Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Plant Biology

A systems view of nitrogen nutrient and metabolite responses in Arabidopsis

Elena A Vidal[1] and Rodrigo A Gutiérrez[1,2]

Nitrogen (N) is an essential macronutrient available to plants mainly as nitrate in agricultural soils. Besides its role as a nutrient, inorganic and organic N sources play key roles as signals that control genome-wide gene expression in Arabidopsis and other plant species. Genomics approaches have provided us with thousands of genes whose expression is modulated in response to N treatments in Arabidopsis. Recently, systems approaches have been utilized to map the complex molecular network that plants utilize to integrate metabolic, cellular, and developmental processes to successfully adapt to changing N availability. The challenge now is to understand the molecular mechanisms underlying N regulation of gene networks and bridge the gap between N sensing, signaling, and downstream physiological and developmental changes. We discuss recent advances in this direction.

Addresses
[1] Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile
[2] Department of Biology, New York University, 100 Washington Square East, 1009 Main Building, New York, NY 10003, United States

Corresponding author: Gutiérrez, Rodrigo A (rgutierrez@bio.puc.cl)

Current Opinion in Plant Biology 2008, **11**:1–9

This review comes from a themed issue on
Cell Signalling and Gene Regulation
Edited by Jason Reed and Bonnie Bartel

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2008.07.003

Introduction

Nitrogen (N) is an essential macronutrient and a key factor limiting agricultural productivity. Plants exhibit sophisticated adaptive mechanisms to cope with variations in soil N availability. Plant growth and developmental aspects such as root architecture [1], leaf development [2], seed dormancy [3], and flowering [4] can be dramatically affected by the source and/or amount of N supplied to plants. Because of its importance to agricultural productivity, an enormous amount of work on the biochemical and physiological aspects of N metabolism has been performed. Despite this solid groundwork, a detailed understanding of the molecular mechanisms underlying N sensing, N signaling, and growth and developmental responses to changes in N availability is still lacking. Although some of the components of the regulation of N metabolic genes are conserved between plants and other organisms (e.g. PII protein, TOR kinases), thus far the mechanisms of N signaling in other organisms have not directly extrapolated to plants (for example see [5–7]).

It is now clear that plants utilize multiple mechanisms to respond to environmental variations in N supply (e.g. localized nitrate patches in the soil, uniform high nitrate and N starvation) [8•]. Genomics, bioinformatics, and systems approaches are uncovering a complex regulatory network involving both transcriptional and post-transcriptional mechanisms to integrate plant responses to changes in nitrate availability. This review focuses on the advances provided by these approaches, as well as recent advances related to mechanisms involved in N responses in *Arabidopsis thaliana*. For a more detailed description of the topics covered in this essay please refer to the many excellent reviews published recently [4,9,10•,11–15].

Global regulation of gene expression by N nutrients and metabolites

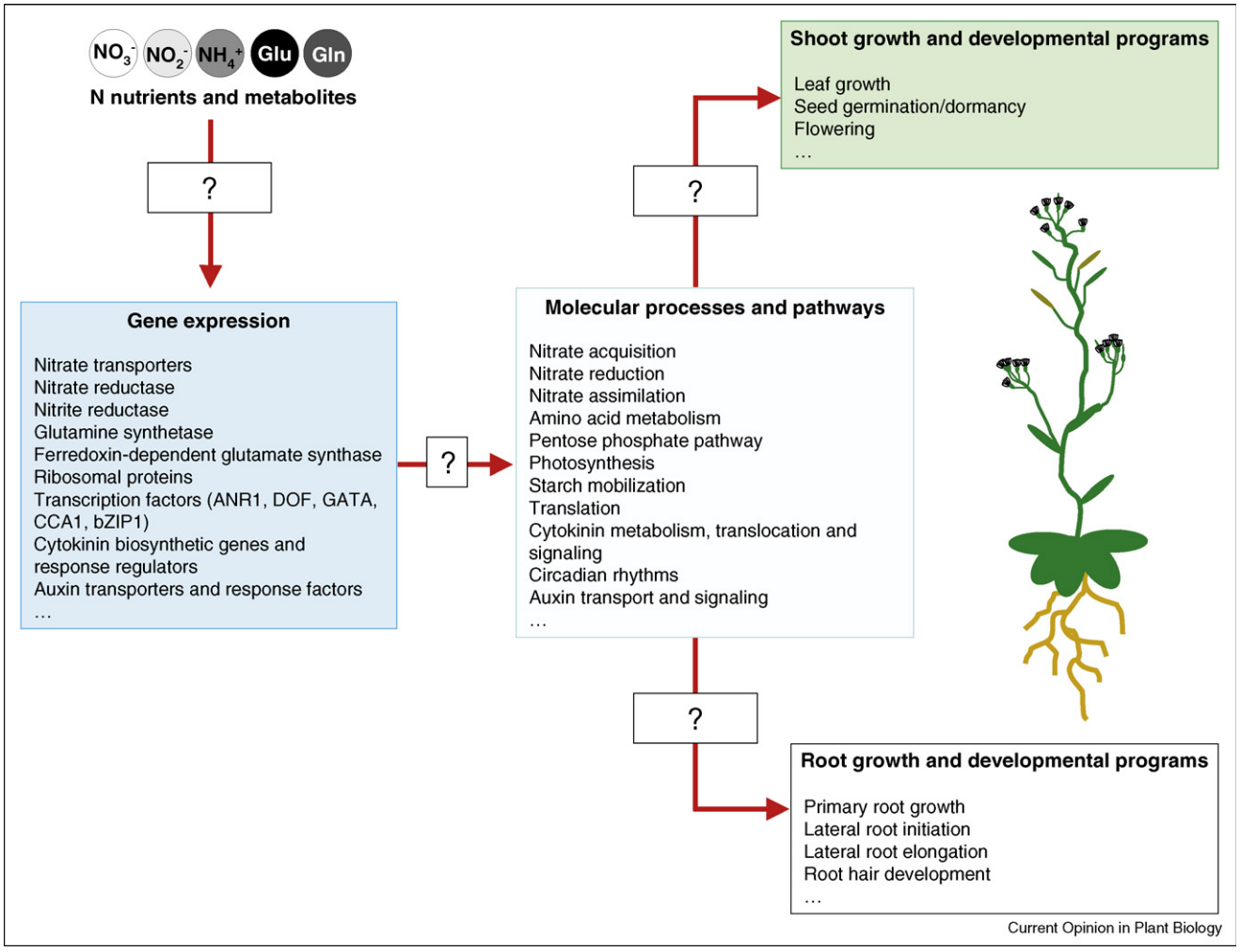
The metabolic, physiological, and developmental adaptations observed in plants in response to changing N supply are at least partly due to changes in gene expression. Nitrate and N metabolites derived from nitrate reduction and assimilation pathways act as signaling molecules to regulate global gene expression in Arabidopsis and other plants. Numerous microarray studies have provided an impressive catalog of N-responsive genes participating in a wide range of processes [16–18,19••,20••,21•,22,23,24••,25] (Figure 1). Together, these studies indicate that a large fraction of the Arabidopsis genome responds to N nutrient or metabolite treatments. Summarizing the extensive literature available on regulation of gene expression by N is beyond the scope of this review. Here we emphasize recent results obtained by genomics and systems approaches.

Regulation of gene expression by nitrate

Nitrate controls the expression of thousands of genes involved in a wide range of plant processes. Many of these genes respond within minutes (20 min of treatment) to nitrate concentrations as low as 100 nM [16,20••,25]. For example, nitrate regulates its uptake and assimilation by regulating nitrate transporters, nitrate reductase, nitrite reductase, as well as pathways for production of reducing equivalents such as the pentose

Please cite this article in press as: Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis, Curr Opin Plant Biol (2008), doi:10.1016/j.pbi.2008.07.003

Figure 1



N metabolite sensing and signaling results in massive changes in gene expression and morphological adaptations to N supply. Boxes with question marks represent molecular mechanisms that are not yet fully understood. N metabolites are sensed by yet unknown cellular components. The N signal is transduced by an unidentified signal transduction pathway, resulting in changes in transcript levels at a global scale. Regulation of gene expression by N metabolites leads to upregulation or downregulation of molecular processes and pathways that control growth and developmental programs in roots and shoots. See text for recent advances toward understanding the factors and mechanisms in these boxes.

phosphate pathway and glycolytic pathways. Nitrate also regulates the expression of genes involved in carbon (C) metabolism, thus coordinating the production of organic acids needed for inorganic N assimilation into amino acids. This close integration between N and C metabolism is essential for normal plant growth and development [26]. More recently, genomics approaches have provided evidence for interactions between C and N that include and extend beyond metabolic pathways [21•,22,23]. Many of the nitrate responsive genes respond in a C-dependent manner, suggesting that C and N signaling pathways interact to regulate gene expression. Other nitrate-regulated processes include secondary metabolism, hormone metabolism and transport, protein synthesis, and signal transduction pathways [16–18].

To distinguish the global effect of nitrate from the signaling effect of metabolites downstream of nitrate reduction and assimilation, Wang *et al.* compared the transcriptome of a nitrate reductase (NR)-null mutant and wild-type shoots and roots in response to nitrate treatments [17]. The lack of NR prevents nitrate reduction and assimilation blocking the production of downstream metabolic signals. Genes that are similarly regulated by nitrate in both wild type and the NR-null mutant are thought to be nitrate-regulated. These experiments demonstrated that nitrate plays a signaling role and identified hundreds of genes that are regulated by the nitrate signal. Nitrate transport, nitrate reduction, and assimilation as well as energy, metabolism, glycolysis and gluconeogenesis, amino acid metabolism, and nitrogen

Please cite this article in press as: Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis, Curr Opin Plant Biol (2008), doi:10.1016/j.pbi.2008.07.003

and sulfur utilization are among the nitrate-regulated processes in Arabidopsis [17].

Genomics approaches have also shown the substantial tissue-specific and cell-specific nature of the plant response to nitrate treatments [17,24••]. From the identified nitrate-regulated genes, 67% are induced and 82% are repressed in only one organ [17]. A detailed analysis of cell-specific responses uncovered a vast and predominantly cell-specific response in Arabidopsis roots [24••]. The increased sensitivity of cell-specific profiling revealed localized regulation of transcripts that was not evident from previous global analyses [24••].

The nitrate sensor and signaling pathway has yet to be identified. However, mounting evidence suggest nitrate transporters (see below) are likely components of a nitrate sensor and/or transducer system in Arabidopsis.

Regulation of gene expression by nitrite and organic N metabolites

In comparison to nitrate responses, we know less about gene expression responses to the addition of other N nutrients or metabolites as well as the tissue-specific or cell-specific nature of these responses. Nitrite, the direct product of nitrate reduction, was recently shown to act as a potent signal for global regulation of gene expression in Arabidopsis roots [20••]. This study showed that over 50% of nitrate-regulated genes, including genes involved in pentose phosphate pathway, carbon metabolism, nitrogen and sulfur metabolism, energy metabolism, and assimilation of ammonium, also responded to nitrite after 20 min of treatment [20••]. Many genes also responded specifically to nitrite, suggesting that nitrite can serve as a signal to coordinate regulation of gene expression in Arabidopsis roots [20••]. Nitrite responses were not mimicked by ammonium treatments and were not affected in the NR-null mutant. These results suggest that Arabidopsis roots are not responding to ammonium (direct product of nitrite reduction) or nitric oxide (nitrite can be converted to nitric oxide by NR) but possess a sensor and signaling system that recognizes nitrite directly [20••].

To uncouple gene responses to inorganic N (e.g. nitrate, nitrite) from those elicited by downstream products of inorganic N assimilation (e.g. glutamate), several treatments were performed with combinations of inorganic N (nitrate and ammonium), glutamate (Glu), and MSX, an inhibitor of glutamine synthetase that blocks ammonia incorporation into amino acids in Arabidopsis seedlings [19••]. This study showed that a significant proportion of N-regulated genes respond to organic N [19••]. Organic N signals induce cell wall biosynthesis and repress amino acid and carbohydrate metabolism. These results suggest feedback regulation by organic N for coordination of N assimilation and other cellular processes [19••]. Although organic N metabolites can elicit a strong response, the nature of the organic N signal remains elusive. Likely candidates are glutamine or Glu. Recently, external L-Glu has been shown to elicit complex changes in the root architecture of Arabidopsis (discussed below) [27•]. Ionotropic Glu-receptor-like proteins phylogenetically conserved in animals, and plants have been identified in Arabidopsis [28]. AtGLR1.1, a putative Glu receptor has been suggested as a regulator of carbon and nitrogen metabolism in Arabidopsis [29]. However, whether Glu can bind to putative plant Glu receptors and initiate a signaling cascade *in vivo* is unclear [30].

Regulation of gene expression by nitrogen limitation or deprivation

Genomics approaches characterized the global plant responses to nitrogen limitation [31,32] or deprivation [18]. Nitrogen deprivation in Arabidopsis coordinated repression of genes involved in photosynthesis, chlorophyll synthesis, plastid protein synthesis, and induction of genes for secondary metabolism as well as a reprogramming of mitochondrial electron transport [18]. By contrast, nitrate limitation induced expression of genes involved in protein degradation and the biosynthesis of anthocyanin and phenylpropanoid pathways and repressed genes functioning in photosynthesis and in the synthesis of nitrogenous macromolecules such as chlorophyll, proteins, amino acids, and nucleotides [31,32]. Numerous genes responding to nitrogen limitation and deprivation encode transcription factors, signal transduction components, and proteins required for hormone synthesis and response. The role of these regulatory factors in the plant response to nitrogen limitation or deprivation remains to be investigated.

Systems approaches to understand N regulation of gene expression

The thousands of genes identified to date that are regulated by N nutrients and metabolites pose a significant challenge for interpretation. Integrating this amount of data is problematic but certainly necessary for understanding how plants adapt to changes in N supply at the molecular level. One simple strategy to integrate genomics data generated by different groups used the new Sungear tool [33]. This analysis identified context-independent genes and pathways that respond to nitrate under a variety of conditions [34]. By contrast, other processes such as protein synthesis are context-dependent, responding only under a subset of conditions [34]. Data from the NR-null mutant suggest context-independent genes respond directly to nitrate, and context-dependent genes may be regulated by downstream nitrogen metabolites [17]. Integrative network biology is another promising strategy to achieve data integration [35]. The first comprehensive multinetwork model of plant molecular interactions was built recently to analyze the processes modulated by different N sources and identify new regulatory mechanisms [19••,21•]. This

Please cite this article in press as: Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis, Curr Opin Plant Biol (2008), doi:10.1016/j.pbi.2008.07.003

multinetwork model integrates information about metabolic pathways and other molecular interactions such as known and predicted protein–protein, protein–DNA and miRNA–RNA interactions [21•]. Network models can be used as scaffolds to interpret expression data and have provided new specific hypotheses for regulatory mechanisms of the plant response to changes in N availability that were not obvious from simple gene expression analysis or classic genetic approaches. In one study, network analysis of expression data in Arabidopsis roots identified molecular machines, cellular processes, and regulatory networks that are modulated by C and/or N [21•]. For example, hormone regulatory subnetworks were found, highlighting the role of different phytohormones, auxin most prominently, in the root response to C and N [21•]. Also, a possible role for microRNA-regulated networks in the nitrate response emerged from this analysis [21•]. In a separate study, network analysis was used to identify key regulatory factors involved in controlling gene expression in response to organic N (discussed below) [19••]. Network approaches have also recovered other known regulatory mechanisms involved in the plant response to changes in N availability. We envision that integrative network biology and other systems approaches in combination with genomics and genetic approaches will play a more prominent role in generating integrated dynamic models of plant responses to N in the near future.

Multiple mechanisms and levels of control mediate N responses in plants

Genomics has provided us with a myriad of N-regulated genes, many of which are probably part of the N sensing and signaling pathways. The challenge now is to elucidate the N sensing mechanism(s) and identify the signaling networks involved in plant N responses. As discussed below, it is clear that multiple mechanisms and levels of control are involved in plant responses to N nutrient and metabolites.

Regulation at the transcriptional level

ANR1, a root-specific MADS-box protein, was the first transcription factor identified as playing a role in plant developmental N responses [1]. Using reverse genetics, it was shown that plants with reduced *ANR1* levels were impaired in lateral root (LR) production in response to a localized nitrate supply [1]. *ANR1* was initially found to be induced by nitrate in root cultures [1]. It was later shown that *ANR1* was induced by N starvation and repressed by nitrate resupply [36], suggesting a role as a feedback regulator of LR growth rates by the N status of the plant.

Other transcription factors involved in N regulation include members of the GATA and DOF families. GATA transcription factors are key regulators of N metabolism in yeast [37]. There are 30 GATA transcription factors in the Arabidopsis genome. The *GATA*, *Nitrate-inducible*, *Carbon metabolism-involved* (*GNC*) gene was shown to be induced by nitrate in shoots and required for normal regulation of C metabolism in Arabidopsis [38]. These results suggested a possible role for *GNC* as an integrator of N and C metabolism [38]. In another report, overexpression of the maize transcription factor DOF1 in Arabidopsis and potato plants led to overaccumulation of amino acids, changes in organic acid metabolism, increase in N content and overexpression of genes in N and C metabolism [39]. These results also suggested a role for DOF1 in coordinating C and N responses. Promoter deletion analysis of the *NRT2.1* gene promoter fused to the GUS reporter gene showed that a 150-bp region of this promoter is important for regulation of *NRT2.1* by nitrate and sucrose [40]. Interestingly, this region contains GATA, DOF as well as other putative response elements, strengthening the possible connection between N and/or C regulation of gene expression by GATA and DOF transcription factors in Arabidopsis.

In a recent report, network analysis revealed an organic N-regulated subnetwork that included the myb-family transcription factor *CCA1*, *bZIP1*, and *GLK1* [19••]. CCA1, one of the master regulators of the circadian clock, was shown to coordinate the response of nitrate assimilatory genes to organic N by direct binding to the promoters of *bZIP1* (which in turn regulates *ASN1* expression), glutamine synthetase 1.3 and glutamate dehydrogenase 1 [19••]. This is the first report of an organic N-responsive transcription factor that directly regulates the expression of genes involved in N assimilation in Arabidopsis. Moreover, CCA1 provides a mechanistic link for integrating N nutrition and circadian regulation of gene expression in Arabidopsis.

Regulation at the post-transcriptional level

Post-transcriptional regulation of mRNA stability by microRNAs has been shown to be important for phosphate and sulfate deprivation responses in plants [41–43]. Recent studies indicated that microRNAs also play a role in plant N responses. Microarray studies identified that many microRNA targets are regulated by nitrate and/or sucrose treatments, suggesting microRNA-mediated regulation of gene expression by nitrate and/or sucrose [21•,24••]. One of these studies characterized the role of miR167 and one of its targets, *AUXIN RESPONSE FACTOR 8* (*ARF8*), in the control of LR development in response to nitrate [24••]. Nitrate treatments repressed miR167 in pericycle cells, leading to an increase in *ARF8* transcript. The transcript levels of *ARF6*, another miR167 target, are not regulated by nitrate in pericycle cells, despite miR167 repression [24••]. *ARF8* induction in pericycle cells triggered an increase in the ratio between initiating and emerging LRs, suggesting *ARF8* controls a key developmental checkpoint between lateral root initiation and emergence [24••].

Please cite this article in press as: Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis, Curr Opin Plant Biol (2008), doi:10.1016/j.pbi.2008.07.003

N treatments have also been shown to regulate the transcript levels of proteins involved in protein synthesis and degradation, several protein kinases and protein phosphatases [16–18,21•] suggesting a role for N in mediating changes at the translational and/or post-translational level. Recently, a putative methyltransferase *OSU1/QUAD2/TSD2* has been identified that is required for normal C:N balance in Arabidopsis [44]. It has been shown that OSU1 suppresses *ASN1* expression in response to low C:N ratios, but the molecular mechanisms of action of OSU1 over *ASN1* or its putative target is unknown [44]. Evidence for post-translational regulation by N also comes from experiments focusing on the switch from N sufficient to N deficient conditions. The Arabidopsis nitrogen limitation adaptation (*nla*) mutant is defective in developing the nitrogen limitation adaptive responses [45]. *NLA* codes for a RING-type ubiquitin ligase that functions as a positive regulator controlling Arabidopsis adaptation to nitrogen limitation [45]. However, the targets for NLA or other factors involved in potential post-translational control remain to be identified.

Bridging the gap between N sensing, signaling, and developmental adaptations to N availability

One of the most striking examples of plant developmental plasticity to changing environmental conditions is the modulation of root system architecture in response to N supply [8•]. Under homogeneous conditions in Arabidopsis, high external N concentrations reduce primary as well as lateral root elongation. By contrast, LR elongation is induced under N-limiting conditions. LR density is relatively constant across a range of N concentrations. Localized zones of high N concentration promote rather than suppress LR growth. Genetic and genomics analyses are beginning to unravel the regulatory and signaling pathways mediating these responses (Figure 2). Phytohormones are key players in the developmental adaptations plant utilize to cope with changing N availability.

Nitrogen: hormone interactions

Phytohormones are key regulators of plant growth and developmental processes. Genetic and physiological evidence indicates that N status and availability trigger morphological changes by modulating hormone homeostasis and/or signaling. Two hormones have been implicated in N responses as signals communicating N availability between roots and shoots: cytokinin and auxin. Cytokinin has been implicated in communicating root nitrate availability to the shoot [46]. Nitrate supplementation to plant roots results in a rapid increase in cytokinin levels and its translocation into the xylem vessels [46]. Nitrate is able to upregulate the transcript levels of *IPT3*, a key enzyme for cytokinin biosynthesis [17,47]. Two main signaling pathways of nitrate responses have been proposed: a nitrate-specific pathway and a cytokinin-dependent pathway [48]. Nitrate-specific genes are involved in nitrate assimilation and synthesis of amino acids and nucleotides, and cytokinin-dependent genes are related to developmental programs and increased capacity for protein synthesis [10•].

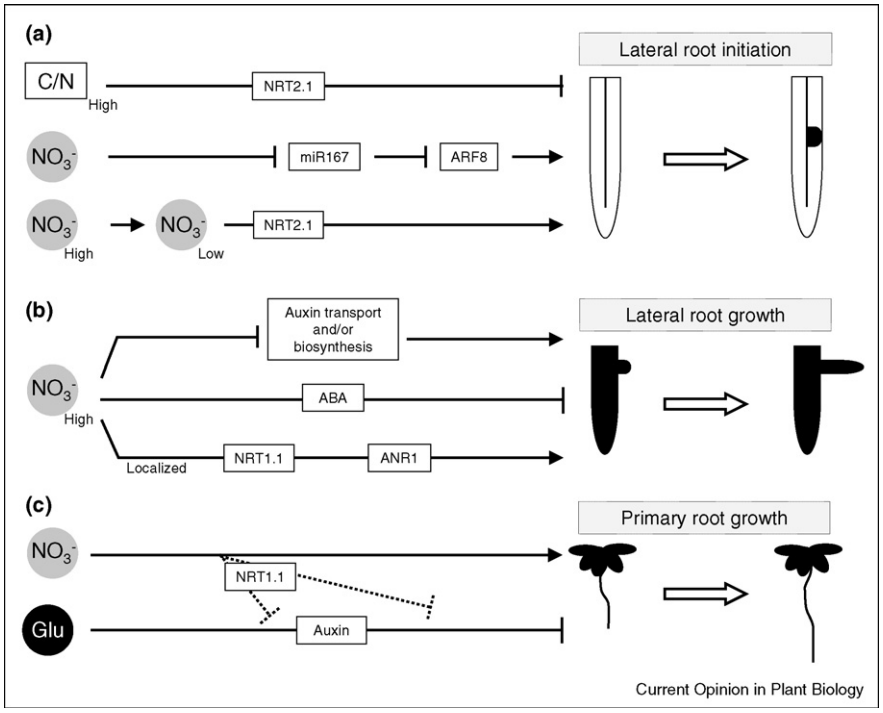
As we have already stated, there is an evident auxin:nitrate connection that arises from network analysis of nitrate-regulated genes [21•]. Auxin has been proposed as the long-range signal from shoot to root mediating high nitrate systemic inhibition of LR growth just after their emergence [49,50]. This is a logical connection given that auxin is the main hormone regulating LR development [51]. Auxin root tissue concentration increases in Arabidopsis seedlings transferred from high nitrate (50 mM) to low nitrate concentrations (0.1 mM) as compared to plants grown continuously in high nitrate [49]. This increase in auxin levels correlates with the release of LRs from high nitrate inhibition when transferred to low nitrate [49]. This result suggests that high nitrate supply might be inhibiting auxin biosynthesis or its translocation from shoot to roots [49,50]. However, ABA has also been implicated in high nitrate repression of LR growth [8•,52] by a pathway that is independent of auxin [53].

Lateral root development: the role of nitrate transporters

Although the function of NRT1.1 and NRT2.1 as receptors for nitrate remains to be demonstrated, several independent results suggest nitrate transporters play a signaling role in LR development in response to external nitrate. Examples of dual-function nutrient transporters/sensor proteins that can modulate downstream events in response to nutritional cues are common in other organisms [54]. The first evidence for the involvement of nitrate transporters in LR development came from a genetic screen for mutants that were able to develop LRs in a medium with a high sucrose/nitrate ratio [55]. High sucrose/nitrate medium represses LR initiation in wild-type plants, probably by inhibiting auxin transport to the roots [55]. The *lateral root initiation 1* (*lin1*) mutant was isolated for its capacity to overcome the high sucrose/nitrate repression on LRs. The *lin1* mutant was later shown to carry a missense mutation in the *NRT2.1* gene [56••]. The *lin1* mutant exhibited compromised NRT2.1 function and reduced nitrate uptake [56••]. However, the observed phenotype in LR initiation was independent of nitrate transport as it could not be rescued by increasing external nitrate [56••]. These results suggest that the increase in lateral root initiation is caused by abrogating a function of NRT2.1 that is unrelated to nitrate uptake. Besides its role as a repressor of LR initiation in high sucrose/nitrate medium, *NRT2.1* also regulates LR development in response to changes in nitrate availability [57••]. Intriguingly, this role in promoting LR initiation for NRT2.1 under low nitrate appears to be opposite to the observed function under high sucrose to nitrate ratio.

Please cite this article in press as: Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis, Curr Opin Plant Biol (2008), doi:10.1016/j.pbi.2008.07.003

Figure 2



Regulatory and signaling pathways mediating root adaptations to N supply. This figure summarizes described root adaptations to N supply and the identified factors involved. At least in some cases, these adaptations are mediated by independent molecular pathways. Future work will identify the exact relationship between the different pathways described. **(a)** Lateral root initiation is repressed by high C:N ratios, and this repression requires the nitrate transporter NRT2.1 [55,56••]. NRT2.1 is also involved in lateral root initiation induced by transfer from high to low nitrate concentrations [57••]. Nitrate repression of miR167 increases *ARF8* levels and this causes an increase in the ratio of initiating vs. emerging lateral roots [24••]. **(b)** High nitrate causes a systemic inhibition of lateral root development just after their emergence from the primary root [64]. This is probably caused by nitrate inhibition of auxin biosynthesis and/or its translocation from shoot to root [49,50]. An ABA-dependent pathway mediating high nitrate inhibition of lateral root growth has also been reported [8•,52]. Lateral root growth is induced by localized nitrate supply and this is mediated by a signaling pathway including the dual-affinity *NRT1.1* transporter and the MADS-box transcription factor *ANR1* [61]. **(c)** Nitrate stimulates primary root growth [62]. Primary root growth is inhibited by exogenous Glu [27•]. The effect of Glu involves a possible interaction with the auxin signaling pathway [27•]. Nitrate antagonizes the effect of Glu via a pathway that requires NRT1.1 [62]. The mechanism of repression, and whether it is upstream or downstream of auxin signaling, is unknown [62].

The reason for the apparent discrepancy is not clear (e.g. constant growth in sucrose/nitrate versus sudden decrease in external nitrate or different plant ecotypes) [8•]. Further experiments will be necessary to understand the role of NRT2.1 in LR development. NRT2.1 modulation is partly mediated by the nitrate transporter NRT1.1, as lack of this transporter suppresses the feedback repression of NRT2.1 caused by high nitrate [58]. NRT1.1 is a dual-affinity transporter [59], member of the peptide transporter family [60]. NRT1.1 has also been implicated in LR responses, in LR elongation in response to localized nitrate-rich patches [61]. This is an essential developmental response as it allows preferential root development where nitrate is abundant in the heterogeneous soil environment. The role of NRT1.1 has been analyzed using a split-root system, where one side of the root system is grown in high nitrate concentration (10 mM NO_3^-) and the other side is grown in low nitrate concentration (0.05 mM NO_3^-) to emulate soil nitrate patchiness. NRT1.1 mutant plants showed reduced LR length than WT plants in the high nitrate medium and increased LR length in the low nitrate medium. These results suggest that the NRT1.1 mutant does not impair LR growth *per se* but modifies LR length between both sides of the root system. This response is independent of nitrate transport by NRT1.1, suggesting NRT1.1 also acts by sensing nitrate availability. NRT1.1 works upstream of the MADS-box transcription factor *ANR1*, as NRT1.1 mutant plants have low levels of *ANR1* regardless of the external nitrate concentration [61]. *ANR1* accumulation has been shown to be essential for LR development [1]; therefore, NRT1.1 somehow participates in nitrate sensing that triggers root elongation mediated by *ANR1* (Figure 2).

Primary root growth effects in response to external Glu

External L-Glu was shown to inhibit primary root growth by inhibiting mitotic activity at the root apical meristem

Please cite this article in press as: Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis, Curr Opin Plant Biol (2008), doi:10.1016/j.pbi.2008.07.003

by a pathway that probably involves the hormone auxin [27•]. Putative sensors for Glu availability are the ionotropic Glu-receptor-like proteins [28]. However, root system architecture changes in response to L-Glu were not affected in the presence of antagonists of mammalian Glu receptors, suggesting these receptors are not involved in this response [27•]. Nitrate has been recently shown to stimulate primary root growth in Arabidopsis [62]. Interestingly, nitrate can also antagonize the inhibition of primary root growth caused by external L-Glu, and this requires an intact NRT1.1 [62]. Replacing the Thr 101 of NRT1.1 for an Ala compromises high but not low affinity nitrate transport [63]. The observation that overexpression of this NRT1.1^{Thr101Ala} mutant was unable to alleviate L-Glu inhibition of primary root growth provided new evidence suggesting that NRT1.1 is involved in nitrate sensing.

Conclusions

N sensing and signaling enables organisms to regulate their metabolism, growth, and development in response to N availability. With the advent of genomics technologies, our understanding of N regulation of gene expression has grown considerably over the past few years. Nitrate, nitrite, and other N metabolites regulate gene expression and a vast variety of processes at the cellular level. Although classic genetic approaches (i.e. phenotypic and gene expression analyses of mutants) have characterized important components of N signaling pathways, the global vision of the plant circuitry provided by systems approaches has proven useful to generate new hypotheses and discovering novel regulatory networks controlled by N. Systems approach should now help us gain an integrated understanding of the molecular networks controlled by the different regulatory components characterized, and the way different pathways interact in the plant N response. Moreover, combining systems, genomics, and genetic approaches should not only provide new insights for the function of known factors but also accelerate the discovery of new elements to fully map the sequence of events that lead from N perception to genome-wide expression changes to physiological, morphological, and/or developmental responses in plants. Our view is that systems approaches will be key in the coming years to understanding how the different mechanisms elicited by N nutrients and metabolites are integrated at the cell, organ, and organism level for optimal plant growth and development in a changing environment.

Acknowledgements

We would like to thank Dr Nigel Crawford, Dr Alain Gojon, Felipe Aceituno, and Pamela Naulin for critical reading and helpful comments. Research in RAG's laboratory in this area is supported by grants from FONDECYT (1060457), ICGEB (CRPCHI0501), and ICM-MIDEPLAN (MN-PFG P06-009-F). We apologize to the authors of many publications that we were unable to cite owing to space constraints.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Zhang H, Forde BG: **An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture**. *Science* 1998, **279**:407-409.

2. Walch-Liu P, Neumann G, Bangerth F, Engels C: **Rapid effects of nitrogen form on leaf morphogenesis in tobacco**. *J Exp Bot* 2000, **51**:227-237.

3. Alboresi A, Gestin C, Leydecker MT, Bedu M, Meyer C, Truong HN: **Nitrate, a signal relieving seed dormancy in Arabidopsis**. *Plant Cell Environ* 2005, **28**:500-512.

4. Stitt M, Muller C, Matt P, Gibon Y, Carillo P, Morcuende R, Scheible WR, Krapp A: **Steps towards an integrated view of nitrogen metabolism**. *J Exp Bot* 2002, **53**:959-970.

5. Hsieh MH, Lam HM, van de Loo FJ, Coruzzi G: **A PII-like protein in Arabidopsis: putative role in nitrogen sensing**. *Proc Natl Acad Sci U S A* 1998, **95**:13965-13970.

6. Ferrario-Méry S, Bouvet M, Leleu O, Savino G, Hodges M, Meyer C: **Physiological characterisation of Arabidopsis mutants affected in the expression of the putative regulatory protein PII**. *Planta* 2005, **223**:28-39.

7. Menand B, Desnos T, Nussaume L, Berger F, Bouchez D, Meyer C, Robaglia C: **Expression and disruption of the Arabidopsis TOR (target of rapamycin) gene**. *Proc Natl Acad Sci U S A* 2002, **99**:6422-6427.

8. • Zhang H, Rong H, Pilbeam D: **Signalling mechanisms underlying the morphological responses of the root system to nitrogen in *Arabidopsis thaliana***. *J Exp Bot* 2007, **58**:2329-2338.
An excellent review on root morphological responses to N supply.

9. Coruzzi G, Bush DR: **Nitrogen and carbon nutrient and metabolite signaling in plants**. *Plant Physiol* 2001, **125**:61-64.

10. • Sakakibara H, Takei K, Hirose N: **Interactions between nitrogen and cytokinin in the regulation of metabolism and development**. *Trends Plant Sci* 2006, **11**:440-448.
This is an excellent review that summarizes the known interactions between nitrate and cytokinin signaling in gene regulation and developmental processes.

11. Forde BG: **The role of long-distance signalling in plant responses to nitrate and other nutrients**. *J Exp Bot* 2002, **53**:39-43.

12. Miller AJ, Fan X, Orsel M, Smith SJ, Wells DM: **Nitrate transport and signalling**. *J Exp Bot* 2007, **58**:2297-2306.

13. Schachtman DP, Shin R: **Nutrient sensing and signaling: NPKS**. *Annu Rev Plant Biol* 2007, **58**:47-69.

14. Crawford NM: **Nitrate: nutrient and signal for plant growth**. *Plant Cell* 1995, **7**:859-868.

15. Stitt M: **Nitrate regulation of metabolism and growth**. *Curr Opin Plant Biol* 1999, **2**:178-186.

16. Wang R, Okamoto M, Xing X, Crawford NM: **Microarray analysis of the nitrate response in Arabidopsis roots and shoots reveals over 1000 rapidly responding genes and new linkages to glucose, trehalose-6-phosphate, iron, and sulfate metabolism**. *Plant Physiol* 2003, **132**:556-567.

17. Wang R, Tischner R, Gutierrez RA, Hoffman M, Xing X, Chen M, Coruzzi G, Crawford NM: **Genomic analysis of the nitrate response using a nitrate reductase-null mutant of Arabidopsis**. *Plant Physiol* 2004, **136**:2512-2522.

18. Scheible WR, Morcuende R, Czechowski T, Fritz C, Osuna D, Palacios-Rojas N, Schindelasch D, Thimm O, Udvardi MK, Stitt M: **Genome-wide reprogramming of primary and secondary metabolism, protein synthesis, cellular growth processes, and the regulatory infrastructure of Arabidopsis in response to nitrogen**. *Plant Physiol* 2004, **136**:2483-2499.

Please cite this article in press as: Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis, Curr Opin Plant Biol (2008), doi:10.1016/j.pbi.2008.07.003

19. Gutierrez RA, Stokes TL, Thum K, Xu X, Obertello M, Katari MS, •• Tanurdzic M, Dean A, Nero DC, McClung CR *et al.*: **Systems approach identifies an organic nitrogen-responsive gene network that is regulated by the master clock control gene CCA1**. *Proc Natl Acad Sci U S A* 2008, **105**:4939-4944.
Using a glutamine synthetase inhibitor, the authors distinguish global gene responses to organic N from inorganic N. Using a systems approach the authors discover a novel organic N regulatory network that links N assimilation and the circadian clock in plants.

20. Wang R, Xing X, Crawford N: **Nitrite acts as transcriptome signal •• at micromolar concentrations in Arabidopsis roots**. *Plant Physiol* 2007, **145**:1735-1745.
The authors analyze genome-wide responses to nitrite, discovering that nitrite is a signal for regulation of gene expression at least as potent as nitrate. Evidence is provided to support the idea of a nitrite signaling system.

21. Gutierrez RA, Lejay L, Dean A, Chiaromonte F, Shasha DE, • Coruzzi GM: **Qualitative network models and genome-wide expression data define carbon/nitrogen-responsive molecular machines in Arabidopsis**. *Genome Biol* 2007, **8**:R7.
A systems approach is used to study nitrogen and carbon responses for the first time in Arabidopsis. A qualitative multinetwork model of the Arabidopsis metabolic and regulatory network is constructed and used to query quantitative microarray data obtained from treating Arabidopsis roots with combinations of sucrose and nitrate. Specific interacting genes in biological modules that respond to carbon and nitrogen treatments are identified.

22. Palenchar PM, Kouranov A, Lejay LV, Coruzzi GM: **Genome-wide patterns of carbon and nitrogen regulation of gene expression validate the combined carbon and nitrogen (CN)-signaling hypothesis in plants**. *Genome Biol* 2004, **5**:R91.

23. Price J, Laxmi A, St Martin SK, Jang J-C: **Global transcription profiling reveals multiple sugar signal transduction mechanisms in Arabidopsis**. *Plant Cell* 2004, **16**:2128-2150.

24. Gifford ML, Dean A, Gutierrez RA, Coruzzi GM, Birnbaum KD: **Cell-•• specific nitrogen responses mediate developmental plasticity**. *Proc Natl Acad Sci U S A* 2008, **105**:803-808.
A cell sorting approach is used to analyze for the first time cell-specific nitrate responses in Arabidopsis. A nitrate-responding biological module including miR167, its target, ARF8, and 126 potential ARF8 targets is identified in pericycle cells. The authors show this module controls lateral root initiation.

25. Wang R, Guegler K, LaBrie ST, Crawford NM: **Genomic analysis of a nutrient response in Arabidopsis reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate**. *Plant Cell* 2000, **12**:1491-1510.

26. Huppe HC, Turpin DH: **Integration of carbon and nitrogen metabolism in plant and algal cells**. *Annu Rev Plant Physiol Plant Mol Biol* 1994, **45**:577-607.

27. Walch-Liu P, Liu L-H, Remans T, Tester M, Forde BG: **Evidence • that l-glutamate can act as an exogenous signal to modulate root growth and branching in *Arabidopsis thaliana***. *Plant Cell Physiol* 2006, **47**:1045-1057.
Authors show that exogenous L-Glu is able to elicit complex morphological changes in the Arabidopsis root system, pointing at a signaling role of Glu that is independent of Arabidopsis Glu receptors and probably involves interaction with the auxin signaling pathway.

28. Lam HM, Chiu J, Hsieh MH, Meisel L, Oliveira IC, Shin M, Coruzzi G: **Glutamate-receptor genes in plants**. *Nature* 1998, **396**:125-126.

29. Kang J, Turano FJ: **The putative glutamate receptor 1.1 (AtGLR1.1) functions as a regulator of carbon and nitrogen metabolism in *Arabidopsis thaliana***. *Proc Natl Acad Sci U S A* 2003, **100**:6872-6877.

30. Forde BG, Lea PJ: **Glutamate in plants: metabolism, regulation, and signaling**. *J Exp Bot* 2007, **58**:2339-2358.

31. Bi YM, Wang RL, Zhu T, Rothstein SJ: **Global transcription profiling reveals differential responses to chronic nitrogen stress and putative nitrogen regulatory components in Arabidopsis**. *BMC Genomics* 2007, **8**:281.

32. Peng M, Bi YM, Zhu T, Rothstein SJ: **Genome-wide analysis of Arabidopsis responsive transcriptome to nitrogen limitation and its regulation by the ubiquitin ligase gene *NLA***. *Plant Mol Biol* 2007, **65**:775-797.

33. Poultney CS, Gutierrez RA, Katari MS, Gifford ML, Paley WB, Coruzzi GM, Shasha DE: **Sungear: interactive visualization and functional analysis of genomic datasets**. *Bioinformatics* 2007, **23**:259-261.

34. Gutierrez RA, Gifford ML, Poultney C, Wang R, Shasha DE, Coruzzi GM, Crawford NM: **Insights into the genomic nitrate response using genetics and the Sungear Software System**. *J Exp Bot* 2007, **58**:2359-2367.

35. Gutierrez RA, Shasha DE, Coruzzi GM: **Systems biology for the virtual plant**. *Plant Physiol* 2005, **138**:550-554.

36. Gan Y, Filleur S, Rahman A, Gotensparre S, Forde B: **Nutritional regulation of *ANR1* and other root-expressed MADS-box genes in *Arabidopsis thaliana***. *Planta* 2005, **222**:730-742.

37. Liao WL, Ramon AM, Fonzi WA: ***GLN3* encodes a global regulator of nitrogen metabolism and virulence of *C. albicans***. *Fungal Genet Biol* 2008, **45**:514-526.

38. Bi YM, Zhang Y, Signorelli T, Zhao R, Zhu T, Rothstein S: **Genetic analysis of Arabidopsis GATA transcription factor gene family reveals a nitrate-inducible member important for chlorophyll synthesis and glucose sensitivity**. *Plant J* 2005, **44**:680-692.

39. Yanagisawa S, Akiyama A, Kisaka H, Uchimiya H, Miwa T: **Metabolic engineering with Dof1 transcription factor in plants: Improved nitrogen assimilation and growth under low-nitrogen conditions**. *Proc Natl Acad Sci U S A* 2004, **101**:7833-7838.

40. Girin T, Lejay L, Wirth J, Widiez T, Palenchar PM, Nazoa P, Touraine B, Gojon A, Lepetit M: **Identification of a 150 bp *cis*-acting element of the *AtNRT2.1* promoter involved in the regulation of gene expression by the N and C status of the plant**. *Plant Cell Environ* 2007, **30**:1366-1380.

41. Fujii H, Chiou T-J, Lin S-I, Aung K, Zhu J-K: **A miRNA involved in phosphate-starvation response in Arabidopsis**. *Curr Biol* 2005, **15**:2038-2043.

42. Jones-Rhoades MW, Bartel DP: **Computational identification of plant microRNAs and their targets, including a stress-induced miRNA**. *Mol Cell* 2004, **14**:787-799.

43. Bari R, Pant BD, Stitt M, Scheible W-R: **PHO2, micro RNA399 and PHR1 define a phosphate signaling pathway in plants**. *Plant Physiol* 2006, **141**:988-999.

44. Gao P, Xin Z, Zheng ZL: **The OSU1/QUA2/TSD2-encoded putative methyltransferase is a critical modulator of carbon and nitrogen nutrient balance response in Arabidopsis**. *PLoS ONE* 2008, **3**:e1387.

45. Peng M, Hannam C, Gu H, Bi YM, Rothstein SJ: **A mutation in *NLA*, which encodes a RING-type ubiquitin ligase, disrupts the adaptability of Arabidopsis to nitrogen limitation**. *Plant J* 2007, **50**:320-337.

46. Takei K, Takahashi T, Sugiyama T, Yamaya T, Sakakibara H: **Multiple routes communicating nitrogen availability from roots to shoots: a signal transduction pathway mediated by cytokinin**. *J Exp Bot* 2002, **53**:971-977.

47. Takei K, Ueda N, Aoki K, Kuromori T, Hirayama T, Shinozaki K, Yamaya T, Sakakibara H: **AtIPT3 is a key determinant of nitrate-dependent cytokinin biosynthesis in Arabidopsis**. *Plant Cell Physiol* 2004, **45**:1053-1062.

48. Sakakibara H: **Nitrate-specific and cytokinin-mediated nitrogen signaling pathways in plants**. *J Plant Res* 2003, **116**:253-257.

49. Walch-Liu P, Ivanov II, Filleur S, Gan Y, Remans T, Forde BG: **Nitrogen regulation of root branching**. *Ann Bot (Lond)* 2006, **97**:875-881.

50. Forde BG: **Local and long-range signaling pathways regulating plant responses to nitrate**. *Annu Rev Plant Biol* 2002, **53**:203-224.

51. Bhalerao RP, Eklof J, Ljung K, Marchant A, Bennett M, Sandberg G: **Shoot-derived auxin is essential for early lateral**

Please cite this article in press as: Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis, Curr Opin Plant Biol (2008), doi:10.1016/j.pbi.2008.07.003

root emergence in Arabidopsis seedlings. *Plant J* 2002, **29**:325-332.

52. Signora L, De Smet I, Foyer CH, Zhang H: **ABA plays a central role in mediating the regulatory effects of nitrate on root branching in Arabidopsis**. *Plant J* 2001, **28**:655-662.

53. De Smet I, Signora L, Beeckman T, Inze D, Foyer CH, Zhang H: **An abscisic acid-sensitive checkpoint in lateral root development of Arabidopsis**. *Plant J* 2003, **33**:543-555.

54. Forsberg H, Ljungdahl PO: **Sensors of extracellular nutrients in *Saccharomyces cerevisiae***. *Curr Genet* 2001, **40**:91-109.

55. Malamy JE, Ryan KS: **Environmental regulation of lateral root initiation in Arabidopsis**. *Plant Physiol* 2001, **127**:899-909.

56. 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 U S A* 2005, **102**:13693-13698.

lin1, a mutant insensitive to high carbon/nitrogen ratio inhibition of lateral root development is identified as carrying a mutation in the *NRT2.1* gene. The authors show that lateral root development is induced in the mutants relative to WT plants independently of nitrate external supply. This work suggests NRT2.1 is involved in sensing nitrate.

57. 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**:909-921.

Using a split-root system, where one side of the root system is grown in high nitrate concentration and the other side is grown in low nitrate concentration to emulate soil nitrate patchiness, the authors show that NRT1.1 is involved in lateral root elongation in nitrate rich patches. Evidence provided indicates NRT1.1 works upstream of *ANR1*. This response is independent of nitrate transport by NRT1.1, suggesting NRT1.1 is involved in sensing nitrate availability.

58. Krouk G, Tillard P, Gojon A: **Regulation of the high-affinity NO_3^- uptake system by NRT1.1-mediated NO_3^- demand signaling in Arabidopsis**. *Plant Physiol* 2006, **142**:1075-1086.

59. Liu K-H, Huang C-Y, Tsay Y-F: **CHL1 is a dual-affinity nitrate transporter of Arabidopsis involved in multiple phases of nitrate uptake**. *Plant Cell* 1999, **11**:865-874.

60. Tsay, Chiu, Tsai, Ho, Hsu: **Nitrate transporters and peptide transporters**. *FEBS Lett* 2007, **581**:2290-2300.

61. Remans T, Nacry P, Pervent M, Filleur S, Diatloff E, Mounier E, Tillard P, Forde BG, Gojon A: **The Arabidopsis NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches**. *Proc Natl Acad Sci U S A* 2006, **103**:19206-19211.

62. Walch-Liu P, Forde BG: **Nitrate signalling mediated by the NRT1.1 nitrate transporter antagonises L-glutamate-induced changes in root architecture**. *Plant J* 2008, **54**:820-828.

63. Liu KH, Tsay YF: **Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation**. *EMBO J* 2003, **22**:1005-1013.

64. Zhang H, Jennings A, Barlow PW, Forde BG: **Dual pathways for regulation of root branching by nitrate**. *Proc Natl Acad Sci U S A* 1999, **96**:6529-6534.

Please cite this article in press as: Vidal EA, Gutiérrez RA. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis, Curr Opin Plant Biol (2008), doi:10.1016/j.pbi.2008.07.003