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Isolation and characterization of two cDNAs encoding for compartment specific isoforms of *O*-acetylserine (thiol) lyase from *Arabidopsis thaliana*

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Abstract cDNAs encoding for two isoforms of *O*-acetylserine (thiol) lyase (OAS-TL), which catalyzes the synthesis of cysteine, have been isolated from *Arabidopsis thaliana*. Secondary structure together with expression patterns derived during photomorphogenesis indicate cellular localizations in the cytosol and plastids, thus allowing a direct comparison of compartment-specific forms within one species. The cytosolic OAS-TL complemented an *E. coli* auxotrophic mutant lacking cysteine synthesis. Both isoforms are represented by small gene families. They are expressed under all conditions investigated and were observed to increase in expression in plants grown with limited sulfate supply.

Key words: Cysteine synthesis; Sulfate assimilation; *O*-Acetylserine (thiol) lyase; *Arabidopsis thaliana*

1. Introduction

The synthesis of cysteine represents the essential process of integration of reduced sulfur into the metabolism of the plant cell. Free or bound sulfide reacts in the final step of sulfate assimilation with *O*-acetylserine to form cysteine and acetate [1]. The reaction is catalyzed by *O*-acetylserine (thiol) lyase (OAS-TL, EC 4.2.99.8; also called cysteine synthase) which has been purified from plants such as spinach [2,3], bell pepper [4], onion [5], and chive [6]. The enzyme consists of a homodimer with subunits of 32–36 kDa, each carrying one pyridoxal phosphate as a cofactor. It has recently been cloned from bell pepper [4], spinach [7–9,10] and wheat [11]. OAS-TL activity has been shown to exist in about equal amounts in the chloroplasts, the cytosol, and to a minor extent in mitochondria in leaves of spinach as well as in heterotrophic tissue of cauliflower [12,13]. Therefore, plants can synthesize cysteine in all compartments where protein synthesis and turnover takes place.

Besides its role in sulfate assimilation, little is known about the function of OAS-TL, particularly the non-chloroplast isoforms. For one, OAS-TL may use free sulfide that is released from catabolism of proteins and breakdown of storage compounds such as glucosinolates, which exist in *Arabidopsis thaliana* in more than 20 different forms [14]. Furthermore, OAS-TL was shown to synthesize not only cysteine but also cysteine derivatives and heterocyclic β-substituted alanins in spinach and chive [2,6]. These functions may be attributed to cytosolic isoforms as well as OAS-TL in proplastids and chloroplasts.

We are interested in the compartment-specific role of OAS-TL in the genetic model plant *Arabidopsis thaliana*. To this end cDNAs encoding a cytosolic and a plastid localized OAS-TL have been isolated and characterized with respect to genomic organization and enzymatic activity. The expression of the corresponding genes is described in organs, during greening and at sulfur deficiency.

2. Materials and methods

2.1. PCR amplification, cDNA screening and DNA sequencing analysis

A cDNA library constructed from poly(A)$^+$ RNA from aerial parts of non-flowering *Arabidopsis thaliana* cv. Columbia in the vector λZAP (Stratagene, La Jolla) was used as template in PCR and cDNA screening. For the generation of OAS-TL DNA fragments four synthetic degenerate oligonucleotide primers were designed:
F14 (18-mer; 5′-GGATCCGTC/G/TAAIGACA/CGG/CATT/A/AGG-3′), F15 (18-mer; 5′-GGATCCGAGCCT/C/GACC/GAT/GC/GGGTAA-3′), R16 (18-mer; 3′-CGA/T/G/CTTC/TCCITACTCT/CCCTTCGAA-5′) and R17 (18-mer; 3′-ACA/C/T/GGGICCG/A/CGAG/AATT/CTGGTTCGAA-5′).

The PCR reaction was performed according to [15]. All DNA sequences were determined on both strands by the dideoxy method using sequenase polymerase (US Biochemicals, Cleveland).

cDNA library screenings were performed with the ^{32}P-random prime labeled PCR products as probes. Hybridization of the filters (Hybond N, Amersham, Braunschweig) was carried out according to [16] with 10 μg/ml herring sperm DNA at 55°C. Washing steps were performed in 2 ×, 1 × and 0.5 × SSC, respectively, and 0.1% SDS at 55°C. Plasmids pBS-SK$^-$ carrying the cDNA inserts At.OAS.5-8 and At.OAS.7-4 were isolated from positive phages via in vivo excision. Standard molecular techniques, buffers and bacterial media were carried out according to [17]. DNA and protein analysis was performed with Macvector (IBI, New Haven) and Entrez (NCBI, Bethesda) sequence analysis software.

2.2. Heterologous complementation and determination of enzymatic activity

The insert of pAt.OAS.5-8 was isolated by *Xho*I restriction between position 65 of the cDNA and 672 of the polylinker and cloned into the *Sal*I site of pEXP2 [18], thereby regaining an ATG start-codon in sense orientation to the *Taq* promoter. The resulting plasmid pEXP5-8 was transformed into the cysteine auxotroph *E. coli* strain NK3 (*ΔtrpE5 leu6 thi hsdR hsdM cysK cysM*; generously provided by Dr. N. Kredich, Duke University, NC, USA) and selected on M9 agar plates supplemented with IPTG, leucine, tryptophan and thiamine.

For determination of OAS-TL activity a saturated culture of transformed NK3 was used to inoculate 1:100 LB medium containing 100 μg/ml ampicillin. 10 mM IPTG was added after one hour and the

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Abbreviations: OAS-TL, *O*-acetylserine (thiol) lyase; IPTG, isopropyl-β-D-thiogalactoside.

The sequences reported in this paper have been deposited in the EMBL database under Accesion Numbers X80376 (At.OAS.5-8) and X80377 (At.OAS.7-4).

SSDI 0014-5793(94)00872-8

A. At.OAS.5-8

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ATT TTC TTC AAA ACG ATT CGG GTC AGG TTA TTG ACT TTC TCA TTC AGT  48
GAA GCT TGA ATC ATG GCC TCG AGA ATT GCT AAA GAT GTG ACT GAA TTG  96
                M  A  S  R  I  A  K  D  V  T  E  L
ATT GGG AAC ACC TTA TTG GTG TAT TTG AAC AAT GTT GCT GAA GGA TGT 144
 I  G  N  T  L  L  V  Y  L  N  N  V  A  E  G  C
GTT GGT CGT GTT GCT GCT AAG CTT GAG ATG ATG GAA CCG TGC TCT AGT 192
 V  G  R  V  A  A  K  L  E  M  M  E  P  C  S  S
GTC AAA GAC AGG ATT GGT TTT AGT ATG ATT TCT GAT GCT GAG AAG AAG 240
 V  K  D  R  I  G  F  S  M  I  S  D  A  E  K  K
GGT CTT ATC AAA CCA GGA GAG AGT GTG CTG ATT GAG CCA ACA AGT GGG 288
 G  L  I  K  P  G  E  S  V  L  I  E  P  T  S  G
AAC ACT GGA GTT GGG ATT GCA TTC ACG GCA GCT GCC AAA GGC TAC AAG 336
 N  T  G  V  G  I  A  F  T  A  A  A  K  G  Y  K
CTT ATT ATT ACA ATG CCA GCT TCT ATG AGT ACT GAG AGA AGA ATC ATT 384
 L  I  I  T  M  P  A  S  M  S  T  E  R  R  I  I
CTC TTA GCT TTT GGA GTT GAG TTG GTT TTA ACT GAC CCA GCT AAG GGC 432
 L  L  A  F  G  V  E  L  V  L  T  D  P  A  K  G
ATG AAA GGA GCT ATC GCA AAG GCG GAA GAG ATT TTG GCG AAA ACA CCC 480
 M  K  G  A  I  A  K  A  E  E  I  L  A  K  T  P
AAT GGT TAC ATG CTT CAG CAG TTT GAG AAC CCT GCC AAC CCT AAG ATC 528
 N  G  Y  M  L  Q  Q  F  E  N  P  A  N  P  K  I
CAC TAT GAG ACT ACG GGA CCT GAG ATA TGG AAA GGC ACT GGT GAC AAA 576
 H  Y  E  T  T  G  P  E  I  W  K  G  T  G  D  K
ATC GAC ATC TTT GTT TCT GGG ATT GGT ACT GGC ATT ACA GGT GCT GGG 624
 I  D  I  F  V  S  G  I  G  T  G  I  T  G  A  G
AGT ATC TTA AAG AAC AGA ACG GCA AAC GTC AAG CTG TAT GGA GTG GAG 672
 S  I  L  K  N  R  T  A  N  V  K  L  Y  G  V  E
CCA GTT GAA AGT GCT ATT CTA ATC GGT GGG AAG CCA GGT CCT CAC AAG 720
 P  V  E  S  A  I  L  I  G  G  K  P  G  P  H  K
ATT CAA GGG ATA GGA GCT GGT TTT ATA CCA AGT GTA TTG AAT GTT GAT 768
 I  Q  G  I  G  A  G  F  I  P  S  V  L  N  V  D
CTT ATT GAC GAA GTT GTT CAG GTT TCA AGT GAT GAA TCC ATT GAC ATG 816
 L  I  D  E  V  V  Q  V  S  S  D  E  S  I  D  M
GCA AGG CAG CTT GCT CTT AAA GAA GGC TTT CTT GTG GGA ATA TCA TCC 864
 A  R  Q  L  A  L  K  E  G  F  L  V  G  I  S  S
GGT GCA GCA GCT GCT GCA GCA ATT AAA CTT GCA CAG AGG CCA GAA AAC 912
 G  A  A  A  A  A  A  I  K  L  A  Q  R  P  E  N
GCT GGG AAG CTA TTT GTG GCG ATA TTC CCG AGT TTC GGG GAG AGG TAT 960
 A  G  K  L  F  V  A  I  F  P  S  F  G  E  R  Y
CTA TCA ACG GTA CTT TCG ATG CGA CAA GGA AAG AAG CGG AAG CCA TGA 1008
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CCT TCG AGG CTT GAA CAT TCT CCA TTT CTT CTT AAG AGA CGC CAA AAT 1056
AAA AGA GAT GTT CAG TTT CTC CTA TAG AGA CTC TTC ATC TTT AGT TAC 1104
ATT GCG TCT TTG CTT CCA TCT GTA TCT TCT CTT GTG TGC CAA TAA AAG 1152
TCA AAC TAG ATT TTC TCT GTT TCT TTT GTG AAC CAC TTG CTT GTT AAT 1200
GAA GTT TAA ATT TGC TAA AAA AAA AAA AAA AAA A 1234
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B. At.OAS.7-4

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AAA GAG CAA GTC ATG GCG GCG ACA TCT TCC TCT GCT TTT CTC CTT AAT  48
                M  A  A  T  S  S  S  A  F  L  L  N
CCG TTG ACT TCT CGC CAC CGT CCT TTC AAA TAC TCG CCA GAG CTC TCT  96
 P  L  T  S  R  H  R  P  F  K  Y  S  P  E  L  S
TCT CTC TCC TTA TCC TCT CGA AAG GCT GCT GCT TTC GAT GTT TCC TCA 144
 S  L  S  L  S  S  R  K  A  A  A  F  D  V  S  S
GCT GCT TTC ACG CTC AAG AGA CAG AGC CGG AGT GAT GTT GTG TGC AAG 192
 A  A  F  T  L  K  R  Q  S  R  S  D  V  V  C  K
GCT GTA TCT ATC AAG CCA GAA GCT GGT GTT GAA GCG CTC AAT ATC GCC 240
 A  V  S  I  K  P  E  A  G  V  E  A  L  N  I  A
GAT AAC GCC GCT CAG CTT ATT GGG AAA ACT CTG ATG GTG TAC TTG AAC 288
 D  N  A  A  Q  L  I  G  K  T  L  M  V  Y  L  N
AAT GTA CGT CAA GGC TGT GTT GCA AGT GTT GCT GCT AAG CTT GAG ATC 336
 N  V  R  Q  G  C  V  A  S  V  A  A  K  L  E  I
ATG GAA CCA TGT TGC AGT GTC AAG GAT AGG ATT GGG TAC AGT ATG ATT 384
 M  E  P  C  C  S  V  K  D  R  I  G  Y  S  M  I
ACT GAT GCT GAA GAG AAA GGA CTT ATA ACA CCT GGA AAG AGT GTT CTT 432
 T  D  A  E  E  K  G  L  I  T  P  G  K  S  V  L
GTG GAA TCT ACG AGT GGG AAC ACA GGG ATT GGC CTT GCA TTC ATT GCT 480
 V  E  S  T  S  G  N  T  G  I  G  L  A  F  I  A
GCT TCA AAA GGC TAT AAG CTT ATC TTG ACG ATG CCT GCG TCC ATG AGT 528
 A  S  K  G  Y  K  L  I  L  T  M  P  A  S  M  S
TTG GAA AGG CGG GTT CTT TTG AGG GCA TTT GGA GCT GAG CTT GTG TTA 576
 L  E  R  R  V  L  L  R  A  F  G  A  E  L  V  L
ACT GAA CCT GCA AAA GGT ATG ACT GGA GCA ATT CAG AAG GCT GAA GAA 624
 T  E  P  A  K  G  M  T  G  A  I  Q  K  A  E  E
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 P  A  N  P  K  I  H  Y  E  T  T  G  P  E  I  W
GAA GAT ACA AGA GGC AAA ATC GTC ATA TTG GTT GCG GGG ATT GGA ACT 768
 E  D  T  R  G  K  I  V  I  L  V  A  G  I  G  T
GGT GGA ACT ATC ACT GGT GTT GTC GAT TCA TTA AAG AAA GCA AAA CCT 816
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 E  L  K  V  I  G  V  E  P  T  E  S  L  Y  F  L
GTG GAA AAC CCC GGA CCT CAC AAG ATT CAA GGA ATT GGA GCT GGA TTT 912
 V  E  N  P  G  P  H  K  I  Q  G  I  G  A  G  F
GTA CCC AAG AAT TTG GAT CTG GCT ATT GTA GAT GAA TAC ATA GCG ATT 960
 V  P  K  N  L  D  L  A  I  V  D  E  Y  I  A  I
TCC AGT GAG GAA GCT ATT GAA ACC TCG AAG CAA CTA GCT CTC CAG GAA 1008
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GGC TTG TTG GTT GGT ATA TCT TCT GGA GCT GCT GCT GCT GCT GCA ATC 1056
 G  L  L  V  G  I  S  S  G  A  A  A  A  A  A  I
CAG TTT GAT AAG AGA CCT GAA AAT GCC GGG AAA CTC ATA GCC GTT GTG 1104
 Q  F  D  K  R  P  E  N  A  G  K  L  I  A  V  V
TTC CCG AGC TTC GGG GAA CGT TAC CTC TCG ACC CAG CTT TTC CAG TCG 1152
 F  P  S  F  G  E  R  Y  L  S  T  Q  L  F  Q  S
ATT CGA GAA GCC TGC GAG CAA ATG CAG CCC GAG CTT TGA TTT TCT TCT 1200
 I  R  E  A  C  E  Q  M  Q  P  E  L
GTA ATT TCG TGA CCA ACA AGG AAC CTC TTC TTT GAT TTA TTG CTG ATT 1248
GTT TTT CTT CTA ATA GAC GTT TCT GCA GAA ATC TCT ACG TCT ACT GTG 1296
TAT ATT TAC AAT TCG CAA CGC AAT TTG GTG TTT GTT ATT AAT AAA GGC 1344
ATA AGA GGT TTT GGT CAA AAA AAA AAA AAA AAA A                   1378
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Fig. 1. Complete cDNA sequence and translated amino acid sequence of the open reading frames of (A) At.OAS.5-8 and (B) At.OAS.7-4. In-frame TGA stop-codons are marked bold, putative polyadenylation motifs are underlined.

culture then grown for 12 h. The cells were harvested by centrifugation, resuspended in 20 mM HEPES-KOH, pH 7.9, 60 mM KCl, 5% glycerol, 0.1 mM EDTA, 2 mM DTT, 1 mM PMSF and lysed by 3 freeze/thaw cycles. OAS-TL activity was found in the supernatant of a subsequent centrifugation of 10 min at 10,000 × *g*. The enzymatic determination of OAS-TL was according to Gaitonde's ninhydrin method as in [19].

2.3. DNA and RNA hybridization analysis

Arabidopsis plants for nucleic acid isolation were grown in culture vessels (Duchefa, Haarlem, NL) with 50 ml of 0.5 × MS medium [20] at 24°C and 9 h light/15 h dark with a light intensity of 40 W/m^2. Following extraction 10 μg of total RNA were resolved on a 1.2% agarose gel containing formaldehyde, transferred to Hybond N and UV cross-linked according to [21]. Genomic DNA was isolated as described in [22]. Hybridization procedures for both Northern and Southern analysis were as described for library screening. Hybridization and washes were carried out at 65°C with a final step of 0.2 × SSC, 0.1% SDS for 30 min.

3. Results and discussion

3.1. Isolation of cDNAs encoding for OAS-TL from Arabidopsis

The isolation of cDNAs encoding for OAS-TL from *Arabidopsis thaliana* was started with the generation of homologous DNA fragments that could be used to screen a DNA library. As an existing OAS-TL cDNA from spinach chloroplasts [10] did not cross-hybridize with *Arabidopsis* in DNA and RNA hybridization experiments (data not shown), a PCR approach was chosen. Two degenerate oligonucleotide primer pairs were designed that corresponded to highly conserved regions of OAS-TL sequences from *E. coli*, spinach and bell pepper [4,7,10,23]. PCR amplification with phage DNA from an *Arabidopsis* leaf λZAP cDNA library yielded two products that strongly hybridized with a cDNA probe of spinach plastid

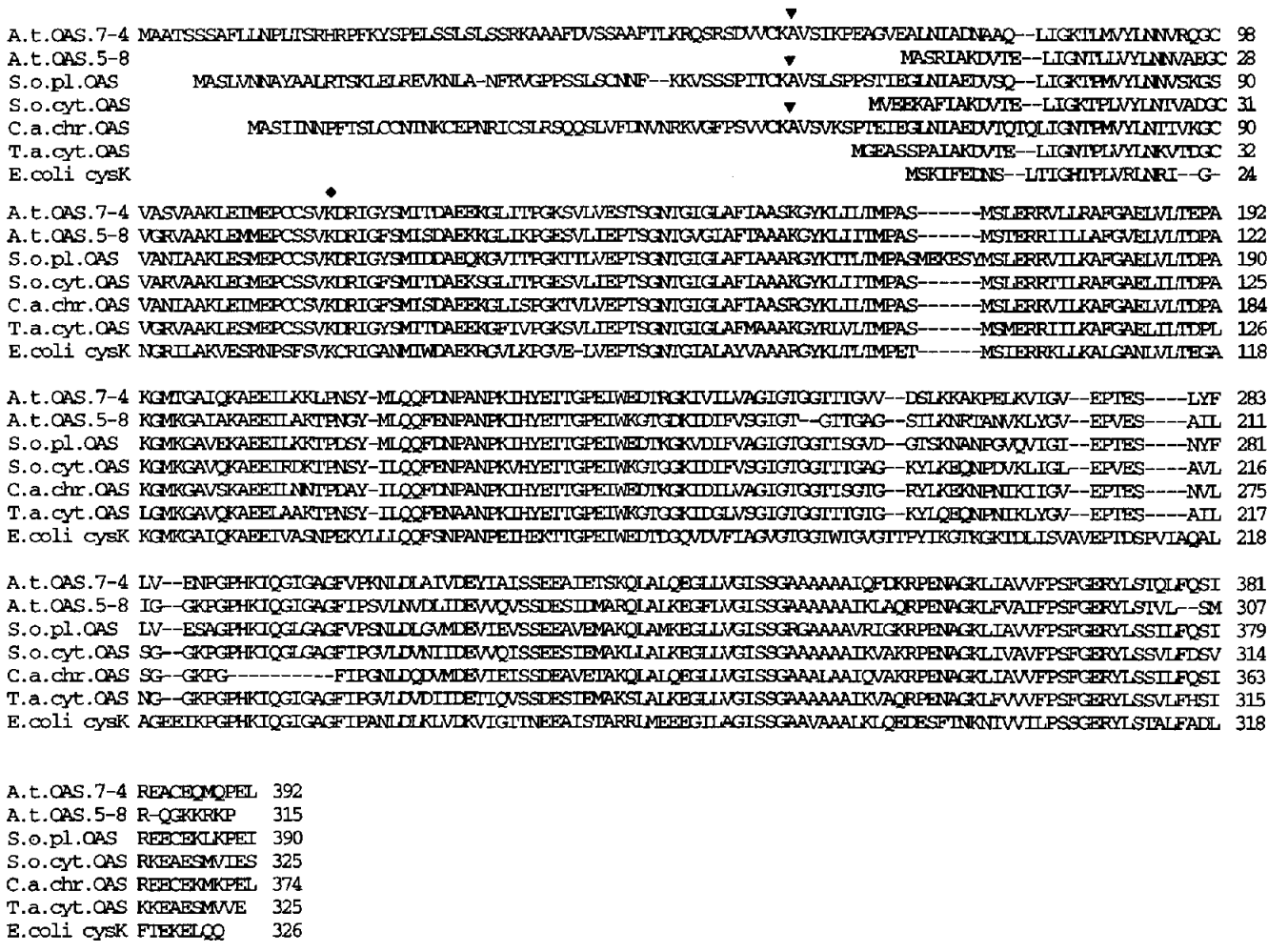

Fig. 2. Amino acid alignment of At.OAS.5-8 and At.OAS.7-4 with *O-acetylserine* (thiol) lyase sequences from *Spinacea oleracea* plastid (S.o.pl.OAS), (S.o.cyt.OAS), *Capsicum annuum* chromoplast (C.a.chr.OAS), putative cytosolic *Triticum aestivum* (T.a.cyt.OAS) and the *E. coli cysK* gene (*E.coli*.cysK). ▼ = putative transit peptide cleavage site; ◆ = consensus Lysine residue responsible for pyridoxal phosphate binding; - = gap introduced to increase alignment homology.

OAS-TL. The two cloned PCR products, termed At.PCR.5 and At.PCR.7, respectively, were 241 and 170 bp in size and showed 74% and 86% nucleic acid homology to the spinach plastid OAS-TL clone.

The ^{32}P-labeled PCR products At.PCR.5 and At.PCR.7 were used in two independent screenings of 150,000 plaques each of the *Arabidopsis* λZAP cDNA library from which they had been amplified. Four clones were isolated with At.PCR.5 and one clone with At.PCR.7. Sequencing of the 5′ and 3′ ends showed that the four At.PCR.5 inserts differed only in length. The longest one, termed At.OAS.5-8, was used for further investigation together with the clone from the second screening, At.OAS.7-4 (Fig. 1). The cDNA sequences differed by less than 5% when aligned to the corresponding PCR products.

At.OAS.5-8 carried an insert of 1,234 bp with a continuous open reading frame of 315 amino acids starting from position 61, thus encoding for a protein with a predicted molecular weight of 33.242 Da (Fig. 1A). Two partial sequences deposited in the *Arabidopsis* dbEST collections, T13908 and ATTC0319, were found to be 95.1% and 89.8% identical to At.OAS.5-8, respectively. Clone At.OAS.7-4 was 1,378 bp in length. Starting from position 13 it had the capacity to encode a protein of 392 amino acids with a derived molecular mass of 41,877 Da (Fig. 2B). The flanking sequences of the putative start codons of both clones were in good agreement with the plant translation initiation consensus motif [24].

3.2. Analysis of the derived amino acid sequences

A comprehensive alignment of OAS-TL amino acid sequences from plants and *E. coli* [4,7,9–11,23] reveals several common structural features (Fig. 2). Two sequences from spinach plastid OAS-TLs [8,9] as well as *cysM* from *E. coli* [23] were very similar to some of the presented sequences and were therefore not included here. Both *Arabidopsis* proteins contain a lysine residue that is highly conserved between all known OAS-TLs and that has been shown to be the functionally active pyridoxal binding site of a cytosolic OAS-TL [25].

The size of the At.OAS.5-8 protein corresponds exactly to the cytosolic and mature OAS-TL proteins and in terms of amino acid identity is more homologous to the spinach and wheat cytosolic forms (81% identity) than to the plastid isoforms of spinach and bell pepper (64%). The stop-codon that

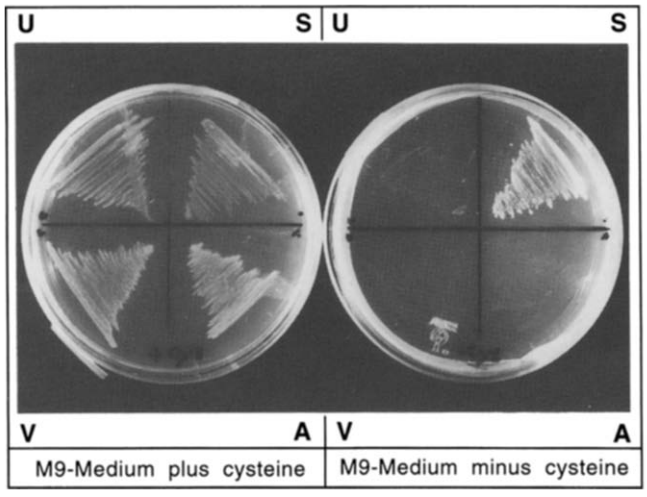

Fig. 3. Complementation of the *cysK/cysM* mutant *E. coli* NK3 with recombinant *Arabidopsis* OAS-TL5-8. Bacterial cells without plasmid (U) or transformed with pEXP2 wildtype vector (V), pEXP5-8 in antisense orientation (A), or pEXP5-8 in sense orientation (S) were plated on minimal medium ± cysteine.

immediately preceeds the putative ATG start codon excludes the possibility of a missing 5′ sequence that could encode for an amino-terminal organelle transit sequence. At.OAS.5-8 therefore is likely to represent a cytosolic OAS-TL isoform.

In contrast, At.OAS.7-4 corresponds well to the plastid OAS-TLs from spinach and bell pepper (76% identity) and shares less homology with the cytosolic isoforms (70%). The start of the putative mature protein sequence is marked by a plastid transit peptide cleavage motif that has been confirmed by determination of the N-terminal amino acids of purified mature OAS-TLs from spinach and bell pepper plastids [3,4]. Accordingly, a molecular weight of 35,387 Da was derived for the mature *Arabidopsis* protein. The first 60 amino acids of At.OAS.7-4 up to the consensus cleavage site have only limited sequence homology to the transit peptides of the two plastid OAS-TLs, but structural analysis with respect to hydrophobicity and amphiphilicity showed almost identical profiles. It is rich in hydroxylated amino acid residues, has an uncharged amino-terminal domain and a positively charged central domain, although no amphiphilic sheet is predicted for the carboxy terminus. These features make it unlikely to represent a mitochondrial targeting sequence [26].

3.3. *Complementation of the cysteine auxotroph* E. coli *NK3 and enzymatic activity of OAS-TL5-8*

The identity of the protein encoded by cDNA clone At.OAS.5-8 as an *O*-acetylserine (thiol) lyase was verified by genetic complementation and in vitro enzyme assay (Fig. 3). Since the putative startcodon at position 61 was preceeded by a TGA stopcodon, the cDNA was cloned into the expression vector pEXP2 [18] yielding the plasmid pEXP5-8. The protein was expressed with an amino terminal fusion of 19 amino acids of the T4 lysozyme gene that replaced the first two amino acids of the authentic protein. When pEXP.5-8 was transformed into the cysteine synthase-deficient *E. coli* strain NK3 the cells were promoted from auxotrophic to prototrophic growth, whereas untransformed, pEXP2 wildtype, and pEXP5-8 antisense transformed cells were unable to grow on M9 medium without cysteine.

Cysteine synthesizing activity was demonstrated in lysates of pEXP5-8 transformed NK3 cells by formation of cysteine from *O*-acetylserine and sulfide. NK3 cells transformed with pEXP2 as a control contained no detectable OAS-TL activity. With pEXP5-8 a rate of cysteine formation of 1.49 nkat per mg protein was determined. For comparison, the total extractable OAS-TL activity from leaves of 6-week-old *Arabidopdsis* plants was 0.04 nkat per mg protein. Substrate affinities of the recombinant protein were calculated from Lineweaver–Burke plots with varying concentrations of *O*-acetylserine and Na_2S (not shown). K_M values were determined as 8.81 ± 0.75 mM for *O*-acetylserine and 0.067 ± 0.011 mM for Na_2S, respectively. This is in agreement with data from authenic OAS-TL preparations [2–4,6] and indicates unchanged properties of the protein in the heterologous expression system. Thus, At.OAS.5-8 in fact encoded for a cysteine synthesizing activity that can be efficiently expressed in *E. coli* and determined without measurable background.

3.4. *Genomic organization of the cytosolic and plastid OAS-TL isoforms*

Genomic DNA of *Arabidopsis thaliana* was restricted with a combination of enzymes that were known to cut within the cDNAs (*Hin*dIII, *Xho*I, *Pst*I in At.OAS.5-8; *Hin*dIII in At.OAS.7-4) and some without such recognition sites, respectively (*Eco*RI, *Bam*HI for At.OAS.5-8; *Eco*RI, *Xho*I, *Pst*I, *Bam*HI for At.OAS.7-4). Both cDNAs were hybridized to the same DNA blot to allow direct comparison of the labeled fragments (Fig. 4).

The expected restriction sites could be confirmed giving rise to at least two fragments. Additional sites such as *Bam*HI in At.OAS.5-8 and *Xho*I in At.OAS.7-4 appeared to occur within intron sequences of the gene.

The two restriction patterns showed no similarities in fragment sizes which might indicate at least no closed linkage at the genome. If so, the isoforms are probably not a result of a gene duplication. This would be supported by the limited sequence

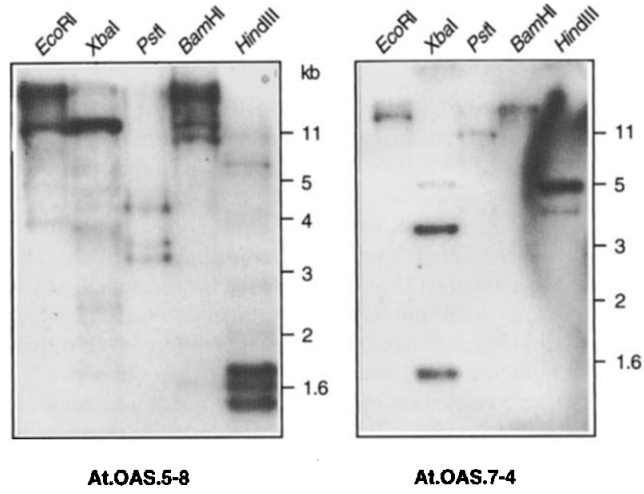

Fig. 4. Southern analysis of the genomic organization of the cytosolic and plastid OAS-TL isoforms in *Arabidopsis thaliana* cv. Columbia. The same DNA blot was hybridized consequently with the entire cDNA of either At.OAS.5-8 or At.OAS.7-4 as a probe.

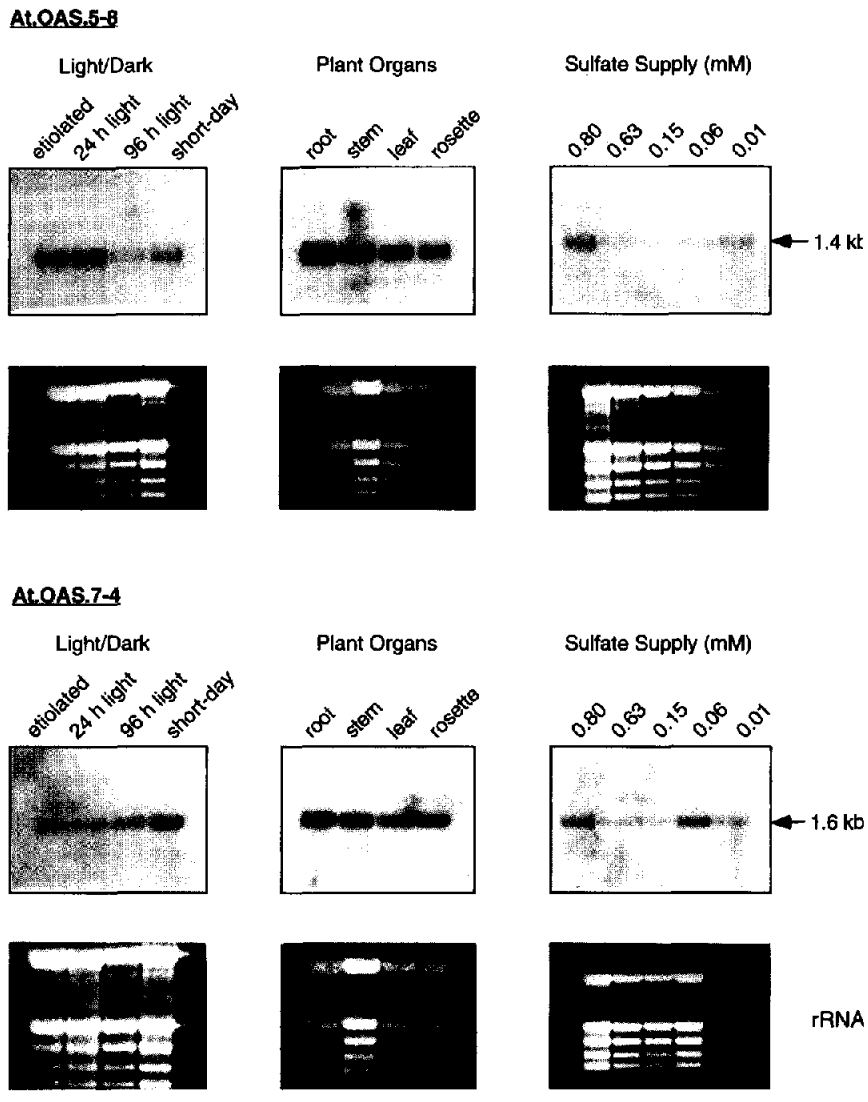

Fig. 5. Expression pattern of the cytosolic OAS-TL5-8 and the plastid OAS-TL7-4 as indicated by Northern analysis. For light/dark and sulfate supply experiments whole plants were extracted. Etiolated seedlings were grown in the dark for 7 days, transfered to light for 24 h or 96 h and compared to plants grown for 10 days in the dark or 10 days at short day. Ethidium bromide stained ribosomal RNAs are shown for each experiment in order to control the loaded RNA samples.

homology of cytosolic and plastid OAS-TLs. Number and size of the genomic fragments as well as the relative intensity of some of the fragments indicated more than one locus for each of the isoforms.

3.5. Expression analysis of the two OAS-TL isoforms

The abundance of OAS-TL mRNA has so far only been investigated for plastid isoforms in leaves and roots of spinach and during bell pepper fruit development [4,9]. Therefore, the expression pattern of At.OAS.5-8 and At.OAS.7-4 was investigated with respect to plant organs, light and sulfate supply (Fig. 5). Both OAS-TL forms were found to be expressed at all conditions tested, however with different abundance. The blots routinely had to be exposed for 8 days to obtain decent signals. For At.OAS.5-8 a 1.4 kb RNA was detected and At.OAS.7-4 gave rise to an mRNA of 1.6 kb

Light did not significantly influence the level of At.OAS.5-8 but rather decreased its mRNA in relation to total RNA from illuminated plants. In contrast, light clearly induced an increase of At.OAS.7-4 mRNA (Fig. 5B). The kinetics of the induction of mRNA fit with a nuclear encoded plastid stroma localized enzyme that is not directly related to photosynthesis as is, e.g. the small subunit of ribulose-1,5-bisphophate carboxylase [27,28].

At.OAS.5-8 was most abundant in roots and also present in leaf tissue as can be expected for a cytosolic gene product. At.OAS.7-4 was similarly expressed although highest mRNA levels would be expected in leaves. However, mRNA of a chloroplast OAS-TL was also well detectable in roots of spinach [9]. The observed pattern in *Arabidopsis* was probably a consequence of the tissue culture-like growth conditions. Low light intensity and the presence of sucrose in the culture medium together can cause a limited development of photosynthetic capacity.

It is interesting to note that both isoforms are expressed in heterotrophic tissue and probably both give rise to functional proteins as has been shown for pea root proplastids and OAS-TL isoforms in cauliflower inflorescence [13,29]. This could indicate an active sulfate assimilation even in the absence of photosynthetic activity and would confirm an additional function of OAS-TL in recycling of catabolic sulfide from protein turnover or glucosinolate breakdown as well as synthesis of compounds other than cysteine [2,6].

Since the assimilation of sulfate is the predominant function at least of the chloroplast OAS-TL, the expression of both isoforms was investigated under conditions of sulfur deficiency (Fig. 3C). With decreasing amounts of sulfate in the growth medium the typical symptom of leaf chlorosis occured and at 0.01 mM sulfate the plants hardly developed after germination (data not shown). This phenotype is assumed to be a consequence of stress induced damage to the photosynthetic apparatus as has been shown for spinach [30,31]. Under these conditions a 1.5–2-fold increase in At.OAS.5-8 and At.OAS.7-4 mRNA was detected as compared to rRNA indicating an adaptation to sulfur deficiency during plant development. The fact that both compartmental forms react to the same extent suggests that they have the same function at sulfur starvation, i.e. to recycle all available sulfide that can be released from storage compounds and glutathione for protein synthesis and maintenance of the photosynthetic apparatus.

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