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 relative to available cellular reductants, the primary step in assimilation requires its activation via an ATP-dependent reaction (2). This reaction is catalyzed by ATP sulfurylase (ATP: sulfite adenyltransferase, EC 2.7.7.4) and leads to the formation of adenosine 5'-phosphosulfate (APS)\(^1\) (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 proplastids (10). Although ATP sulfurylase 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 (MAT\(a\), his3, leu2, ade2, trpl, ur3) and the mutant strains CC371–4C (MAT\(a\), his3, leu2, ur3, met3) and C155 (MAT\(a\), his3, leu2, ur3, met3::URA3), which were grown as described (24), unless otherwise stated. Plasmids were propagated in Escherichia coli.

**EST, expressed sequence tag; bp, base pair(s); kb, kilobase pair(s); MOPS, 3-(N-morpholino)propanesulfonic acid.**
col strain DH5α (Life Technologies, Inc.) in classical media selecting with ampicillin (25).

Plant Material—Oilsed rape (B. napus Metzger var Drakkar) seeds were grown as described in the yeast shuttle expression vector pFL61, 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 pM3-4 (24) in plasmid pEMBLY23 (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 pFL61 subcloned into pBluescript SK-5 (Stratagene).

Isolation of an Arabidopsis cDNA Encoding an ATP Sulfurylase—The yeast mutant CC371-4C was transformed with 5 instructions (Qiagen Inc.). For sequencing, the insert was excised from mid pEMBLYe23 (pNaCl, 0.3M trisodium citrate, pH 7.0, before transfer of RNA to nitrocellulose strain DH5α) using 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) as described (37); duplicates of each sample and nonspecific controls of 50 µg of E. coli tRNA were loaded. After hybridization, as described above, blots were exposed to preflashed film (38) for 50–70 h at –80°C with intensifying screens. Quantification of Northern blots was carried out using the Imager system (Appligene) in conjunction with the National Institutes of Health Image program (39).

Plasmid 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 µm Tris-HCl, pH 8.0, 10 mM EDTA, 2 mM DTT followed by centrifugation at 4°C for 10 min at 7,800 × g. ATP sulfurylase activity measurements were carried out on sulfate-free dialyzed fractions (8) by molybdoysis (3). The pyrophosphate released from ATP was determined according to Ref. 40.

Yeast ATP Sulfurylase Assay—Yeast cells were grown at 28°C in appropriate culture solutions to an absorbance of 1.0 measured at 650 nm. Extraction was essentially as described (41) using cell breakage with glass beads and a modified extraction buffer, 100 mM Tris-HCl, pH 7.5, 0.2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10% (w/v) glycerol. The supernatant was desalted on a Sephadex G-25 M column (Pharmacia PD-10) equilibrated with extraction buffer minus phenylmethylsulfonyl fluoride. ATP sulfurylase activity was measured according to Ref. 42.

RESULTS

Isolation of an Arabidopsis 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. [35S]Sulfate uptake assays were performed as described (44), at 0.1 mM Na2SO4 final concentration, and was initiated by addition of 0.33 µCi of carrier-free [35S]Na2SO4 (ICN Biomedicals) to each uptake measurement tube. Uptake duration was between 30 s and 3 min, where uptake is known to be linear. Sulfate uptake was stopped by isotopic dilution with ice-cold 0.5 mM MgSO4, vacuum filtration through a glass microfiber filter (GF/C, Whatman) applied to a prechilled stainless steel filtration device, and radioactivity retained on the filters was counted.

Protein Assays—Protein content of extracts were measured by the method (45) method of Lowry using bovine serum albumin as a standard.

Reagents—Chemicals were purchased from Sigma or Merck and were “AnalAR” grade or the highest grade available. Restriction and modifying enzymes were obtained from Life Technologies, Inc.

Isolation of an Arabidopsis cDNA Encoding an ATP Sulfurylase—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 Arabidopsis cDNA library in a yeast expression vector (27) was employed to obtain an ATP sulfurylase cDNA obtained 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 colonies were obtained and analyzed further. Using 5-fluoro-orotic acid, uracil and methionine auxotrophs 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 Deduced 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 ATP sulfurylase cDNAs recently cloned from animals, Mus musculus (20) and Pteroglossus cau po (21) (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 isomorph purified from spinach (SLIDPDSGLSLIDLVPENER) (5) is identical for the first 8 amino acids to ASA1 amino acids 63–70 (underlined in Fig. 1). Three blocks of homology with functional features have been proposed for ATP sulfurylase sequences. Two conserved blocks (Fig. 1, blocks I and IV) could be involved in sulfate binding (15). The third block contains part of the sequence GXXKXXG which corresponds to a phosphate-binding loop motif and may be an ATP-binding region (14) (Fig. 1, block III). In addition to these blocks, the ASA1 coding region contains a putative tyrosine phosphorylation site at amino acid 286, asterisked in Fig. 1, block II. This site conforms to the consensus motif (R/K)XXX(D/E)XXX (Prosite accession number P500007) and is also present in the other higher eukaryote ATP sulfurylase sequences (Fig. 1, block II).

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 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.

Properties of Yeast Expressing pASA1—When yeast CC371-4C or its parental strain, the ATP sulfurylase activity was 30% lower than when pFL61 alone was expressed in yeast CC371-4C rescued by pFL61-ASA1, although restored in media containing sulfate as the sole sulfate source, had a rate that was 2.8 × 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 pEMBLYe23 (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 selec-
homocysteine as the sole sulfur source) in the presence of 4 mM sodium selenite, only the wild-type W303-1A and the mutant complemented by the yeast gene (CC371-4C + pM3-32) were unable to grow, whereas the mutant complemented by the A. thaliana ATP sulfurylase cDNA (CC371-4C + pFL61-ASA1) grew as well as the noncomplemented Met3+ mutant (Fig. 4). To investigate whether the selenate growth effect was related to sulfate transport, [35S]sulfate influxes were measured (Table 1). The heterologous expression of pFL61-ASA1 partly restored the sulfate uptake function to the mutant, but to less than 20% of the sulfate uptake capacity of the yeast control. When the mutant was complemented by the homologous MET3 ATP sulfurylase gene, the [35S]sulfate influx was almost 4-fold higher than in the yeast control.

Sulfur Starvation Effects on the Abundance of ASA1 Transcripts in Roots and Leaves—Northern blots of total RNA from A. thaliana leaves and roots identified an ASA1 hybridizing transcript of approximately 1.9 kb (Fig. 5). This transcript was most abundant in leaves, and on sulfur starvation the transcript abundance increased, but only in roots (1.8-fold). As it is difficult to grow A. thaliana in the hydroponic conditions required to impose short term sulfur starvation, B. napus, a related Brassicaceae with high sulfate requirements, was compared to A. thaliana for its suitability in further studies. Northern blots of B. napus total RNA hybridized to an ASA1 probe also revealed a transcript of ~1.9 kb (Fig. 5). An increase in transcript abundance was observed in both cotyledons and roots on sulfur deprivation, 1.3-fold and 1.5-fold, respectively.

Analysis of the Effect of Sulfur Starvation on ATP Sulfurylase Expression in B. napus—Analyses of both relative RNA abundance and ATP sulfurylase activity were carried out on organs of 8-day-old B. napus seedlings grown in sulfate-limiting conditions for 2 and 4 days. Total RNA extracts from hypocotyls also contain ASA1 hybridizing transcripts of ~1.9 kb (Fig. 6, upper panel). The patterns of relative ASA1 RNA abundance and ATP sulfurylase activity up to 2 days sulfate starvation are comparable in roots and cotyledons, highest in cotyledons, and the largest relative increase in roots (Fig. 6, A, B, and C). However, after 4 days of sulfate deprivation, although the ATP sulfurylase activity had increased, the ASA1 RNA abundance decreased in both cotyledons and roots. Hypocotyls showed different expression profiles on sulfur starvation: no significant changes in the transcript relative abundance was observed, and a specific activity decrease after 2 days was followed by an increase.

**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
soluble protein was isolated from cotyledons (roots (B. napus) is expressed in the wildtype strain. Expression of activity, suggesting that more activity than required for growth curve of the mutant complemented with the homologous gene is Met3 compared to when it was expressed in the Met3 strain and in the reduced or no ATP sulfurylase activity. In contrast, the activity measured for pM3-32 in the Met3 strain was 20-fold higher than that when the plasmid complemented the mutant strains and 4-fold greater than the noncomplemented Met3 strain which supports the suggestion that, in the wild type strain, ATP sulfurylase activity levels are not growth-limiting.

Yeast strains which have the met3 mutation have been shown to be defective in sulfate transport as well as in ATP sulfurylase activity (44). These mutants, unlike wild type strains, can grow on media containing selenate, a toxic analogue of sulfate (Fig. 4). Comparison of strains, expressing either the homologous or heterologous ATP sulfurylases, by growth on selenate-containing media showed that only the strain expressing the homologous protein was able to grow (Fig. 4). This was confirmed to result from reduced [35S]sulfate transport capacity (Table I) and demonstrates that ASA1 does not fully complement the met3 mutant phenotype. This suggests that the observed differences in growth kinetics (Fig. 3) probably result from the low sulfate uptake rate in the pF61-ASA1 complemented mutant rather than differences in expression level or structure. The loss of sulfate transport when the ATP sulfurylase gene is mutated indicates that structural interactions, which are essential for efficient sulfate uptake, may exist in yeast between some sulfate transporters and ATP sulfurylase. In plants, such an interaction between the sulfate transporter and ATP sulfurylase may not exist or may depend on specific recognition domains which are absent or different from those of yeast. Alternatively, the presence of the transit peptide may affect protein conformation and this interaction. Interestingly, a yeast mutant defective in sulfate transport exhibits normal ATP sulfurylase activity (49). Interaction between the sulfate transporter and the first enzyme in the sulfate metabolic pathway may be advantageous as it could result in a degree of sulfate channeling from the transporter into the sulfate binding and activation site of the ATP sulfurylase. This could push the unfavorable thermodynamic equilibrium of the ATP sulfurylase reaction toward forming the APS product. Such a channeling mechanism has been suggested in plants for the cysteine synthase complex (50, 51) and for the animal bifunctional ATP sulfurylase/adenosine 5'-phosphosulfate kinase (7, 20, 21).

Sequence analysis indicates that ASA1 has a 62-amino acid putative transit peptide. Homology of the proposed mature ASA1 protein to N-terminal sequence of a spinach chloroplast isozyme (5) suggests its chloroplastic location. In addition to blocks of homology common to all eukaryotic ATP sulfurylase sequences, a putative tyrosine phosphorylation site was identified (Fig. 1, block II) which is conserved only in the higher eukaryote sequences. This site is situated between the conserved block proposed to form part of the catalytic site (Fig. 1, block I) (15) and a region of homology including the putative phosphate binding loop sequence (Fig. 1, block III) (14). Translational control of enzyme activity through tyrosine phosphorylation has been demonstrated extensively in animals. In plants, a tyrosine kinase has only recently been cloned (52), and the regulation of a plant enzyme by tyrosine phosphorylation has just been demonstrated in vivo (53). Prokaryotic ATP sulfurylases have been found to contain a GTP binding motif and to be stimulated by GTP (2). GTP has little effect on the activity of plant, yeast, and fungal enzymes (15) and, in common with other eukaryotic ATP sulfurylase sequences, no GTP preference for polyadenylation in response to the second site after the open reading frame (46). Yeast Met3 mutants complemented with ASA1 had a lower growth rate than wild type or the mutant complemented by the homologous gene. This result may reflect differences between constitutive expression of ASA1 by the phosphoglycerate kinase promoter and expression of the yeast ATP sulfurylase gene by its own promoter. Alternatively, the presence of a chloroplast transit peptide or differences in primary sequence between the yeast and ASA1 could significantly alter the activity of the plant enzyme. Putative cytosolic and chloroplastic forms of ATP sulfurylase have been cloned from potato by functional complementation (16); however, only the activity of the cytosolic form expressed in yeast was presented. Activity measurements for all complemented met3 mutants were lower than for the noncomplemented Met3 strain, but as the growth curve of the mutant complemented with the homologous gene is similar to that of the wild type (Fig. 3) there does not seem to be any growth limitation due to insufficient ATP sulfurylase activity, suggesting that more activity than required for growth is expressed in the wild type strain. Expression of ASA1 in the Met3 strain reduced the ATP sulfurylase activity by 30% compared to when it was expressed in the met3 mutants and reduced the activity of the noncomplemented Met3 strain by 50% (Table I). This result implies that the plant ATP sulfurylase protein or cDNA has an effect on either the yeast protein activity or the yeast gene expression. For example, ATP sulfurylase is a homomultimeric protein in plants (5, 19) and yeast (9), and it is possible that in the non-mutant Met3 strain the co-expressed yeast and plant proteins form chimeric structures with reduced or no ATP sulfurylase activity. In contrast, the activity measured for pM3-32 in the Met3 strain was 20-fold higher than that when the plasmid complemented the mutant strains and 4-fold greater than the noncomplemented Met3 strain which supports the suggestion that, in the wild type strain, ATP sulfurylase activity levels are not growth-limiting.
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, T42953, 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 perhaps 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 sulfate 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, 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 multi-gene 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 sulfate starvation. This indicates that the initial response to sulfate starvation is probably at the transcriptional level. Subsequently relative SA1 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 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 A. thaliana cDNA library, and Prof. R. J. Fer (University of Gainesville) for the A. thaliana actin probe. We are grateful to Dr. H. Sentenac for useful discussions and to P. Bousquet for technical assistance.

REFERENCES