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Molecular and Developmental Biology of Inorganic Nitrogen Nutrition

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INTRODUCTION

Unique among the major mineral nutrients, inorganic N is available to plants in both anionic and cationic forms (NO_3^- and $NH4^+$, respectively). The relative abundance of these two ions in natural soils is highly variable and to a large degree depends on the relative rates of two microbial processes: mineralisation (the release of NH_4^+ from organic N) and nitrification (the conversion of NH_4^+ to NO_3^-) (Marschner, 1995). In well-aerated soils nitrification is rapid, so that NH_4^+ concentrations are low and NO_3^- is the main N source, but in waterlogged or acidic soils nitrification is inhibited and NH_4^+ accumulates. Most plants (including Arabidopsis) seem to be able to use either form of N, although exceptions to this rule are known (e.g. Kronzucker et al., 1997).

Nitrogen's importance in plant biology extends far beyond its role as a nutrient. It is now clear that several different N compounds, including NO_3^-, NH_4^+ and some of the products of their assimilation, exert strong regulatory effects on both metabolic and developmental pathways (Redinbaugh and Campbell, 1991; Crawford, 1995; Forde and Clarkson, 1999; Stitt, 1999; Zhang and Forde, 2000; Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001). Both the biochemical and the regulatory aspects of inorganic N nutrition with emphasis on Arabidopsis will be considered in this chapter.

NITRATE ASSIMILATION

The assimilation of NO_3^- involves just a few steps: uptake, reduction to nitrite, reduction to NH_4^+ then incorporation into amino acids (Figure 1). Though simple, this pathway often provides the bulk of net nitrogen input, and, as a result, is tightly regulated to insure that NO_3^- assimilation is integrated into overall metabolism and growth of the plant. Many excellent reviews are available on this topic. These include recent reviews on NO_3^- uptake (Crawford and Glass, 1998; Daniel-Vedele et al., 1998; Forde and Clarkson, 1999; Crawford et al., 2000; Forde, 2000; Galvan and Fernandez, 2001; Glass et al., 2001; Williams and Miller, 2001) and on NO_3^- reduction and its regulation (Campbell, 1999; Kaiser et al., 1999; Mendel and Schwarz, 1999; Stitt, 1999; Zimmer and Mendel, 1999; Crawford et al., 2000; Tischner, 2000; Campbell, 2001; Coruzzi and Bush, 2001; MacKintosh and Meek, 2001). In this section, we focus on Arabidopsis after reviewing the major principles of NO_3^- assimilation that have been uncovered by work with plants and eukaryotic algae in general.

Figure 1. Nitrate assimilation pathway. NRT: nitrate transporters; NR: nitrate reductase; NiR; nitrite reductase.

Nitrate uptake

The first step in the assimilation of NO_3^- is usually uptake from the soil solution (reviewed by Glass and Siddiqi, 1995; Crawford and Glass, 1998; Forde and Clarkson, 1999; Galvan and Fernandez, 2001; Glass et al., 2001; Williams and Miller, 2001). Nitrate concentrations vary widely with typical levels in agricultural soils ranging from 1-10 mM. Uptake occurs in the outer cell layers of the root by active transport processes mediated by H^+ cotransport. In other words, the energy stored in the plasma membrane H^+ gradient drives NO_3^- transport into epidermal and cortical cells. Such activity can be followed by measuring the depletion of NO_3^- from the media surrounding roots or by monitoring the changes in electrical potential across the plasma membrane with microelectrodes as NO_3^- uptake coincides with a depolarization of the plasma membrane, i.e. becomes more positive inside (Miller et al., 2001). An excellent and extensive electrophysiological study using Arabidopsis root hairs has been reported (Meharg and Blatt, 1995). Such experiments uncovered two general systems that account for NO_3^- uptake: a high affinity system, whose K_m for NO_3^- ranges from 10-100 μM, and a low affinity system, which typically shows linear or nonsaturable kinetics and is most evident above 500 μM NO_3^-. Both systems show depolarization in response to NO_3^- indicating that both are active and involve cotransport. The high and low affinity systems correspond to the two classical mechanisms, Mechanism I and Mechanism II, respectively, described by Epstein, who first showed ion uptake in plant roots obeyed Michaelis-Menten kinetics (Epstein, 1953; Epstein, 1972).

An important aspect of NO_3^- uptake is that it is regulated (reviewed by Crawford and Glass, 1998; Daniel-Vedele et al., 1998; Forde and Clarkson, 1999; Forde, 2000; Galvan and Fernandez, 2001; Glass et al., 2001; Williams and Miller, 2001). Various environmental and internal signals influence the rate of uptake into roots. Nitrate itself acts as an inducer. When plants are deprived of NO_3^- (either by starving them of nitrogen or by providing them with another form of nitrogen such as NH_4^+ or an amino acid), they retain a basal or constitutive level of NO_3^- uptake that has both high and low affinity components (abbreviated as cHATS and cLATS, respectively). Upon exposure to NO_3^-, uptake rates increase. The characteristics of such induced activity (abbreviated iHATS) indicate that it is a high affinity system distinct from cHATS. In addition to NO_3^- induction, uptake shows feedback inhibition and responds to signals from the shoot. Nitrate uptake decreases when plants are fed NH_4^+ or high levels of NO_3^-. Uptake also responds to circadian rhythms, reduced carbon, and shoot nitrogen demand. A classic example of shoot demand comes from split root experiments where one half of the roots from a single plant are placed in one chamber and the other half are placed in a second chamber (Figure 2). The first half is monitored for NO_3^- uptake in a solution with ample NO_3^-. If the remaining half of the roots is placed in a solution with low NO_3^-, uptake rates increase in the first half. It is thought that the shoots sense a reduction in overall nitrogen availability when the second group of roots is placed in low NO_3^- solutions. The shoots then respond by sending a signal to the roots to increase NO_3^- uptake.

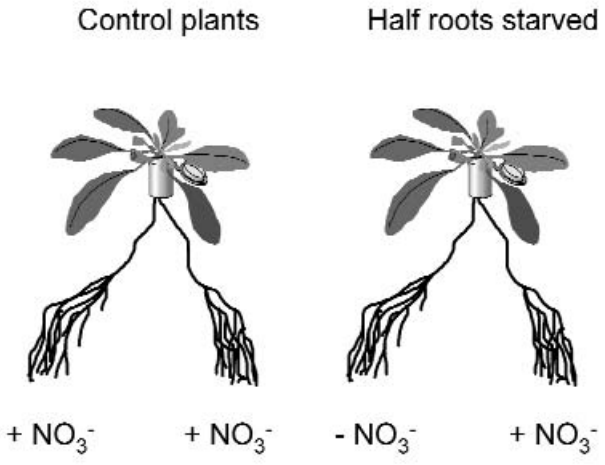


Figure 2. Schematic diagram showing a split-root experiment. Figure was adapted from (Cerezo et al., 2001).

Nitrate reduction

Once NO_3^- has been taken up into the cytosol, it can be (1) mobilized into the xylem for transport to the shoot, (2) transported into the vacuole for storage or (3) reduced to nitrite, a reaction that is catalyzed by NO_3^- reductase (NR), a cytosolic enzyme that uses NAD(P)H for reductant (Campbell, 1999; Campbell, 2001). Nitrate reduction is the first committed step in the NO_3^- assimilation pathway and is regulated at both transcriptional and post-translational levels. Both the NO_3^- reductase genes (NIA) and the NR protein respond to many signals (Crawford, 1995; Kaiser et al., 1999; Stitt, 1999; Tischner, 2000; MacKintosh and Meek, 2001). As is the case for NO_3^- uptake, NO_3^- serves a key inducer that rapidly activates transcription of the NIA

genes. Sucrose, light and cytokinin enhance NIA induction while NH_4^+ and amino acids repress expression. NIA genes also respond to circadian rhythms.

Another well understood mechanism of NR control occurs at the protein level (Kaiser et al., 1999; MacKintosh and Meek, 2001). NR produces nitrite, which is toxic to plants if it accumulates. Nitrite is transported into the chloroplasts in green tissues (plastids in non-green tissues), where it is reduced to NH_4^+ by nitrite reductase (NiR) using reduced ferredoxin. If nitrite reduction is slowed (e.g. caused by a reduction in light, reduced carbon or oxygen), nitrite levels could increase to toxic levels if NR activity is not inhibited. Plants have a rapid and reversible mechanism for doing just this. NR is phosphorylated by one of several kinases on a key serine in the first hinge region, which separates the Mo-cofactor and heme-binding regions of NR. NR has three redox centers (FAD, heme and the Mo-cofactor) that are bound by three distinct regions of the protein (Campbell, 1999; Campbell, 2001). The Mo-cofactor transfers electrons to NO_3^- while the heme shuttles electrons from a FAD prosthetic group to the Mo-factor. If NR is phosphorylated in the hinge 1 region, it can bind a 14-3-3 dimer, which in turn inactivates the enzyme. Removal of the 14-3-3 dimer or dephosphorylation of NR by a phosphatase results in reactivation of the enzyme. Exposure of plant to light, high CO_2 and oxygen levels leads to most of the NR being active while darkness, low CO_2 levels and hypoxia result in most of the NR being inactive.

Nitrate assimilatory mutants of Arabidopsis

In the NO_3^- assimilation field, Arabidopsis was the first plant used for mutant isolation. In the early seventies, a graduate student Fietje Braaksma isolated chlorate resistant mutants (chl) of Arabidopsis in the lab of W.J. Feenstra (Braaksma and Feenstra, 1973). Chlorate is the chlorine analog of NO_3^-, which, when taken up and reduced to chlorite, is toxic to plants. Chlorate resistant mutants had been identified in fungi and were shown to be defective in NO_3^- uptake, reduction and regulation (Cove, 1979; Marzluf, 1981; Crawford and Arst, 1993), but no such mutants had been described in plants. In her initial report, Braaksma described three mutants, two of which were allelic. B1 and B3 (now called *chl1* or *nrt1.1*) had normal levels of NR activity but reduced levels of chlorine accumulation (a measure of ClO_3^- uptake). B2-1 (now called *chl2*) had much reduced NR activity. These data uncovered two genes, one involved in NO_3^- reduction and another in chlorate uptake. After Braaksma and Feenstra's initial publication, additional chl mutants were described in Arabidopsis (Braaksma and Feenstra, 1982; Braaksma and Feenstra, 1982), in cells lines of Nicotiana (Muller and Grafe, 1978) and in barley plants (Kleinhofs et al., 1978). As these and other mutants were characterised in angiosperms, they were all found to fall into two classes: those having mutations in NIA (R) genes and those having mutations in CNX genes needed for the synthesis of the Mo-cofactor (Crawford, 1992; Warner and Kleinhofs, 1992; Crawford and Arst, 1993; Hoff et al., 1994; Mendel, 1997). Both classes have reduced NR activity. No mutations were found in NO_3^- uptake or regulatory genes except for *chl1*.

Further work on the molecular genetics of NO_3^- reduction in Arabidopsis revealed that it had two NIA genes and several CNX genes (Crawford, 1992). The NIA2 gene, which encodes for most of the NR activity in the plant (85-90%), corresponds to the *chl3* locus at position 56 in the middle of chromosome 1 (Crawford et al., 1988; Wilkinson and Crawford, 1991). The NIA1 gene encodes for 10-15% of the NR activity and is located near the end of chromosome 1 at position 116 near the GL2 gene (Cheng et al., 1988; Wilkinson and Crawford, 1993). Both NIA genes are regulated by NO_3^- and circadian rhythms and are expressed in leaves and roots (Cheng et al., 1988; Crawford et al., 1988; Cheng et al., 1991; Pilgrim et al., 1993). Such duplication of genes is found over much of the Arabidopsis genome (Blanc et al., 2000), and the NIA genes provided an early example of this. It is interesting that even though there is genetic redundancy, one can still isolate chlorate resistant mutants in which only the NIA2 gene is inactivated. This is because the two genes have diverged enough so that NIA2 accounts for most of the NR activity. NIA2 (*chl3*) mutants still show some chlorate sensitivity due to NIA1, but they are much less sensitive than wildtype plants. The residual NR activity (10-15% wildtype) from the NIA1 gene is sufficient for normal growth on NO_3^- as the sole nitrogen source (Crawford, 1995). For such a key enzyme, this result was surprising but was found to be true in other plants as well (reviewed in (Crawford, 1995)). When it was discovered that NR is post-translationally regulated, an explanation for this puzzling result arose. In wildtype tobacco plants, the NR is not 100% active when measured in the presence of magnesium but oscillates between 20-90% of full potential activity, which is measured in the presence of EDTA (Scheible et al., 1997a). In NR mutants, the extent of inactivation is reduced so that the reduced NR levels in the mutant are compensated by less inactivation of NR (Scheible et al., 1997a). It is not until NR activity levels drop below 10% of wildtype levels that one observes nitrogen deficiencies in the mutants. In Arabidopsis, a *nia1nia2* double mutant has been described with a deletion of the NIA2 gene and a point mutation in the NIA1 gene that has only 1% of wildtype level of activi-

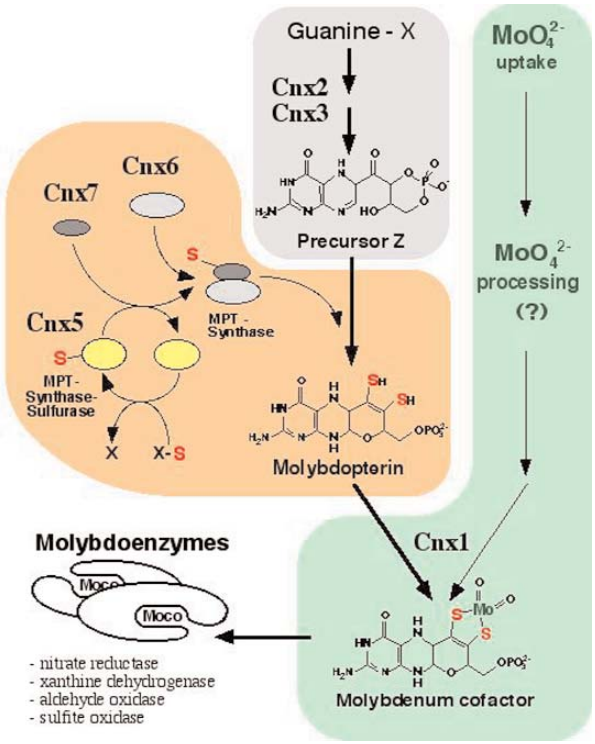


Figure 3. Schematic diagram showing the Moco synthesis pathway. Picture taken from (Mendel, 1997).

ty (Wilkinson and Crawford, 1993). This mutant has grows poorly on NO_3^- alone but does show some growth.

The other class of NR-deficient mutants is cnx. Four cnx mutants have been identified in Arabidopsis: *chl2*, *chl4* (B25), *chl6* (B73) and chl7 (Crawford, 1992). An interesting feature of these mutants is that three of them (*chl2*, *chl6* and *chl7*) are unable to grow on 0.1 mM tungstate, a useful tool for testing the loss of Mo-cofactor (LaBrie et al., 1992). Further work has lead to the identification of six genes required for synthesis of the Mo-cofactor in Arabidopsis (Mendel, 1997) (Figure 3). The early steps convert a guanosine nucleotide into an intermediate called Precursor Z. These steps are mediated by the products of the CNX2 and CNX3 genes (Hoff et al., 1995). Precursor Z is converted into molybdopterin by the CNX5, CNX6 and CNX7 products (Mendel, 1997). Molybdate is then added to molybdopterin by the two domain protein CNX1, which is homologous to the mammalian neuroprotein Gephyrin and binds the cell's cytoskeleton as does Gephyrin (Stallmeyer et al., 1995; Kuper et al., 2000; Schwarz et al., 2000). The CNX1 gene is the same as CHL6 (originally named B73) (Schwarz et al., 2000).

The NRT1 family of nitrate transporters

Unlike NO_3^- reduction, the molecular genetics of NO_3^- uptake had been hampered by the fact that no uptake mutants were known in plants for sometime except for *chl1* in Arabidopsis (in the new nomenclature the gene is called *AtNRT1.1*). Ironically, this was the first chlorate-resistant mutant ever identified in plants, and mutations at this locus are found more frequently than in any other gene in chlorate selections. The original work on *chl1* indicated that it was strongly inhibited in chlorate uptake and partially defective in NO_3^- uptake (Doddema et al., 1978). The latter defect appeared to be a reduction in low affinity but not high affinity NO_3^- uptake activity (Doddema and Telkamp, 1979). Subsequent cloning of the gene from a T-DNA-tagged line revealed that it encoded a hydrophobic protein with twelve membrane spanning regions indicative of a cotransporter (Tsay et al., 1993a). Expression of the CHL1 protein in Xenopus oocytes showed it was a NO_3^- transporter (Tsay et al., 1993a) with, surprisingly, biphasic kinetics (Huang et al., 1996; Liu et al., 1999). These experiments along with studies of NO_3^- uptake in whole plants demonstrated that CHL1 is a dual affinity transporter contributing to both HATS and LATS (Huang et al., 1996; Touraine and Glass, 1997; Wang et al., 1998; Liu et al., 1999). CHL1's contribution, however, depends on the environmental conditions used for plant growth. One key effector is NH_4^+. Overall NO_3^- uptake is reduced when plants are grown with NH_4NO_3 as compared to KNO3; however, CHL1's contribution is the greatest when plants are grown with NH_4^+; that is, *chl1* mutants show the largest reduction in uptake when grown with NH_4^+ than without (Huang et al., 1996; Touraine and Glass, 1997). Another critical parameter is pH. CHL1 is more highly expressed at acidic pHs (Tsay et al., 1993a) and makes a greater contribution to cHATS at pH 5.5 than pH 7.0 (Wang et al., 1998). CHL1 expression also shows diurnal variation, and the reduction in the dark can be reversed by supplying sucrose indicating that carbon signals affect CHL1 (Lejay et al., 1999). Nitrogen metabolites, however, do not appear to repress CHL1 as depriving Arabidopsis of nitrogen does not increase CHL1 expression (Lejay et al., 1999).

Recent experiments have shown that CHL1 expression is targeted to actively growing regions of roots and shoots (Guo et al., 2001). High levels of expression are found in root tips, lateral root primordia, young leaves and flower buds. Little expression over background is found in mature leaves, flowers and the apical meristem. In the mature parts of the root, intermediate expression is found in the stele and little in the outer layers of the root. *chl1* mutants also are defective in growth of nascent organs. Thus, the function of CHL1 is not simply to take up nitrate from the

Table 1. NRT1 family of nitrate transporters

NRT1 Gene	Organism	Substrate	Regulation[a]	Identity[b]	Accession#
AtNRT1.1 (CHL1)	Arabidopsis	NO_3^-	Inducible	100%	L10357
AtNRT1.2 (NTL1)	Arabidopsis	NO_3^-	Constitutive	36%	AF073361
AtNRT1.3 (NTP3)	Arabidopsis	Unknown	Constitutive	49%	AJ131464
AtNRT1.4 (NTP2)	Arabidopsis	Unknown	Constitutive	54%	AJ011604
AtPTR2B	Arabidopsis	Peptides	N/A	39%	L39082
BnNRT1.2	Brassica napus	NO_3^- & basic AA	Inducible	91%	U17987

[a] Response to nitrate. [b]Percent identity to AtNRT1.1 (CHL1) at the amino acid level.
N/A=not applicable

soil by the main body of the root but also to support the growth of nascent and actively growing organs in both roots and shoots.

After cloning CHL1, four additional genes with similar sequences were found in Arabidopsis (Table 1). *AtNRT1.2* (originally named *NTL1*) was cloned by homology to *CHL1* and found to be 36% identical at the amino acid level (Huang et al., 1999). Further work showed that it is a low affinity NO_3^- transporter (K_m of 6 mM in oocytes) that is constitutively expressed in root epidermal and root hair cells. This finding indicates that AtNRT1.2 is a component of cLATS. A second gene AtPTR2B encodes a peptide transporter (Rentsch et al., 1995; Song et al., 1996). In fact, almost all the *CHL1*-related genes which have been described from bacteria, yeast and mammals have been identified as transporters of small oligopeptides including di- and tri-peptides (Steiner et al., 1995; Graul and Sadee, 1997; Crawford and Glass, 1998). Why transporters with specificity to NO_3^- should be related to peptide transporters is not clear. A most interesting protein, NRT1.2 from *Brassica napus*, transports histidine and NO_3^- (Zhou et al., 1998), indicating that this protein retains substrate specificities found in all members of the family. This protein is also the most similar to CHL1 being 91% identical. The entire gene family has been named PRT for peptide transporter and POT for H^+ -dependent oligopeptide transporter. This family was tentatively placed in the Major Facilitator Superfamily (MFS) of transporters (Pao et al., 1998) but now is its own family (Saier, 2000). The PTR and POT names are misleading as they do not include the NO_3^- transporters in the family. Two other genes in this family have been cloned from Arabidopsis and named NTP2 (now called AtNRT1.4) and NTP3 (now called AtNRT1.3) (Hatzfeld and Saito, 1999), but their functions are not known, and they do not appear to be nitrate-regulated (Glass et al., 2001).

The NRT2 family of transporters

The second family of NO_3^- transporter genes (*NRT2*) was first discovered in Aspergillus. A chlorate-resistant mutant called *crnA* was identified with a defect in NO_3^- uptake (Brownlee and Arst, 1983). It was subsequently cloned and shown to be NO_3^-—regulated and NH_4^+-repressed and to encode a transport protein (Unkles et al., 1991). Subsequently, two related genes (NAR-3 and NAR-4) were isolated from Chlamydomonas (Quesada et al., 1994). NAR-3 (CrNrt2.1) was shown to encode a high affinity, bi-specific NO_3^-/NO_2^- transporter while NAR-4 (CrNrt2.2) encodes a high affinity NO_3^-—specific transporter (Galvan and Fernandez, 2001). The NAR-3 protein is about 30% identical to CRNA. Independently, a barley cDNA clone (BCH1) was identified by PCR amplification using degenerate primers corresponding to two conserved motifs found in a small group of MFS transporters that included CRNA (Trueman et al., 1996; Trueman et al., 1996). BCH1 (now called HvNRT2.1) is 32% identical to CRNA and 50% identical to CrNRT2.1. Subsequently, NRT2 genes have been identified in six plant species as well as *Chlamydomonas, Aspergillus, Hansenula and E. coli* (reviewed by Crawford and Glass, 1998; Daniel-Vedele et al., 1998; Forde, 2000; Galvan and Fernandez, 2001; Glass et al., 2001; Williams and Miller, 2001). These genes are found in the NO_3^-/NO_2^- porter (NNP) family within the major facilitator superfamily (Saier, 2000).

In Arabidopsis a total of 7 *NRT2* genes have been found (Table 2) (Forde, 2000; Glass et al., 2001; Okamoto et al., 2001). The first two identified were *AtNRT2.1* and *AtNRT2.2* ((Filleur and Daniel-Vedele, 1999; Zhuo et al., 1999) and Genbank Accession # AF019748 and Z97058). These genes are about 1 kb apart in a tail to tail orientation on chromosome 1 at position 10-12 cM, not far from *CHL1. NRT2*.1 shows strong regulated expression in roots (Filleur and Daniel-Vedele, 1999; Lejay et al., 1999; Zhuo et al., 1999; Cerezo et al., 2001) while little mRNA is detect-

Table 2. Arabidopsis NRT2 family of nitrate transporters

NRT2 Gene	Chromosome	Substrate	Regulation[a]	Identity[b]	Accession#
AtNRT2.1	1	NO_3^-	Inducible	100%	AF019748
AtNRT2.2	1	Unknown	Inducible	87%	AF019749
AtNRT2.3	5	Unknown	Transient Rep.	68%	AB015472
AtNRT2.4	5	Unknown	Constitutive	82%	AB015472
AtNRT2.5	5	Unknown	Constitutive	44%	AL163792
AtNRT2.6	3	Unknown	Constitutive	68%	AL353992
AtNRT2.7	1	Unknown	Repressed	56%	AC012187

[a] Response to nitrate. [b] Percent identity to AtNRT2.1 at amino acid level.
Data for this table was a kind gift of Mamoru Okamoto and Anthony Glass (Okamoto et al., 2001).

ed for *NRT2.2* (Zhuo et al., 1999). *NRT2.1* is induced by NO_3^- or by nitrogen starvation and is repressed by NH_4^+, amino acids (especially arg) and by growth on high levels of NO_3^-. These properties are all characteristic of the inducible HATS system, which is NO_3^-—inducible and feedback repressed by nitrogen metabolites or high NO_3^- levels. NRT2 has been proposed to be a component of the iHATS in plants (reviewed by (Forde, 2000)).

Direct demonstration that *NRT2.1* is component of iHATS has come from mutant and heterologous expression studies. Arabidopsis transgenic lines containing antisense constructs of *AtNRT2.1* (Forde, 2000) and an Arabidopsis deletion mutant removing all of *NRT2.1* and part of *NRT2.2* (Filleur et al., 2001) have been constructed and shown to be defective in HATS but not LATS. Furthermore, the NRT2.1/2.2 mutant is defective in iHATS activities (i.e. in NO_3^-—inducible, starvation-derepressible and NH_4^+-repressible high affinity uptake) (Cerezo et al., 2001). The CRNA protein has been functionally expressed in Xenopus oocytes and found to have two transport mechanisms: a H^+ -coupled mechanism with a K_m for NO_3^- of 24 μM and a passive mechanism with a K_m for NO_3^- of 126 μM (Zhou et al., 2000b). It was suggested that under conditions of high external NO_3^- concentrations the passive mechanism would provide an energetic advantage to the fungus by enabling it to absorb NO_3^- without the energetically expensive coupling to H^+ fluxes. Whether plant NRT2 transporters function in similar ways is still unknown. The Chlamydomonas NRT2.1 protein expresses high affinity NO_3^- transport activity in Xenopus oocytes but only if co-expressed the a second protein (NAR2), indicating that this system requires two components (Zhou et al., 2000a).

Overview of nitrate transporter regulation and function

Additional aspects of overall transporter regulation have emerged from Arabidopsis. Both *CHL1* and *NRT2.1* display diurnal rhythms having low levels of mRNA at night (Lejay et al., 1999). The low levels at night can be enhanced by the addition of sucrose. These two genes are also NO_3^-—responsive but *CHL1* does not display the feedback regulation that *NRT2.1* does as it is not repressed by NH_4^+ treatment nor induced by nitrogen starvation (Filleur and Daniel-Vedele, 1999; Lejay et al., 1999). CHL1 does respond to pH as its mRNA levels are enhanced at more acidic pH (Tsay et al., 1993a).

With all this information, it has become possible to associate a given gene with a NO_3^- uptake system. The two genes for which we have the most physiological data are CHL1 and NRT2.1. Present data indicate that NRT2.1 is a major component of iHATS that would be especially significant when plants are provided low levels or are deprived of nitrogen. The role of CHL1 is more complicated. The expression patterns of this gene and the properties of the CHL1 protein suggest that it could contribute to all three uptake systems in that it is expressed in the absence of NO_3^-, its expression is enhanced by NO_3^- and it has dual affinity uptake kinetics. Measurements of uptake in whole plants have indicated that CHL1 does contribute to cHATS and LATS, but its role in iHATS is controversial (Wang et al., 1998; Liu et al., 1999; Cerezo et al., 2001; Glass et al., 2001). These experiments have also indicated that CHL1's contribution depends heavily on the presence of NH_4^+, which would also repress NRT2.1 expression. In the presence of NH_4^+, *chl1* mutants have significantly reduced

NO_3^- uptake activity compared with wildtype plants for both cHATS and LATS. This is true for plants grown with or without NO_3^-. Thus, unlike NRT2.1, it is difficult to assign CHL1 to one particular system. It is also appears that cHATS, iHATS and LATS are not static but dynamic systems whose composition changes depending on the environmental conditions in which it is grown and on the developmental stage of the plant. Other Arabidopsis transporters that are known to contribute to the NO_3^- uptake systems are NRT2.1, a component of iHATS (Filleur and Daniel-Vedele, 1999; Cerezo et al., 2001; Filleur et al., 2001), NRT1.2, whose properties indicate it is a constitutive component of LATS (Huang et al., 1999), and CHL8, whose gene has not been cloned but which contributes to cHATS (Wang and Crawford, 1996).

Nitrate regulation of gene expression

As can be seen from the above discussion, NO_3^- is not only a substrate for the NO_3^- assimilatory pathway, but it is also a key signal. One of the most studied aspects of NO_3^- signalling is induction of gene expression (reviewed by (Redinbaugh and Campbell, 1991; Crawford, 1995; Koch, 1997; Stitt, 1999; Tischner, 2000; Wang et al., 2000; Coruzzi and Bush, 2001). Many genes involved in different aspects of nitrogen and carbon metabolism are induced by NO_3^-. This effect can be observed by treating plants that are either starved for nitrogen or grown with a reduced source of nitrogen such as NH_4^+ or amino acids. Genes that respond to NO_3^- have been grouped based on their metabolic niche as follows: NO_3^- assimilation, NH_4^+ assimilation, ferredoxin reduction, oxidative pentose phosphate pathway and organic acid synthesis. Nitrate also mobilises carbon into organic acids by suppressing starch synthesis.

The primary NO_3^- assimilatory genes (NIA, NII and NRT) all respond to NO_3^- (Tang and Wu, 1957; Cheng et al., 1986; Crawford et al., 1986; Melzer et al., 1989; Cheng et al., 1991; Gowri et al., 1992; Aslam et al., 1993; Tischner et al., 1993; Koch, 1997; Stitt, 1999; Tischner, 2000). The NIA genes have been the most studied and respond within minutes to NO_3^- at concentrations as low as 10 μM. In addition to NII, there are a group of responsive genes that contribute to nitrite reduction by providing reductant (encoding ferredoxin (Fd), ferredoxin NADP+ oxidoreductase (FNR), 6-phosphogluconate dehydrogenase (6PGDH) and 6-phosphogluconate dehydrogenase (6PGDH) and S-adenosyl-L-methionine-dependent uroporphyrinogen III methyltransferase (UPM1), which makes a prosthetic group for the NiR enzyme) (reviewed by (Stitt, 1999; Wang et al., 2000)). Genes involved in NH_4^+ assimilation, encoding specific isoforms of glutamine synthetase and glutamate synthase, are also induced (reviewed by (Lam et al., 1996; Koch, 1997; Stitt, 1999)). Responsive genes involved in starch and organic acid metabolism encode phosphoenolpyruvate carboxylase (PEPC, involved in organic acid metabolism), which is induced, and ADP-glucose pyrophosphorylase (agpS2, involved in starch synthesis), which is repressed by NO_3^- (Scheible et al., 1997b). Other genes that encode organic acid metabolic enzymes—cytosolic pyruvate kinase (PKc), citrate synthase (CS), and NADP-isocitrate dehydrogenase (ICDH1)—respond to NO_3^- (Scheible et al., 1997bb).

A microarray analysis of NO_3^-—responsive genes has been reported for Arabidopsis (Wang et al., 2000). Using glass slides with approximately 5500 Arabidopsis genes/clones, mRNA from plants treated with low NO_3^- (250 μM) for short times (20 min) and with high NO_3^- (5-10 mM) for longer times (2 hrs) was analysed to detect both rapid response genes and responsive genes in general. Besides detecting many of the genes described above, new responsive genes were identified. Two genes of the nonoxidative branch of the pentose phosphate pathway—transketolase and transaldolase—were induced. Other metabolic genes that were induced included malate dehydrogenase, asparagine synthetase, histidine decarboxylase and two methyltransferases. Several other very interesting genes were induced including a myb transcription factor gene, a tonoplast high capacity calcium antiporter gene CAX1, the senescence-associated gene SAG21 and the nonsymbiotic hemoglobin gene AHB1. Several genes were found to be repressed by NO_3^- including the NH_4^+ transporter gene AMT1;1. The general conclusions that came from this and other studies are the following. First, the most responsive genes are those that are involved directly or indirectly with nitrite reduction (encoding NiR, Fd, FNR, UMP1, G6PDH, 6PGDH, transketolase and transaldolase). Nitrite is toxic to plants so that it makes sense that the genes critical for nitrite reduction would be rapidly induced when NO_3^- is present. Second, different genes show different response patterns indicating that multiple signalling and regulatory events are occurring as plants take up and assimilate NO_3^-. For example, some genes show reduced expression after 2 hrs in low NO_3^- while others do not. Key signals that may be dictating expression levels are NO_3^-, reduced nitrogen and carbon metabolites. As NO_3^- gets depleted by assimilation or is accumulated in the vacuole, some genes will sense the drop in NO_3^- and begin to diminish their expression. In addition, the products of NO_3^- assimilation including NH_4^+ and amino acids, especially glutamine and glutamate, are also thought to feedback repress genes in the pathway (Hoff et al., 1994; Dzuibany et al., 1998; Tischner, 2000). Carbon metabolites sucrose (Cheng et al., 1992) and

malate (Muller et al., 2001) also have been shown to serve as signals.

With such elaborate signalling, it is clear that regulatory networks exist to control NO_3^- assimilation and integrate it with nitrogen and carbon metabolism. Our understanding of these networks is in its infancy. The discussion below is restricted to NO_3^- signalling. Several promoter elements have been identified that are needed for NO_3^- induction of NiR (Rastogi et al., 1993; Rastogi et al., 1997) and NR (Lin et al., 1994; Hwang et al., 1997) genes in tobacco (reviewed by Rothstein and Sivasankar, 1999). With the use of inhibitors, it has been shown that NO_3^- induction is a primary response (i.e. not blocked by protein synthesis inhibitors (Gowri et al., 1992; Redinbaugh and Campbell, 1993; Ritchie et al., 1994; Redinbaugh and Campbell, 1998)) and involves calcium (Sakakibara et al., 1996; Sakakibara et al., 1997; Sueyoshi et al., 1999) as well as kinases and phosphatases (Champigny and Foyer, 1992; Sakakibara et al., 1997; Sueyoshi et al., 1999). There have been two reported attempts to find NO_3^- regulatory mutants in Arabidopsis. The first selected for mutants that were resistant to chlorate after a transient treatment with low NO_3^- (Lin and Cheng, 1997). A mutant (cr88) was found that was defective in light reception and was designated as a hy mutant (Lin and Cheng, 1997; Cao et al., 2000). The other was a screen using transgenic plants containing the tobacco NiR promoter fused to GUS (Leydecker et al., 2000). This screen produced cnx mutants that overproduced NiR mRNA in the absence of NO_3^-. Thus, no regulatory mutants defective in NO_3^- induction have been reported. However, a NO_3^-—regulated gene (*ANR1*) has been identified which encodes a MADS box transcription factor that is involved in nitrate regulation of root development. This gene is discussed in more detail below (Zhang and Forde, 1998).

AMMONIUM UPTAKE

Arabidopsis, like many plants, does not grow well with NH_4^+ as its sole source of N (e.g. (Wilkinson and Crawford, 1993)), probably reflecting its adaptation for growth in aerobic soils, where NO_3^- is the main form of inorganic N and NH_4^+ concentrations rarely exceed 50 μM (Marschner, 1995). In culture, however, Arabidopsis grows best on a mixed N source (NH4NO3) and under these conditions it may even absorb the NH_4^+ preferentially (Gazzarrini et al., 1999).

The relevance of the NH_4^+ uptake system extends beyond acquiring NH_4^+ from the soil; significant leakage of NH_4^+ occurs from root cells, even when NO_3^- is the N source, so that an uptake system with a high affinity for NH_4^+ is important for conserving valuable N supplies. As discussed in detail elsewhere (von Wiren et al., 2001), NH_4^+ transport is also important for a variety of processes in the shoot, which include unloading of NH_4^+ from the xylem, absorption of atmospheric NH_3 and retrieval of photorespiratory NH_3 released into the leaf apoplast. A number of recent reviews covering all aspects of NH_4^+ uptake in plants are available (Glass et al., 1997; Forde and Clarkson, 1999; Howitt and Udvardi, 2000; von Wiren et al., 2000a; von Wiren et al., 2001).

Kinetics and Energetics of NH_4^+ Uptake

In common with other plants, the kinetics of NH_4^+ uptake in Arabidopsis are biphasic, being separable into a high-affinity system (HATS) and a low-affinity system (LATS) (Rawat et al., 1999). The LATS only becomes evident at external NH_4^+ concentrations above 1 mM (Rawat et al., 1999). The high-affinity system displays typical Michaelis-Menten kinetics (Rawat et al., 1999; Shelden et al., 2001), while the LATS has linear (non-saturable) kinetics (Rawat et al., 1999), consistent with it being mediated by a passive transport system such as a channel (see below). The affinity of the HATS for its substrate, as estimated from $^{13}NH_4^+$ labelling studies, falls within the range reported in other plants (Rawat et al., 1999). For seedlings maintained on NH4NO3, the estimated K_m values for NH_4^+ were from 85 to 168 μM, compared with values in the range 20-360 μM for other plant species (von Wiren et al., 2001). Using an electrophysiological method, a similar K_m value of 46 ± 5 μM was obtained for $NH4NO_3^-$grown Arabidopsis seedlings, but seedlings that had been N-starved or grown on NO_3^- alone for 3 d gave K_m values of 7-8 μM (Shelden et al., 2001).

It is now generally accepted that NH_4^+, rather than the unprotonated species NH_3, is the transported form (Forde and Clarkson, 1999; von Wiren et al., 2001). However the precise mechanism of NH_4^+ influx across the plasma membrane is still not fully resolved. Based on typical estimates for cytosolic NH_4^+ concentrations and the electrical potential across the plasma membrane, thermodynamic considerations imply that in the range of external NH_4^+ concentrations that roots are exposed to in well-aerated soils (i.e. <50 μM) the uptake of NH_4^+ will require the input of energy (Forde and Clarkson, 1999). This could be achieved, for example, by a high-affinity H+ /NH_4^+ co-transporter that coupled NH_4^+ influx to the H+ gradient generated by the plasma membrane H+ pump. The importance of the H+ gradient in driving NH_4^+ influx by the

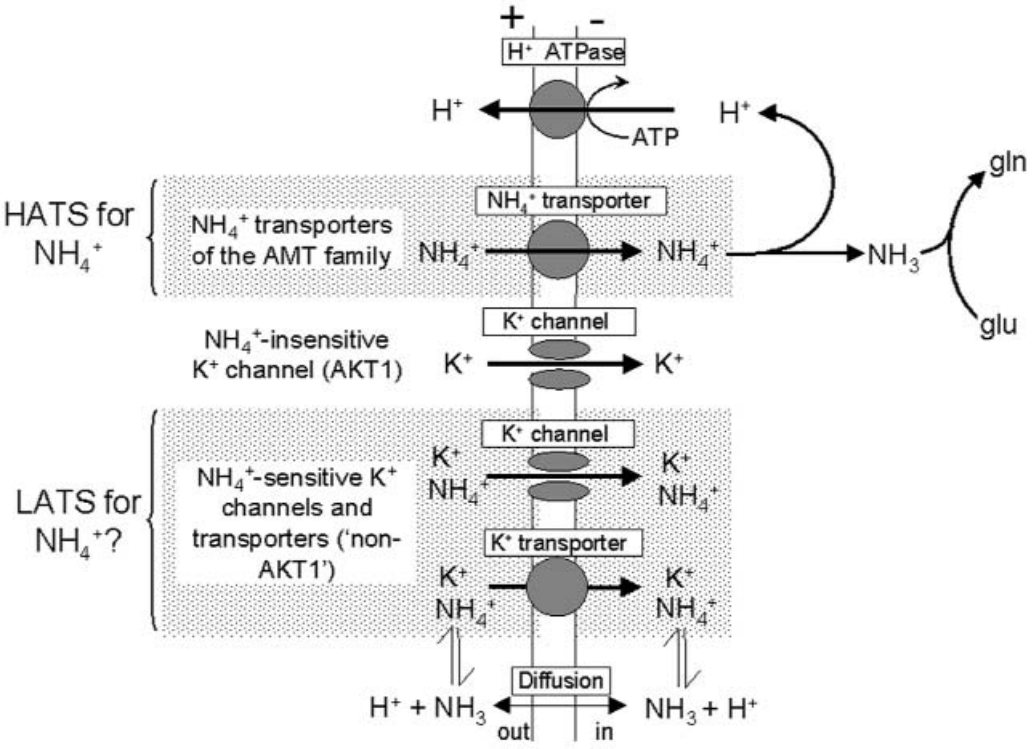


Figure 4. **Possible mechanisms for high- and low-affinity NH_4^+ uptake by Arabidopsis roots**. High-affinity NH_4^+ influx is thought to be catalysed by members of the AMT transporter family (e.g. AtAMT1.1) and is dependent on the H+ gradient generated by the plasma membrane H+-ATPase. The membrane proteins responsible for low-affinity NH_4^+ uptake are still unidentified but are likely to include K+ channels and transporters that do not discriminate against NH_4^+. Diffusion of the uncharged species NH_3 across the plasma membrane is unlikely to contribute significantly to influx, but may be a major route for NH_3/NH_4^+ efflux. See text for further discussion.

HATS in rice roots has been demonstrated using protonophores and ATPase inhibitors (Wang et al., 1993), but direct evidence for H+ /NH_4^+ symport in plant roots is still lacking. In the bacterium Corynebacterium glutamicum, the amt NH_4^+ transporter (which is homologous to higher plant NH_4^+ transporters) has been proposed to operate via an NH_4^+ uniport mechanism that is driven by the membrane potential (Siewe et al., 1996).

The net uptake of any ion is the result of the opposing processes of influx and efflux, and under some conditions it is known that NH_4^+ efflux can have a significant influence on net NH_4^+ uptake (Forde and Clarkson, 1999). Rates of NH_4^+ efflux in Arabidopsis (Rawat et al., 1999) and in other plants (Wang et al., 1993; Kronzucker et al., 1995) are positively correlated with the cytosolic NH_4^+ concentration. When Arabidopsis seedlings were grown on NH_4^+-rich medium (1 mM NH_4^+) the rate of NH_4^+ efflux was estimated to be as much as 35% of the influx rate (Rawat et al., 1999). As discussed elsewhere (Forde and Clarkson, 1999), because the efflux of the protonated form of NH_4^+ is likely to be thermodynamically uphill, it is thought that it is actually NH_3 that diffuses passively from the cells and that this is then protonated in the external solution giving the false impression that the ionic form had crossed the plasma membrane (Figure 4).

Regulation of NH4+ Uptake: the Physiological Evidence

It has been established for a wide range of plant species that the HATS for NH_4^+ (but not the LATS) is highly regulated according to the N nutrition of the plant (Forde and

Clarkson, 1999; von Wiren et al., 2001). A number of studies have now confirmed that the same is true in Arabidopsis (Gazzarrini et al., 1999; Rawat et al., 1999; Shelden et al., 2001). For example, transferring Arabidopsis plants from high NH4NO3 concentrations to low led to a 12-fold increase in HATS activity (Rawat et al., 1999), and when N-depleted plants were resupplied with NH4NO3, the NH_4^+ influx rate declined rapidly (Rawat et al., 1999). In the same experiments, the LATS activity was independent of the external NH_4^+ supply and the plant's N status.

As already noted above, the kinetic properties of the HATS can also be affected by changes in N nutrition. In rice, a positive correlation was found between the external NH_4^+ supply and the affinity of the HATS for NH_4^+ (Wang et al., 1993). While Rawat et al., working with Arabidopsis, found no simple relationship between the ambient N supply and the affinity of the HATS for NH_4^+ (Rawat et al., 1999), a more recent study reported that when Arabidopsis seedlings were grown for 3 d in the absence of NH_4^+ (whether N-deprived or with NO_3^- as sole N source) the K_m for NH_4^+ declined from 46 μM to <10 μM (Shelden et al., 2001). A possibly critical difference between the two studies is that in the former case the 'low N' plants were grown on 0.1 mM NH4NO3 and so were not fully NH_4^+-deprived. Perhaps the very high-affinity HATS is synthesised or activated only when the external NH_4^+ concentration is very low or zero.

Table 3. AMT1 family of NH_4^+ transporters

AMT1 Gene	Tissue specificity[a]	N regulation[b]	K_m for methylNH_4^+[c]	K_i for NH_4^+[c]	AGI code[d]
AtAMT1.1	R≥S [1,2,4][e]	+++ [1,3,4]	65 μM [2] 8 μM [1] 32 μM [4]	5-10 μM [2] 0.5 μM [1] 1-10 μM [4]	At4g13510
AtAMT1.2	R>>S [1,4]	constitutive [1,4]	24 μM [1] 36 & 3000 μM [4]	~35 μM [1] 1-10 μM [4]	At1g64780
AtAMT1.3	R [1]	+ [1]	11 μM [1]	~35 μM [1]	At3g24300
AtAMT1.4	?	?	?	?	At4g28700
AtAMT1.5	?	?	?	?	At3g24290
AtAMT2	R<S [5]	++ (in root) [5]	not transported [5]	?	At2g38290

[a]Based on distribution of RNA transcripts. R = roots, S = shoots.
[b]N regulation of mRNA abundance.
[c]Substrate affinities determined from yeast expression studies.
[d]AGI locus name (see the MIPS *Arabidopsis thaliana* database at http://mips.gsf.de/proj/thal/)
[e]References: [1] Gazzarrini et al. (1999); [2] Ninnemann et al. (1994); [3] Rawat et al. (1999); [4] Shelden et al. (2001); [5] Sohlenkamp et al. (2000).

NH_4^+ influx in Arabidopsis roots is diurnally regulated, reaching a maximum at the end of the light period and declining markedly with the onset of darkness (Gazzarrini et al., 1999). This suggests that the C status of the plant may have a regulatory effect on HATS activity that is somehow transmitted from shoot to root.

Structure and Biochemical Properties of the AMT Family of NH_4^+ Transporters

The Arabidopsis AtAMT1.1 gene was the first NH_4^+ transporter gene to be cloned from higher plants (Ninnemann et al., 1994). Subsequently, two closely related cDNAs (AtAMT1.2 and AtAMT1.3) were isolated by homology and shown to be able, like AtAMT1.1, to complement an NH_4^+ transport mutant of yeast (Gazzarrini et al., 1999). The completed Arabidopsis genome sequence reveals that there are two further members of the AMT1 family (AtAMT1.4 and AtAMT1.5) (Table 3), but no further data are available on these. A sixth gene (AtAMT2), which is more distantly related to the other AMT genes, has been identified and partially characterised (Sohlenkamp et al., 2000).

The AMT genes encode hydrophobic proteins of 475-514 amino acids and belong to a probably ubiquitous gene family (the AMT/MEP family), which has known members in bacteria, archaea, fungi, plants and animals (Saier et al., 1999). All functionally characterised members of the family catalyse NH_4^+ uptake and most also transport the NH_4^+ analogue, methylammonium. Figure 5 illustrates the phylogenetic relationships between the Arabidopsis AMT transporters and selected members of the wider AMT/MEP family. The AtAMT1 genes seem to fall into three subfamilies, one that includes AtAMT1.1, AtAMT1.3 and AtAMT1.5 (the AtAMT1.1 subfamily), and two others that have single members, AtAMT1.2 and AtAMT1.4. The tomato LeAMT1.1 and the Lotus japonicus LjAMT1.1 genes belong to the AtAMT1.1 subfamily, while the tomato LeAMT1.2 gene is most closely related to AtAMT1.2. The close relationship between AtAMT1.3 and AtAMT1.5, together with their occurrence adjacent to each other within a 6 kb region of chromosome 3, suggests that they represent a relatively recent gene duplication. The AtAMT2 gene is the most divergent of the Arabidopsis AMT sequences, being more closely related to the yeast and bacterial and yeast AMT/MEP genes than to the plant ones (Figure 5).

The tomato LeAMT1.3 transporter has an N-terminus that is 20 amino acids shorter than the other AMT1 polypeptides and two untranslated open reading frames are found in its 5'-untranslated region, something often associated with regulatory genes (von Wiren et al., 2000b). Surprisingly, there appears to be no direct ortholog of LeAMT1.3 in the Arabidopsis genome.

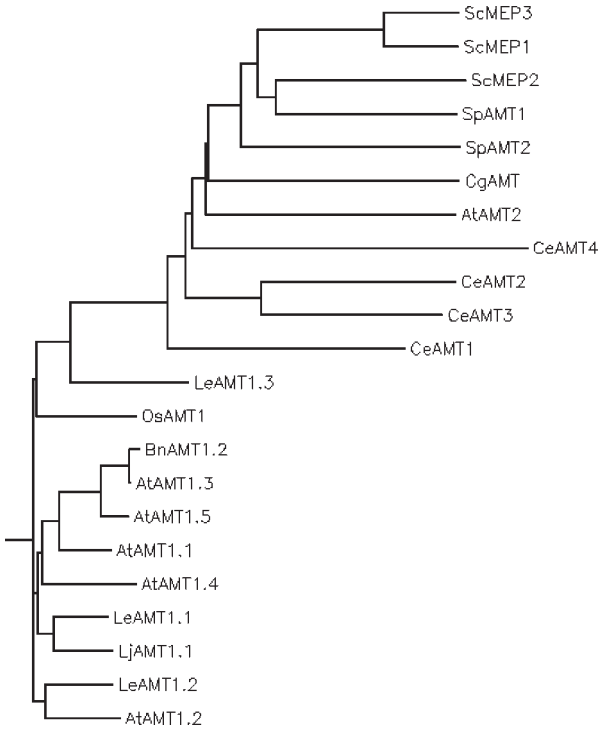
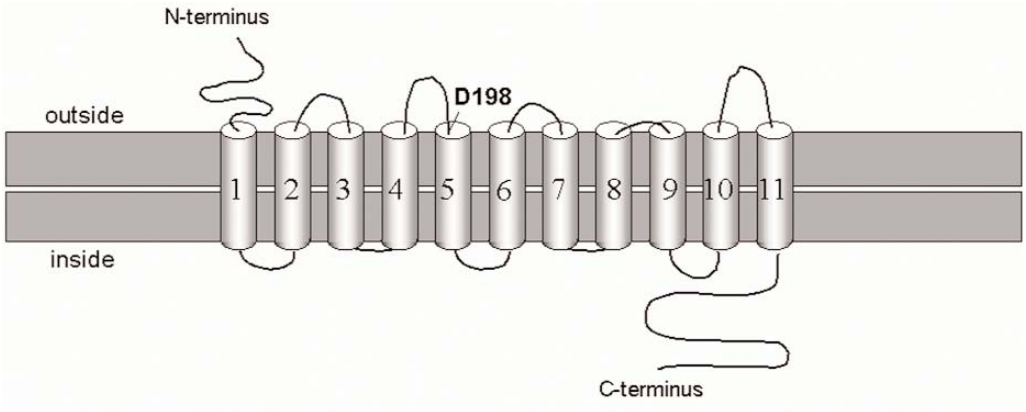


Figure 5. Phylogenetic tree for the Arabidopsis AMT transporters and selected members of the AMT/MEP family. The sequences were aligned using the CLUSTAL W program (Thompson et al., 1994) and the rooted tree constructed using DRAWGRAM (Felsenstein, 1993). The accession numbers for the AtAMT sequences are given in Table 3. The other sequences, with their accession numbers, are from *Saccharomyces cerevisiae* (ScMEP1, PIR:S46225; ScMEP2 PIR:S51089; ScMEP3 PIR:S69027), *Schizosaccharomyces pombe* (SpAMT1, PIR:T50244; SpAMT2, GB:CAB83006), *Caenorhabditis elegans* (CeAMT1, GB:P54145; CeAMT2, GB:Q20605; CeAMT3, GB:Q21565; CeAMT4, GB:AAA83579), rice (OsAMT1. GB:AAB58937), *Cornyebacterium glutamicum* (CgAMT, GB:CAA63770), *Lotus japonicus* (LjAMT1.1, GB:AAG24944), tomato (LeAMT1.1, GB:X92854; LeAMT1.2, GB:CAA64475; LeAMT1.3, GB:AAG11397) and *Brassica napus* (BnAMT1.2, GB:AAG28780).

Computer-based predictions that the E. coli AMTB protein has 12 transmembrane domains, with the N- and C-termini located on the interior face of the membrane, were recently confirmed using fusions with marker proteins

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MSCSATDLAVLLGPNATAAANYICGQLGDVNNKFIDTAFAIDNTYLLFSA    50
YLVFSMQLGFAMLCAGSVRAKNTMNIMLTNVLDAAAGGLFYYLFGYAFAF   100
GSPSNGFIGKHYFGLKDIPTASADYSNFLYQWAFAIAAAGITSGSIAERT   150
QFVAYLIYSSFLTGFVYPVVSHWFWSVDGWASPFRTDGDLLFSTGAIDFA   200
GSGVVHMVGGIAGLWGALIEGPRLGRFDNGGRAIALRGHSASLVVLGTFL   250
LWFGWYGFNPGSFNKILVTYETGTYNGQWSAVGRTAVTTTLAGCTAALTT   300
LFGKRLLSGHWNVTDVCNGLLGGFAAITGGCSVVEPWAAIICGFVAALVL   350
LGCNKLAEKLKYDDPLEAAQLHGGCGAWGLIFTALFAQEKYLNQIYGNKP   400
GRPHGLFMGGGGKLLGAQLIQIIVITGWVSATMGTLFFILKKMKLLRISS   450
EDEMAGMDMTRHGGFAYMYFDDDESHKAIQLRRVEPRSPSPSGANTTPTP   500
V*
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Figure 6. Schematic model for the membrane topology of AtAMT1.1. The model (above) is redrawn from (Thomas et al., 2000) and the predicted locations of the eleven transmembrane domains are indicated in red on the AtAMT1.1 amino acid sequence (below). The precise start and end points of these transmembrane domains in AtAMT1.1 were taken from the predictions in the Arabidopsis Membrane Protein Library (http://www.cbs.umn.edu/arabidopsis). A conserved aspartate residue suggested as a possible $NH4^+$ binding site (Thomas et al., 2000) is indicated on the topological model..

(Thomas et al., 2000). However, sequence alignments indicate that the most N-terminal of the 12 transmembrane domains in AMTB is not present its plant or fungal homologues, so that an 11 transmembrane topology is predicted for the eukaryotic members of the AMT/MEP family (Thomas et al., 2000). The implication that the N-terminus of the eukaryotic AMT/MEP proteins must be on the outside of the membrane has been confirmed experimentally for the yeast MEP2 protein (Marini and Andre, 2000). Figure 6 shows a 2-D representation of the likely membrane topology of AtAMT1.1 transporter. According to this model, the majority of positively charged amino acids on the AtAMT1.1 polypeptide are on the cytoplasmic side of the membrane (Thomas et al., 2000), consistent with the 'positive-inside' rule (Von Heijne, 1992).

The kinetic properties of some of the Arabidopsis AMT transporters have been studied in the yeast heterologous expression system (Ninnemann et al., 1994; Gazzarrini et al., 1999; Sohlenkamp et al., 2000; Shelden et al., 2001). The ability of most AMT proteins to transport methylammonium enables (14C)methylammonium to be conveniently used to assay transport activity. The pH optimum for methylammonium uptake by yeast cells expressing AtAMT1.1 was 7, with only low rates of uptake at pH 9, consistent with NH_4^+ being the substrate rather than NH_3 (Ninnemann et al., 1994). Transport activity was dependent on an energy source (glucose) and was sensitive to protonophores (which collapse the H+ gradient across the plasma membrane) and to inhibitors of the H+ pump (Ninnemann et al., 1994). AtAMT1.1 displayed Michaelis-Menten kinetics for methylammonium uptake, with a K_m of 65 μM. As expected, NH_4^+ acted as a strong competitive inhibitor of methylammonium uptake (K_i of <10 μM), but AtAMT1.1 showed no affinity for K+ (Ninnemann et al., 1994). Thus AtAMT1.1 was demonstrated to be an energy-dependent high-affinity NH_4^+ uptake system.

Subsequent studies have used the same yeast expression system to examine the kinetic properties of AtAMT1.1, AtAMT1.2, AtAMT1.3 and AtAMT2 (see Table 3 for a summary). Gazzarrini and colleagues reported that of

the three AtAMT1 proteins, AtAMT1.1 had the highest affinity for NH_4^+ (K_m 0.5μM) (Gazzarrini et al., 1999). In contrast, a more recent study (Shelden et al., 2001) found no significant difference between AtAMT1.1.and AtAMT1.2 in their affinity for NH_4^+ and reported that AtAMT1.2 showed biphasic kinetics for methylammonium uptake, with K_m values of 36 μM and 3 μM.

AtAMT2 expressed in yeast was unable to transport methylammonium, making analysis of its biochemical properties difficult. However, it was established that yeast cells expressing AMT2 were able to use NH_4^+ as a N source and to deplete NH_4^+ from medium containing concentrations <100 μM, although at a slower rate than cells expressing AtAMT1.1 (Sohlenkamp et al., 2000).

Regulation of the AMT Genes

In addition to their differing biochemical properties, the AMT genes also show contrasting patterns of mRNA expression (Table 1). All four AMT genes so far analysed are expressed in roots (Ninnemann et al., 1994; Gazzarrini et al., 1999; Rawat et al., 1999; Sohlenkamp et al., 2000; Shelden et al., 2001), but only AMT1.1 and AMT2 are also expressed strongly in shoots (Gazzarrini et al., 1999; Sohlenkamp et al., 2000; Shelden et al., 2001). In roots, AtAMT1.1 and AtAMT2 are highly responsive to the NH_4^+ supply, both being strongly repressed in the presence of high external NH_4^+ concentrations (Rawat et al., 1997; Gazzarrini et al., 1999) (Sohlenkamp et al., 2000; Shelden et al., 2001). AtAMT1.3 is similarly regulated, but to a lesser degree, while AtAMT1.2 is constitutively expressed (Gazzarrini et al., 1999; Shelden et al., 2001).

Of the three N-responsive genes, it is AtAMT1.1 that responds to the N supply in a manner that most closely parallels the changes in HATS activity (Gazzarrini et al., 1999; Rawat et al., 1999; Shelden et al., 2001). For example, when plants were transferred from high NH_4^+ concentrations to low, AtAMT1 mRNA levels began to increase within 1 h and continued to increase over a 24 h period concurrently with an increase in $13NH_4^+$ influx rates (Rawat et al., 1999). Similarly AtAMT1.1 mRNA levels in roots increased markedly during a 2 d period after the N supply was withdrawn, as did the $15NH_4^+$ influx rate, but there was no significant increase in AtAMT1.3 mRNA abundance during the same period (Gazzarrini et al., 1999). Nevertheless, these correlations need to be treated with caution since there are indications that post-transcriptional as well as transcriptional controls can operate in the regulation of NH_4^+ transporter activity (see below).

There is evidence that glutamine is a key regulatory metabolite involved in the feedback regulation of AtAMT1.1 expression (Rawat et al., 1999). Strong negative correlations were found between glutamine concentrations in root extracts and both $13NH_4^+$ influx rates and AtAMT1.1 transcript levels. Furthermore, the repressive effect of NH_4^+ on AtAMT1.1 expression could be relieved by blocking the conversion of NH_4^+ to glutamine with methionine sulfoximine (MSX, an inhibitor of glutamine synthetase activity).

Are Post-Transcriptional Controls Involved in the Regulation of AMT Transporters?

There are several lines of evidence pointing to the possibility that regulation of the NH_4^+ transporters is complex and operates at multiple levels. Gazzarrini and colleagues found that when Arabidopsis plants were transferred from NH4NO3 to KNO3, the NH_4^+ influx rate increased over the following three days without any change in AtAMT1.1, AtAMT1.2 or AtAMT1.3 mRNA levels (Gazzarrini et al., 1999). Under similar conditions it was reported that the K_m for NH_4^+ of the HATS changed from 46 μM to 7 μM (Shelden et al., 2001). Only two explanations for these observations seem possible: either one of the other AMT genes is responsible for these changes in the activity and kinetic properties of HATS (and is regulated by the N supply in a very different way from AtAMT1.1, AtAMT1.2 or AtAMT1.3), or regulation of AMT expression at some post-transcriptional level is occurring.

In experiments where MSX was used to block NH_4^+ assimilation and NH_4^+ consequently failed to cause a significant decline in AtAMT1.1 transcript levels, there was nevertheless a 30% decrease in $13NH_4^+$ influx rates (Rawat et al., 1999). Further evidence for post-transcriptional controls comes from experiments where the resupply of NH4NO3 to N-starved plants consistently led to a more rapid decline in NH_4^+ influx rates than in AtAMT1.1 mRNA abundance (Rawat et al., 1997), even though AtAMT1.1 is the most highly responsive of the AMT gene family (Gazzarrini et al., 1999); (Rawat et al., 1999; Sohlenkamp et al., 2000; Shelden et al., 2001).

All of these observations are consistent with a role for the NH_4^+ ion in the post-transcriptional regulation of the HATS (Rawat et al., 1999), perhaps by directly inhibiting AMT transporter activity or by inhibiting the synthesis or post-translational processing of the AMT proteins. A

model for the feedback regulation of AtAMT1.1 by glutamine and NH_4^+ is shown in Figure 7.

Concluding Remarks on AMT Transporter Function

The current evidence strongly suggests that one or more of the AtAMT genes encode the HATS for NH_4^+ in Arabidopsis roots. Their differing affinities for NH_4^+ and responsiveness to N, and the varied patterns of expression within the plant, suggest that they perform different, but perhaps overlapping, physiological functions. The high-affinity of AtAMT1.1 for NH_4^+ and the close correlation between AtAMT1.1 mRNA levels and the HATS activity has made this member of the AMT family the strongest candidate for being a major component of the high-affinity NH_4^+ uptake system, particularly in NO_3^-—fed and N-deprived plants. The very high affinity of the AtAMT1.1 transporter expressed in yeast (Table 3), and its strong derepression in N-deprived roots, suggests that it accounts for the highest affinity component of the HATS (K_m of 8 μM) that has only been detected in fully NH_4^+-deprived roots (Shelden et al., 2001).

Nevertheless, since AtAMT1.2, AtAMT1.3 and AtAMT2 are also expressed in roots, and since each has been shown to have the capacity to transport NH_4^+ in the concentration range typical of the HATS (Table 3), it is likely that they too contribute to the HATS. The extent to which each contributes seems certain to vary with such factors as the rate and nature of the external N supply, the time of day and the N and C status of the plant. Examination of the TIGR Gene Index for Arabidopsis ESTs (Quackenbush et al., 2001) reveals that the Columbia root EST collection contains two cDNAs for AtAMT1.1, four for AtAMT1.2 and one for AtAMT1.4, which may give some indication of the relative levels of expression of the AMT genes in $NH4NO_3^-$ grown plants of this ecotype.

The physiological roles of the different AtAMT transporters will become clearer once we have more information about their subcellular localizations and their cellular distributions within the root and shoot. Most important will be the analysis of mutants and transgenic lines in which the expression of single and multiple AtAMT genes is modified.

Low-affinity NH_4^+ uptake

The non-saturable kinetics of NH_4^+ influx at external NH_4^+ concentrations above 1 mM suggest that it is likely to be occurring by diffusion, but it is only at high external pH that diffusion of NH_3 is likely to contribute much to the low-

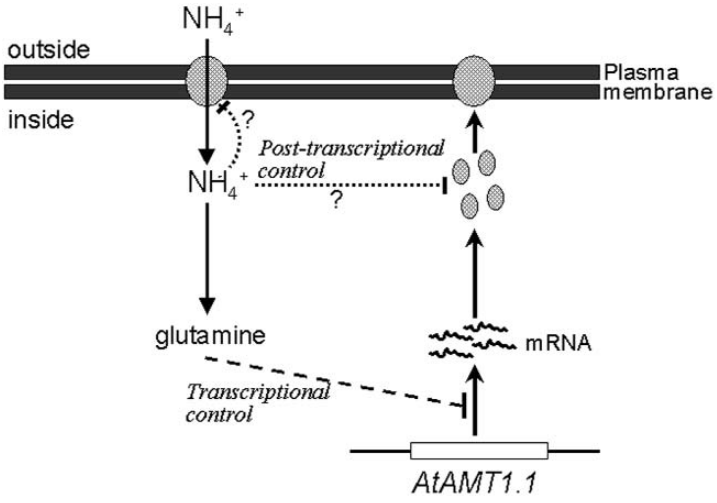

Figure 7. Model for the feedback regulation of the *AtAMT1.1* gene by N metabolites. While glutamine may repress *AtAMT1.1* expression at the transcriptional level, NH_4^+ may act post-transcriptionally (e.g. on mRNA translation or targeting of the protein) or may have direct effects on the activity or kinetic properties of the AtAMT1.1 transporter. Model based on that proposed by (Rawat et al., 1999).

affinity phase. In yeast, one member of the AMT/MEP family (Mep3p) has a very low affinity for NH_4^+ (1.4-2.1 mM), so that within this one family a full range of substrate affinities is possible. The only indication so far that a member of the AtAMT family may contribute to the LATS is the report that AtAMT1.2 has a dual affinity for methylammonium when expressed in yeast, the low affinity phase having a K_m of 3 mM (Shelden et al., 2001).

Low-affinity NH_4^+ uptake might also occur as a by-product of the activity of K+ channels or K+ transporters (Forde and Clarkson, 1999; von Wiren et al., 2001). K+ uptake systems in maize roots included both NH_4^+-sensitive and NH_4^+-insensitive components (Vale et al., 1987). Analysis of a mutant carrying an insertion in the gene for an inward-rectifying K+ channel (AKT1) has confirmed that a similar situation exists in Arabidopsis (Spalding et al., 1999). Furthermore, it was shown that the NH_4^+-sensitive component corresponded to the non-AKT1 fraction of K+ transport activity (Spalding et al., 1999). One class of K+ channels that could contribute to low-affinity NH_4^+ influx are the voltage-independent non-selective channels (VICs; (Amtmann and Sanders, 1999)). Evidence that these are permeable to NH_4^+ has come from lipid bilayer studies on a voltage-independent K+ channel from rye roots (White, 1996) and from in situ analysis of VICs in the plasma membrane of maize root cortical cells (A. Amtmann, unpublished results). Which genes encode VICs are still unknown. Figure 4 summarises the possible pathways for high and low-affinity NH_4^+ transport across the plasma membrane.

Ammonium sensing

In yeast, the NH_4^+ transporter Mep2p acts as a sensor for environmental NH_4^+ in regulating the initiation of the process of pseudohyphal differentiation (Lorenz and Heitman, 1998). Thus Mep2p belongs to a class of membrane transporters in yeast that also serve as sensors for metabolites; other examples include the Snf3p and Rgt2p glucose transporters (Ozcan et al., 1996) and the Ssy1p and Ptr3p amino acid transporters (Klasson et al., 1999), which each sense the extracellular levels of their respective substrates. Often transporters that function as nutrient sensors have extended N- or C-terminal domains that may serve as signalling domains. None of the AMT sequences possess any additional domains that might suggest a role other than in solute transport, but since this also true of Mep2p it is still a possibility worthy of investigation that one or more of the AMT permeases function as NH_4^+ sensors.

NITROGEN REGULATION OF PLANT DEVELOPMENT

It is well known that the N supply can have major effects on many aspects of plant development (Redinbaugh and Campbell, 1991; Crawford, 1995; Bloom, 1997; Stitt, 1999). Root developmental processes often reported to be N-regulated include root branching, root diameter, root hair density and/or length, and (in legumes) numbers of N-fixing root nodules (Forde and Lorenzo, 2001). Above ground processes that in many species are subject to N regulation include leaf growth, shoot branching and flowering time (Stitt, 1999). The term ‘trophomorphogenesis’ has been proposed to describe the changes in plant morphology arising from variations in the availability or distribution of nutrients in the environment (Forde and Lorenzo, 2001). Trophomorphogenesis, like photomorphogenesis, is an example of developmental plasticity, which is a characteristic feature of plant development. Arabidopsis, like other fast-growing plant species, displays a high degree of developmental plasticity in response to environmental signals and is therefore an excellent subject in which to study the molecular basis of these morphogenetic responses, a subject that has until recently been neglected. In the past few years a number of studies have examined the effects of the N supply on Arabidopsis development, with an emphasis on roots, and some progress is now beginning to be made in identifying components of the signalling pathways involved and their relationship to signalling pathways for plant hormones. Some of this work has been reviewed in detail elsewhere (Zhang and Forde, 2000).

Nitrate Stimulation of Lateral Root Growth

A series of classical studies in the 1970’s examined the effects of NO_3^- and NH_4^+ on root development in barley (Hordeum vulgare) (Drew et al., 1973; Drew, 1975; Drew and Saker, 1975). Using sand cultures or flowing nutrient solutions, Drew and colleagues found that locally concentrated supplies of NO_3^-, NH_4^+ or phosphate stimulated root branching specifically within the nutrient-rich zones. The response consisted of a stimulation of both lateral root initiation and lateral root growth in the zone of treatment. In contrast, growth of the seminal roots was little affected by the external nutrient supply (Drew et al., 1973), showing

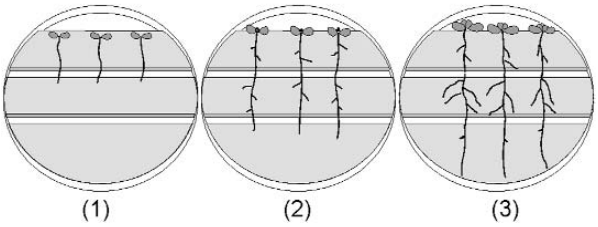

Figure 8. Segmented agar plate (SAP) method for applying localised nutrient treatment to Arabidopsis roots. Figure adapted from (Zhang and Forde, 1998). To prevent diffusion of nutrients between different treatment zones, agar plates are divided into three segments using a sterile scalpel. For localised NO_3^- treatments, the agar contains a basal supply of N (10 μM NH_4NO_3) and the middle segment is supplemented with either 1 mM KNO_3 or (as control) 1 mM KCl. At the start of the experiment (1), seedlings with roots *ca* 2 cm long are placed on the top segment, with just a few mm of the primary root apex resting on the middle segment. The plates are incubated vertically while the primary roots grow over the surface of the middle (treated) segment and onto the bottom segment (2). Around 10-12 d after seedling transfer (3), lateral root lengths in each segment are measured.

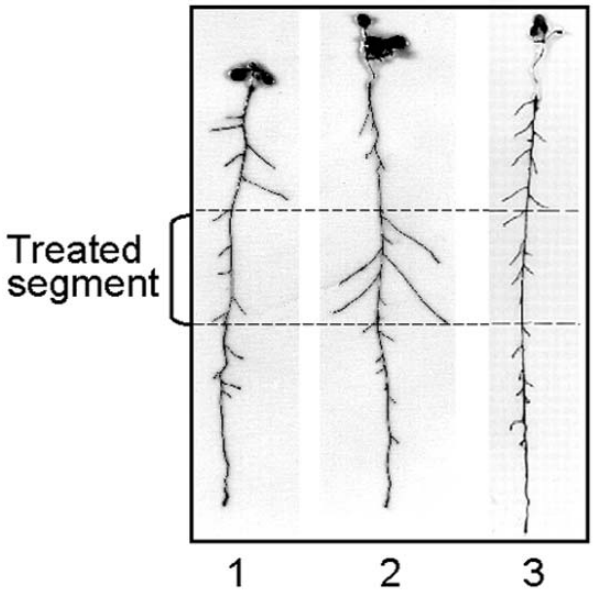

Figure 9. Localised stimulation of lateral root growth by NO_3^-. Seedling 1 (control): wild-type seedling grown on segmented agar plate containing low NO_3^- (10 μM NH_4NO_3) in all three segments; Seedling 2: wild-type seedling exposed to a localised supply of 1 mM KNO_3; Seedling 3: transgenic line S10 (in which expression of the *ANR1* MADS-box gene has been down-regulated by co-suppression) exposed to a localised supply of 1 mM KNO_3 (Zhang and Forde, 1998).

that different types of root meristem within one root system can have very different sensitivities to nutrients.

The development of a technique for applying nutrients in a localised fashion to Arabidopsis seedlings growing on vertical agar plates (Figure 8) allowed this phenomenon to be investigated in a species more amenable to molecular genetics (Zhang and Forde, 1998). Initial studies showed that localised NO_3^- treatments did indeed stimulate lateral root elongation (2- to 3-fold), specifically within the NO_3^-—rich zone (Figure 9). The stimulation occurred even if the NO_3^- treatments were delayed until after the lateral roots had emerged (Zhang and Forde, 2000), showing that the NO_3^- does not need to be present during initiation or maturation of lateral root primordia for it to be effective, as has also been shown in wheat (Hackett, 1972). As in barley and other species, primary root growth in Arabidopsis is remarkably insensitive to changes in the NO_3^- supply (Zhang and Forde, 1998).

Neither the number of lateral roots initiated (Zhang and Forde, 1998), nor the rate of progression through the early stages of lateral root development (Zhang and Forde, 2000), are modified by localised NO_3^- treatments. The absence of any significant effect on lateral root initiation differs from the situation in barley and wheat, where the localised response to NO_3^- consisted of both an increase in elongation rates and an increase in numbers of laterals (Hackett, 1972; Drew et al., 1973). In Arabidopsis it was shown that even relatively low concentrations of NO_3^- (50-100 μM) in the NO_3—enriched zone were sufficient to elicit the response (Zhang et al., 1999). The absence of any significant increase in the size of mature cells in the NO_3^-—stimulated roots established that the increased growth rates were due to increased meristematic activity in the lateral root tip (Zhang et al., 1999).

When localised supplies of NH_4^+ or glutamine were substituted for NO_3^-, no stimulation of lateral root growth was observed (Zhang et al., 1999), indicating that the roots were responding to the NO_3^- ion itself rather than to NO_3^- as a source of N. Further evidence for this was obtained using a *nia1nia2* mutant which is deficient in NR and hence unable to use NO_3^- as an effective N source (Wilkinson and Crawford, 1993); this mutant showed a wild-type response to a localised NO_3^- treatment (Zhang and Forde, 1998). Thus, just as NO_3^- is a signal for the induction of the nitrate assimilatory pathway (see above), it is also a signal for increased root proliferation. It seems that individual lateral root tips have a mechanism for monitoring the external NO_3^- concentration and for modifying their meristematic activity accordingly (Zhang and Forde, 1998; Zhang et al., 1999).

Genes Involved in Nitrate Stimulation of Lateral Root Growth

A possible component of the signal transduction pathway linking external NO_3^- to changes in meristematic activity has been identified during a screen for NO_3^- regulated genes in Arabidopsis roots (Zhang and Forde, 1998). Amongst the novel NO_3^-—inducible sequences identified was ANR1, a member of the MADS box family of transcription factors (Zhang and Forde, 1998). There are at least 82 MADS box genes in the Arabidopsis genome (Riechmann et al., 2000) and most of those that have so far been functionally characterised are involved in controlling floral organogenesis or other processes related to the reproductive phase of growth (Ng and Yanofsky, 2001). ANR1 belongs to a group of nine MADS box genes so far known to be expressed in Arabidopsis roots (Alvarez-Buylla et al., 2000), but most of these are of unknown function. When transgenic lines were generated in which ANR1 expression was down-regulated (by antisense or co-suppression), their lateral roots were found to have lost the ability to respond to localised supplies of NO_3^- (Figure 9). It was proposed that ANR1 is a component of the sensory pathway linking NO_3^- to the stimulation of lateral root growth (Zhang and Forde, 1998).

Auxin is well known as an important regulator of root growth (Leyser, 1997), and experiments using auxin-response mutants of Arabidopsis have provided evidence for an overlap between NO_3^- signalling and auxin signalling in the control of lateral root elongation (Zhang et al., 1999). Three auxin-response mutants (aux1, axr2 and axr4) were tested for their sensitivity to localised supplies of NO_3^-: While aux1 and axr2 showed responses that were not significantly different from the wild-type, the axr4 mutant showed a phenotype similar to the ANR1 down-regulated lines (Zhang et al., 1999). Although the AXR4 gene has not been identified at the molecular level, its location on chromosome 1 (Hobbie and Estelle, 1995) establishes it as distinct from ANR1 (which is on chromosome 2). Unusual among auxin-response mutants, the phenotype of axr4 mutants is restricted to roots and does not involve cross-resistance to other plant hormones such as cytokinin or ethylene (Hobbie and Estelle, 1995). Apart from reduced gravitropism, the root phenotype of the axr4-2 mutant is remarkably like the wild-type, making its altered sensitivity to NO_3^- stimulation of lateral root elongation of particular interest. A model for the NO_3^- signalling pathway leading to increased meristematic activity in the lateral root tip is shown in Figure 10.

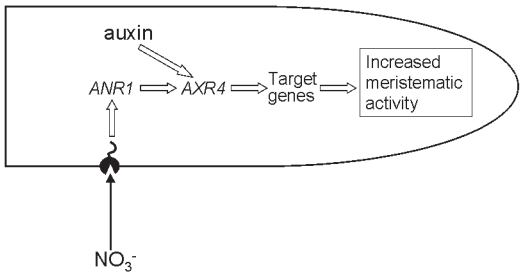

Figure 10. Model for NO_3^- signalling pathway leading to localised stimulation of lateral root growth. The external NO_3^- supply is monitored by individual lateral root tips and the signal is transduced *via* the NO_3—inducible *ANR1* gene and the auxin-sensitivity gene *AXR4* to produce increased meristematic activity. The identity and sub-cellular location of the NO_3^- receptor/sensor are unknown, as is the mechanism for induction of *ANR1*. ANR1 has tentatively been placed upstream of ANR1 in the signal transduction pathway. The model is based on that proposed by (Zhang et al., 1999). See text for further details.

Nitrate as an Inhibitor of Early Lateral Root Development

Nitrate has also been found to have an inhibitory effect on lateral root development (Scheible et al., 1997c; Zhang and Forde, 1998; Stitt, 1999; Zhang et al., 1999). In Arabidopsis this consists of a delayed outgrowth of the lateral root primordium when roots are exposed to NO_3^- concentrations above 1 mM (Zhang et al., 1999). At 50 mM, the highest NO_3^- concentration tested, two-week-old seedlings developed primary roots of the same length as at 1 mM, and had a similar number of lateral root primordia, but most or all of the laterals were less than 0.5 mm in length. This inhibitory effect of NO_3^- appears to be very different in nature from its stimulatory effect (Zhang et al., 1999; Zhang and Forde, 2000). Firstly, while the stimulatory effect acts on elongation of mature laterals, the inhibitory effect acts specifically on immature LRs during a discrete phase around the time of their emergence from the primary root. Secondly, the high external NO_3^- concentration does not act directly on the developing lateral; if only a portion of the primary root was exposed to 50 mM NO_3^-, the inhibitory effect was much diminished and what inhibitory effect remained was distributed along the root (Zhang et al., 1999). Thus the effect seems to depend on the NO_3^- absorbed by the plant rather than the external NO_3^- concentration per se.

Using the NR-deficient *nia1nia2* mutant it was shown that the assimilation of NO_3^- was not required for the inhibitory effect, and furthermore that lateral root development in the mutant was rather more sensitive to NO_3^- inhibition than the wild-type (Zhang et al., 1999). Nitrate reductase-deficient mutants growing on NO_3^- are known to accumulate high levels of tissue NO_3^-, particularly in the leaves e.g. Warner and Huffaker, 1989), so this observation is consistent with the notion that NO_3^- levels inside the plant are responsible for generating the inhibitory effect on lateral root outgrowth. A similar phenomenon has been reported in tobacco, where NR deficient lines growing on NO_3^- display a marked inhibition of root growth (Scheible et al., 1997b; Scheible et al., 1997c) due to a reduction in lateral root frequency (Stitt, 1999). Here the evidence from metabolite analysis and split root experiments has lead to the conclusion that the inhibition of root growth is due to a signal from the shoot triggered by NO_3^- accumulation in the shoot (Scheible et al., 1997c). In Arabidopsis (but not in tobacco), an increase in the sucrose concentration in the medium led to a partial alleviation of the inhibitory effect of NO_3^- on root branching (Scheible et al., 1997c; Zhang et al., 1999). This would suggest that either the balance between the carbon and nitrogen status of the plant is being monitored, or that sucrose itself has a signalling role that acts antagonistically with the NO_3^- signal (Smeekens, 2000).

Why outgrowth of the lateral root primordium should be particularly sensitive to inhibition by NO_3^- is unclear. From other work it is known that the stage just after its emergence is a critical step in the development of a lateral root (Cheng et al., 1995; Malamy and Benfey, 1997a; Malamy and Benfey, 1997b). Although the lateral root meristem appears to be fully formed prior to emergence, emergence itself takes place through cell expansion rather than cell division (Malamy and Benfey, 1997b). It is only after emergence that activation of the lateral root meristem occurs and growth of the mature lateral root begins (Cheng et al., 1995; Malamy and Benfey, 1997b). Thus there may be a developmental process between differentiation of the lateral root meristem and its activation that is particularly sensitive to high NO_3^- concentrations. An alternative explanation could lie in the timing of the establishment of symplastic connections between the phloem of the primary root and the lateral root primordium. Since a shoot-derived signal may be involved, these connections could be necessary for transmission of the signal to the developing primordium. A study using fluorescent dyes and confocal microscopy has elegantly demonstrated for Arabidopsis that early in its development the lateral root is symplastically isolated and that the earliest functional symplastic connections with the lateral root primordium occur around the time of emergence from the cortex (Oparka et al., 1995).

The finding that NO_3^- can either inhibit or stimulate lateral root production, depending on its external distribution and its accumulation within plant tissues, has led to a 'dual pathway' model for NO_3^- regulation of root branching in Arabidopsis (Zhang et al., 1999). This model, illustrated in Figure 11, depicts one signalling pathway (the localised stimulatory pathway) which responds to the external NO_3^- concentration at the lateral root tip and regulates increase

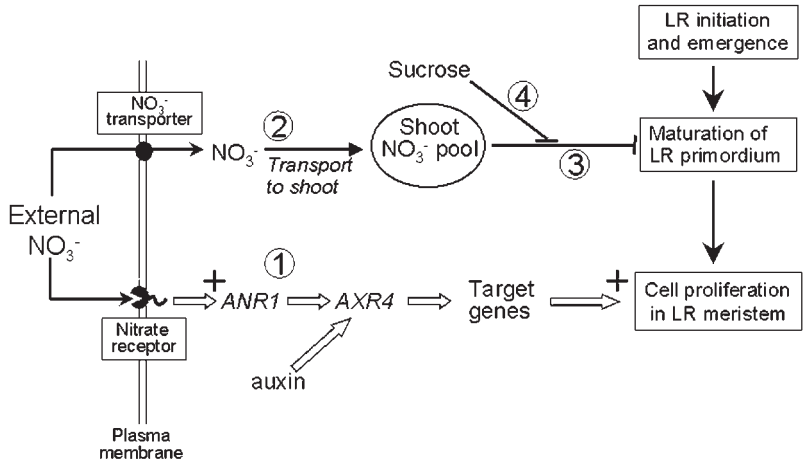

Figure 11. 'Dual pathway' model for NO_3^- regulation of root branching by nitrate. (1). Localised stimulatory pathway responding to changes in the external NO_3^- supply (see Figure 10). (2) Systemic inhibitory pathway, requiring the uptake and transport of NO_3^- to the shoot. (3) Maturation of the lateral root primordium is under the control of shoot-derived inhibitory signal that becomes prevalent at high shoot NO_3^- concentrations. (4) An increased supply of sucrose is able to alleviate the inhibitory effect of high NO_3^- supplies. The model is based on that proposed by (Zhang et al., 1999). See text for further details.

meristematic activity specifically in that root tip, and a second signalling pathway (the systemic inhibitory pathway) which responds to the internal NO_3^- status of the plant and regulates progress through the latter stages of lateral root maturation. These two antagonistic pathways for regulation of root branching can be seen as providing a mechanism by which the plant can modify its root architecture in a manner that integrates information about the spatial distribution of NO_3^- in the soil and the plant's current N status (Zhang et al., 1999). Thus the plant could modulate the intensity of its proliferative response to a localised NO_3^- supply according to its demand for N. This would allow the allocation of resources within the plant to be optimised by ensuring that the strongest response to a localised NO_3^- supply occurred when the plant was N-depleted (Zhang et al., 1999). Increased lateral root proliferation is only one component of a plant's response to localised NO_3^- supplies; a much faster response is seen at the metabolic level, where NO_3^- influx by roots located within a NO_3^-—rich patch is rapidly induced (Laine et al., 1995; Laine et al., 1998). The up-regulation of the NO_3^- influx system can be seen as a short-term adaptive response to localised NO_3^-, with stimulation of lateral root development being the longer-term component of the response. It is known that the NO_3^- influx system is regulated by signals originating in the shoot (Laine et al., 1995), and it remains to be seen whether the same signals are involved in regulating lateral root development.

Ammonium Inhibition of Root Growth

Ammonium, when supplied to plants as the sole N source, often inhibits plant growth, particularly in unbuffered media when acidification of the rhizosphere becomes a problem (von Wiren et al., 2001). However, a novel aspect of NH_4^+ inhibition of root growth has been reported in Arabidopsis (Cao et al., 1993). In these studies it was found that if K+ was omitted from the medium, and NH_4^+ concentrations were high (>3 mM) seedling root growth was dramatically inhibited, even when NO_3^- was present. This inhibition could be reversed if low levels of K+, Rb+ or Cs+ ions were added, and the protective effect of K+ was shown not to be due to an inhibition of NH_4^+ uptake. The further finding that three auxin-response mutants (aux1, axr1 and axr2) were resistant to NH_4^+ inhibition of root growth led to the suggestion that this unusual response to NH_4^+ was mediated by effects on hormone metabolism or signalling. Since it is unlikely that an Arabidopsis plant will often be exposed in nature to the required combination of high NH_4^+ and very low K+ concentrations, it is unclear what the physiological significance of this intriguing phenomenon may be; however, it is an example in Arabidopsis showing the role of auxin in nutrient effects on root growth.

Nitrogen Regulation of Shoot Growth and Development

It has been established for Arabidopsis that a higher rate of N supply leads to an increased allocation of resources to shoot growth (Schulze et al., 1994), leading to increased shoot:root ratios, which is the morphological response commonly seen in many other plant species (Ericsson, 1995). Analysis of metabolite pools showed that there was a striking correlation between the concentrations of NO_3^- and organic N in rosette leaves and shoot growth rates (Schulze et al., 1994). In tobacco, transgenic lines with low NR activity have helped to demonstrate the role of the shoot NO_3^- pool as a signal in regulating shoot-root allocation (Scheible et al., 1997c).

Other studies have highlighted the importance of the short-term N supply and root-to-shoot signalling in the regulation of leaf growth (McDonald and Davies, 1996). Recent studies with tobacco found that the rate of cell division and cell elongation in leaves fell markedly within 24 h of transferring plants from NO_3^- to NH_4^+ (Walch-Liu et al., 2000). This effect was not due to limitation of carbohydrates or N, nor to a lack of osmotica or water. A possible role for NO_3^- as a signal was inferred from evidence that it was absence of NO_3^- rather than the presence of NH_4^+ that was responsible for the reduction in leaf growth (Walch-Liu et al., 2000). A decline in the zeatin + zeatin riboside fraction in xylem exudates which paralleled that in leaf expansion rates led to the suggestion that NO_3^- may be involved in a signal transduction chain that regulates leaf morphogenesis by modulating the flux of cytokinin from root to shoot (Walch-Liu et al., 2000).

Although leaves of NO_3^-—grown Arabidopsis plants are larger than those of NH_4^+-grown plants (e.g. (Oostinder-Braaksma and Feenstra, 1973)), the possible short-term modulation of leaf expansion by the NO_3^- supply has not been studied in Arabidopsis. However it is interesting to note that some members of the Arabidopsis family of response regulators (ARRs) have been shown to be induced in leaves by either the direct application of cytokinins or the re-supply of NO_3^- to roots of N-starved plants (Taniguchi et al., 1998). It has been proposed that the ARR genes are part of a cytokinin-mediated signalling pathway which enables gene expression in leaves to be modulated by the inorganic N supply to the roots

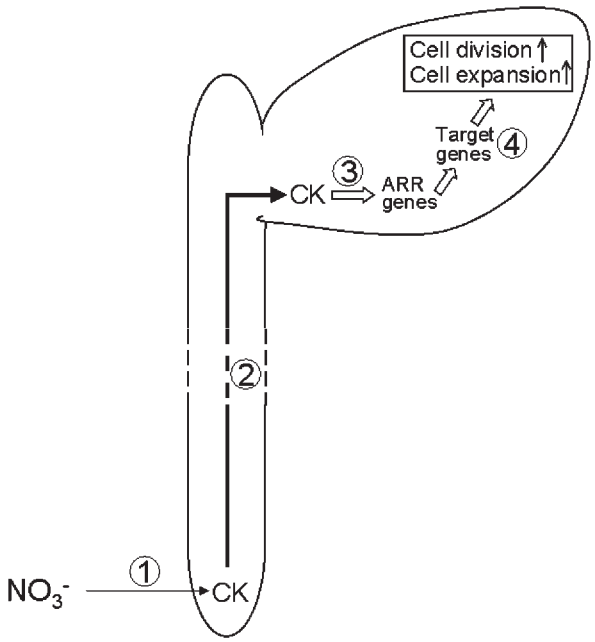

Figure 12. Model for the control of leaf expansion by the NO_3^- supply to the root. (1) The external supply of NO_3^- stimulates cytokinin (CK) production in the root. (2) CKs are transported to the shoot via the xylem. (3) CKs stimulate expression of members of the ARR (Arabidopsis response regulator) family of transcription factors. (4) The ARR transcription factor(s) activate a set of target genes to stimulate cell division and cell expansion in the leaf. See text for further details.

(Sakakibara et al., 2000). The finding that one of the cytokinin-regulated genes (ARR5) is expressed in shoot apical meristems (D'Agostino et al., 2000), would be consistent with a possible role in regulating leaf expansion in response to the NO_3^- supply. A tentative model for such a NO_3^-—cytokinin signalling pathway is depicted in Figure 12.

In many species, increased N fertilisation is reported to delay flowering (Bernier et al., 1993). In one study, flowering time in Arabidopsis was found to be only slightly delayed by a high rate of N supply (6 mM compared to 0.1 mM NH4NO3) (Schulze et al., 1994). In this study, seed set was much more sensitive to the N supply than flowering time, with the number of seeds per plant being over 10-fold greater under the high N regime. Importantly, by switching plants from high N to low N and vice versa at the end of the vegetative phase of growth, it was shown that it was the N supply during the reproductive phase that was critical in determining seed numbers (Schulze et al., 1994). It is not yet known which are the key N pools responsible for regulating leaf growth and seed set in Arabidopsis.

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