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Minireview

Molecular mechanisms underlying nitrate responses in plants

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SUMMARY

Nitrate is an important source of inorganic nitrogen. Nitrate modulates many plant metabolic, physiological, and developmental processes. This minireview highlights recent findings on the intricate molecular wiring that allows plants to adapt to environmental nitrate conditions. We focus on the role of regulatory pathways and their components — transporters, receptors, second messengers, kinases, and transcription factors — in mediating plant metabolic and developmental responses to nitrate. Work is still needed to identify missing components of the nitrate signaling pathway and their interplay with known and well-characterized master regulators and to validate their molecular interactions to explain the complexity of phenotypical responses to nitrate. Understanding how plants perceive nitrate and transduce it into responses at the molecular level is crucial to optimize nitrogen-use efficiency, improve crop yield and mitigate the adverse environmental impacts of fertilizer overuse in a changing world.

Introduction

Nitrogen (N) is a key nutrient for plants and a component of essential macromolecules, such as proteins and DNA. Changes in N availability significantly impact plant growth and development[1]. Thousands of tons of N-based fertilizers are required on a yearly basis to sustain crop production. However, plants only take up a fraction of the N applied as fertilizer; the rest is released to the atmosphere or lost as excess run-off that pollutes water bodies[2]. Overuse of N-fertilizers contributes to eutrophication and global warming[2]. Significant effort has been devoted to elucidate the signaling pathways underlying molecular N-responses. Understanding these processes provides targets to engineer new N-use-efficient crop varieties and more cost-effective N-fertilizing programs, which benefit agriculture and environmental protection. For instance, *Os*NLP4, the rice homolog of NLP7 — an important regulator of inorganic N responses in *Arabidopsis thaliana*[3,4] — was tested as a tool to improve rice yield and N-use efficiency[5]. *Os*NLP4 induced the expression of genes involved in N uptake and assimilation in rice[5], and overexpressing *Os*NLP4 enhanced rice yield and N-use efficiency[5].

Nitrate is a preponderant source of N in aerobic soils[2], and can also act as a molecular signal mediating short-term (minutes to hours) and long-term (days) responses in plants[1,6]. Many regulatory components of the nitrate signaling pathway have been reported in the last decade, including receptors, kinases, and transcription factors (TFs)[6–10]. Also, the sequence of molecular events triggered by its perception has been described, including mechanisms of nitrate perception, the release of second messengers, post-translational modifications of nitrate regulators, and transcriptional responses[4,7,8,10,11]. The nitrate signaling pathway induces or represses genes involved in nitrate uptake, reduction, and N-assimilation, leading to changes in the N status of the plant[12–14]. Moreover, nitrate signaling interacts with phytohormone pathways to modulate developmental responses, including germination[15], flowering[16], branching[17], shoot apical meristem dynamics[18], vegetative growth[19–21] and root system architecture modifications involving primary roots[22–24], lateral roots[22,25–27] and root foraging[28,29].

This minireview summarizes recent research that expands our knowledge about the molecular mechanisms underlying nitrate responses in *Arabidopsis*. We review nitrate uptake and perception, the effect of nitrate on intracellular signaling, fine-tuning of transcriptional control, and molecular events connecting nitrate signals with developmental responses. The increased availability of genome- and transcriptome-wide sequencing techniques, improved bioinformatics applications and databases, site-directed genome editing tools, and high-quality genomes for various plant species makes it possible and more straightforward to translate the knowledge obtained in *Arabidopsis* to crop-improvement strategies. For example, genetic variation of the nitrate transporter NRT1.1 or the nitrate reductase gene *NIA2* in rice determines N-use efficiency divergence of *indica* versus *japonica* varieties[30,31]. A number of excellent recent reviews expand or cover other aspects[1,2,6,32–34].

Regulation of nitrate uptake at a molecular level

Nitrate uptake, a critical step in N acquisition, is performed by a transport system located in the membrane of plant root cells[2,6].

In *Arabidopsis*, nitrate is incorporated into the roots mainly by transporters belonging to either the low-affinity NRT1 family or the high-affinity NRT2 family[2,6]. Among transporters belonging to these families, NRT1.1 is the only transporter that can mediate low- and high-affinity nitrate uptake[2,6]. The switch from low-affinity to high-affinity state depends on phosphorylation of threonine residue 101 (Thr101) by CIPK23 kinase[7,35]. This phosphorylation also impacts NRT1.1 plasma membrane dynamics and intracellular trafficking[26]. A similar mechanism to control transport affinity has been recently proposed for the NRT2.1 transporter, which is essential for high-affinity nitrate transport[36]. The serine residue 501 (Ser501) in the NRT2.1 carboxy-terminus is phosphorylated under increasing nitrate concentrations, leading to NRT2.1 inactivation[37]. Notably, plants with a mutated version of NRT2.1 that mimics a constitutive phosphorylation of Ser501 showed a comparable phenotype to NRT2.1-null plants in response to nitrate[37].

In addition to post-translational control, nitrate uptake is also adjusted through transcriptional control of genes encoding nitrate transporters. For example, the expression of *NRT2* transporters is regulated by N and carbon availability[38]. Recently, the TFs TGA3, MYC1, and bHLH093 were shown to be involved in transcriptional modulation of the NRT2 transporters in response to N and carbon[39]. Furthermore, the interaction between TGA3 and MYC1 with the *NRT2.4* and *NRT2.5* promoters was validated through a yeast-1-hybrid assay[39].

The ability of NRT1.1 to transport auxin allows plants to adapt to changes in nitrate availability also by coordinating the transport of nitrate and this phytohormone[25,40]. Under nitrate deficiency, the NRT1.1 transporter drives auxin away from the root tip, suppressing the growth of lateral root[25]. In contrast, NRT1.1-mediated auxin transport is inhibited in response to nitrate supply, leading to auxin accumulation and lateral root growth[25]. This mechanism has also been associated with the phosphorylation of Thr101[25,26,40]. Interestingly, the crosstalk between nitrate and auxin signaling goes beyond transport and involves NRT1.1-dependent transcriptional regulation of auxin-related genes[27]. For example, recent studies have shown that under low nitrate conditions, the auxin biosynthetic and transport *TAR2* and *LAX3* genes are transcriptionally repressed in an NRT1.1-dependent manner which leads to the repression of lateral root primordium development[27]. This repression is alleviated in response to high nitrate availability, and lateral root primordium development is promoted[27].

Molecular events in nitrate signal transduction

Once nitrate is perceived in root cells, a cascade of molecular events transmits the signal to the nucleus (Figure 1). The signal transduction pathway involves nitrate perception by NRT1.1[7]. In addition to NRT1.1, another study recently showed that NRT1.13 cannot transport nitrate, although it plays a role in modulating flowering time and branching under low-nitrate conditions. This evidence suggests that NRT1.13 may play a role as a new nitrate receptor (Figure 1A)[9].

After nitrate perception by NRT1.1, Ca^{2+} concentration rises in the cytoplasm, and the CPK10/30/32 Ca^{2+}-activated kinases phosphorylate NLP7[8,11] — an important regulator of nitrate transcriptional responses in *Arabidopsis*[3,4] (Figure 1A) — leading to its nuclear retention[4,8] and the activation of transcriptional responses (Figure 1A). The molecular mechanism mediating the Ca^{2+} influx upon nitrate perception was recently investigated in more detail. Under low nitrate conditions, the cyclic nucleotide-gated channel protein CNGC15 is inactivated by forming a heterocomplex with NRT1.1 in the plasma membrane[10]. In contrast, CNGC15 dissociates from the transceptor under high nitrate conditions and functions as a Ca^{2+} channel that allows the influx of Ca^{2+} into the cells (Figure 1A)[10]. This study also showed that CNGC15 is required for the nuclear retention of NLP7[10].

Regulatory events rapidly propagate the nitrate signal at a transcriptional level once transduced to the nucleus. Over the last few years, a TF hierarchy has been established as a mechanism for transcriptional control and rapid signal propagation within minutes of nitrate exposure (Figure 1B–D)[4,13,14,41,42]. In one study, genes responding to a combination of nitrate and ammonium treatments were identified in roots and shoots in a high-resolution manner from 5 to 120 minutes[14]. The authors binned genes according to the first time they were regulated, and found unique *cis*-regulatory elements enriched at each time point, implicating a cascade of TFs that consecutively governs gene expression in response to N[14].

A hierarchical network mediating transcriptional responses to nitrate was constructed in a separate study using genome-wide mapping of TF-binding sites in accessible chromatin regions[41]. The construction of this network was achieved using DNase I enzyme treatments. DNAse I cleaves accessible chromatin regions known as DNase I hypersensitive sites that are associated with RNA polymerase II localization and active transcriptional regulation[41,43,44]. DNase I hypersensitive sites were mapped by sequencing the products of DNase I digestion[41,43,44], and TF binding sites were detected within these areas because TF occupancy blocks DNAse I digestion[41,43,44]. The highest layer of this network, in which TFs with more connections are present, is composed of factors not regulated by nitrate at the gene expression level[41]. Such primary TFs are predicted to control nitrate-responsive factors in lower network layers (Figure 1B). This network layout explains immediate changes in gene expression and rapid amplification of the nitrate signal to affect diverse biological processes[41].

A regulatory TF hierarchy as a mechanism of transcriptional control has emerged from various studies. The genome-wide targets of 33 N-responsive TFs were identified and classified as direct or indirect targets in a recent analysis[42]. It was observed that the TFs were connected to their indirect targets through the direct connection with other TFs[42]. This study developed and used a network walking approach that connects TFs to their direct or indirect targets in root cells combining data from *in planta* experiments and TARGET[42] — a cell-based assay that validates direct factor–target interactions based on TF-induced changes in gene expression[45].

An integrative gene regulatory network analysis performed using publicly available data of TF–target interactions positioned NLP7 as one of the most influential TFs for nitrate uptake, reduction, and assimilation[34]. Indeed, NLP7 modulates the expression of approximately 60% of nitrate-responsive genes[4], including important TFs involved in nitrate responses such as LBD37, LBD38 and LBD39[4,8]. These members of the LBD family of TFs are negative regulators of nitrate-responsive genes[46]. The

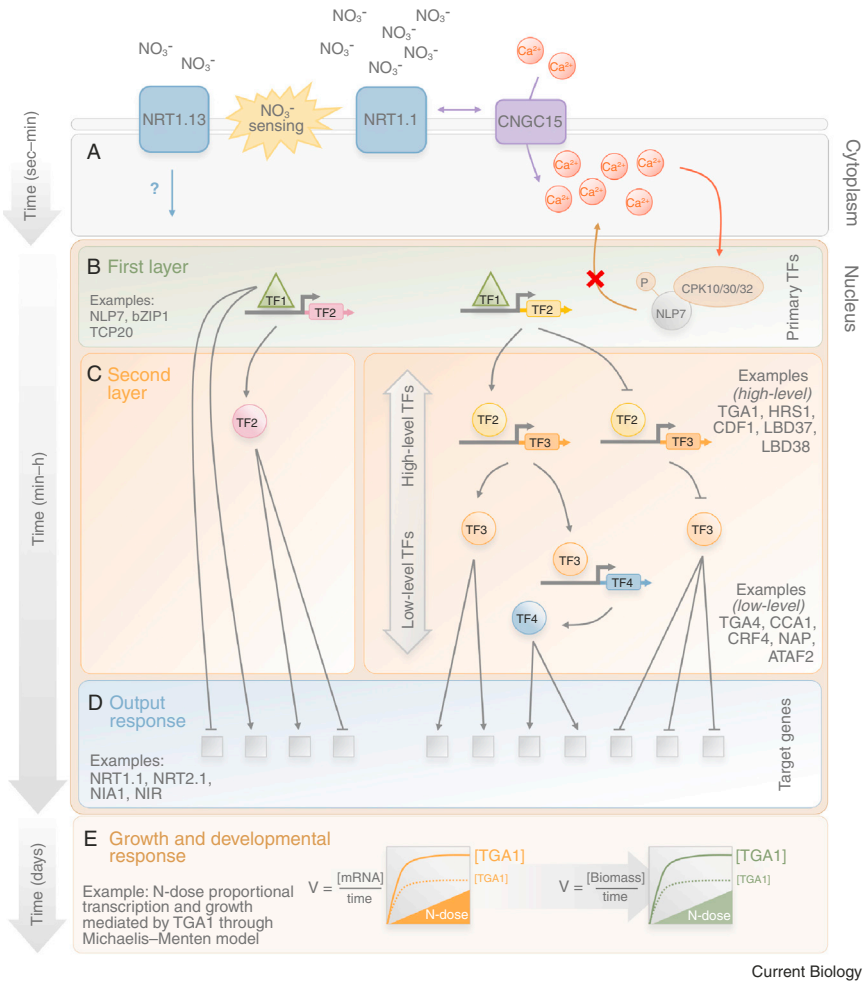

Figure 1. A hierarchical cascade of molecular events and interactions underlies fine-tuning of phenotypical responses to nitrogen.

(A) Under high-nitrate conditions, NRT1.1 perceives nitrate signal, and CNCG15 dissociates from the transceptor, allowing Ca^{2+} influx into the cell. NRT1.13 was recently identified as a nitrate receptor that modulates flowering and branching under low-nitrate conditions. The signaling pathway downstream of NRT1.13 is unknown. (B) Increased intracellular Ca^{2+} concentration activates CPK10/30/32 kinases which phosphorylate NLP7. Phosphorylated NLP7 is retained in the nucleus to coordinate early transcriptional responses to nitrate. Once the nitrate signal is transduced, a set of primary TFs (TF1) are activated to mediate the early nitrate response. Primary TFs such as NLP7 are recruited or retained into the nucleus, for instance, by post-translational modifications, where they can rapidly regulate gene expression (arrows: gene induction, edges with a perpendicular line: gene repression). These TFs are not themselves transcriptionally regulated by nitrate. (C, left) Primary TFs in the first layer regulate (D) target genes in the output response directly or through intermediary regulators (TF2). (C, right) In addition, recent evidence shows that primary TFs trigger and spread the nitrate transcriptional response through a second layer of regulation consisting of a hierarchical cascade of high and low-level TFs (TF2, TF3, TF4) (high and low-level TFs are classified according to gene network displayed in Vidal *et al.*[34]). (D) Target genes in the output response modulate the plant phenotype, including plant N-uptake and N-metabolism changes. These genes can be regulated by a different number of regulation layers composed of high- and low-level TFs. (E) More layers of complexity have been recently identified in the growth and developmental responses downstream of the output transcriptional response. For instance, the rates of transcription of TGA1 and its targets increase with N-dose and is explained by the Michaelis–Menten model. This regulatory complexity influences the phenotype, giving a molecular basis for plant adaptation to N levels.

expression of key genes for nitrate uptake and assimilation is higher in *lbd37*, *lbd38*, or *lbd39* mutants than in wild-type plants[46]. Interestingly, NLP7 is not itself transcriptionally induced or repressed by nitrate but is activated by post-translational modification[4,8,13].

Besides NLP7, other members of the NLP family have a function in regulating gene expression triggered by nitrate as well. NLPs can be divided into three subgroups — NLP1 to NLP5, NLP6/NLP7, and NLP8/NLP9[47]. All NLPs bind to a 43 bp nitrate-responsive *cis*-element using yeast 1 hybrid analysis[47]. Overexpression of NLP2 and NLP6 in the absence of nitrate does not induce nitrate responsive genes, suggesting that post-translational modification of NLP2 and NLP6 is necessary for nitrate induction of gene expression, similar to NLP7[47,48].

A recent study demonstrated that NLP7 triggers a temporal transcriptional cascade in response to N, using the TARGET system[13]. The authors captured direct and indirect NLP7 targets, and observed that NLP7 directly regulates early N-responsive TFs (high-level TFs), which amplify the NLP7-initiated transcriptional cascade by regulating late responsive TFs (low-level TFs) (Figure 1C)[13]. Interestingly, NLP7 binds transiently to its direct targets. This finding is reminiscent of a 'hit and run' model of transcription previously proposed for bZIP1 — a TF that also regulates early responses to N — and supports a model where primary TFs initiate a cascade of gene expression in response to nitrate[13,49].

The integrative gene regulatory network analysis of TF–target interactions mentioned above also positioned TGA1 as one of the most influential TFs in the nitrate response[34]. In fact, 97% of the genes regulated by TGA1 and its homolog TGA4 are nitrate-responsive, including the nitrate transporter genes NRT2.1 and NRT2.2[22]. In addition, *tga1 tga4* mutants are impaired in primary root and lateral root growth after a long-term nitrate treatment[22].

Recently, it was shown that TGA1 also modulates the rates at which N regulates gene expression[12]. In this study, the authors investigated how plant root transcriptomes change in response to increasing N concentrations over time, and identified thousands of genes whose expression changed as a function of the amount of supplied N (N-dose). Interestingly, the authors found that the Michaelis–Menten model — a classic equation conceived to describe enzyme reaction rates as a function of substrate concentration — could explain changes of genome-wide transcript levels in response to N-dose. Both transcriptome

and plant growth N-dose-dependent changes fit the Michaelis–Menten model[12], meaning that adjusting gene-regulation kinetics would have a proportional impact on plant growth. Indeed, overexpression of TGA1 led to increasing gene expression rates that in turn correlated with increasing plant growth rates (Figure 1E)[12]. Both direct and indirect TGA1 targets follow Michaelis–Menten kinetics, suggesting that the N-dose signal is propagated downstream of the hierarchy of TFs controlled by TGA1[12].

Transcription factors cooperate to mediate nitrate transcriptional responses

Along with transcriptional cascades, gene expression and cellular responses to nitrate are influenced by TFs acting with other TF partners[13,24,42]. TF–TF interactions are functionally relevant to connecting transcriptional regulation with developmental responses to nitrate. For instance, NLP7 physically interacts in the nucleus with its homolog NLP6 and TCP20 — a nitrate regulator for the systemic nitrate foraging response[29]. NLP6 and NLP7 interact with TCP20 and bind to the promoter of the nitrate sentinel gene *NIA1*[24]. This interaction is required to sustain *NIA1* expression[24]. In addition, NLP6/7 physically interacts with TCP20 to modulate the expression of the cell-cycle gene *CYCB1;1*, which is induced in *nlp6/7* and *tcp20* mutants under N-starvation conditions, leading to a reduced cell number in the meristem and shorter primary root than in wild-type plants[24].

Direct and indirect targets of TFs involved in N signaling were identified in a recent study, and it was proposed that these TFs can act in opposite ways — induce or repress transcription — depending on the *cis*-motif context of the target gene[13,42]. The data suggest this dual TF action could operate via direct binding to a *cis*-element or through an indirect interaction via binding to a partner TF. Similarly, another study demonstrated that genes induced by NLP7 are enriched in the NLP7 *cis*-motif, while repressed genes are not[13]. These results suggest that the directionality of gene regulation in response to N is determined by direct interaction of TFs with promoters or by indirect interaction through TF partners. Furthermore, gene induction correlated with *de novo* TF recruitment to DNA in response to nitrate, while repression is not associated with *de novo* TF recruitment or disengagement to the target genes[41]. These findings suggest that nitrate-elicited repression might be related to TFs engaged to their target promoters before the stimuli and post-transcriptional regulation[42].

Cis-regulatory elements might also determine the different TF binding kinetics (stable, transient, and highly transient)[13,49]. For example, genes induced and stably bound by NLP7 are enriched only in the NLP7 *cis*-motif, while transient and highly transient targets are enriched in the NLP7 *cis*-motif as well as in other *cis*-motifs like ERF and GATA elements[13]. In contrast, the three classes of genes repressed by NLP7 (stable, transient, and highly transient) are not enriched in the NLP7 *cis*-motif. These genes are, however, enriched in other *cis*-motifs such as W-box, ABRE-like, MYB and G-box elements[13]. These results suggest that *cis*-elements and TF partners determine not only the direction of gene expression but also TF binding kinetics.

Early plant development is fine-tuned by environmental nitrate

Nitrate signaling controls developmental programs, with direct consequences on plant structure and function. Therefore, researchers have extensively studied the role of nitrate in different developmental processes of the plant life cycle. For example, nitrate concentration and distribution in the soil modulate primary and lateral root length and root-hair density[32]. Nitrate also reshapes the shoot system architecture by affecting shoot apical meristem size, plastochron ratio, branching, and leaf growth[17,18,21]. Processes such as germination and flowering are also modulated by nitrate[15,16]. It is important to note that developmental responses to nitrate have been explored primarily in adult or reproductive stages. Early post-germinative developmental responses to nitrate have only recently been researched.

A recent study showed that nitrate promotes cotyledon growth five days after germination (Figure 2A)[19]. By tracking cell area for seven days using cotyledon live-imaging and measuring ploidy levels by flow cytometry, the authors found that this growth process is a result of modulating cell expansion and endoreplication processes[19]. Also, the authors found that cell area and ploidy changes required the cell-cycle inhibitor LGO[19]. Nitrate increased the number of cells expressing *LGO* as early as three days after germination, and *lgo* mutants lost their ability to increase cell area and ploidy in response to nitrate[19]. Surprisingly, *lgo* mutants reached the same organ size as wild-type plants in response to nitrate availability by promoting cell proliferation regardless of their high disruption in cell expansion and ploidy profiles[19]. Interestingly, this mechanism of nitrate control over shoot development was also observed in true leaves[19].

Also, regarding first true leaf development during early post-germinative growth, it recently was observed that mitotic activity in leaf primordium is activated by inorganic N sources (nitrate and ammonium) through ROP2–TOR signaling (Figure 2B)[20]. True leaf growth was observed to be completely inhibited in the absence of inorganic N sources. This observation was correlated with decreased activity of the TOR kinase five days after germination, and the complete abolishment of cell proliferation in true leaf primordium after seven days by growing plants under an N-free medium. Also, it was observed that re-supplying inorganic N to nine-day-old N-starved seedlings reactivates true leaf development. Moreover, the authors observed that a constitutively active version of the ROP2 GTPase maintained TOR and mitotic activity in the absence of inorganic N sources, suggesting that it plays a role upstream of TOR signaling. Interestingly, although a battery of different amino acids was able to supply the absence of external N by activating TOR signaling, the authors showed that nitrate- and ammonium-elicited TOR activation is independent of N assimilation[20].

Some aspects of the signaling pathways upstream of LGO and ROP2–TOR are partially described to date. For instance, although NRT1.1 and NLP7 mutants (*chl1-5* and *nlp7*, respectively) had diminished vegetative area and ploidy levels, it remains to be elucidated whether this phenotype is associated with impaired *LGO* expression[19]. Furthermore, *chl1-5* and *nlp7* mutant plants were smaller than wild-type or *lgo* mutants, suggesting that both *chl1-5* and *nlp7* mutants cannot compensate, as *lgo* mutant plants to reach normal organ size in response to nitrate[19]. This result indicates that the impact of the NRT1.1/NLP7 signaling pathway on early post-germinative shoot growth interplays with others pathways to determine the final organ size[19]. In contrast, the NRT1.1 transceptor is not required for

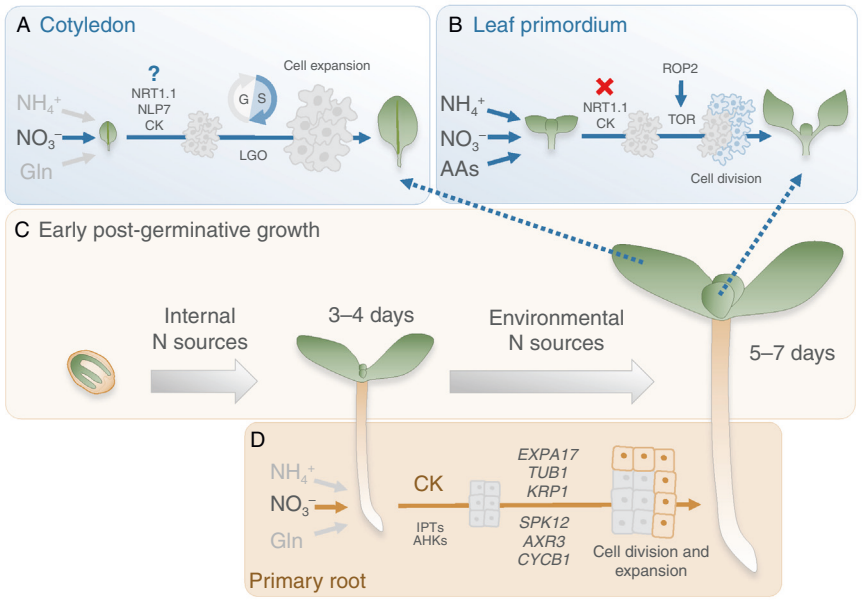

Figure 2. Early plant development is finely tuned by environmental nitrate.
(A) Nitrate promotes early post-germinative cotyledon growth by increasing cell area and ploidy through the cyclin-dependent kinase inhibitor LGO. This cell-cycle regulator is involved in the onset of cell endoreplication. Nitrate-elicited cotyledon growth was not observed in response to other N-sources such as ammonium (NH_4^+) and glycine (Gln). Canonical regulators of the nitrate signaling pathway such as NRT1.1 and NLP7 are partially involved in this response. Also, evidence at later developmental stages suggests that CK — a long-distance and systemic mediator of nitrate responses — may also mediate nitrate-elicited early shoot growth. (B) Nitrate and other N-sources such as ammonium and amino acids (AAs) promote true-leaf primordium growth through cell division. The ROP2 GTPase and the TOR kinase (ROP2–TOR module) support this cellular response, while nitrate transceptor NRT1.1 and CK do not play a role in it. (C) Primary root growth is triggered by nitrate in crosstalk with CK during early plant development. Both cell division and expansion in the meristematic and elongation zones are involved in this response, respectively. The transcriptional regulation of genes involved in cell division (*KRP1*, *SPK12*, *AXR3*, *CYCB1*) and expansion (*EXPA17*, *TUB1*) in the primary root in response to nitrate depends on CK biosynthetic (*IPTs*) and signaling (*AHKs*) genes. (D) Post-embryonic plant development is dependent on internal seed N-sources until three to four days after germination, a period in which plants do not respond to external N-sources yet. Then, plants begin to perceive external N-sources. Developmental responses are verified as early as five to seven days after germination.

the activation of the ROP2–TOR signaling pathway[20]. These results lend support to the idea of the existence of different signaling pathways that contribute to coordinate nitrate-elicited responses that are not discovered yet. Experimental designs and systems biology analyses directed to early developmental stages can help uncover these molecular mechanisms[19]. These tools have already helped unravel the complex wiring behind short-term responses to nitrate in adult plants and identify new players of these responses[12–14,22,41,42]. Also, the possible involvement of other known nitrate signaling pathway regulators such as TGA1 in the control of developmental responses remains to be evaluated.

Concerning root development, recently, cytokinin (CK) was shown to be required for early post-germinative primary growth in response to nitrate[23]. CK is a well-known, long-distance, and root-derived signal mediating systemic responses to nitrate[28]. Mutants defective in CK biosynthesis and perception are impaired in primary root growth as early as seven days after germination[23]. This phenotype was attributed to a decrease in cell division and expansion in the meristematic and elongation zones, respectively, and the deregulation of genes involved in cell elongation (*EXPA17*, *TUB1*) and division (*KRP1, SPK12, AXR3*) (Figure 2C)[23].

The concept of long-distance and systemic CK-mediated nitrate signaling was further characterized recently in adult plants using a split-root system[28]. In this experiment, the root is divided into two portions that grow on physically separated sectors of a vertical agar plate. Thus, different parts of the same root can be simultaneously exposed to contrasting nitrate regimes. Using this system, it was concluded that root uptake and biomass in one root portion depend on integrating root-derived CK signals in the shoot coming from the other root portion exposed to a specific nitrate treatment — a root-to-shoot-to-root signal[28]. The response to nitrate availability characterized through the split-root system is noticeably long-distance since the phenotype observed implies shoot and root communication[28]. However, it remains to be elucidated if primary root growth[23] is due to local — that is, in the vicinity of the perception site — or long-distance CK signaling in the context of early post-germinative root developmental responses to nitrate. It is unclear if a root-to-shoot signal is involved in this primary root phenotype[23]. Nevertheless, despite the signaling pathway underlying early post-germinative primary root growth in response to nitrate is not deciphered yet[23], the nitrate and CK interplay for this phenotype is apparent and already operative as early as seven days after germination[23].

Current evidence invites exploring the view that CK could also act as a long-distance signal to the early shoot developmental responses to nitrate. For instance, CK mutants are impaired in rosette growth and modify their shoot gene expression pattern in response to nitrate in adult plants[28], raising the question of whether this phenotype is replicable in seedlings. In addition, the root phenotype discussed above[23] denotes that CK and nitrate interplay is already operative seven days after germination, matching the time in which nitrate-elicited cotyledon and primordia growth is observed[19,20]. Further research has yet to establish if CK plays a role in long-distance signaling in LGO-dependent cell expansion and endoreduplication[19] in response to nitrate during early development. Indeed, recent research provides some insight into this question, showing the extent of cell proliferation and expansion and CK signaling interplay during leaf morphogenesis[50]. Interestingly, even though CK signaling is required for nitrate-mediated meristem dynamics in adult plants[18], CK could not replace inorganic N sources in

activating ROP2–TOR signaling in leaf primordium nine days after germination[20].

Conclusions

Understanding the signaling pathways that transduce the nitrate signal into phenotypical responses is an active field of research. Here, we described new aspects of the molecular mechanisms that fine-tune nitrate uptake and nitrate-elicited transcriptional and developmental responses: the post-transcriptional and post-translational control of nitrate transporter activity, the hierarchical structure of N signaling, and the cooperation of TF partners for gene regulation. Also, we reviewed regulatory pathways established soon after germination that connect external N-signaling to early shoot development.

Though much has been learned over the past several years, many questions remain. We have yet to establish how plants exploit a restricted universe of regulatory components to calibrate organ-specific responses to nitrate. The transcriptional control mechanisms discussed here, such as the hierarchy of TFs amplifying transcriptional responses and partner TF cooperation, are mainly associated with short-term responses to nitrate treatments in roots. Less is known about how nitrate signaling operates over the long-term and in interaction with other environmental signals. Moreover, less attention has been given to nitrate signaling in above-ground organs. Identifying components of nitrate signaling and how they operate in each plant tissue and at each developmental stage is critical to identify new molecular targets to engineer crop varieties with better N-use efficiency and improved yield.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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