CHL1 Functions as a Nitrate Sensor in Plants

Cheng-Hsun Ho,[1,2] Shan-Hua Lin,[2] Heng-Cheng Hu,[2] and Yi-Fang Tsay[1,2,*]
[1]Molecular Cell Biology, Taiwan International Graduate Program, Institute of Molecular Biology, Academia Sinica, and Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan
[2]Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan
*Correspondence: yftsay@gate.sinica.edu.tw
DOI 10.1016/j.cell.2009.07.004

SUMMARY

Ions serve as essential nutrients in higher plants and can also act as signaling molecules. Little is known about how plants sense changes in soil nutrient concentrations. Previous studies showed that T101-phosphorylated CHL1 is a high-affinity nitrate transporter, whereas T101-dephosphorylated CHL1 is a low-affinity transporter. In this study, analysis of an uptake- and sensing-decoupled mutant showed that the nitrate transporter CHL1 functions as a nitrate sensor. Primary nitrate responses in CHL1T101D and CHLT101A transgenic plants showed that phosphorylated and dephosphorylated CHL1 lead to a low- and high-level response, respectively. In vitro and in vivo studies showed that, in response to low nitrate concentrations, protein kinase CIPK23 can phosphorylate T101 of CHL1 to maintain a low-level primary response . Thus, CHL1 uses dual-affinity binding and a phosphorylation switch to sense a wide range of nitrate concentrations in the soil, thereby functioning as an ion sensor in higher plants.

For a video summary of this article, see the PaperFlick file with the Supplemental Data available online.

INTRODUCTION

Plants acquire most of their essential nutrients from the soil. Ions in the soil not only serve as essential nutrients, but also function as signal molecules regulating plant development, gene expression, and metabolism. The ability to sense changes in soil ion concentrations and to respond metabolically to these changes is vital for nonmobile plants to survive in harsh conditions and to sustain maximal growth in nutrient-sufficient conditions. However, the plasma membrane ion sensor that detects these nutrient changes in the soil has not yet been identified in higher plants.

Nitrogen is a key limiting factor for plant growth and crop productivity. For most plants, nitrate is the primary nitrogen source (Crawford, 1995). To be assimilated, it has to be taken up from the soil and converted into ammonium by nitrate reduc-tase and nitrite reductase, and then into amino acid by enzymes such as glutamate synthase (Crawford, 1995). In addition to being an essential nutrient, nitrate also serves as a signaling molecule. For instance, it is known to regulate root architecture, stimulate shoot growth, delay flowering, regulate abscisic acid-independent stomata opening, and relieve seed dormancy (Walch-Liu et al., 2005).

The best known nitrate-induced response is the primary nitrate response (Redinbaugh and Campbell, 1991), in which gene expression of nitrate assimilatory enzymes and nitrate transporters, such as CHL1 and NRT2.1, is rapidly induced (within 0.5 to 1 hr) by nitrate (Wang et al., 2003). A normal primary nitrate response is seen in nitrate reductase mutants (Deng et al., 1989) and in the presence of protein synthesis inhibitors (Redinbaugh and Campbell, 1993), indicating that nitrate itself is responsible for the response and that de novo protein synthesis is not required. The molecular identities of the signaling components in the nitrate response are just beginning to be determined. For example, the transcription factor ANR1 is involved in the nitrate regulation of root architecture (Zhang and Forde, 1998), and recent studies have shown that the transcription factor NLP7 (Castaings et al., 2009) and the calcineurin-like protein (CBL)-interacting protein kinase CIPK8 (Hu et al., 2009) are positive regulators of the primary nitrate response.

In contrast to nitrate signaling, the molecular mechanism of nitrate uptake is well characterized. Three families of nitrate transporters, *AtNRT1* with 53 members, *AtNRT2* with 7, and *AtCLC* with 7, have been identified in *Arabidopsis* (De Angeli et al., 2009; Forde, 2000; Tsay et al., 1993). Two *AtNRT1* genes (*AtNRT1.1* [*CHL1*] and *AtNRT1.2*) and two *AtNRT2* genes (*AtNRT2.1* and *AtNRT2.2*) are involved in nitrate uptake. Nitrate concentrations in the soil can vary by four orders of magnitude from micromolar to millimolar. To cope with this wide range of concentrations, plants have evolved two nitrate uptake systems: a high-affinity system, with a K_M of about 50 μM, and a low-affinity system, with a K_M of about 5 mM. AtNRT2.1 and AtNRT2.2 are involved in high-affinity uptake (Li et al., 2007; Little et al., 2005) and AtNRT1.2 in low-affinity uptake (Huang et al., 1999), whereas AtNRT1.1 (CHL1) functions as a dual-affinity transporter involved in both high- and low-affinity uptake (Liu et al., 1999; Wang et al., 1998). CHL1 functions as a high-affinity nitrate transporter when T101 is phosphorylated and as a low-affinity nitrate transporter when T101 is dephosphorylated (Liu and Tsay, 2003). The phosphorylation of CHL1 at T101, triggered by changes in external