Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 15134–15139, December 1998
Plant Biology

The *Arabidopsis* CHL1 protein plays a major role in high-affinity nitrate uptake

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ABSTRACT **The *CHL1* (*NRT1*) gene of *Arabidopsis* encodes a nitrate-inducible nitrate transporter that is thought to be a component of the low-affinity (mechanism II) nitrate-uptake system in plants. A search was performed to find high-affinity (mechanism I) uptake mutants by using chlorate selections on plants containing *Tag1* transposable elements. Chlorate-resistant mutants defective in high-affinity nitrate uptake were identified, and one had a *Tag1* insertion in *chl1*, which was responsible for the phenotype. Further analysis showed that *chl1* mutants have reduced high-affinity uptake in induced plants and are missing a saturable component of the constitutive, high-affinity uptake system in addition to reduced low-affinity uptake. The contribution of CHL1 to constitutive high-affinity uptake is higher when plants are grown at more acidic pH, conditions that increase the level of *CHL1* mRNA. *chl1* mutants show reduced membrane depolarization in root epidermal cells in response to low (250 μM) and high (10 mM) concentrations of nitrate. Low levels of nitrate (100 μM) induce a rapid increase in *CHL1* mRNA. These results show that CHL1 is an important component of both the high-affinity and the low-affinity nitrate-uptake systems and indicate that CHL1 may be a dual-affinity nitrate transporter.**

Since the discovery that plant-nutrient uptake is carrier-mediated and obeys Michaelis–Menten kinetics (reviewed in ref. 1), intensive efforts have been devoted to identify and characterize the components of plant ion-uptake systems (reviewed in ref. 2). Many of these systems actively transport ions into root cells creating electrical responses across the plasma membrane. These systems have both high-affinity and low-affinity components (corresponding to uptake mechanisms I and II, respectively) and are regulated in response to internal or environmental signals such as nutrient treatment or starvation. Driving nutrient uptake is the proton gradient established by the plasma-membrane proton ATPase, which maintains electrical gradients typically between −100 and −250 mV in plant roots.

Nitrate is an important nutrient for plants, and its uptake has been studied extensively (reviewed in refs. 3–8). Nitrate uptake is driven by the cotransport of two protons that initially depolarize the plasma membrane, making it more positive inside the cell (refs. 5, 9, and 10 and references therein). This mechanism applies to both high-affinity and low-affinity systems, as nitrate-induced depolarizations occur over a wide range of nitrate concentrations. The high-affinity system shows typical Michaelis–Menten kinetics with Michaelis constant (K_m) in the 10- to 100-μM range. Both constitutive and nitrate-inducible components have been proposed for the high-affinity system (reviewed in refs. 4, 7, and 8). The low-affinity system shows linear kinetics above 0.5 mM and no nitrate-induction in plants such as barley (reviewed in refs. 4, 7, and 8).

Efforts to identify the components of the nitrate-uptake system have found two gene families called *NRT1* and *NRT2* (reviewed in refs. 6–8). The *NRT2* family encodes high-affinity nitrate transporters identified in fungi (11), algae (12), and yeasts (13). Higher plants also have *NRT2* genes whose expression is root-specific and nitrate-inducible (reviewed in refs. 7, 8, and 14). A gene in the *NRT1* family was first identified as a chlorate-resistant mutant of *Arabidopsis* called *chl1* (15). This mutant showed defects in low-affinity nitrate transport (16–18). The wild-type (WT) *CHL1* gene was cloned and shown to encode a transporter with low-affinity nitrate-uptake activity (K_m = 8 mM) in *Xenopus* oocytes (17, 19) and to be expressed in epidermal, cortical, and endodermal cells of the root (17). *CHL1* is nitrate-inducible and thus does not fit the original model for the low-affinity system, which shows no evidence of induction in plants such as barley (4). To resolve this paradox, it was proposed that the low-affinity system has two components: CHL1, which serves as an inducible component, and another component (possibly a CHL1-related protein) that is constitutively expressed (17, 18, 20).

To further our understanding of nitrate uptake, especially of the high-affinity system, mutants that are resistant to low concentrations of chlorate were identified and characterized. Chlorate is the chlorine analog of nitrate, and it is taken up and then reduced to toxic chlorite by nitrate reductase. *chl1* mutants were identified originally as being resistant to high levels (2 mM) of chlorate in the presence of millimolar concentrations of nitrate (15). We have identified mutants that are resistant to low levels (100–500 μM) of chlorate (21). Two such mutants, *chl8–1* and *chl8–2* (originally called *nrt2–1* and *nrt2–2* but renamed *chl8*), were shown to be defective in constitutive, high-affinity nitrate uptake (10). This uptake defect was pH sensitive and was restricted to nitrate concentrations below 2 mM. Above 2 mM nitrate, *chl8* plants took up as much nitrate as the parent when plants were grown in the absence of nitrate. The mutants also showed little membrane depolarization in response to 250 μM nitrate (high-affinity range) but had normal responses to 10 mM nitrate (low-affinity range). The phenotype of *chl8* plants is very different from that of *CHL1* plants, as described below.

We have identified a third, high-affinity uptake mutant. This mutant was obtained from a line of *Arabidopsis* that originated from a *chl1-6* (*chl1*::*Tag1*) mutant, which has an active transposable element called *Tag1* located at *chl1* (22). The newly isolated, third, high-affinity mutant is defective in both high-affinity and low-affinity nitrate uptake. After further characterization, it was discovered that the mutation causing the high-affinity uptake defect was a *Tag1* insertion in *chl1*. This

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Abbreviations: HATS, high-affinity uptake system; LATS, low affinity-uptake system; WT, wild-type.
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finding led us to reexamine the role of *chl1* in high-affinity uptake. Our findings are described below.

MATERIALS AND METHODS

Plant Materials and Growth Conditions. *Arabidopsis chl1* mutants are as follows: *chl1-1* (15, 16); *chl1-2* (*chl1*::*T-DNA*), *chl1-3* (19), *chl1-4* (19), and *chl1-5* (deletion mutant; ref. 19); and *chl1-6* (*chl1*::*Tag1*; ref. 22).

The chlorate selection originally used to identify high-affinity uptake mutants was performed in vermiculite/perlite soil irrigated with nutrient medium containing 100 μM NH_4NO_3, 5 mM $(NH_4)_2SO_4$, 0.5 mM $KClO_3$, and other nutrients as described (21). Resistant seedlings were rescued with nutrient medium containing 5 mM NH_4NO_3 and no chlorate. Subsequent chlorate selections were performed on Petri dishes with 0.2 mM $KClO_3$, 50 μM NH_4NO_3, 6 mM ammonium succinate, 0.5% sucrose, 0.5% agarose (pH 6), and other nutrients as described (21). Chlorate-sensitive revertants of *chl1*::*Tag1* were identified by using the Petri-dish selection and rescued on plates containing fresh medium with 5 mM NH_4NO_3 and no chlorate.

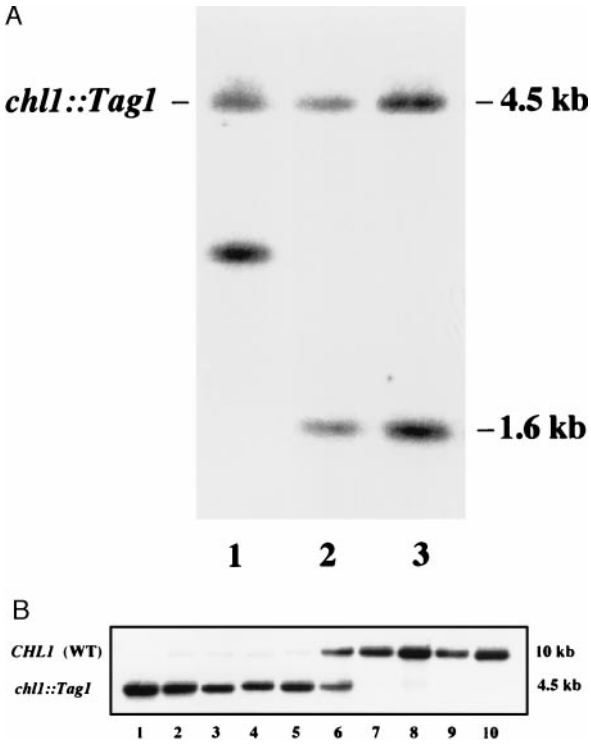

FIG. 1. DNA blot analysis of *chl1*::*Tag1* (ctg6) and its revertant lines. (*A*) Southern blot of genomic DNA from three mutants probed with *Tag1* is shown. DNA was prepared from the original *chl1-6* (*chl1*::*Tag1*) mutant (lane 1), from the high-affinity uptake mutant ctg6 (lane 2), and from ctg6bc, which had been backcrossed three times to WT Columbia plants (lane 3). DNA was digested with *Hin*dIII and hybridized to a radiolabeled, 1.6-kb *Hin*dIII fragment of *Tag1*. (*B*) DNA blot with *CHL1* probe is shown. Genomic DNA was prepared from the original *chl1-6* (*chl1*::*Tag1*) mutant (lane 1) and from progeny of a chlorate-sensitive revertant of ctg6 (lanes 2–10). DNA was prepared from homozygous, chlorate-resistant progeny (with genotype (*chl1*::*Tag1*); lanes 2–5), a heterozygous plant (lane 6), and homozygous, chlorate-sensitive plants (lanes 7–10). Plants with no intact *CHL1* genes (lanes 1–5) were defective in high-affinity nitrate uptake; plants with a WT allele of *CHL1* (lanes 6–10) had WT high-affinity nitrate uptake. Genomic DNAs were digested with *Hin*dIII and hybridized with a radiolabeled 885-bp fragment from the 5′-end of a *CHL1* cDNA clone (19). DNA preparation and blot analysis were performed as described in *Materials and Methods*.

For uptake studies, plants were grown submerged in 2 ml of liquid culture for 5 days as described (10). For one set of experiments, plants were grown hydroponically in 10 ml of growth medium with 25 mM NH_4NO_3 in 100-ml glass beakers for 7 days with agitation (swirled at 60–80 rpm) and constant illumination. Seeds were supported on "floats" of cheese cloth wrapped around tops of cut-off Eppendorf tubes.

Ion-Uptake Assays. For ion-uptake assays, 5-day-old seedlings were washed twice with 4 ml of 10 mM K_2HPO_4 (pH 6) and then resuspended in 4 ml of fresh nutrient medium containing 5 mM K_2HPO_4, 5 mM Mes (pH 6.0), and KNO_3 for 2–3 h with agitation as described (10). The medium was replaced with 2 ml of fresh medium, and then nitrate uptake was determined by measuring the disappearance of nitrate from the nutrient solution. Samples (50 μl) were analyzed by HPLC with a Vydac 300IC405 anion exchange column (Hesperia, CA) and by monitoring the absorbance of the flow-through at 210 nm as described (23). Phosphate and sulfate uptake were determined for plants grown in liquid culture with 5 mM NH_4NO_3 for 10 days as described (10). Seedlings were blotted dry and weighed to determine fresh weight. Experiments were performed in triplicate except as indicated; in the figures, error bars less than the diameter of the symbols were omitted.

Nucleic Acid Methods. DNA and RNA preparations and gel blots were performed as described (19) with hybridizations at 42°C for 16 h with 50% formamide. The *NRT2* DNA was obtained by PCR amplification of *Arabidopsis* genomic DNA by using oligonucleotides 5′-CAATGGGTGATTCTACTGG-TGAG-3′ and 5′-GCACCATAGCCACAACGGCAG-3′ obtained from a sequence provided by Brian Forde. The ends of the DNA clone were sequenced (data not shown) and found to be identical to the *AtNRT2* sequence ACH1 (GenBank accession no. AF019748).

Membrane-Potential Measurements. *Arabidopsis* seedlings were grown for 4 days with media containing 5 mM NH_4NO_3 as described (10). Membrane-potential changes of root epidermal cells in response to treatment with $CsNO_3$ were measured with microelectrodes as described (10). Cesium was used as the counterion to nitrate in these experiments to block background activity from K channels.

RESULTS

A chlorate selection for high-affinity nitrate-uptake mutants was initially performed in pots containing 100 μM nitrate, 500 μM chlorate, and 5 mM $(NH_4)_2SO_4$ as described in *Materials and Methods*. Seeds from a *chl1-6* mutant (a *chl1*::*Tag1* mutant in the Landsberg *erecta* background) were used for the selection. This line has *Tag1* elements that excise at high rates so that most of the progeny are *chl1* revertants. After the chlorate selection, 5–10% of the seedlings survived, and one, named ctg6, was examined further. The mutant was backcrossed three times to WT Columbia plants, which have no *Tag1* elements, to produce ctg6bc. Both ctg6 and ctg6bc have two *Tag1* insertions with one corresponding to the insertion at *chl1* (Fig. 1*A*). Nitrate-uptake assays indicated that ctg6bc has low nitrate-uptake activity at 250 μM nitrate (Fig. 2*A*) but normal sulfate and phosphate uptake at 250 μM and 25 mM concentrations (data not shown).

To determine whether the uptake defect in ctg6 was unstable (i.e., caused by a transposon insertion), we searched for a chlorate-sensitive revertant among the progeny of ctg6 as described in *Materials and Methods*. A revertant was found and rescued. Nitrate-uptake assays showed that high-affinity nitrate uptake was restored in the revertant (Fig. 3; compare bars labeled "Ctg6bc" and "Rev"). The revertant was selfed, and progeny seed was collected and planted. DNA from each line was examined by Southern blot analysis with *CHL1* DNA as probe, and the chlorate-resistance phenotype of their progeny

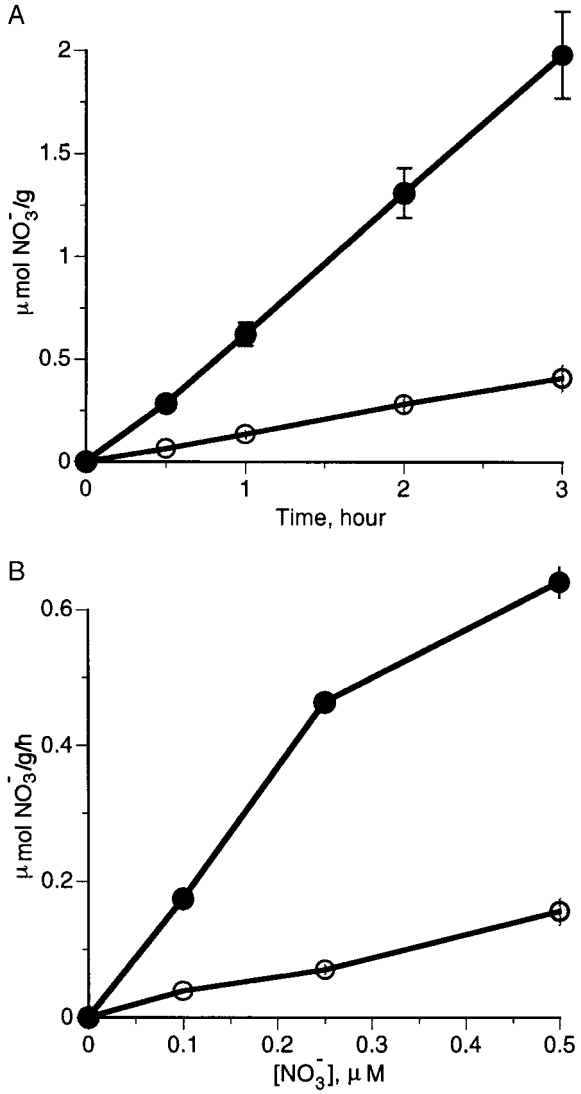

FIG. 2. High-affinity nitrate uptake in plants grown in ammonium nitrate. Seedlings were grown with 5 mM NH_4NO_3 and analyzed for nitrate uptake while they were submerged in liquid with 250 μM KNO_3 (pH 6.0) as described in *Materials and Methods*. (*A*) Time courses of nitrate uptake for WT and ctg6 (*chl1-6*) plants. (*B*) Nitrate-uptake rate as a function of nitrate concentration in the high-affinity range for WT and *chl1-5* plants. Open circles, *chl1* plants; closed circles, WT plants.

was examined. All homozygous chlorate-resistant lines had a *Tag1* insertion at *chl1* (Fig. 1*B*, lanes 2–5), whereas the homozygous revertant lines had the WT *CHL1* allele containing no *Tag1* at *chl1* (Fig. 1, lanes 7–10). A heterozygous line had both the *chl1:Tag1* and the WT *CHL1* DNA bands (lane 6). We concluded that the chlorate resistance and high-affinity nitrate-uptake defect were caused by a *Tag1* insertion at *chl1*.

These results were surprising as they indicated that CHL1 is involved in high-affinity nitrate uptake. To test this hypothesis further, a new set of experiments was performed. First, high-affinity nitrate uptake in the *chl1* deletion mutant *chl1-5* was examined. Assays with plants grown in ammonium nitrate showed that the reduction in nitrate uptake at 250 μM for *chl1-5* is the same as for the ctg6 mutant (data not shown). Further analysis showed that reduced nitrate uptake in *chl1-5* occurs from 0.1 mM to 0.5 mM for plants grown in ammonium nitrate (Fig. 2*B*). Second, additional *chl1* alleles were examined for high-affinity uptake defects (i.e., the original ethyl methanesulfonate-generated *chl1-1* mutant, ref. 15; a T-DNA insertion mutant *chl1-2*, ref. 19; and two γ-ray-induced mutants, *chl1-3* and *chl1-4*, ref. 19). All *chl1* mutants show substantially reduced levels of high-affinity nitrate uptake in contrast to the small reduction noted for the *nia2* NR mutant G5 (also called *chl3–5*) (Fig. 3). Finally, measurements of membrane potentials in root epidermal cells showed that nitrate-induced depolarizations in *chl1-5* mutants were about half the amplitude in WT plants at 250 μM nitrate (18.8 $\pm$ 2.6 mV for *chl1* vs. 41.3 $\pm$ 4.7 mV for WT) and 10 mM nitrate (42.5 $\pm$ 4.6 mV for *chl1* vs. 80 $\pm$ 5.2 mV for WT). The depolarization was followed by the characteristic recovery of the membrane potential (data not shown) described for *Arabidopsis* (9, 10). These findings confirm that a mutation in *chl1* significantly reduces high-affinity nitrate uptake.

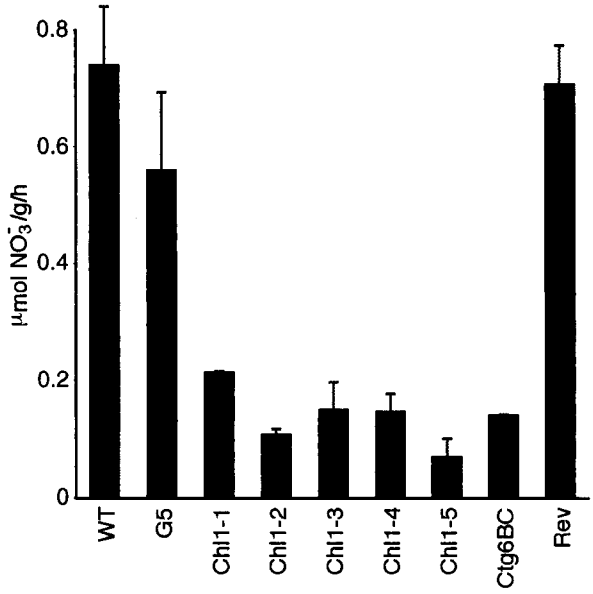

FIG. 3. Nitrate uptake by different *chl1* mutants. Alleles of *chl1* are shown on the *x* axis. Growth and assay conditions were the same as those described for Fig. 2. Mutants are described in *Materials and Methods*. "Rev" refers to a revertant of ctg6. G5 refers to the *nia2* NR deletion mutant *chl3–5* (30).

We next examined uptake kinetics in the high-affinity range (0–150 μM) to determine whether CHL1 has a saturable component with a K_m below 100 μM, characteristic of the high-affinity uptake system (HATS) or shows linear kinetics characteristic of the low-affinity uptake system (LATS). For these experiments, uninduced plants (i.e., plants grown on ammonium succinate without nitrate) were used so that only constitutive HATS activity would be apparent. We found that, under these conditions, nitrate uptake by uninduced *chl1* mutants is much less than that of WT plants at 50 μM nitrate, and the effect is more pronounced as the medium becomes more acidic (Fig. 4). These results indicate that CHL1 is a major component of constitutive HATS and that its contribution increases with decreasing pH of the growth medium. The increase in CHL1 contribution correlates with the increase in *CHL1* mRNA levels after acidification of the growth medium as reported (19). A more extensive kinetic analysis showed that constitutive uptake activity is substantially reduced in *chl1* mutants in nitrate as low as 10 μM (Fig. 5 *A* and *B*) and up to 10 mM nitrate (Fig. 5*C*). The CHL1-specific activity is calculated by subtracting the *chl1* from WT activities (shown in Fig. 5 *A* and *B*); this difference shows a saturation between 50 and 100 μM (data not shown). A K_m value of 38 μM was estimated for the high-affinity saturable component of CHL1 when KNO_3 was used in the uptake medium (Fig. 5*A*).

In addition to its contribution to constitutive HATS, we suspected that CHL1 might contribute to the inducible HATS, because *CHL1* is induced by high (10–25 mM) nitrate treat-

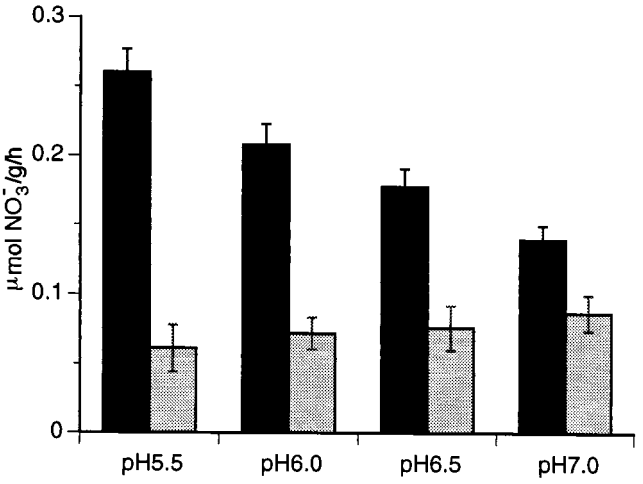

FIG. 4. Nitrate-uptake rates as a function of pH. Seedlings were grown in liquid culture with 12 mM ammonium succinate without nitrate for 7 days at pH 6.5 as described in *Materials and Methods*. Seedlings were then preincubated with fresh medium (at the indicated pH) containing 5 mM ammonium succinate for 3 h. The preincubated seedlings were then washed with 5 ml of growth medium containing 50 μM KNO_3 and no ammonium succinate at indicated pH values. Uptake assays were performed by resuspending seedlings in 2 ml of uptake medium at the indicated pH (same constituents as wash medium in previous step) and monitoring loss of nitrate from the medium after 30 min as described in *Materials and Methods*. The histogram shows nitrate-uptake activity by WT (black) and *chl1-5* (grey) plants at the indicated pH.

ments (19). To test this hypothesis, we examined the expression of *CHL1* in response to low nitrate levels. Treating uninduced plants with 100 μM nitrate leads to a dramatic increase in *CHL1* mRNA levels within 30 min in WT plants (Fig. 6, lanes 1–6), whereas similar treatments with no nitrate result in only a slight increase in *CHL1* mRNA (Fig. 6, lanes 7–10). For these experiments, plants were grown at pH 6.5 where *CHL1* mRNA levels are low in uninduced plants. As expected, no *CHL1* mRNA was found in the *chl1-5* deletion mutant (Fig. 6, lanes 11–14). These experiments show that *CHL1* is induced by nitrate at low concentrations.

We next compared the nitrate induction of *CHL1* with that of *AtNRT2*, an *Arabidopsis* gene of the *NRT2* family, which is most likely a component of the inducible HATS (refs. 8, 24, and 25; B. Forde and A. Glass, personal communication). A genomic clone for an *AtNRT2* gene was obtained as described in *Materials and Methods*. *AtNRT2* mRNA levels in *Arabidopsis* roots have been found to increase within 30 min after treatment with 1 mM nitrate (H. Zhang and B. Forde, personal communication). Under our conditions (treatment with 100 μM nitrate), *CHL1* mRNA increased about 1 h earlier than *NRT2* (Fig. 6, lanes 1–6). Thus, *CHL1* is induced at the same nitrate levels and at least as fast as *NRT2*. *NRT2* mRNA levels also increased in the *chl1-5* deletion mutant after nitrate induction, showing that loss of *CHL1* did not impeded the nitrate induction of *NRT2*.

Finally, we examined uptake activity at 50 μM nitrate by plants induced with 100 μM nitrate. Uptake levels increase approximately threefold after 4 h of nitrate treatment in WT plants (Fig. 7). CHL1 activity (obtained from plotting the difference between WT and mutant activities) showed an increase then a decline over this time period (Fig. 7). Thus, an increase in HATS activity after nitrate induction correlates with an increase in *CHL1* mRNA levels and is partially dependent on *CHL1*. Interestingly, no increase in WT or CHL1 uptake activity was observed in the low-affinity range (2.5 mM) under these same conditions (data not shown).

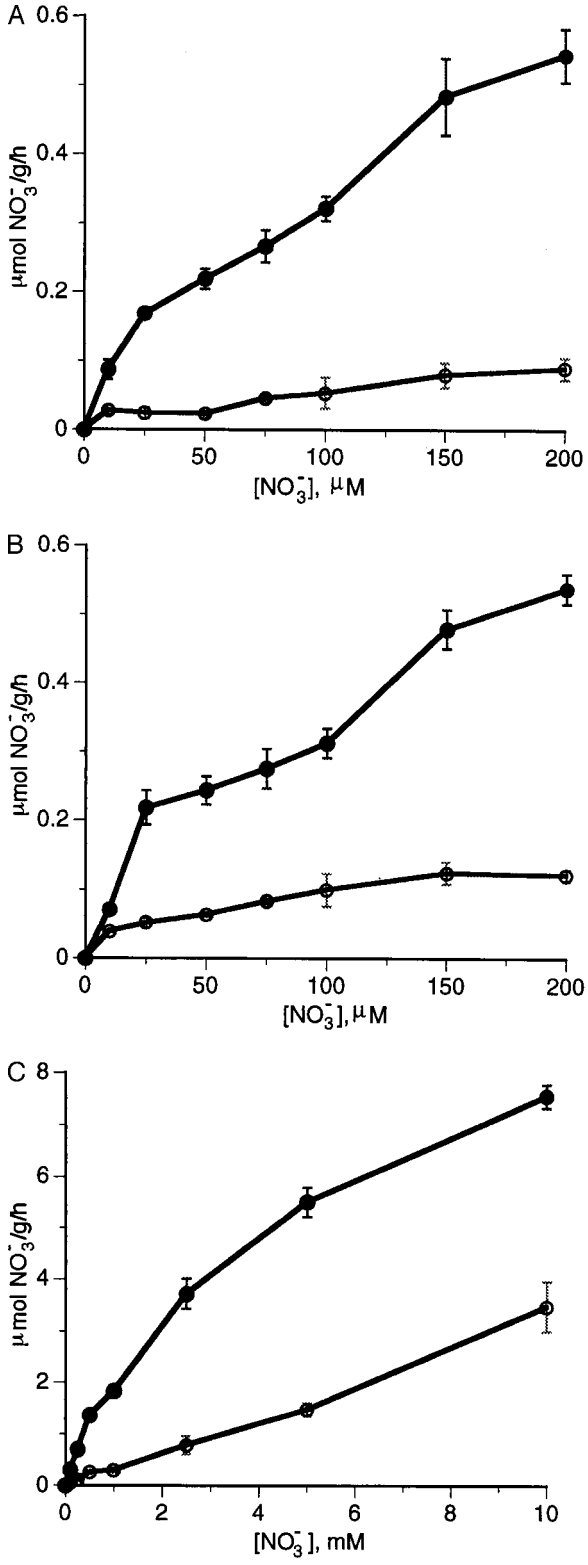

FIG. 5. Constitutive nitrate-uptake activity in WT and *chl1* plants. Seedlings were grown in liquid culture with 12 mM ammonium succinate without nitrate for 7 days at pH 6.5 (see *Materials and Methods*) and then preincubated with fresh medium (5 mM ammonium succinate and no nitrate) at pH 5.5 for 3 h. Seedlings were then washed with 5 ml of wash/uptake medium (as described in Fig. 4) and then resuspended in 2 ml of uptake medium with different concentrations of either KNO_3 (*A* and *C*) or NH_4NO_3 (*B*) at pH 5.5. Nitrate-uptake activity was monitored for 15–45 min as described in *Materials and Methods*. The graphs show uptake kinetics in the high-affinity range (*A* and *B*) and low-affinity range (*C*). Open circles, *chl1-5* plants; closed circles, WT plants.

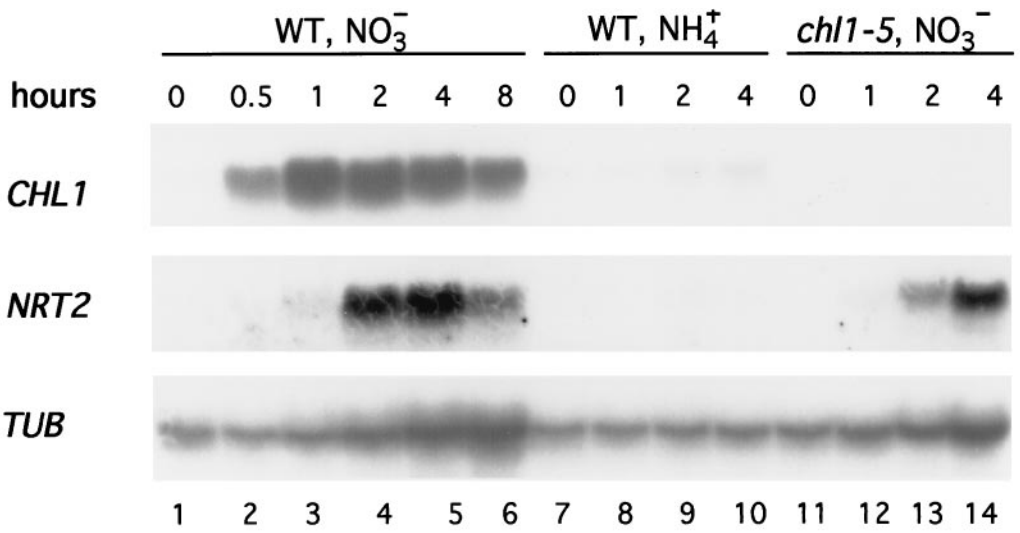

FIG. 6. RNA blot analysis of WT and *chl1-5* plants. Whole seedlings were grown in liquid culture with 12 mM ammonium succinate at pH 6.5 for 5 days as described in *Materials and Methods*. At time zero, equal volumes (2 ml) of fresh medium containing 0.2 mM KNO_3 (to give a final nitrate concentration of 0.1 mM; lanes 1–6 & 11–14) or 0.2 mM KCl (final concentration of 0.1 mM; lanes 7–10) were added to the culture. At times indicated in the figure, seedlings were rinsed with distilled water, blotted dry, and frozen in liquid nitrogen. Total RNA was prepared from whole seedlings, analyzed on 1.4% formaldehyde agarose gels, blotted onto a filter, hybridized with radiolabeled *CHL1* cDNA (*Top*), *NRT2–1* genomic DNA (*Middle*), or β-tubulin cDNA (*Bottom*), and autoradiographed.

DISCUSSION

Our results show that under certain environmental conditions, CHL1 is essential for most of the nitrate-uptake activity in *Arabidopsis*. CHL1 makes a major contribution to HATS activity in plants grown with NH_4NO_3 and in uninduced plants. The extent of its contribution depends on the pH of the medium; the more acidic the medium, the more CHL1 contributes to uptake. CHL1 activity (as assessed by comparing activities in WT and *chl1* deletion mutants) also shows a high-affinity, saturable component with an approximate K_m of 38 μM in addition to its more linear low-affinity activity.

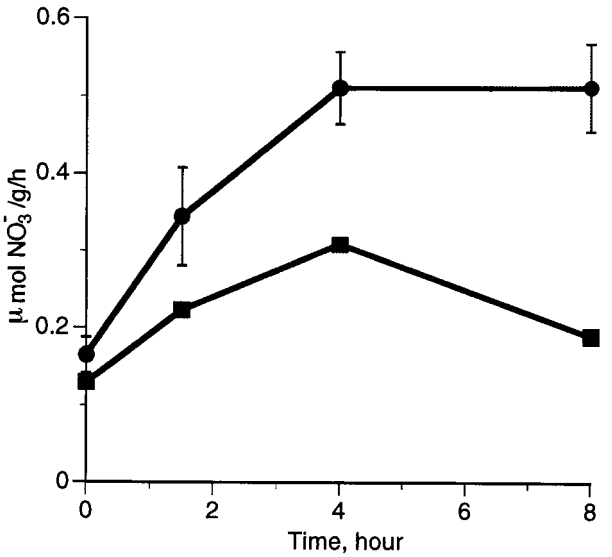

FIG. 7. Nitrate induction of HATS activity for WT and *chl1-5* plants. Seedlings were grown on ammonium succinate without nitrate and treated with 0.1 mM KNO_3 as described for Fig. 6. After treatment, at the indicated times, nitrate uptake was measured for 30 min in the presence of 50 μM KNO_3 as described in *Materials and Methods*. The graph shows uptake activity for WT plants (circles) and the difference between WT and *chl1-5* plants (i.e., contribution by CHL1; squares).

Considering the history of *CHL1*, these findings are remarkable. Since 1979, CHL1 was considered to be involved only in low-affinity uptake based on the *chl1* phenotype (16). In 1993, the *CHL1* gene was cloned and shown to encode a transporter with nitrate-uptake activity in *Xenopus* oocytes (19). Subsequent reports showed that CHL1 has low-affinity nitrate-uptake activity in *Xenopus* oocytes with a K_m of about 8 mM (17); *chl1* mutants have greatly reduced low-affinity nitrate uptake when plants are grown with NH_4NO_3 but much less so when grown on KNO_3 without ammonium (17, 18). *chl1* mutants showed no defects in HATS when grown on KNO_3; however, high-affinity uptake was not measured for plants grown with NH_4NO_3 (18). Therefore, it was surprising to find that a *chl1* mutation in our ctg6 mutant was responsible for resistance to low concentrations of chlorate (250–500 μM) and for reduced HATS activity. All subsequent experiments confirmed that *CHL1* is involved in high-affinity nitrate uptake: (*i*) multiple alleles of *chl1* show the HATS defect, whereas a revertant of *chl1*::*Tag1* does not, (*ii*) nitrate-induced depolarizations across root epidermal membranes at low nitrate concentrations are reduced in *chl1* mutants, and (*iii*) micromolar concentrations of nitrate can induce *CHL1* gene expression.

Given that CHL1 is a significant component of HATS, why was the high-affinity defect in *chl1* mutants not observed previously? The contribution of CHL1 to HATS depends on the growth medium. For LATS, it was found that the uptake defect in *chl1* mutants is partially to completely dependent on the presence of ammonium the growth medium (17, 18). We find the same ammonium dependence for HATS as well. *chl1* mutants grown with NH_4NO_3 show dramatic reductions in HATS activity compared with WT (Figs. 2–5); however, for plants grown with KNO_3, uptake of 100 μM nitrate was slightly higher in *chl1* mutants compared with WT plants (data not shown), a drastically different result than that obtained with plants grown with NH_4NO_3. Other parameters, including the age of the plant, the pH of the medium, and the nature of the conditions (submerged vs. hydroponic) were also tested but with no effect; *chl1* mutants grown with NH_4NO_3 always took up much less nitrate than WT plants (data not shown). Thus, the presence or absence of ammonium determines the con-

tribution that CHL1 makes to high-affinity and low-affinity uptake.

Our present findings add to the list of functions of CHL1 by showing that it is a nitrate-and acid-inducible component of HATS, making its most significant contributions to constitutive HATS when plants are grown at acidic pH and to inducible HATS when plants are grown on NH_4NO_3. The pH effect is the result of prolonged exposure of plants (from hours to days) to acidic pH during their growth and not to the immediate effect of acid on nitrate uptake. We propose that the addition of nitrate or the acidification of the medium results in enhanced expression of *CHL1*, which in turn leads to greater nitrate uptake by the plant through this transporter. The pH response might also explain the ammonium effect on the contribution of CHL1 to uptake, because the assimilation of ammonium generates protons that acidify the rhizosphere, which then could enhance *CHL1* expression.

Given our results, one might ask how CHL1 contributes to both HATS and LATS when these systems seem to work by different mechanisms. We favor the proposal that CHL1 is a dual-affinity transporter with two distinct K_ms for nitrate, one at $\approx$40 μM and the other at $\approx$8 mM. Examples of other, potentially dual-affinity transporters in plants include *AKT1*, which is thought to provide a mechanism for low-affinity potassium uptake in plants but is also required for high-affinity potassium-uptake-dependent growth of *Arabidopsis* in the presence of ammonium (26). Other examples are the potassium transporter genes from the *AtKUP1/HAK1* family. The products of these genes show HATS activity in *E. coli*, LATS activity in plant cell culture, and dual-affinity potassium-uptake activity in yeast (27–29). These findings show the complexities of nutrient-uptake systems and the importance of continued physiological and molecular studies to elucidate the components that are involved.

We thank Brian Forde, Degen Zhuo, and Anthony Glass for providing sequence information for *NRT2* and Wilbur Campbell for help with data analysis. This work was funded by National Institutes of Health Grant GM40672.

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