Whether do plant cells sense nitrate changes without a sensor?

Yu-Fan Fu†, Lin-Bei Xie†, Xin-Yue Yang, Zhong-Wei Zhang and Shu Yuan*

College of Resources, Sichuan Agricultural University, Chengdu, China

KEYWORDS

nitrate transporter NRT1.1, transcription factor NLP7, nitrate sensor, nitrate signaling, adenosine monophosphate-activated protein kinase

NRT1.1-dependent and NRT1.1-independent nitrate signaling pathways

Nitrogen is a key nutrient macroelement. Nitrate is the most abundant inorganic form of N in soils for plant absorption, and works as a signaling molecule that regulates multiple growth and developmental processes (Fredes et al., 2019; Vidal et al., 2020; Li et al., 2021), such as root elongation (Zhang et al., 2019), leaf expansion (Yang et al., 2022) and flowering (Yuan et al., 2016; Zhang et al., 2021). However, nitrate signaling in plant cells remains largely unknown. Both a proton-coupled transporter NRT1.1 (CHL1; Ho et al., 2009) and the NIN-like protein (NLP) transcription factor NLP7 (Liu et al., 2022) have been suggested as nitrate sensors.

The nitrate transporter NRT1.1 is a dual-affinity nitrate transport controller the primary nitrate response (nitrate signaling), in which expressions of nitrate assimilation genes and nitrate transporter genes are induced rapidly by nitrate treatments (Fredes et al., 2019; Vidal et al., 2020; Li et al., 2021), such as root elongation (Zhang et al., 2019), leaf expansion (Yang et al., 2022) and flowering (Yuan et al., 2016; Zhang et al., 2021). However, nitrate signaling in plant cells remains largely unknown. Both a proton-coupled transporter NRT1.1 (CHL1; Ho et al., 2009) and the NIN-like protein (NLP) transcription factor NLP7 (Liu et al., 2022) have been suggested as nitrate sensors.

The nitrate transporter NRT1.1 is a dual-affinity nitrate transceptor controlling the primary nitrate response (nitrate signaling), in which expressions of nitrate assimilation genes and nitrate transporter genes are induced rapidly by nitrate treatments (Ho et al., 2009). NRT1.1 facilitates not only nitrate uptake but also auxin transport. Nitrate treatments repress NRT1.1-mediated auxin uptake, indicating that the nitrate signaling via NRT1.1 is correlated with a regulation of auxin transport (Krouk et al., 2010). The T101 residue of NRT1.1 could be phosphorylated by calcineurin B-like interacting protein kinase 23 (CIPK23; Ho et al., 2009). The phosphorylation state of NRT1.1 plays an important role in regulating lateral root development by modulating nitrate-mediated basipetal auxin export and nitrate-dependent signal transduction (Zhang et al., 2019). Thus, NRT1.1 is a master switch that integrates nitrate signaling/transport and auxin signaling/transport. However, mutation of NRT1.1 promotes both lateral root growth and auxin accumulation in these roots at low nitrate levels, but not at high levels (Krouk et al., 2010) And the null chl1-5 mutant resembled wild-type plants when grown on medium with nitrate as the sole nitrogen source (Liu et al., 2022). The activation of
primary nitrate response genes were only partially repressed in chl1-5 mutant (Liu et al., 2022). Thus, there should be NRT1.1-independent nitrate signaling pathways.

**NLP7 is insensitive to nitrate changes, when the cytosol nitrate concentrations are higher than 1 mM**

The NIN-like protein (NLP) transcription factor NLP7 functions as a master switch, which controls the expression of a large number of genes in response to nitrate changes (Liu et al., 2017). Recently, Liu et al. (2022) indicated that nitrate directly binds to NLP7, and NLP7 is derepressed upon nitrate perception via its N terminus. Transcriptome reprogramming in primary nitrate responses triggered by nitrate was abolished in nlp7 mutant (Liu et al., 2022). However, none of the previously-reported receptors have been identified as transcription factors so far. For all known receptors (sensors), downstream of binding with signaling molecules, there are usually multiple elements involved in the signaling pathway, such as mitogen-activated protein kinase (MAPK) cascade (Liu, 2012). If nitrate acts on NLP7 directly, there will be no crosstalk with other signals and no possibility of positive or negative feedback regulations, which does not conform to the evolutionary law.

The dissociation constant $K_d$ value of NLP7 binding to nitrate was about 50 μM (Liu et al., 2022). However the $K_d$ values of phytohormone receptors range from 4 nM to 50 nM, which are about 1000 times lower than that of NLP7 (Table 1). Even for the nonspecific amino-acid receptors, glutamate receptor-like (GLR) channels, their $K_d$ value can be as low as 0.33–5.5 μM (Alfieri et al., 2020), which are still 9–150 times lowers than that of NLP7 (Table 1). Therefore, NLP7 is not a specific nitrate receptor.

A nitrate transporter in the cyanobacterium *Synechococcus*, NrtA, was shown to bind nitrate and nitrite with a high affinity ($K_d = 0.3 \mu M$; Maeda and Omata, 1997). Comparatively, another nitrate transporter in *Staphylococci*, NreA, was shown to bind nitrate with a low affinity ($K_d = 22 \mu M$; Niemann et al., 2014; Table 1). Thus, NLP7 may have a function as a nonspecific transporter with low micromolar affinity. Similarly, Ethylene Insensitive2 (EIN2) contains the 12-transmembrane domain of the NRAMP family of metal transporters, but has no capacity for metal transport. Instead, EIN2 functions an essential ethylene signaling component in higher plants (Alonso et al., 1999). NLP7 shares some similarity with cyanobacterial nitrate transporters (Liu et al., 2022), however, in higher plants, it has developed a new function, serving as a transcription factor. This suggests that the role of NLP7 as a nitrate transporter might have been diminished largely from its ancient roots.

On the other hand, the contents of nitrate in plant cells are very high, even under low N conditions (North et al., 2009). For instance, when the N level in MS medium was reduced to 1/20, the whole seedling nitrate content only reduced from 10 mM to 7 mM (Fu et al., 2020; Yang et al., 2022). Another study demonstrated that, by the time the barley roots had been out of nitrate for 24 h, the cytosol nitrate contents in root epidermal cells only decreased from 4.6 mM to 4.0 mM, and the cytosol nitrate contents in root cortical cells only decreased from 3.7 mM to 2.9 mM (van der Leij et al., 1998), when the nitrate-starvation signal has been already triggered. Nitrate concentration is 1 to 5 mM in the cytosol and 5 to 75 mM in the vacuole under nitrogen sufficient conditions. Under nitrogen deficient conditions, the nitrate concentration of cytosol was maintained stable by export of nitrate from the vacuole. However, the stored nitrate in vacuoles can only lasted for two

| TABLE 1 | Dissociation constant ($K_d$) values of phytohormone, amino-acid receptors (sensors) and nitrate transporters. |
| --- | --- | --- | --- | --- |
| **Signaling molecule** | **Receptor (Sensor)** | **$K_d$** | **Reference** |
| Auxin (Indole-3-acetic acid; IAA) | Transport Inhibitor Response 1 (TIR1) | 18 nM | Calderon Villalobos et al., 2012 |
| Cytokinin (CTK) | Histidine Kinases (HK) | 4.0 nM to trans-zeatin | Stolz et al., 2011 |
| Gibberellin (GA) | GA Insensitive Dwarf 1 (GID1) | 30 nM to GA$_3$ | Nakajima et al., 2006 |
| Abscisic acid (ABA) | Protein Phosphatases Type 2C (PP2C) | 38 nM | Santiago et al., 2009 |
| Ethylene (ET) | Ethylene Receptor 1 (ETR1) | 1.24 μ/liter | McDaniel and Binder, 2012 |
| Salicylic acid (SA) | Non-expessor of Pathogenesis Related protein 4 (NPR4) | 50 nM | Wang et al., 2020 |
| Jasmonic acid (JA) | Coronatine-Insensitive 1 (COI1) | 48 nM | Sherd et al., 2010 |
| Amino acid (AA) | Glutamate receptor-like (GLR) channels (nonspecific receptors in plants) | 0.33 μM to Cys; 2.2 μM to Glu; 5.5 μM to Gly | Alfieri et al., 2020 |
| *Synechococcus* sp. Strain PCC 7942 | Nitrate transporter A (nrtA) | 0.3 μM | Maeda and Omata, 1997 |
| *Staphylococcus carnosus* | Nitrate regulatory element A (NreA) | 22 μM | Niemann et al., 2014 |
| Arabidopsis thaliana | Nitrate transporter NRT1.1 | 1 mM | Parker and Newstead, 2014 |
Nitrate signaling through AMPK pathways may not require a nitrate sensor

We speculate that either the nitrate binding capacity of NLP7 is not related to its transcriptional activation activity, or that, even if it is related, the change of nitrate at high concentrations (> 1 mM, when NLP7 is saturated bound with nitrate) may be mainly perceived through other proteins. Nitrate represses ferredoxin-NADP⁺-oxidoreductase (FNRI) expression, thereby contributing to declines in NADPH/NAD⁺ and ATP/AMP ratios, which in turn activates AMPK (Krouk et al., 2010) and modulates its nuclear abundance (Yuan et al., 2016). KIN10 phosphorylates NLP7 to induce its cytoplasmic retention and the subsequent degradation (Wang et al., 2021a). Under the normal growth condition, nitrate activates NRT1.1-CNGC15 (cyclic nucleotide-gated channel protein 15) complex to produce NO₃⁻ specific Ca²⁺ signature (Wang et al., 2021b), which results in the NLP7 phosphorylation by Ca²⁺-sensor protein kinases (CPKs) at Ser205, thereby triggering NLP7 nuclear localization, which regulates downstream gene expression and promotes plant growth (Liu et al., 2017). Nevertheless, under nitrate-deficient conditions, KIN10 is induced to phosphorylate Ser125 and Ser306 of NLP7 protein, which increases its cytoplasmic localization and the
subsequent degradation, therefore repressing nitrate-regulated gene expression and inhibiting growth (Wang et al., 2021a). These pathways may not require a nitrate sensor (like NRT1.1), but may be regulated through changes in cellular nitrate levels. So far, the nitrate signaling panorama is still incomplete. The role of KIN11 and its correlation with KIN10 in nitrate signaling are still unclear. The association between CPKs and AMPK (who phosphorylate each other) requires further studies. And the relationship between NRT1.1 (sensor)-dependent nitrate signaling and NRT1.1-independent nitrate signaling also needs further investigations.

Author contributions

SY conceived the project. Y-FF, L-BX, X-YY, and Z-WZ performed the literature search. SY wrote the manuscript with input from Y-FF, L-BX, X-YY, and Z-WZ. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Sichuan Province Youth Science and Technology Innovation Team (20CXTD0062) to SY and the Applied Basic Research Program of Sichuan Province (2020YJ0410) to Z-WZ.

Acknowledgments

We thank Miss. Hannah Elizabeth Levengood (Purdue University, USA) for the linguistic assistance during the preparation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed by any product that may be evaluated in this article, or endorsed by the publisher.
Nakajima, M., Shimada, A., Takashi, Y., Kim, Y. C., Park, S. H., Ueguchi-Tanaka, M., et al. (2006). Identification and characterization of arabidopsis gibberellin receptors. *Plant J.* 46, 880–889. doi:10.1111/j.1365-313X.2006.02748.x

Niemann, V., Koch-Singenstreu, M., Neu, A., Nilkens, S., Götz, F., Unden, G., et al. (2014). The NreA protein functions as a nitrate receptor in the staphylococcal nitrate regulation system. *J. Mol. Biol.* 426, 1539–1553. doi: 10.1016/j.jmb.2013.12.026

North, K. A., Ehlting, B., Kopriva, A., Rennenberg, H., and Kopriva, S. (2009). Natural variation in arabidopsis adaptation to growth at low nitrogen conditions. *Plant Physiol. Biochem.* 47, 912–918. doi:10.1016/j.plaphy.2009.06.009

Parker, J. L., and Newstead, S. (2014). Molecular basis of nitrate uptake by the plant nitrate transporter NRT1.1. *Nature* 507, 68–72. doi:10.1038/nature13116

Santiago, J., Rodrigues, A., Saez, A., Rubio, S., Antoni, R., Dupeux, F., et al. (2010). Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. *Nature* 468, 400–405. doi:10.1038/nature09430

Stolz, A., Riefler, M., Lomin, S. N., Achazi, K., Romanov, G. A., and Schmülling, T. (2011). The specificity of cytokinin signalling in *Arabidopsis thaliana* is mediated by differing ligand affinities and expression profiles of the receptors. *Plant J.* 67, 157–168. doi: 10.1111/j.1365-313X.2011.04584.x

van der Leij, M., Smith, S., and Miller, A. (1998). Remobilisation of vacuolar stored nitrate in barley root cells. *Planta* 205, 64–72. doi: 10.1007/s004250050297

Vidal, E. A., Alvarez, J. M., Araus, V., Riveras, E., Brooks, M. D., Krouk, G., et al. (2020). Nitrate in 2020: thirty years from transport to signaling networks. *Plant Cell* 32, 2094–2119. doi: 10.1105/tpc.19.00748

Wang, X., Feng, C., Tian, L., Hou, C., Tian, W., Hu, B., et al. (2021b). A transceptor-channel complex couples nitrate sensing to calcium signaling in arabidopsis. *Mol. Plant* 14, 774–786. doi: 10.1016/j.molp.2021.02.005

Wang, H., Han, C., Wang, J. G., Chu, X., Shi, W., Yao, L., et al. (2021a). Regulatory functions of cellular energy sensor SnRK1 for nitrate signalling through NLP7 repression. *Nat. Plants* 8, 1094–1107. doi: 10.1038/s41477-022-01236-5

Yang, X. Y., Zhang, Z. W., Fu, Y. F., Feng, L. Y., Li, M. X., Kang, Q., et al. (2022). Shade avoidance 3 mediates crosstalk between shade and nitrogen in arabidopsis leaf development. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.809913

Yuan, S., Zhang, Z. W., Zheng, C., Zhao, Z. Y., Wang, Y., Feng, L. Y., et al. (2016). Arabidopsis cryptochrome 1 functions in nitrogen regulation of flowering. *Proc. Natl. Acad. Sci. U.S.A.* 113, 7661–7666. doi: 10.1073/pnas.1602804113

Zhang, X., Cui, Y., Yu, M., Su, B., Gong, W., Baluška, F., et al. (2019). Phosphorylation-mediated dynamics of nitrate receptor NRT1.1 regulate auxin flux and nitrate signaling in lateral root growth. *Plant Physiol.* 181, 480–498. doi: 10.1104/pp.19.00346

Zhang, S., Zhang, Y., Li, K., Yan, M., Zhang, J., Yu, M., et al. (2021). Nitrogen mediates flowering time and nitrogen use efficiency via floral regulators in rice. *Curr. Biol.* 31, 671–683.e5. doi: 10.1016/j.cub.2020.10.095