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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*

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

From the École 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 enzymes. 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: sulf fate adenyllyltransferase, 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 plastids (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 homologous, 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 (MATa, his3, leu2, ade2, trp1, ura3) and the mutant strains CC371–4C (MATa, leu2, ura3, met3) and C155 (MATa, his3, leu2, ura3, 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.
ATP Sulfurylase from A. thaliana

coli 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 prepared by replacing MgSO₄ by the corresponding chloride salt. Organs were harvested after 8 days of growth. A. thaliana organs essentially as (mutant type erreta 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 temperature 20°C, 70% relative humidity, and 90% constant 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 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) was 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 and subcloned into pBluescript SK+ (Stratagene) by the spheroplast protocol (25).

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 × 10⁶ cells μg⁻¹) by the spheroplast protocol (28). The transformation mixture was plated on 2% Bacto-agar selective medium (29). This library was prepared from mRNA extracted from mRNA derived from 

Northern blotting was carried out on sulfate-free dialyzed fractions (8) by molybdolysis (3). The pyrophosphate released from ATP was determined according to Ref. 42.

Yeast ATP Sulfurylase Assay—Yeast cells were grown at 28°C in suitable culture solutions to an absorbance of 1.0 measured at 650 nm. Extraction was essentially as described (41) using cell breakage glass beads and a modified extraction buffer, 100 mM Tris-HCl, pH 7.5, 0.2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10% (v/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.

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

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 sulfate as 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 isolating with ice-cold 0.5 mM MgSO₄, vacuum filtration through a glass microfiber filter (GF/C, Whatman) applied to a pre-chilled stainless steel filtration device, and radioactive retention on the filters was counted.

Protein Assays—Protein content of extracts were measured by the 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.

**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 sulfate as 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 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) at 46 (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 (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 isoform purified from spinach (SLIDPDGGSLIDLIVPENER) (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 GXXXKX 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 confirms to the consensus motif (R/K)XXX(D/E)XXX (Prosite accession number PS000007) and is also present in the other higher eukaryote ATP sulfurylase sequences (Fig. 1, block II).
ATP Sulfurylase from A. thaliana

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**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 milliunits initial absorbance, measured at 650 nm, in synthetic media supplemented with 0.4 mM sodium selenate as the sole sulfur source and the required auxotrophy factors. Symbols correspond as follows: □, Met^3^- strain W303-1A; □, met3 mutant strain CC371-4C; □, met3 mutant strain CC371-4C transformed with pFL61-ASA1; △, met3 mutant strain CC371-4C transformed with pM3-32.

**TABLE I**

<table>
<thead>
<tr>
<th>Yeast strains</th>
<th>ATP sulfurylase a</th>
<th>[35S]Sulfate influxes b</th>
</tr>
</thead>
<tbody>
<tr>
<td>W303-1A</td>
<td>538</td>
<td>46.0</td>
</tr>
<tr>
<td>W303-1A + pM3-32 a</td>
<td>2329</td>
<td>ND</td>
</tr>
<tr>
<td>W303-1A + pFL61-ASA1 a</td>
<td>243</td>
<td>ND</td>
</tr>
<tr>
<td>CC371-4C</td>
<td>356</td>
<td>7.9</td>
</tr>
<tr>
<td>CC371-4C + pFL61-ASA1 a</td>
<td>113</td>
<td>178.5</td>
</tr>
<tr>
<td>C155</td>
<td>3</td>
<td>ND</td>
</tr>
<tr>
<td>C155 + pFL61-ASA1</td>
<td>317</td>
<td>ND</td>
</tr>
</tbody>
</table>

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homocysteine as the sole sulfur source) in the presence of 4 mM sodium selenate, 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 I). 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.

**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

Fig. 5. Northern blot analysis of total RNA from A. thaliana
and B. napus with and without sulfate. Total RNA (10 μg) from plants grown in the presence (+) or absence (−) of sulfate (S) was isolated from leaves (L), cotyledons (C), or roots (R) and electrophoresed on an agarose (1.2%) gel, transferred onto a nitrocellulose filter, and then hybridized with 32P-labeled DNA fragments either ASA1 or actin.

Fig. 6. Northern blot analysis and ATP sulfurylase activity of B. napus organs grown with and without sulfate. Total RNA or soluble protein was isolated from cotyledons (Co), hypocotyls (H), and roots (R) after growth in the presence (+) or absence of sulfate for 4 (−) days. RNA (10 μg) was electrophoresed on an agarose (1.2%) gel, transferred onto a nitrocellulose filter, and then hybridized with 32P-labeled DNA fragments either ASA1 or actin. A, the relative abundances of ASA1 hybridizing transcripts in these organs, determined from RNA slot-blot analysis, standardized to actin hybridization, and compared for each organ to the sulfate (+) value. Duplicate slots were carried out for each sample. B, the relative ATP sulfurylase specific activities in these organs compared for each organ to the sulfate (+) value. C, the ATP sulfurylase specific activities measured in these organs, expressed as nmol min⁻¹ mg⁻¹ of protein, are the mean of three replicates.

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...
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, R28919, 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,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

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2 C. Deswarte, A. Borchers, H. Logan, and J.-C. Davidian, unpublished results.
having different response profiles to sulfate 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.

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ATP Sulfurylase from A. thaliana 12233