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Article Addendum

Nitrate Signaling and the Two Component High Affinity Uptake System in Arabidopsis

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Addendum to:

Characterisation of a Two Component High Affinity Nitrate Uptake System in Arabidopsis*: Physiology and Protein-Protein Interaction*

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ABSTRACT

Nitrate transporters are important for nitrogen acquisition by plants and in algae some require two gene products, *NRT2* and *NAR2,* for function. The *NRT2* family was already described and the recent identification of a family of the *NAR2*-type genes in higher plants showed that there was a homologue in *Arabidopsis*, *AtNAR2.1*. Using heterologous expression in yeast and oocytes we showed that the two *Arabidopsis* AtNRT2.1 and AtNAR2.1 proteins interacted to give a functional high affinity nitrate transport system (HATS). The gene knock out mutant *atnar2.1-1* is deficient specifically for HATS activity and the resulting growth phenotype on low nitrate concentration is more severe than for the *atnrt2.1-1* knock out mutant. Physiological characterisation of the plant N status and gene expression revealed a pattern that was characteristic of severe nitrogen deficiency. Consistent with the down regulation of *AtNRT2.1* expression, the *atnar2.1-1* plants also displayed the same phenotype as *atnrt2.1* mutants in lateral root (LR) response to low nitrate supply. Using *atnar2.1-1* plants constitutively expressing the NpNRT2.1 gene, we now show a specific role for AtNAR2.1 in LR response to low nitrate supply. AtNAR2.1 is also involved in the repression of LR initiation in response to high ratios of sucrose to nitrogen in the medium. Therefore the two component system itself is likely to be involved in the signaling pathway integrating nutritional cues for LR architecture regulation. Using a green fluorescent protein-NRT2.1 protein fusion we show the essential role of AtNAR2.1 for the presence of AtNRT2.1 to the plasma membrane.

INTRODUCTION

Nitrate uptake by plants is a fundamental aspect of their nutrition and several families of transporters have been identified.[1,2] For one of these families, the NRT2s, a second protein has been shown to be necessary for the nitrate transport function in *Xenopus* oocytes[3,4] and in planta using gene knock-out mutants.[5,6] Earlier research using the green alga *Chlamydomonas* had identified that a second gene was important for high affinity nitrate transport and it was named *Nar2.*[7] Now it has been shown that the interaction of the two components actually occurs between the two proteins.[5] Gene knock-out *Arabidopsis* plants that lack normal levels of mRNA for the second protein component, AtNAR2.1, are unable to take up nitrate in the high affinity range at low external concentrations.[5,6]

When plants are grown on low concentrations of nitrate (0.2 mM), even though the actual amount of nitrate present may be large by volume, the *atnar2.1-1* mutant plants cannot access this pool and show all the physiological symptoms of nitrogen (N) deficiency. Wild type plants under these conditions grow normally as they would when supplied with much higher nitrate concentrations. The growth phenotype of *atnar2.1-1* plants was so strong when they were grown on low nitrate concentrations (0.2 mM nitrate), that the mutant plants (*atnar2.1-1*) provide a powerful tool to unravel high affinity nitrate uptake function by other transport proteins. These knock-out mutants also display a root architecture phenotype in responses to low nitrate supply. By crossing the *atnar2.1-1* mutant with other plants we may also have a complementation test for the ability of other proteins to substitute for the responses of roots to low nitrate supply.

Lateral root assays to identify if the tobacco NpNRT2.1 gene can compensate for AtNRT2.1 and is AtNAR2.1 essential? Changes in lateral root (LR) growth are a sensitive and easily measurable response of plants to N limitation.[8] Recently these assays were used to demonstrate a central role for the nitrate transporter AtNRT2.1 in the morphological and physiological responses of roots to changes in N supply.[9,10]

Either disruption of the production of mRNA[9] or a point mutation that decreases the transport activity of the AtNRT2.1[10] was shown to interfere with the response of LR growth to changes in N supply. For both these types of mutants, measurements of ^{15}N-nitrate influx showed that the plants had impaired high affinity uptake, but in addition AtNRT2.1 has some role in LR initiation that was independent of this transport function. The authors suggested a direct stimulatory role for AtNRT2.1 in LR initiation under N-limited conditions[9] and a repressive role under high sucrose/ low nitrate conditions.[10] When gene knock-out plants that lack either the NRT2.1 (*atnrt2.1-1*) or NAR2.1 (*atnar2.1-1*) genes were compared in LR growth assays under low nitrate supply we found that there were some small but significant differences between the two types of mutant.[5] For both mutants the response of the root system can be attributed to lower nitrate uptake rates creating a stronger N deprivation status. After 4 d under low nitrate conditions, the *atnar2.1-1* mutant cannot sustain an increased growth rate[5] while no limitation has been reported for the *atnrt2.1-1* mutant.[9] This result is probably due to a shortage of N occurring earlier in *atnar2.1-1* than *atnrt2.1-1* due to a greater limitation of the HATS. In addition, the *atnar2.1-1* mutant displays the same inhibition of LR initiation on the newly developed primary root as the *atnrt2.1-1* mutant. This phenotype could result from the decreased expression of *AtNRT2.1* in the *atnar2.1-1* mutant. The root specific constitutive expression of *NpNRT2.1* can restore the HATS in the *atnrt2.1-1* mutant,[11] but not in the *atnar2.1-1* mutant[5] leading to the conclusion that AtNAR2.1 is essential for a functional HATS. With the *RolDNpNRT2.1X atnar2.1-1* plants we generated a tool decoupling the HATS activity and the NRT2.1 gene expression in the roots.[5] Therefore we tested the role of the NRT2.1 protein itself in the LR initiation when the plants are transferred to low nitrate supply.

The important result in these assays was the number of new LRs produced on the newly developed primary root.[5] Data for *RolDNpNRT2.1X atnar2.1-1* plants in comparison to the *RolDNpNRT2.1* over-expresser, *atnar2.1-1* mutant and wild type are shown (see Fig. 1). On 10 mM nitrate, the LR numbers were not significantly different between the genotypes (Fig. 1), but on 0.5 mM, both *RolDNpNRT2.1X atnar2.1-1* and *atnar2.1-1* plants had significantly lower numbers of new LRs when compared with wild type and *RolDNpNRT2.1* plants. Plants constitutively expressing *NpNRT2.1* could not compensate for the lack of *AtNAR2.1* for the LR initiation response during N limitation. Therefore, either the tobacco gene cannot functionally replace the *Arabidopsis* gene *AtNRT2.1* for the LR response while it can for the HATS activity,[11] or both genes *NRT2.1* and *AtNAR2.1* are required for the LR response as well as for a functional HATS. The second idea is consistent with the hypothesis that the AtNAR2.1 proteins may be involved in targeting the NRT2.1s proteins to the plasma membrane.[5] In this case even if the NpNRT2.1 protein is constitutively expressed in the root, the proteins cannot be properly targeted to the plasma membrane in the *atnar2.1-1* background and the LR response cannot be restored.

The role of AtNAR2.1 in the repression of LR initiation in response to nutritional cues. Both proteins AtNRT2.1 and AtNAR2.1 are essential for a functional HATS and also for stimulation of LR development in response to low nitrate. In the next experiment we tested if like *AtNRT2.1*, *AtNAR2.1* was also involved in the repression of LR initiation by nutritional cues.[10] Plants were grown on high ratios of sucrose to nitrogen (7.5% sucrose), 1 mM NH_4Cl and decreasing concentrations of KNO_3 from 5 mM to 0 mM (Fig. 2).

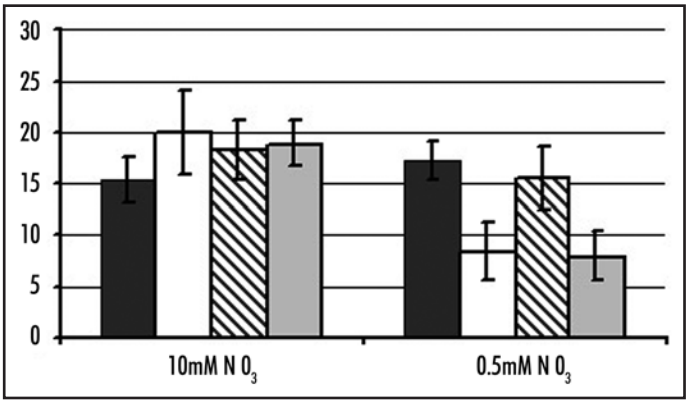

Figure 1. Number of new lateral roots on new primary roots. WT (WS, black bars), mutant (*atnar2.1-1*,white bars), overexpresser (*WSRolDNpNRT2.1*, cross-hatched bars) and cross (*RolDNpNRT2.1X atnar2.1-1*, grey bars) plants were grown on vertical agar plates on 10 mM NO_3^- for 7 d and then transferred to either 0.5 or 10 mM NO_3^- for a further 6 d (13 d of total growth). *Materials and Methods* described previously.[5] Mean values ± SD of 12 seedling are shown. On 0.5 mM nitrate both *atnar2.1-1* and *RolDNpNRT2.1X atnar2.1-1* show significantly less new lateral roots when compared with wild type and *WSRolDNpNRT2.1*. Statistical comparisons of means between treatments or genotypes were performed by the pooled Student's t test using the Sigmaplot software (Systat Software UK, London, UK).

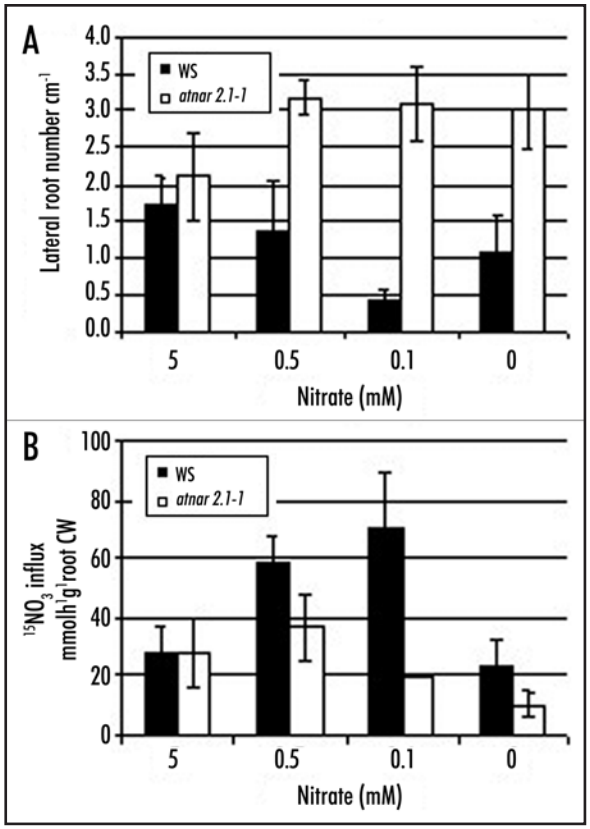

Figure 2. High sucrose/low nitrate represses lateral root initiation in WT but not *atnar2.1*. (A) WT (WS, black) and mutant (*atnar2.1-1*, white) seedlings were grown for 13 days on media containing 7.5% sucrose, 0.1 mM NH_4Cl, and various nitrate concentration (0, 0.1, 0.5, 5 mM KNO_3).[10] KCl was used to balance total salt concentrations between media. The values are means of the number of visible lateral roots per cm of primary root length ± SD of 12 seedlings. (B) Root $^{15}NO_3^-$ influx measured at 0.2 mM. The values are means ± SD of three replicates (pooling four to eight plants).

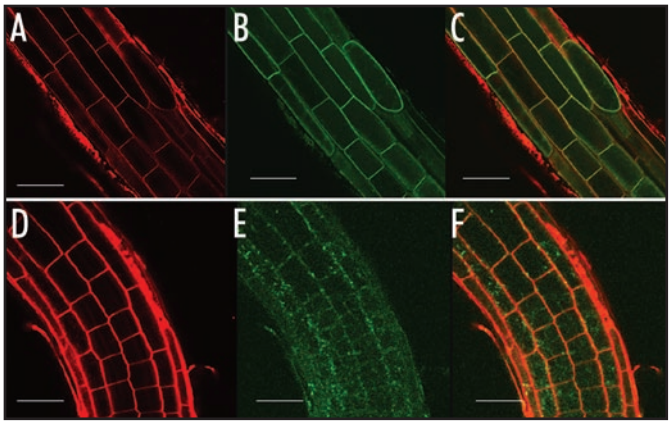

Figure 3. GFP-NRT2.1 localization in plants expressing the P_{35S}-*GFP-NRT2.1* transgene in wild-type background (p43NRT2.1 plants) (A–C) and in the *nar2.1-1* mutant background (*nar2.1-1*/p43NRT2.1 plants) (D–F). Plants were grown in horizontal Petri dishes for 7 days with 1% sucrose. Root cells were stained for 10 min with the endocytic tracer FM4-64, *Materials and Methods* described previously.[12] GFP emission was measured on p43NRT2.1 plants (Ws wild-type transformed with the construct P_{35S}-*GFP-NRT2.1)* (B), and in *nar2.1-1*/p43NRT2.1 plants resulting from a cross between the *nar2.1-1* mutant and p43NRT2.1 (E). (A) and (D) represent background images of the corresponding plants and (C) and (F) overlays of the GFP and marker images. Bar = 40 µm.

In the high affinity range of nitrate concentrations, LR initiation is gradually repressed in the WT as nitrate concentration is decreasing (5 mM to 0.5 mM and 0.1 mM), while in the *atnar2.1-1* mutant, LR initiation is induced and maintained at the same level (Fig. 2A). Nitrate uptake activity is induced in the WT as the concentration is decreasing, but repressed in the *atnar2.1-1* mutant (Fig. 2B). As was observed for the *atnrt2-1* mutants,[10] the LR phenotype is independent of the amount of nitrate absorbed because *atnar2.1-1* displays the phenotype even on 0 mM nitrate supply (Fig. 2). Therefore *AtNAR2.1* is also essential for the repression of LR initiation in response to nutritional cues.

AtNRT2.1 localisation in the atnar2.1-1 background: a role for AtNAR2.1 The AtNRT2.1 localisation at the plasma membrane has recently been elucidated using a constitutive expression of the AtNRT2.1-green fluorescent protein (GFP) fusion in the WT background.[12,13] In order to investigate the role of AtNAR2.1 in the targeting of AtNRT2.1 to the plasma membrane the 35SAtNRT2.1-GFP transformed plants were crossed with the *atnar2.1-1* mutant. The resulting plants are constitutively expressing the AtNRT2.1-GFP protein in the *atnar2.1-1* background. While the GFP fluorescence associated with the AtNRT2.1 protein is nicely localised at the plasma membrane in the WT background (Fig. 3B), the fluorescence is lower and diffused throughout the cell in the *atnar2.1-1* background (Fig. 3E). In addition, as revealed by l-scanning, the signal is due to an autofluorescence mechanism rather that a true GFP fluorescence. AtNRT2.1 is not properly targeted to the plasma membrane in the *atnar2.1-1* mutant. Therefore AtNAR2.1 is likely to be involved in the stability of AtNRT2.1 possibly through the targeting process.

Both AtNRT2.1 and AtNAR2.1 proteins are involved in the regulation of the LR architecture independently of their uptake function. AtNRT2.1 has been suggested to have a role as a nitrate sensor.[10] It is likely that AtNAR2.1 also participates in this signaling pathway in a way that is consistent with a role in the targeting of AtNRT2.1 to the plasma membrane.

FINAL NOTE

Gene nomenclature–Three names for the same gene. Naming genes is complicated and one of the two components of the HATS has had three different names. The gene was actually first named *WR3* as it was identified as a wound-inducible gene in Arabidopsis.[14] When the gene family was found to be important for higher plant nitrate transport[4] it was named *AtNAR2.1* after the related gene in *Chlamydomonas*, *CrNAR2*. Finally, in a recent paper *AtNAR2.1* was renamed *AtNRT3.1*.[7]

References

1. Williams LE, Miller AJ. Transporters responsible for the uptake and partitioning of nitrogenous solutes. Ann Rev Plant Physiol Plant Mol Biol 2001; 52:659-88.
2. Orsel M, Krapp A, Daniel-Vedele F. Analysis of the NRT2 nitrate transporter family in Arabidopsis: Structure and gene expression. Plant Physiol 2002; 129:886-96.
3. Zhou JJ, Fernandez E, Galvan A, Miller AJ. A high affinity nitrate transport system from *Chlamydomonas* requires two gene products. FEBS Letts 2000; 466:225-7.
4. Tong Y, Zhou JJ, Li Z, Miller AJ. A two-component high-affinity nitrate uptake system in barley. Plant J 2005; 41:442-50.
5. Orsel M, Chopin F, Leleu O, Smith SJ, Krapp A, Daniel-Vedele F, Miller AJ. Characterisation of a two component high affinity nitrate uptake system in Arabidopsis; physiology and protein-protein interaction. Plant Physiol 2006; 142:1304-17.
6. Okamoto M, Kumar A, Li W, Wang Y, Siddiqi MY, Crawford NM, Glass ADM. High-affinity nitrate transport in roots of *Arabidopsis* depends on expression of the *NAR2*-Like gene *AtNRT3.1*. Plant Physiol 2006; 140:1036-46.
7. Quesada A, Galvan A, Fernandez E. Identification of nitrate transporter genes in *Chlamydomonas reinhardtii*. Plant J 1994; 5:407-19.
8. Zhang H, Forde BG. Regulation of Arabidopsis root development by nitrate availability. J Exp Bot 2000; 51:51-9.
9. Remans T, Nacry P, Pervent M, Girin T, Tillard P, Lepetit M, Gojon A. A central role for the nitrate transporter NRT2.1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. Plant Physiol 2006; 140:909-21.
10. Little DY, Rao H, Oliva S, Daniel-Vedele F, Krapp A, Malamy JE. The putative high-affinity nitrate transporter NRT2.1 represses lateral root initiation in response to nutritional cues. Proc Natl Acad Sci USA 2005; 102:13693-8.
11. Filleur S, Dorbe MF, Cerezo M, Orsel M, Granier F, Gojon A, Daniel-Vedele F. An *Arabidopsis* T-DNA mutant affected in *Nrt2* genes is impaired in nitrate uptake. FEBS Letts 2001; 489:220-4.
12. Chopin F, Wirth J, Dorbe MF, Lejay L, Krapp A, Gojon A, Daniel-Vedele F. The Arabidopsis nitrate transporter AtNRT2.1 is targeted to the root plasma membrane (submitted to Functional Plant Biology).
13. Wirth J, Chopin F, Santoni V, Viennois G, Tillard P, Krapp A, Lejay L, Daniel-Vedele F, Gojon A. Control of nitrate uptake activity at the NRT2.1 protein level in *Arabidopsis thaliana*: Unexpected structural complexity and lack of fast responses to environmental changes (submitted to Plant Cell).
14. Titarenko E, Rojo E, Leon J, Sanchez-Serrano J. Jasmonic acid-dependent and -independent signaling pathways control wound-induced gene activation in *Arabidopsis thaliana*. Plant Physiology 1997; 115:817-26.