Plant Signaling & Behavior 6:9 1286-1289; September 2011; ©2011 Landes Bioscience

Multiple roles of nitrate transport accessory protein NAR2 in plants

Huimin Feng,[1,†] Xiaorong Fan,[1,†] Ming Yan,[1] Xiaoqin Liu,[1] Anthony J. Miller[2] and Guohua Xu[1,*]

[1]State Key Laboratory of Crop Genetics and Germplasm Enhancement; College of Resources and Environmental Sciences; Nanjing Agricultural University; Nanjing, China;
[2]Disease and Stress Biology Department; John Innes Center; Norwich Research Park; Norwich, UK

[†]These authors contributed equally to this work.

Key words: NAR2, NRT2, nitrate transporter, root

Two component high affinity nitrate transport system, NAR2/NRT2, has been defined in several plant species. In Arabidopsis, AtNAR2.1 has a role in the targeting of AtNRT2.1 to the plasma membrane. The gene knock out mutant *atnar2.1* lacks inducible high-affinity transport system (IHATS) activity, it also shows the same inhibition of lateral root (LR) initiation on the newly developed primary roots as the *atnrt2.1* mutant in response to low nitrate supply. In rice, OsNAR2.1 interacts with OsNRT2.1, OsNRT2.2 and OsNRT2.3a to provide nitrate uptake over high and low concentration ranges. In rice roots OsNAR2.1 and its partner NRT2s show some expression differences in both tissue specificity and abundance. It can be predicted that NAR2 plays multiple roles in addition to being an IHATS component in plants.

Function of NAR2 as a Nitrate Accessory Protein

The plant NAR2-type genes were first named *WR3* identified by expression that was induced by wounding[1] and pathogens.[2] The involvement of NAR2 in the high-affinity transport system (HATS) for nitrate was initially identified genetically using mutants in *Chlamydomonas reinhardtii*, in which *NAR2* is next to *NRT2.1* in the nitrate-related gene cluster.[3,4] Functional analysis of the CrNAR2/CrNRT2.1 components for nitrate HATS was demonstrated in Xenopus oocytes.[5,6] Numerous genes belonging to the *NAR2* family have been identified during recent years in several plants species. At present, three very similar barley *NAR2* genes (*HvNAR2.1–HvNAR2.3*),[7] two Arabidopsis *NAR2* genes (*AtNAR2.1* and *AtNAR2.2*),[8] and two rice *NAR2s* genes (*OsNAR2.1* and *OsNAR2.2*),[9] have been characterized. However, not all NAR2 family members demonstrated a function in two-component nitrate HATS. For example, among three barley *NAR2* genes, only HvNAR2.3 formed a functional unit with HvNRT2.1.[7] In rice OsNAR2.1, but not OsNAR2.2, functions in rice two-component nitrate HATS.[9]

Clarifying the function of NAR2 family members in nitrate transport requires the identification of partner pairs and the specificity of their interaction. In Arabidopsis, besides the well-characterized interaction with AtNRT2.1, AtNAR2.1 showed weak interaction with AtNRT2.3 in yeast, but the co-expression of AtNAR2.1 and AtNRT2.3 did not show nitrate uptake activity in oocytes.[8] In rice, OsNAR2.1 can cooperate with both OsNRT2.1/2.2 and OsNRT2.3a in yeast,[9] and the interaction provided NO_3^- uptake both in oocytes and rice over low to high concentration ranges.[10] In tobacco *NpNRT2.1*, which is an ortholog of *AtNRT2.1*, can complement the *atnrt2.1-1* mutant, but not the *atnar2.1* mutants.[11] The finding suggests that NpNRT2.1 can function in nitrate transport, but it is not known if NpNRT2.1 needs an accessory protein for its function.

The NAR2s are a small proteins, possibly containing a single TM domain in CrNAR2 and two TM domains in HvNAR2.3.[5,7] The NRT2s are typical carrier-type proteins, containing 12 putative TM domains.[8] The NAR2 protein plays a role in establishing HATS activity by targeting NRT2 to the plasma membrane.[12,13] In the *rnc1* mutant of Arabidopsis, it was suggested that an aspartate residue in the central loop of NAR2 was important for HATS activity.[14] Immunological results demonstrated an oligomer, proposed to contain a tetramer consisting of two subunits each of AtNRT2.1 and AtNAR2.1, was active in HATS.[13] In barley, a direct protein-protein interaction was shown to occur between the HvNRT2.1 C-terminal, S463A, and the HvNAR2.3 central region.[12] However, it is difficult to determine if this serine is a key residue because it is not conserved in AtNRT2.1 and HvNRT2.1 C-terminal.[12] In rice roots we detected some differences in the tissue specific expression of OsNAR2.1 and its interacting NRT2 members.[10] *OsNAR2.1* expression was strongest in the epidermal cells and much lower in the cortical and stelar cells (**Fig. 1**), while *OsNRT2.1*, *OsNRT2.2* and *OsNRT2.3* were expressed abundantly in the stelar cells.[10]

It was shown that the expression of *AtNAR2.1*, *AtNRT2.1* and *AtNRT2.2* in Arabidopsis was perfectly coordinated with nitrate HATS regulation: induction by low external nitrate concentration and sudden N deprivation, and suppression by high nitrate

*Correspondence to: Guohua Xu; Email: ghxu@njau.edu.cn
Submitted: 04/27/11; Revised: 06/09/11; Accepted: 06/10/11
DOI: 10.4161/psb.6.9.16377

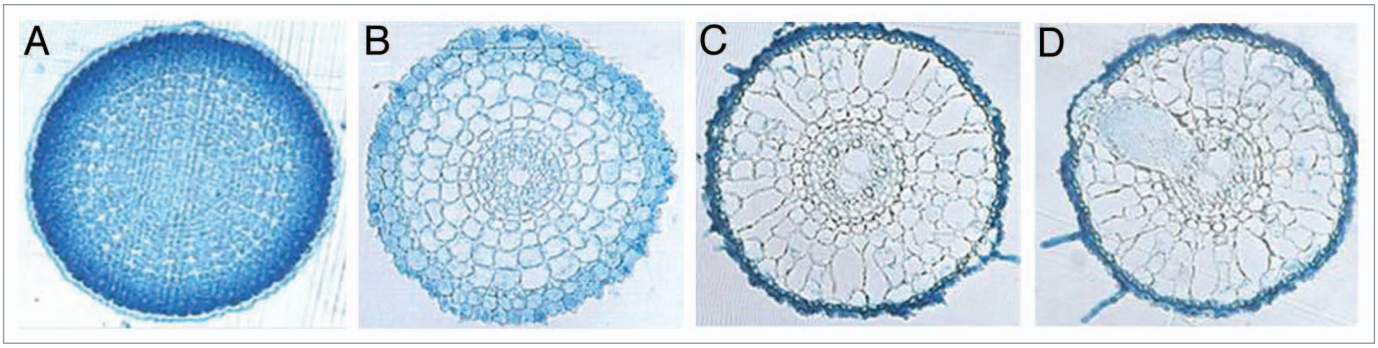

Figure 1. Root cross sections of plants transformed with *OsNAR2.1* promoter-*GUS* genes. The expression specificity of *OsNAR2.1* in the root of ten-day-old seedlings (*Oryza sativa* L. ssp. *Japonica cv.* Nipponbare) cultured with de-ionized water containing 25 mgL^{-1} hygromycin. Transverse sections are taken from approximately 0.5 mm (A), 1.5 mm (B), 1 cm (C) and 1.5 cm (D) from the root tip.

supply.[8,15,16] In rice roots the expression of *OsNAR2.1, OsNRT2.1*, *OsNRT2.2* and *OsNRT2.3a* genes was induced by both low and high nitrate concentrations.[9] In the Arabidopsis *atnar2.1* knockout mutant, the expression of *AtNRT2.1* decreased when compared with WT grown at 0.2 mM nitrate, while it increased at 6 mM nitrate.[8] The expression level of *AtNRT1.1* did not change significantly between the *atnar2.1* and wild type plants under a high nitrate concentration.[8] In rice, RNAi-knockdown of *OsNAR2.1* suppressed the expression of *OsNRT2.1*, *OsNRT2.2* and *OsNRT2.3a* very largely, but not the expression of *OsNRT1.1*, *OsNAR2.2*, *OsNRT2.3b* and *OsNRT2.4*, at both 0.2 mM and 5 mM nitrate supply levels.[9] It is interesting to know if there are the large different responses of NAR2/NRT2 members to the change of N supply among the genotypes of each rice or Arabidopsis species. If not, the expressional discrepancies of NAR2/NRT2 members between Arabidopsis and rice could link to the evolutionally adaptation of the plants to varied N form and concentrations in upland and flooded paddy soils.

Role of NAR2.1 in Root Development

Local stimulation of lateral root growth was shown to be due to a specific NO_3^- signaling pathway, involving the putative MADS-box transcription factor ANR1.[17] NRT1.1 acts upstream of ANR1 in the NO_3^- signaling pathway governing lateral root growth.[18] It has also been shown that *AtNRT2.1* plays a crucial role in root branching independently of its NO_3^- uptake activity.[19,20] The *atnar2.1* and *atnrt2.1-1* mutants display the same inhibition of lateral root (LR) initiation on the newly developed primary roots.[8,11] The tobacco gene *NpNRT2.1* cannot functionally replace the Arabidopsis gene *AtNRT2.1* for the LR response while it can restore the HATS activity under nitrogen (N) limitation.[8,11] Compared with the *RolDNpNRT2.1* overexpresser (the plants overexpressing *NpNRT2.1* gene) and wild type, *RolDNpNRT2.1X atnar2.1* plants (the *atnar2.1* mutant complemented with the *NpNRT2.1* gene) and *atnar2.1* mutant had significantly lower numbers of new LRs under a low nitrate concentration supply.[11] The Arabidopsis *atnar2.1* mutants showed decreased growth and lower numbers of new lateral roots (LR) only under a low nitrate supply condition.[8,11]

Does *NAR2* Gene Regulate the Expression of Partner *NRT2* s?

Members of the NRT2 family showed a variety of tissue expression patterns and regulation[10] (**Table 1**). The expression patterns of some *NAR2* and *NRT2* family members of different species show common responses to different N forms, they are induced by nitrate and repressed by feedback regulation from N metabolites[10] (**Table 1**). Furthermore, HATS activity and transcript levels of *NAR2.1* and *NRT2.1* in Arabidopsis and barley plants are repressed significantly by high nitrate concentrations.[12,15] Interestingly, in rice the expression patterns of *NAR2.1* and *NRT2s* in roots treated with high nitrate are similar to those treated with low concentrations.[9] At present, no trans-acting factors (TFs) that are absolutely necessary to regulate nitrate-responsive transcription have been identified in higher plants. We found a nitrate regulated element (NRE) in the *OsNAR2.1* promoter[10] had a highly similar NRE to one located in the Arabidopsis *NIR* promoter, with only one base changed from A to C.[10,21] However there was no such conserved sequence in the -1,000 bp promoter regions of *OsNRT2* genes in rice, and *AtNAR2.1/AtNAR2* genes in Arabidopsis.[10] One possibility is that *OsNAR2.1* can more directly sense nitrate than the *OsNRT2s* in rice plants. However, because the identity of the TFs or NRE(s) is still uncertain, another possibility is that *OsNAR2.1* and *OsNRT2s* have their own independent and perhaps rice specific NREs.

The overexpression of *NpNRT2.1* in tobacco plants failed to yield any increase in nitrate uptake.[22] It may be that the overexpression of *NRT2.1*, without *NAR2.1*, is unlikely to increase nitrate uptake. *AtNAR2.1* expression is similar in wild-type and *atnrt2.1-1* plants growing under differing supplies of nitrate, while *AtNRT2.1* expression is repressed in *atnar2.1* mutants under low nitrate concentration.[8] When both *atnar2.1* and *atnrt2.1* mutants were supplied with low nitrate concentrations (0.2 or 0.5 mM), the *atnar2.1-1* mutant has a more severely stunted growth phenotype than the *atnrt2.1-1* mutant.[8] After 4 d supplied with low nitrate, the *atnar2.1-1* mutant cannot sustain an increased growth rate while no limitation was reported for the *atnrt2.1* mutant.[8] An explanation is probably that *atnar2.1-1* mutants suffer more N deficiency than *atnrt2.1-1*, indicating

Table 1. Literature summary of the tissue expression and regulation of some NRT2s in higher plants

Gene (Accession number)	Expression		Regulation
	Characterization	Technique	
AtNRT2.1/2.2 (AF019748/AF01979)	Root cortical and epidermal cells	RT-PCR; GUS/GFP fusion	NO_3^-, sucrose and light induction; NH_4^+ and amino acids repression
AtNRT2.3 (AB015472)	Mainly in roots	RT-PCR	NO_3^- induction in shoots
AtNRT2.4 (AB015472)			Modest NO_3^- induction
AtNRT2.6 (AL353992)			-
AtNRT2.5 (AC012187)			NO_3^- repression; Glucose induction
AtNRT2.7 (AL163792)	Shoots; Embryo; seeds	RT-PCR; GUS/GFP fusion	NO_3^- insensity
ZmNRT2.1 (AY129953)	Root epidermis and cortex	RT-PCR; In situ hybridization	NO_3^- induction; Glucose down- and sucrose upregulation
ZmNRT2.2 (AY659965)	Root endodermis, centralcylinder and lateral primordia		
NpNRT2 (Y08210)	Strong in epidermis and endodermis close to the root tip, week in the epidermis and lateral root primordia in the mature part of the root	RNA gel blot; In situ hybridization	NO_3^- induction
HvNRT2.1	Root	RNA gel blot	NO_3^- and NO_2^- induction
OsNAR2.1 (AP008208)	Strongest in root epidermis and lower in cortex and stele	RT-PCR; GUS fusion	NO_3^-, light and sucrose upregulation; NH_4^+ repression
OsNRT2.1 (AP008519)	Whole roots; leaves; week in hulls and anthers		
OsNRT2.2 (AP008519)	Whole roots; leaves; strong in hulls and anthers		
OsNRT2.3a (AP003245)	Root stele; leaves; seed scutellum		
OsNRT2.3b (AP003245)			light and sucrose upregulation
OsNRT2.4 (AP004614)	Adventitious root primordial; leaves; ends of the hull and in vascular tissue of the anther		NO_3^-, light and sucrose upregulation; NH_4^+ repression

that AtNAR2.1 had a greater role in N uptake than AtNRT2.1.[8] Some studies have suggested that AtNRT2.1 might act as a nitrate transceptor[23] like AtNRT1.1.[24] Because of the lack of confirmed nitrate sensing motifs, it is difficult to determine which gene in the NAR2/NRT2 partnership acts as the nitrate sensor/signal transducer.

Acknowledgments

Research in China was funded by the National Basic Research Program 973 (2011CB100300), the National Natural Science Foundation, Jiangsu Natural Science Foundation (BK2010227) and transgenic project (2008ZX08001-0052, 009ZX08009-126B).

References

1. 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 Physiol 1997; 115:817-26; PMID:9342878; DOI:10.1104/pp.115.2.817.
2. Marois E, Van den Ackerveken G, Bonas U. The Xanthomonas type III effector protein AvrBs3 modulates plant gene expression and induces cell hypertrophy in the susceptible host. Mol Plant Microbe Interact 2002; 15:637-46; PMID:12118879; DOI:10.1094/MPMI.2002.15.7.637.
3. Quesada A, Galvan A, Fernandez E. Identification of nitrate transporter genes in *Chlamydomonas reinhardtii*. Plant J 1994; 5:407-19; PMID:8180624; DOI:10.1111/j.1365-313X.1994.00407.x.
4. Galván A, Quesada A, Fernandez E. Nitrate and nitrate are transported by different specific transport systems and by a bispecific transporter in *Chlamydomonas reinhardtii*. J Biol Chem 1996; 271:2088-92; PMID:8567664.
5. Zhou JJ, Fernandez E, Galvan A, Miller AJ. A high affinity nitrate transport system from Chlamydomonas requires two gene products. FEBS Lett 2000; 466:225-7; PMID:10682832; DOI:10.1016/S0014-5793(00)01085-1.
6. Zhou JJ, Trueman LJ, Boorer KJ, Theodoulou FL, Forde BG, Miller AJ. A high affinity fungal nitrate carrier with two transport mechanisms. J Biol Chem 2000; 275:39894-9; PMID:10984478; DOI:10.1074/jbc.M004610200.
7. Tong Y, Zhou JJ, Li Z, Miller AJ. A two-component high-affinity nitrate uptake system in barley. Plant J 2005; 41:442-50; PMID:15659102; DOI:10.1111/j.1365-313X.2004.02310.x.
8. Orsel M, Chopin F, Leleu O, Smith SJ, Krapp A, Daniel-Vedele F, et al. Characterisation of a two component high affinity nitrate uptake system in Arabidopsis: physiology and protein-protein interaction. Plant Physiol 2006; 142:1304-17; PMID:17012411; DOI:10.1104/pp.106.085209.
9. Yan M, Fan XR, Feng HM, Miller AJ, Shen QR, Xu GH. Rice OsNAR2.1 interacts with OsNRT2.1, OsNRT2.2 and OsNRT2.3a nitrate transporters to provide uptake over high and low concentration range. Plant Cell Environ 2011; 34:1360-72; PMID:21486304; DOI:10.1111/j.1365-3040.2011.02335.x.
10. Feng H, Yan M, Fan XR, Li BZ, Shen QR, Miller AJ, et al. Spatial expression and regulation of rice high-affinity nitrate transporters by nitrogen and carbon status. J Exp Bot 2011; 62:2319-32; PMID:21220781; DOI:10.1093/jxb/erq403.
11. Orsel M, Chopin F, Leleu O, Krapp SS, Daniel-Vedele F, Miller AJ. Nitrate Signaling and the Two Component High Affinity Uptake System in Arabidopsis. Plant Signal Behav 2007; 2:260-2; PMID:19704673; DOI:10.4161/psb.2.4.3870.
12. Ishikawa S, Ito Y, Sato Y, Fukaya Y, Takahashi M, Morikawa H, et al. Two-component high-affinity nitrate transport system in barley: membrane localization, protein expression in roots and a direct protein-protein interaction. Plant Biotechnol 2009; 26:197-205 DOI:10.5511/plantbiotechnology.26.197.
13. Yong Z, Kotur Z, Glass ADM. Characterization of an intact two-component high-affinity nitrate transporter from Arabidopsis roots. Plant J 2010; 63:739-48; PMID:20561257; DOI:10.1111/j.1365-313X.2010.04278.x.
14. Kawachi T, Sunaga Y, Ebato M, Hatanaka T, Harada H. Repression of nitrate uptake by replacement of Asp105 by asparagine in AtNRT3.1 in *Arabidopsis thaliana* L. Plant Cell Physiol 2006; 47:1437-41; PMID:16980702; DOI:10.1093/pcp/pcl010.
15. Okamoto M, Kumar A, Li W, Wang Y, Siddiqi MY, Crawford NM, et al. High-affinity nitrate transport in roots of Arabidopsis depends on expression of the *NAR2*-Like gene *AtNRT3.1*. Plant Physiol 2006; 140:1036-46; PMID:16415212; DOI:10.1104/pp.105.074385.

16. Fraisier V, Gojon A, Tillard P, Daniel-Vedele F. Constitutive expression of a putative high-affinity nitrate transporter in *Nicotiana plumbaginifolia*: evidence for post-transcriptional regulation by a reduced nitrogen source. Plant J 2000; 23:489-96; PMID:10972875; DOI:10.1046/j.1365-313x.2000.00813.x.
17. Zhang H, Forde BG. An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. Science 1998; 279:407-9; PMID:9430595; DOI:10.1126/science.279.5349.407.
18. Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E, et al. The Arabidopsis *NRT1.1* transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. Proc Natl Acad Sci USA 2006; 103:19206-11; PMID:17148611; DOI:10.1073/pnas.0605275103.
19. 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; PMID:16157886; DOI:10.1073/pnas.0504219102.
20. Remans T, Nacry P, Pervent M, Girin T, Tillard P, Lepetit M, et al. 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; PMID:16415211; DOI:10.1104/pp.105.075721.
21. Konishi M, Yanagisawa S. Identification of a nitrate-responsive cis-element in the Arabidopsis *NIR1* promoter defines the presence of multiple cis-regulatory elements for nitrogen response. Plant J 2010; 63:269-82; PMID:20444232; DOI:10.1111/j.1365-313X.2010.04239.x.
22. Filleur S, Dorbe MF, Cerezo M, Orsel M, Granier F, Gojon A, et al. An Arabidopsis T-DNA mutant affected in *Nrt2* genes is impaired in nitrate uptake. FEBS Lett 2001; 489:220-4; PMID:11165253; DOI:10.1016/S0014-5793(01)02096-8.
23. Gojon A, Krouk G, Perrine-Walker F, Laugier E. Nitrate transceptor(s) in plants. J Exp Bot 2011; 62:2299-308; PMID:21239382; DOI:10.1093/jxb/erq419.
24. Ho CH, Lin SH, Hu HC, Tsay YF. CHL1 functions as a nitrate sensor in plants. Cell 2009; 138:1184-94; PMID:19766570; DOI:10.1016/j.cell.2009.07.004.