Cloning of a cDNA encoded by a member of the Arabidopsis thaliana ATP sulfurylase multigene family. Expression studies in yeast and in relation to plant sulfur nutrition

Helen M. Logan, Nicole Cathala, Claude Grignon, Jean-Claude Davidian

To cite this version:

Helen M. Logan, Nicole Cathala, Claude Grignon, Jean-Claude Davidian. Cloning of a cDNA encoded by a member of the Arabidopsis thaliana ATP sulfurylase multigene family. Expression studies in yeast and in relation to plant sulfur nutrition. Journal of Biological Chemistry, American Society for Biochemistry and Molecular Biology, 1996, 271 (21), pp.12227-12233. 10.1074/jbc.271.21.12227. hal-02696531

HAL Id: hal-02696531
https://hal.inrae.fr/hal-02696531
Submitted on 1 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Cloning of a cDNA Encoded by a Member of the Arabidopsis thaliana ATP Sulfurylase Multigene Family

EXPRESSION STUDIES IN YEAST AND IN RELATION TO PLANT SULFUR NUTRITION*

(Received for publication, December 29, 1995, and in revised form, February 28, 1996)

Helen M. Logant, Nicole Cathala, Claude Grignon, and Jean-Claude Davidian

From the Ecole Nationale Supérieure Agronomique de Montpellier, Laboratoire de Biochimie et Physiologie Végétales, Institut National de la Recherche Agronomique, CNRS (ura 573), 34060 Montpellier, France

An Arabidopsis thaliana ATP sulfurylase cDNA (ASA1), encoding a putative chloroplastic isoform, has been cloned by functional complementation of a Saccharomyces cerevisiae (met3) ATP sulfurylase mutant which also has a poor sulfate transport capacity. Homologous complementation of the yeast mutant with the ATP sulfurylase gene restores both ATP sulfurylase function and sulfate transport. Heterologous complementation restores only ATP sulfurylase function as demonstrated by low \(^{35}S\) sulfate influx measurements and selenate resistance. A structural relationship between ATP sulfurylase and sulfate membrane transporters in yeast is proposed. The sequence of ASA1 is homologous to deduced plant and animal ATP sulfurylase sequences. Analyses indicate a potential tyrosine phosphorylation site which is unique to higher eukaryote sequences. ASA1 is specified by a single copy gene that is part of a multigene family in A. thaliana. At least two ASA1 copies are found in Brassica napus plants. ASA1 transcripts were found in all organs examined, with the highest transcript abundance and ATP sulfurylase activity in leaves or cotyledons. Absence of sulfate from culture media transiently increased B. napus transcript abundance, indicating that initially, the response to sulfate deprivation is transcriptionally regulated.

Sulfur is an essential mineral nutrient for plant and animal growth which, in its reduced form, is incorporated into sulfur amino acids, other sulfur-containing metabolites, and coenzymes. In its oxidized form, it is incorporated into sulfolipids which are the major components of the chloroplast membrane (1). In both plants and microorganisms, active uptake of sulfate through specific transporters is followed by reduction to sulfide. As sulfate has a very low oxidation/reduction potential through specific transporters is followed by reduction to sulfide. As sulfate has a very low oxidation/reduction potential, the primary step in assimilation requires its activation via an ATP-dependent reaction (2). This reaction is catalyzed by ATP sulfurylase (ATP: sulfate adenylyltransferase, EC 2.7.7.4) and leads to the formation of adenosine 5′-phosphosulfate (APS) (1). The equilibrium for the formation of this product is thermodynamically unfavorable and, as the efficiency of APS removal by subsequent reactions seems insufficient for energetic compensation, shift of the thermodynamic balance through subcellular compartmentalization or substrate channeling may occur (2).

ATP sulfurylase has been purified from a wide range of sources and characterized extensively at the biochemical level from plants (3–5), animals (6, 7), and fungi (8, 9). In plants, leaves are considered to be the main site of sulfur assimilation with ATP sulfurylase activity found predominantly in chloroplasts and at low levels in the cytosol (4, 5). All the enzyme activities involved in assimilatory sulfate reduction have also been detected in plant root plastids (10). Although ATP sulfurylates isoforms with different biochemical properties have been purified from higher plants, no specific cellular function has been attributed to any of them.

An ATP sulfurylase gene was first cloned from Saccharomyces cerevisiae (11). Genes have subsequently been cloned from prokaryotes (12–14) and another lower eukaryote (15), and several cDNAs have been cloned from plants (16–19) and animals (20, 21). The prokaryotic enzymes are homodimers with a catalytic subunit and a subunit that acts as a stimulatory GTPase (2). The yeast and plant enzymes are homooligomers, dimeric and tetrameric (5, 11, 19), and do not respond to GTP (15). In animals, ATP sulfurylase and APS kinase reside on a single bifunctional protein (7, 20, 21).

Using an Arabidopsis thaliana cDNA library, we have functionally complemented an ATP sulfurylase-defective yeast mutant. We report the isolation of the corresponding ATP sulfurylase cDNA clone that restores yeast methionine heterotrophy and ATP sulfurylase activity. The clone was sequenced and found to be identical with one of the three previously cloned A. thaliana ATP sulfurylases (18, 19), except for differences in the 3′ sequence. Comparison of yeast mutants complemented by the heterologous cDNA and homologous gene led us to propose the existence of a structural relationship between ATP sulfurylase and membrane sulfate transporters in yeast. ATP sulfurylase activity has been shown to increase under sulfur limiting conditions in plants (22, 23), but the mechanism by which this response is induced has not been determined. We have carried out ATP sulfurylase expression studies in relation to sulfur availability in A. thaliana and Brassica napus.

EXPERIMENTAL PROCEDURES

Strains and Media—The following strains were used in this study, S. cerevisiae wild type W303-1A (MATα, his3, leu2, ade2, trp1, ura3) and the mutant strains CC371–4C (MATα, his3, leu2, ade2, trp1, ura3) and C135 (MATα, his3, leu2, ura3, met3::URA3), which were grown as described (24), unless otherwise stated. Plasmids were propagated in Escherichia coli HB101 E. coli Rec A (SBYES) M15 (EMP, 12227-12228).
col. strain DH5α (Life Technologies, Inc.) in classical media selecting with ampicillin (25).

Plant Material—Oilseed rape (B. napus Metzger var Drakkar) seeds were grown as already described (26). The complete nutrient solution (+S) contains 2 mM MgSO4 and the sulfate free medium (−S) was replaced by replacing MgSO4 by the corresponding chloride salt. Organ cultures were harvested after 8 days of culture. A. thaliana, B. napus organ essentially as (mutant type ecotype of the ecotype Landsberg) seeds were disinfected and sown in a sterile plastic box, on 3 layers of nylon mesh (1 mm2) supported over the surface of a sterile nutrient +S solution identical with that used for B. napus, and aerated with filtered air. Growth conditions included a 16-h photoperiod, constant day and night 20°C temperature and 70% humidity, and 250 μmol m−2 s−1 photosynthetically active radiation. After 3 weeks of sterile culture, plants were transferred either to fresh +S or −S nutrient media for 2 days, after which organs were harvested.

Plasmids and cDNA Library—The A. thaliana cDNA library was constructed in the yeast shuttle expression vector pFLe1 which bears the yeast URA3 marker and the constitutive phosphoglycerate kinase promoter (27). This library was prepared from mRNA extracted from complete two-leaf stage seedlings, including roots. The yeast ATP sulfurylase gene which had been subcloned from pMa3-4 (24) was in plasmid pMBLYz23 (pM3-32), which bears the yeast URA3 gene. Plasmids were prepared using Qiagen kits according to the manufacturer’s instructions (Qiagen Inc.). For sequencing, the insert was excised from pFLe1 by digestion with KpnI and subcloned into plasmid SK + (Stratagene). Isolation of an Arabidopsis cDNA Encoding an ATP Sulfurylase—The yeast mutant CC371-4C was transformed with 5 μg of the Arabidopsis cDNA library (6 × 107 cells μg−1) by the spheroplast protocol (28). The transformation mixture was plated on 2% Bacto-agar selective Difco yeast nitrogen base supplemented with 0.1 mM histidine, then incubated at 28°C for several days. The A. thaliana colonies were picked, plated on yeast nitrogen base, which contains 25 mM ammonium sulfate as the sole sulfur source, and incubated at 28°C for several days. Preparation of both the ura3 and met3 functions were confirmed using 5-fluoro-orotic acid (29) and appropriate selective culture media. Plasmid DNA was recovered from complemented yeast transformants as described (30). The plasmid pFL61-ASA1 was reintroduced into the ATP sulfurylase-defective S. cerevisiae strains CC371-4C and C155 using a lithium chloride transformation procedure (31).

DNA Sequencing—The sequence of the ASA1 cDNA was determined from both strands by the dideoxy chain termination method with a double-stranded DNA template and the Taq dye primer cycle sequencing kit (Applied Biosystems). Sequences were compared using the GenBank data bases (R90; EMBL, R39; Swiss-Prot, R30) by the spheroplast protocol (27).

Nucleic Acid Hybridization Analysis—For Southern blot hybridization, genomic DNA was prepared from B. napus (coryledons from 8-day-old seedlings) or A. thaliana (pregerminated 4-day-old seedlings) as described (35). DNA was digested with restriction enzymes according to the manufacturer’s instructions, and the resulting fragments were separated by electrophoresis in a 0.8% (w/v) agarose gel followed by denaturation of DNA and transfer to BA85 nitrocellulose filters (Schleicher & Schuell) (25). The filter was then hybridized at 42°C with ASA1 cDNA fragments random prime-labeled (T, Quickprime kit, Pharmacia) with 32P-DCTP (3000 Ci mmol−1) (ICN Biomedicals) at 1.25 × 106 dpm μg−1 for 16–24 h. Formamide (50% v/v) containing prehybridization and hybridization solutions were prepared as described (25), with E. coli tRNA (100 μg ml−1) used as a blocking agent. Filters were washed twice at room temperature for 5 min and twice at 42°C for 15 min in 30 mM NaCl, 3 mM trisodium citrate, pH 7.0, 0.1% (w/v) SDS, to remove nonspecific hybridization. Blots were then autoradiographed for 12–110 h at −80°C with intensifying screens.

Plant ATP Sulfurylase Assay—Soluble protein extracts were obtained from 5 fresh weight of each B. napus organ essentially as described (3) except for initial extraction. Crude extracts were obtained by grinding the organs to a fine powder in liquid N2, then adding 4 ml of ice-cold extraction buffer per g fresh weight of tissue, 100 mM Tris-HCl, pH 8.0, 10 mM EDTA, 2 mM DTT followed by centrifugation at 4°C for 10 min at 7800 × g. ATP sulfurylase activity measurements were carried out on sulfate-free dialyzed fractions (8) by method of (3). The pyrophosphate released from ATP was determined according to Ref. 42.

RESULTS

Isolation of an A. thaliana ATP Sulfurylase cDNA—Yeast strains CC371-4C and C155 have both been shown to be defective in ATP sulfurylase activity (24) as a result of point or insertion mutations, respectively, and are unable to grow on media where sulfate is the sole sulfur source. Heterologous complementation using an A. thaliana cDNA library in a yeast expression vector (27) was employed to obtain an ATP sulfurylase done using strain CC371-4C. Initial transformation with selection for uracil auxotrophies resulted in only 500 complemented colonies. These were recovered for secondary screening and replated on media containing sulfate as the sole sulfur source; 4 positive clones were obtained and analyzed further. Using 5-fluoro-orotic acid, uracil and methionine auxotrophies were confirmed to arise from plasmid complementation and not recombination (29). Plasmids were recovered from yeast into E. coli and amplified. Restriction analysis of the four plasmids indicated that they were identical, thus one representative was taken. The strain CC371-4C was retransformed with this plasmid and complementation of methionine and uracil auxotrophies was confirmed. The cDNA done was termed ASA1 (ATP sulfurylase Arabidopsis 1).

Sequence Analysis of cDNA and Comparison of Deducible Amino Acid Sequence with Other ATP Sulfurylases—Analysis of the insert from pFL61-ASA1 indicated a 1431-bp open reading frame in the 1662-bp cDNA which would encode a polypeptide of 476 amino acids with a calculated molecular mass of 53,604 Da (Fig. 1). The predicted polypeptide is encoded by bases 59 to 1489 with the 3′-untranslated region containing a putative polyadenylation signal AATAAT (46) at base 1643.
ASA1 is homologous to the sequence AtMet3-1 (18) and APS2 (19). ASA1 has a 5'-leader sequence 40 bp longer than that of AtMet3-1 (the data base accession for APS2 contains only 3' sequence at present) and the 3' sequences differ apparently through polyadenylation in response to an earlier signal in ASA1. The proposed amino acid sequence of mature ASA1 shows lower homology to the other A. thaliana ATP sulfurylase cDNA sequences APS1 (17) and APS3 (19) (67% identity) than they exhibit to each other (89% identity). Comparison to Solanum tuberosum StMet3-1 and StMet3-2 sequences showed slightly higher identity (74% and 69%, respectively) (16). Nonetheless, these values are higher than those obtained with ATP sulfurylase cDNAs recently cloned from animals, Mus musculus (22) and Urechis caupo (23) (59% identity).

Features of Deduced Amino Acid Sequence—The first 62 amino acids of the ASA1 open reading frame have features which correspond to a transit peptide, also suggested for APS2 (19), as this region is rich in hydroxylated amino acids, contains no acidic amino acids, and has a hydrophobic valine residue at position 59 in agreement with the chloroplast transit peptide cleavage-site motif (47, 48). Additionally, the N-terminal sequence of a chloroplast ATP sulfurylase isoform purified from spinach (SLIDPDGGSLIDLVPEN) (5) is identical for the noncomplemented met3 strain (CC371-4C and C155) containing pFL61-ASA1 and B. napus homologous genes present in its genome. The proposed amino acid sequence of mature ASA1, encoding the yeast ATP sulfurylase, in the vector pEMBL Ye23 (pM3-32), showed essentially wild type growth kinetics. The ability of these strains to grow on sulfate containing media correlated with ATP sulfurylase activities measured in their extracts (Table I). The activities measured for both Met3 strains (CC371-4C and C155) containing pFL61-ASA1 were 66% higher than those measured from extracts of CC371-4C complemented by pM3-32, but 37% lower than those of the wild type (W303-1A). When ASA1 was expressed in the Met3 strain, the ATP sulfurylase activity was 30% lower than when it was expressed in the met3 mutants and 50% of that in the noncomplemented Met3 strain (Table I).

In addition to the lack of ATP sulfurylase activity, yeast strains which have the met3 mutation have been shown to be defective in sulfate transport (44). In order to ascertain the effect of complementation on sulfate transport, we used selenium, as a toxic structural analogue of sulfate, in drop assays. When the strains were grown on sulfatetless media (containing

**Fig. 1. Alignment of A. thaliana ASA1 with other eukaryotic ATP sulfurylase amino acid sequences.** The amino acids of the open reading frame encoded by the A. thaliana ASA1 cDNA are compared to the ATP sulfurylase sequences APS1 (17) and APS3 (A. thaliana) (19), StMet3-1 and StMet3-2 (S. tuberosum) (16), MET3 (S. cerevisiae) (11), and APS (P. chrysogenum) (15), and U. caupo (21) represents the sequence of bifunctional ATP sulfurylase/APS kinase sequence for the homology blocks, discussed in the text, are shown. Roman numerals refer to blocks of homology. Boxed areas represent regions where all but one of the sequences are identical or all the sequences show similarity. Positions where all residues are identical are shaded. Numbers at the ends of lines indicate the position of the most 3' amino acid relative to the start of the protein. * indicates the position of putative tyrosine phosphorylation and ‡ indicates the ASA1 putative transit peptide cleavage site. Residues underlined correspond to those identical with the N-terminal sequence from purified spinach ATP sulfurylase (5). Dashes indicate gaps in the sequence to yield the best alignment.

Genomic Organization of ASA1—Southern blot analyses of A. thaliana and B. napus genomic DNA digested with four restriction enzymes were carried out in order to estimate the copy number of ASA1 in their respective genomes. The ASA1 probe hybridized to 2 fragments in the A. thaliana EcoRV and BamHI digested DNA, which both have a restriction site in the cDNA, and to one fragment in both the EcoRI- and HindIII-digested DNA (Fig. 2). This indicates that ASA1 is encoded by a single copy gene in A. thaliana. Three to five genomic DNA fragments hybridized to the ASA1 probe in B. napus at high stringency (Fig. 2), suggesting that there are at least two or three copies of ASA1 homologous genes present in its genome. Repulsion of yeast EMBL Ye23 pM3-32 containing ASA1 encoding yeast CC371-4C rescued by pFL61-ASA1, although restored in media containing sulfate as the sole sulfur source, had a rate that was 2.8 x lower than that of wild type, with a doubling time of ~440 min (Fig. 3). No growth was observed for the mutant containing the vector pFL61 alone. The same mutant expressing the gene encoding the yeast ATP sulfurylase, in the vector pEMBL Ye23 (pM3-32), showed essentially wild type growth kinetics. The ability of these strains to grow on sulfate containing media correlated with ATP sulfurylase activities measured in their extracts (Table I). The activities measured for both Met3 strains (CC371-4C and C155) containing pFL61-ASA1 were 66% higher than those measured from extracts of CC371-4C complemented by pM3-32, but 37% lower than those of the wild type (W303-1A). When ASA1 was expressed in the Met3 strain, the ATP sulfurylase activity was 30% lower than when it was expressed in the met3 mutants and 50% of that in the noncomplemented Met3 strain (Table I).
ATP Sulfurylase from A. thaliana

Fig. 2. Southern blot analysis of the genomic DNA from A. thaliana and B. napus. The genomic DNA (10 μg) was digested by restriction enzymes (E, EcoRI; H, HindIII; B, BamHI; RV, EcoRV), separated by agarose gel (0.8%) electrophoresis, transferred onto nitrocellulose, and then hybridized with 32P-labeled ASA1 DNA fragments.

Fig. 3. Growth curve of yeast strains transformed with ASA1 or MET3. Yeast were grown from 50 milliliter initial absorbance, measured at 650 nm, in synthetic media supplemented with 0.4 mM sodium sulfate as the sole sulfur source and the required auxotrophy factors. Symbols correspond as follows: ●, Met3 mutant strain W303-1A; ▲, met3 mutant strain CC371-4C transformed with pFL61-ASA1; △, met3 mutant strain CC371-4C transformed with pM3-32.

TABLE I
ATP sulfurylase activity and [35S]sulfate influx measurements in yeast

| Yeast strains* | Activitya | [35S]Sulfate influxb |
|----------------|-----------|---------------------|
| W303-1A        | 538       | 46.0                |
| W303-1A + pM3-32c | 2329     | ND                  |
| W303-1A + pFL61-ASA1c | 243     | ND                  |
| CC371-4C       | 0         | 0                   |
| CC371-4C + pFL61-ASA1c | 356    | ND                  |
| CC371-4C + pM3-32c | 113      | 178.5               |
| C155           | 3         | ND                  |
| C155 + pFL61-ASA1c | 317     | ND                  |

* Relevant strain phenotypes: W303–1A (MET3+, ura3), CC371–4C (met3, ura3), C155 (met3:URA3).

a The ATP sulfurylase activity and [35S]sulfate influx are the mean of three or four independent experiments, respectively. The results are all expressed as nmol·min⁻¹·mg⁻¹ protein. ND indicates not determined.

b ATP sulfurylase activity was measured in the forward direction by molybdoysis.

c Unidirectional [35S]sulfate influxes were measured for 3 min in 0.1 mM MgSO₄ at 30 °C.

d pM3–32 and pFL61-ASA1 represent plasmids containing the yeast gene and the plant cDNA ATP sulfurylase clones, respectively.

DISCUSSION

In this paper we report the cloning, by functional complementation of a yeast mutant, of a cDNA which encodes an A. thaliana ATP sulfurylase (ASA1). The sequence of this clone is identical with the previously reported AtMet3-1 (18) and APS2 cDNAs (19), except that the latter part of its 3′-untranslated sequence is different. Heterogeneity at the 3′ ends of mRNA encoded by a single plant gene has been shown to result from polyadenylation of the transcripts at multiple sites (46). The poly(A) of the two ASA1 homologous sequences and an homologous EST (accession number Z26572) are located downstream of the ASA1 poly(A) which seems to agree with the observed
preference for polyadenylation in response to the second site
binding motif is found in the ASA1 sequence. Labeling of cysteine residues in P. chrysogenum has identified two buried cysteine residues which may have important structural functions, one of which is in a region whose sequence is conserved in A. nidulans and S. cerevisiae (15). Although this cysteine is not conserved in the plant sequences, there is a conserved cysteine in the plant ATP sulfurylases which belongs to a highly conserved block at the 3′ end (Fig. 1, block V).

Genomic Southern analysis (Fig. 2) demonstrated that ASA1 is encoded by a unique gene in agreement with the results of Murillo and Leustek (19) for APS2. Under low stringency conditions, an additional 2–3 fragments were detected in each A. thaliana genomic DNA digest (not shown) indicating at least 2 related ATP sulfurylase genes. This agrees with the cloning of two different full-length A. thaliana ATP sulfurylase cDNAs, APS1 and APS3 (17, 19). Southern blots probed with these cDNAs indicate that they are also single copy genes, suggesting that the ATP sulfurylase gene family in A. thaliana has just these three members (19). Our data base searches have, however, identified six additional ASA1 homologous A. thaliana ESTs (accession numbers T21966, T24953, R29819, T88260, T45338, and T21042) which appear to encode one or several related ATP sulfurylases. Analyses using the deduced protein sequence from the 6 combined sequences showed 62, 68, and 72% identity, respectively, to ASA1, APS1, and APS3. These values are sufficiently different to suggest a fourth ATP sulfurylase gene in A. thaliana which may not cross-hybridize, even under low stringency conditions. As the six ESTs were all identified from the same systematic sequencing program (54), they may represent an organ or treatment-specific ATP sulfurylase form. In A. thaliana, the existence of a fifth ATP sulfurylase gene expressed in the cytosol might be expected since cell fractionation and enzyme activity studies on spinach revealed a cytosolic form (4). In addition, a cDNA without a transit peptide has been cloned from S. tuberosum (16). Therefore, the A. thaliana ATP sulfurylase family probably consists of at least four chloroplastic and/or one cytosolic isoform.

The identification of at least 2 ASA1-like gene copies in B. napus is consistent with the allotetraploid state of this species (55).

Northern blot analyses of A. thaliana and B. napus identified a transcript of ~1.9 kb in all organs examined, with highest expression in leaves (Fig. 5). An APS1 probe also identified a transcript (1.85 kb) in leaf and root total RNA (17) which was most abundant in leaves. An increase in transcript abundance in roots of both A. thaliana and B. napus was observed on sulfur deprivation, 1.8-fold and 1.5-fold, respectively, indicating that the expression of the ASA1 gene responds to the availability of sulfate. The apparent absence of response in A. thaliana leaves to sulfate starvation seems in contrast to the 1.3-fold increase observed in B. napus. This can be attributed to the difference in the age of the organs used, 9-day-old cotyledons compared to 3-week-old leaves, and is more likely to reflect the higher sulfur requirement of the young organs. ATP sulfurylase specific activity has been shown to be high in young leaves, decreasing as the leaves mature (23, 56) indicating that the sulfur requirement of mature leaves is low.

As effects of sulfate starvation on the relative abundance of ASA1 hybridizing transcripts in B. napus were similar to those observed in A. thaliana, although the cDNA probe was heterologous, the use of B. napus in these studies was considered appropriate. ATP sulfurylase activity and Northern analysis carried out on the same B. napus organs showed similar patterns up to two days of sulfate starvation with the largest relative increase in roots and highest in cotyledons (Fig. 6, A and C). Specific activities increased in all organs with sulfur starvation, although in hypocotyls after 2 days they decreased then increased. This indicates that in these organs initial changes in enzyme activity on sulfur starvation are probably transcriptionally regulated. Increased ATP sulfurylase activity in response to the absence of sulfate in the external media has previously been observed in higher plants (22, 23). In M. atropurpureum, this difference was also found to be largest in roots, with only a slight initial activity increase in leaves. Large increases in relative transcript abundance for the recently cloned plant sulfate transporters have also been observed for roots (49). Sulfate translocation studies have demonstrated that roots are the predominant sulfur sink during sulfate deprivation (23). These results, together might indicate that roots have priority for sulfate utilization. Such a priority could be envisaged to improve plant survival under sulfate limiting conditions by augmenting the sulfur foraging ability of roots.

After 4 days of sulfate deprivation, although the enzyme activity had increased, the RNA abundance decreased in both cotyledons and roots. A difference between relative ATP sulfurylase activity and RNA abundance profiles was also observed for hypocotyls (Fig. 6, A and B). B. napus probably expresses multiple forms of ATP sulfurylases whose combined enzyme activities might differ, on sulfur starvation, from the profile of one transcript type. The observed differences could, however, result from translational control, but as the observed increases in activity, observed on deprivation, are greater than increases in ASA1 RNA abundance, simultaneous increases in expression of several transcript types seems likely.

The increase in transcript abundance observed in B. napus leaves suggests that the absence of sulfate in the root external media is perceived by leaves. Sulfate uptake and transport from roots to shoots have been shown to be inhibited by glutathione (57) which has been proposed to act as a quantitative signal informing the plant of its sulfur status (58). Glutathione levels have been found to decrease on sulfate starvation,2 and this could induce the changes in transcript abundance on sulfate starvation.

CONCLUSIONS

We have cloned a cDNA which encodes an A. thaliana ATP sulfurylase (ASA1) with a putative chloroplast transit peptide. ASA1 is encoded by a single copy gene that is part of a multigene family in A. thaliana, probably consisting of at least four members. Yeast mutants deficient in ATP sulfurylase also lack sulfate uptake capacity. Comparison of these mutants transformed with the heterologous ASA1 plant cDNA to those transformed with the homologous yeast MET3 ATP sulfurylase gene showed that, although ATP sulfurylase activity is completely restored, the sulfate uptake ability is not fully complemented. We propose a model involving structural interaction between the yeast plasma membrane sulfate carrier and the cytosolic ATP sulfurylase. We plan to verify this model in yeast and examine the possibility of such an interaction in plants.

A putative tyrosine phosphorylation site was found in the ASA1 sequence which is conserved, but only in higher eukaryote ATP sulfurylases. This tyrosine is situated between two homology blocks proposed to correspond to part of the catalytic site and bind ATP (14, 15). Investigation of this region with regard to possible regulation and catalytic functions should be informative. Northern blot analysis showed that ASA1 is expressed in all A. thaliana and B. napus organs examined with highest expression in leaves or cotyledons, respectively. The relative RNA abundance and ATP sulfurylase activity in B. napus were found to increase in cotyledons, and to

---

2 C. Deswarte, A. Borchers, H. Logan, and J.-C. Davidian, unpublished results.
a greater degree in roots, after 2 days of sulfur starvation. This indicates that the initial response to sulfate starvation is probably at the transcriptional level. Subsequently relative ASA1 RNA abundance decreases whereas ATP sulfurylase activity continues to increase, this could be as a result of translational control or other members of the ATP sulfurylase gene family having different response profiles to sulfate starvation.

Acknowledgments—We are very grateful to Dr. Y. Surdin-Kerjan, Dr. D. Thomas, and Dr. H. Cherest (CNRS, CGM, Gif-sur-Yvette, France) for their help and discussions, and for providing us with the \textit{S. cerevisiae} strains and the plasmid pM3-32, we are also indebted to Dr. Y. Surdin-Kerjan for critical reading of the manuscript. We thank Dr. M. Minet and Prof. F. Lacroute (CNRS, CGM, Gif-sur-Yvette, France) for providing the \textit{A. thaliana} cDNA library, and Prof. R. J. Ferl (University of Gainesville) for the \textit{A. thaliana} actin probe. We are grateful to Dr. H. Sentenac for useful discussions and to P. Bousquet for technical assistance.

REFERENCES

1. Schmid, A., and Jäger, K. (1992) Plant Mol. Biol. 192, 325–349
2. Lehri, T. S. (1993) Crit. Rev. Biochem. Mol. Biol. 28, 15–42
3. Osslund, T., Chandler, C., and Segel, I. H. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 10300–10308
4. Leyh, T. S. (1993) Mol. & Gen. Genet. 243, 248–252
5. Renosto, F., Patel, H. C., Martin, R. L., Thomassian, C., Zimmerman, G., and Segel, I. H. (1993) Arch. Biochem. Biophys. 105, 323–324
6. Yu, M., Martin, R. L., Jain, S., Chen, L. J., and Segel, I. H. (1992) Arch. Biochem. Biophys. 296, 164–174
7. Lyle, S., Stanisz, J., Wang, K., and Schwartz, N. B. (1994) Biochemistry 33, 5920–5925
8. De Vito, P. C., and Dreyfuss, J. (1964) Anal. Biochem. 187, 1089–1098
9. Renosto, F., Martin, R. L., Wailes, L. A., Daley, L. A., and Segel, I. H. (1990) J. Biol. Chem. 265, 10293–10300
10. Brunold, C., and Suter, M. (1987) Planta 170, 228–234
11. Cherest, H., Kerjan, P., and Surdin-Kerjan, Y. (1987) Mol. & Gen. Genet. 210, 307–311
12. Lehri, T. S., Vogt, T. F., and Suen, Y. H. (1992) J. Biol. Chem. 267, 10405–10410
13. Schwedock, J., and Long, S. R. (1989) Mol. Plant-Microbe Interact. 2, 181–194
14. Klawonn, D., and Nison, D. C. (1994) J. Bacteriol. 176, 3723–3729
15. Foster, B. A., Thomas, S. M., Mahr, J. A., Renosto, F., Patel, H. C., and Segel, I. H. (1994) J. Biol. Chem. 269, 19777–19786
16. Klein, D., Pfleger, R., Willmitzer, L., and Schwitz, J. W. (1994) Plant J. 6, 105–115
17. Leustek, T., Murolo, M., and Cervantes, M. (1994) Plant Physiol. 105, 897–902
18. Klein, D., Riesmeier, J. W., and Willmitzer, L. (1995) Plant Physiol. 107, 653–654
19. Murolo, M., and Leustek, T. (1995) Arch. Biochem. Biophys. 323, 195–204
20. Li, H., Deyrup, A., Mensch, J. R. Jr., Donowicz, M., Konstantinidis, A. K., and Schwartz, N. B. (1995) J. Biol. Chem. 270, 3945–3949
21. Rosenblath, E., and Leustek, T. (1995) Gene (Amst.) 165, 243–248
22. Brunold, C., Suter, M., and Lavandy, P. (1987) Physiol. Plant. 70, 168–174
23. Bell, C. I., Clark, D. T., and Crabbe, M., and F. Lacroute, F. (1993) Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley Interscience, New York
24. Hawkesford, M. J., Davidian, J.-C., and Grignon, C. (1993) Planta 190, 297–304
25. Minet, M., Dufour, M.-E., and Lacroute F. (1992) Plant J. 2, 417–422
26. Hinnen, A., Hinks, J. B., and Fink, G. R. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 1929–1933
27. Breton, A., and Surdin-Kerjan, Y. (1992) Mol. Cell Biol. 9, 1387–1391
28. Crabeel, M., Messenguy, F., Lacroute, F., and Glaesner, N. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 5026–5030
29. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) J. Bacteriol. 153, 163–168
30. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) J. Bacteriol. 153, 163–168
31. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) J. Bacteriol. 153, 163–168
32. Schwidock, J., and Long, S. R. (1989) J. Bacteriol. 171, 1719–1727
33. Schwartz, N. B. (1995) Arch. Biochem. Biophys. 269, 10405–10410