Nitrate transport: a key step in nitrate assimilation
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The nitrate assimilation pathway has been the matter of intensive research during the past decade. Many genes involved in low and high affinity nitrate uptake have been identified in fungi, algae and, more recently, in plants. The plant genes so far isolated are transcriptionally regulated; their inducibility by nitrate seems to be a common feature, shared by their homologs in fungi and algae. A number of questions remain to be elucidated regarding the physiological roles of these transporters and the regulation of their expression.

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Abbreviations
HATS high affinity transport system
LATS low affinity transport system
NiR nitrite reductase
NR nitrate reductase

Introduction
Nitrate is the major source of nitrogen for the vast majority of plants. It is first reduced to nitrite by nitrate reductase (NR), nitrite being further reduced into ammonium by nitrite reductase (NiR). Nitrate assimilation has been the matter of many studies and has been reviewed by Hoff et al. [1], Crawford [2] and more recently by Daniel-Vedele and Caboche [3] and Campbell [4]. Of particular interest, is the study of the post-transcriptional control of nitrate reductase activity. This enzyme represents a key step in nitrate metabolism which involves phosphorylation.

Nitrate itself also has to be considered as a signal; recent data suggest that nitrate may be a signal for the regulation of carbon metabolism, for example by modulating the expression of genes involved in the biosynthesis of organic acids [5*]. Nitrate also functions as a morphogenetic signal, governing shoot:root balance [6**]. Very recently, the Arabidopsis thaliana ANR1 gene was isolated on the basis of its nitrate inducibility. This gene belongs to the MADS box family, whose members code for transcription factors mostly expressed in flowers, affecting the floral organ identity. ANR, specifically expressed in roots, was shown to be a key determinant of developmental plasticity in A. thaliana roots in response to nitrate [7**].

The duality of nitrate, as both a nutrient and a signal, reinforces the need to increase our knowledge of the two primary steps in nitrate acquisition, namely nitrate sensing and nitrate uptake. In this review, we will begin by describing the physiology of nitrate uptake and then go on to discuss the genes involved in low and high affinity transport, and how nitrate transport and reduction is regulated. We will then highlight the many questions that have been brought to light by recent advances in these areas.

Physiology of nitrate uptake
Nitrate is the common form of inorganic nitrogen in most soils and the available concentration can vary enormously depending on pH and oxygen availability [8]. Active nitrate transport across the plasmalemma of epidermal and cortical cells of the root is the first step in nitrate acquisition and use.

Net uptake of nitrate is the difference between its influx and efflux across the plasma membrane. On the basis of physiological studies of nitrate influx, three different types of electrogenic H+/NO3− symporters have been postulated to exist [9]. At high external nitrate concentration, a low affinity transport system (LATS, Km>0.5 mM) operates and appears to be constitutively expressed and essentially unregulated. At low external concentrations (0–0.5 mM), two high affinity transport systems (HATS, 5<Km<200µM) operate, one of these being constitutive whereas the other is induced by nitrate [10]. Nitrate efflux may also be a substrate-inducible process that requires the synthesis of both RNA and protein(s). Unlike the uptake system, however, the efflux system, once induced, is relatively stable in the presence of inhibitors of RNA and protein synthesis as long as nitrate or nitrite is present [11].

Feedback regulation of nitrate uptake appears to be highly complex, and nitrate is capable of exercising feedback regulation of its own influx [12]. Finally, nitrate uptake is regulated at the whole plant level by nitrogen demand [13], presumably mediated by a signal such as amino acids translocated from shoots to roots.

Genes involved in low affinity nitrate transport (LATS)
Chlorate, a nitrate analog which is toxic when reduced to chlorite by nitrate reductase, has been widely used to select for mutants impaired in nitrate uptake or metabolism. The genes encoding nitrate transporters have been renamed according to the recommended nomenclature: Nrt (nitrate transport) 1 or 2 (for low and high affinity transport, and how nitrate transport and reduction is regulated. We will then highlight the many questions that have been brought to light by recent advances in these areas.

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T-DNA tagged populations allowed Tsay and collaborators [14] to isolate a disrupted allele of Chl1, renamed Nrt1:1At according to the agreed nomenclature [15], and to demonstrate its role in chloride toxicity. Nrt1:1At protein contains 12 putative transmembrane-spanning segments. The expression of Nrt1:1At is preferentially located in root cells, and is pH dependent and nitrate inducible. In addition, expression of the Nrt1:1At protein in Xenopus oocytes exposed to nitrate at acidic pH leads to a depolarization of the membrane potential, which is also the initial response observed when plant cells are exposed to nitrate, to an inward current or to an accumulation of nitrate.

The role of Nrt1:1At in low affinity nitrate uptake is still a matter of debate. Nitrate uptake, measured by nitrate depletion in the medium, is found to be lower in the wild-type and the mutant are similar [16**]. In addition, expression of the Nrt1:1At protein located in root cells, and is pH dependent and nitrate inducible. In contrast, expression of Nrt1:1At is inducible. In addition, expression of the Nrt1:1At protein in Xenopus oocytes exposed to nitrate at acidic pH leads to a depolarization of the membrane potential, which is also the initial response observed when plant cells are exposed to nitrate, to an inward current or to an accumulation of nitrate.

Recently, Nrt1:1At was used as a heterologous probe to isolate homologues from tomato [18]. Interestingly, two different but homologous genes were isolated from a root hair-specific tomato cDNA library: Nrt1:1Le is expressed in root hairs as well as other root tissues under all nitrogen treatments applied and may correspond to a constitutively expressed LAT5. In contrast, Nrt1:2Le mRNA accumulation is restricted to root hairs that had been exposed to nitrate. Functional studies remain to be performed, however, to determine the transport characteristics of these two proteins.

**Genes involved in high affinity nitrate transport (HATS)**

Recent progress made in isolating genes involved in the HATS in higher plants originates from the molecular analysis of nitrate uptake in fungi and algae.

The crnA mutant of Aspergillus nidulans was isolated on the basis of chlorate resistance: this mutant is defective in nitrate uptake at the conidiospore and young mycelium stages. The corresponding gene was isolated [19] and it encodes a 507 amino acid protein, containing 12 putative membrane-spanning domains [20]. In the green alga, Chlamydomonas reinhardtii, restoration of nitrate transport in a mutant strain was achieved by co-transformation with the genes nar-2 and Nrt2:1Cr or nar-2 and Nrt2:2Cr but not with single plasmids containing the individual genes. In addition, in contrast with nar-2 whose function is not yet identified, the deduced amino acid sequences of Nrt2:1Cr and Nrt2:2Cr showed significant similarity with the A. nidulans CRNA protein (Figure 1 [21]). Physiological studies of the complemented strains suggest that three HATS operate in C. reinhardtii: one, encoded by a gene sharing strong similarities with the other members of the family (A Quesada, J Hidalgo, E Fernandez, unpublished data), is specific for nitrite; a second one is encoded by nar2/Nrt2:2Cr specific for nitrate; and a third one encoded by nar2/Nrt2:1Gr, which is bispecific for both anions [22].

CRNA, Nrt2:1Cr and Nrt2:2Cr share a common structural motif of 12 transmembrane α-helical segments and a number of conserved sequence motifs [23]. They belong to the major facilitator superfamily which contains five clusters of transport proteins, including drug-resistant proteins, sugar facilitators, facilitators for Kreb's cycle intermediates, phosphate ester-phosphate antiporters and a distinct group of oligosaccharide-proton symporters [23].

A PCR approach using degenerate oligonucleotides derived from these conserved motifs allowed Truean and collaborators to isolate barley cDNAs (Nrt2:1He and Nrt2:2He) encoding polypeptides similar to the algal genes Nrt2:1Gr and Nrt2:1Gr. They have lesser, but significant, similarity to the CRNA protein (Figure 1). In the case of Nrt1:1At, these genes are expressed preferentially in barley roots and are induced by nitrate [24**]. Recently, a full length cDNA, Nrt2:1Np, was isolated from the dicot species Nicotiana plumbaginifolia, also using a PCR approach [25]. The expression of this gene is root-specific, nitrate-inducible and negatively regulated by nitrogen metabolites. This last observation is especially striking as it suggests that the high affinity transporter is under the same regulatory control operating on nitrate and nitrite reduction. Furthermore, nitrate inducibility of Nrt2:1Np in plants starved of a nitrogen source is only transient; after a rapid initial induction, the Nrt2:1Np level decreases even though the internal nitrate concentration remains high (A Krapp et al., unpublished data). This suggests that the process of nitrate uptake by root cells is tightly controlled by the plant nitrogen status.

In contrast to algae, there is not yet direct evidence for the physiological role of the plant Nrt2 genes. A first interesting correlation, however, comes from the study of a methylammonium resistant mutant of N. plumbaginifolia [26]. This mutant, MeaR, is impaired in the negative feedback regulation of nitrate uptake by methylammonium. It is also impaired in the repression mechanism of Nrt2:1Np expression by methylammonium (A Krapp et al., unpublished data). A Hansenula polymorpha nitrate transport mutant was recently isolated [27] and, consistent
with a nitrate transport function, the Nrt2:1Hc cDNA from barley was able to complement this yeast mutant (M Dunn and B Forde, abstract 682, 5th International Congress of Plant Molecular Biology, Singapore, 21–27 September 1997).

In the future, two parallel approaches should give further insights into the function of the plant Nrt genes. The physiological characterization of transgenic plants specifically overexpressing or underexpressing these genes will be of great interest. Loss-of-function mutations affecting these genes would be extremely useful, but cannot be easily screened for, due to the redundancy of such genes; two copies exist in N. plumbaginifolia [25], and between 7 and 10 related genes are found in barley [24*]. This goal can be achieved in the model species Arabidopsis thaliana, however, using a reverse genetic approach with PCR experiments performed on a T-DNA insertional library. For this purpose, we have recently isolated by differential display a full length cDNA from A. thaliana, Nrt2:1At, which we presume to code for one of the HATS elements (Figure 1; S Filleur, F Daniel-Vedele, unpublished data).

Regulation of nitrate transport and reduction

It has been known for a long time that the nitrate assimilatory pathway is under tight regulation by the available nitrate and reduced nitrogen. Several of the LATS- and HATS-related genes, apart from being root specific, are also inducible by nitrate, and there is evidence that at least one HATS-related gene, Nrt2:1Np, is also repressible by reduced nitrogen ([25], A Krapp et al., unpublished data). This suggests that regulatory mechanisms are shared by components of the transport system and of the assimilatory pathway.

In algae, the Nrg1 and Nrg2 genes are proposed to be responsible for a specific negative control by ammonium (or its derivatives) of the expression of proteins involved in nitrate assimilation, namely NR, NiR and nitrate transport.
transporters [28]. It will be interesting to test whether MeaR loci are related to these regulatory elements.

The search for such regulatory loci has led to the identification of AreA/Nit2 and NirA/Nit4 in fungi [29]. In these organisms, the regulation of NR and NiR genes is governed, on the one hand, by pathway-specific control genes, NirA/Nit4 for A. nidulans and Neurospora respectively, involved in nitrate inducibility. On the other hand, AreA/Nit2, the major positive control genes are involved in N-metabolite repression.

It has turned out to be more of a challenge to identify their structural characteristics to that of NRT1 and NRT2 transport, the beginning of a long story?

Conclusion: genes involved in nitrate transport, the beginning of a long story?

Nrt1 and Nrt2 genes define two classes of membrane proteins, probably involved in low and high affinity nitrate transport respectively. At the molecular level, it is still unclear whether they function alone as nitrate transporters or as part of a more sophisticated transport system involving other polypeptides. A number of questions remain unanswered regarding their physiological roles and their regulation.

What are the molecular components of the constitutive low affinity transport system? Is there an Nrt1 related gene such as the Nrt1:1Le in tomato? In A. thaliana, an Nrt1:1At homolog, NTL1, fits the criteria of a second, constitutive low affinity nitrate transporter [16••]. It has also been shown that Nrt1:1At is able to transport peptides (T Müller, personal communication). Is this latter observation of physiological significance? Finally, are these low affinity transporters also involved in nitrate efflux?

The observation of differential turnover rates for the uptake and efflux does not support this hypothesis, but suggests rather that influx and efflux are independent processes [11]. It would be of great interest to identify the proteins involved in nitrate efflux and to compare their structural characteristics to that of NRT1 and NRT2 proteins.

One class of mutants impaired in constitutive high affinity nitrate transport has been identified [35]. These mutants do not affect inducible, high affinity or constitutive, low affinity nitrate uptake. To which family does the corresponding gene belong?

Is there, as found for algae, a two-component high affinity transport system in plants? If so, does a homolog of the nar-2 gene exist in plants?

Is there a specific role for each copy of the crnA related genes found in N. plumbaginifolia or barley?

A common feature to all these plant genes is their preferential expression in roots. One additional challenge for the future will be identifying the molecular components of nitrate translocation in shoots and its storage in the vacuole.

Note added in proof

The papers referred to in the text as (A Quesada, J Hidalgo, E Fernandez, unpublished data) and (A Krapp et al., unpublished data) have now been accepted for publication [36,37].

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


These authors have investigated the effects of nitrate on carbon and nitrogen metabolism in wild-type tobacco plants and low nitrate reductase expressing transformants. These experiments allowed the distinction between events triggered by nitrate itself and changes produced more indirectly as a result of nitrate assimilation. Nitrate initiates an extensive program of gene expression, resulting in co-ordinate alterations in the activities of enzymes in several metabolic pathways.


Tobacco genotypes with low expression of nitrate reductase resemble a nitrate-deficient wild-type with respect to their growth rates and content of organic nitrogenous compounds, but accumulate high levels of nitrate. Nitrate accumulation in the shoots is accompanied by inhibition of root growth and an increase in the shoot/root ratio.


In the course of screening Arabidopsis roots for nitrate-inducible genes, a cDNA clone was identified that has homology to the MADS Box family of transcription factors. The expression of the gene in antisense orientation leads to an increased sensitivity of lateral roots to nitrate inhibition, when nitrate is ubiquitously supplied, and to an inhibition of lateral root elongation.
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by localized applications of nitrate. These results demonstrate that nitrate acts as a signal in the development of the plant root system.


In vivo uptake measurements show that the amount of nitrate taken up by chl1 mutants was less than that in the wild-type plants and this uptake deficiency was effectively corrected in 3SS:CHL1 transgenic plants of the chl1 mutant. CHL1 mRNA is found primarily in the epidermal cells near the root tip and in cells beyond the epidermis when moving basipetally. The low affinity transport system of Arabidopsis is proposed to be comprised of both inducible and constitutive elements encoded by two different genes.


24. Trueman LJ, Richardson A, Forde B: Molecular cloning of higher plants homologues of the high affinity nitrate transporters of Chlamydomonas reinhardtii and Aspergillus nidulans. Gene 1996, 175:223-231. Degenerate oligonucleotides corresponding to crmA were used in PCR to amplify related sequences from barley root RNA. Two full-length cDNAs were isolated and found to code for hydrophilic proteins with twelve predicted transmembrane domains. NARK from E. coli, CRNA, NRT2:1Cr and barley homologs are members of a subclass within the major facilitator superfamily. The expression of the barley gene was induced by nitrate but not by ammonium. This paper describes for the first time the molecular cloning of putative high affinity nitrate transporters from higher plants.


The expression of two Arabidopsis thaliana cDNAs was found to restore growth on ammonium containing medium of a yeast strain, mutated in both the Gdh1 gene encoding the NADPH-glutamate dehydrogenase and the regulatory Gln3 gene. The two clones are homologous to each other and define, with the SCARECROW gene, a new family of putative transcription factors. Their expression in plants appears to be constitutive with respect to nitrogen source or organ specificity.


33. Peng J, Carol P, Richards DE, King KE, Cowling R, Murphy GP, Harberd NP: The Arabidopsis GAI gene defines a signalling pathway that negatively regulates gibberelins responses. Genes Develop 1997, 11:3194-3205. This reports the molecular cloning of GAI and a closely related gene GRS, both of which are involved in gibberelin signaling. The authors demonstrate that, like the pathway that operates as a negative regulator of the ethylene response, a negative and derepressible regulatory system is the mechanism by which gibberelin regulates plant development. It is rather surprising that the GAI and GRS genes correspond to genes that are able to restore growth on ammonium (RGA1 and RGA2) of a yeast mutant strain [31].


