Molybdenum (Mo) is an essential element for living organisms, however no molybdate transporter has been identified in eukaryotes. Here, we report the identification of a molybdate transporter, MOT1, from Arabidopsis thaliana. MOT1 is expressed in both roots and shoots, and the MOT1 protein is localized, in part, to plasma membranes and to vesicles. MOT1 is required for efficient uptake and translocation of molybdate and for normal growth under conditions of limited molybdate supply. Kinetics studies in yeast revealed that the $K_M$ value of MOT1 for molybdate is $\approx 20$ nM. Furthermore, Mo uptake by MOT1 in yeast was not affected by coexistent sulfate, and MOT1 did not complement a sulfate transporter-deficient yeast mutant strain. These data confirmed that MOT1 is specific for molybdate and that the high affinity of MOT1 allows plants to obtain scarce Mo from soil.

Results and Discussion

In our effort to characterize the A. thaliana mutant bor-1 (18, 19), we determined the concentrations of 10 elements in shoots of A. thaliana plants grown hydroponically in a standard medium (20). We found that bor-1 specifically reduces boron concentration in rosette leaves but not the other elements examined. In this analysis, we also noticed that the concentrations of Mo, but not those of the other nine elements examined, differed by approximately 3-fold between the accessions Col-0 and Ler (Fig. 1A). A similar result has also been reported (21).

To understand the genetic mechanism that controls Mo concentration in shoots, Col-0 × Ler recombinant inbred lines (22) were grown, and their Mo concentrations were determined. The results indicated that the trait is mostly regulated by a single locus on chromosome 2 (Fig. 1B). Further genetic analysis of Col-0 × Ler R1 lines and F2 lines delimited the locus in a 172-kb region of chromosome 2 between polymorphisms located at 10,887,652 bp (F13B15.02) and 11,060,616 bp (F17B15.01) (Fig. 1C). This region is predicted to contain 36 genes including a gene (At2g25680) annotated as Suhr5:2 (representing a member of sulfate transporter) (13). However, to our knowledge, no sulfate transport activity has been demonstrated for this protein. A transcriptome study indicated that the accumulation of At2g25680 transcripts is not affected by sulfur deficiency, unlike the transcripts of other major sulfate transporters (24). Mo is taken up from soil by plants in the form of molybdate (MoO$_4^{2-}$), which is chemically similar to sulfate (7). We speculated that At2g25680 was a molybdate transporter, and the causal gene of the difference in Mo concentration in shoots between the two accessions. Analysis of the nucleotide sequences of this intronless gene in Col-0 and Ler identified two differences (25). One is a single nucleotide substitution at position 1,286 (relative to the initiation codon). In Col-0 and Ler, this nucleotide was A and T, respectively, corresponding to an amino acid residue difference of Asp$^{229}$ in Col-0 and Val$^{229}$ in Ler. The other is a deletion/insertion of a 53-bp sequence just upstream of the initiation codon. The nucleotide sequence corresponding to −27 to −79 upstream of the start codon in Col-0 is not present in Ler. These differences were inferred to affect activity of the translation product and the gene expression level, respectively.

Author contributions: H. Tomatsu, J.T., and T.F. designed research; H. Tomatsu, J.T., H. Takahashi, A.W.-T., and T.F. performed research; N.S. contributed new reagents/analytic tools; H. Tomatsu, J.T., and T.F. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Present address: Department of Nutritional Science, University of Wisconsin, 1415 Linden Drive, Madison, WI 53706.

**To whom correspondence should be addressed. E-mail: atoru@cc.u-tokyo.ac.jp.

This article contains supporting information online at www.pnas.org/cgi/content/full/0706373104DC1.

© 2007 by The National Academy of Sciences of the USA

An Arabidopsis thaliana high-affinity molybdate transporter required for efficient uptake of molybdate from soil

Hajime Tomatsu*†, Junpei Takano*‡, Hideki Takahashi§, Akiko Watanabe-Takahashi‡, Nakako Shibagaki¶, and Toru Fujiwara***

*Biotechnology Research Center and †Graduate School of Agricultural and Life Sciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan; ‡RIKEN Plant Science Center, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan; ¶Department of Plant Biology, Carnegie Institution of Washington, 260 Panama Street, Palo Alto, CA 94305; and §Solution-Oriented Research for Science and Technology (SORST), Japan Science and Technology Corporation, Cho-ku, Tokyo 103-0027, Japan

Edited by Maarten J. Chrispeels, University of California at San Diego, La Jolla, CA, and approved October 1, 2007 (received for review July 7, 2007)
To confirm that At2g25680 is the gene responsible for the differences in shoot Mo concentrations, two independent transgenic plant lines carrying transferred DNA (T-DNA) inserted in At2g25680 were obtained from the Arabidopsis Biological Re-

source Center at Ohio State University (Columbus, OH). Sequence analysis of the junction of the T-DNA with the genomic DNA confirmed that the lines SALK_118311 and SALK_069683

Fig. 1. Difference in Mo concentration between Col-0 and Ler and quantitative trait loci mapping. (A) Comparison of elemental concentrations in shoots of A. thaliana Col-0 and Ler. Col-0 and Ler plants were grown hydroponically in a standard medium (20) for 18 days. The concentration of each element in Ler plants is shown relative to those in Col-0 plants. Averages and standard deviations are shown. n = 5. (B) Quantitative trait loci mapping of Mo concentration. Logarithm of odds (LOD) score curves of chromosomes 1–5 were obtained from the interval-mapping methods by using QGENE software (23). The highest LOD scores on each chromosome are presented. (C) Mapping of MOT1. 16 recombinant inbred lines (Col-0 × Ler) containing recombination between the markers mi238 and er were scored for Mo concentrations and genetic markers. The RI mapping data referred to the data published by the Nottingham Arabidopsis Stock Centre. The RI lines were sorted into two classes based on their shoot Mo concentrations (high, Col-0 type; low, Ler type). The class of each was compared with the genotype in each marker, and the number of mismatches was counted. The arrow and number indicate the position of genetic markers and the count of mismatches. To identify the single locus correlated with the shoot Mo concentration, Col-0 × Ler F₂ lines were analyzed in the same manner. The concentration of each element in L. plants is shown relative to those in Col-0 plants. Averages and standard deviations are shown; n = 5. (D) Effect of Mo deprivation on plant growth. Wild-type and mot1 mutants. The seeds harvested from the plants grown on rockwool supplied with standard medium (20) without molybdate were used for this growth test. Plants were grown on solid standard medium with an added 14 mM KNO3 with and without 170 mM molybdate. The shoot fresh weights of plants grown for 15 days were measured, and averages and standard deviations are shown; n = 4. (E) The root length of plants grown for 8 days. Averages and standard deviations are shown; n = 4.
carry T-DNAs in the coding and promoter regions of At2g25680, respectively (Fig. 2A). These lines will be referred to as mot1-1 and mot1-2 (for molybdate transporter). Considering the position of the T-DNA insertion, the mot1-1 mutant is unlikely capable of producing a normal At2g25680. The levels of At2g25680 transcript accumulation in mot1-2 mutant plants were found to be $\approx 50\%$ of that in wild-type plants, in both shoots and roots (Fig. 2B). Accumulation of At2g25680 transcripts in Ler was also reduced to $\approx 50\%$ and $20\%$ of the levels in shoots and roots, respectively, of Col-0 (Fig. 2B). It is possible that the deletion in the promoter region of At2g25680 in Ler is the cause of the reduced accumulation of At2g25680 transcripts in this accession.

We next determined the Mo concentration in shoots and roots of the mutant lines. The lines were grown in the presence of $170$ nM molybdate for 5 weeks, after which the Mo concentrations in shoots and roots were determined (Fig. 2C). The Mo concentrations in shoots of the mot1-1 and mot1-2 mutant plants were reduced to $10\%$ and $20\%$, respectively, of that in the wild type, and, in roots, the Mo concentrations were reduced to $20\%$ and $25\%$ of that in the wild type. These results indicate that At2g25680 is the determinant of the Mo concentration in both roots and shoots. For further confirmation, the Mo concentration in shoots of F$_2$ progeny from crosses between mot1-1 and mot1-2 were determined and found to be $<20\%$ of that in wild-type plants (Fig. 2C). Mo concentrations were also determined in shoots of individual F$_3$ progeny from crosses between the mot1-1 mutant and Col-0. Among the 41 F$_3$ plants examined, 4 plants were found to be homozygous for the T-DNA insertion. Each of the four lines had Mo concentrations $\approx 10\%$ of that in the wild type. In all of the other F$_3$ plants, each of which carried the intact At2g25680 gene, the Mo levels in shoots were similar to that in wild type (data not shown). These results further confirm that At2g25680 is the causal gene for the phenotype. We therefore named the At2g25680 gene MOT1.

Because shoots and roots of mot1-1 and mot1-2 mutant plants contain reduced levels of Mo, we postulated that the growth of the mutants would be affected under conditions of limited Mo supply. To test this hypothesis, the mot1 mutant, Col-0, and Ler plants were grown together on medium (20) with and without supplementation of Mo (Fig. 2D). Because Mo stored in seeds may influence plant growth, the seeds used for these tests were harvested from plants grown on rockwool supplied with the standard medium without molybdate. When compared with growth under conditions of normal Mo supply, growth under Mo limitation reduced shoot growth to $35\%$, $80\%$, and $55\%$ in mot1-1, mot1-2 mutants and Ler plants, respectively (Fig. 2E). In contrast, the shoot fresh weight of Col-0 did not change significantly ($P > 0.05$). Root lengths were also reduced to $\approx 70\%$, $75\%$, and $65\%$ in mot1-1, mot1-2 mutants and Ler plants, respectively (Fig. 2F). These results suggest that MOT1 is required for efficient growth of both roots and shoots under conditions of limited Mo supply. It is likely that plant growth on medium without supplementation of Mo is supported by Mo contamination in the medium or Mo stored in seeds. The levels of Mo contamination in our media were found to be $<5$ nM (data not shown).

To investigate regulation of MOT1 gene expression by Mo availability, we performed quantitative RT-PCR to quantify MOT1 mRNA from plants grown under sufficient or limited Mo conditions. Plants were grown on the solid standard medium with and without molybdate for 14 days, and total RNA was isolated from shoots and roots. Under Mo limitation, MOT1 expression in shoots was decreased to $50\%$ of that in plants supplied with sufficient Mo. MOT1 expression in roots was slightly but significantly reduced under Mo limitation (Fig. 3A).

We then examined the tissue specificity of MOT1 expression in transgenic Col-0 plants expressing the β-glucuronidase (GUS) under control of the MOT1 promoter. Plants were grown on the solid standard medium or on rockwool supplied with the Mo-supplemented standard medium. In 7-day-old seedlings, GUS activity was observed in roots and petioles of cotyledons (Fig. 3B) and in all cells in the mature portion of roots (Fig. 3C). In plants at the reproductive stage, GUS activity was observed in...
cells is hypothesized to be stationary level of Mo concentration in cells expressing MOT1, which is linearly up to 15 min, and a steady-state level duration of the experiment (Fig. 4). The cells were incubated with 170 nM molybdate for 15 min. Averages and standard deviations are shown; n = 4 (A and C) or 3 (B).

Fig. 4. Transport properties of MOT1. (A) Time course of molybdate uptake in S. cerevisiae cells expressing MOT1 (filled circles) or cells containing the empty vector (open circles). (B) Lineweaver–Burk plot of molybdate uptake in S. cerevisiae cells expressing MOT1 under various Mo conditions. (C) Molybdate uptake in S. cerevisiae cells expressing MOT1 cloned from Col-0 or Ler. The cells were incubated with 170 nM molybdate for 15 min. Averages and standard deviations are shown; n = 4 (A and C) or 3 (B).

Mesophylls and petioles of leaves, stamen, and calyx in flowers and siliques (Fig. 3D–F). These expression patterns were observed consistently in five independent transgenic lines. These results suggest that MOT1 is expressed mostly throughout the plant body and may be important for Mo uptake in various types of cells in both roots and shoots.

We next investigated subcellular localization of MOT1. GFP-MOT1 was expressed in tobacco (Nicotiana tabacum L.) BY-2 cells were cultured under control of the cauliflower mosaic virus 35S RNA promoter, and GFP fluorescence was observed by using a laser scanning confocal microscope. Typical images of tobacco cells expressing GFP-MOT1 (Fig. 3G) or GFP alone (Fig. 3H) are shown. Fluorescence was observed in dot-like structures and in the periphery of the cells bombarded with GFP-MOT1. Compared with the pattern observed with free GFP, fluorescence of GFP-MOT1 cells is more confined to near the plasma membrane (see right-hand side of the cells in Fig. 3G and H). These results suggest that MOT1 is localized to the plasma membrane and the endomembrane presumably in the secretory and/or endocytic pathways.

To examine the molybdate transport activity of the protein, MOT1 was expressed in the yeast Saccharomyces cerevisiae. Yeast transformants cultured in Mo-free SD medium (26) to the midlog phase were transferred to synthetic defined (SD) medium containing 170 nM molybdate, and after various exposure times, Mo concentrations in cells were determined. In cells containing the empty vector, the Mo concentration remained low for the duration of the experiment (Fig. 4A, open circles). In contrast, in cells expressing MOT1, the Mo concentrations increased linearly up to 15 min, and a steady-state level [\(\approx 10 \mu g\cdot g^{-1}\cdot DW\) as Mo, which is \(\approx 100\ nmol\cdot g^{-1}\cdot dry\ weight\ (DW)\), was attained within 45 min (Fig. 4A, filled circles). The dry weight of yeast cells is hypothesized to be \(\approx 30\%\) of fresh weight (26). The stationary level of Mo concentration in cells expressing MOT1 was estimated to be roughly 30 \(\mu M\); >100-fold higher than that in the medium used for the uptake study. These results indicated that MOT1 is a molybdate transporter capable of transporting molybdate against a concentration gradient.

We then investigated the molybdate transport kinetics of MOT1. Yeast transformants expressing MOT1 were exposed to SD medium containing various concentrations of Mo for 15 min, and total Mo concentrations in the cells were determined. Kinetics analysis of the results showed that \(K_m\) and \(V_{max}\) values for molybdate uptake of yeast expressing MOT1 were 21 ± 4 nM and 0.5 ± 0.1 \(\mu g\cdot g^{-1}\cdot DW\cdot min^{-1}\), respectively (Fig. 4B). To our knowledge, 20 nM is the lowest \(K_m\) values of the mineral–nutrient transporters in plants reported (11–17) thus far.

In planta, the Mo concentrations in roots and shoots of Ler were lower than those of Col-0 (Fig. 2C). We measured the Mo concentrations in cells expressing MOT1 from Ler and found that MOT1 is a molybdate transporter capable of transporting molybdate against a concentration gradient.
that the Ler type MOT1 also has molybdate transport activity, but Mo levels in cells were lower than that of cells expressing MOT1 from Col-0 (Fig. 4C). It is possible that the amino acid substitution in MOT1 in Ler affects molybdate transport activity.

Because the chemical properties of molybdate are similar to sulfate, we investigated the effect of coexistent sulfate on molybdate uptake by MOT1. Yeast transformants expressing MOT1 were cultured in Mo-free SD medium (26) to the midlog phase and were transferred to SD medium containing 170 nM molybdate and 20, 100, 500, or 2,500 μM sulfate. The cells were incubated for 15 min, and Mo concentrations in cells were determined. No significant differences were found in their Mo concentrations (Fig. 5A).

Because MOT1 is similar to sulfate transporters, we examined molybdate transport activity of a sulfate transporter from A. thaliana. Yeast cells expressing Sultr1;2, a high-affinity sulfate transporter required for sulfate uptake from soil, were exposed to molybdate, and Mo concentrations in cells were determined. Unlike that observed for MOT1, expression of Sultr1;2 did not cause high accumulation of Mo (Fig. 5B). We also examined the possible sulfate transport activity of MOT1. MOT1 was expressed in yeast mutant CP154-7B (27, 28), which is defective in two high-affinity sulfate transporters, and growth of the transformants were compared on the media containing sulfate as a sole source of sulfur. Expression of MOT1 did not complement the growth defect of the mutant strain on –Met media, whereas Sultr1;2 complemented the defect (Fig. 5C). This result suggests that MOT1 does not transport sulfate to the extent of high-affinity transporters. It is likely that MOT1 and sultr1;2 are specific to molybdate and sulfate, respectively. It is possible that MOT1 evolved from an ancestral sulfate transporter gene and diverged to obtain a molybdate-specific transport function.

Taken together, the present results demonstrate that MOT1 is a high-affinity transporter essential for plants to take up scant molybdate from soil. MOT1 is expressed in the whole-cell layer in root, especially at higher levels in endodermis and stele cells. These results suggest that MOT1 accumulates molybdate in the stele cells, and different transporters may exist for loading accumulated molybdate to xylem. In addition, MOT1 is expressed in the shoot, and Mo concentrations in the shoots of mot1 mutants were decreased. These facts suggest that MOT1 enhances molybdate uptake from soil into root cells for utilization and also for translocation to shoots.

Materials and Methods

Plant Materials. Col-0 and Ler of A. thaliana (L.) Heynh were derived from our laboratory stock. The SALK.118311 (mot1-1) and SALK.069683 (mot1-2) lines were obtained, and homogenous lines for the T-DNA insertion were selected by using gene-specific primers in combination with the T-DNA left border-specific primer. The resulting PCR products were sequenced to determine the location of the T-DNA insertion. The seeds used for growth tests were harvested from the plants grown on rockwool supplied with standard medium (20) without molybdate.

Mapping of MOT. Eighteen recombinant inbred lines (Col-0 × Ler) (22) were scored for shoot Mo concentration for quantitative trait loci analysis. Shoot Mo concentration in Col-0 × Ler F2 lines were determined for fine mapping, and 13 F2 lines with recombinations between two MOT1 flanking markers were used to further delimit the MOT1 locus.

Quantitative RT-PCR. Total RNA was isolated from plant materials and subjected to quantitative RT-PCR analysis (29). The sizes of the amplified fragments were confirmed by gel electrophoresis.

Plasmid Construction and Plant Transformation. Details of the construction are described in supporting information (SI) Text. Plant transformations with Agrobacterium and particle bombardment were carried out as described (30).

Expression of MOT1 in S. cerevisiae and Molybdate Transport Assay. The MOT1 ORF was amplified by PCR and cloned into the yeast expression vector pYX222x (27), which contains the triose phosphate isomerase promoter for constitutive expression of MOT1. The S. cerevisiae strains BY4741 and CP154-7B (27) were transformed with the pYX222x vector containing MOT1 or A. thaliana Sultr1;2 (27). For time-course analysis of molybdate uptake, cells were transferred to the medium supplemented with 24 nM hexaammonium heptamolybdate, and shaken at 30°C for 0, 5, 10, 15, 20, 30, 45, or 60 min. For kinetic analysis of molybdate uptake, cells were transferred to the medium supplemented with 7, 8, 10, 12, 16, 24, 97 or 194 nM hexaammonium heptamolybdate, and shaken at 30°C for 15 min. Then, cells were harvested by centrifugation and washed twice with ice-cold deionized water. Yeast pellets were oven-dried, and their dry weights were determined. The experiment was performed on three or four independent transformants.

Analysis of Nutrient Concentration. Plant and yeast samples were prepared as described (30). Nutrient concentrations in the samples were determined as described (19).

We thank Y. Kawara and K. Aizawa for excellent technical assistance. We acknowledge Drs. Y. Niwa (University of Shizuoka, Shizuoka, Japan), K. Schumacher (Universität Tübingen, Tübingen, Germany), G. Schaaf (Universität Hohenheim, Hohenheim, Germany), M. D. Curtis, and U. Grossniklaus (Universität Zürich, Zürich, Switzerland) for providing plasmids; Dr. Akiko Yamamoto (Mie University, Mie-ken, Japan) for providing OGENE software; Nottingham Arabidopsis Stock Centre (Loughborough, U.K.) for providing seeds of the RI lines. We thank Dr. Emilio Fernández for sharing unpublished information about their recently identified molybdate transporter from Chlamydomonas reinhardtii, Drs. T. Uemura and T. Ueda for detailed advice on transformation of Arabidopsis cultured cells, and K. Miwa and D. Goto for critical reading of the manuscript. This work was supported, in part, by grants for Scientific Research on Priority Areas from the Japanese Ministry of Education, Culture, Sports, and Science, by a Grant-in-Aid for Scientific Research (B), from a 21st Century Center of Excellence project, and from the Greentechno Project by the Ministry of Agriculture of Japan (to T.F.).