Cysteine biosynthesis in plants: isolation and functional identification of a cDNA encoding a serine acetyltransferase from *Arabidopsis thaliana*

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Abstract **A cDNA encoding for serine acetyltransferase which catalyzes the committing step of cysteine biosynthesis has been cloned from *Arabidopsis thaliana*. The plant protein has a predicted molecular weight of 32.8 kDa and shows up to 43% of amino acid homology to bacterial serine acetyltransferases. It complements a serine acetyltransferase negative *E. coli* mutant and can be enzymatically determined in the heterologous host. The corresponding mRNA is predominantely expressed in light exposed tissue and represents one of at least two related genes.**

Key words: Cysteine synthesis; Serine acetyltransferase; Sulfate assimilation; Complementation; *Arabidopsis thaliana*

1. Introduction

The assimilation of sulfate represents one of the basic metabolic pathways in photoautotrophic organisms. In plants sulfur serves in oxidized forms in compounds such as sulfolipids and glucosinolates, but mainly is used in its reduced form in amino acids, vitamins, cofactors, and others [1,2]. The biochemical and molecular dissection of photosynthetic sulfate reduction [3,4] resulted in the isolation of cDNAs of several of the involved enzymes [5–9]. The final step of sulfate assimilation consists in the integration of free or bound sulfide into cysteine [3] catalyzed by *O*-acetylserine (thiol) lyase (OAS-TL) and has been extensively analyzed [10–15].

The activation of serine, a key reaction in the cysteine biosynthetic pathway, however, has been investigated at the molecular level only in prokaryotes [16–20]. The reaction is catalyzed by serine acetyl transferase (SATase) which acetylates serine with acetyl-coenzyme A to form *O*-acetylserine (OAS). OAS together with sulfide functions as substrate for cysteine synthesis by OAS-TL. In bacteria SATase and OAS-TL form a bifunctional complex called cysteine synthetase [21,22]. The bacterial SATase is constitutively expressed but is sensitive to feedback inhibition by cysteine [23].

In plants, SATase also exists in a complex with OAS-TL [24–26] and has only recently been purified to homogeneity from spinach chloroplasts [26]. The enzyme has been reported to be localized in plastids [26,27], the cytosol [27] and in the mitochondria [28,29]. In physiological experiments the availability of *O*-acetylserine has been found to be rate limiting for cysteine synthesis [30,31].

To gain an insight in the molecular properties and regulation of cysteine synthesis in plants we have isolated a cDNA encoding a SATase from *Arabidopsis thaliana*. The recombinant protein has been functionally identified and the genomic organization and expression are presented.

2. Materials and methods

2.1. Nucleic acid and computer analysis

Poly(A)$^+$ RNA from aerial parts of non-flowering *Arabidopsis thaliana* cv. Columbia plants was used to prepare a cDNA expression library in λZAP (Stratagene, La Jolla) that served as a template in PCR reactions and a source in cDNA screening procedures. PCR amplification of the 3′-end of the SATase sequence (SAT-EST) was performed as in [32] with two specific oligonucleotides as primers: SAT 36 (25-mer; 5′-TTTCTAGACTTCGCCGTCGATATTC-3′) and SAT 37 (25-mer; 3′-GATACCTGGTCTGTAGCGAGCTCTT-5′). The resulting product was cloned into pBS-SK$^+$ and sequenced from both strands by the didesoxy method using sequenase polymerase (US Biochemicals, Cleveland). cDNA library screening was as described in [15] except that hybridization and washing steps were performed at 60°C and that the PCR product SAT-EST was ^{32}P-labeled by PCR as a probe.

All standard molecular procedures and bacterial media were according to [33]. Cloning steps were performed with the *E. coli* strain XL-1 Blue (Stratagene). For DNA and protein analysis the Macvector sequence analysis software (IBI, New Haven) was employed. Homology searches of the EMBL/Genbank and expressed sequencing tags (EST) databases were carried out with the Entrez alignment program (NCBI, Bethesda). The EST clone 108J19T7 (accession number T23000) was obtained by courtesy of Dr. T. Newman at the Arabidopsis Biological Resource Center at Ohio State University, Columbus.

DNA and RNA hybridization analysis were carried out as described in [15]. RNA amounts blotted on nylon filters were subsequently hybridized with SATase cDNA and the 18S rRNA specific clone pPO-55 [34] to allow direct comparisons between lanes.

2.2. Complementation experiments and SATase activity determination

The SATase deficient *E. coli* strain EC1801 (*ΔtrpE5 leu-6 thi hsdR$^-$ hsdM$^+$ cysE*) was generously provided by Dr. N.M. Kredich, Duke University, Durham. For the complementation of SATase deficiency the first start codon of the cDNA SAT1–6 in pBS-SK$^-$ was cloned in frame with the reading frame of the *lacZ* gene. pSAT1–6 was restricted with *Not*I and *Eco*RI and protruding ends were filled with Klenow DNA polymerase. The religated plasmid ΔSAT1–6 was again restricted with *Eco*RI and then transformed via electroporation into EC1801. Transformed colonies were selected on LB medium with ampicillin and then transfered to VB [35] minimal medium that was supplemented with IPTG, glucose, leucine, tryptophane and thiamine.

The enzymatic activity of SATase was determined in a modified assay according to [36] by coupling with *O*-acetylserine (thiol) lyase and quantification of formed cysteine. Growth conditions, induction and protein extraction of bacteria were described elsewhere [15]. Protein extracts were desalted by gel filtration and 1 mg of soluble protein was assayed in a total volume of 0.5 ml that contained 50 mM Tris-HCl pH 7.6, 1 mM EDTA, 20 mM L-serine, 0.1 mM coenzyme A, 10 mM acetylphosphate, 5 units of phosphotransacetylase (Boehringer, Mannheim) and 5 mM Na_2S. The required excess of endogenous bacte-

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Abbreviations: SATase, serine acetyltransferase; OAS-TL, *O*-acetylserine (thiol) lyase; EST, expressed sequencing tag; IPTG, isopropyl-β-D-thiogalactoside.

The sequence reported in this paper has been deposited in the EMBL database under accession number X82888.

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rial OAS-TL activity was verified by separate OAS-TL enzyme determination [15]. The SATase reaction assay was preincubated for 10 min at 30°C to allow production of acetyl coenzyme A. The SATase reaction was started by addition of protein extract and continued for 20 min at 30°C.

2.3. Plant growth conditions

Arabidopsis thaliana cv. Columbia plants were cultivated under sterile conditions in a growth chamber at 24°C and under a 9 h light/15 h dark cycle with a light intensity of 40 W/m^2. The plants were grown on B5 medium with 1% (w/v) sucrose at a regular sulfate concentration of 2.006 mM and without addition of vitamins or hormones either in culture vessels with 0.4% (w/v) gelrite or in Erlenmeyer flasks with 50 ml liquid medium.

3. Results and discussion

3.1. Search and cloning of cDNAs encoding for SATase from Arabidopsis

Functional identity between procaryotic and eucaryotic proteins often correlates with significant structural similarity. Substantial amino acid as well as nucleic acid homology to their counterparts in bacteria and yeast has been shown for several enzymes from *Arabidopsis* that catalyze reactions of sulfate assimilation [7–9,15]. The *Arabidopsis* database of expressed sequencing tags was therefore searched at low stringency with the nucleic acid sequence of the *Salmonella typhimurium* SATase gene *cysE* [19] as a template. The EST accession T23000 matched the 3′ end of the *Salmonella* sequence with 64% similarity. In order to verify this homology the corresponding clone was obtained with the support of the Arabidopsis Biological Research Center and sequenced completely. The full sequence of this clone was found to be only 498 bp which covered approximately half of the coding region of the *Salmonella* gene.

The general presence of this sequence in *Arabidopsis* was proved by amplification and sequencing of a 380 base pair PCR fragment from an *Arabidopsis* λZAP cDNA library with primers derived from EST T23000. In order to obtain a full length cDNA this library was screened with the PCR product as a probe. The isolated clones SAT1–2, SAT2–1, SAT3–1 and SAT1–6 differed for about 300 bp in length. Sequencing of 5′ and 3′ ends revealed that SAT2–1 had the highest homology to

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1          GGCACGAGGACGATGAATCTGGCTTTCGTTAC ATG AAC TAC TTC CGT TAT
1                                           M   N   Y   F   R   Y

51  CCT GAT CGA TCT TCC TTC AAT GGA ACC CAG ACC AAA ACC CTC CAT ACT
7   P   D   R   S   S   F   N   G   T   Q   T   K   T   L   H   T

99  CGT CCT TTG CTT GAA GAT CTC GAT CGC GAC GCT GAA GTC GAT GAT GTT
23  R   P   L   L   E   D   L   D   R   D   A   E   V   D   D   V

147 TGG GCC AAA ATC CGA GAA GAG GCT AAA TCT GAT ATC GCC AAA GAA CCT
39  W   A   K   I   R   E   E   A   K   S   D   I   A   K   E   P

195 ATT GTT TCC GCT TAT TAT CAC GCT TCG ATT GTT TCT CAG CGT TCG TAT
55  I   V   S   A   Y   Y   H   A   S   I   V   S   Q   R   S   Y

243 GAA GCT GCG TTG GCG AAT ACT TTA TCT GTT AAA CTC AGC AAT TTG AAT
71  E   A   A   L   A   N   T   L   S   V   K   L   S   N   L   N

291 CTT CCA AGC AAC ACG CTT TTC GAT TTG TTC TCT GGT GTT CTT CAA GGA
87  L   P   S   N   T   L   F   D   L   F   S   G   V   L   Q   G

339 AAC CCA GAT ATT GTT GAA TCT GTC AAG CTA GAT CTT TTA GCT GTT AAG
103 N   P   D   I   V   E   S   V   K   L   D   L   L   A   V   K

387 GAG AGA GAT CCT GCT TGT ATA AGC TAC GTT CAT TGT TTC CTT CAC TTT
119 E   R   D   P   A   C   I   S   Y   V   H   C   F   L   H   F

435 AAA GGC TTC CTC GCT TGT CAA GCG CAT CGT ATT GCT CAT GAG CTT TGG
135 K   G   F   L   A   C   Q   A   H   R   I   A   H   E   L   W

483 ACT CAG GAC AGA AAA ATC CTA GCT TTG TTG ATC CAG AAC AGA GTC TCT
151 T   Q   D   R   K   I   L   A   L   L   I   Q   N   R   V   S

531 GAA GCC TTC GCT GTT GAT TTC CAC CCT GGA GCT AAA ATC GGT ACC GGG
161 E   A   F   A   V   D   F   H   P   G   A   K   I   G   T   G

579 ATT TTC GCG CTA GAC CAT GCT ACG GCT ATT GTG ATC GGT GAG ACG GCG
177 I   F   A   L   D   H   A   T   A   I   V   I   G   E   T   A

627 GTT GTG GGG AAC AAT GTT TCG ATT CTC CAT AAC GTT ACG CTT GGA GGA
193 V   V   G   N   N   V   S   I   L   H   N   V   T   L   G   G

675 ACG GGG AAA CAG TGT GGA GAT AGG CAC CCG AAG ATT GGC GAT GGG GTT
209 T   G   K   Q   C   G   D   R   H   P   K   I   G   D   G   V

723 TTG ATT GGG ACT TGT ATT TTG GGG AAT ATC ACG ATT GGT GAA GGA GCT
225 L   I   G   T   C   I   L   G   N   I   T   I   G   E   G   A

771 AAG ATT GGT GGG GGG TCG GTG GTG TTG AAA GAG GTG CCG GGG GGT ACG
241 K   I   G   G   G   S   V   V   L   K   E   V   P   G   G   T

819 ACG GTT GTT GGA AAT CCG GCG AGG TTG CTT GGT GGT AAA GAT AAT CCG
257 T   V   V   G   N   P   A   R   L   L   G   G   K   D   N   P

867 AAA ACG CAT GAC AAG ATT CCT GGT TTG ACT ATG GAC CAG ACG TCG CAT
273 K   T   H   D   K   I   P   G   L   T   M   D   Q   T   S   H

915 ATA TCC GAG TGG TCG GAT TAT GTA ATT TGA AAAAGTCTTTGTTTTTGGTTGTT
289 I   S   E   W   S   D   Y   V   I   *

968 GTTGTTGTTTATGGCTTTTCATTGTTCTCTCGGCTTCTTCTGTTATTGAAGCTGGTGAGGTAT

1031 ATGATATGCGATGATGCTGTTTCCATAAAAAAAAAAAAAAAAAAA
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Fig. 1. DNA sequence of the cDNA clone SAT1–6 from *Arabidopsis thaliana*. The derived amino acid sequence of the longest open reading frame is shown in italics.

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Arabidopsis t.  MNYFRYPDRSSFNGTQTKTLHTRPLLEDLDRDAEVDDVWAKIREEAKSDIAKEPIVSAYYHASIVSQRSYEAALANTLSVKLSNLNLPSNTLFDLFSGVL  100
Salmonella t.                                MPECEELEI..KN.KA..RALADC..MLASF...TLLKHENLGS..SYM.AN..ASPIM.AIAIREVVEEAY   72
Buchnera a.                                  MCSLEELEL-.NM.KHK.QKILK....L.NF.QK..LNHKKLSHS.SCI..D...TSMISEKDIYNI.NKIY   71

                                                                                                  ..          ..
Arabidopsis t.  QGNPDIVESVKLDLLAVKERDPACISYVHCFLHFKGFLACQAHRIAHELWTQDRKILALLIQNRVSEAF-AVDFHPGAKIGTGIFALDHATAIVIGETAV  199
Salmonella t.   AAD.EMIA.AAC.IQ..RT....VDK.STPL.YL...H.L..Y..G.W..NKG.RA..IFL..Q..VS.-Q..I..A....R..M-.....G..V.....  170
Buchnera a.     AN.IS.IN..VK.IK.ASQ...VVKH.LTPL.YL...H.LE.Y.LS.Y..NIK.YE.SAYL.S.I.TV.-S..I..A.S..S..M-.....G.....GVI  169
Bacillus s.        MFFRML.E.IDT.FDQ...AR..FEVI.TYS.LH.IW......A.YKRKFYF..R..-SQ..RF.TGIEI....T..RRF.-I..GMGV.....CE   94
Azotobacter c.   MSLLAQWREDIRCVE.....ARTTFEVLTTYP.VH.IMLY.L..R..RPNALPRPAAV-V.ARLVS-N..I....V..ARF.-I..GACV......E   94

                 ..    ..    ..          ..    .    .    ..    ..    ..    ..    .    ..
Arabidopsis t.  VGNNVSILHNVTLGGTGKQCGD-RHPKIGDGVLI--GTCILGNITIGEGAKIGGGSVVLKEVPGGTTVVGNPARLLGGKDNPKTHDKIPGLTMDQTSHIS  296
Salmonella t.   IEDD....QS........TS..-.....RE..M.GA.AK.....EV.R.....A.....QP..PH..AA.V...IV-..--.-GS..-.SMD...HFNGI  264
Buchnera a.     IE.D...F.S.......SNT.KN...I.RKN.T.GA.AK.....EV.Q.V.V.A..I...NI.PFV....V..KIIKKIKNSNKNLFQKEKK          261
Bacillus s.     I....TVFQG........EK.K-...T.K.DA..AT.AKV..S..V...S...A.....HD..DFS....I.G.VVVQNGKKVRR.LNHQDLPDPVADRF  193
Azotobacter c.  I.RD.TLY.G......TGAK.K-...TL..V..VGA.AK...P....AN.RV.AN...VQD..E.C....I.GKVVKLREAGQ-LNPY-.IDL.HHLIPD  191

Arabidopsis t.  EWSDYVI                                                                                            303
Salmonella t.   HHTFEYGDGI                                                                                         274
Bacillus s.     KSLEQQILELKAELEDRKERINQK                                                                           217
Azotobacter c.  PVGKAIACLLERIDSLEKRVEAGGLVAAAASSTFYEGCNPDNSICETNLRRSAPWSSGRPRRPAHAGDRVSGRAKGSD                        269
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Fig. 2. Alignment of the derived amino acid sequence of *Arabidopsis* SAT1–6 and serine acetyltransferases from *Salmonella typhimurium* [19], *Buchnera aphidicola* [20], *Bacillus subtilis* [16] and *Azotobacter chroococcum* [17]. Amino acids identical to *Arabidopsis* are indicated by peroids. Dots above the *Arabidopsis* sequence mark the positions one and two of the transferase hexamer motif [37].

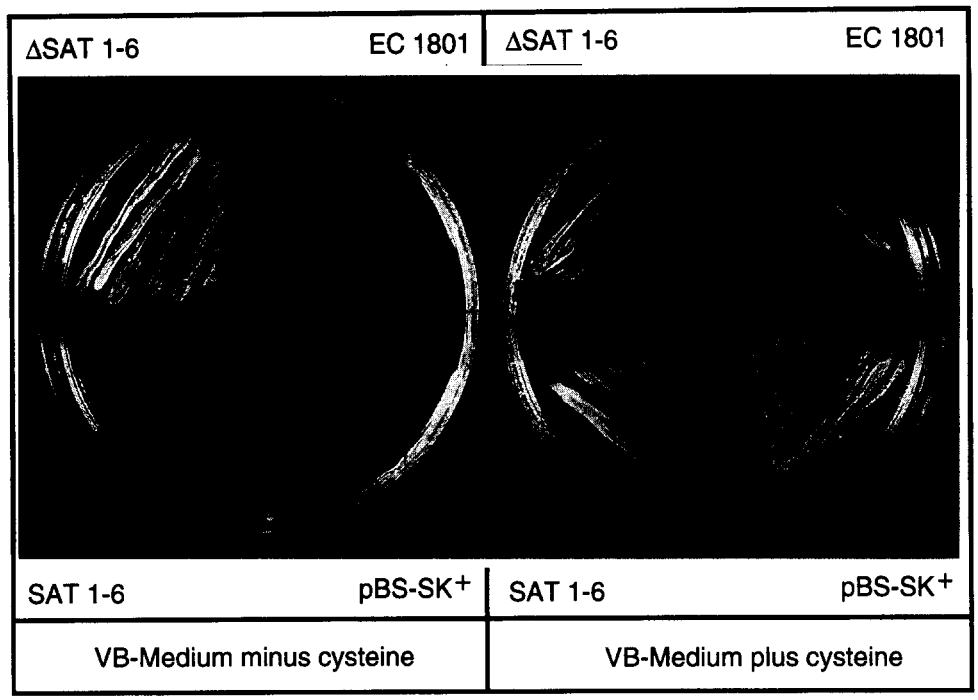

Fig. 3. Complementation of the serine acetyltransferase (*cysE*) deficient *E. coli* strain EC1801. ΔSAT1–6, *Arabidopsis* SAT1–6 in frame with *lacZ*; SAT1–6, *Arabidopsis* SAT1–6 out of frame with *lacZ*; pBS-SK$^+$, wild-type plasmid; EC1801, mutant strain without plasmid.

EST T23000, but was clearly shorter than the other 3 clones. SAT1–2, SAT3–1 and SAT1–6 were identical, with SAT1–6 being the only one that exceded the 5′ end of the *Salmonella* SATase gene (Fig. 1). At the nucleic acid level the homolgy between *Salmonella* and the complete sequence of *Arabidopsis* SAT1–6 was found to be 49% for the overlapping regions. The *Arabidopsis* cDNA carried a continous open reading frame starting with an ATG from nucleotide position 33 to 941 that potentially encodes for a protein of 303 amino acids with a calculated molecular weight of 32818 Dalton and a pI of 6.18. This molecular weight correlates with those found for the purified SATases from *Allium tuberosum* and spinach [25,26]. In this reading frame there was no stop codon 5′ of the ATG and no other methionine appears in the translated amino acid sequence. From these data, however, it can not be excluded that the coding region extends further upstream of the cDNA.

3.2. *Amino acid analysis and comparison of* Arabidopsis *SAT1–6*

An amino acid comparison of the derived amino acid sequence of *Arabidopsis* SAT1–6 with SATase sequences of bacterial origin [17,19,20] is presented in Fig. 2 in the order of the observed homolgy. It is interesting to note that SAT1–6 exceeds the amino termini of the four bacterial proteins for at least 28 amino acids, but is shorter at the carboxy terminal end, in particular when compared with the *Azotobacter* sequence. The amino acid identity of *Arabidopsis* SAT1–6 to the bacterial SATases ranges between 36% and 43% for the overlapping central region. The *Salmonella* sequence is closest related to *Arabidopsis* both in terms of homology and size.

Since the bacterial proteins catalyze the same reaction it seems reasonable to assume that the functional domain is confined to the overlapping central region [17,20]. Within this region a putative transferase domain has been proposed [37] which consists of imperfect repeated hexamer motifs of the sequence I/L/V G X G/D/N A/A X. Indeed these repeats can be found in the corresponding region of the *Arabidopsis* SAT1–6 sequence that, in accordance with the bacterial transferases, has a good probability to form β-sheet structures.

The amino terminal 28 amino acid extension of SAT1–6 could represent an organelle target sequence since a part of the SATase activity is known to be localized in the chloroplast [26, 27]. Although the domain has a 22% serine/threonine content and carries a positive net charge it does not fully correspond to the typical features of transit peptides as defined in [38–40]. The lack of an extensive carboxy terminal domain such as in *Azotobacter* might be due to a specialized role of the described *Azotobacter* SATase encoded by the *nifP* gene [17]: strains with

Table 1
Serine acetyltransferase activities of bacterial protein extracts

Enzyme assay	Protein extract	nmol cysteine (mg protein · 20 min^{-1})
Minus Na_2S	EC 1801 with pΔSAT	0
Minus serine	EC 1801 with pΔSAT	0.2
Minus protein	–	0.5
ΔSAT	EC 1801 with pΔSAT	51.0
Bluescript cysE mutant	EC 1801 with pBS-SK$^+$	0.5
Bluescript cysE wild type	XLI-Blue with pBS-SK$^+$	72.1

Specific activities of recombinant and endogenous SATases represent mean values from measurements of three independent experiments each. Standard deviations were lower than ± 10%.

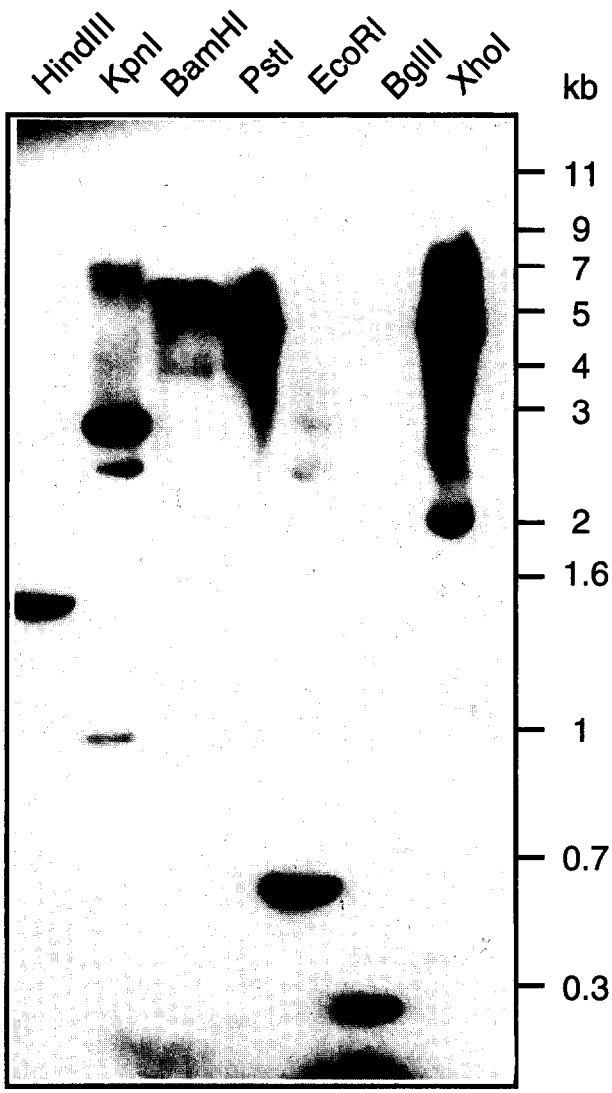

Fig. 4. Southern analysis of *Arabidopsis thaliana* cv. Columbia DNA. 8 μg of genomic DNA per lane was hybridized with the complete cDNA SAT1–6.

an inactivated *nifP* locus seem to exhibit a phenotype only in response to nitrogen supply.

3.3. *Functional complementation of an* E. coli *SATase mutant with* Arabidopsis *SAT1–6*

The functional identity of the protein encoded by the cDNA SAT1–6 was confirmed by heterologous complemetation of the serine acetyltransferase negative *E. coli* strain EC1801 (Fig. 3). The open reading frame of SAT1–6 was cloned in frame with the *lacZ* gene of pBS-SK$^-$ yielding ΔSAT1–6 and, after induction with IPTG, the protein was expressed as an aminoterminal fusion with β-galactosidase. EC1801 was transformed with ΔSAT1–6 and prototrophic colonies were selected with ampicillin and subsequently on minimal medium without cysteine. All colonies that carried pΔSAT1–6 were able to grow in the absence of cysteine, whereas controls with EC1801 cells that were not transformed or either transformed with pSAT1–6 (not in frame) or pBS-SK were unable to grow without addition of cysteine.

The identity of the *Arabidopsis* ΔSAT1–6 encoded protein as a serine acetyltransferase was further verified by determination of the enzymatic activity in a coupled assay with O-acetylserine (thiol) lyase which was provided by the bacteria (Table 1). Serine acetyltransferase activity could be demonstrated only from EC1801 cells which expressed ΔSAT1–6. Neither the β-galactosidase fusion part nor the amino terminal domain of the *Arabidopsis* protein appeared to prevent the catalytic activity, although it was lower than the *cysE* encoded activity in *E. coli* XL-1 Blue. Therefore, the cDNA SAT1–6 represents a serine acetyltransferase from *Arabidopsis thaliana* which is able to provide the substrate *O*-acetylserine for cysteine biosynthesis.

3.4. *Genomic organization and expression pattern of the* Arabidopsis *SATase SAT1–6*

Genomic DNA from *Arabidopsis thaliana* cv. Columbia was restricted with 7 restriction enzymes (Fig. 4) and subjected to Southern analysis. Performed at high stringeny the hybridization patterns and intensities suggest the presence of at least two genes that are structurally not too closely related. It is possible that the cDNA SAT2–1 represents such an isoform since it was only about 75% homologous to SAT1–6 at the nucleic acid level. The existence of a second SATase protein is also indicated by localization experiments with spinach leaves where cytosolic and chloroplast SATase activities have been demonstrated [27].

The expression of SAT1–6 was investigated by Northern analysis under various aspects (Fig. 5). In order to compare signals obtained from different tissues and conditions the amounts of 18S rRNA were determined on the same filters. The complete cDNA of SAT1–6 hybridized with a RNA of about 1.1 kb which suggests that SAT1–6 represents a full length cDNA with respect to the open reading frame. Hybridization with RNA isolated from etiolated seedlings and etiolated ones that were transfered to the light showed an increase of SAT1–6 mRNA in the light treated tissue. Comparison of SAT1–6 mRNA from roots and shoots clearly demonstrated a predominant expression in the green tissue. These findings indicate a possible chloroplast localization for the SAT1–6 protein, since light seems to determine the SAT1–6 mRNA levels. It is important to note, however, that SAT1–6 is also expressed at low levels in heterotrophic tissue as has been found for the plastid isoform of OAS-TL from *Arabidopsis* [15].

The expression of the *Arabidopsis* SAT is also influenced by the sulfate supply of the plant. Twenty hours after transfer of

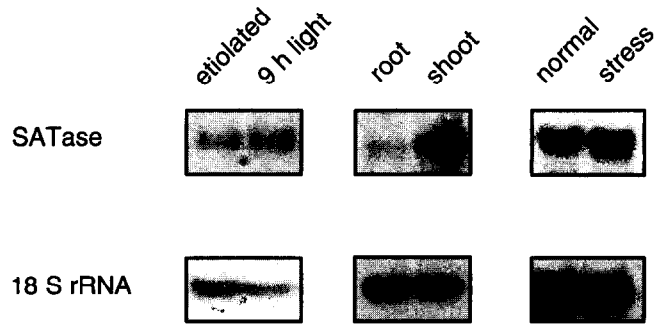

Fig. 5. Northern analysis of *Arabidopsis* SAT1–6. The entire cDNA was hybridized with 10 μg of total RNA from the tissues as indicated. Left, etiolated seedlings; center, roots and aerial parts from light grown seedlings; right, whole plants grown at 2.006 mM sulfate (normal) and after transfer to 0.006 mM sulfate (stress).

plants from 2 mM sulfate to 0.006 mM sulfate an 1.5- to 2-fold increase of SAT1–6 mRNA was observed when compared to 18S rRNA. This result was confirmed in independent experiments and correlates with the data for the *Arabidopsis* OAS-TLs [15]. The cDNA presented here thus encodes for a SATase that might be plastid localized and whose expression is regulated by light and sulfur deficiency.

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