Nitrate regulation of metabolism and growth

Mark Stitt

Recent research shows that signals derived from nitrate are involved in triggering widespread changes in gene expression, resulting in a reprogramming of nitrogen and carbon metabolism to facilitate the uptake and assimilation of nitrate, and to initiate accompanying changes in carbon metabolism. These nitrate-derived signals interact with signals generated further downstream in nitrogen metabolism, and in carbon metabolism. Signals derived from internal and external nitrate also adjust root growth and architecture to the physiological state of the plant, and the distribution of nitrate in the environment.

Introduction

Nitrate fertilisation leads to higher levels of amino acids and protein and increased growth [1,2,3•*,4•*,5], and also to changes in carbon metabolism including increased levels of organic acids and decreased levels of starch [6•*,7••], to changes in phytohormone levels [1,2], and to changes in allocation and phenology including a decreased root-shoot ratio [6••], altered root architecture [1,6•*,8•], and delayed flowering [5], tuber initiation and senescence [1,2]. These far-reaching changes underline the important role played by the signalling mechanisms that regulate metabolism and development in response to the availability of nitrogen.

Signals might be derived from nitrate itself, from metabolites formed during nitrate assimilation, or, more indirectly, as a result of changes in other cell constituents or the overall rate of growth. In addition, signals originating from downstream events may exert negative feed-back regulation on earlier steps in nitrate acquisition. In a given situation, it is likely that several signals interact to condition the observed response. In microbes, nutrients typically induce genes required for their uptake and metabolism. It has been known since the early 1970’s that nitrate uptake [1,3•*,4•,9] and nitrate reductase (NIA) activity [2,10] increase after adding nitrate. The following review asks whether signals derived from nitrate regulate metabolic and physiological processes in higher plants, and then considers how nitrate-signalling interacts with signals generated further downstream in metabolism.

Nitrate as a regulator of genes for primary metabolism

Using mutants with low expression of NIA, the external and internal nitrate concentration can be varied independently of the rate of nitrate assimilation and the resulting changes in the levels of downstream metabolites and growth rate [6•*,7••,11,12,13•,14,15••]. Complementary approaches to identify nitrate-regulated processes include investigating the effect of nitrate on transcript levels in presence of inhibitors of nitrate or ammonium metabolism [12,16••], and investigation of transcripts that increase rapidly after adding nitrate [17–19,20••]. Table 1 summarises genes shown to be induced by nitrate and Figure 1 summarises the relevant metabolic pathways.

Nitrate induces genes encoding the high (NRT2) [13•,14,15••,21] and low (NRT1) [14,15••,22,23] affinity nitrate uptake systems, for nitrate reductase (NIA) [7••,11,12,16••,18], nitrate reductase (NIR) [7••,12,16••], and the enzymes required for ammonium assimilation via the GOGAT pathway (GLN2, GLN1, GLU) [7••,17,24•]. The increase in transcript is accompanied by increased rates of nitrate uptake [13•,15••,21,25*], increased NIA protein and activity [7••,11,12], and increased activity of NIR and glutamine synthetase [7••].

Nitrate assimilation requires synthesis of organic acids, especially α-oxoglutarate which acts as the acceptor for ammonium in the GOGAT pathway, and malate which acts as a counter-anion and substitutes for nitrate to prevent alkalisation (Figure 1). Nitrate leads to a marked increase of transcripts encoding proteins involved in the synthesis of these organic acids (PK, PPC, CS, ICDH-I; see Table 1) [7••], an increase in the activity of the corresponding enzymes [7••], and an accumulation of malate and α-oxoglutarate [7••]. Fumarase, which catalyses a reaction in a section of the tricarboxylic acid cycle that is not required during nitrate assimilation, is not induced by nitrate (A Krapp, W-R Scheible, M Stitt, unpublished data).

Increased synthesis of organic acids requires diversion of carbon from carbohydrate synthesis. Accordingly, nitrate represses AGPS, encoding the regulatory subunit of ADP-glucose pyrophosphorylase, a key enzyme in the starch synthesis pathway [7••], leading to decreased activity of ADP-glucose pyrophosphorylase and a marked depletion of starch ([7••]; W-R Scheible, A Krapp, M Stitt, unpublished data). Interestingly, sucrose phosphate synthase (SPS) is not repressed by nitrate [7••], indicating that sucrose production continues. This may be important, because amino acid export and utilisation requires continued synthesis of sucrose. These results open up new strategies to engineer carbon partitioning to starch accumulation.
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Nitrate and nitrite reduction consume NADH in the cytoplasm and reduced ferredoxin in the plastid, respectively. Reducing equivalents can be provided directly or indirectly by photosynthetic electron transport in leaves in the light, but in the dark and in non-photosynthetic organs they are supplied by respiratory metabolism. Addition of nitrate leads to rapid induction of relevant genes in roots, including the oxidative pentose phosphate pathway enzyme 6-phosphogluconate dehydrogenase [20**], ferredoxin [26**] and ferredoxin:NAD oxidoreductase [19].

Thus, nitrate leads to rapid changes in the levels of a wide range of transcripts encoding enzymes in nitrogen and carbon metabolism. This allows a reprogramming of nitrogen metabolism. Nitrate induces genes required for nitrate accumulation, ammonium accumulation, the synthesis of carbon acceptors like α-oxoglutarate, the synthesis of organic acids like malate to maintain pH balance, and, in non-photosynthetic tissues, also induces genes required to generate redox equivalents during respiratory metabolism. In parallel, nitrate represses genes required for starch synthesis. For abbreviations, see Table 1.

Figure 1

Nitrate-regulated genes in primary metabolism. Nitrate induces genes required for nitrate accumulation, ammonium accumulation, the synthesis of carbon acceptors like α-oxoglutarate, the synthesis of organic acids like malate to maintain pH balance, and, in non-photosynthetic tissues, also induces genes required to generate redox equivalents during respiratory metabolism. In parallel, nitrate represses genes required for starch synthesis. For abbreviations, see Table 1.

Table 1

<table>
<thead>
<tr>
<th>Physiological function</th>
<th>Gene</th>
<th>Protein</th>
<th>Regulated in leaf</th>
<th>Regulated in root</th>
<th>References</th>
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</thead>
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<tr>
<td>Nitrate uptake</td>
<td>NRT1</td>
<td>high affinity NO₃ transporter</td>
<td>+</td>
<td>+</td>
<td>[13*,14,15**,21]</td>
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<tr>
<td></td>
<td>NRT2</td>
<td>low affinity NO₃ transporter</td>
<td>+</td>
<td>+</td>
<td>[14,15**,22,23]</td>
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<tr>
<td>Nitrate assimilation</td>
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<td>nitrate reductase</td>
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<td>+</td>
<td>[7**,11,12,16**,18]</td>
</tr>
<tr>
<td></td>
<td>NII</td>
<td>nitrite reductase</td>
<td>+</td>
<td>+</td>
<td>[7**,11,16**]</td>
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<tr>
<td>Ammonium assimilation</td>
<td>GLN1</td>
<td>plastid glutamine synthetase</td>
<td>+</td>
<td>+</td>
<td>[7**,17]</td>
</tr>
<tr>
<td></td>
<td>GLN2</td>
<td>cytosolic glutamine synthetase</td>
<td>+</td>
<td>+</td>
<td>[7**,17,24**]</td>
</tr>
<tr>
<td></td>
<td>GLU</td>
<td>GOGAT</td>
<td>+</td>
<td>+</td>
<td>[7**]</td>
</tr>
<tr>
<td>Organic acid metabolism</td>
<td>PPC</td>
<td>phosphoenolpyruvate carboxylase</td>
<td>+</td>
<td>+</td>
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<td>cytosolic pyruvate kinase</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>CS</td>
<td>citrate synthase</td>
<td>+</td>
<td>+</td>
<td>[7**]</td>
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<tr>
<td></td>
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<td>NADP-isocitrate dehydrogenase</td>
<td>+</td>
<td>+</td>
<td>[7**]</td>
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<td>Redox metabolism</td>
<td>PGD</td>
<td>6-phosphogluconate dehydrogenase</td>
<td>?</td>
<td>+</td>
<td>[20**]</td>
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<tr>
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<td>FNR</td>
<td>ferredoxin:NAD oxidoreductase</td>
<td>?</td>
<td>+</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>FD</td>
<td>ferredoxin</td>
<td>?</td>
<td>+</td>
<td>[26**]</td>
</tr>
<tr>
<td>Starch synthesis</td>
<td>AGPS</td>
<td>ADP-glucose pyrophosphorylase (regulatory subunit)</td>
<td>–</td>
<td>?</td>
<td>[7**]</td>
</tr>
</tbody>
</table>
and carbon metabolism, to facilitate the assimilation of nitrate and its incorporation into amino acids. It will also contribute to the regulation of metabolism in limiting nitrogen [7••,27•] and in elevated CO₂, where faster growth often leads to a marked decrease of nitrate [27•,28•], in particular it may explain why starch accumulates even though sugars often remain low in these conditions. Further work is needed to investigate if nitrate-derived signals affect the post-translational regulation of pathway metabolism, and whether further cellular processes are also regulated by nitrate, including the posing of photosynthetic electron transport, starch re-mobilisation, storage protein expression, and the synthesis of secondary metabolites.

Interaction with downstream events in nitrogen metabolism

Nitrate-signalling interacts with signals generated further downstream in nitrogen metabolism and carbon metabolism. This is illustrated by the three general observations. First, whereas nitrate addition leads to a transient induction of genes involved in nitrate uptake and assimilation and organic acid synthesis in wild-type plants, it results in a sustained overexpression of these genes in low NIA mutants [7••,12•,13•]. Secondly, whereas wild-type plants show pronounced diurnal changes of transcripts levels encoding enzymes involved in nitrate, ammonium and organic acid biosynthesis and diurnal changes of the corresponding enzyme activities that correlate with diurnal fluctuations of the sugar, amino acid and organic acid pools ([29•]; A Krapp, W-R Scheible, M Stitt, unpublished data), NIA-deficient mutants have constitutive and high levels of these transcripts and enzyme activities throughout the day and night [11,29•]. Third, addition of glutamine, asparagine or other amino acids inhibits nitrate uptake [3•,4•] and nitrate assimilation [12•].

In prokaryotes and fungi, glutamine and α-oxoglutarate (see below) play a dominating role in regulating metabolism. The metabolites involved in downstream nitrogen-signalling have not been characterised as clearly in plants. In leaves, the endogenous level of ammonium or glutamine often correlates negatively with the NIA transcript level [7••,12•,16••,29•,30•], and ammonium or glutamine addition typically lead to a decrease of the transcripts for NRT2 [13•,14,21], NRT1 [15••,23], NIA [12,16••,29•,31] and NII [31]. Inhibitor experiments indicate that glutamine rather than ammonium is the metabolite responsible for the repression of NIA [12•]. Glutamine and asparagine also repress NIA in maize roots [32•].

Two recent studies, however, point towards more a complicated situation. Split-root experiments with castor bean plants indicate that feed-back inhibition of nitrate uptake involves a shoot-derived signal that does not require increased levels of amino acids in the phloem [33•]. In a new approach to manipulate glutamine levels in vivo, where glu-deficient Arabidopsis mutants were transferred from high to low CO₂, the resulting over-accumulation of glutamine was not accompanied by a decrease of NIA transcript [34••]. As there was a small decrease of leaf nitrate, it was proposed that the exogenous glutamine used in previous experiments acts indirectly, by inhibiting nitrate uptake [34••]. Alternative explanations would be that NIA is repressed by a specific glutamine pool which is not affected in the glu mutants, or that the increase of glutamine is compensated for by changes in other unidentified metabolite pools.

Nitrogen metabolism is complicated in leaves of C-3 plants by fluxes of ammonium around the photorespiratory cycle, which can be 10-fold higher than the net rate of nitrate assimilation. It will be interesting to learn how changes of compounds related to ammonium assimilation can be used as a reliable indicators for nitrogen status, when their turnover is dominated by an unrelated process that reflects the rate of photosynthesis and the stomatal aperture.

Interaction with sugar signalling

In many cases, sugars exert complementary effects to nitrate on gene expression. Sugars induce NRT1 and NRT2 [15••], NIA, GLN1, pyruvate kinase, PPC, and ICDH1 [35,36], and stimulate the post-translational activation of NIA [2,32•,37]. The complementary effects of nitrate and sugars are illustrated by the demonstration that both have to be supplied to achieve high rates of nitrate assimilation and amino acid synthesis in detached leaves [38•]. High sucrose stimulates the conversion of nitrate to glutamine and, especially, the conversion of glyceralate-3-phosphate (the immediate product of photosynthesis) to α-oxoglutarate [38•]. When sugars are low they exert a strong negative regulation on NIA expression, that over-rides the signals derived from nitrogen metabolism [39•]. It is not yet known whether these effects of sugars on nitrate metabolism are mediated via the currently discussed signalling mechanisms involving hexokinase, or by other means [35,36].

Interaction with pH

Nitrate assimilation leads to alkalisation, and ammonium assimilation leads to acidification. Whereas roots can exchange protons directly or indirectly with the soil, assimilation of nitrate in leaves requires the synthesis and export of malate to the roots, where it is decarboxylated. Intriguingly, alkalisation leads to post-transcriptional inactivation of NIA [40••]. The mechanism involves phosphorylation and binding of 14-3-3 proteins, which is reminiscent of the mechanism for activation of the plastidial ATPase [37]. It will be fascinating to learn more about the regulatory interactions between cellular pH, organic acid metabolism, and nitrogen metabolism. Indeed, changes in pH might provide a far more pressing reason for rapid regulation of nitrate and ammonium metabolism than accumulation of ammonium, glutamine and other amino acids, as these can accumulate to relatively high levels in plants without harmful effects.
Nitrate and the regulation of shoot-root allocation and root architecture

It has been known for a long time that root growth and architecture is modified by nitrogen fertilisation. High nitrate preferentially inhibits root growth, leading to a decrease of the root:shoot ratio [1,41] and decreasing the frequency of lateral roots [1,42]. Local application of nitrate to roots of nitrogen-limited plants, on the other hand, leads to localised proliferation of lateral roots [43] (Figure 2). As a consequence, nitrogen limited plants forage a larger soil volume for nitrogen-containing nutrients, and direct their root growth in response to the spatial distribution of nutrients in the soil. Recent research is revealing that signals derived from nitrate trigger these adaptive changes in root growth and architecture.

NIA-deficient tobacco transformants growing on high nitrate develop an abnormally high shoot:root ratio [6**], even though they are severely deficient for amino acids and protein. The inhibition of root growth involves a decrease in the number of growing lateral roots [8*], correlates with nitrate accumulation in the plant, and was shown, in split-root experiments, to be due to an unidentified signal generated in the shoot [6**]. High external nitrate also leads to a decrease in the number of growing lateral roots in Arabidopsis, especially in NIA-deficient mutants [44**,45**].

When NIA-deficient Arabidopsis is grown on low nitrate, and nitrogen supplied to a small region of the root, nitrate leads to proliferation of lateral roots at this site, whereas other nitrogen sources were ineffective [44**]. Similar results were obtained in split-root experiments with NIA-deficient tobacco, where it was also shown that amino acids and proteins decreased in the root sector where growth had been stimulated [6**].

Thus, signals derived from internal and external nitrate interact to modulate root growth and architecture, and allow efficient exploitation of the nutrient supply in the atmosphere. The role of nitrate in regulating other important whole plant processes like flowering and senescence still has to be investigated. It is also intriguing that hypernodulating mutants have been identified where the increased nodule number is due to loss of a shoot-dependent nitrate-repression of module formation, and also show a pleiotropic increase of root lateral frequency when they are grown in the absence of Rhizobium [46–49].

Mechanisms for nitrogen signalling in prokaryotes, fungi and plants

The molecular mechanism(s) of nitrate-sensing in higher plants are still unknown. It can be anticipated, however, that several mechanisms are involved. The cytosolic nitrate concentration is maintained constant [50,51*] whereas high concentrations of nitrate are accumulated in and re-mobilised from the vacuole. In leaves, the levels of nitrate-induced transcripts like NIA decrease after illumination, and re-fertilisation with high nitrate concentrations later in the light period leads to re-induction of NIA, even though the overall pool of nitrate remains high throughout the day [30*]. It, therefore, appears necessary to postulate separate sensing systems to monitor incoming nitrate, to maintain a nitrate homeostasis in the cytosol and, possibly, to monitor vacuolar nitrate.

In bacteria, the best-characterised nitrate-sensing mechanism regulates an operon that contains genes for a set of electron transport components and an anaerobiosis-specific NIA which catalyses electron transfer to a nitrate terminal acceptor. Very low concentrations of extracellular nitrate are sensed via two functionally overlapping sensors NARX/NARQ and NARL/NARP [52,53]. Nitrate or nitrite lead to transfer of phosphate from the autophosphorylating histidine kinase sensor components (NARX, NARQ) to the corresponding DNA-binding response element (NARL, NARP). Homologous systems occur in higher plants, but it is not known any if any of them represent functional homologs for nitrate-sensing. Intriguingly (see below), several are induced by nitrate.

Carbon–nitrogen interactions are regulated via sensing of downstream metabolites in E. coli. Glutamine and α-oxoglutarate are sensed in E. coli via a cascade involving the bifunctional uridylic transferase/uridyl removing enzyme (UT–UR), the regulatory PII protein that is modulated by uridylation and deuridylation, the bifunctional kinase/phosphatase NR1I and the transcription factor NR1. This cascade exerts transcriptional and post-translational regulation on glutamine synthetase. UT–UR and PII probably represent the sensor, as they are constitutively expressed [54], and their activity is regulated by metabolites [55,56]. There is recent evidence in E. coli and other species [57,58] including cyanobacteria [59], however, for a
PII homolog that is transcriptionally regulated by metabolites. The cyanobacterial PII homolog is post-translationally regulated by phosphorylation instead of uridylation, and may be involved in the post-transcriptional regulation of nitrate uptake [60]. A nuclear-encoded PII homolog (GLB1) was recently identified in Arabidopsis [61••]. GLB1 lacks the uridylation site at Tyr-51 and, in analogy to other newly discovered PII homologs, is induced by sucrose and repressed by glutamine, asparagine and aspartate. GLB1 is located in the plastid, indicating a role in sensing or regulating ammonium assimilation in the plastid, and raising fascinating problems with respect to the communication between the plastid and the nucleus.

In filamentous fungi [62,63], nitrate reductase (here designated NIT3) expression is regulated by two positive acting transcription factors (NIT4 and NIT2 in Neurospora crassa, NIRA and AREA in Aspergillus nidulans). NIT4/NIRA is a member of the GAIA family and binds to an unusual asymmetrical nucleotide sequence [64] to mediate a pathway-specific induction of nitrate uptake and NIT3. NIT2/AREA is member of the GATA family of transcription factors and acts positively in the absence of preferred nitrogen sources like ammonium and glutamine to induce genes required to utilise alternative nitrogen sources like nitrate, and to catabolise amino acids. When preferred nitrogen sources are present these pathways are repressed, because NIT2/AREA is inactivated. An NIT2 homolog positively regulates expression of nitrate transport and assimilation in the absence of ammonium in Chlamydomonas [65]. The function of a tobacco NIT2 homolog [66] is still unclear. Recently, NIT2 binding sequences have been reported in the spinach NII promoter [67••], and two further putative transcription factors designated RGA1 and RGA2 have been isolated via functional complementation of a GLN3-deficient yeast mutant (the yeast homolog of AREA/NIT2) [68••]. As discussed in [69], these two genes are identical with GAI and RGA1, that negatively modulate gibberellin-induced changes during development.

The repertoire of sensing mechanisms will probably grow. In Chlamydomonas, the nitrate-induction of genes encoding nitrate and nitrite transporter components is modified by constitutive expression of NIA [70], probably due to regulation exerted by nitrite (E Frenandez, personal communication). Two novel regulatory genes RGA1 and RGA2 required for the ammonium modulation of NIA expression have also been identified in Chlamydomonas [71]. One fascinating recent development is the realisation that glutamine-tRNA isoform acts as a indicator for nitrogen source quality in yeast, and regulates the uptake and use of non-preferred nitrogen sources and sporulation independently of changes in the rate of protein synthesis [72•]. Transplant proteins can also act in yeast [73–76].

Recent studies have elucidated novel downstream signalling components in nitrate-signalling pathways in higher plants. It has been demonstrated, using reverse genetics, that a nitrate-induced transcription factor ANR1 plays an important role in the stimulation of lateral root growth by localised application of nitrate, and in the inhibition of lateral root growth by high nitrate [44••]. ANR1 encodes a MADS-box transcription factor, a family of proteins that act as molecular switches during development, and represents the first example where expression is environmentally regulated. The stimulatory and inhibitory effects of nitrate on lateral root growth are exerted just after the lateral initials emerge from the primary root [45••], and are blocked in the axr4 auxin-resistant mutants [45••], indicating a fascinating cross-talk with hormonal regulation of root development. Lateral root growth clearly provides an exciting model system for molecular analysis of an interplay between an endogenous developmental programme and signals that modulate it in response to the physiological state of the plant and the environmental conditions.

Recent work on nitrate-induced gene expression in maize [24••,77••,78] is providing information about downstream components in two further nitrogen-signalling pathways. Nitrate addition to detached leaves rapidly induces NIA, NII, GS2, ferredoxin-dependent-GOGAT and NADH-dependent-GOGAT [24•], whereas PPC (which in a C-4 plant is essential for photosynthesis as well as net organic acid synthesis) is not induced by nitrate addition to detached leaves, but is induced when nitrate or ammonium are added to the roots of whole plants [77••]. The nitrate-specific induction of the nitrate assimilation pathway in leaves may involve calcium and protein phosphorylation [24•] via protein kinase C [78]. Induction of PPC in whole plants, on the other hand, is associated with increased levels of cytokinins in the xylem sap and an increase of the transcript for a cytokinin induced protein (pZmCIP1) in leaves [77••]. The increase of the PPC and pZmCIP1 transcripts could be mimicked by feeding cytokinins to detached leaves [77••]. pZmCIP1 encodes a nitrogen- and cytokinin-induced homolog of the response element of bacterial two component signalling systems. A small family of response regulator proteins (ARR3–ARR7) showing a similar cytokinin- and nitrate-dependent expression has recently been identified in Arabidopsis [79••]. It will be fascinating to learn which endogenous ligands interact with these cytokinin and nitrogen-induced sensors, and what phenotypes result when their expression is disrupted.

Thus, although molecular analysis of nitrate-sensing in plants is in its infancy, there are tantalising hints of interactions with fundamental processes in plant metabolism and development. Whereas nitrate has only been implicated in pathway-specific regulation in bacteria and fungi, it may have a more far-reaching role in higher plants. This may reflect the importance of nitrate as a nitrogen source for many plants and habitats, and inherent differences between plants and microbes including their ability to store large amounts of nitrate, possible complicating effects of photorespiration, and the need to modulate allocation and development in a multi-cellular
organism. Although progress will certainly draw on analyses of nitrogen-signalling in microbes, appropriate and novel approaches will also be needed in higher plants. The recent advances in identifying a wider range of nitrate-regulated genes and nitrate-dependent changes in plant growth and development provide important tools to address these problems, as well the application of multi-parallel gene expression, and the exploitation of insertion mutants and other novel sources of genetic variability including activation-tagged lines and the natural biological diversity available in ecotypes and inbred recombinant lines.

Conclusions

Nitrate sensing provides one of the mechanisms whereby plants sense nitrogen availability in the environment and their internal nitrogen status. At a cellular level, it leads to reprogramming of metabolism to allow nitrate to be assimilated and incorporated into organic compounds, and at a whole plant level it modulates allocation and development to allow root growth and nutrient uptake to respond to spatial and temporal fluctuations in the availability of nitrate. We can expect that molecular analysis of nitrate-signalling will provide further important insights into the networking of metabolism, and into the mechanisms whereby plant development is modulated by response to changes in the environment.

Acknowledgements

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References and recommended reading

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

• of special interest

** of outstanding interest


See annotation for [*].


Together with [39], two excellent reviews of the classic studies of the physiology of nutrient uptake, and their relation to the recent advances following the cloning of genes encoding nitrate transport systems.


In a sequel to [8*], low-NIA tobacco transformants were grown on nutrient agar at various nitrate supplies in the presence or absence of sucrose. The inhibition of root growth when nitrate accumulates in the plant was accompanied by a marked decrease in the frequency of growing lateral roots, which was not relieved by including sucrose in the medium.


Investigations in wild-type tobacco and low-NIA mutants growing at various nitrogen supplies. The use of mutants and transgenic tobacco transformants showed that NRT2:1Np (encoding a high affinity nitrate transporter) is induced by nitrate, and repressed by ammonium or a product of ammonium metabolism.


Transcript levels for NRT2:1 and NRT1 (encoding a high and low affinity nitrate transporter) and nitrate uptake rates were studied in wild-type Arabidopsis and low-NIA mutants growing at various nitrogen supplies. Diurnal changes and the response to added sugar were also analysed. Nitrate induces both transporters, whereas ammonium or products of ammonium assimilation repress both transporters, and sugars lead to increased expression of both transporters. Further, it is shown that the changes in transcript levels correlate with changes in the rates of nitrate uptake.


Inhibition of nitrate assimilation by addition of tungsten led to increased expression of NIA and NII but did not alter expression of GS2 and GLU, indicating that downstream products of nitrate assimilation exert feed-back control on the nitrate assimilation pathway, but not on the ammonium assimilation pathway.


Addition of nitrate to maize plants leads to a rapid increase of transcript encoding 6-phosphogluconate dehydrogenase via a mechanism that does not require the intervening synthesis of new proteins. Longer treatments lead to an increase of 6-phosphogluconate protein and activity. This is the first report directly linking nitrate to regulation of the oxidative pentose phosphate pathway, which is required to supply reducing equivalents for nitrate assimilation in roots.


Addition of nitrate to maize leaves leads to rapid induction of NIA, NII, plasto-m GLN2 and GLU4, via a mechanism that does not require an intervening expression of new proteins. The effects of chelators, calcium analogs and protein kinase and phosphatase inhibitors indicate a role for Ca2+ and protein phosphorylation in the signalling mechanism.


Increased expression of NIA leads to increased nitrate uptake, whereas decreased expression leads to accumulation of nitrate in the plants. These results indicate that a downstream metabolite of nitrate metabolism regulates nitrate uptake, and provide direct evidence that changes in the rate of nitrate assimilation in the leaves has consequences for the rate of nitrate uptake in the roots. Possible molecular mechanisms for this interaction are presented in [15].


Reduction of nitrate to ammonium in the roots requires reduced ferredoxin for the plastid-localised NIT1 step. This paper shows that nitrate induces a non-photosynthetic ferredoxin genes in maize roots. Together with [20], it shows that nitrate re-programmes root cells to increase the delivery of reduced equivalents in the appropriate form to support nitrate assimilation.


This study of the effect of elevated CO2 on transcripts and activities of enzymes in nitrogen and carbon metabolism, on metabolite levels and growth of plants growing at several nitrogen supplies shows that accumulation of photosynthesis the increased accumulation of starch in elevated CO2 are triggered by changes in the nitrogen status, including nitrate. This challenges the widely-held view that these changes in carbon metabolism are caused by sugar-regulation of gene expression.


It was well established in several species that mutants with a 50–90% decrease in maximum NIA activity grow at similar rates to wild-type plants. This study of tobacco mutants shows that they compensate by altering the diurnal regulation of NIA protein levels and post-translational regulation of NIA, whereas the diurnal changes of NIA transcript levels were not markedly modified. Comparison of the in vivo changes in NIA protein and activation and the endogenous changes in glutamine as well as the effect of feeding exogenous glutamine provide indirect evidence that glutamine or related metabolites exert feed-back regulation on the activation and stability of NIA protein.


NIA transcript typically declines dramatically during the photoperiod, even when the leaves still retain a considerable nitrate pool. One finding in this article is that re-irrigation with a high nitrate concentration late in the photoperiod partially reverses this inhibition, indicating that the influx rather than the total pool of nitrate is critical for the regulation of NIA expression.


In roots of maize seedlings, NIA transcript and NIA activity are increased by sugars and reduced by amino acids including glutamine and asparagine.


It has been widely assumed that amino acids moving in the phloem from the shoot to the roots are responsible for an inhibition of nitrate uptake. This article shows, using a split-root system, that addition of nitrate to one root sector leads to inhibition of nitrate uptake in the other root sector, even though the levels of amino acids in the low-nitrogen sector do not increase.


It has been frequently proposed, on the basis of correlative studies and the effects of inhibitors, that glutamine, or a closely related metabolite, exerts negative feed-back regulation on NIA expression. In this study, Arabidopsis mutants deficient in Fd-GOGAT were grown in the presence of 2% CO2 to suppress photorespiration, and then shifted to air levels of CO2. Despite a large accumulation of glutamine, NIA transcript responded as in wild-type plants. The authors conclude that glutamine does not exert feed-back regulation on NIA, and argue that the repression reported in earlier studies after adding exogenous glutamine may be an indirect effect, due to glutamine inhibiting nitrate uptake.


A provocative review of the role of protein phosphorylation and of 14-3-3 proteins in regulating primary metabolism and proton pumping at the plasmalemma, that highlights possible interactions between carbon metabolism, nitrogen metabolism and pH regulation.


It was well established that sugar addition leads to an increase of NIA transcript and activity in detached leaves, but the situations in which sugars exert...
an over-riding influence on nitrate assimilation were previously unclear. By investigating the effects of an increasingly long dark period in combination with petiole cooling to inhibit phloem export, it is shown that when sugars fall below a critical level (5–10 µmol/g fresh weight) they strongly inhibit NIA expression and activity, over-riding signals derived from nitrate and nitrogen metabolism. Measurements of amino acid levels indicate that sugars also exert a global inhibition on the amino acid biosynthesis pathways.


In studies investigating the stimulatory effect of anaerobiosis on post-translational regulation of nitrate reductase, it was shown that the activation is due to acidification, rather than to changes in the energy status. It is also shown that the decline of NIA activity in prolonged darkness can be prevented by sugar addition.


A MADS-box transcription factor ANR1 was identified in a screen for nitrate-induced changes, and its function investigated in antisense Arabidopsis transformants. The overall frequency of lateral root formation in wild-type Arabidopsis (see also [84] and [87]) decreases when plants are grown in high nitrate, whereas local application of nitrate leads to increased lateral root proliferation at the site of application. Antisense inhibition of ANR1 expression increased the sensitivity with which high nitrate inhibited lateral root proliferation at the site of application. Antisense inhibition of high nitrate, whereas local application of nitrate leads to increased lateral root growth and the rates of nitrate uptake when nitrate is restricted to only one part of the root system. *J Exp Bot* 1976, 26:79-90.

44 Zhang H, Forde BG: *An Arabidopsis MADS-box gene controlling nutrient-induced changes in root architecture*. Science 1998, 279:407-409. A MADS-box transcription factor ANR1 was identified in a screen for nitrate-induced changes, and its function investigated in antisense Arabidopsis transformants. The overall frequency of lateral root formation in wild-type Arabidopsis (see also [84] and [87]) decreases when plants are grown in high nitrate, whereas local application of nitrate leads to increased lateral root proliferation at the site of application. Antisense inhibition of ANR1 expression increased the sensitivity with which high nitrate inhibited lateral root proliferation at the site of application. Antisense inhibition of high nitrate, whereas local application of nitrate leads to increased lateral root growth and the rates of nitrate uptake when nitrate is restricted to only one part of the root system. *J Exp Bot* 1976, 26:79-90.


The mechanisms whereby nitrate modulates lateral root formation are further investigated. High nitrate does not inhibit initiation but, rather, acts at the stage where they emerge from the root, apparently delaying final activation of the meristem. This inhibition is accentuated in low-NIA mutants, indicating that tissue nitrate levels are involved in generating the signal.


51 Van de Leij M, Smith S, Miller AJ: Nitrate accumulation in the vacuole is remobilised after removal of nitrate from the external nutrient medium, and that this remobilisation occurs without the significant decrease of the cytosolic nitrate concentration or NIA activity. As a result, the nitrate concentration in the xylem only decreases gradually after depletion of external nitrate.


This is the first report of a PII protein in plants. It was identified from an EST in *Arabidopsis*, and is shown to be a nuclear encoded protein whose sequence indicates a plastid localisation. It differs from the classical E. coli PII system but resembles several recently reported PII homologs in E. coli and other organisms in showing transcriptional regulation in response to sugars and nitrogen metabolites (see [57-59,60]) rather than being regulated post-translationally via unification/der-unification (see [84-86]).


Following earlier investigations showing that a 130 bp upstream region of the spinach nii promoter suffices to confer nitrate-induction of gus expres- "sion, a 50 bp region was identified by deletion studies that contained two adjacent gca elements, and bound in vitro a fusion protein containing the zinc finger domain of *neurospora crassa* nii2. These results indicate that nii expression is mediated by nitrate-specific binding of trans-acting factors to sequences that are preserved between fungi and plants.


Two Arabidopsis cDNA sequences were identified that are able to functionally complement a yeast gln3 gln1 mutant. GLN3 is related to the positive control genes that mediate repression of nitrate assimilation when prefered nitrogen sources such as ammonium or glutamine are available – *ARA* and *NIT2* in *Aspergillus nidulans* and *Neurospora crassa*, respectively. The *Arabidopsis* genes (termed RGA1 and RGA2, restore growth on ammonium) are members of a multigene family whose other members include SCARECROW that regulates pattern formation in roots. They are identical (see [69]) to GAI and RGA, that are involved in gibberelin signal transduction.


This intriguing article reports that a glutamine tRNA isoform is involved in nitrogen-signalling in yeast. Mutants lacking this isoform impair nitrogen sensing without impairing protein synthesis, leading to pseudohyphal growth, sporulation even in the presence of preferred nitrogen sources.


Addition of nitrate or ammonium to the roots of intact maize plants leads to induction of *PPC* in the leaves, whereas addition of nitrogen sources to the leaves does not. This paper implicates the synthesis of cytokinins in the roots and cytokinin-induction of a response-regulator homolog ZmCIp1 in the leaf signalling pathway in the signalling pathway.


In a sequel to [74], five response regulator homologs are identified in *Arabidopsis* whose expression is increased by addition of nitrate or cytokinins. The identity of the ligand for the response regulators is still unknown, but they could represent a self-amplification loop or a change in sensitivity to further unidentified signalling components.