Molecular and Biochemical Evidence for the Involvement of Calcium/Calmodulin in Auxin Action*

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The use of 35S-labeled calmodulin (CaM) to screen a corn root cDNA expression library has led to the isolation of a CaM-binding protein, encoded by a cDNA with sequence similarity to small auxin up RNAs (SAURs), a class of early auxin-responsive genes. The cDNA designated as ZmSAUR1 (Zea mays SAURs) was expressed in Escherichia coli, and the recombinant protein was purified by CaM affinity chromatography. The CaM binding assay revealed that the recombinant protein binds to CaM in a calcium-dependent manner. Deletion analysis revealed that the CaM binding site was located at the NH2-terminal domain. A synthetic peptide of amino acids 20–45, corresponding to the potential CaM binding region, was used for calcium-dependent mobility shift assays. The synthetic peptide formed a stable complex with CaM only in the presence of calcium. The CaM affinity assay indicated that ZmSAUR1 binds to CaM with high affinity (Kd ~15 nM) in a calcium-dependent manner. Comparison of the NH2-terminal portions of all of the characterized SAURs revealed that they all contain a stretch of the basic α-amphiphilic helix similar to the CaM binding region of ZmSAUR1. CaM binds to the two synthetic peptides from the NH2-terminal regions of Arabidopsis SAUR-AC1 and soybean 10A5, suggesting that this is a general phenomenon for all SAURs. Northern analysis was carried out using the total RNA isolated from auxin-treated corn coleoptile segments. ZmSAUR1 gene expression began within 10 min, increased rapidly between 10 and 60 min, and peaked around 60 min after 30 μM α-naphthaleneacetic acid treatment. These results indicate that ZmSAUR1 is an early auxin-responsive gene. The CaM antagonist N-(6-aminoheptyl)-5-chloro-1-naphthalenesulfonamide hydrochloride inhibited the auxin-induced cell elongation but not the auxin-induced expression of ZmSAUR1. This suggests that calcium/CaM do not regulate ZmSAUR1 at the transcriptional level. CaM binding to ZmSAUR1 in a calcium-dependent manner suggests that calcium/CaM regulate ZmSAUR1 at the post-translational level. Our data provide the first direct evidence for the involvement of calcium/CaM-mediated signaling in auxin-mediated signal transduction.

Plant hormone auxin plays a central role in growth and development by controlling cell division, cell elongation, and cell differentiation (1–4). Auxin-induced cell elongation, one of the fastest hormonal responses known, has been used widely as a model system to study the mechanism of auxin action (5–7). A vast array of cellular responses to external and internal stimuli such as light and hormones involves Ca2+ as a second messenger (8–12). Calmodulin (CaM), a ubiquitous Ca2+-binding protein in eukaryotes, is a primary intracellular Ca2+ receptor that transduces the second messenger Ca2+ signal by binding to and altering the activity of the variety of other proteins (9, 13–15).

Recent evidence indicates that there is a close relationship between the mechanism of auxin action and calcium signaling, but the interaction between them has been controversial and is still unresolved (3, 16). The effect of auxin on changes in cellular calcium levels has been obtained using calcium-sensitive fluorescent dyes or Ca2+-sensitive microelectrodes. Felle (17) reported a decrease in free calcium in cells after auxin treatment, whereas Gehring et al. (18) observed an increase in calcium levels after auxin treatment. Depletion of calcium in tissues using calcium chelators and CaM inhibitors has implicated a role for calcium in the auxin signal transduction. Raghothama et al. (19) found that CaM antagonists such as chlorpromazine, trifluoperazine, fluphenazine, and W-7 inhibited the auxin-induced elongation of oat and corn coleoptile segments. Gonzalez-Daros et al. (20) observed that some, but not all, CaM inhibitors tested could inhibit auxin-induced medium acidification by oat coleoptile segments. Similarly, Reddy et al. (16) observed that the calcium chelator EGTA and calcium channel blocker D-600 inhibited auxin-induced elongation of pea epicotyl segments. Auxin has been linked with Ca2+ transport (21), release of Ca2+ from membrane vesicles (22), and phosphatidylinositol hydrolysis (23). It was proposed that calcium acts as a second messenger in the transduction of the hormone signal (8–12, 23). Auxin-calcium interaction in cellular processes could be regulated through CaM; however, no direct molecular and biochemical evidence for this interaction has been reported so far.

Here we report the isolation and characterization of a novel CaM-binding protein that is encoded by a corn homolog of SAURs (small auxin up RNAs); it is designated as ZmSAUR1 (Zea mays SAURs). SAURs belong to one group of the early auxin-response genes (4). Many early auxin-responsive genes have been cloned and characterized (2, 4, 24–27). SAURs are one of the gene families in higher plants which have been well characterized. Initially isolated from soybean (28), SAUR genes have also been characterized from several dicots such as mung

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‡ The abbreviations used are: CaM, calmodulin; NAA, α-naphthaleneacetic acid; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; SAUR, small auxin up RNA; W-5, N-(6-aminohexyl)-1-naphthalenesulfonamide hydrochloride; W-7, N-(6-aminohexyl)5-chloro-1-naphthalenesulfonamide hydrochloride.

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bean (29), Arabidopsis (30), and apple (31). In all cases examined, SAUR genes encode short transcripts with highly conserved open reading frames that accumulate rapidly and specifically after auxin treatment. Soybean SAUR gene transcription can be detected as soon as 2.5 min after the application of auxin (28, 32). We have demonstrated that corn ZmSAUR1 is a rapid auxin-responsive gene as well. The results described here provide direct molecular and biochemical evidence for the involvement of the Ca²⁺/CaM messenger system in auxin action.

**EXPERIMENTAL PROCEDURES**

Preparation of [³⁵S]Labeled CaM—[³⁵S]-Labeled recombinant CaM was prepared as described (33) using a potato CaM PCI6 cloned into the pET-3b expression vector (34). The specificity of [³⁵S] labeled CaM was about 0.5 × 10⁶ cpm/μg.

Screening the cDNA Expression Library and DNA Sequence Analysis—A corn root cDNA expression library (LAPII) prepared in our laboratory was screened using [³⁵S]-labeled PCI6 as described (33). Several positive clones were isolated from 2 × 10⁶ recombinant phages. The cDNA clones were sequenced on both strands. DNA sequences were analyzed using CGG version 8.0 and 9.0 software (35).

Growth and Auxin Treatment of Corn Plants—Corn (Zea mays L. cv. Merit) seeds were sown in plastic trays filled with vermiculite and kept in the dark for about 5 days at 24 ± 3°C. The dark-grown coleoptiles were harvested at the 8-mm segmentation, excluding the 3-mm tip, were excised. Coleoptile segments were transferred into a beaker containing distilled water and kept floating for a 4-h period. Sets of 10 presoaked coleoptile segments were transferred into Petri dishes containing 10 ml of incubation medium consisting of 10 mM KH₂PO₄ (pH 6.3), 1.5% w/v sucrose, 10 mM sodium citrate, and 0.1% v/v ethanol. NAA (Sigma), W-7 (Sigma), and W-5 (Sigma) treatments were carried out as described (19). After auxin treatment, the lengths of the coleoptile segments were measured using a ruler under a dim green light, or the samples were frozen in liquid nitrogen for RNA extraction.

Southern Blot Analysis—Corn genomic DNA was extracted as described (36). 10 μg of DNA was digested with various restriction enzymes, separated by electrophoresis on 0.8% agarose gel, and transferred to Hybond N nylon membrane (Amersham Pharmacia Biotech) with 0.4 m NaOH. Southern blot analysis was carried out as described earlier (28) using a ZmSAUR1 probe covering the complete coding region from nucleotide 37 to 480 (see Fig. 1).

RNA Isolation and Northern Analysis—Total RNA was isolated from frozen tissue essentially as described (37). RNA samples (50 μg) were denatured and separated on 1.5% formaldehyde-agarose gels. After transfer to Hybond N filters, the blots were hybridized using [³²P]-labeled ZmSAUR1 cDNA fragment 3'-1249 and washed as described earlier (38). Blots were stripped and reprobed with a fragment of Arabidopsis 18 S rDNA, accession no. X16077, nucleotides 158–1669.

Construction of DNA Templates Coding for Full and Truncated ZmSAUR1 Proteins—Templates coding for wild type ZmSAUR1 and deletion mAC and mAN were produced by PCR amplification from the original cDNA with ZmSAUR1-specific oligonucleotides containing appropriate restriction sites (Neoe at the 5'-end and BamHI at the 3'-end) for cloning into the downstream of the His₆ tag in a pET-14b expression vector (Novagen, Inc.). The wild type and deletion mutant proteins of ZmSAUR1 were expressed in Escherichia coli vector (Novagen, Inc.). The wild type and deletion mutant proteins of ZmSAUR1 were expressed in Escherichia coli (39). The nucleotide sequence of all cloned fragments derived by PCR amplification was determined after cloning into the pET-14b vector, using oligonucleotides designed for sequencing from both sides of the pET-14b cloning sites as primers. The 35S-recombinant CaM-binding assay—Wild type and truncated ZmSAUR1 proteins were extracted and purified essentially as described (40). The amount of protein was estimated by the Bradford (67) method using a protein assay kit (Bio-Rad). Proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), electrotransferred onto a polyvinylidene difluoride membrane (Millipore), and treated with 35S-labeled recombinant CaM with 1 μM CaCl₂ or 2 μM EGTA in a total volume of 50 μl as described (40).

**Peptide Binding to CaM**—The synthetic peptides were prepared using an Applied Biosystems peptide synthesizer 431A in the Laboratory of Bioanalysis and Biotechnology, Washington State University. Samples containing 240 pmol (4 μg) of bovine CaM (Sigma) and different amounts of purified synthetic peptides in 100 μl Tris-HCl (pH 7.2) and either 1 μM CaCl₂ or 2 μM EGTA in a total volume of 50 μl were incubated for 1 h at room temperature. The samples were analyzed by non-denaturing PAGE as described (41).

**RESULTS**

Using the CaM binding screening approach, nine positive clones from a corn root cDNA expression library were obtained. One of these clones had high affinity to CaM. DNA sequencing indicated that the 760-base pair cDNA clone contained a partial coding region and a full 3'-untranslated region plus a poly(A) tail. The clone was designated as ZmSAUR1 because it has high homology to soybean SAUR genes. To get the full clone, a cDNA-specific primer as indicated in Fig. 1 and the vector specific T3 primer were used for PCR, and the longest amplified fragment was sequenced. The PCR fragment was 60 base pairs longer than the cDNA clone at the 5'-end. An in-frame methionine residue was found in the downstream region of an in-frame stop codon (Fig. 1). Thus, the ZmSAUR1 cDNA with the full coding region and 3'-untranslated region was obtained.

Fig. 1 shows the nucleotide sequence and the deduced amino acid sequence of ZmSAUR1. The cDNA codes for a polypeptide of 147 amino acids flanked by a 320-base pair untranslated region at the 3'-end and a 36-base pair untranslated region at the 5'-end. The calculated molecular mass and the isoelectric point of the ZmSAUR1 polypeptide are 16.6 kDa and 7.22, respectively. The soybean SAUR genes encode proteins around 10 kDa in size with an isoelectric point between 6 and 7. Like other characterized SAURs (28–31), the amino acid sequence does not contain a typical signal sequence, endoplasmic reticulum retention signal, or N-glycosylation signal, suggesting that the ZmSAUR1 protein does not enter the secretory pathway. However, it is possible that the ZmSAUR1 protein is a nuclear protein because it contains two short regions of basic amino acids (amino acids 33–37 and 67–69) that may form a bipartite nuclear localization signal (42).

The deduced amino acid sequence of ZmSAUR1 is aligned in Fig. 2 with those of soybean SAUR 10A5, 15 A (32), mung bean SAUR ARG7 (29), and Arabidopsis SAUR-AC1 (30). The size of ZmSAUR1 is larger than other SAURs. However, searching the Arabidopsis genomic sequences, a ZmSAUR1 homolog, SAUR-A2, with an even larger molecular mass, was found (Fig. 2).
difference lies in the NH\textsubscript{2}-terminal 54 amino acids and about 30 amino acids in the COOH terminus of ZmSAUR1, where soybean and other plant SAURs have less similarity. In contrast, the sequences are highly similar within the central portion (from 55 to 117 in ZmSAUR1) in all SAURs. Between these residues, ZmSAUR1 is 70.6% similar (58.8% identical) to the soybean 10A5 and 72.5% similar (54.9% identical) to Arabidopsis SAUR-AC1. Thus it seems likely that the central conserved portion of these proteins is most important for whatever function they fulfill.

To study further the properties of ZmSAUR1, the ZmSAUR1 protein was expressed in E. coli, using the pET-14b expression vector. The recombinant protein was present mainly in the soluble fraction. The following two experiments proved that CaM binds to the ZmSAUR1 protein. First, the 18.8-kDa fusion protein (16.6-kDa ZmSAUR1 plus 2.2-kDa NH\textsubscript{2}-terminal His\textsubscript{6} tag) was purified by CaM affinity chromatography to near homogeneity as judged by SDS-PAGE (data not shown). Second, \textsuperscript{35}S-labeled CaM binds to ZmSAUR1 protein only in the presence of Ca\textsuperscript{2+} (Fig. 3). After adding 2 mM EGTA, no CaM binding was observed, suggesting that CaM binding to ZmSAUR1 is calcium-dependent. The proteins from E. coli transformed with the pET-14b vector did not show any CaM binding (data not shown).

To map the CaM binding region of ZmSAUR1, two mutants were prepared (Fig. 4A). The mutant m\textsubscript{3}C lacks the COOH-terminal 81 amino acid residues, which includes the conserved central portion; the mutant m\textsubscript{3}N lacks the NH\textsubscript{2}-terminal 66 residues. The wild type ZmSAUR1 and the two mutants were expressed in E. coli and purified as described (see "Experimental Procedures"). These proteins were used for \textsuperscript{35}S-CaM binding assays in the presence and absence of Ca\textsuperscript{2+}. The binding of CaM to wild type and mutant m\textsubscript{3}C was similar, whereas CaM did not bind to the mutant m\textsubscript{3}N (Fig. 4A), indicating that a CaM binding region is restricted to the 66 amino acids of the NH\textsubscript{2} terminus, where SAURs showed the least similarity. CaM binding to wild type and m\textsubscript{3}C of ZmSAUR1 was prevented by the addition of 2 mM EGTA (data not shown), indicating an absolute requirement of Ca\textsuperscript{2+} for CaM binding.

CaM is a protein capable of recognizing the basic amphiphilic \alpha-helical domain of the target proteins (14, 41, 47). Helical wheel projection of the peptide sequences predicted that the CaM binding region was restricted further to amino acids 20–45 of ZmSAUR1. A peptide with 26 residues corresponding to the amino acids 20–45 was incubated with bovine CaM, and complex formation was assessed by nondenaturing PAGE in the presence or absence of Ca\textsuperscript{2+}. The results showed that the peptide is capable of forming a stable complex with CaM in the presence of Ca\textsuperscript{2+} (Fig. 4C) but not in the absence of Ca\textsuperscript{2+} (data not shown). Several ratios of peptide to CaM were used. In the absence of the peptide, there was a single band reflecting the pure CaM. As the peptide was added, another band of low mobility appeared, representing the peptide-CaM complex. When the ratio of peptide to CaM was equal, the CaM band disappeared, and the intensity of the peptide-CaM complex increased. At a peptide to CaM molar ratio of 1.5, no free CaM was detected. At higher ratios (up to 2.5), no new band appeared on the gel, nor did the peptide-CaM complex band change its intensity, suggesting...
that multivalent complexes were absent. These observations indicate that the peptide binds to Ca\(^{2+}\)/CaM with a 1:1 stoichiometry.

Based on the primary structure of SAURs in Fig. 2, it seems that ZmSAUR1 is very divergent isofrom with a longer NH\(_2\)-terminal domain. However, using the helical wheel projection method, we found that the NH\(_2\)-terminal portions in all of the SAURs listed in Fig. 2 as well as other SAURs in the database are CaM-binding proteins. To prove this, two SAURs, 10A5 and SAUR-AC1, were synthesized, and a similar gel mobility shift assay showing CaM binding to the synthetic peptides, corresponding to amino acids 2–24 of 10A5 (left) and 2–19 of SAUR-AC1 (right). The amino acid sequences of the peptides are listed on the top. Increasing amounts of the peptide (peptide/CaM molar ratios indicated) were incubated with 240 pmol of bovine CaM with 1 mM CaCl\(_2\); samples were separated by nondenaturing PAGE. Arrows indicate the positions of the free CaM and the peptide-CaM complex.

peptides formed a stable complex with CaM, visualized as a larger size band instead of the smaller size band of CaM itself (Fig. 5B). Moreover, the two peptides bind to Ca\(^{2+}\)/CaM with a 1:1 stoichiometry, too; however, only one CaM band was detected in the presence of EGTA (data not shown).

CaM binding affinity of ZmSAUR1 was studied using different concentrations of \(^{35}\)S-labeled CaM. To eliminate nonspecific CaM binding, bovine serum albumin was used as a negative control. The average background count was subtracted from the counts of ZmSAUR1 protein samples when calculating the specific binding. Binding of labeled CaM to ZmSAUR1 saturated at concentrations above 100 nM (Fig. 6), indicating the presence of a saturable high affinity binding site in ZmSAUR1. From Scatchard plot analysis of the saturation curve, the dissociation constant (\(K_d\)) of CaM for ZmSAUR1 was estimated to be about 15 nM. The binding of CaM to ZmSAUR1 was blocked completely in the presence of 2 mM EGTA. Scatchard analysis also indicated that ZmSAUR1 has a single CaM binding site (Fig. 6).
tiles in the medium without the NAA application elongated only about 5%. To study the auxin induction kinetics of Zm-SAUR1 expression, corn coleoptile segments were collected at different times after incubation in the medium with 10 mM NAA for RNA preparation. Northern analyses indicated that the level of ZmSAUR1 is undetectable if NAA was not applied (Fig. 7B). Treatment with NAA led to a significant induction of the ZmSAUR1 with a size of 0.8 kilobase, which coincides with the cDNA size of ZmSAUR1. The induction began within 10 min, a sharp increase occurred between 20 and 60 min, with half-maximal after 30 min and saturation in 60 min. This kinetics of auxin induction is similar to Arabidopsis SAUR-AC1 (30), in contrast to soybean SAUR mRNAs, in which the induction happened in 2.5 min and peaked at 15 min (28, 32). This demonstrates that ZmSAUR1 is indeed an early auxin-responsive gene.

Earlier studies in our laboratory revealed that W-7, a CaM antagonist, inhibited auxin-induced coleoptile elongation. However, its structural homolog W-5 (same as W-7 but lacking a Cl), which is 10 times less active as a CaM antagonist with the same membrane affinity, did not inhibit cell elongation significantly (19). To study the effect of W-7 on the ZmSAUR1 expression in response to auxin, coleoptile segments were treated with auxin in the presence and absence of W-7. The presence of 200 μM W-7 in the medium with 10 μM NAA totally inhibited auxin-induced elongation of coleoptiles (Fig. 8A); however, similar levels of auxin-induced ZmSAUR1 expression were detected in both treatments containing NAA plus W-7 and NAA plus W-5 (Fig. 8B). Thus blocking the function of CaM does not affect the auxin-induced ZmSAUR1 expression, suggesting that CaM does not regulate ZmSAUR1 at the transcriptional level. The results also suggest that Ca2+/CaM interferes with auxin-induced cell elongation; however, we cannot exclude the possibility that W-7 inhibits the auxin-induced cell elongation by blocking the function of CaM, as well as inhibiting the other enzymes such as calcium-dependent protein kinase (43) and mitochondrial pyruvate dehydrogenase (44).

SAUR is a multigene family in soybean (28, 32) and Arabidopsis (27, 30). Based on a data base search, more than 30 Arabidopsis SAUR genes that have been found to date are scattered throughout genome. Genomic Southern analysis of corn genome is shown in Fig. 9. A probe covering the Zm-SAUR1 coding region hybridizes to two or three bands of corn genomic DNA under high stringency conditions. Under low stringency conditions of hybridization, two or three additional

**Fig. 6.** Saturation curve of 35S-CaM binding to purified Zm-SAUR1. E. coli-expressed ZmSAUR1 protein (4 pmol) was spotted on an Immobilon membrane (Millipore) and incubated with different amounts of 35S-labeled CaM. After washing in the buffer without 35S-CaM, radioactivity of the filter was measured using a liquid scintillation counter. The amount of bound CaM at each point was represented as a percent of the maximal binding. The inset shows a Scatchard plot of data indicating that binding ratio of CaM to SAUR is 1.1. Bound/free and bound CaM are expressed as B/F and B, respectively.

**Fig. 7.** Effect of auxin (10 μM NAA) on elongation of corn coleoptile segments and the induction of ZmSAUR1. A, three sets of 10 coleoptile segments were floated in test solution in the dark for time periods indicated on the top (min). Vertical bars represent S.E. B, autoradiograms of Hybond N1 filter hybridized successively with 32P-labeled ZmSAUR1 cDNA fragment 37–480 (Fig. 1) and 18 S rDNA fragment 158–1669 (accession number X16077).

**Fig. 8.** Effect of CaM antagonist (W-7) on auxin-induced cell elongation and expression of ZmSAUR1 in corn coleoptile. A, three sets of 10 coleoptile segments were floated in test solution with or without 10 mM NAA plus W-7 or W-5 for 1 h in the dark. Vertical bars represent S.E. B, autoradiograms of Hybond N1 filter hybridized successively with 32P-labeled ZmSAUR1 cDNA fragment 37–480 (Fig. 1) and 18 S rDNA fragment 158–1669 (accession number X16077).
bands were detected (data not shown). Thus there are about two ZmSAUR1 gene loci and two more ZmSAUR1-related gene loci in the corn genome. It is possible that in one locus there could be several similar SAUR genes arranged in tandem. For example, a cluster of SAURs, including five closely related genes, was found in the soybean genome (32).

**DISCUSSION**

CaM contains four Ca$^{2+}$ binding sites and is highly conserved among plants and animals. Upon Ca$^{2+}$ binding, CaM undergoes conformational changes, which in turn transmit the Ca$^{2+}$ signal by binding to and activating numerous target proteins (9, 13–15). In contrast to CaM sequences, which show considerable conservation in plants and animals, the CaM binding domains of CaM targets show extreme variability in sequence. However, the majority of known target sites for CaM are composed of a stretch of 12–30 contiguous amino acids with positively charged amphiphilic characteristics and a propensity to form an α-helix upon binding to CaM. This affords a tremendous potential for variability in the primary sequence, i.e. target diversity, for CaM binding sites (14, 40, 41, 45). Based on the notion of the structural feature of CaM binding domain, the CaM binding region was mapped onto the NH$_2$-terminal domain of ZmSAUR1 (Fig. 4A).

Amino acid residues 32–45 in the NH$_2$ terminus of ZmSAUR1 protein formed an α-helix with a hydrophobic face as opposed to a basic, hydrophilic face (Fig. 4B). Particularly, amino acids 35–37 in ZmSAUR1 protein have a Trp-Lys-Lys motif, which is present in other known CaM targets (41, 46). The Trp in the Trp-Lys-Lys motif can be replaced by residues with large hydrophobic side chains such as Leu and Phe, and the Lys can be replaced by Arg (41). Therefore, we predicted that the CaM binding site should be in the NH$_2$-terminal domain. A synthetic peptide (amino acids 20–45) from the NH$_2$-terminal domain, with the α-amphiphilic helix (Fig. 5A), includes a Val-Arg-Arg motif in amino acids 9–11 (Fig. 2). Similarly, amino acids 3–16 in Arabidopsis SAUR-AC1 can also form an α-amphiphilic helix (Fig. 5A). The synthetic peptides corresponding to these regions from soybean 10A5 and Arabidopsis SAUR-AC1 bind to CaM in the presence of calcium, but not with EGTA (Fig. 5B).

Thus it can be concluded that most SAUR proteins are CaM-binding proteins.

The SAUR genes have been isolated from several dicots such as soybean (28), mung bean (29), Arabidopsis (30), and apple (31). Isolation of the ZmSAUR1, an SAUR homolog, from corn, a monocot, indicates structural conservation and functional significance among different SAURs in higher plants. The structural feature of SAUR proteins appears to have at least two common domains. The central portions of SAURs share a high amino acid homology (Fig. 2); therefore they could be functional domains for this type of protein (32). The NH$_2$-terminal domain, with the α-amphiphilic helical structure, can act as a regulatory domain to respond to the Ca$^{2+}$/CaM signaling. The CaM binding sites or closely juxtaposed regions in other characterized CaM-binding proteins often function as the autoinhibitory or pseudo-substrate domains. This region maintains the targets in an inactive state in the absence of the calcium signal (14) such as in plant glutamate decarboxylase (47), animal CaM kinase II (48), and plant Ca$^{2+}$/CaM-dependent protein kinase (40, 49).

The exact function of SAUR proteins is still unknown. However, in all cases examined, SAUR genes encode short transcripts with highly conserved open reading frames that have been localized to tissues that are targets of auxin-induced cell elongation (27, 50). In excised soybean-elongating hypocotyl sections, auxin-induced cell elongation was observed after a lag of about 12 min (51, 52). The induction of ZmSAUR1 by auxin occurred within 10 min. The rapid response of SAUR induction in response to auxin has been reported earlier. For example, soybean SAUR expression in the hypocotyl sections was detected within 5 min after auxin application (28, 32). Therefore, it is believed that SAURs play a role in rapid plant growth response such as cell elongation. Their spatial and temporal expression patterns also suggest that they may be involved in auxin-induced cell elongation (53, 54). For example, in gravity-stimulated soybean seedlings or transgenic tobacco, SAUR expression is maximal on the side of the plant where cells are destined to elongate and generate the tropic curvature (27, 53). Moreover, several auxin- and gravity-response mutants of Arabidopsis, e.g. axr2-1, exhibit decreased accumulation of SAUR-AC1 mRNA in elongating etiolated seedlings (30, 55).

Tagawa and Bonner (56) reported that application of calcium and other ions altered the response of coleoptile tissues to auxin. Calcium is also known to affect auxin binding to corn coleoptile membrane preparations (57). Cohen and Nadler (58) demonstrated that calcium played a role in auxin-induced acidification of coleoptile cells. More recently, much physiological evidence demonstrates that calcium and calcium-binding proteins such as CaM affect cell elongation in corn and oat coleoptiles, pea epicytols, and soybean hypocotyls. It is believed that Ca$^{2+}$ and Ca$^{2+}$/CaM are involved in regulating various developmental events, including cell elongation in plants (3, 8–15). Rapid changes in the cytosolic Ca$^{2+}$ concentration were detected in corn coleoptiles after auxin treatment. Such changes were noticeable in both epidermal and cortical cells within 5 min after auxin application (18). It was suggested that the increase of cytosolic Ca$^{2+}$ would precede the change in SAURs and that Ca$^{2+}$ may therefore be a secondary messenger involved in the accumulation of such SAURs (59). However, the results described here suggest that Ca$^{2+}$/CaM do not affect the transcription of SAUR, but they may play a role in cell elongation (Fig. 8). These results are consistent with an earlier study in this laboratory of the effects of calcium on auxin action (16). Depletion of calcium by a calcium chelator, calcium ionophore, or calcium channel blocker inhibited the auxin-induced cell elongation of pea epicytols but not the induction of pIAA4/5 and...
Calmodulin Binds to SAUR Proteins

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