論 文 の 内 容 の 要 旨

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論文題目

Identification and Characterization of Arabidopsis Molybdate Transporters

（シロイヌナズナのモリブデン酸輸送体の同定と解析）

Molybdenum（Mo）is an essential trace element for living organisms; however, molybdate transporter has not been identified in eukaryotes. The present thesis describes the first identification of a gene encoding eukaryotic molybdate transporter, MOT1, from *Arabidopsis thaliana* and the characterization of *MOT2*, a *MOT1*-like gene. *MOT1* transcript is accumulated in both roots and shoots. The growth of *mot1-1* and *mot1-2* mutants was suppressed under Mo limited conditions, suggesting that MOT1 is essential for efficient molybdate uptake from soils. Molybdate uptake assay using yeast expressing MOT1 revealed that MOT1 is a high-affinity molybdate transporter. The analysis of a transferred DNA insertion line for *MOT2* suggested that MOT2 is involved in molybdate translocation from roots to shoots. This thesis provides the basis for understanding the molecular mechanisms of Mo transport in plants.

Introduction

Molybdenum（Mo）is an essential element for all living organisms. Mo is a transition element, and is used by several enzymes that participate in reduction and oxidation reactions. In molybdenum-requiring enzymes（molybdoenzymes）, except for bacterial nitrogenase, Mo is bound to pterin as a form of Mo-cofactor（Moco）. In plants, four enzymes, nitrate reductase, aldehyde oxidase, sulfite oxidase, and xanthine oxidase are known as molybdoenzymes. Nitrate reductase catalyzes reduction of nitrate to nitrite, the first step of nitrate assimilation to ammonia and amino acids. Aldehyde oxidase is involved in an oxidation reaction that leads to the synthesis of abscisic acid. Plants that are incapable of using Moco are shown to be defective in nitrate reduction and abscisic acid biosynthesis.

Plants take up Mo from soil as molybdate（MoO_4^{2-}）. Molybdate is a weak Lewis acid, and the availability of Mo depends on soil pH. Mo deficiency is a widespread agricultural problem, especially in acid soils.

Regarding to the mechanism of Mo uptake from environment, molybdate transporters（ModABC）, which belong to the ATP-binding cassette protein superfamily, have been described in eubacteria and archaea. The ATP-binding cassette protein superfamily exists in eukaryotes; however, molybdate-specific transporters in eukaryotes have never been reported. In this thesis, I identified the first eukaryotic molybdate transporter as the causal gene of the difference of Mo concentrations in *Arabidopsis thaliana* two accessions, Col-0 and L*er*. Identified transporter was not an ATP-binding cassette type protein.

Chapter 1 An *Arabidopsis thaliana* high-affinity molybdate transporter required for efficient uptake of molybdate from soil

Dr. Takano determined the concentrations of 10 elements in shoots of *A. thaliana* plants grown hydroponically in a standard medium and noticed that the concentrations of Mo, but not the other nine elements examined, differed by approximately 3-fold between the accessions Col-0 and L*er*. Based on the results of quantitative trait loci mapping of Mo concentration carried out by Dr.

Takano, I mapped the causal gene of the difference in Mo concentration in shoots between the two accessions in detail. I identified the causal gene (*At2g25680*) that encodes a sulfate transporter-like protein, and named this gene *MOT1* (molybdate transporter 1).

Analysis of the nucleotide sequences of this intronless *MOT1* gene in Col-0 and L*er* identified two differences; single nucleotide substitution resulting in amino acid sequence change, and deletion of a 53-bp sequence just upstream of the initiation codon of *MOT1* in L*er*. The *MOT1* transcripts were detected in both roots and shoots, and molybdate induced their accumulation. In the transgenic plants expressing β-glucuronidase (GUS) under the control of the *MOT1* promoter, strong GUS signal was observed in the endodermis and stele. In tobacco cultured cells transiently expressing green fluorescent protein-MOT1 (GFP-MOT1) fusion protein, GFP-MOT1 fusion protein was localized, in part, to plasma membranes and to vesicles.

Mo concentrations in both roots and shoots of the mutants carrying transferred DNAs in the coding region (referred to as *mot1-1*) and promoter region (referred to as *mot1-2*) of *MOT1*, were less than 30% of that in wild-type plants. When compared with fresh weight of the *mot1-1* and *mot1-2* plants grown on the standard medium supplemented with 170 nM molybdate, fresh weight on the standard medium without molybdate supply was reduced to 35% and 80%, respectively. On the other hand, fresh weights of wild-type plants grown on standard medium supplied with or without molybdate were not significantly different. These results indicate that MOT1 is required for efficient uptake and/or 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 approximately 20 nM. Furthermore, molybdate 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 suggest that the high affinity of MOT1 allows plants to obtain scarce Mo from soil.

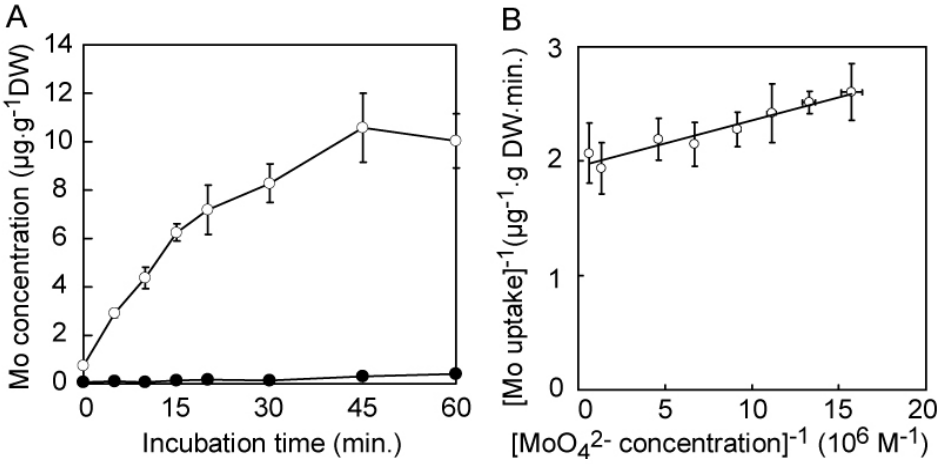

Figure 1 Transport properties of MOT1. (*A*) Time course of molybdate uptake in *S. cerevisiae* cells expressing MOT1 (open circles) or cells containing the empty vector (filled circles). The cells cultured with medium without molybdate were incubated with medium containing 170 nM molybdate for various times. Mo concentrations in cells were measured. (*B*) Lineweaver-Burk plot of molybdate uptake in *S. cerevisiae* cells expressing MOT1 cultured with medium supplemented with 7, 8,

Chapter 2 *Arabidopsis thaliana* molybdate transporter 2 (MOT2) is required for efficient translocation of molybdate

Homology search in the sequence databases suggests presence of genes similar to MOT1 in plants and fungi. At1g80310 is the most similar (Identity, 51%; Similarity, 82%) to MOT1 in Arabidopsis genome at amino acid sequence level and named MOT2. In this chapter, I examined whether MOT2 is involved in the transport of molybdate.

MOT2 is expressed both in roots and shoots. Molybdate did not induce *MOT2* transcript accumulation. In the transgenic plants expressing GUS under the control of the *MOT2* promoter, strong GUS signal was observed in the veins of leaves. In the cells of roots of the transgenic plants expressing GFP-MOT2 under the control of the cauliflower mosaic virus 35S RNA promoter, GFP-MOT2 fusion protein was localized to plasma membranes.

When plants grown on the standard medium without molybdate supply, the growth of the mutant plants carrying transferred DNA in the coding region of *MOT2* (*mot2-1*) were reduced to less than 40% of that of wild-type plants. Mo concentrations in shoots of *mot2-1* mutant plants

grown on standard medium supplemented with 170 nM molybdate were less than 45% of that of wild-type plants. However, Mo concentrations in roots of *mot2-1* mutant plants were approximately 2-fold higher than that of wild-type plants. Furthermore, in plants grown on standard medium without molybdate supply, Mo concentrations in shoots of *mot2-1* mutant plants were not different from that of wild-type plants, although the shoot growth of mutants was suppressed. Taken together, these results suggest that MOT2 is involved in molybdate translocation from roots to shoots in *A. thaliana*.

Figure 2 Mo concentrations in roots and shoots. Plants were grown for 3 weeks with medium containing 170 nM molybdate. *mot2-1* and *mot1-1* are mutant plants that carry transferred DNA in the coding region of *At1g80310*（*mot2-1*）or *At2g25680*

Conclusion

MOT1 is a molybdate importer expressing in both roots and shoots. MOT1 is a high-affinity and molybdate specific transporter for taking up scarce molybdate from soil efficiently. In the shoot, MOT1 is expressed in various tissues.

MOT2 is expressed in both roots and shoots, especially in the veins of leaves. The phenotype of *mot2-1* mutant suggests that MOT2 is essential for molybdate translocation from roots to shoots, efficiently.

Taken together, I established that *A. thaliana* has at least two types of molybdate transporters; one for uptake（MOT1-type）and the other for translocation（MOT2-type）of molybdate.

References

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