Note

Differential Accumulation of Transcripts Encoding Sulfur Assimilation Enzymes upon Sulfur and/or Nitrogen Deprivation in Arabidopsis thaliana

Yube Yamaguchi, Tatsuo Nakamura, Emiko Harada, Nozomu Koizumi, and Hiroshi Sano*

Research and Education Center for Genetic Information, Nara Institute of Science and Technology, 8916-5 Takayama-cho, Ikoma, Nara 630-0101, Japan

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Expression of nine genes encoding enzymes involved in the sulfur assimilation pathway was examined by RNA blot hybridization. Significantly increased levels of transcripts encoding ATP sulfurylase and APS reductase were apparent under sulfur deprivation. However, in the absence of nitrogen, their responsiveness to sulfur deprivation was markedly reduced. Results suggest that the sulfur assimilation pathway is regulated at the transcriptional level by both nitrogen and sulfur sources.

Key words: APS reductase; Arabidopsis thaliana; ATP sulfurylase; nitrogen source; sulfur assimilation

Extracellular sulfate is taken up by sulfate transporters and then activated by ATP to form APS in a reaction catalyzed by ATP-sulfurylase. APS is believed to be directly reduced by APS-reductase to sulfite, which is further reduced to sulfide by sulfite reductase. The resulting sulfide is used for cysteine biosynthesis by cysteine synthase using O-acetylserine, which is synthesized from serine by serine acetyltransferase. From physiological studies, sulfate assimilation has been shown to be coordinated with nitrate assimilation. In order to understand the crosstalk between sulfur and nitrogen assimilation, we examined first whether or not regulation of sulfur assimilation occurs at the mRNA level, and if so whether or not availability of sulfur and/or nitrogen influences their transcript levels.

Seeds of A. thaliana (ecotype Columbia) were germinated on agar medium at 25°C under continuous light. After one week, seedlings were transferred to, and grown in, liquid medium containing 1% sucrose at 25°C under continuous light with gentle shaking. Two-week-old plants were transferred to nutrient stress media containing 1% sucrose of the following compositions: sulfate-deficient medium containing MgCl₂ instead of MgSO₄, or nitrogen-deficient medium with KCl and CaCl₂ in place of KNO₃ and Ca(NO₃)₂, respectively. Plants were further cultivated in stress medium at 25°C under continuous light or continuous dark condition, and harvested on days 0.5, 1, 4, and 6.

Nine cDNA probes (Table 1) were prepared from a cDNA library of A. thaliana by PCR amplification. The sequences were derived from ATST1 (sulfate transporter), APS (chloroplastic ATP sulfurylase), PRH19 (chloroplastic APS reductase), akn (chloroplastic APS kinase), sir (chloroplastic sulfite reductase), cyS-3A (cytosolic cysteine synthase), cpAACS1 (chloroplastic cysteine synthase) (GenBank X81973), mtAACS1 (mitochondrial cysteine synthase) (GenBank X81698) and SAT-c (cytosolic serine acetyltransferase).

Total RNA from the whole plant body was analyzed by RNA blot hybridization. The hybridization signals were measured by a BAS-2000 Image Analyzer with normalization with respect to the signal from ribosomal RNA stained with ethidium bromide. Accumulation of mRNA transcripts was estimated using a relative ratio to the value at time zero (Fig. 1).

ATST1, encoding a sulfate transporter which was mainly expressed in above-ground tissues, was little influenced by nutrient stress and light conditions. However, mRNA of another sulfate transporter gene,

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### Table 1. Genes Used in This Study

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Enzyme name</th>
<th>Localization</th>
<th>Reference</th>
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<tr>
<td>ATST1</td>
<td>sulfate transporter</td>
<td>unknown</td>
<td>Yamaguchi et al., 1997</td>
</tr>
<tr>
<td>APS3</td>
<td>ATP sulfurylase</td>
<td>chloroplast</td>
<td>Murillo et al., 1995</td>
</tr>
<tr>
<td>PRH19</td>
<td>APS reductase</td>
<td>chloroplast</td>
<td>Gutierrez-Marcos et al., 1996</td>
</tr>
<tr>
<td>akn</td>
<td>APS kinase</td>
<td>chloroplast</td>
<td>Arx et al., 1994</td>
</tr>
<tr>
<td>sir</td>
<td>sulfite reductase</td>
<td>chloroplast</td>
<td>Bruhl et al., 1996</td>
</tr>
<tr>
<td>cyS-3A</td>
<td>cysteine synthase</td>
<td>cytosol</td>
<td>Barroso et al., 1995</td>
</tr>
<tr>
<td>cpAACS1</td>
<td>cysteine synthase</td>
<td>chloroplast</td>
<td>GenBank X81698</td>
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<tr>
<td>mtAACS1</td>
<td>cysteine synthase</td>
<td>mitochondrial</td>
<td>GenBank X81973</td>
</tr>
<tr>
<td>SAT-c</td>
<td>serine acetyltransferase</td>
<td>chloroplast</td>
<td>Howarth et al., 1997</td>
</tr>
</tbody>
</table>

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* To whom correspondence should be addressed. Hiroshi Sano, Fax: +81-743-72-5659; E-mail: sano@gtc.aist-nara.ac.jp

Abbreviations: APS, adenosine 5′-phosphosulfate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate
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**Fig. 1.**

- **Light**
  - *ATST1*
  - *APS3*
  - *AKN*
  - *PRH19*
  - *SIR*

- **Dark**
  - *ATST1*
  - *APS3*
  - *AKN*
  - *PRH19*
  - *SIR*

Relative mRNA level vs. Time (day)
AST68, was increased up to 9-fold in roots when plants were exposed to sulfate deprivation. It was suggested that higher plants respond to sulfur deficiency by increasing the capacity to take up sulfate via root tissue.

During sulfur starvation, mRNA of APS3, which encodes ATP sulfurylase, was increased 4-fold under continuous darkness. Under the continuous light condition, however, the level of mRNA was stably maintained. It is conceivable that the expression of APS3 may be under the control of both light and sulfur concentration. This speculation is partly agreeable to a report that, under sulfur deficiency the extractable activity of ATP sulfurylase...
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in green tissues remains little changed from control values, while it is increased in heterotrophically grown cells. Because the nutrient solution used in our study contained 1% sucrose, sulfur stress under continuous darkness may reflect the heterotrophic condition. Additional two isoforms of plastidic ATP sulfurylase were reported. The mRNA level of ASAl was increased with sulfur starvation, but that of APS1 was not affected.

An accumulation of PRH19 mRNA encoding APS reductase increased more than 5-fold with sulfur starvation in comparison to the controls. This is consistent with previous reports where PRH19 mRNA showed significant increases in plant roots exposed to sulfur deprivation. APS reductase and APS kinase function at the branching point of the primary and the secondary metabolic pathways of sulfur, and compete for APS as a substrate. Since there was no increase in akn mRNA encoding APS kinase, it was conceivable that, when plants are exposed to sulfur deprivation, they selectively increase APS reductase activity to give priority to primary metabolism over secondary metabolism.

The mRNA level of sir, encoding the chloroplastic sulfite reductase, was not influenced by nutrient stress or light conditions, indicating sir to function in a house-keeping mode at ATST1.

Under sulfur starvation, the mRNA level of cpACS, encoding the chloroplastic cysteine synthase, was consistent under continuous light, but increased slightly under continuous darkness. The mRNA for cys-3A encoding the cytosolic cysteine synthase increased 2.5-fold under continuous light, and 2-fold under continuous darkness. This is consistent with a previous report showing cys-3A to respond to sulfur deprivation. Accumulation of mtACS1 mRNA, encoding the mitochondrial cysteine synthase, was not influenced by either nutrient stress or light conditions.

Accumulation of SAT-m and SAT-c mRNAs, encoding mitochondrial and cytosolic serine acetyltransferase, respectively, was not influenced by sulfur deprivation. Transcripts of SAT-p, which encodes the chloroplastic isoform of serine acetyltransferase, were reported to be greatly increased under sulfur starvation. However, we found in this study that SAT-c increased up to 2-fold with nitrogen starvation.

Our experiments have shown that, plants were deprived of sulfur, the mRNA levels for PRH19 and APS3 were greatly increased. When only nitrogen was withdrawn, their mRNA levels did not change. However when both nitrogen and sulfur were withdrawn, their mRNA levels were only somewhat increased. This was not due to the suspension of protein synthesis by nitrogen starvation, because the accumulation of mRNAs did not decrease during nitrogen starvation in all cases. This is consistent with a biochemical experiment, in which cysteine synthase enzymatic activity in A. thaliana increased during sulfur deprivation but not during the double stress of sulfur and nitrogen deprivation. It was thus concluded that higher plants require a nitrogen source to properly respond to sulfur starvation.

To date, multiple isoforms are known for genes encoding enzymes involved in the sulfur assimilation pathway. Although we did not analyze all of these isoforms in this study, our results together with published observations may reflect a common feature of sulfur assimilation, in which only limited genes respond transcriptionally to sulfur deprivation. However, for the appropriate response of these genes, a sufficient nitrogen source was found to be necessary. This suggests that a well tuned regulation mechanism functions in crosstalk between sulfur and nitrogen assimilation. The question of how sulfur and nitrogen stresses are transmitted to the sulfur assimilation pathway remains to be solved.

Acknowledgments
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References
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