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# The *Arabidopsis* NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches

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**Localized proliferation of lateral roots in NO<sub>3</sub><sup>-</sup>-rich patches is a striking example of the nutrient-induced plasticity of root development. In *Arabidopsis*, NO<sub>3</sub><sup>-</sup> stimulation of lateral root elongation is apparently under the control of a NO<sub>3</sub><sup>-</sup>-signaling pathway involving the ANR1 transcription factor. ANR1 is thought to transduce the NO<sub>3</sub><sup>-</sup> signal internally, but the upstream NO<sub>3</sub><sup>-</sup> sensing system is unknown. Here, we show that mutants of the NRT1.1 nitrate transporter display a strongly decreased root colonization of NO<sub>3</sub><sup>-</sup>-rich patches, resulting from reduced lateral root elongation. This phenotype is not due to lower specific NO<sub>3</sub><sup>-</sup> uptake activity in the mutants and is not suppressed when the NO<sub>3</sub><sup>-</sup>-rich patch is supplemented with an alternative N source but is associated with dramatically decreased ANR1 expression. These results show that NRT1.1 promotes localized root proliferation independently of any nutritional effect and indicate a role in the ANR1-dependent NO<sub>3</sub><sup>-</sup> signaling pathway, either as a NO<sub>3</sub><sup>-</sup> sensor or as a facilitator of NO<sub>3</sub><sup>-</sup> influx into NO<sub>3</sub><sup>-</sup>-sensing cells. Consistent with this model, the *NRT1.1* and *ANR1* promoters both directed reporter gene expression in root primordia and root tips. The inability of *NRT1.1*-deficient mutants to promote increased lateral root proliferation in the NO<sub>3</sub><sup>-</sup>-rich zone impairs the efficient acquisition of NO<sub>3</sub><sup>-</sup> and leads to slower plant growth. We conclude that *NRT1.1*, which is localized at the forefront of soil exploration by the roots, is a key component of the NO<sub>3</sub><sup>-</sup>-sensing system that enables the plant to detect and exploit NO<sub>3</sub><sup>-</sup>-rich soil patches.**

adaptive root development | ANR1 signaling pathway | plant growth | nitrate sensing | nitrogen nutrition

Their lack of mobility means that plants have to modify their organ development to enhance their ability to capture light and edaphic resources. In particular, the plasticity of root development plays an important role in the adaptive responses of plants to the large spatial and temporal changes in the availability of water and mineral ions (1, 2). Many species have evolved mechanisms allowing them to detect a nutrient-rich patch in their root environment and to promote lateral root (LR) growth preferentially within those patches (3–5). This foraging behavior is crucial because it determines the efficiency with which plants compete with their neighbors and other organisms for the use of limiting mineral resources (6, 7). Although it must somehow involve systems for external nutrient sensing, molecular data unraveling the mechanisms of this adaptive response to spatial heterogeneity of nutrient availability are largely missing. Concerning nitrate (NO<sub>3</sub><sup>-</sup>), the main N source for plant nutrition, studies with *Arabidopsis thaliana* have shown that LR proliferation in NO<sub>3</sub><sup>-</sup>-rich patches mostly involves enhanced LR elongation (5). Stimulation of LR elongation by localized high NO<sub>3</sub><sup>-</sup> supply is not a nutritional effect because of improved N assimilation in these roots but results from the action of a specific NO<sub>3</sub><sup>-</sup>-signaling pathway (5). The *ANR1* MADS box gene, encoding a putative transcription factor, was shown to play a key

role in this signaling pathway because its underexpression prevents the local stimulation of LR elongation by high NO<sub>3</sub><sup>-</sup> availability (5). The precise role of *ANR1* is not clarified yet. However, its expression was shown to be regulated by N availability in a complex way, being induced by NO<sub>3</sub><sup>-</sup> and repressed by high N supply, depending on the plant growth conditions (5, 8). These data suggest that *ANR1* itself is controlled by an upstream NO<sub>3</sub><sup>-</sup>-sensing system, which has been postulated to involve an unknown plasma membrane NO<sub>3</sub><sup>-</sup> receptor located in the root tip (9, 10).

The aim of the present studies was to investigate the possibility that the role of NO<sub>3</sub><sup>-</sup> receptor in the *A. thaliana* root tip could be performed by the NRT1.1 (formerly CHL1) NO<sub>3</sub><sup>-</sup> transporter. In *Arabidopsis thaliana*, the known NO<sub>3</sub><sup>-</sup> transporters are encoded by two genes families, namely *NRT1* (belonging to the large *PTR* family of 53 transporter genes) and *NRT2* (7 members). To date, only four of these putative transporters (*NRT1.1*, *NRT1.2*, *NRT1.4*, and *NRT2.1*) have been functionally characterized *in planta* (11–14), with three being involved in root NO<sub>3</sub><sup>-</sup> influx: *NRT1.1* as dual-affinity transporter (11, 15, 16), *NRT1.2* as a low-affinity transporter (12), and *NRT2.1* as a major component of the high-affinity uptake system (13, 17). Three main reasons guided us to select *NRT1.1* as the most promising candidate for our study. First, *NRT1.1* is highly expressed in young tissues, and especially in root tips (18), suggesting a specific role for this transporter in the early acquisition of NO<sub>3</sub><sup>-</sup> when growing roots enter soil areas yet unexplored by the plant. Second, mutants of *NRT1.1* (*chl1* mutants) were shown to display a root development phenotype under very specific conditions (i.e., reduced LR emergence at low external NO<sub>3</sub><sup>-</sup> concentration and low external pH) that could not be related to *NRT1.1* NO<sub>3</sub><sup>-</sup> transport activity (18). Neither the physiological significance of this phenotype nor the mechanisms responsible are understood, but this raised the question of a putative role of *NRT1.1* in root development. Most importantly, both a recent report (19) and our previous work (20) suggested a signaling function of *NRT1.1* in other key responses of the plant to changes in external N

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Abbreviations: HN, high NO<sub>3</sub><sup>-</sup> medium; LN, low NO<sub>3</sub><sup>-</sup> medium; LR, lateral root.

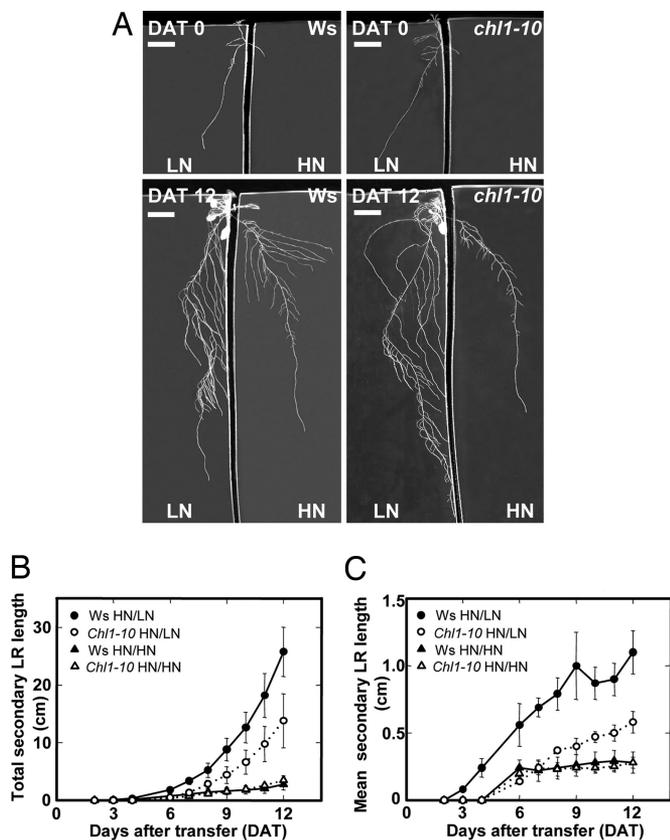
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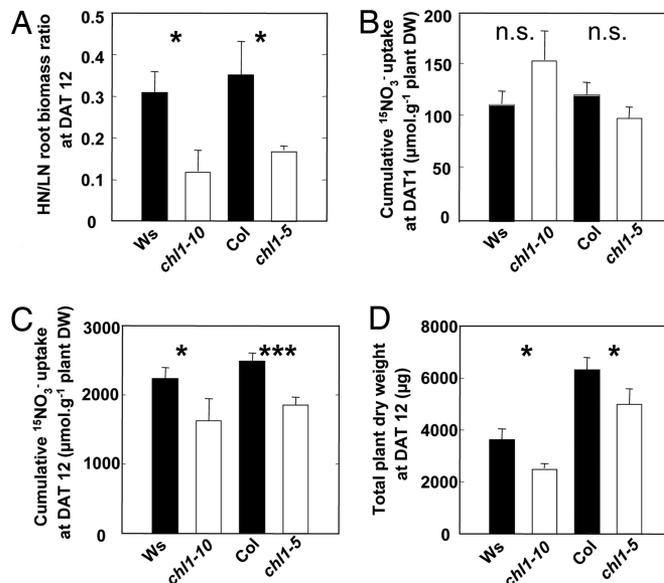
**Fig. 1.** Mutation of *NRT1.1* alters LR elongation in a nitrate-rich patch. (A) Response of the root system architecture to a localized high  $\text{NO}_3^-$  supply (HN; 10 mM) in wild-type (Ws) and *NRT1.1* mutant (*chl1-10*) of *Arabidopsis*. LN, low  $\text{NO}_3^-$  medium (0.05 mM). DAT, day after transfer (Scale bars: 1 cm.) (B and C) Stimulation of LR growth in the  $\text{NO}_3^-$ -rich patch with total (B) and mean (C) length of second-order LRs on the HN side. DAT, day after transfer. Errors bars represent SEM ( $n = 10-14$ ).

availability, i.e., the relief of seed dormancy by  $\text{NO}_3^-$  and the regulation of the high-affinity  $\text{NO}_3^-$  uptake system, respectively. Indeed, *chl1* mutants show a constitutive overexpression of the high-affinity  $\text{NO}_3^-$  uptake system, related to a profound alteration of the feedback repression of *NRT2.1* expression by  $\text{NH}_4^+$  or high  $\text{NO}_3^-$  supply.

In the present paper, we have analyzed the response of LR growth to a localized  $\text{NO}_3^-$  supply in *chl1* mutants to determine whether *NRT1.1* also contributes to this aspect of the adaptive response of the plant to  $\text{NO}_3^-$  availability. We show that *NRT1.1* plays a key role in triggering the stimulation of LR elongation in  $\text{NO}_3^-$ -rich patches and provide evidence that this aspect of *NRT1.1* function is linked to the ANR1-signaling pathway.

## Results

**Mutants at the *NRT1.1* Locus Show Reduced LR Growth Within a Nitrate-Rich Patch.** To mimic the situation where the tip of a growing LR enters a  $\text{NO}_3^-$ -rich patch, we have devised a split-root experimental system by using segmented vertical agar plates (Fig. 1A) and transferred 9-d-old *Arabidopsis* seedlings with only the apical part of one first-order LR positioned on high  $\text{NO}_3^-$  medium (HN; 10 mM  $\text{NO}_3^-$ ) and the rest of the root system placed on low  $\text{NO}_3^-$  medium (LN; 0.05 mM  $\text{NO}_3^-$ ). After 12 d, the single LR on HN medium displayed a dramatic proliferation response in Ws wild-type plants (Fig. 1A), with both a strongly increased elongation of this first-order LR and the appearance of a large number of visible second-order LRs (>0.5 mm). None of these responses were found in control plants transferred to



**Fig. 2.** Mutation of *NRT1.1* does not alter specific nitrate uptake activity but reduces growth of plants subjected to localized nitrate supply. (A) Ratio between root dry biomass in HN (10 mM) and LN (0.05 mM) patches in two *NRT1.1* mutant alleles (*chl1-10* and *chl1-5*) and related wild-types (Ws and Col) after 12 d of growth. (B and C) Cumulative  $^{15}\text{NO}_3^-$  uptake by the roots on  $^{15}\text{NO}_3^-$ -labeled HN patch expressed on the total plant dry weight basis either 1 d (B) or 12 d (C) after transfer to HN/LN medium. (D) Total plant dry biomass 12 d after transfer to HN/LN medium. DAT, day after transfer. Errors bars represent SEM ( $n = 6-14$ ). Means for WT and *chl1* mutants significantly different (Student *t* test) at: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . n.s., not significant ( $P > 0.1$ ).

systems with an even distribution of  $\text{NO}_3^-$  between the two sides [either HN/HN or LN/LN; supporting information (SI) Fig. 6]. As a consequence, the total second-order LR length measured on the first-order LR on HN medium (taken as an indicator of the proliferation response) was 10-fold higher in plants subjected to local high  $\text{NO}_3^-$  supply (LN/HN) than in HN/HN control plants (Fig. 1B). In the *chl1-10* mutant, a T-DNA knockout mutant of *NRT1.1* (20), the overall LR growth response in the  $\text{NO}_3^-$ -rich patch was strongly attenuated, with only a 4-fold increase in total second-order LR length on HN medium as compared with controls (Fig. 1A and B). This was found to be almost entirely due to a 50% reduction of the mean length of these second-order LRs in *chl1-10* as compared with Ws plants (Fig. 1A and C). Neither the final length of the first-order LR on HN medium nor the total number of visible second-order LRs were affected by the *NRT1.1* mutation (SI Fig. 7). This LR phenotype of the *chl1-10* mutant was specific for plants under localized high  $\text{NO}_3^-$  supply because no restriction of root growth was recorded in *chl1-10* plants growing on homogeneous medium with 10 mM  $\text{NO}_3^-$  (SI Fig. 8, see also HN/HN controls in Fig. 1B and C).

**The LR Growth Phenotype of the *NRT1.1*-Deficient Mutants Is Not Due to Decreased Root Nitrate Uptake Activity.** We considered the possibility that the altered root growth response in *chl1-10* may be due to reduced  $\text{NO}_3^-$  uptake activity. Both plant growth and  $\text{NO}_3^-$  uptake from the HN side therefore were measured in *chl1-10* and in another *NRT1.1*-deficient mutant, *chl1-5* (11), and their corresponding wild-types (Ws and Col, respectively). After 12 d of treatment, the mutations at the *NRT1.1* locus strongly hampered the increased root biomass allocation to the HN side that was observed in wild-type plants (Fig. 2A). Most importantly, this was not associated with an impaired  $^{15}\text{NO}_3^-$

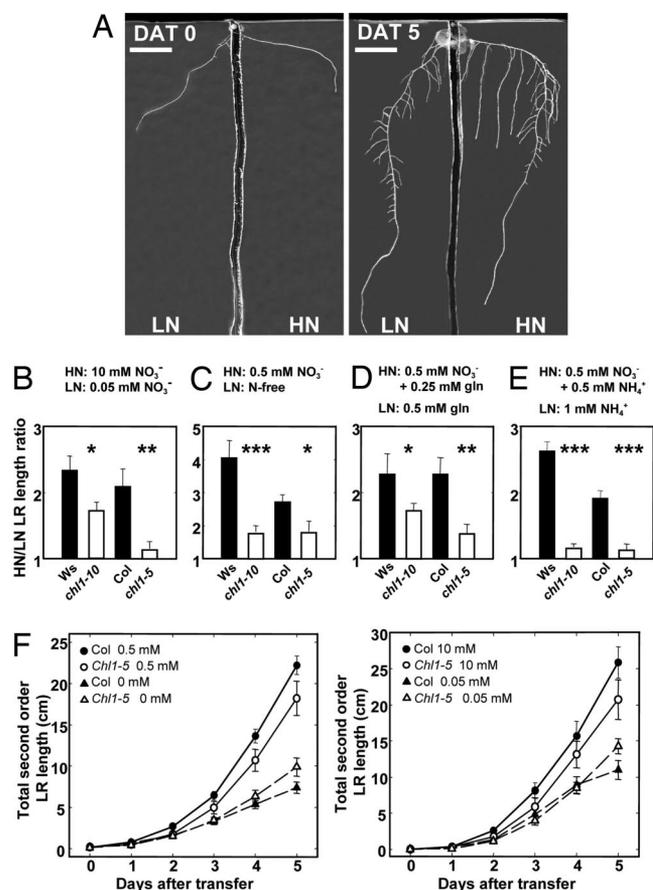
uptake from the  $^{15}\text{N}$ -labeled HN side in the *chl1* mutants during the first day after transfer (Fig. 2B). However, at the end of the 12-d treatment, both cumulative  $^{15}\text{NO}_3^-$  uptake and total plant biomass were reduced by  $\approx 25\%$  in *chl1* mutants as compared with the wild-types (Fig. 2C and D). Most of the reduction in final biomass of the mutants was accounted for by a 35% slower growth of the shoot during the treatment (SI Table 1).

It is important to note that cumulative  $^{15}\text{NO}_3^-$  uptake from the HN side in the *chl1* mutants (expressed on a total plant weight basis) was reduced by only 25% compared with the wild-types (Fig. 2C), whereas root biomass on this HN side was reduced by at least 50% (Fig. 2A). This shows that the specific  $\text{NO}_3^-$  uptake activity (expressed on a root weight basis) of the HN roots has been increased in *chl1* mutants, but not enough to compensate for the strongly reduced growth of these roots compared with wild-types. Taken together, these data indicate that the much less pronounced LR growth response of the *chl1* mutants was not the consequence of a defect in specific  $\text{NO}_3^-$  uptake activity. However, it does result after 12 d in a net reduction in the cumulative N uptake from the  $\text{NO}_3^-$ -rich patch compared with the wild-types, which accounts for the reduced biomass accumulation of the *chl1* mutants.

**The Phenotype of *NRT1.1*-Deficient Mutants Is Associated with Altered Nitrate Signaling.** A limitation of the split-root system depicted in Fig. 1A is that HN and LN sides markedly differed in nature and size at the beginning of the treatment, and that the architecture of the LN side rapidly becomes too complex to be easily assessed. Thus, to investigate more specifically the differential growth between the HN and LN sides of the root system, a simplified split-root device was set up, where the root system of 9-d-old plants was pruned to only the two most basal LR positions on LN or HN medium, respectively (Fig. 3A). In wild-type plants, preferential root growth on the HN side was marked particularly in this case (Fig. 3B), because after only 5 d of treatment, total second-order LR length already was between 2- and 4-fold greater on the HN than on the LN side. A similar number of second-order LRs developed on both the HN and the LN sides, but the mean length of these roots was much higher on the HN side. This behavior was found equally whether the  $\text{NO}_3^-$  concentration on the HN side was 10 mM or only 0.5 mM (Fig. 3B and C). In both cases, preferential second-order LR growth on the HN side was attenuated strongly in both *chl1* mutants (Fig. 3B and C), demonstrating the key role of *NRT1.1* in governing this adaptive root development response in a wide range of  $\text{NO}_3^-$  availability situations. As was the case with 10 mM  $\text{NO}_3^-$ , specific  $\text{NO}_3^-$  uptake activity of the roots on the 0.5 mM  $\text{NO}_3^-$  HN side was not reduced by *NRT1.1* mutation (SI Fig. 9).

Significantly, the LR growth response on the HN side, and its alteration in *chl1* mutants, also was observed when the spatial heterogeneity of  $\text{NO}_3^-$  availability was compensated for by the addition of glutamine or  $\text{NH}_4^+$  to the medium, to yield the same total N concentration on both HN and LN sides (Fig. 3D and E). This confirms previous evidence that  $\text{NO}_3^-$  stimulation of LR growth is due to the  $\text{NO}_3^-$  ion itself and is not a response to higher N availability *per se* (9). However, it also shows that the altered response of *chl1* mutants was not due to any deficiency in their ability to use  $\text{NO}_3^-$  as a nutrient, because supply of an alternative N source did not restore LR growth on the HN side to the level of the wild-type. In agreement with the above conclusions, the LR proliferation response to localized supply of  $\text{NH}_4^+$  was much less pronounced than with  $\text{NO}_3^-$  (compare SI Fig. 10 with Fig. 3B–E), and was not affected by *NRT1.1* mutation (SI Fig. 10).

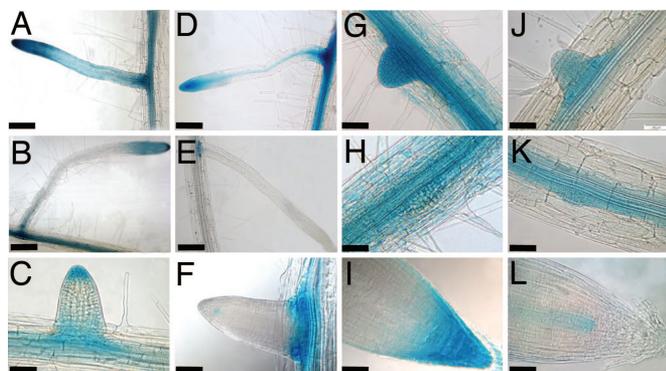
Furthermore, the time course analysis of LR growth on both HN and LN sides revealed that the attenuation of the LR growth response to localized  $\text{NO}_3^-$  supply in *chl1* mutants was due to both decreased LR growth on the HN side and increased LR growth on the LN side (Fig. 3F and data not shown for *chl1-10*).



**Fig. 3.** The root phenotype of *chl1* mutants is due to altered sensing of nitrate. (A) Response of the root system architecture to a localized high  $\text{NO}_3^-$  availability in *Arabidopsis* plants with a root system pruned to two first-order LRs, placed for 5 d either on high (10 mM, HN) or low (0.05 mM, LN)  $\text{NO}_3^-$  medium, respectively. DAT, day after transfer to HN/LN medium (Scale bars: 1 cm.). (B–E) Effect of N composition of HN and LN media on the ratio at DAT5 between total second-order LR length in HN and LN sides of the root system. Means for WT and *chl1* mutants significantly different (Student's *t* test) at: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . (F) Time course of second-order LR growth in plants subjected to heterogeneous  $\text{NO}_3^-$  supply (HNLN) with HN at either 0.5 or 10 mM and LN at 0 or 0.05 mM, respectively. Errors bars represent SEM ( $n = 8–15$ ).

This indicates that under uneven  $\text{NO}_3^-$  availability, mutation of *NRT1.1* does not impair LR growth *per se* but strongly modifies the distribution of LR growth between the two sides of the root system.

***NRT1.1* Regulates *ANR1* Expression.** Collectively, the above results show that the LR growth phenotype of the *chl1* mutants resembles that of transgenic lines underexpressing *ANR1* (i.e., reduced LR elongation in a  $\text{NO}_3^-$ -rich patch). This suggests that the function of *NRT1.1* in regulating LR growth may involve the *ANR1*-dependent  $\text{NO}_3^-$ -signaling pathway. To investigate this hypothesis, we compared both the localization of *NRT1.1* and *ANR1* expression in the roots, and the levels of *ANR1* mRNA in wild-type and *chl1* mutants. Under most conditions, GUS activity in transgenic *pNRT1.1::GUS* and *pANR1::GUS* lines was found to colocalize in the same tissues of the roots (Fig. 4), namely, apex and base of LRs (Fig. 4A–F), young emerging LRs (Fig. 4G and J), LR primordia (Fig. 4H and K), and apex of the primary root (Fig. 4I and L). Quite often, both *pNRT1.1* and *pANR1* also were found to be active in the stele (Fig. 4A–K). In some instances, *pANR1* activity could not be detected in the apex



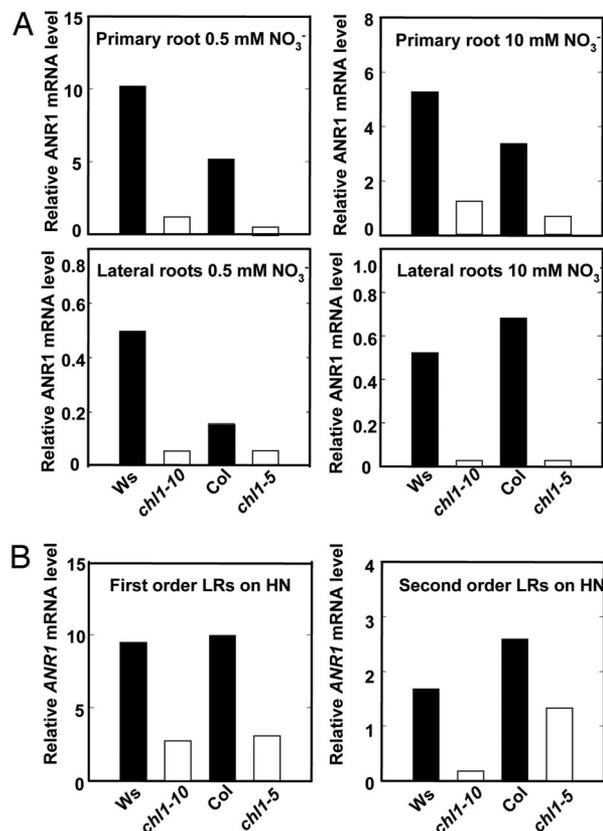
**Fig. 4.** Spatial localization of *NRT1.1* and *ANR1* expression. (A–C and G–I) Histochemical localization of GUS activity in *pNRT1.1::GUS* plants. (D–F and J–K) Histochemical localization of GUS activity in *pANR1::GUS* plants. GUS activity was visualized in LRs (A–F), emerging LR primordia (G and J), un-emerged LR primordia (H and K), and primary root apex (I and L). Plants were grown on 0.5 mM  $\text{NO}_3^-$  (B, C, and E–L) or on 0.1 mM  $\text{NO}_3^-$  plus 0.5 mM gln (A and D) as the N source. (Scale bars: A, B, D, and E 150  $\mu\text{m}$ ; C and F–L, 50  $\mu\text{m}$ .)

of mature LRs (Fig. 4E), suggesting that *ANR1* expression may be down-regulated in the LR tip at a relatively late stage of development. Interestingly, when investigated in the apical part (1–1.5 cm) of both primary and LRs of plants uniformly supplied with  $\text{NO}_3^-$ , *ANR1* transcript levels were found to be dramatically reduced in *chl1* plants as compared with wild-types, regardless of the external  $\text{NO}_3^-$  concentration in the medium (Fig. 5A). Similar results were obtained in plants under localized supply of  $\text{NO}_3^-$ , where *NRT1.1* mutation strongly reduced *ANR1* mRNA accumulation in the apex of first-order LRs and in second-order LRs growing on the HN side (Fig. 5B). The *ANR1* transcription regulation was confirmed by the comparison of *pANR1::GUS* expression in either wild-type or *chl1* genetic background (SI Fig. 11). Because a high level of *ANR1* expression is required to stimulate LR elongation in  $\text{NO}_3^-$ -rich patches (5), this provides a convincing molecular explanation for the altered LR growth response of the *chl1* mutants.

## Discussion

The detection by the plant of a  $\text{NO}_3^-$ -rich patch in the root environment is the first step in a crucial developmental response that leads to preferential LR growth in the zone where this essential nutrient is most abundant (3–5). Using a different experimental approach involving a split root system, we confirm here previous evidence that, in *Arabidopsis*, this response mainly relies on the stimulation of LR elongation (5) and that it is not a nutritional effect, but results from specific local  $\text{NO}_3^-$  signaling (9).

Our data indicate that *NRT1.1* has a major role in the  $\text{NO}_3^-$ -signaling pathway, leading to increased rates of LR elongation. Both *NRT1.1*-deficient *chl1* mutants showed a strongly decreased LR colonization of the  $\text{NO}_3^-$ -rich patch, resulting in a reduced ability of the plant to efficiently exploit this localized nutrient resource. Four major arguments support the hypothesis that the LR growth phenotype of the *chl1-5* and *chl1-10* mutants is due to altered local  $\text{NO}_3^-$  signaling and not to impaired N acquisition. First, the defect in LR growth was observed only when the  $\text{NO}_3^-$  supply was localized and not when  $\text{NO}_3^-$  was uniformly supplied. Second, no decrease in specific root  $\text{NO}_3^-$  uptake activity was observed in the *chl1* mutants. Third, addition of an alternative N source such as glutamine or  $\text{NH}_4^+$  in the HN side was unable to restore normal LR growth in the *chl1* mutants. Fourth, both *chl1* mutants displayed a dramatically altered expression of *ANR1*, a major component of the local  $\text{NO}_3^-$ -signaling pathway triggering LR elongation in  $\text{NO}_3^-$ -rich patches (5).



**Fig. 5.** Effect of *NRT1.1* mutation on *ANR1* expression. (A) Relative *ANR1* mRNA levels in the apical 10–15 mm of primary and LRs of wild-type and *chl1* mutant plants, grown on homogenous medium containing either 0.5 or 10 mM  $\text{NO}_3^-$  as the N source. The values are the means from two replicate experiments. (B) Relative *ANR1* mRNA levels in the apical 10–15 mm of first order or in second-order LRs of wild-type and *chl1* mutant plants transferred for 3 d to heterogenous HN/LN medium (10/0.05 mM  $\text{NO}_3^-$ ). The data presented are those obtained for the roots the in HN side.

The observation that mutations at the *NRT1.1* locus did not reduce specific root  $\text{NO}_3^-$  uptake activity at either 0.5 mM or 10 mM external  $\text{NO}_3^-$  concentration fits well with previous functional characterization of this transporter. Indeed, Touraine and Glass (21) showed that *NRT1.1* mutation has little impact on low-affinity  $\text{NO}_3^-$  uptake (i.e., at external concentrations >1 mM) when  $\text{NO}_3^-$  is the sole N source. The same is true for high-affinity  $\text{NO}_3^-$  uptake (i.e., at external concentrations <1 mM), because it is now well documented that *NRT2.1*, and not *NRT1.1*, is the main transport system for root  $\text{NO}_3^-$  uptake in this situation (13, 17, 22–24). Thus, together with the previous report that *chl1* mutants display an altered root growth under very specific conditions even in the absence of added  $\text{NO}_3^-$  in the medium (18), this indicates that the consequences of *NRT1.1* mutation on root architecture cannot simply be explained by lowered N acquisition by the plant. Thus, it is not surprising that the altered LR growth phenotype of *chl1* mutants could not be rescued by glutamine or  $\text{NH}_4^+$  supply. However, the effects of *NRT1.1* on LR growth remain in line with its surprising functional transport properties. Indeed, *NRT1.1* is an unusual dual-affinity transporter (15, 16), shifting from low to high affinity for  $\text{NO}_3^-$  in response to posttranslational regulation by phosphorylation (25). This may explain why a LR growth phenotype is found for *chl1* mutants independently of whether the actual  $\text{NO}_3^-$  concentration in the  $\text{NO}_3^-$ -rich patch is in the low (0.5 mM) or high (10 mM) range. Finally, the observation that mutation of *NRT1.1* prevents normal expression of *ANR1* is most important,

because it provides a clear indication of altered  $\text{NO}_3^-$  signaling in *chl1* mutants. Although the precise role of ANR1 is not clarified yet, it has been reported that a high level of *ANR1* transcript accumulation is required to yield the LR growth response (5). This suggests that NRT1.1 directs preferential LR elongation in  $\text{NO}_3^-$ -rich patches because it contributes to activating the ANR1-mediated  $\text{NO}_3^-$ -signaling pathway through modulation of *ANR1* mRNA accumulation.

To account for our observations, we propose that NRT1.1 acts in external  $\text{NO}_3^-$  sensing, and is located upstream of ANR1 in the  $\text{NO}_3^-$  signaling pathway that triggers increased LR elongation in  $\text{NO}_3^-$ -rich patches. The question rises whether NRT1.1 actually is a  $\text{NO}_3^-$  sensor generating a signal transduced internally by the ANR1 pathway or is the specific transporter providing the  $\text{NO}_3^-$  signal to an internal sensor triggering the ANR1 pathway. The fact that specific  $\text{NO}_3^-$  uptake activity is not reduced in the *chl1* mutants as compared with wild-types supports the first hypothesis, because it indicates that entry of the  $\text{NO}_3^-$  signal into the roots is not prevented by *NRT1.1* mutation. Accordingly, other aspects of  $\text{NO}_3^-$  signaling, such as  $\text{NO}_3^-$  induction of *NRT2.1*, are not altered by an *NRT1.1* mutation (20) and  $\text{NO}_3^-$  induction of *NRT2.1* is not affected in an ANR1 knockout mutant (8).

Well documented in yeast, the idea that members of membrane transporter families may fulfill a nutrient-signaling function also has been proposed in plants (26). In *Arabidopsis*, the *NRT2.1* high-affinity  $\text{NO}_3^-$  transporter recently has been shown to be implicated in the modulation of LR initiation, also in a way that cannot be explained by changes in root  $\text{NO}_3^-$  uptake activity (27, 28). Furthermore, a  $\text{NO}_3^-$ -signaling role for NRT1.1 already has been proposed to account for the fact that its mutation strongly alters the normal regulation of *NRT2.1* expression in preventing repression of this gene by high N provision to the plant (20). Alternatively, one could argue that a possible very localized defect in  $\text{NO}_3^-$  transport (e.g., at the root tip) of the *chl1* mutants, which cannot be unraveled by our macroscopic  $^{15}\text{NO}_3^-$  uptake measurements, may prevent  $\text{NO}_3^-$  reaching the internal sensor. Expression of *ANR1* initially was shown to be  $\text{NO}_3^-$  inducible (5). Thus, a possibility would be that NRT1.1 is responsible for supplying locally the  $\text{NO}_3^-$  inducer for expression of *ANR1*. However, this explanation may be too simplistic because recent data indicate that regulation of *ANR1* is much more complex and that, as commonly observed for several other N-related genes, it is also repressed by high N provision to the plant (8). Whatever the precise role of NRT1.1, it appears to be functionally related to the ANR1-signaling pathway and may either provide or transduce the  $\text{NO}_3^-$  signal to this pathway. Moreover, the importance of NRT1.1 as a central player in the integrated responses of the plant to nutrient cues is highlighted, because it governs both key metabolic (*NRT2.1* uptake system) and developmental (LR elongation) adaptive responses to changes in external  $\text{NO}_3^-$  availability.

The predominant expression of *NRT1.1* in all root tips (Fig. 4 and SI Fig. 12) is of major significance because it indicates that this transporter is localized at the forefront of the soil exploration by the root system, a strategic place for scanning the mineral environment at the periphery of the rooted area and identifying gradients in external nutrient availability (10, 29). Furthermore, the overlap between *NRT1.1* and *ANR1* expression in LR primordia and LR apices is consistent with the conclusion that the local  $\text{NO}_3^-$  signaling triggering the LR growth response in  $\text{NO}_3^-$ -rich patches stimulates cell production in LR meristems (9). This also provides an explanation for the role of NRT1.1 in modulating the distribution of LR growth within the root system, because it suggests that this transporter specifically favors meristem activity on the HN side, thus increasing the sink strength of this part of the root system, to the detriment of the roots in LN side. Accordingly, *NRT1.1* mutation not only reduces LR elongation on the HN side but also increases it on the LN side

(Fig. 3 B–F). However, we cannot formally rule out the reverse hypothesis, i.e., that the primary action of NRT1.1 is to repress meristem activity on the LN side, indirectly favoring root growth on the HN side.

Finally, it must be recalled that mutation of *NRT1.1* markedly attenuates, but does not totally suppress, the LR growth response in  $\text{NO}_3^-$ -rich patches. This indicates that part of this response may involve other signaling pathways, independent of NRT1.1 and possibly of ANR1. Interestingly, several putative  $\text{NO}_3^-$  transporter genes other than *NRT1.1* were found to be expressed in root tips (30). This does not necessarily mean that these transporters also participate to  $\text{NO}_3^-$  sensing in relation with root development. However, the fact that both NRT1.1 and NRT2.1, the first  $\text{NO}_3^-$  transporters identified in higher plants, now are reported to have a signaling function may indicate a more general role of  $\text{NO}_3^-$  transporters, and by analogy of other ion transporters, in external nutrient sensing. Furthermore, several other MADS box genes have been shown to be regulated in the roots by N provision to the plant (8), thus providing interesting candidates for the investigation of additional signaling pathways involved in the adaptive responses of the plant to the changes in external N availability. We anticipate that a wider investigation of mutants defective in ion transporters or other MADS box genes (particularly those expressed in root tips or LR primordia) will provide important insight into the mechanisms enabling the plant to modulate its root development for improved efficiency and higher competitiveness in nutrient acquisition.

## Methods

**Plant Material.** The *Arabidopsis thaliana* Heynh ecotypes used in this study were Wassileskija (Ws) and Columbia (Col-0). The *NRT1.1* mutants were the *chl1-5* mutant in a Col-0 background (11) and the *chl1-10* T-DNA insertion mutant in a Ws background (20). Transgenic *Arabidopsis* lines used for histochemical studies carried the following promoter-reporter gene fusions: *pNRT1.1::GUS* (18) and *pANR1::GUS*. For the *pANR1::GUS* construct, a 2,957-bp fragment located upstream of the translation initiation codon (–2,963 to –6) of *ANR1* was cloned into the BamHI site in pBI101.3 (Clontech Laboratories, Palo Alto, CA), and Col-0 was transformed by the floral dip method (31) by using *Agrobacterium tumefaciens* GV3101. The *pANR1::GUS* construct has been introduced in *chl1-5* background by crossing.

**Plant Growth.** Basal medium contained 0.5 mM  $\text{CaSO}_4$ , 0.5 mM  $\text{MgCl}_2$ , 1 mM  $\text{KH}_2\text{PO}_4$ , 2.5 mM Mes (2-[morpholino]ethanesulphonic acid; Sigma, Saint Quentin, France) (pH 5.8), 50  $\mu\text{M}$   $\text{NaFeEDTA}$ , 50  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 12  $\mu\text{M}$   $\text{MnCl}_2$ , 1  $\mu\text{M}$   $\text{CuCl}_2$ , 1  $\mu\text{M}$   $\text{ZnCl}_2$ , and 0.03  $\mu\text{M}$   $\text{NH}_4\text{MoO}_4$ . This basal medium was supplemented with  $\text{KNO}_3$  as a sole nitrogen source at the concentrations indicated for each individual experiment. The  $\text{K}^+$  concentration was adjusted to 10 mM by addition of  $\text{K}_2\text{SO}_4$  in all media with  $\text{KNO}_3$  concentrations <10 mM. *Arabidopsis* seeds were surface sterilized for 10 min in 1 ml of 50% (vol/vol) ethanol containing 2% (wt/vol) Bayrochlor (Bayrol, Mundolsheim, France), followed by five washes with 100% ethanol and drying in a laminar air flow. Sterilized seeds were planted with a sterile toothpick in 12 × 12 cm transparent plates on 40 ml of solid medium (1% Difco Bacto agar; BD Biosciences, Sparks, MD) containing 10 mM  $\text{NO}_3^-$ . After storing for 2 d at 4°C in the dark, plates were incubated vertically in a growth chamber at 22°C, with a 16 h/8 h light/dark regime and a light intensity of 230  $\mu\text{moles}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$ . Plantlets growing on the surface of the agar were transferred at various time points as indicated to fresh growth media (at five plants per plate) containing various  $\text{NO}_3^-$  concentrations.

The experimental device for localized high  $\text{NO}_3^-$  supply was established by using segmented agar plates where two patches of agar were separated by a trench. The high  $\text{NO}_3^-$  concentration on

