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Characterization of the Auxin-Inducible *SAUR-AC1* Gene for Use as a Molecular Genetic Tool in *Arabidopsis*[1]

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The small auxin up RNA (*SAUR*) genes were originally characterized in soybean, where they encode a set of unstable transcripts that are rapidly induced by auxin. In this report, the isolation of a *SAUR* gene, designated *SAUR-AC1*, from *Arabidopsis thaliana* (L.) Heynh. ecotype Columbia is described. The promoter of the *SAUR-AC1* gene contains putative regulatory motifs conserved among soybean *SAUR* promoters, as well as sequences implicated in the regulation of other genes in response to auxin. The transcribed region is approximately 500 bp in length and contains no introns. Highly conserved sequences located within the *SAUR-AC1* transcript include the central portion of the coding region and a putative mRNA instability sequence (DST) located in the 3′ untranslated region. Accumulation of *SAUR-AC1* mRNA is readily induced by natural and synthetic auxins and by the translational inhibitor cycloheximide. Moreover, several auxin- and gravity-response mutants of *Arabidopsis* exhibit decreased accumulation of the *SAUR-AC1* mRNA in elongating etiolated seedlings. In particular, in the *axr2-1* mutant the *SAUR-AC1* transcript accumulates to less than 5% of wild-type levels. These studies indicate that *SAUR-AC1* will be a useful probe of auxin-induced gene expression in *Arabidopsis* and will facilitate the functional analysis of both transcriptional and posttranscriptional regulatory elements.

A number of plant mRNAs have been identified that accumulate rapidly following induction with natural or synthetic auxins (Theologis, 1986). In excised elongating soybean hypocotyls, one family of transcripts, known as the small auxin up RNAs (*SAUR*s), begins to accumulate within 2.5 min after auxin application (McClure and Guilfoyle, 1987). Although the function of the *SAUR* gene products is unknown, the transcripts have been localized to tissues that are targets for auxin-induced cell elongation (Gee et al., 1991). The appearance of the *SAUR*s before auxin-induced cell elongation is observed (Vanderhoef and Stahl, 1975; McClure and Guilfoyle, 1987) suggests that they may contribute to the process (McClure et al., 1989). A correlation between *SAUR* gene expression and cell elongation has also been observed during the gravitropic response. In gravity-stimulated seedlings, an asymmetric accumulation of the *SAUR* transcripts is evident before visible bending of the plants occurs (McClure and Guilfoyle, 1989). The *SAUR* transcripts accumulate in the cells that are destined to elongate, presumably due to a rapid redistribution of endogenous auxin (McClure and Guilfoyle, 1989; Li et al., 1991). Their rapid disappearance from cells that are not targeted for enhanced elongation indicates that the *SAUR* transcripts are highly unstable (McClure and Guilfoyle, 1989).

The accumulation of the soybean *SAUR* transcripts in response to auxin is due at least in part to transcription (Franco et al., 1990) and is likely to involve regulatory sequences in the promoter region (Li et al., 1991). However, the instability of the transcripts must presumably be due to sequences downstream of the promoter. One good candidate is the downstream element designated DST (McClure et al., 1989), which is conserved in the 3′ UTR of plant *SAUR* genes (reviewed in Green, 1993). Recently, tandem copies of this sequence were shown to destabilize reporter transcripts in tobacco (Newman et al., 1993). At present, there is no evidence that auxin affects the function of DST sequences or the stability of the *SAUR* transcripts (Li et al., 1991; Newman et al., 1993). Therefore, if DST sequences act to destabilize the *SAUR* transcripts, they may do so constitutively so as to allow the *SAUR* mRNA level to adjust rapidly in response to decreases and increases in the auxin concentration (Newman et al., 1993).

In an effort to elucidate mechanisms of auxin signal transduction, a number of mutants have been isolated that have altered responses to auxin (Estelle, 1992) and/or defects in gravitropism (e.g. Bullen et al., 1990), a process considered to be controlled by auxin. Many of these mutants have been isolated in *Arabidopsis thaliana* because of the widely recognized advantages of this system for molecular genetics (Somerville, 1989). The analysis of auxin-responsive gene expression could provide a particular advantage in the characterization of these mutants because many of their physiological traits are tedious to score quantitatively, or develop too slowly to provide insight into the early events in auxin action. The main reason that auxin-responsive gene expression, as a means to characterize mutants, has received a minimal amount of attention to date is the paucity of well-

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Abbreviations: CHX, cycloheximide; SAUR, small auxin up RNAs; UTR, untranslated region.

characterized auxin-regulated genes of *Arabidopsis* that are suitable for these studies.

We were interested in isolating a *SAUR* gene from *Arabidopsis* that could be used as a molecular probe for the analysis of gravity-response and auxin-response mutants. Another goal was to characterize the structure and expression of the gene as a means of identifying potentially important regulatory features of the *SAUR*s that are conserved or that differ between soybean and *Arabidopsis*. In this report, we describe the isolation and characterization of the auxin-inducible *SAUR-AC1* gene of *Arabidopsis*. This gene shares many structural features with its soybean counterparts, including the presence of a DST sequence in the 3′ UTR. The identification of auxin- and gravity-response mutants with defects in *SAUR-AC1* expression indicates that this gene should be an effective tool for studying auxin signal transduction in *Arabidopsis*.

MATERIALS AND METHODS

Isolation and Sequencing of *SAUR-AC1* Genomic and cDNA Clones

Primers corresponding to two highly conserved regions of the soybean *SAUR* open reading frames were synthesized as follows: 5′-GCAGTCTATGT(T/C)GGAGA-3′ and 5′-CA(T/A)GG(T/A)ATTGTGAG(G/A)CC-3′. Amplification of DNA sequences flanked by these primers was accomplished by two sequential PCR experiments using genomic DNA of *Arabidopsis thaliana* (L.) ecotype Columbia as template. The initial amplification was carried out in a volume of 100 μL and contained 100 ng of heat-denatured *Arabidopsis* genomic DNA, 163 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 μM of each dNTP, and 2.5 units of Taq DNA polymerase (Perkin Elmer). Reactions were subjected to 25 cycles of 94°C for 1 min, 37°C for 2 min, and 72°C for 3 min. After a final 7-min incubation at 72°C, the PCR products were separated on a low-melting temperature agarose gel and the major product (130–150 bp) was excised and used for a second round of PCR under the same conditions as above. The major PCR product was gel purified and blunt-end ligated into a plasmid vector, and the clones were sequenced using the dideoxy method of Sanger et al. (1977). The deduced amino acid sequence of one of the PCR clones was found to be 78% identical to the soybean consensus and was used as a probe to isolate the corresponding gene designated *SAUR-AC1*. The gene resides on an *Eco*RI fragment of genomic DNA approximately 6 kb in length isolated from an *A. thaliana* (L.) ecotype Columbia genomic library that was kindly provided by Drs. Carrie Schneider and Chris Somerville. A cDNA clone identical to positions +45 to +485 was isolated from an *Arabidopsis* cDNA library described previously (Taylor and Green, 1991) that was kindly provided by Drs. Alex Gasch and Nam-Hai Chua. Both the cDNA clone and the genomic clone were sequenced on both strands to generate the sequence shown in Figure 1.

Growth and Auxin Treatment of *Arabidopsis* Plants

Wild-type and mutant etiolated seedlings of *A. thaliana* were grown in complete darkness for 7 to 10 d in Petri dishes

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-416  tag tca aat ttt cct ttc ttg cca cag aat ttt ttt gaa ggc ttt tgt
-368  gac ttt gtc agt ata taa tca aat cat gtg gtc ctg tct ttt gaa tat
-320  cat tga gaa gaa ggt atg act taa tca gtg ata gag act tgt ggt tca
-272  tta ggt aca caa ctt cat gtc cct aca taa tct ata gag aga ttt ggc
-224  atg tga tgt ctt tga gac aaa taa gcc cca atg aag tta tca tgt agg
-176  aca tca tta gac act aac ttc tca gac acc att tat tga ttt gtt ctt
-128  ggt tta gta tct aca acc caa agc cat gtg ctc tgt aat taa aaa gaa
 -80  aaa cct caa aaa tac tta atc att tct ttc ata cgt tcg ttt ata taa
 -32  caa gta act aaa ccc ctt tag att cac aaa ctA AAG AAG ATC CAA TTT

  17  TAA AAT CTC AAA GCT TTC TCC AAG ACT AAG AAA CAT TTA AGC TTC AGG

                                 Met Ala Phe Leu Arg Ser Phe Leu Gly Ala
  65  AAA ACA TAA GGG AAA ATA ATG GCT TTT TTG AGG AGT TTC TTG GGT GCT

      Lys Gln Ile Ile Arg Arg Glu Ser Ser Ser Thr Pro Arg Gly Phe Met
 113  AAG CAA ATT ATT CGA AGG GAA TCA TCG TCG ACA CCA AGA GGA TTC ATG

      Ala Val Tyr Val Gly Glu Asn Asp Gln Lys Lys Lys Arg Tyr Val Val
 161  GCG GTC TAT GTA GGA GAG AAT GAT CAG AAG AAG AAG AGA TAT GTG GTG

      Pro Val Ser Tyr Leu Asn Gln Pro Leu Phe Gln Gln Leu Leu Ser Lys
 209  CCG GTT TCA TAC TTA AAC CAG CCT TTG TTT CAA CAA CTG TTG AGT AAA

      Ser Glu Glu Glu Phe Gly Tyr Asp His Pro Met Gly Gly Leu Thr Ile
 257  TCT GAG GAA GAG TTT GGT TAT GAT CAT CCA ATG GGC GGC TTA ACA ATA

      Pro Cys His Glu Ser Leu Phe Phe Thr Val Thr Ser Gln Ile Gln  *
 305  CCA TGT CAT GAA TCT TTG TTC TTC ACA GTC ACA TCT CAG ATA CAA TGA

 353  AGT ACT ATA CTA CAA CAT TTC CAT ATT TTT TTT AGA TTG TTA GCT AAT

 401  TTC CCC TGG AGA TAA TTG TAA ATT GTT TCA ATG AGA GGA ATA TAC AAT

 449  ACA TAG ATC GTA ATT GAT CAA TGC GTA TTT GCA TGT Taa tac att tgt

 497  gtc ttg tac caa aaa aag gaa tta tac att tgt gtc att taa ctc tgg
 545  aca cca tac att tcg tca tta cag tga aac ggc aga att tga aca ctc
 593  att att ctg gtt ggt agt tat ttc cat att tct caa aga aca ttt atg
 641  tga cta tta tca ttc ctt gcg aca act gta ata atg aga aaa ctt ggt
 689  att ttt ttt ggc tct tca tat aag ttg ttt aaa ata ggt ttc gaa gcc
 737  caa agc cca taa att aaa cgc cta aca ttc acg cgc tct ttg act atg
 785  gtt gct tag gaa cag atg cgc gtg ggg aag ttg gca ccg ttt ttt ctc
 833  ttg ctt aca tct att ttt ttt tct taa acg tct att tat ttg ctt tac
 881  gtc att gta acg ttt gtt tgt ttt ctc tgt atc gtt agt tgt tgt aca
 929  ctt gta cta tgg acg ttg aac tgc ggt tag tcc ggt tca gct agc att
 977  ttt taa atg tac atc tat ttt ctt att gat tat gtg tat aat gtt att
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Figure 1. The nucleotide and deduced amino acid sequences of the *SAUR-AC1* gene. Capitalized nucleotides indicate transcribed sequences. Numbers on the left refer to the nucleotide sequence. The first nucleotide of the *SAUR-AC1* mRNA is numbered +1. The sequence for the putative TATA box (−39) and promoter sequences (Z element, −51; DUE element, −84; NDE element, −105; A box, −255) found in other auxin-regulated promoters, and in the *SAUR-AC1* promoter, are boxed. A DST element in the 3′ UTR is indicated by shading. Two possible poly(A) signals are underlined. All nucleotide positions designate the 5′-proximal nucleotide of the corresponding sequence.

(80 × 25 mm) containing AGM medium (4.3 g/L Murashige-Skoog salts [Sigma], 3% [w/v] Suc, 1× Murashige-Skoog vitamins [Sigma], 2.5 mM Mes, pH 5.7, 8 g/L phytagar [Gibco]) under sterile conditions. The aerial portions of the seedlings (material above the agar surface) were cut into 2- to 3-mm sections and incubated in KPSC (10 mM potassium phosphate, pH 6, 2% [w/v] Suc, 50 μM chloramphenicol) medium for 4 h to deplete endogenous auxins (McClure and Guilfoyle, 1987). Samples were transferred to fresh buffer with or without 50 μM 2,4-D or 10 μM IAA for 1 h at 28°C and then frozen in liquid nitrogen.

S1 Nuclease Protection Analysis

The 5′ end of the *SAUR-AC1* mRNA was analyzed by S1 nuclease protection as described previously (Newman et al.,

1993), with minor modifications. The probe was a single-stranded DNA covering the sequence from −414 to +141 in Figure 1 that was labeled according to Nagy et al. (1987). A primer complementary to the sequence from +119 to +141 was used to prime synthesis of the probe and generate a sequencing ladder to size the protection products. Twenty micrograms of total RNA from *Arabidopsis* seedlings treated with 2,4-D as described above were used for the S1 nuclease protection reaction.

RNA Isolation and Northern and Southern Analyses

Total RNA was isolated from frozen tissue essentially as described by Newman et al. (1993), except that a second phenol extraction was performed after solubilization of the lithium chloride precipitate. RNA samples were denatured and separated on formaldehyde/agarose gels. Following transfer to Biotrace RP (Gelman Sciences, Ann Arbor, MI), the blots were prehybridized and hybridized as described by Taylor and Green (1991) using a *SAUR-AC1* probe covering the complete coding region from positions +56 to +356 in Figure 1. Blots were subjected to successive 20-min washes at 65°C with 1× SSC, 0.1% SDS, and 0.5× SCC, 0.1% SDS. Radioactive bands were then quantified with a phosphorimager. Blots were stripped and reprobed with a fragment of the coding region from the gene encoding the highly expressed translational factor eIF4A (Taylor et al., 1993). Accumulation of the *SAUR-AC1* mRNA was normalized by calculating the ratio of the *SAUR-AC1* signal to the eIF4A internal standard for each individual sample. High-stringency southern hybridization was as described by Taylor and Green (1991) using the same *SAUR-AC1* coding region probe and wash conditions as described above for northern blots. For low-stringency experiments, Southern blots were washed at 37°C in 1× SSC, 0.1% SDS for 8 h.

RESULTS

Structure of the *SAUR-AC1* Gene

The soybean *SAUR* genes contain several stretches of highly conserved amino acids and no introns (McClure et al., 1989). Based on these observations, we devised a strategy to generate an *Arabidopsis SAUR* probe from genomic DNA using the PCR. Oligonucleotide primers corresponding to the most highly conserved regions of the soybean *SAUR* genes were synthesized as described in "Materials and Methods" and used to amplify *Arabidopsis* genomic DNA. A major amplification product of the expected size (about 140 bp) was isolated, reamplified, and cloned into a plasmid vector. The inserts from 25 clones were partially or completely sequenced, but only one was found to contain sequences homologous to the soybean *SAUR* genes. This insert was used as a probe to isolate genomic and cDNA clones of the corresponding gene, which was designated *SAUR-AC1* (for *Arabidopsis* Columbia *SAUR* gene 1).

Figure 1 shows the nucleotide and deduced amino acid sequence of *SAUR-AC1*. Similar to soybean *SAUR* genes (McClure et al., 1989), *SAUR-AC1* potentially encodes a small (89 amino acid) polypeptide and contains no introns. Approximately 400 bp of the promoter region and 500 bp 3′ of the gene have been sequenced, allowing for the identification of a TATA box at −39, and two putative poly(A) signals (underlined in Fig. 1) located at +439 and +446 [37 and 44 bp, respectively, upstream of the poly(A) addition site of the cDNA clone].

The calculated molecular mass and isoelectric point of the *SAUR-AC1* polypeptide are 10 kD and 9.2, respectively. The amino acid sequence does not contain a typical signal sequence, ER retention signal, or *N*-glycosylation signal, suggesting that the *SAUR-AC1* polypeptide does not enter the secretory pathway. However, it is possible that *SAUR-AC1* is a nuclear protein because it contains two short regions of basic amino acids (amino acids 18–23 and 47–50, as numbered in Fig. 2) that may form a bipartite nuclear localization signal (Raikhel, 1992).

The deduced amino acid sequence of *SAUR-AC1* is aligned in Figure 2 with that of the soybean *SAUR* consensus (McClure et al., 1989) and a mung bean *SAUR* cDNA, designated *ARG7* (Yamamoto et al., 1992), that was recently isolated. The soybean *SAUR* genes are least similar upstream of amino acid 31 (McClure et al., 1989), and the *SAUR-AC1* gene shows little similarity to the soybean or *ARG7* sequences in this region. In contrast, the sequences are highly similar within the region amplified initially with PCR from amino acids 38 to 87. Between these residues, *SAUR-AC1* is 78% identical to the soybean SAUR consensus and 80% identical to the *ARG7* amino acid sequence. Over the same region, the nucleotide sequence of *SAUR-AC1* is 75% identical to that of *ARG7* and 74% identical to the soybean prototype, *SAUR-15A* (McClure et al., 1989): There were two mismatches between the *SAUR-AC1* nucleotide sequence and each of the two primers used for PCR, which could explain the low frequency of SAUR clones isolated in that experiment (<5%). Beyond amino acid 87, three of the five amino residues most highly conserved among the soybean *SAUR* genes are also found in *SAUR-AC1*. There are conservative changes at the other two positions. The overall size of the coding region is similar for all *SAUR* genes.

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                                1                   25                  50                  75                  100
                                .                   .                   .                   .                   .
SAUR CONSENSUS (Soybean)        MGFRlpGIRk-aSf--anq----asskavdvp-KGYlaVYVGe---KmrRFvIPVSYlNqPSFQdLLsQAEEEFGYdHPmGGLTIPCsEdvFq-iTscL--N
                                * •    :*   **  * *     **     * :*::******   * :*:*:******** ** *** :****************** • :*  :** :  :
SAUR-AC1                        MAF----LR---SFLGAKQIIRRESSS---TP-RGFMAVYVGENDQKKKRYVVPVSYLNQPLFQQLLSKSEEEFGYDHPMGGLTIPCHESLFFTVTSQI--Q
                                * *    :*   *   *::     **    :* :*::******* •  *:***** ******* *** :********** ****** ****  :** :  *
ARG7 (Mung bean)                MGFRLPGIRKTLS---ARNE---ASSKVLDAPPKGYLAVYVGEN-MK--RFVIPVSHLNQPLFQDLLSQAEEEFGYDHPM-GLTIPCSEDLFQHITSCLSAQ
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Figure 2. Alignment of the deduced amino acid sequences of *SAUR-AC1*, the consensus sequence for the soybean *SAUR* genes (McClure et al., 1989), and the *ARG7* cDNA from mung bean (Yamamoto et al., 1992). Identical amino acids are marked with asterisks and similar amino acids with colons. Similar amino acids are grouped as: A, S, T; N, Q; D, E; I, L, M, V; H, K, R; and F, W, Y.

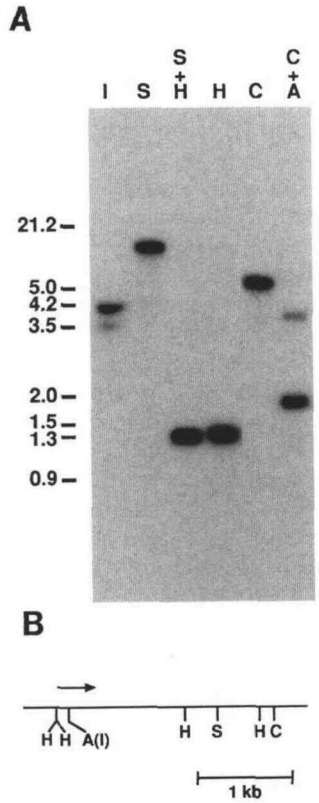

Figure 3. Southern blot hybridization analysis of the *SAUR-AC1* gene. A, Samples of 10 μg of *Arabidopsis* genomic DNA were cut with *Hin*cII (I), *Sac*I (S), *Sac*I, and *Hin*dIII (S+H), *Hin*dIII (H), *Cla*II (C), or *Cla*II and *Acc*I (C+A) as indicated. The blot was hybridized to a ^{32}P-labeled probe covering the *SAUR-AC1* coding region as described in "Materials and Methods." Size markers in kb are indicated on the left. B, Partial restriction map of the *SAUR-AC1* gene derived from the genomic clone and its nucleotide sequence. Restriction enzyme abbreviations are as in A. The arrow indicates the position of the *SAUR-AC1* coding region.

The promoter region of *SAUR-AC1* contains several sequence motifs that are homologous to those found in the soybean *SAUR* genes and other auxin-inducible genes (see boxes in Fig. 1). These include the first 6 bp of the 8-bp A box and the last 7 bp of the 10-bp Z element, implicated in auxin induction of the pea PS-IAA4/5 and PS-IAA6 promoters (Ballas et al., 1993) and nos promoter (An et al., 1990), respectively. *SAUR-AC1* also contains a 12/14-bp match with the DUE element, and a 12/16-bp match with the NDE element, which are the most highly conserved sequences in soybean *SAUR* promoters (McClure et al., 1989). Thus, there are many candidates for auxin-responsive elements upstream of the *SAUR-AC1* TATA box.

In Southern analyses shown in Figure 3, a probe covering the *SAUR-AC1* coding region hybridizes to a single band of genomic DNA unless the coding region contains a site for the restriction enzyme used (e.g. *Hin*cII and *Acc*I). The lack of detection of other *SAUR* genes in these hybridizations, which were performed at high stringency, indicates that this probe is specific for the *SAUR-AC1* gene under these conditions. Several faint bands can be detected in most digests if low-stringency conditions are used (data not shown). This is consistent with the observation that other *SAUR* genes are present in *Arabidopsis* (Guilfoyle et al., 1992), but the sequences of these genes and their regulatory properties have not been described.

Structure of the *SAUR-AC1* mRNA

To map the 5′ end of the *SAUR-AC1* mRNA, a single-stranded probe covering the region between −414 and +141 was annealed to total RNA from 2,4-D-treated seedlings and digested with S1 nuclease, as described in "Materials and Methods." Figure 4 shows the S1 protection products electrophoresed adjacent to a sequencing ladder of the probe. The 5′ ends are clustered within a 7-bp region, with start sites at two A residues (see asterisks in Fig. 4) being favored. The A residue designated as +1 in Figure 1 corresponds to the most 5′ of the two A's.

The 3′ end of the *SAUR-AC1* mRNA shown in Figure 1 (at +485) was deduced from the position of poly(A) addition within the cDNA clone. Since only one cDNA clone was isolated, the presence of additional 3′ ends cannot be ruled out; however, if they exist they may be located nearby, because the two putative poly(A) signals are adjacent to each other at about the expected distance (Mogen et al., 1992) from the poly(A) site of the cDNA. Perhaps the most interesting sequence motif conserved among the 3′ ends of *SAUR*

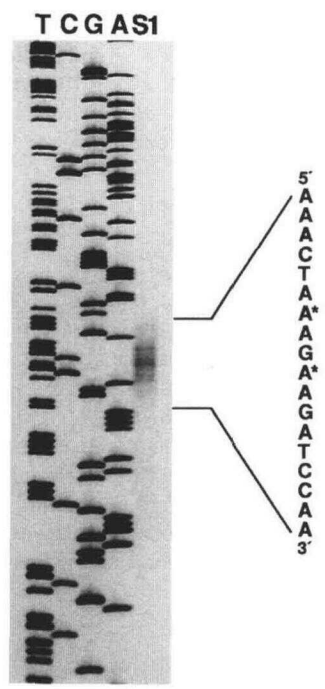

Figure 4. S1 protection analysis of the 5′ end of the *SAUR-AC1* transcript. Transcriptional start sites were detected by alignment of the protected DNA fragment with a sequencing ladder of the sense strand of the *SAUR-AC1* DNA. The major protection products in the S1 lane are marked with asterisks on the antisense strand sequence to the right.

genes is the DST element implicated in mRNA instability. One feature of DST sequences is that they consist of three highly conserved sequences separated by two more variable sequences (McClure et al., 1989; Newman et al., 1993). As shown in Figure 5, *SAUR-AC1* contains a DST element with these characteristics that is located 10 bases upstream of the poly(A) addition site of the transcript (see Fig. 1).

Induction of *SAUR-AC1* Expression in Wild-Type and Mutants of *Arabidopsis*

Most studies of *SAUR* gene expression in soybean have been conducted using etiolated elongating soybean hypocotyl sections that respond rapidly to auxin treatment (McClure and Guilfoyle, 1987; McClure et al., 1989). In an effort to use an analogous system to investigate the effect of auxin on *SAUR-AC1*, we performed a series of induction experiments using segments of etiolated *Arabidopsis* seedlings as described in "Materials and Methods." Seedlings were incubated for 4 h in buffer to deplete endogenous auxin (McClure et al., 1989) and were then treated with either the synthetic auxin 2,4-D or the natural auxin IAA for 1 h. The level of *SAUR-AC1* is below the level of detection following the depletion step as shown in Figure 6, and the same was true in untreated seedlings (data not shown). Treatment with either 2,4-D or IAA led to a significant induction of the *SAUR-AC1* mRNA. The effect of the protein synthesis inhibitor CHX was also investigated because of the inductive effect of CHX on the soybean *SAUR* genes (Franco et al., 1990). A very large increase in *SAUR-AC1* expression was observed following a 1-h treatment with CHX as shown in Figure 6. Treatment with 2,4-D plus CHX induced only slightly more *SAUR-AC1* mRNA than CHX alone. This is in contrast to the superinduction of *SAUR* mRNA that occurs in soybean seedlings subjected to treatment with 2,4-D and CHX (Franco et al., 1990).

One of the main reasons for isolating an *Arabidopsis SAUR* gene is its potential use as a molecular probe to study signal transduction in a system amenable to molecular genetics. Therefore, it was of interest to investigate whether known mutants of *Arabidopsis* exhibit deficiencies in the expression of *SAUR-AC1*. We chose to focus on mutants with altered auxin and gravity responses because the influence of both of these stimuli on *SAUR* expression in soybean has been well documented.

Figure 7A shows a comparison of *SAUR-AC1* expression in wild-type and two auxin-resistant mutants of *Arabidopsis*

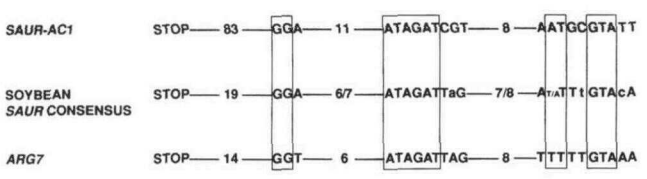

Figure 5. Alignment of DST sequences found in *SAUR* genes. Nucleotides that are identical in *SAUR-AC1*, *ARG7*, and the soybean consensus are boxed. The number of nucleotides in the variable regions separating each box or separating the DST elements from their respective stop codons are indicated.

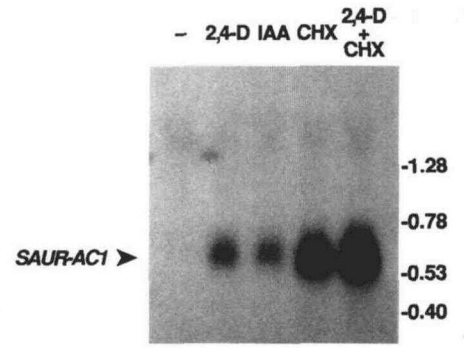

Figure 6. Northern analysis of *SAUR-AC1* mRNA levels in etiolated seedlings of *Arabidopsis*. Seedlings were depleted for endogenous auxin and then subjected to one of the following 1-h treatments: none (–); 50 μM 2,4-D (2,4-D); 10 μM IAA (IAA); 70 μM CHX (CHX); or 50 μM 2,4-D, 70 μM CHX (2,4-D+CHX). Samples of 20 μg of total RNA were separated on a formaldehyde gel and blotted to nylon membrane. The blot was hybridized with a ^{32}P-labeled probe covering the *SAUR-AC1* coding region as described in "Materials and Methods."

following 2,4-D treatment for 1 h. The most severe effect on transcript accumulation was exhibited by seedlings of the *axr2–1* mutant, where little or no expression was routinely observed. As shown in Figure 7C, when expression was observed in *axr2–1*, the level was less than 5% of the wild-type level. The *aux1–7* mutant induces about 45% less *SAUR-AC1* mRNA than the wild type.

Defects were also observed when gravity-response mutants (Bullen et al., 1990; Bullen, 1992) were assayed for *SAUR-AC1* expression. Seedlings of two of these mutants, *mg20* and *mg421*, exhibit reduced induction of *SAUR-AC1* mRNA in response to 2,4-D, whereas seedlings of the third mutant (*mg65*) induced wild-type levels of the mRNA (Fig. 7, B and C). None of the gravity-response or auxin-response mutants in this study expressed detectable *SAUR-AC1* mRNA in etiolated seedlings without 2,4-D treatment (data not shown).

DISCUSSION

The isolation and characterization of the *SAUR-AC1* gene of *Arabidopsis* described in this report serves two important purposes. First, because *Arabidopsis* is rather distantly related to the legumes from which the other reported *SAUR* genes derive, the structural features conserved in *SAUR-AC1* should suggest which characteristics are of importance to *SAUR* genes in general. Second, the identification of an auxin-regulated *SAUR* gene of *Arabidopsis* provides a means to exploit the unique genetic resources of this model system so that the nature and mechanisms of auxin responses can be investigated at the molecular level.

Within the promoter regions of the soybean *SAUR* genes, the most prominent conserved elements are the NDE and DUE elements, both of which are also found in the *SAUR-AC1* promoter, albeit in the opposite order. Several other sequence motifs that have been implicated in auxin-responsive expression, either experimentally or on the basis of sequence conservation, are also present upstream of the

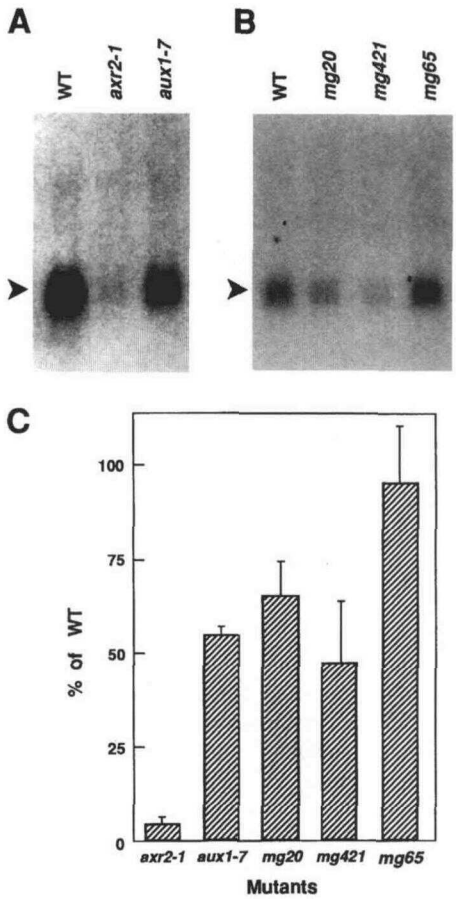

Figure 7. Expression of *SAUR-AC1* in auxin-resistant and gravity-response mutants of *Arabidopsis*. A, *SAUR-AC1* mRNA accumulation in wild-type and auxin-resistant mutants of *Arabidopsis* ecotype Columbia. B, *SAUR-AC1* mRNA accumulation in wild-type and gravity-response mutants of *Arabidopsis* ecotype Estland. Northern blots contained 20 μg of total RNA per lane from wild-type or mutant seedlings following 2,4-D treatment as described for Figure 6. C, Histogram of the relative accumulation of *SAUR-AC1* mRNA in 2,4-D-treated, etiolated seedlings of *Arabidopsis* mutants expressed as a percentage of the expression in the appropriate wild-type parent. The results represent the data average ± SD from three independent experiments, each of which has been normalized to an endogenous internal standard as described in "Materials and Methods."

SAUR-AC1 transcription start site. In addition to the A box and the Z element shown in Figure 1, the *SAUR-AC1* promoter contains multiple copies of both the R and CCATT elements conserved among other auxin-inducible genes (Gielen et al., 1984; Slighton et al., 1986; Conner et al., 1990; Hagen et al., 1991). In the future, it should be possible to employ deletion and gain of function experiments to delineate the contribution of each of these sequence motifs to the transcriptional control of *SAUR-AC1*.

Another potentially important sequence, the DST element, was identified in the 3′ UTR of the *SAUR-AC1* transcript. DST sequences have also been identified downstream of the other SAUR coding regions. As in the case of *SAUR-AC1*, the cDNA sequence of *ARG7* (Yamamoto et al., 1992) demonstrates that the DST sequence falls within the transcribed region. The other *SAUR* mRNAs have not been mapped, but based on the proximity of the DST sequences to the coding regions in the soybean *SAUR* genes, it is likely that the DST elements are transcribed as part of the mRNAs in these cases as well. DST sequences have been proposed to contribute to the instability of the soybean *SAUR* transcripts (McClure et al., 1989; Li et al., 1991), and recently it was shown that DST sequences can destabilize both β-glucuronidase and globin reporter transcripts in tobacco (Newman et al., 1993). These observations indicate that the DST element within the *SAUR-AC1* mRNA may function as a determinant of mRNA instability. The alignment of DST sequences among SAUR genes (see Fig. 5) demonstrates that the ATAGAT and GTA sequences in the second and third blocks are invariant and thus may be important functional determinants, a hypothesis that can now be tested.

The time required for a transcript to reach a new steady state (higher or lower) after a change in transcription is dependent on the stability of the mRNA. A common characteristic of unstable transcripts is that they can be induced or repressed rapidly. The marked induction of the *SAUR-AC1* mRNA within 1 h in response to natural and synthetic auxins is therefore consistent with the transcript having a short half-life. In addition, many unstable transcripts can be induced by CHX, as is *SAUR-AC1*. At present it is unknown whether the CHX effect occurs because (a) a labile *trans*-acting factor required for rapid mRNA degradation or transcriptional repression is depleted, or (b) rapid degradation of the *SAUR* mRNA requires translation in *cis*. The soybean *SAUR* genes are also induced by CHX, but this induction differs from that of *SAUR-AC1* in two respects. First, for the soybean *SAUR* genes, the mRNA accumulation following CHX treatment is only slightly greater than that following 2,4-D treatment. However, in the case of *SAUR-AC1*, CHX is a much stronger inducer of mRNA accumulation than is 2,4-D. The second difference, which is particularly striking, relates to the lack of superinduction of *SAUR-AC1* when CHX and 2,4-D treatments are combined. With the soybean *SAUR* genes, a prominent superinduction effect was observed under the same conditions (Franco et al., 1990). This distinction indicates that the mechanisms by which CHX and/or 2,4-D act to induce *SAUR-AC1* and the soybean *SAUR* genes are likely to differ.

One of the most promising approaches to the study of plant regulatory mechanisms is the isolation and analysis of mutants. In *Arabidopsis*, a number of mutants have been isolated that exhibit altered responses to hormones and to external stimuli that are presumed to be mediated by hormones. Although most of these mutants have been thoroughly characterized physiologically, in many cases the molecular analysis has been limited by the lack of availability of suitable molecular probes. The work described in this report demonstrates that the *SAUR-AC1* gene should provide a useful molecular marker for the study of both gravity- and auxin-response mutants because seedlings of several of these mutants have observable defects in *SAUR-AC1* expression. The auxin-responsive mutants investigated in this study, *aux1-7* and *axr2-1*, were both isolated as auxin resistant, but they differ from each other with respect to many other characteristics, such as their inheritance pattern, resistance to

other hormones, response to gravity, and patterns of growth (Pickett et al., 1990; Wilson et al., 1990). Therefore, it is not surprising that the *aux1-7* and *axr2-1* mutations have different effects on *SAUR-AC1* expression. The accumulation of the *SAUR-AC1* transcript in response to 2,4-D is almost completely blocked in etiolated seedlings of *axr2-1*, whereas only a modest reduction is evident in *aux1-7* seedlings. At present it is unknown whether the residual effect of the *aux1-7* mutation on *SAUR-AC1* expression is due to incomplete inactivation of the *AUX1* gene in this mutant or because the mutation alters a pathway that is not totally responsible for induction of *SAUR-AC1* by auxin.

A modest reduction in expression of *SAUR-AC1* is also seen with two of the three gravity-response mutants analyzed. Although all of the mutants studied (including the auxin-resistant mutants) have some defect in gravitropism, this defect does not appear to correlate directly with *SAUR-AC1* mRNA levels in the mutant seedlings. In *mg20*, gravitropism is normal in root but altered in stem (Wilson et al., 1990; Bullen, 1992), whereas in *aux1-7* the opposite is true. *mg421* exhibits defects in both root and stem gravitropism (Bullen, 1992), yet *mg20*, *aux1-7*, and *mg421* have similar effects on *SAUR-AC1* expression in seedlings (Fig. 7C). This lack of correlation also extends to *mg65* and *axr2-1*, both of which affect the gravity response in roots and shoots. Little or no *SAUR-AC1* expression was detected in *axr2-1* seedlings, whereas mRNA accumulation was induced normally in *mg65*. However, it should be noted that defects in gravitropism may be more likely to affect the distribution of the *SAUR-AC1* mRNA than its overall accumulation in the seedling. Work done with soybean *SAUR* genes has demonstrated that the redistribution of *SAUR* expression can be visualized using tissue printing (McClure and Guilfoyle, 1989) or promoter fusion studies (Li et al., 1991). Similar studies with the *SAUR-AC1* gene may now be designed to reveal more subtle defects among the mutants affecting the gravity response.

The most obvious difference among the mutants studied in this report has to do with growth. With the exception of *axr2-1*, all of the mutants exhibit a normal stature. In contrast, *axr2-1*, which is the most deficient in *SAUR-AC1* expression, displays an extreme dwarf phenotype and very short internodes (Wilson et al., 1990). Examination of the cellular anatomy of *axr2-1* has indicated that the primary reason for the shortened internodes is a reduction in cell elongation rather than cell number (Timpte et al., 1992). Although the rapid induction of cell elongation by auxin is well documented, it has been difficult to attribute the elongation deficiency in *axr2-1* to a defect in auxin action because this mutant is highly pleiotropic (Wilson et al., 1990). However, SAUR expression is highly correlated with the rapid effects of auxin on cell elongation. Therefore, the strong effect of *axr2-1* on *SAUR-AC1* expression and on elongation lends support to the possibility that the growth defect in *axr2-1* is the direct result of a defect in auxin action.

SAUR-AC1 may be useful not only in the evaluation of known phenotypes of auxin and gravity-response mutants, but may also detect phenotypes that are not morphologically evident. Moreover, the *SAUR-AC1* gene will likely be an expedient tool in evaluating double mutants for epistatic relationships and thus aid in efforts to elucidate how the components that comprise auxin signal-transduction chains interact. Conversely, it is also likely that the aforementioned mutants can be used to enhance future expression studies of the *SAUR-AC1* gene as well as studies of SAUR function.

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