, 24:1 (vol/vol), was added and incubated for an additional 20 min. To the aqueous phase 0.1 vol of 10% (wt/vol) hexadecyltrimethylammonium bromide containing 0.7 M NaCl was added and the aqueous phase was reextracted with chloroform/isoamyl alcohol. Total nucleic acids were precipitated by adding 2 vol of 95% (vol/vol) ice-cold ethanol and kept overnight at −20°C. The nucleic acid pellet was collected, dried, and dissolved in diethyl

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*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (accession no. J04559).

pyrocarbonate-treated water. Four volumes of ice-cold 4 M LiCl was added to the nucleic acid solution and kept on ice overnight. Total RNA was collected, washed with 2 M LiCl, dissolved in diethyl pyrocarbonate-treated water, and ethanol precipitated. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose column essentially as described (23).

Construction and Screening of cDNA Library. Poly(A)$^+$ RNA from potato stolon tips was used as a template for synthesis of double-stranded cDNA. cDNA was synthesized according to the method of Gubler and Hoffman (24), blunt-ended using T4 polymerase (25), and cloned into *Eco*RI site of λgt10 by the procedure of Huynh *et al.* (26), except that excess linkers were separated by centrifugation through a Centricon. Recombinant λ phage DNA was packaged *in vitro* using Stratagene *in vitro*-packaging extract (Gigapack Plus) according to the manufacturer's instructions and plated on *Escherichia coli* C600 hfl$^-$. The cDNA library was screened according to Maniatis *et al.* (27). The filters were washed for three 10-min periods in 2× SSC/0.1% SDS at room temperature (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). The final wash was performed at 55°C in 0.5× SSC/0.1% SDS at 55°C for 1 hr.

RNA Blot Analysis. Poly(A)$^+$ RNA or total RNA was separated by electrophoresis on formaldehyde-containing agarose gels and blotted onto nitrocellulose filters (27). The immobilized RNA was hybridized to labeled calmodulin cDNA (pPCM-1). The hybridization was performed at 42°C in formamide-containing buffer for 24 hr (27). The washing conditions were the same as described above under the library screening section, unless indicated otherwise.

Preparation of Labeled Probes. cDNA inserts were isolated and labeled according to Feinberg and Vogelstein (28) using a Pharmacia oligolabeling kit.

DNA Sequencing and Analysis. Both strands of the cDNA insert from pPCM-1 were sequenced using the Sanger dideoxynucleotide chain-termination method (29). Computer software developed by the University of Wisconsin Genetics Group was used for sequence analysis.

RESULTS

Isolation of Potato Calmodulin cDNA Clones Using 270-Base-Pair Chicken Calmodulin cDNA as a Probe. To understand the possible role of calmodulin in the tuberization process, we isolated a plant calmodulin cDNA and used it as a probe to study calmodulin gene expression. Since there is a high degree of homology (90%) between chicken and plant calmodulin at the amino acid level, we used a chicken cDNA clone (270 base pairs containing part of the coding region from amino acid 10) as a probe. Initial experiments were to determine (*i*) whether chicken calmodulin cDNA hybridizes to plant mRNA and (*ii*) what was the abundance of mRNA that hybridizes to chicken cDNA in various parts of the potato plant. To answer these questions poly(A)$^+$ RNA was isolated from various parts of the potato plant (roots, stem, stolon, stolon tips, and small potatoes) and analyzed by Northern analysis (27) with the chicken cDNA probe. The RNA blot analysis revealed that chicken cDNA hybridizes to a single species of mRNA under the conditions used. Furthermore, stolon tips showed the highest expression of calmodulin (data not shown). Based on these results, poly(A)$^+$ mRNA from stolon tips was used to prepare the cDNA library. Screening of 20,000 independent recombinants yielded 16 positive signals. The cDNA from λPCM-1, which contained the longest insert, was subcloned into the *Eco*RI site of pUC13 (pPCM-1) and used for further characterization.

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        10                  30                  50                  70                  90
TTTTGAGGAGAGAGATAAAACTCAAATCACATACATATTAGTACTTAACTGTACAGCTGTTTCTAGGAGTACGAAAAAAAATGGCAGAGCAGCTGACGGA
                                                                                    MetAlaGluGlnLeuThrGl
       110                 130                 150                 170                 190
GGAGCAGATCGCCGAGTTCAAGGAAGCTTTTAGCCTTTTCGACAAGGATGGCGATGGCTGTATTACTACCAAGGAGTTGGGAACAGTGATGAGATCACTT
uGluGlnIleAlaGluPheLysGluAlaPheSerLeuPheAspLysAspGlyAspGlyCysIleThrThrLysGluLeuGlyThrValMetArgSerLeu
       210                 230                 250                 270                 290
GGTCAGAATCCCACTGAAGCTGAACTACAGGATATGATCAGTGAAGCTGATGCTGATCAGAATGGAACCATTGATTTTCCAGAGTTCTTGAATCTGATGG
GlyGlnAsnProThrGluAlaGluLeuGlnAspMetIleSerGluAlaAspAlaAspGlnAsnGlyThrIleAspPheProGluPheLeuAsnLeuMetA
       310                 330                 350                 370                 390
CACGTAAGATGAAGGACACTGATTCTGAGGAGGAACTCAAAGAGGCTTTCAAGGTTTTCGATAAAGATCAGAATGGCTTTATTTCTGCAGCTGAGCTTCG
laArgLysMetLysAspThrAspSerGluGluGluLeuLysGluAlaPheLysValPheAspLysAspGlnAsnGlyPheIleSerAlaAlaGluLeuAr
       410                 430                 450                 470                 490
TCATGTAATGACAAACCTTGGAGAGAAGCTGACTGATGAAGAGGTGGATGAGATGATCCGAGAGGCAGATATTGATGGTGATGGGCAAGTTAATTATGAG
gHisValMetThrAsnLeuGlyGluLysLeuThrAspGluGluValAspGluMetIleArgGluAlaAspIleAspGlyAspGlyGlnValAsnTyrGlu
       510                 530                 550                 570                 590
GAGTTTGTCCGTATGATGCTTGCCAAGTGATGGCTTAAGATTCTCTTAGCTACTGTGTAATTTATGATGGCCGCTTAGTTACTACAACTTCTAGCTGGCA
GluPheValArgMetMetLeuAlaLysEnd
       610                 630                 650                 670                 690
GTTATATTCTGTTCTGTTAAGACAAACAAATGTGTCGTATGGTTTTACTAGCATCTAGACTCCTTTCAGTTTTATGTTAACTTATGGACTACAGGTGTAT
       710                 730                 750                 770                 790
GCTGCTTTAGTCCCTTGCCCATCCAGAGGGGAAAAGAAAAGGAGAAATTAGATAGTTCATTCGTAATATCTTGTTAGTTGCCTTGGTTTCTTTCCAGGCT
       810                 830                 850                 870                 890
ACAGTCTGTATGTGTTTCACTGTCTCTAACTGTTATATTTCCCGTTTCTGGTTTTCGCTTTTATCACTGATTAATGGGAAGGTCAAATGCAAGGTGATTA
       910
TTTCCTAAAAAAAAAAAAAAAA
```

FIG. 1. Nucleotide sequence of potato calmodulin cDNA clone pPCM-1. The numbers indicate nucleotide positions. The initiation and termination codons are underlined. The amino acid sequence derived from nucleotide sequence is shown below the nucleotide sequence.

Nucleotide Sequence of pPCM-1. The complete nucleotide sequence of potato calmodulin clone pPCM-1 is shown in Fig. 1. The 922-base-pair sequence contains the entire coding region and 80 nucleotides of 5′ and 376 nucleotides of 3′ untranslated regions. The amino acid coding region starts at position 81, ends at position 527, and codes for 148 amino acids. No other plant calmodulin nucleotide sequence is available, but, in comparing the nucleotide sequence of potato calmodulin with that of chicken calmodulin (13) (Fig. 2), a high degree of homology (78%) was found, with more than 78% homology in the calcium-binding domains. The 5′ and 3′ untranslated region of potato and chicken calmodulin showed 34% and 39% homology, respectively.

Comparison of deduced amino acid sequence of potato calmodulin with other plant and animal calmodulin amino acid sequences revealed minor differences. The potato calmodulin sequence differed from the chicken sequence in 14 amino acid residues, 4 of which, positions 26 (Thr → Cys), 59 (Gly → Gln), 96 (Gly → Gln), and 99 (Tyr → Phe), are in the proposed calcium-binding loops. The substitutions in potato calmodulin at positions 26, 70, and 96 were also found in spinach and wheat calmodulin (31, 32). However, the substitutions at positions 55, 59, and 146 are unique to the potato sequence. Comparison of nucleotide sequence of potato calmodulin with that of chicken calmodulin at positions where unique substitutions occurred revealed the changes GTA → GCT, GGC → CAG, ACA → CTT in amino acid codons 55, 59, and 146. Like other plant calmodulin, potato calmodulin has a single tyrosine residue at position 138. The second tyrosine residue found at the 99 position in animal calmodulin is substituted with phenylalanine. Unlike spinach and wheat calmodulin, potato calmodulin does not have a valine inserted between positions 143 and 144 but has a valine at position 136 (31, 32). Also, the asparagine inserted between positions 8 and 9 in wheat calmodulin was not found in potato calmodulin (32).

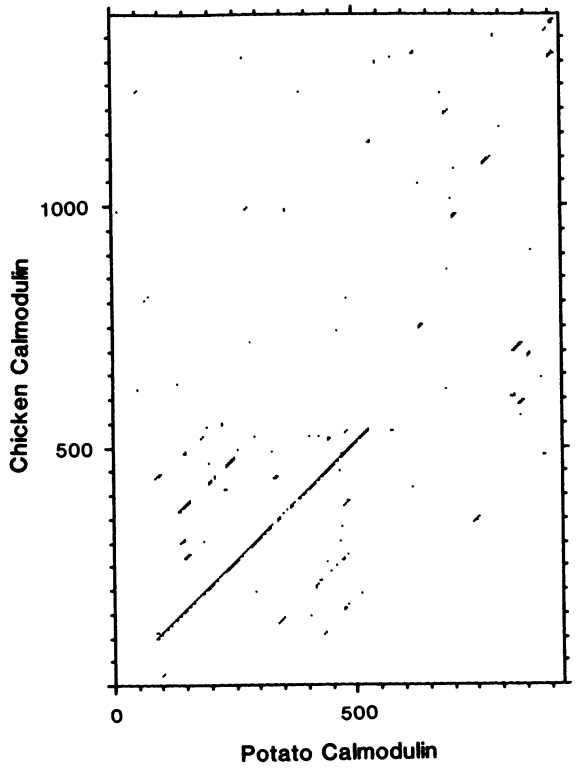

FIG. 2. Comparison of the nucleotide sequence of potato calmodulin with that of chicken calmodulin using a dot plot. The dot plot was generated by using the enhanced graphic matrix procedure of Maizel and Lenk (30) with a window size of 21 bases and a stringency of 14.0.

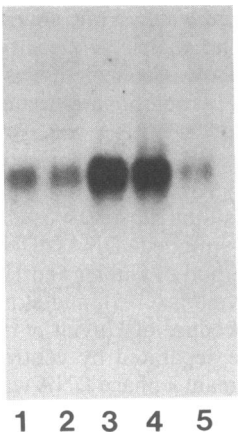

FIG. 3. Northern blot analysis of poly(A)$^+$ RNA hybridized with pPCM-1. Two micrograms of poly(A)$^+$ RNA isolated from different parts of potato plant was electrophoresed on formaldehyde-containing agarose gels, transferred to a nitrocellulose filter, and hybridized to ^{32}P-labeled potato calmodulin cDNA of pPCM-1. The filters were washed and exposed to x-ray film. Lanes: 1, stem; 2, small developing potatoes (<1 cm in diameter); 3, stolon tip; 4, stolon; 5, root.

Expression of Calmodulin in Various Potato Tissues. To study the level of calmodulin mRNA, poly(A)$^+$ mRNA from potato plant (stem, roots, stolon, stolon tips, and young potatoes) was isolated, electrophoresed, transferred onto nitrocellulose filters, and hybridized with the potato cDNA probe. As shown in Fig. 3, a single species of calmodulin mRNA was detected in all the tissues under the conditions used. However, a high level of calmodulin was observed in stolon tips. The level of calmodulin mRNA in stolon tips was 10 times more than in roots and 5 times more than in stems. However, small tubers (about 1 cm) contained 5 times less calmodulin mRNA as compared to solon tips. These results show that the expression of calmodulin goes up significantly during tuberization.

Effect of Auxin on Calmodulin Gene Expression. In strawberry fruit, auxin produced by the achenes controls receptacle growth, and the production of auxin by the achenes is dependent on pollination (20, 33, 34). It is well established that the growth of the pollinated fruit can be stopped at any stage by deachening the pollinated fruit, and growth can be reinitiated by exogenous application of auxin (20, 35, 36). The facts that (*i*) auxin promotes growth in strawberry fruit, (*ii*) auxin regulates gene expression, and (*iii*) calmodulin is involved in the growth process led us to investigate the effect of auxin on calmodulin gene expression. RNA was isolated from deachened fruits that were treated with or without auxin and probed with the calmodulin probe. As shown in Fig. 4, a higher level of calmodulin mRNA was detected within 24 hr of auxin treatment. However, the level of calmodulin mRNA was found to be the same in both auxin-treated and control fruits up to 12 hr. We have also probed for the level of calmodulin mRNA during the development of pollinated and unpollinated fruits. Pollinated fruits contained higher levels of calmodulin mRNA as compared to unpollinated fruits, indicating a positive correlation between the level of auxin and calmodulin mRNA (Fig. 5).

Expression of Calmodulin mRNA in Merit Corn Root Tips. Roots of Merit corn require light to develop gravitropic sensitivity. Several reports indicate the involvement of calcium and calmodulin in light-induced gravitropism (21, 37, 38), and light has been shown to increase the level of calmodulin in Merit corn root tips (38). However, it is not known at what step calmodulin gene expression is affected by

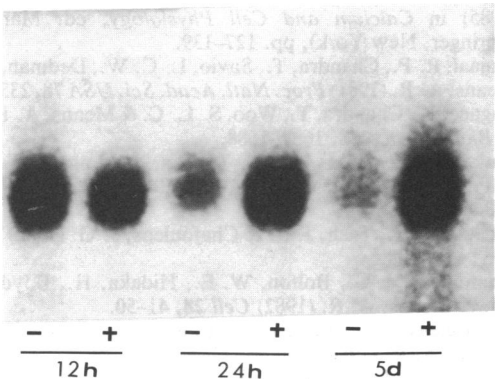

FIG. 4. Effect of auxin on the expression of calmodulin mRNA. Fully opened flowers were hand-pollinated and allowed to grow for 7 days. On day 7 fruits were deachened and lanolin paste alone or containing 1 mM naphthaleneacetic acid in 1% dimethyl sulfoxide was applied to deachened fruits. The fruits were harvested at desired times, frozen in liquid N_2, and used for RNA isolation. (*A*) Total RNA (25 μg) from the fruits alone (−) or fruits treated with auxin (+) was electrophoresed, blotted, and probed with pPCM-1. Hybridization and washing conditions were performed as described (23).

light. To study the effect of light on the level of calmodulin mRNA, poly(A)$^+$ RNA from dark- and light-treated root tips was probed with pPCM-1. As shown in Fig. 6, light-treated root tips showed a detectable increase in calmodulin mRNA. The observed increase in calmodulin mRNA suggests that at least part of the light-induced increase in calmodulin protein could be due to higher levels of mRNA in light-treated roots. However, the light-induced increase in calmodulin protein is about 300%, as compared to a 30–50% increase at the mRNA level. Translational control or the length of the root tip segments that were used in the experiments could be the reason for the difference in the light-induced increase in calmodulin mRNA and protein. In our experiments, we used 2-mm root tip segments, whereas Stinemetz *et al.* (38) used 1-mm segments.

DISCUSSION

cDNA clone pPCM-1 containing sequences complementary to potato calmodulin mRNA has been isolated. Nucleotide sequence analysis shows that pPCM-1 contains the entire coding sequence for calmodulin. The predicted amino acid sequence shows that the potato calmodulin is similar to other plant and animal calmodulins, with a few exceptions (4, 7, 10). Some of the unique changes that are found in spinach and

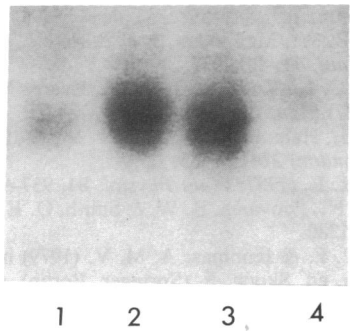

FIG. 5. Expression of calmodulin in 3- and 5-day-old unpollinated and pollinated fruits. Fully opened flowers were either pollinated or left unpollinated by emasculating the anthers on the day of flower opening. Fruits were collected after 3 and 5 days, frozen in liquid N_2, and used for RNA isolation. Total RNA (25 μg) was probed with potato calmodulin cDNA probe (pPCM-1). Lanes: 1, 3-day unpollinated; 2, 3-day pollinated; 3, 5-day pollinated; 4, 5-day unpollinated.

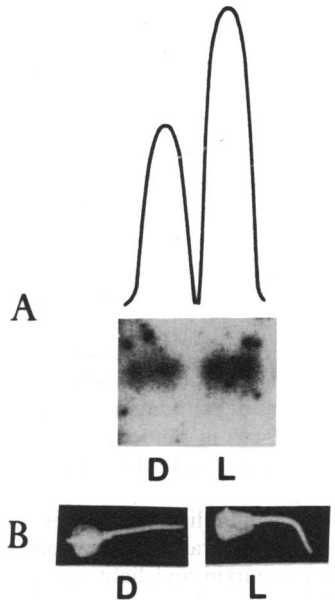

FIG. 6. (*A*) Calmodulin mRNA in Merit corn root tips that were left in dark (D) or exposed to light (L). Seedlings (48 hr old) were kept either in dark or exposed to light for 40 min. Then the root tips (2 mm) were excised, frozen immediately in liquid N_2, and used for poly(A)$^+$ RNA isolation. RNA (2 μg) was probed with radiolabeled pPCM-1. The autoradiogram and densitometer scanning results are presented on the lower and upper part of the figure, respectively. (*B*) Light-dependent gravitropism in Merit corn roots. Forty-eight-hour-old corn (*Zea mays* L. cv. Merit) seedlings were kept in the dark (D) or exposed to light for 10 min and left in the dark for 6 hr (L).

wheat calmodulin are found in potato. Potato calmodulin amino acid sequence showed an overall homology of 98% and 95% with plant (spinach) and animal (chicken) calmodulin, respectively. A high degree of homology was also found between the nucleotide sequence of the coding region of potato and chicken calmodulin (Fig. 2). A very high degree of similarity in amino acid sequence of calcium-binding domains of calmodulin in phylogenetically divergent species, such as bacteria, plant, and animals (4, 7, 10, 39), indicates (*i*) that this protein plays a fundamental role in all living organisms ranging from bacteria to humans and (*ii*) that there was considerable evolutionary pressure to conserve the sequence of this protein.

Northern blot analysis shows that potato calmodulin cDNA clone hybridizes to a single species of mRNA corresponding to ≈1.3 kilobases. In animal cells, cytoplasmic mRNA contains calmodulin transcripts with different lengths, and these transcripts are derived from different polyadenylylation sites (10). In barley, four transcripts were found to hybridize to an animal calmodulin probe (40). Of four mRNAs, a 650-nucleotide transcript showed highest thermal stability. It has been suggested that the different transcripts that are found to hybridize represent either different mRNA species or transcripts that code for other related calcium-binding proteins. The apparent difference that we see between barley and potato in the number of species of mRNA that hybridize to the calmodulin probe could be due to hybridization and washing conditions.

Among the various parts of the potato plant tested for abundance of calmodulin mRNA, stolon tips showed the highest level of calmodulin mRNA. Calmodulin mRNA in stolon tips is found to be 10-fold higher than in the roots. In potato plants, the tuberization process is regulated by a number of factors (19, 41, 42). Using single-node leaf cuttings with an intact axillary bud from a tuberizing mother plant, we have shown that the tuberization is mediated by calcium (19).

Depletion of calcium in the leaf cuttings prevented tuberization, and replenishment of calcium to depleted leaf cuttings restored the tuberization process. Furthermore, calmodulin antagonists inhibited tuberization, indicating the involvement of calmodulin in this calcium-mediated process. Although we do not know at the present time how calmodulin is involved in the regulation of tuberization, the fact that calmodulin mRNA increases during tuberization further supports a critical role for calmodulin in the tuberization process.

Calmodulin is found in all parts of the plant (4, 7). However, the level of calmodulin was different in different parts of the plant (4, 7, 43–45). In most cases the amount of calmodulin was determined by an assay of calmodulin activity. In our earlier studies, we have shown that plants contain endogenous inhibitors of calmodulin activity (46, 47). Hence, the observed differences in calmodulin activity in the different parts of the plant may not reflect the level of expression of the calmodulin gene. With the plant calmodulin probe, the expression of the calmodulin gene can be studied in detail.

Our experiments with strawberry fruit and corn roots show that signals such as auxin and light can affect the level of calmodulin mRNA. Until now, several auxin-regulated cDNA clones have been isolated from various auxin-responsive systems. However, the identity or function of most of these auxin-induced proteins is unknown. The observation that calmodulin gene expression is affected by auxin provides insight into the mechanism of auxin action. By determining the level of calmodulin in dark- and light-treated root tips, Stinemetz *et al.* (38) also reported that light increases calmodulin. However, in barley leaves, light has been shown to decrease calmodulin mRNA by a factor of 2 (40). The observed increase in calmodulin mRNA in our system by auxin and light could be due to an increased rate of transcription or regulation of posttranscriptional events, such as processing and/or stability of mRNA.

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1. Thimann, K. V. (1977) *Hormone Action in the Whole Life of Plants* (Univ. of Massachusetts Press, Amherst).
2. Leopold, A. C. & Kriedemann, P. E. (1975) *Plant Growth and Development* (McGraw-Hill, New York).
3. Hepler, P. K. & Wayne, R. O. (1985) *Annu. Rev. Plant Physiol.* **36,** 397–439.
4. Poovaiah, B. W. & Reddy, A. S. N. (1987) *CRC Crit. Rev. Plant Sci.* **6,** 47–103.
5. Poovaiah, B. W., Reddy, A. S. N. & McFadden, J. J. (1987) *Physiol. Plant.* **69,** 569–573.
6. Roux, S. J. & Serlin, B. S. (1987) *CRC Crit. Rev. Plant Sci.* **5,** 205–236.
7. Roberts, D. M., Lukas, T. J. & Watterson, D. M. (1986) *CRC Crit. Rev. Plant Sci.* **4,** 311–339.
8. Veluthambi, K. & Poovaiah, B. W. (1984) *Science* **223,** 167–169.
9. Ranjeva, R. & Boudet, A. M. (1987) *Annu. Rev. Plant Physiol.* **38,** 73–93.
10. Means, A. R., Lagace, L., Simmen, R. C. M. & Putkey, J. A. (1985) in *Calcium and Cell Physiology*, ed. Marme, D. (Springer, New York), pp. 127–139.
11. Mujaal, R. P., Chandra, T., Savio, L. C. W., Dedman, J. R. & Means, A. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2330–2334.
12. Lagace, L., Chandra, T., Woo, S. L. C. & Means, A. R. (1983) *J. Biol. Chem.* **258,** 1684–1688.
13. Putkey, J. A., Ts'us, K. F., Tanaka, T., Lagace, L., Stein, J. P., Lai, E. C. & Means, A. R. (1983) *J. Biol. Chem.* **258,** 11864–11870.
14. Means, A. R., Tash, J. S. & Chafouleas, J. G. (1982) *Physiol. Rev.* **62,** 1–38.
15. Chafouleas, J. G., Bolton, W. E., Hidaka, H., Boyd, A. E., III, & Means, A. R. (1982) *Cell* **28,** 41–50.
16. Chafouleas, J. G., Lagace, L., Bolton, W. E., Boyd, A. E. & Means, A. R. (1984) *Cell* **36,** 73–81.
17. Chafouleas, J. G., Pardue, R. L., Brinkley, B. R., Dedman, J. R. & Means, A. R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 996–1000.
18. Zendegui, J. G., Zielinski, R. E., Watterson, D. M. & van Eldik, L. J. (1984) *Mol. Cell. Biol.* **4,** 883–889.
19. Balamani, V., Veluthambi, K. & Poovaiah, B. W. (1986) *Plant Physiol.* **80,** 856–858.
20. Veluthambi, K. & Poovaiah, B. W. (1984) *Plant Physiol.* **75,** 349–353.
21. Reddy, A. S. N., McFadden, J. J., Friedmann, M. & Poovaiah, B. W. (1987) *Biochem. Biophys. Res. Commun.* **149,** 334–339.
22. Murray, M. G. & Thompson, W. F. (1980) *Nucleic Acids Res.* **8,** 4321–4325.
23. Theologis, A., Huynh, T. V. & Davis, R. W. (1985) *J. Mol. Biol.* **183,** 53–68.
24. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25,** 263–269.
25. Watson, C. J. & Jackson, J. F. (1985) in *DNA Cloning: A Practical Approach*, ed. Glover, D. M. (IRL, Oxford), Vol. 1, pp. 79–88.
26. Huynh, T., Young, R. A. & Davis, R. W. (1985) in *DNA Cloning: A Practical Approach*, ed. Glover, D. M. (IRL, Oxford), Vol. 1, pp. 49–78.
27. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
28. Feinberg, A. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
29. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
30. Maizel, J. V. & Lenk, R. P. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7665–7669.
31. Lukas, T. J., Iverson, D. B., Schleicher, M. & Watterson, D. M. (1984) *Plant Physiol.* **75,** 788–795.
32. Toda, H., Yazawa, M., Sakiyama, F. & Yagi, K. (1985) *J. Biochem.* **98,** 833–842.
33. Nitsch, J. P. (1955) *Plant Physiol.* **30,** 33–39.
34. Dreher, T. W. & Poovaiah, B. W. (1982) *J. Plant Growth Regul.* **1,** 267–276.
35. Mudge, K. W., Narayanan, K. R. & Poovaiah, B. W. (1981) *J. Am. Soc. Hortic. Sci.* **106,** 80–84.
36. Reddy, A. S. N. & Poovaiah, B. W. (1987) *Biochem. Biophys. Res. Commun.* **147,** 885–891.
37. Poovaiah, B. W., McFadden, J. J. & Reddy, A. S. N. (1987) *Physiol. Plant.* **71,** 401–407.
38. Stinemetz, C. L., Kuzmanoff, K. M., Evans, M. L. & Jarrett, H. W. (1987) *Plant Physiol.* **84,** 1337–1342.
39. Swan, D. G., Hale, R. S., Dhillon, N. & Leadlay, P. F. (1987) *Nature (London)* **329,** 84–85.
40. Zielinski, R. E. (1987) *Plant Physiol.* **84,** 937–943.
41. Arteca, R. N., Poovaiah, B. W. & Smith, O. E. (1979) *Science* **205,** 1279–1280.
42. Wareing, P. F. & Jennings, A. M. V. (1979) in *Plant Growth Substances*, ed. Skoog, F. (Springer, Berlin), pp. 293–300.
43. Muto, S. & Miyachi, S. (1984) *Z. Pflanzenphysiol.* **114,** 421–431.
44. Muto, S. (1982) *FEBS Lett.* **147,** 161–164.
45. Dauwalder, M., Roux, S. J. & Hardison, L. (1986) *Planta* **168,** 461–470.
46. Paliyath, G. & Poovaiah, B. W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2065–2069.
47. Paliyath, G. & Poovaiah, B. W. (1985) *Plant Cell Physiol.* **26,** 201–209.