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Strong Cellular Preference in the Expression of a Housekeeping Gene of *Arabidopsis thaliana* Encoding S-Adenosylmethionine Synthetase

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S-Adenosylmethionine serves as a methyl group donor in numerous transmethylation reactions and plays a role in the biosynthesis of polyamines and ethylene. We have cloned and sequenced an S-adenosylmethionine synthetase gene (*sam*-1) of *Arabidopsis thaliana*. The deduced polypeptide sequence of the enzyme has extensive homology with the corresponding enzymes of *Escherichia coli* and yeast. Genomic hybridization indicates the presence of two adenosylmethionine synthetase genes per haploid *Arabidopsis* genome. RNA gel blot analysis shows that adenosylmethionine synthetase mRNA levels are high in stems and roots, correlating well with the higher enzyme activity in stems, compared with leaves. Histochemical analysis of transgenic *Arabidopsis* plants transformed with a chimeric β-glucuronidase gene, under the control of 748-base pair 5′ sequences of the *sam*-1 gene, demonstrates that the gene is expressed primarily in vascular tissues. In addition, high expression was observed in sclerenchyma and in the root cortex. A hypothesis for the strong cellular preference in the expression of the *sam*-1 gene is presented.

INTRODUCTION

S-Adenosylmethionine synthetase (AdoMet synthetase or ATP:L-methionine *S*-adenosyltransferase, EC 2.5.1.6) is an enzyme that catalyzes the only known biosynthesis of *S*-adenosylmethionine (AdoMet) by the following reaction (for review, see Tabor and Tabor, 1984):

$$\text{Methionine} + \text{ATP} \xrightarrow[K^+]{Mg^{2+}} \text{AdoMet} + \text{PPi} + \text{Pi}$$

In all living organisms AdoMet serves as a cofactor in a variety of reactions. It acts as a methyl group donor in numerous highly specific transmethylations involving various kinds of acceptor macromolecules, such as proteins, lipids, polysaccharides, and nucleic acids. AdoMet also serves, after decarboxylation by AdoMet decarboxylase, as a propylamine group donor in the biosynthesis of polyamines. In plants, AdoMet is a precursor molecule in the biosynthesis of the phytohormone ethylene (Yang and Hoffman, 1984). In addition, AdoMet is believed to play a regulatory role in the synthesis of methionine and other aspartate-derived amino acids. Known examples of such regulation in plants by AdoMet are the feedback inhibition of AdoMet synthetase and the allosteric stimulation of threonine synthetase (Giovanelli *et al.*, 1980).

AdoMet synthetase has been studied extensively in bacteria, yeast, and animal systems. In *Escherichia coli* the enzyme consists of four identical subunits (Markham et al., 1980). *Met*K, the structural gene for the enzyme, has been cloned and sequenced (Markham et al., 1984). In *Saccharomyces cerevisiae* AdoMet synthesis is catalyzed by two isoenzymes encoded by two different genes (*sam*-1 and *sam*-2), which have been cloned recently (Cherest et al., 1978; Thomas and Surdin-Kerjan, 1987). Three isoenzymes, α, β, and γ, have been identified in mammalian tissues (Abe et al., 1980; Suma et al., 1986). In plants, AdoMet synthetase activity has been shown in barley and pea (Mudd, 1960; Aarnes, 1977). In these studies the plant enzymes seem to have the same properties as the yeast and mammalian AdoMet synthetase: requirement of Mg^{2+} and a monovalent cation and inhibition by tripolyphosphate. No AdoMet synthetase genes from higher eukaryotes have been cloned yet.

In this paper we present the characterization of an AdoMet synthetase gene, *sam*-1, of *Arabidopsis thaliana*. We show that the gene is expressed at high levels in stems and roots and identify the cell types in which the gene is expressed most abundantly.

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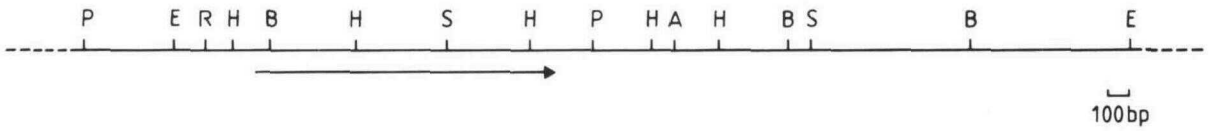

Figure 1. Restriction map of the *sam*-1 Gene.

Arrow indicates the transcribed region. A, AvaI; B, BglII; E, EcoRI; H, HindIII; P, PstI; R, EcoRV; S, SalI.

RESULTS

Isolation of the *sam*-1 Gene and RNA Gel Blot Analysis

A genomic sublibrary of *A. thaliana* containing 1145 randomly chosen cosmid clones was differentially screened with cDNA probes of poly(A)$^+$ RNA preparations from different organs of *Arabidopsis* (Simoens et al., 1988). One of the clones, pATC9A1, was chosen for further investigation because it reproducibly gave strong hybridization signals with cDNA probes of stem and callus poly(A)$^+$ RNA (Simoens et al., 1988).

To localize the gene on the pATC9A1 plasmid, several restriction digests were blotted and hybridized with a cDNA probe of stem poly(A)$^+$ RNA. Figure 1 shows a restriction map of the gene. Different restriction fragments containing the gene were subcloned in the riboprobe system vector pGem1 (see "Methods").

The expression pattern of the gene in the different organs of *Arabidopsis* was determined by RNA gel blot hybridizations. Riboprobes transcribed from the 0.78-kb BglII-SalI fragment of the coding region (Figure 1) were hybridized with RNA gel blots containing total RNA isolated from leaves, stems, roots, inflorescences, seed pods, and callus tissue of *Arabidopsis*. A very abundant transcript of approximately 1.3 kb was detected in the RNA of stems, roots, and callus tissue (Figure 2A). The transcript is 10 to 20 times less abundant in leaf, seed pod, and inflorescence RNA.

Sequence Analysis of the *sam*-1 Gene

Figure 3 shows the sequence of a 2560-bp PstI-AvaI fragment containing the gene. The sequence contains one large open-reading frame of 1182 bp, coding for a putative protein of 394 amino acids, starting from the ATG at position +9 to a stop codon at position +1191. This putative protein has a calculated molecular size of 43,149 D.

The significance of the open-reading frame was further underlined by analysis of a homologous cDNA clone. A cDNA library of poly(A)$^+$ RNA from mature *Arabidopsis* plants was constructed as described in "Methods." Upon screening of 10,000 cDNA clones, 18 clones hybridized with a riboprobe transcribed from the 0.78-kb BglII-SalI fragment containing part of the open reading frame. A cDNA clone, pATCC9A1-2, containing an insert of 1258 bp was chosen for sequence analysis. Figure 3 shows that the cDNA sequence is colinear and 98% identical to the genomic sequence. Since the cDNA and the genomic bank are derived from different varieties of *A. thaliana* (see "Methods"), the 2% mismatches can be attributed to gene polymorphism between the two varieties. Screening of the NBRF data bank (release 14) with the protein sequence encoded by the open reading frame as a probe revealed extensive homology with the AdoMet synthetase protein sequences of *E. coli* and yeast (Figure 4). The deduced amino acid sequence of the *Arabidopsis* AdoMet synthetase is 49% homologous to the *E. coli* and 57% to the yeast AdoMet synthetase polypeptide (Markham et al., 1984; Thomas and Surdin-Kerjan, 1987). The highest degree of conservation between the three compared sequences is found in two domains, one at the amino terminus (between residues 1 and 49) and another one in the middle of the polypeptide (between residues 237 and 289).

The transcription start of the *sam*-1 gene was determined by S1 mapping. A 5′-labeled 295-bp EcoRV-BglII fragment was hybridized to RNA from leaves, stems, and

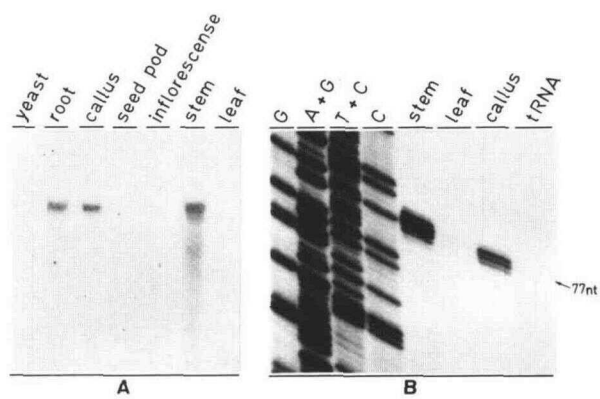

Figure 2. Expression Analysis and Transcription Start Mapping of the *sam*-1 Gene.

(A) RNA gel blot with 20 μg of total RNA from different organs of *Arabidopsis* hybridized with the 0.78-kb BglII-SalI fragment of the *sam*-1 gene as a probe. The hybridization was performed as described in "Methods."

(B) Transcription start mapping of the *sam*-1 gene. Fifty μg of total RNA from stem, leaf, and callus tissue, and 50 μg of tRNA were hybridized with the 295-bp EcoRV-BglII fragment of the *sam*-1 gene labeled at the 5′ end of the BglII site. The hybrids were digested subsequently with S1 nuclease and after ethanol precipitation the samples were run on a 6% DNA-sequencing gel next to a Maxam and Gilbert sequencing ladder as a size marker (for details, see "Methods").

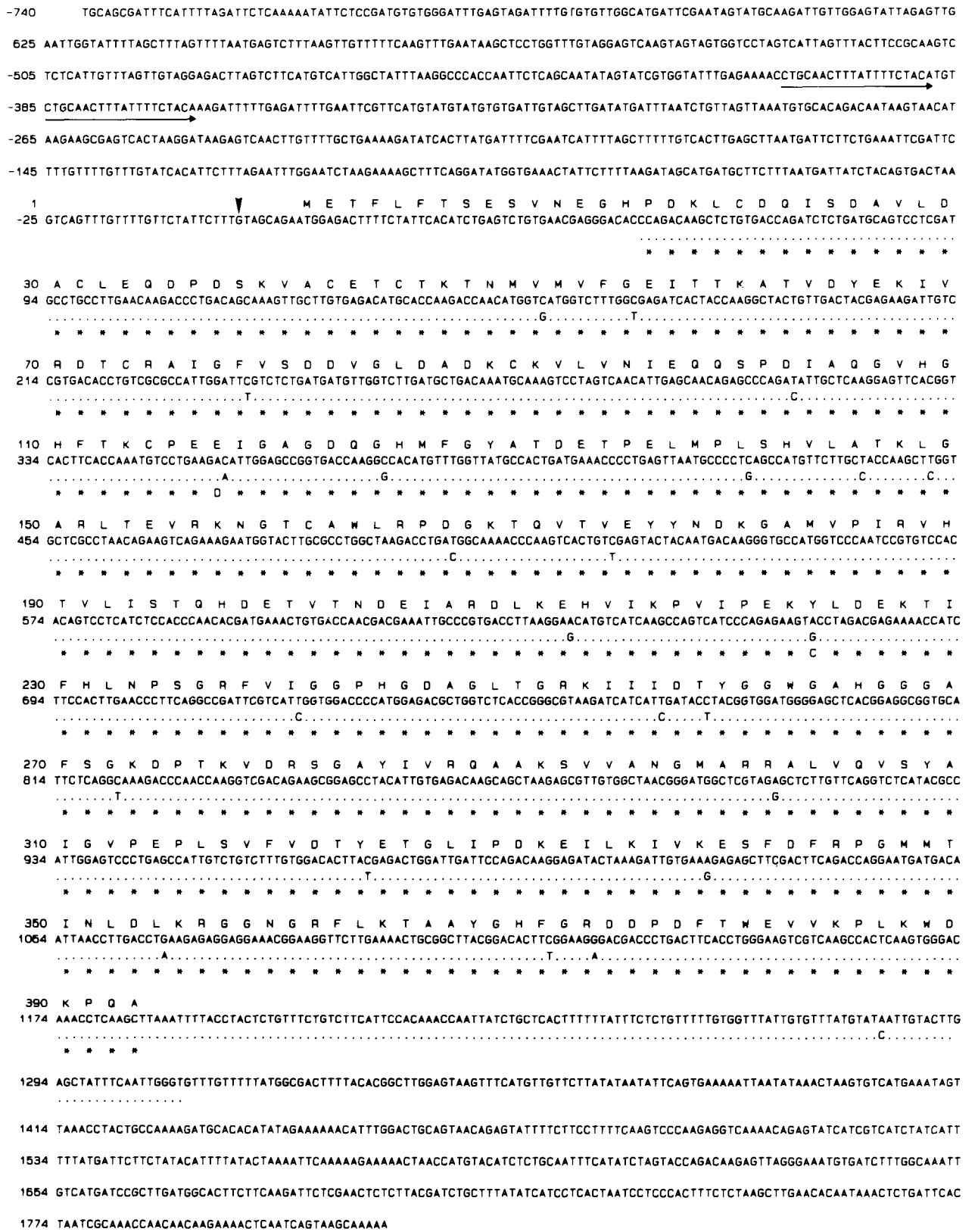

Figure 3. Nucleotide Sequence of the *sam*-1 Gene.

The deduced amino acid sequence is indicated above the nucleotide sequence. The homology with the cDNA clone pATCC9A1-2 is represented below the *sam*-1 gene sequence by dots. Only the mismatches with the *sam*-1 sequence are indicated. The amino acid sequence deduced from the cDNA clone is shown below the cDNA sequence. Amino acids identical with those deduced from the *sam*-1 gene are indicated with asterisks. The transcription start site of the *sam*-1 gene is indicated with a vertical arrow. The direct repeat of 20 bp is underlined with horizontal arrows.

Figure 4. Comparison of the Polypeptide Sequences Encoded by the *sam*-1 Gene of *Arabidopsis thaliana* (3) and Yeast (2) (Thomas and Surdin-Kerjan, 1987) and the *Met*K Gene of *E. coli* (1) (Markham et al., 1984).

Amino acids homologous in all three sequences are boxed. The sequences have been aligned using the Intelligenetics version 5.1 computer program.

callus tissues. This probe was predominantly protected for 77 bp after S1 nuclease treatment (Figure 2B). The hybridization signals of the minor protected bands vary between experiments. The amount of protected fragments using the different RNA preparations is consistent with the results obtained by RNA gel blot analysis. The leader sequence of the *sam*-1 gene is short since the translation start codon is located 9 nucleotides downstream from the transcription start (Figure 3). Such a short leader sequence is not unprecedented. A leader sequence of 8 nucleotides has been reported before for a gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase (*rbcS*-3B) from tomato (Sugita et al., 1987). No obvious TATA box is present at position −30 from the transcription initiation site. From position −408 to −366, the promoter region contains a direct repeat of 20 nucleotides separated by 3 nucleotides.

DNA Gel Blot Analysis

To determine whether this AdoMet synthetase gene (*sam*-1) is unique in the *Arabidopsis* genome, a BglII-SalI riboprobe containing 0.78 kb of coding sequence was hybridized to different restriction digests of genomic DNA. Using the restriction enzymes PstI, BglII, and EcoRI, which do not cut within the 0.78-kb BglII-SalI fragment, two hybridizing bands per digest were observed using moderately stringent hybridization conditions (Figure 5A). Using more stringent conditions, only one band per digest was observed (Figure 5B). The size of these at high stringency hybridizing bands is in agreement with the restriction map of the *sam*-1 gene. The data indicate that the *sam*-1 gene is a member of a gene family consisting of two different copies. A detailed analysis of the second AdoMet synthetase gene will be described elsewhere. Because the RNA

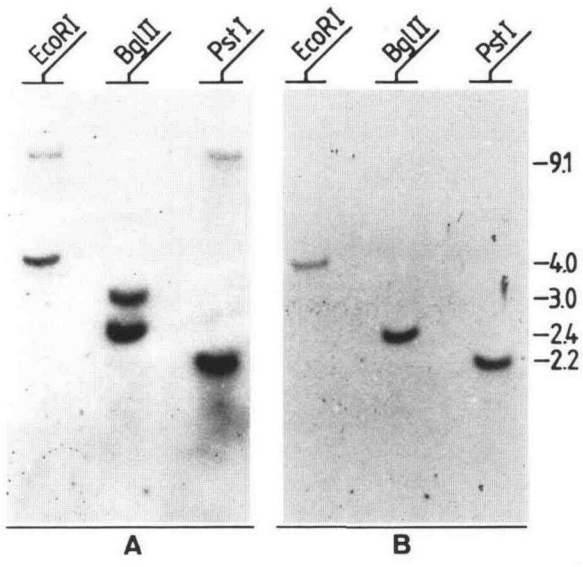

Figure 5. *Arabidopsis* DNA Digests Hybridized with the *sam*-1 Gene.

(A) A genomic DNA gel blot of *A. thaliana* DNA digested with EcoRi, BglII, and PstI was hybridized with a riboprobe transcribed from the 0.78-kb BglII-SalI fragment of the *sam*-1 gene as described in "Methods." The final wash was carried out in 1 × SSC, 0.5% SDS at 68°C followed by autoradiography.

(B) The same hybridization, washed at higher stringency. Final wash was carried out in 0.1 × SSC, 0.5% SDS at 68°C followed by autoradiography.

gel blot hybridization (Figure 2A) has been performed using moderately stringent hybridization conditions, the expression pattern observed in Figure 2A reflects the steady-state mRNA levels derived from both genes.

AdoMet Synthetase Activity in Stems and Leaves of *Arabidopsis*

To determine whether the levels of *sam* mRNA in the different organs are reflected at the protein level, AdoMet synthetase assays were performed as described in "Methods," using stem and leaf extracts. For each reaction 100 μg of crude protein extract was used. Control reactions containing all reactants, except ATP, were used to correct for background activity. The amount of incorporation of ^{35}S-methionine into AdoMet is presented in Table 1. The results show that stem extract contains approximately 10 times more AdoMet synthetase activity than leaf extract. This corresponds closely with the 10 to 20 times higher levels of AdoMet synthetase mRNA in stems compared to leaves as seen by RNA gel blot analysis (Figure 2A).

Expression of a Chimeric Gene in Transgenic *Arabidopsis*

The abundance of a mRNA reflects both the efficiency of transcription and the mRNA stability. To determine the contribution of the *sam*-1 promoter region to the pattern of gene expression, a chimeric gene was constructed consisting of 748 bp of promoter and 5′-untranslated *sam*-1 sequences fused to the initiation codon of the *E. coli* β-glucuronidase gene (*gus*). As a control, a chimeric gene was constructed consisting of the *gus* structural gene under the control of the promoter of the *ats*1a gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase of *Arabidopsis* (Krebbers et al., 1988). The chimeric genes together with a selectable marker gene were then inserted between the T-DNA borders of the binary vector pGSC1706 yielding the plasmids pGUSSAM-1 and pGUSATS-1 (Figure 6). The construction strategy of these plasmids is described in "Methods."

Transgenic *A. thaliana* (var. Columbia) plants were obtained by incubating root pieces with *Agrobacterium* containing these binary vectors (Valvekens et al., 1988). From the root callus, several kanamycin-resistant plants were regenerated for each construct and analyzed for β-glucuronidase (GUS) activity in the leaves, stems, and roots as described in "Methods." The plants transformed with pGUSSAM-1 show 10 to 140 times more GUS activity in stems and roots than in leaves (Table 2). The level of activity in root compared to stem is variable. By contrast, plants transformed with pGUSATS-1 show very little expression in roots and equally high expression in stems and leaves, indicating that the GUS activity reflects the properties of the promoter and not merely the stability of the messenger or the protein. Therefore, it is concluded that the 748-bp 5′ sequences of the *sam*-1 gene are at least partially responsible for the high amount of AdoMet synthetase mRNA in stems and roots.

For each chimeric construct, pGUSSAM-1 and

Table 1. In Vitro Incorporation of ^{35}S-Methionine into AdoMet Using Stem and Leaf Protein Extracts

	Incorporation		
	+ATP	−ATP	Net
	cpm		
Experiment 1			
Leaf Extract	278,516	31,993	246,523
Stem Extract	2,662,277	51,600	2,610,677
Experiment 2			
Leaf Extract	272,124	35,334	236,790
Stem Extract	2,530,000	31,853	2,498,147

Reactions proceeded for 1 hr at 24°C using 100 μg of crude protein extract (see "Methods").

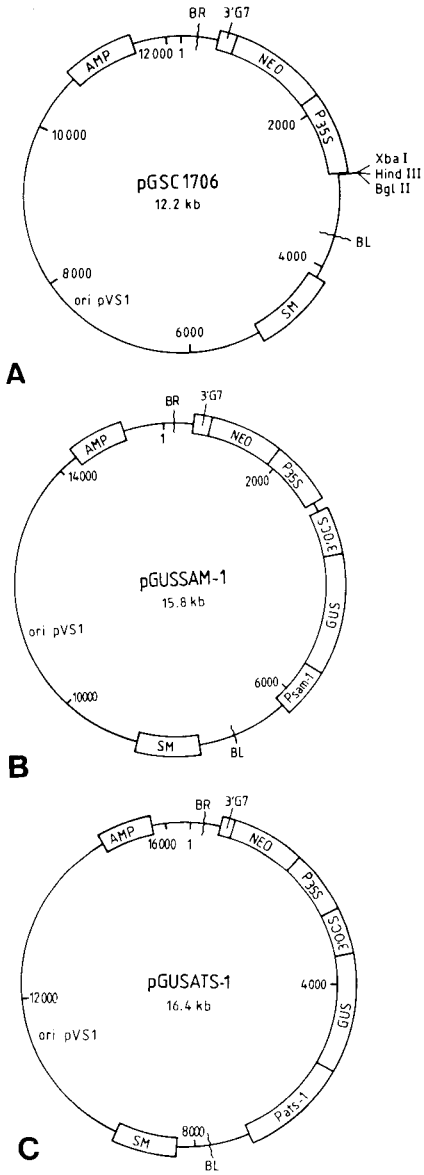

Figure 6. Maps of the *Agrobacterium* Plasmids Containing the Chimeric P*sam*-1-*gus* and P*ats*-1-*gus* Genes.

Construction procedure is described in "Methods." AMP, ampicillin resistance gene; BL, left border of the T-DNA; BR, right border of the T-DNA; GUS, β-glucuronidase gene; NEO, neomycin phosphotransferase II gene; P35S, cauliflower mosaic virus 35S promoter (Odell et al., 1985); SM, streptomycin resistance gene; 3′G7, 3′-untranslated end of the T-DNA gene 7 (Velten and Schell, 1985); 3′OCS, 3′-untranslated end of the T-DNA octopine synthase gene; oripVS1, origin of replication from the *Pseudomonas aeruginosa* plasmid pVS1 (Deblaere et al., 1987).

(A) The binary vector pGSC1706.

(B) pGUSSAM-1, containing the chimeric gene P*sam*-1-*gus*-3′*ocs* cloned into pGSC1706. P*sam*-1, 748 bp of the 5′-untranslated region of the *sam*-1 gene.

(C) pGUSATS-1, containing the chimeric gene P*ats*-1-*gus*-3′*ocs* cloned into pGSC1706. P*ats*-1, 1730 bp of the 5′-untranslated region of the *ats*-1 gene.

pGUSATS-1, two representative transgenic plants were chosen for histochemical analysis (Jefferson et al., 1987). At the macroscopic level, leaves of pGUSSAM-1-transformed plants show clear GUS activity in the veins (Figure 7A). This observation shows that the GUS substrate (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid; *X*-Gluc) can easily diffuse through veins. Using leaves of pGUSATS-1-transformed plants, GUS activity was detected only in regions where the leaf had been cut before supplying the GUS substrate (Figure 8A).

To study the expression of the chimeric genes at the cellular level, histological preparations were made from the stained regions of leaf, stem, and root, as described in "Methods." Almost all of the visible GUS activity in pGUSSAM-1-transformed leaves was detected in the vascular tissues: xylem parenchyma cells and phloem cells (Figure 7B). Stems of these transformed plants show high GUS activity in the phloem, in the parenchyma cells of the xylem, in the sclerifying parenchyma cells between the vascular bundles, and in the pericyclic cells (Figure 7C). In both leaf and stem, a cell layer surrounding the vascular bundle also shows visible GUS activity. When examining roots of pGUSSAM-1-transformed plants, most GUS activity is detected in the phloem and the parenchyma cells of the cortex and the secondary xylem (Figure 7, E and F).

By comparison, plants containing the pGUSATS-1 chimeric gene show very high levels of activity in the mesophyll cells of the leaf (Figure 8B). In the stem most GUS activity is found in the cortex parenchyma (Figure 8D). Considerable GUS activity is also detected in the vascular tissues of both stem and leaf. However, the activity in the vascular tissues appears to be lower than in pGUSSAM-1-transformed plants. This might explain why, at the macroscopical level, the veins of pGUSATS-1-transformed leaves show only GUS activity at the wound surface (Figure 8A). Most leaf and stem sections of pGUSATS-1-transformed plants show only a few epidermal cells with GUS activity, presumably representing guard cells. Roots have no detectable activity (Figure 8E). By in situ immunofluorescence, Aoyagi et al. (1988) have shown a similar expression pattern of the small subunit of ribulose-1,5-bisphosphate carboxylase in tobacco.

DISCUSSION

In this paper, the sequence of an AdoMet synthetase gene of *A. thaliana* is presented. The polypeptide sequence of AdoMet synthetase is highly conserved even among distantly related organisms. The predicted amino acid sequence, as deduced from the *sam*-1 gene, is 57% homologous to the yeast enzyme and 49% to the *E. coli* enzyme. The homology with the *E. coli* and yeast enzyme extends throughout the entire polypeptide sequence. Since the amino terminus of the polypeptide is particularly highly conserved between *Arabidopsis* and *E. coli*, it is clear that

Table 2. GUS Activity Using 20 μg of Crude Protein Extracts from Leaf, Stem, and Root of Transgenic Plants Transformed with pGUSSAM-1 and pGUSATS-1

	Leaf	Stem	Root
pGUSSAM-1-Transformed Plants			
C1	10	473	535
C6	30	260	ND[a]
C14	3	1020	370
C21	27	309	386
C24	6	350	430
C25	25	710	190
C1000	20	400	1180
pGUSATS-1-Transformed Plants			
B4	1130	980	34
B61	535	523	5
B132	532	320	8

[a] ND, not determined.

Reactions and fluorescence measurements of the GUS product (4-methylumbelliferone) were performed as described by Jefferson et al. (1987). The values shown are the fluorescence values measured after 30 min reaction time. One μM of 4-methylumbelliferone has a fluorescence value of 400.

the *sam*-1 gene does not code for an N-terminal transit peptide. Therefore, it seems unlikely that there would be import of the protein into chloroplasts or mitochondria.

AdoMet is needed in all living cells as methyl group donor. To this extent, the *sam*-1 gene can be considered as a housekeeping gene. Although most housekeeping genes are expected to be expressed constitutively, the *sam*-1 gene is not. RNA gel blot hybridizations of RNA from different organs show a 10- to 20-fold higher expression of the *sam*-1 gene in stems and roots (and callus tissue) compared with leaves, inflorescences, and seed pods. This mRNA distribution corresponds with a 10-fold difference of AdoMet synthetase activity in stems compared to leaves. This suggests that the expression of the enzyme is regulated primarily at the RNA level. To examine the expression pattern of the *sam*-1 gene in detail, a chimeric gene consisting of *sam*-1 promoter sequence fused to the coding region of the *E. coli* β-glucuronidase gene (*gus*) was introduced into the *Arabidopsis* genome. Transgenic plants containing this construct show 10 to 140 times more GUS activity in the stems and roots compared with leaves. These data indicate that the 5′ end of *sam*-1 is the major determinant for the differential expression pattern of the gene. When examining thin sections of roots, stems, and leaves of the transgenic *Arabidopsis* plants, high levels of GUS activity were observed in the xylem and the phloem throughout the whole plant, the sclerenchyma tissue between the vascular bundles in older parts of the stems, and in the parenchyma cells of the root cortex. Plants transformed with the *gus* gene under the control of the promoter of the *ats*1a gene encoding the small subunit of the ribulose-1,5-bisphosphate carboxylase contain, as expected, the highest GUS activity in the chloroplast-containing cells.

The strong cellular preference in the expression of the *sam*-1 gene seems to be, at least partly, correlated with the extent of lignification the tissues are undergoing: xylem, sclerenchyma tissue, and, to a lesser extent, the phloem are known to be highly lignified (Cutter, 1978). High expression of the *sam*-1 gene is presumably essential in lignifying tissues. Lignin is methylated prior to polymerization: the synthesis of each monolignol needs one or two AdoMet molecules (Higuchi, 1981). Consequently, high amounts of AdoMet must be consumed in cells that produce large amounts of lignin. For some enzymes involved in lignin biosynthesis, high expression in lignifying tissues has been shown. Phenylalanine ammonia-lyase, which is a key enzyme in the synthesis of various phenolic compounds, including lignin, is relatively highly expressed in lignifying tissues of various plant stems compared with young undifferentiated tissues (Haddon and Northcote, 1976; Higuchi, 1981). Similarly, it has been shown that the peroxidases, which are involved specifically in lignin biosynthesis (Syr-oxidases), are active mainly in lignifying tissues such as differentiating xylem and fibers (phloem and sclerenchyma) (Catesson et al., 1986). However, this hypothesis does not explain the high expression of the *sam*-1 gene in the root cortex. Since AdoMet also serves for purposes other than lignin biosynthesis, such as ethylene and polyamine biosynthesis and transmethylation of many different types of molecules, other factors might as well be responsible for the high expression of the *sam*-1 gene in certain of the described tissues. In addition, part of the AdoMet produced in the phloem might be transported to other tissues of the plant where enzyme activity is low.

Genomic blot data indicate that there is a second *sam* gene in *Arabidopsis*, a situation that is similar to that in yeast (Thomas and Surdin-Kerjan, 1987). Isolation of the other AdoMet synthetase gene of *Arabidopsis*, *sam*-2, will allow us to study the particular expression pattern of this copy and determine whether both genes are biologically equivalent or whether they are specialized to perform separate tasks. These data can provide greater understanding of the regulatory mechanisms underlying AdoMet synthetase expression in plants.

METHODS

Subclones and Riboprobes

From the originally isolated genomic clone pATC9A1 (Simoens et al., 1988), a 4-kb EcoRI fragment and a 0.78-kb PstI-SalI fragment containing *sam*-1 gene sequences were subcloned in the riboprobe system vector pGem1, yielding the plasmids pATC9A1-1 and pATC9A1-13. Riboprobes were prepared according to the Promega protocol (Promega Biotec).

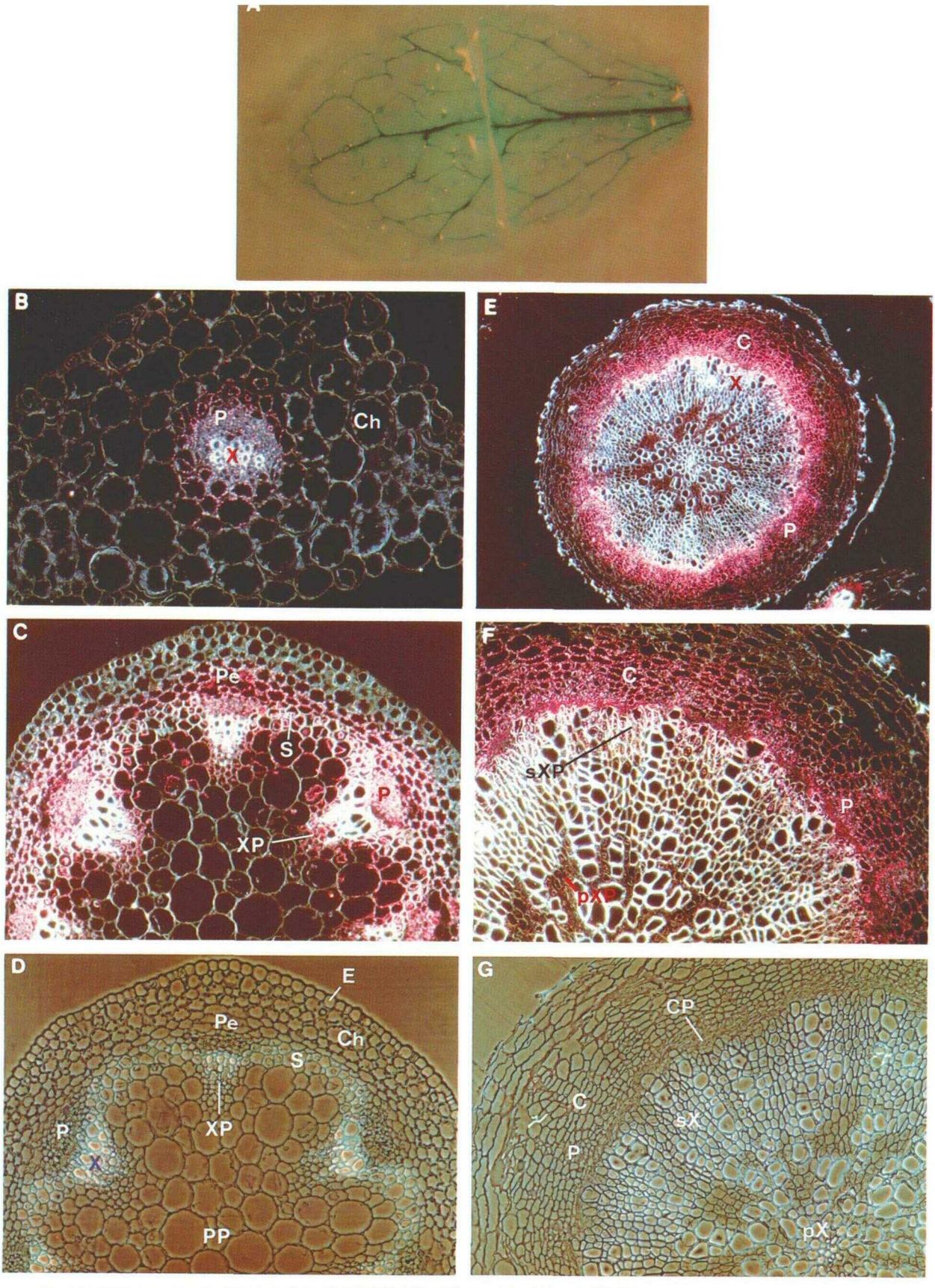

Figure 7. Histochemical Localization of GUS in pGUSSAM-1-Transformed *Arabidopsis* Plants.

(A) Bright-field image of leaf after staining with *X*-Gluc (see "Methods"). The green-blue precipitate represents regions of high GUS activity. Dark-field micrographs of transverse sections through leaf **(B)**, stem **(C)**, and roots **(E and F)**. The red-purple stem represents GUS activity. Phase-contrast image of transverse sections of stem **(D)** and root **(G)**. C, cortex parenchyma; Ch, chlorenchyma cells; CP, cambium and secondary phloem; E, epidermis; P, phloem; Pe, pericyclic cells; PP, pith parenchyma; pX, primary xylem; pXP, primary xylem parenchyma; S, sclerifying parenchyma; sX; secondary xylem; sXP, secondary xylem parenchyma; X, xylem; XP, xylem parenchyma.

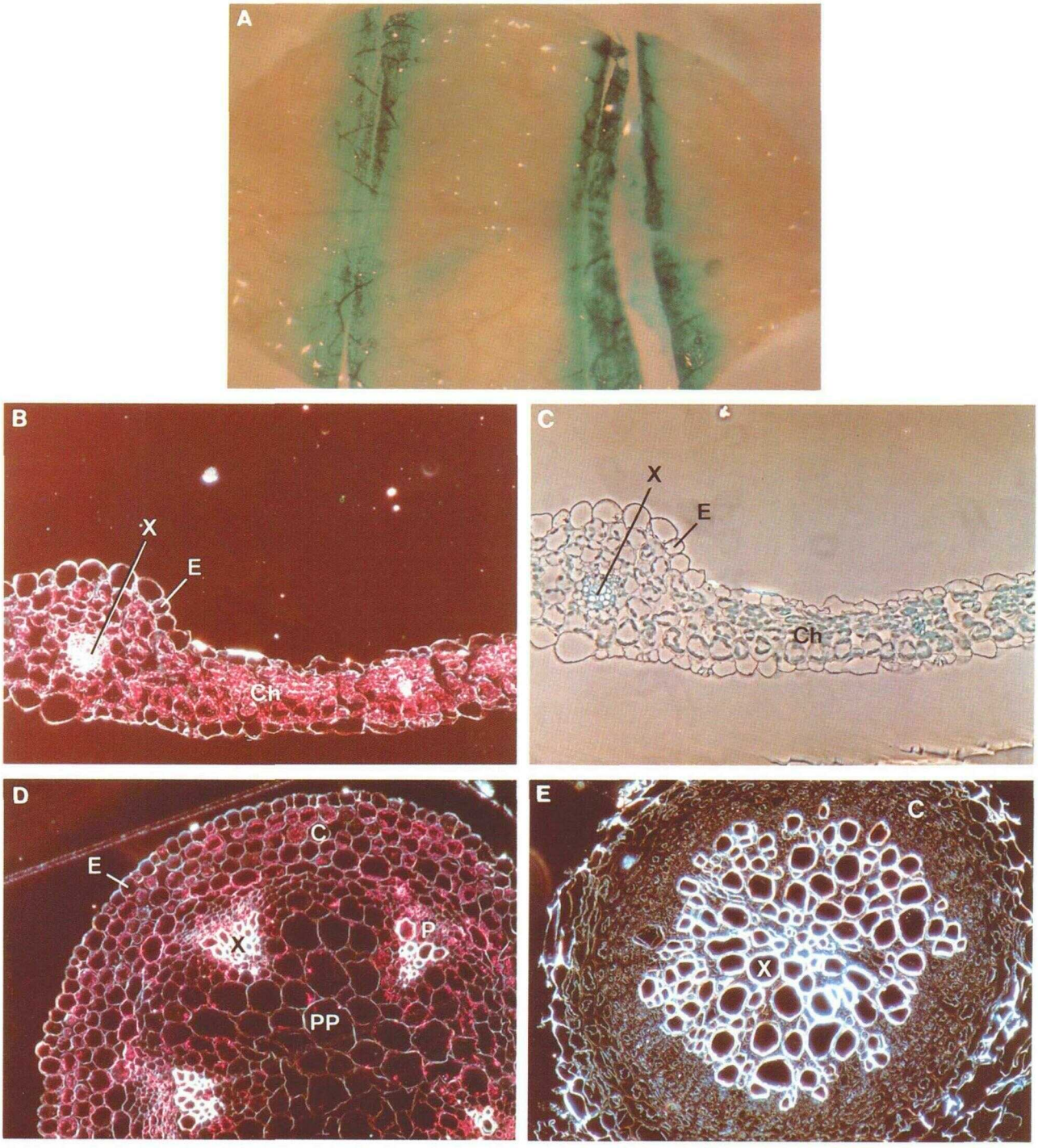

Figure 8. Histochemical Localization of GUS in pGUSATS-1-Transformed *Arabidopsis* Plants.
(A) Bright-field image of leaf after staining with *X*-Gluc (see "Methods"). Before *X*-Gluc incubation, the leaf was cut transversally to allow substrate infiltration. Dark-field micrographs of transverse sections through leaf **(B)**, stem **(D)**, and root **(E)**. Phase-contrast image of a transverse section of a leaf **(C)**. Abbreviations are as in Figure 7.

DNA and RNA Isolation

Total RNAs from different organs of *Arabidopsis thaliana* (var. Columbia) were prepared as described by Simoens et al. (1988). Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose affinity chromatography according to Slater (1984).

DNA was isolated from *Arabidopsis* plants according to the procedure described by Dellaporta et al. (1983). The *A. thaliana* variety used to isolate DNA was obtained from the Max-Planck-Institut für Züchtungsforschung (Köln, Federal Republic of Germany). This variety will be further referred to as var. K85. When using the *sam*-1 gene as a probe, DNA isolated from this variety shows restriction fragment length polymorphism with the DNA isolated from the Columbia variety. The restriction pattern of the *sam*-1 gene isolated from the genomic library (Simoens et al., 1988) is identical to the restriction pattern observed with DNA from the K85 variety.

Nucleic Acid Analysis

For DNA gel blots, DNA was digested with appropriate restriction enzymes and run on 0.7% agarose gels, followed by transfer to nylon filters. Prehybridization and hybridization were carried out in 0.5 M Na_2HPO_4/NaH_2PO_4 (pH 7.2), 3% SDS, 50 μg/ml heparin, and 0.5 mg denatured herring sperm DNA at 68°C using ^{32}P-labeled riboprobes. The final wash was carried out at 68°C in 3 × SSC, 0.5% SDS (low-stringency wash), or in 0.1 × SSC, 0.5% SDS (high-stringency wash), depending on the desired stringency condition, followed by autoradiography.

For RNA gel blots, 20 μg of total RNA was denatured and run on formaldehyde agarose (1.2%) gels, followed by transfer to nylon filters. Prehybridizations and hybridizations were carried out in 5 × SSC, 50% formamide, 5 × Denhardt's solution, 0.5% SDS, and 20 μg/ml denatured herring sperm DNA at 65°C using ^{32}P-labeled riboprobes. The final wash was carried out in 1 × SSC at 68°C.

As size marker, an RNA ladder (Bethesda Research Laboratories) was also run on the gel. After electrophoresis, this lane was submerged in a 10 mg/ml ethidium bromide solution for 30 min and then destained in water overnight.

S1 Mapping

S1 mapping was performed using the method of Berk and Sharp (1977) as modified by Weaver and Weissmann (1979).

Construction and Screening of a cDNA Bank

The poly(A)$^+$ mRNA used to construct the cDNA library was isolated from mature *A. thaliana* var. Columbia plants, grown in a 16-hr light/8-hr dark cycle at 24°C in a greenhouse. The RNA was isolated approximately 10 to 12 hr after the onset of daily illumination. Double-stranded cDNAs were synthesized and then cloned in the PstI site of pUC18 as described by De Loose et al. (1988).

The cDNA library was screened by colony hybridization essentially as described by Maniatis et al. (1982).

AdoMet Synthetase Assays

Proteins were extracted by grinding 1 g of stems and leaves in 0.5 ml of 2 × extraction buffer (100 mM Tris [pH 7.5], 2 mM EDTA, 20% glycerol, 20 mM β-mercaptoethanol, 1 mM dithiothreitol). Debris was removed by centrifugation in an Eppendorf centrifuge at 13,000 rpm for 5 min. The supernatant was used as a crude enzyme preparation. The protein concentration was determined by the method of Bradford (1976) using the kit supplied by the Bio-Rad Laboratories.

The AdoMet synthetase assays were essentially as described by the method of Mudd et al. (1965). One hundred μg of proteins were incubated in 0.25 ml of a reaction mixture containing 100 mM Tris (pH 8.0), 30 mM $MgSO_4$, 10 mM KCl, 20 mM ATP, and 5 mM ^{35}S-methionine (15 μCi). Control reactions contained all reactants except ATP. After incubation for 1 hr at 24°C, the reaction was terminated by adding 2 ml of ice-cold water, and the mixtures were loaded onto 0.67 × 30 cm Dowex AG 50W-X2 cation-exchange columns (NH_4^+ form). Unreacted methionine and ATP were eluted by washing with 10 ml of cold water. Adsorbed AdoMet was eluted with 5 ml of NH_4OH (30%). The NH_4OH was evaporated, 3 ml of universal scintillation liquid (PicoFluor, Packard) were added, and the sample was counted by scintillation spectrometry.

Construction of Chimeric Genes

A cassette was generated containing the *E. coli* β-glucuronidase coding sequence linked to the 3′-untranslated end of the octopine synthase gene. pOCS2 is a pUC19 plasmid (Yanisch-Perron et al., 1985) containing 700 bp of the 3′-untranslated end of the octopine synthase gene. This 3′ end was cloned as a PstI-HindIII fragment in the EcoRI-HindIII sites behind the *gus* coding sequence in pRAJ275 (Jefferson et al., 1987), yielding the plasmid pGUS1.

As a binary T-DNA vector for the transformation experiments, pGSC1706 was used (see Figure 6A). This vector is derived from pGV943 (Deblaere et al., 1987) and contains between the T-DNA borders a chimeric gene consisting of the neomycin phosphotransferase II coding sequence under the control of the cauliflower mosaic virus 35S promoter (Odell et al., 1985) and followed by the 3′-untranslated end of the T-DNA gene 7 (Velten and Schell, 1985). pGSC1706 also contains a polylinker fragment suitable for cloning between the T-DNA borders.

Construction of a Chimeric *Psam1-gus-3′ ocs* Gene

An NcoI restriction site was generated at the translation initiation codon of the *sam*-1 gene by site-directed mutagenesis according to the protocol of P. Stanssens and co-workers (in preparation). To do this, the 1.19-kb EcoRI-SalI fragment of the *sam*-1 gene was cloned in the EcoRI-SalI sites of pMC58 (P. Stanssens and co-workers, in preparation). The 20-mer oligonucleotide 5′-GTCTCCATGGTGCTACAAAG-3′, homologous to the *sam*-1 gene from position +16 to −4, was used to perform the site-directed mutagenesis. The 1.07-bp EcoRV-SalI fragment of the *sam*-1 gene in pATC9A1-13 was then exchanged by the mutagenized EcoRV-SalI fragment yielding the plasmid pATC9A1-13N. Subsequently, 748 bp of the *sam*-1 5′ sequences upstream from

the translation start were fused to the translation start codon of the β-glucuronidase gene by inserting the 751-bp PstI-NcoI fragment of pATC9A1-13N into the polylinker between the PstI and NcoI sites of pGUS1, yielding the plasmid pCRSGUS1.

Construction of a Chimeric *Pats1-gus-3′ ocs* Gene

A fragment containing 1731 bp of 5′ sequences upstream from the translation start of the *Arabidopsis ats*1a gene (coding for the small subunit of ribulose-1,5-bisphosphate carboxylase) (Krebbers et al., 1988) was fused at the translation initiation codon with the coding sequence of the *gus* gene. The pLKAB2 plasmid (D. De Almeida and co-workers, in preparation) containing the *ats*1a gene was cut with Asp^{718}, and the 5′ end was treated with T4 DNA polymerase in the presence of dATP and made blunt by S1 nuclease. Subsequently, the plasmid was cut with EcoRI and the resulting fragment containing 1731 bp of *ats*1a 5′ sequences was cloned into the NcoI site of pGUS1 yielding the plasmid pGUSS1.

Both chimeric P*sam1-gus*-3′ *ocs* and P*ats*1-*gus*-3′ *ocs* genes were cloned as a 3593-bp and 4592-bp PvuII fragment of pCRSGUS1 and pGUSS1, respectively, into the HindIII site of the polylinker of pGSC1706 yielding the plasmids pGUSSAM-1 and pGUSATS-1. These constructs were mobilized to *Agrobacterium* strain C58C1Rif^R containing the *vir* plasmid pGV2260 (Deblaere et al., 1985) by triparental mating (Van Haute et al., 1983).

Transformation of *Arabidopsis*

A. thaliana var. Columbia was transformed using root explants according to the method described by Valvekens et al. (1988).

β-Glucuronidase Assays

Fluorometrical GUS Assay

GUS assays with protein extracts of leaves, stems, and roots were carried out using the fluorometric assay procedure described by Jefferson et al. (1987). Protein concentrations of the various plant extracts were determined by the method of Bradford (1976) using the kit supplied by Bio-Rad Laboratories.

Histochemical GUS Assay

The histochemical localization of GUS in transformed plants was performed essentially as described by Jefferson et al. (1987) with some modifications.

Fresh plant material was cut by hand in small blocks of about 1 mm^3 and immersed immediately in a histochemical reaction mixture containing 0.5 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (*X*-Gluc, Research Organics, Inc., Cleveland, OH) in 100 mM sodium phosphate buffer (pH 7.3). *X*-Gluc was dissolved in ethylene glycol monomethyl ether at 50 mg/ml and then diluted into the reaction mixture. Cutting of larger plant organs into smaller blocks is crucial to allow proper substrate infiltration. Thin roots and small seedlings of *Arabidopsis* can be treated as a whole. The histochemical reaction was performed in the dark at 37°C until a blue indigo color appeared. To stop the reaction, tissues were rinsed several times in 100 mM phosphate buffer followed by fixation in a phosphate-buffered 1% glutaraldehyde solution for 3 hr at 4°C and a final rinse in phosphate buffer.

To allow a better visualization of the indigo stain, tissue blocks were cleared in 70% ethanol overnight to remove chlorophyll. Photographs from whole mounts of tissue blocks were taken at low power magnification using bright-field microscopy.

Thin sections of positively stained plant material were prepared according to the following protocol. After *X*-Gluc staining and rinsing in phosphate buffer, tissue blocks were dehydrated by immersion into a series of aqueous ethanol solutions from 30% to 95% and then in 100% ethanol for 2 hr at room temperature. A gradual infusion with the acrylic resin LR white (London Resin, London, United Kingdom) was carried out using ethanol/LR white mixtures of 2/1, 1/1, and 1/2 for 2 hr each at room temperature. A final infusion with 100% LR white was performed overnight at 4°C. Polymerization was done overnight in gelatin capsules at 60°C. After hardening, the specimens were cut into thin sections using glass knives fitted to a Reichert microtome Ultracut E. Care was taken during sectioning to ensure that only well-stained regions close to the surface of the tissue blocks were processed. Two- to 4-μm sections were picked up dry and transferred on drops of distilled water on a glass slide. Sections were flattened by moving the glass slides carefully in a flame until completely dry. Finally, specimens were mounted without counterstaining with DePeX mounting medium and a coverslip and were allowed to harden overnight. Photographs were taken by dark-field microscopy.

Miscellaneous Techniques

All recombinant DNA techniques not described above were performed according to Maniatis et al. (1982). The DNA sequence was determined on both strands by the procedure of Maxam and Gilbert (1980). Nucleotide comparison analysis was done using the Intelligenetics software package for molecular biologists.

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