Multiple mechanisms of nitrate sensing by Arabidopsis nitrate transceptor NRT1.1

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In Arabidopsis the plasma membrane nitrate transceptor (transporter/receptor) NRT1.1 governs many physiological and developmental responses to nitrate. Alongside facilitating nitrate uptake, NRT1.1 regulates the expression levels of many nitrate assimilation pathway genes, modulates root system architecture, relieves seed dormancy and protects plants from ammonium toxicity. Here, we assess the functional and phenotypic consequences of point mutations in two key residues of NRT1.1 (P492 and T101). We show that the point mutations differentially affect several of the NRT1.1-dependent responses to nitrate, namely the repression of lateral root development at low nitrate concentrations, and the short-term upregulation of the nitrate-uptake gene NRT2.1, and its longer-term downregulation, at high nitrate concentrations. We also show that these mutations have differential effects on genome-wide gene expression. Our findings indicate that NRT1.1 activates four separate signalling mechanisms, which have independent structural bases in the protein. In particular, we present evidence to suggest that the phosphorylated and non-phosphorylated forms of NRT1.1 at T101 have distinct signalling functions, and that the nitrate-dependent regulation of root development depends on the phosphorylated form. Our findings add to the evidence that NRT1.1 is able to trigger independent signalling pathways in Arabidopsis in response to different environmental conditions.

Plants are sessile organisms that face dramatic fluctuations of external mineral nutrient availability. Thus, they have developed sophisticated nutrient sensing systems, which activate physiological and developmental responses that prevent nutrient deficiency or toxicity. To date, most of nutrient sensing systems remain unknown at the molecular level. One exception is the sensing of nitrate (NO₃⁻), which relies largely on ‘transceptors’, that is, membrane NO₃⁻ carriers fulfilling a dual transport/sensing function. The most documented NO₃⁻ transceptor is NRT1.1 of Arabidopsis thaliana, also called CHL1 (ref. 6) or NPF6.3 (ref. 10). Initially characterized as a dual-affinity transporter involved in root uptake and root-to-shoot translocation of NO₃⁻ (refs 11, 12), NRT1.1 was later shown to mediate an impressive number of responses to NO₃⁻ (ref. 8), among which three have been extensively investigated. First, NRT1.1 is required for the ‘primary nitrate response’ (PNR), that is the short-term regulation of gene expression upon first NO₃⁻ supply. This includes the fast induction of many genes of the NO₃⁻ assimilation pathway such as NRT2.1, which encodes a main component of the root NO₃⁻ uptake system. Second, NRT1.1 is also responsible in the longer term for an opposite response of some of these genes (for example NRT2.1) that are feedback repressed by high nitrogen provision. Third, NRT1.1 was shown to promote root branching in response to NO₃⁻ (refs 17, 18), in particular by repressing the emergence of lateral root primordia (LRP) at low NO₃⁻ availability.

How NRT1.1 can control such a large palette of responses to NO₃⁻ is not understood. Two different models have been proposed. On the one hand, the signalling role of NRT1.1 in the PNR was reported to be independent of the transport function of the protein. Indeed, the PNR is unaffected by P492L substitution (chl1-9 mutation) that suppresses NRT1.1 NO₃⁻ transport activity. Furthermore, the PNR is negatively affected by phosphorylation of NRT1.1 at the T101 residue, suggesting that it is the non-phosphorylated form that is predominantly active in NO₃⁻ signalling. On the other hand, the NRT1.1-dependent repression of LRP emergence at low NO₃⁻ availability was explained by auxin transport activity of NRT1.1. We proposed that NRT1.1 mediates NO₃⁻-inhibited auxin transport that exports the hormone out of the LRP at low NO₃⁻ concentration, thus preventing the auxin accumulation in these organs that is required for their proper development.

To date, there is no link between these two NRT1.1 signalling models. In addition, the mechanisms involved in the NRT1.1-dependent feedback repression of gene expression by high nitrogen remain totally obscure. Using Arabidopsis NRT1.1P492L mutant and genotypes expressing the NRT1.1T101A or NRT1.1T101D mutants in an NRT1.1 knockout mutant background, we show here that NRT1.1 modulates LRP development and triggers gene feedback regulation by high nitrogen through mechanisms that are independent of those involved in the PNR, and that the phosphorylated form of NRT1.1 also plays a crucial role in NO₃⁻ signalling and NO₃⁻-dependent auxin transport.

Results

NRT1.1-dependent regulation of lateral root development. The repression exerted by NRT1.1 on lateral root development at low external NO₃⁻ availability is evidenced by the markedly increased lateral root density and length in the chl1-5 knockout mutant of NRT1.1 compared with the Col wild type (WT) in NO₃⁻-free medium, whereas this difference is abolished or dramatically reduced on medium containing 1 mM NO₃⁻ (Fig. 1a–c). We have previously shown that it is NO₃⁻ per se that modulates the repressive action of NRT1.1, because supply of another nitrogen source such as glutamine does not mimic the effect of NO₃⁻ (ref. 19). Both genotypes chl1-9 and T101A expressing the NRT1.1P492L mutant (lacking NO₃⁻ transport activity) or the non-phosphorylatable NRT1.1T101A mutant displayed a chl1-5 phenotype, whereas T101D plants expressing the phosphomimetic NRT1.1T101D mutant showed a WT phenotype (Fig. 1a–c). These results indicate that P492L and T101A but not T101D
substitutions prevented the repressive role NRT1.1 in lateral root development, thus indicating that it is the phosphorylated form of NRT1.1 that is responsible for the NRT1.1-dependent regulation of lateral root development.

**NRT1.1<sup>T101D</sup> transports auxin as the wild-type NRT1.1 protein.**

We have previously proposed that NRT1.1 represses LRP emergence through its auxin transport capacity<sup>19</sup>. The identification of point mutations in NRT1.1 that suppress this repression (such as P492L and T101A, Fig. 1) offers an important opportunity to validate this hypothesis. Therefore, the mutated forms of NRT1.1 were investigated for their auxin influx activity in *Xenopus* oocytes using 2,4-dichlorophenoxyacetic acid (2,4-D) as a substrate and a surrogate to indole-3-acetic acid (IAA)<sup>21</sup>. As reported previously with <sup>3</sup>H-IAA, oocytes injected with WT NRT1.1 cRNA displayed a 75% increase in <sup>3</sup>H-2,4-D accumulation compared with the control (Fig. 2a). Injection with NRT1.1<sup>T101D</sup> cRNA yielded the same outcome, but injection with NRT1.1<sup>T101A</sup> cRNA resulted in a markedly lowered <sup>3</sup>H-2,4-D accumulation, whereas expression of NRT1.1<sup>P492L</sup> failed to increase it over that recorded in control oocytes (Fig. 2a). The facts that NRT1.1<sup>P492L</sup> does not transport auxin and that NRT1.1<sup>T101D</sup> has a markedly higher auxin transport capacity than NRT1.1<sup>T101A</sup> were confirmed by <sup>3</sup>H-IAA uptake assays in tobacco BY-2 cell lines stably transformed with the corresponding genes under the control of an oestradiol-inducible promoter (Fig. 2b and Supplementary Fig. 1). Therefore, we confirm that the mutations preventing or dramatically reducing NRT1.1-mediated auxin transport (P492L and T101A substitution) are also those altering NRT1.1-dependent regulation of LRP emergence (Fig. 1). Furthermore, these data suggest that the P492L substitution not only suppresses transport of NO<sub>3</sub><sup>-</sup> by NRT1.1 but also transport of auxin, and that it is the phosphorylated form of NRT1.1 that is predominantly active in auxin transport.

**NRT1.1<sup>T101D</sup> modulates auxin gradient in LRP as wild-type NRT1.1.**

Next, we investigated the effect of the point mutations on the auxin gradients in LRPs. Indeed, we have previously proposed that NRT1.1 represses emergence of LRPs because its auxin transport activity prevents auxin accumulation in these organs, in the absence but not in the presence of NO<sub>3</sub><sup>-</sup> (ref. 19). To further document this point, we introduced the DR5::GFP auxin biosensor in the various mutant lines. As expected from the very low auxin transport capacity recorded for NRT1.1<sup>P492L</sup> and NRT1.1<sup>T101A</sup>, *chl1-9 × DR5::GFP* and *T101A × DR5::GFP* plants behaved like *chl1-5 × DR5::GFP* plants and exhibited strongly enhanced DR5 activity (up to 300%) in LRPs compared with WT or T101D backgrounds in the absence, but not in the presence, of NO<sub>3</sub><sup>-</sup> (Fig. 2c). Similar results were obtained with independent lines expressing the *DR5::GUS* transgene (Supplementary Fig. 2). These results are consistent with the hypothesis that NRT1.1<sup>P492L</sup> and NRT1.1<sup>T101A</sup> mutants are unable to prevent auxin accumulation in LRPs at low NO<sub>3</sub><sup>-</sup>, and confirm that the effect of NRT1.1 on the local auxin gradients in LRPs is associated with, and most probably due to, its auxin transport activity.

**P492L prevents plasma membrane localization of NRT1.1.**

Altogether, the above results are all in agreement with the conclusion that, due to its capacity to transport auxin, it is the phosphorylated form of NRT1.1 that actually represses LRP development at low NO<sub>3</sub><sup>-</sup> by preventing auxin accumulation in these organs. To further understand why both NRT1.1<sup>P492L</sup> and NRT1.1<sup>T101A</sup> fail to fulfil this function, we checked whether P492L and T101A mutations led to lack of expression or mistargeting of the mutant proteins, by expressing oestradiol-inducible...
NRT1.1–mCherry translational fusions in both chl1-5 mutant plants and tobacco BY2 cells. All four proteins were expressed in LRPs at a similar level (Fig. 3a). However, unlike the other fusion proteins that were correctly addressed at the plasma membrane, NRT1.1P492L displayed a predominant intracellular localization in both root and BY2 cells (Fig. 3b,c). These data provide a relevant explanation for the lack of transport activity recorded for NRT1.1 P492L with both NO₃⁻ and auxin as substrates. They also indicate that reduced auxin transport by NRT1.1T101A is not due to mistargetting, as also suggested by its high NO₃⁻ transport capacity²², but is probably an intrinsic property of the non-phosphorylated form of the protein.

NRT1.1-dependent regulation of NRT2.1 expression. It has been demonstrated¹⁴ that both NRT1.1T101A and NRT1.1P492L can trigger NRT1.1-dependent short-term induction of NRT2.1 expression by NO₃⁻ (illustrative of the PNR), whereas NRT1.1T101D only activates a restricted response. Most surprisingly, we found that the other aspect of the NRT1.1-dependent regulation of NRT2.1 expression, that is its long-term repression under high nitrogen supply, was affected by the point mutations of NRT1.1 in a totally different manner (Fig. 4). As previously shown¹³,¹⁶, the NRT2.1 mRNA level was much higher in the chl1-5 knockout mutant than in Col when grown on 10 mM NH₄NO₃ (Fig. 4a). Both P492L and T101A substitutions
phenocopied knockout mutation of NRT1.1 and prevented repression of NRT2.1 expression by high nitrogen. In comparison, T101D mutation did not markedly alter this repression and only slightly increased NRT2.1 mRNA level over that recorded in WT (Col) plants (Fig. 4a). To determine when the shift between induction and later feedback repression of NRT2.1 occurs and how point mutations of NRT1.1 affect it, we performed time course studies of NRT2.1 transcript accumulation following the first supply of NO$_3^-$ at a high concentration (10 mM). In agreement with a previous study$^{14}$, induction of NRT2.1 after 1 h of NO$_3^-$ treatment was stronger in Col, chl1-9 and T101A than in chl1-5 or T101D (Fig. 4b). However, the relative difference in NRT2.1 transcript accumulation between these genotypes was much lower after 3 h, and NRT2.1 expression was markedly downregulated as early as 6 h after NO$_3^-$ provision in both WT and T101D plants, but not in chl1-5, chl1-9 and T101A plants.
(Fig. 4b). This shows that NRT1.1-dependent repression of NRT2.1 occurs soon after its initial induction, and that unlike the PNR, it requires the phosphorylated form of NRT1.1.

NRT1.1 regulation of transcriptome at high nitrogen. The unexpected finding that induction and repression of NRT2.1 by NRT1.1 involves different mechanisms prompted us to get a genome-wide view of the transcriptome reprogramming triggered by the different forms of NRT1.1 under high nitrogen repressive conditions (10 mM NH₄NO₃). Three independent experiments were performed with the various genotypes (including chl1-12, an additional knockout mutant of NRT1.1). Statistical analysis highlighted 490 differentially regulated probes corresponding to 468 unambiguous genes (Supplementary Table 1, available at http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?&acc=GSE49437), which could be organized in 21 clusters (Fig. 5 and Supplementary Fig. 3). Four clusters (11, 16, 17 and 20, Fig. 5b) were selected for further analysis on the basis of their size, expression pattern and over-represented functional categories. Cluster 20 gathers 155 genes that display NRT2.1-like expression, with upregulation in chl1-5, chl1-12, chl1-9 and T101A genotypes compared with Col and T101D. Interestingly, the functional categories that are found to be enriched in this cluster relate to transport in general and to nitrogen, sulphur and selenium metabolisms (Supplementary Fig. 4). In particular, several nitrogen transport or assimilation genes, known to be nitrogen-regulated, such as NRT1.7, NRT2.4, DUR3 and GNL1.4, belong to this cluster. Clusters 11 and 16 gather 135 genes that show a pattern of expression that is the opposite of NRT2.1, with downregulation in chl1-5, chl1-12, chl1-9 and T101A genotypes compared with Col and T101D (Fig. 5b).
Like cluster 20, clusters 11 and 16 show an over-representation of the transport functional category, but also in genes associated with environment interactions (Supplementary Fig. 4). Note that the NO$_3^-$-inducible transcription factor gene LBD39, known to be a repressor of NRT2.1 expression\cite{23}, was found in cluster 11. Finally, cluster 17 (54 genes) is striking because it displays an over-representation of genes related to extracellular matrix and secreted proteins as well as genes associated with oxydo-reduction reactions (Supplementary Fig. 4), and also because the pattern of expression has never been reported before, as it appears to be a repressor of NRT1.1 that cannot be phenocopied by either NRT1.1P492L\textsuperscript{WT} or NRT1.1T101A\textsuperscript{WT}\textsuperscript{WT}. Dotted lines represent normal responses as recorded for NRT1.1\textsuperscript{WT}. Dotted lines represent attenuated or suppressed responses as compared to NRT1.1\textsuperscript{WT}. NRT1.1\textsuperscript{WT} is not pictured in the plasma membrane to illustrate the predominant intracellular localization of the NRT1.1\textsuperscript{P492L}\textsuperscript{::mCherry} protein. Auxin transport by NRT1.1 is only shown at low NO$_3^-$ because this transport is inhibited at high NO$_3^-$ (ref. 19).

**Discussion**

In this work, we considered three different responses of the plant to NO$_3^-$ that are dependent on NRT1.1 but occur under different experimental conditions: (1) the repression of lateral root development at low NO$_3^-$ availability (Fig. 1), (2) the short-term induction of NRT2.1 (Fig. 4b) that is representative of the PNR\textsuperscript{14}, and (3) the regulation of gene expression by high nitrogen that occurs in the long-term (Figs 4 and 5). Analysis of these responses in the various NRT1.1 point mutants unravels four different ‘modes’ of NRT1.1-dependent signalling (Fig. 6). Our data agree with those...
reported previously\textsuperscript{14}, showing that the short-term induction of NRT2.1 at high NO\textsubscript{3} supply is negatively affected by T101D, but not by P492L and T101A substitutions (Fig. 6a). Furthermore, the long-term regulation of NRT2.1 expression and of genes of clusters 20, 11 and 16 by high nitrogen (Fig. 6b), and the repression of lateral root emergence in the absence of NO\textsubscript{3} (Fig. 6c) show the opposite pattern, with little or no effect of T101D substitution, and loss of signalling resulting from P492L or T101A mutations. Finally, clusters 13 and 17 (>60 genes), are under NRT1.1-dependent regulation that is suppressed by both T101A and T101D mutations, but not by P492L, indicating a yet uncharacterized signalling role of NRT1.1 (Fig. 6d).

The unexpected finding that P492L mutation prevents normal trafficking of NRT1.1, without affecting two of these regulatory ‘modes’ (PNR and regulation of the genes of clusters 13 and 17), suggests that NO\textsubscript{3} signalling by NRT1.1 can partly occur independently of its localization and transport function at the plasma membrane. However, mistargetting of NRT1.1 due to P492L mutation raises the question of whether this substitution actually affects the intrinsic NO\textsubscript{3}− transport capacity of the protein. This point is important because apparent loss of NO\textsubscript{3}− transport by NRT1.1\textsuperscript{P492L} was taken as evidence that transport and signalling functions of NRT1.1 can be uncoupled\textsuperscript{14}, thus supporting the transceptor model for this protein. Nevertheless, it is conceivable from our data that NRT1.1\textsuperscript{P492L} can still transport NO\textsubscript{3}−, but that this ability does not lead to NO\textsubscript{3}− uptake into the cell, because NRT1.1\textsuperscript{P492L} is not at the plasma membrane. In our opinion, these considerations are not sufficient to reject the transceptor model for NRT1.1, for at least two reasons. First, although nothing demonstrates that P492L supresses NO\textsubscript{3}− transport activity of NRT1.1, structural studies on this protein\textsuperscript{26,27} provide some support for this hypothesis. Indeed, although P492 is not part of the NO\textsubscript{3}− recognition site, it is located in the short loop separating transmembrane domains 10 and 11. Therefore, its mutation is expected to alter the structural coordination of these two domains\textsuperscript{26}, and consequently the three-dimensional position of the E476 residue of transmembrane domain 10, which is crucial for the NO\textsubscript{3}− transport mechanism\textsuperscript{27}. Second, P492L is not the only point mutation reported to uncouple transport and signalling functions of NRT1.1. Our own data (Fig. 6) and others’ show that T101A substitution results in loss of NRT1.1-dependent NO\textsubscript{3}− signalling, although it does not prevent NO\textsubscript{3}− transport by NRT1.1\textsuperscript{22}.

The results obtained with the various mutants are fully consistent with our previous proposal that NRT1.1 regulates lateral root development through its auxin transport capacity\textsuperscript{29}. Accordingly, point mutations of NRT1.1 that suppress or inhibit its auxin transport facilitation (P492L and T101A) (Fig. 2a,b) are also those leading to a misregulation of both DRO1 activity in LRPs (Fig. 2c) and emergence of LRPs in response to NO\textsubscript{3}− limitation (Fig. 1). Although it does not constitute a final proof, we believe this correlative evidence provides strong support for our proposal. In contrast, the mechanism involved in the NRT1.1-dependent long-term response to high nitrogen provision remains obscure, and there are no clues indicating that it is associated with the NO\textsubscript{3}−/auxin transport function of NRT1.1, or not. However, there is circumstantial evidence that the feedforward repression of NRT2.1 may be because NRT1.1 acts as an inducer of LBD39, a previously identified negative regulator of NRT2.1 expression\textsuperscript{23}. Accordingly, GLN1.4, which is also under negative control by LBD39 (ref. 23) is repressed by NRT1.1 in the same way as NRT2.1 is. The validation of this hypothesis will nevertheless require further investigation.

Note that T101A and T101D mutations (that suppress and mimic T101 phosphorylation, respectively) inhibit different NRT1.1-dependent signalling responses (Fig. 6). This suggests that phosphorylated and non-phosphorylated forms of NRT1.1 have separate signalling roles. Previous studies have highlighted the crucial impact of T101 phosphorylation for switching between the high- and the low-affinity modes of the protein for NO\textsubscript{3}− transport\textsuperscript{22}. Very recently, crystal structure studies have revealed that T101 phosphorylation controls dimerization and/or structural flexibility of NRT1.1\textsuperscript{26,27}. Here, we show that T101 phosphorylation also directs the action of NRT1.1 towards the activation of specific responses (lateral root development and feedback regulation by high nitrogen), pointing out a crucial signalling function of the phosphorylated form of the protein (Fig. 6). The fact that neither NRT1.1\textsuperscript{T101A} nor NRT1.1\textsuperscript{T101D} can ensure the NRT1.1-dependent regulation of clusters 13 and 17 may appear highly surprising. However, several hypotheses may explain this observation. For instance, this regulation may be directly associated with the dynamic processes of phosphorylation/dephosphorylation of the T101 residue that are impaired by both T101A and T101D mutations. Alternatively, as NRT1.1 forms a dimer\textsuperscript{22}, it is theoretically possible that genes of clusters 13 and 17 are responsive to signalling by an NRT1.1 dimer with only one subunit phosphorylated, a structure that cannot be mimicked in T101A or T101D plants.

In conclusion, our work suggests that the wide range of responses to NO\textsubscript{3}− triggered by NRT1.1 may relate to different NO\textsubscript{3}− transduction mechanisms at the level of the NRT1.1 protein itself that can be separated by specific point mutations. This provides a generalization of observations made on transceptors in yeast\textsuperscript{28}. For instance, the NH\textsubscript{4}+ transceptor Mep2 was reported to mediate two different signalling mechanisms, namely activation of the protein kinase A signalling pathway upon high NH\textsubscript{4}+ supply\textsuperscript{29}, and initiation of the so-called pseudo-hyphal growth in response to NH\textsubscript{4}+ limitation\textsuperscript{30}. These two responses were uncoupled by point mutations in the protein that prevented pseudo-hyphal growth without affecting PKA activation\textsuperscript{29}. Therefore, our work suggests that plants, like yeast, have developed versatile transceptors with multiple sensing/transduction mechanisms, able to activate independent responses, depending on the environmental conditions.

Material and Methods

Plant material. The Arabidopsis thaliana accession used was Columbia (Col-0). The various mutants and transformants were chil-5 (ref. 11) and chil-12 (SALK_034596) knockout nrt1.1 mutants, chil-9 (ref. 14) a point mutant in which the proline 492 of NRT1.1 is replaced with a leucine, T101A and T101D transgenic lines expressing in chil-5 mutant background, modified forms of NRT1.1 in which the threonine 101 is mutated into alanine and aspartate\textsuperscript{14}. Lines expressing DR5:GFP were obtained by crossing the DR5:GFP line\textsuperscript{31} with chil-5, chil-9, T101A or T101D. Transgenic tobacco BY-2 cells and Arabidopsis lines expressing the various NRT1.1 transgenic forms with mCherry under the control of an oestradiol-inducible promoter are detailed in the Supplementary Material and Methods.

Plant culture and root growth analysis. Seedlings were grown and the root system architecture was analysed as described previously\textsuperscript{7}. Basal culture medium was supplemented with the appropriate concentration of KNO\textsubscript{3}, NH\textsubscript{4}NO\textsubscript{3} or NH\textsubscript{4}Cl, respectively. Seeds were obtained by crossing the SALK_034596 nrt1.1::GFP植株 (Invitrogen Life Technologies SAS), and DNase was treated (Qiagen S.A.S. France) and purified using an RNeasy MinElute Cleanup Kit (Qiagen S.A.S. France). Reverse transcription was achieved with 4 µg of RNA in the presence of Moloney Murine Leukaemia Virus reverse transcriptase (Promega, France) after annealing with an anchored poly-dT\textsubscript{(18)} primer. Transcript accumulation was measured by quantitative real-time PCR (LightCycler 480; Roche Diagnostics) using the LightCycler 480 SYBR Green 1 Master kit (Roche Diagnostics). All steps were performed according to the manufacturer’s recommendations. Primers used were CLATHRIN F, 5′-AGCATACTCCTGGTCCAAGAAG-3′, CLATHRIN R, 5′-TCGGCTGTGCACATATCCTC-3′, NRT2-1 F, 5′-AAACGGGCTAAAGGCTT-3′, and NRT2-1 R, 5′-CTGCTTCTCCTGCTCATTT-3′.

Microarray hybridization. Transcripts were realized using GeneChip Arabidopsis ATH1 Genome Array and the GeneChip 3′IVT Express kit (Affymetrix, Cleveland, OH, USA) according to the manufacturer’s instructions. Further details are in the Supplementary Material and Methods.

Microscopy. Confocal imaging of Arabidopsis root cells expressing mCherry fusions were performed using a Leica SP8 Confocal Microscope (Leica, Germany). A laser (561 nm) was used for excitation. The emission light from 580 to 630 nm was
detected with a Leica 63× objective (HCX PL APO 1.4 NA). Green fluorescent protein (GFP) was excited at 470–510 BP filter and detected via a 500–535 BP filter (green) on an Olympus BH2 microscope. GFP quantifications were done with an Axiosvert 200 M microscope (Zeiss, Germany) and coolsnapHQ camera (Photometrics Scientific, USA) and images analysed with MetaFluo V7.7.8.0 software (Molecular Device LLC Sunnyvale, USA). GFP was excited with a HCC-495 filter and detected via a 525/45 BP filter (green). The data represent the fluorescence quantification values in a region of interest (corresponding to the primordium) from which the background fluorescence values measured in the part of the root not expressing DRS5 were subtracted.

AUXIN ACCUMULATION ASSAYS. Oocytes were obtained from Xenopus laevis and injected with cRNA (NRT1.1WT, NRT1.1TMDA, NRT1.1TGSID, NRT1.1PVFS) as described previously.[19] Oocytes were incubated for 30 min in 1 ml of Ringer solution (pH 6) containing 1 μM 2,4-D (100 nM H2-2,4-D; SIGRO, Fleurus, Belgium; diluted with 900 nM cold-2,4-D; Sigma-Aldrich, France) and treated and analysed as described previously.[19] Accumulation assay on BY-2 cells was performed[25] using H1-IAA (2 mM, radioactivity 20 Ci mmol−1, American Radiolabelled Chemicals, USA).

Statistical! Analyses. The data are presented as mean ± s.e., and have been analysed using a one-way ANOVA, followed by a Tukey test.

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Author contributions


Additional information

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Competing interests

The authors declare no competing financial interests.