

Molecular Basis and Regulation of Ammonium Transporter in Rice

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Abstract: Rice grows in flooded paddy fields and takes up ammonium as the preferred nitrogen (N) source. Ammonium uptake is facilitated by a family of integral membrane proteins known as ammonium transporters found in all domains of life. However, the molecular mechanism and functional characteristics of the ammonium transporters (AMT) in rice have not been determined in detail yet. In this review, we report a genome-wide search for AMT genes in rice, resulting in the increase of the number of potential AMT proteins to at least 12, including members of both the alpha and beta sub-groups. Analysis of the predicted protein sequences for the 12 OsAMT proteins identified many conserved phosphorylation sites in both the alpha and beta group members, which could potentially play a role in controlling the activity of the transporters. Present knowledge of the expression of rice AMT genes is also summarized in detail. Future studies should focus on the structural and functional characteristics of OsAMT proteins to provide insight into the mechanism of ammonium uptake and its regulation in rice. Such research could improve utilization and decrease wastage of N fertilizer in rice cultivation.

Key words: rice; ammonium transporter; expression regulation; phosphorylation site

Nitrogen (N) contributes 1% to 5% of the dry weight in higher plants and is one of the most important nutrients for plant growth and reproduction^[1-2]. Ammonium is a major inorganic N source for plants, especially in waterlogged soils^[3-4]. Previous studies have reported that plants take up ammonium by both high- and low-affinity transport systems. The low-affinity system is non-saturable with a K_m in the millimolar concentration range whereas the high-affinity system is saturable and energy-dependent with a K_m in the submillimolar concentration range^[5]. Whilst the low-affinity ammonium transporters have yet to be identified, the proteins responsible for the high-affinity uptake have been identified in many plant species. They belong to a ubiquitous family of membrane proteins known as the ammonium transport (AMT) proteins found in all domains of life, from bacteria to human.

The AMT proteins have been characterized genetically and biochemically from a variety of organisms with the most detailed information coming from studies of the *Escherichia coli* ammonia channel, AmtB. The proteins are homotrimers with a substrate conduction

channel within each monomer, but their mode of action is still not fully understood. Despite their original designation, studies in bacteria suggest that they function as channels through which ammonia (NH_3) moves^[6]. However, studies of plant AMTs have argued that they transport charged (NH_4^+) or co-transport NH_3 with a proton^[7]. Analysis of the predicted amino acid sequences of AMT family members combined with X-ray structures of *E. coli* AmtB, *Archaeoglobus fulgidus* Amt1 and *Nitrosomonas europaea* Rh50 suggest that all ammonia channel proteins are likely to have either 11 or 12 transmembrane helices (TMH)^[8-10]. All three structures elucidated to date have 11 TMHs with an N-out, C-in topology, but analyses of the human rhesus proteins (the animal homologues of the AMT proteins) suggest that in some cases, there may be a 12th N-terminal helix giving an N-in, C-in topology.

In most plants, the AMT proteins constitute a multi-gene family, and many AMT genes in higher plants have been characterized by using functional complementation of a yeast mutant that lacks the native yeast *amt* genes. For dicotyledonous plants, the characterization, regulation and molecular mechanisms of AMT proteins have been well summarized^[2, 11-12]. The model plant species *Arabidopsis thaliana* encodes

Received: 13 July 2009; **Accepted:** 19 October 2009

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six AMT genes: *AtAMT1.1–AtAMT1.5*, *AtAMT2.1* [11,13]. Among them, *AtAMT1.1*, *AtAMT1.2*, *AtAMT1.5* and *AtAMT2.1* are mainly expressed in roots and shoots, *AtAMT1.3* specifically in roots and *AtAMT1.4* in pollen [2, 14-16]. The substrate affinities of these proteins have been calculated to be 50, 234, 61 and 4.5 $\mu\text{mol/L}$ for AMT1.1, AMT1.2, AMT1.3 and AMT1.5, respectively [17]. The transcript levels of AtAMTs were regulated by the available N supply, photosynthetic products and diurnal change [13]. The expressions of *AtAMT1.1* and *AtAMT1.3* were increased under the conditions of N starvation [13], and they contributed about 70% of ammonium influx in N-deficient roots [15,18]. *AtAMT1.2* in roots is localized in the root endodermis and cortex, and facilitates the uptake of ammonium from external environments as well as the translocation of ammonium from roots to shoots.

AMT genes have been isolated from a variety of other dicotyledonous plants including *Lycopersicon esculentum*, *Lotus japonicus* and *Brassica napus*. In *L. esculentum*, *LeAMT1.1*, which is mainly expressed in root hairs, is a high-affinity ammonium transporter and its transport activity has been reported to be concentration- and voltage-dependent, but not affected by protons [7], implying that the transport mechanism is probably independent of protons. Moreover, co-expression of wild-type and mutant tomato AMT genes in *Xenopus laevis* oocytes resulted in the inhibition of ammonium transport in a dominant negative manner, suggesting cross-talk between AMT oligomers and/or heterooligomers [19]. In *B. napus*, two AMT genes, *BnAMT1.1* and *BnAMT1.2* were isolated. *BnAMT1.1* is 87% similar to *AtAMT1.1* and weakly expressed, whereas *BnAMT1.2* is 97% similar to *AtAMT1.3* and strongly expressed in shoots [20]. *LjAMT2.1*, from *L. japonicus*, is constitutively expressed in the whole plant, especially in all major tissues of root nodules, which suggests that *LjAMT2.1* may facilitate the utilization of ammonium exported by nodule cells as a product of N fixation [21].

Rice cultivated in flooded paddy fields is one of the most important staple foods. Rice plants take up ammonium as their preferred N source and are also ammonium-tolerant. Hence the AMT proteins in rice are likely to play a key role in ammonium uptake, and their functions may be more diverse and complex. To

date, ten AMT genes have been identified in rice and assigned to three sub-families, three genes for *OsAMT1*, *OsAMT2* and *OsAMT3*, respectively, and one for *OsAMT4* [22]. However, present knowledge of rice AMTs is less detailed than that of other plants and their functional characteristics are not well understood. With the completion of rice genome sequencing, some AMT genes in rice have again been identified, but some of them now have multiple accession numbers, which results in considerable confusion. In this review, we report the presence of at least 12 AMT genes in rice genome, and update some molecular information and describe their phylogeny. Previous studies of the regulation of *OsAMT* gene expressions have also been summarized. These data provide a foundation for further studying the mechanism of ammonium uptake and functional structure of the AMT proteins in rice. Such research will contribute a theoretical basis to improve N utilization in rice and to decrease wastage and pollution of N fertilizer.

Putative AMT genes in rice

To obtain the putative AMT genes in rice, an annotation search was performed in the TIGR rice genome annotation database (<http://rice.plantbiology.msu.edu/>), the gene expression database for rice (<http://rice.plantbiology.msu.edu/>), and the KOME database (<http://cdna01.dna.affrc.go.jp/cDNA/>). Twelve putative *OsAMT* genes were identified by a search in the TIGR rice genome annotation database, and the NCBI accession numbers of all the 12 genes are listed in Table 1. Of these genes, 10 were previously identified by Suenaga et al [22] and grouped into four subgroups, *OsAMT1* to *OsAMT4*. The accession numbers and nucleotide sequences of *OsAMT1.1* (AF289477), *OsAMT1.2* (AF289478) and *OsAMT1.3* (AF289479) had been updated since 2005. The *OsAMT5.1* also was equal to the *OsAMT5* that was identified by Deng et al [23]. In addition, we identified one more gene, TIGR locus LOC_Os11g01410, which presents 93.7% homology with *OsAMT5.1* and has been designated as *OsAMT5.2*. The 12 AMT genes distribute on rice chromosomes 1 to 5, 11 and 12. Chromosomes 1 and 2 contained three genes each, chromosome 3 contained two genes, and chromosomes 4, 5, 11 and 12 contained one gene each.

Table 1. List of predicted AMT genes in rice.

Gene name	Locus identifier in TIGR	Chromosome	Accession number in NCBI	Protein (aa)	Site of AMT motifs (bp) (Hits by PS01219)	No. of transmembrane regions
<i>OsAMT1.1</i>	LOC_Os04g43070	4	AF289477, NM_001059815, XM_473131	498	191–216	11 (N-out)
<i>OsAMT1.2</i>	LOC_Os02g40710	2	AF289478, NM_001053990	498	193–218	11 (N-out)
<i>OsAMT1.3</i>	LOC_Os02g40730	2	AF289479, NM_001053991	496	191–216	11 (N-out)
<i>OsAMT2.1</i>	LOC_Os05g39240	5	NM_001062336, AB051864	486	187–212	11 (N-out)
<i>OsAMT2.2</i>	LOC_Os01g61510	1	NM_190445	498	196–221	11 (N-out)
<i>OsAMT2.3</i>	LOC_Os01g61550	1	NM_001051237	502	200–225	11 (N-out)
<i>OsAMT3.1</i>	LOC_Os01g65000	1	NM_001051467, AB083582	498	203–228	11 (N-out)
<i>OsAMT3.2</i>	LOC_Os03g62200	3	NM_001058371	480	none	11 (N-out)
<i>OsAMT3.3</i>	LOC_Os02g34580	2	NM_001053632	481	195–220	11 (N-out)
<i>OsAMT4</i>	LOC_Os03g53780	3	AC091811	300	none	4 (N-out)
<i>OsAMT5.1</i>	LOC_Os12g01420	12	NC_008405	459	none	10 or 11 (N-out)
<i>OsAMT5.2</i>	LOC_Os11g01410	11	NC_008404	327	none	8 (N-out)

All the OsAMT proteins match the ammonium transporter domain (Accession No. PF00909) when analyzed using the Pfam program (<http://pfam.sanger.ac.uk/search/sequence>)^[24], supporting the proposal that all of them might have ammonium transport function. The length of the monomer for each of the OsAMT proteins ranged from 459 aa to 502 aa, with the exception of OsAMT4 and OsAMT5.2, which were predicted to be only 300 aa and 327 aa, respectively. All of the ten full length OsAMT proteins can be predicted with the program HMMTOP 2.0 (<http://www.enzim.hu/hmmtop/index.html>) to conform to the topology derived from known AMT crystal structures, namely 11 transmembrane regions with an extracellular N-terminus and an intracellular C-terminus^[25-26] (Table 1). However, OsAMT5.1 was predicted to contain either 10 or 11 transmembrane regions using the programs HMMTOP 2.0 and ConPred II (<http://bioinfo.si.hirosaki-u.ac.jp/~ConPred2/>)^[27-28]. Eight OsAMT proteins included the ammonium transporter signature (PROSITE entry PSO1219)^[29-30] (Table 1), the consensus sequence of which was D-[FYWS]-[AS]-G-[GSC]-x(2)-[IV]-x(3)-[SAG](2)-x(2)-[SAG]-[LIVMF]-x(3)-[LIVMFYWA](2)-x-[GK]-x-R, and it was located in the fifth transmembrane helix (as defined by the *E. coli* AmtB structure).

Studies using the heterologous expression in yeast have shown that OsAMT1.1, OsAMT1.2, OsAMT2.1 and OsAMT5.1 all function as ammonium transporters^[22-23]. Both OsAMT4 and OsAMT5.2 appeared to be partial sequences as they encode only four and eight transmembrane regions, respectively. Further sequence and expression analysis together

with functional studies would be needed to assess any potential function of these two predicted proteins.

Phylogenetic analysis of the AMT family in higher plants

In order to analyze the relationships of the predicted OsAMT proteins, we compared them with the predicted products of other AMTs reported in higher plants: two members in *B. napus*, three in *L. esculentum*, *L. japonicus*, and *T. aestivum*, respectively, and six in *A. thaliana*. The *E. coli* AmtB protein served as a reference in the analysis. Previous phylogenetic analysis of ammonium transporters indicated that they could be divided into two groups^[31]: Amt α group which included *E. coli* AmtB and *A. thaliana* AMT2.1, and Amt β group which included the *A. thaliana* AMT1 proteins.

Our phylogenetic analysis confirmed that all the AMT sequences in higher plants could be assigned to either the Amt α group or the Amt β group (Fig. 1). *E. coli* AmtB is only very distantly related to the plant sequences but is closer to the Amt α group, as expected. The very clear separation of all the OsAMT sequences into either group questions the utility of the previously proposed subdivision of the OsAMT proteins into subfamilies 1, 2, 3, 4 and 5^[22-23]. Given that there is currently no functional data to indicate that these subfamilies have distinct properties or functions, we would argue that there is a strong case for simplifying the present nomenclature so that all OsAMT proteins are either OsAMT1 or OsAMT2.

Heterologous expression studies using an AMT-defective yeast mutant or *Xenopus laevis* oocytes indicated

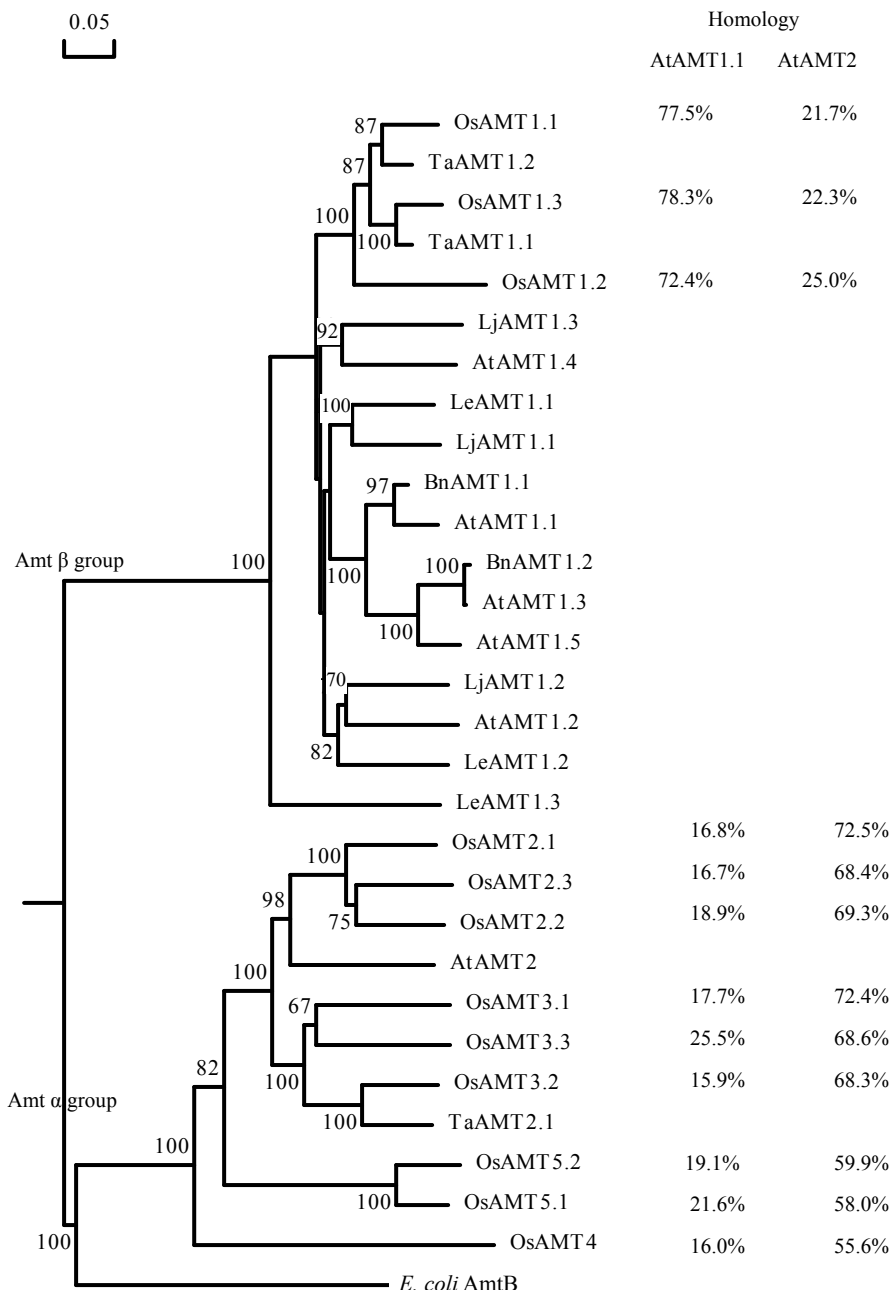


Fig. 1. Phylogenetic tree of AMT proteins in plants.

The phylogenetic tree was constructed by multiple sequences alignment of plant AMT proteins and performed using the DNAMAN 6.0 software with the Neighbor-joining method. Figures for homology alignment were calculated using the DNAMAN 6.0 software. The AMT proteins with the NCBI accession numbers are AtAMT1.1 (CAA53473), AtAMT1.2 (AAD54639), AtAMT1.3 (AAD54638), AtAMT1.4 (CAB81458), AtAMT1.5 (NP_189072), AtAMT2 (NP_181363), TaAMT1.1 (AAS19466), TaAMT1.2 (AAS19467), TaAMT2.1 (AAR87397), LeAMT1.1 (X92857), LeAMT1.2 (CAA64475), LeAMT1.3 (Q9FVN0), LjAMT1.1 (AAG24944.1), LjAMT1.2 (AY135020), LjAMT1.3 (AJ575588), BnAMT1.1 (AF188744), BnAMT1.2 (AF306518) and *E. coli* AmtB (AAD14837). The protein sequences for the rice AMT proteins are consistent with the locus identifier in TIGR (Table 1).

that AMT1 family genes encode high affinity transporters, e.g. AtAMT1.1 to AtAMT1.5 [13, 15, 17, 32], LeAMT1.1 [19] and LjAMT1.1 [33]. Furthermore, almost all the AMT1 genes expressed in roots [7, 13, 15]. Taken together, it is suggested that AMT1 family proteins play a key role in high affinity ammonium uptake

from soil. Indeed, studies have shown that in *Arabidopsis*, AtAMT1.1, AtAMT1.2 and AtAMT1.3 contribute about 30%–40%, 18%–26% and 30% of ammonium influx into roots under the conditions of low external N concentration, respectively [17-18, 34]. However, phylogenetic analysis showed that the

OsAMT1s were relatively distant to the AtAMT1s. This suggests that the function and mechanism of AMT1 family members in rice are diverse from those in *Arabidopsis*.

Studies of AMT α group proteins are more limited. However, *AtAMT2.1*, *OsAMT2.1* and *OsAMT3.1* are all expressed in both root and shoot tissues. *AtAMT2.1* has been shown to be a high-affinity transporter with a K_m of about 20 $\mu\text{mol/L}$ [35]. *OsAMT2.1* required a higher concentration of ammonium than *OsAMT1.1* or *OsAMT1.2* in order to rescue the growth of a yeast *amt* deletion strain (5 mmol/L rather than 1 mmol/L), suggesting that under these conditions, it may have a low affinity for ammonium.

Potential for regulation of plant AMT proteins by phosphorylation of residues within the C-terminal cytoplasmic tail

The flux of substrates through membrane transporters or channels is frequently regulated in response to a combination of substrate availability and the metabolic needs of the cell. In bacteria and archaea, the flux of ammonia through AMT proteins is regulated by reversible interaction of the channel with a cytoplasmic signal transduction protein called GlnK that forms a complex with the channel under the conditions of ammonium sufficiency and prevents further influx [36]. However, GlnK protein in plants is not thought to be involved in regulation of AMT proteins.

Phosphorylation of membrane proteins is a recognized means by which their activities may be regulated, e.g. in the plant aquaporins [37], and it has been suggested that such covalent modification might

also regulate AMT proteins [38]. In *Arabidopsis*, phosphorylation sites in a number of plasmamembrane proteins have been identified and amongst these was AtAMT1.1 [39-41]. Six phosphorylation sites were detected in AtAMT1.1, all of which were located within the C-terminal tail: ⁴⁶⁰T, ⁴⁷⁵S, ⁴⁸⁸S, ⁴⁹⁰S, ⁴⁹²S and ⁴⁹⁶T. The possible significance of these phosphorylation events for control of AMT activity was tested by converting ⁴⁶⁰T of AtAMT1.1 to either alanine or aspartate, the latter being a potential phenocopy of the phosphorylated threonine. The T460D protein was inactive whereas T460A retained activity [10, 42]. Comparable changes to the equivalent threonine residue (⁴⁷²T) in AtAMT1.2 gave similar results: T472D was inactive and T472A was active [42]. The role of the C-terminal cytosolic region of AMT proteins is not well understood but it contains a well-conserved sequence of around 20 amino acids immediately adjacent to the last TMH which forms two single turn helices between which lies a highly conserved glycine residue. ⁴⁶⁰T lies within the first short helix and its phosphorylation in AtAMT1.1 could conceivably change the conformation of the C-terminal region, thereby in some way regulating the protein's activity.

We scanned the sequences of the OsAMT proteins using the NetPhos program in order to identify potential phosphorylation (<http://www.cbs.dtu.dk/services/NetPhos/>) [43] and combined it with the alignment of AtAMT1.1 sequence to look for homologies to the known phosphorylation sites. All three OsAMT1 proteins contain a homologue of AtAMT1.1 ⁴⁶⁰T: ⁴⁵²T for OsAMT1.1, ⁴⁵⁵T for OsAMT1.2 and ⁴⁵³T for OsAMT1.3 (Fig. 2). It has shown that for OsAMT1.2, a mutation causing the T455D change led to the

	T ⁴⁶⁰	
	↓	
AtAMT1.1	KKMKLLRISSEDEMAGMDNTRHGGFAYMYFDDDESHKA. IQLRRVEP.	486
OsAMT1.1	KKLGLLRISAEDETSGMDLTRHGGFAYVYHDEDEHDKS. .GVGGFNL.	477
OsAMT1.2	HRFGLLRVSPATEMEGMDPTCHGGFGYVDEDEGERRRVRAKSAEETAR.	482
OsAMT1.3	NKLGLLRISAEDEMAGMDCLTRHGGFAYAYHDDASGKPDERSVGGFML.	480
OsAMT2.1	GLFIPLRNP. DEQLMGDDAAHGEEAYALWGDGKFDATRHDLSRGG.	461
OsAMT2.2	GLVVP LRNP. DEQLKI GDDAAHGEEAYALWGDGERFDVTRHEGARGGA	471
OsAMT2.3	GLFIP LRVS. DDQLMGDDAAHGEEAYALWGDGKFDVTRPETTRTGG	475
OsAMT3.1	RAVVP LRNP. EEELAI GDDAVHGEEAYALWGDGKFDSTKHGWYSDN.	479
OsAMT3.2	NLLVPLRNP. DDKLEVGDVAHGEEAYALWGDGEMYDVTKHG. . . .	469
OsAMT3.3	SLI LPLRI A. DQELLI GDDAVHGEEAYAI WAEGLNDMTHH. . . .	464
OsAMT4	RVAVP. . . . QLAGGGDAI HGEDAYAVWGDGETYEQYSVHGGGSN.	278
OsAMT5.1	RAVVP LRMT. EDPELLAGDI AVHGEOAYFSSGTCNSLSHET. . . .	452
OsAMT5.2	326
Consensus	

Fig. 2. Conserved phosphorylation sites within the C-terminal cytoplasmic tail of rice AMT transporters.

Potential phosphorylation sites were identified using the NetPhos program and sites that are conserved within phylogenetic groups are indicated by boxes.

functional inactivity (results unpublished), suggesting that a similar regulation by phosphorylation to that proposed in *Arabidopsis* may take place in rice. The other residues identified as phosphorylation sites in AtAMT1.1 are in a region that is not highly conserved in the AMT proteins either of *Arabidopsis* or of rice, so the significance of these sites is presently more difficult to assess. We also identified two more highly conserved potential phosphorylation sites within the C-terminal region: one homologous to ⁴⁴¹S in OsAMT1.1 and one homologous to ⁴⁵³T in OsAMT2 (Fig. 2). However, these have not yet shown to be phosphorylated *in vivo* and are worthy of future study.

Though the C-terminal regions of the OsAMT proteins are quite varied in length, all of them contain the region that would be expected to fold into two short helices and the glycine between them is completely conserved in all cases (Fig. 2). Mutations in this glycine residue have been shown to inactivate the AMT proteins in a number of organisms^[44-47]. Furthermore, when expressing together with a wild-type gene in the same cell, this inactive polypeptide can have an epistatic effect leading to trans-inactivation of the active wild-type protein. It suggests that this phenotype may be a consequence of co-operative effects between the three AMT sub-units which are mediated by the C-terminal region^[47].

A point mutation G456A/D or a variety of mutations that delete part of the cytosolic C tail from Leu446 to the end of the protein led to trans-inactivity of AtAMT1.1 transporter^[10]. Moreover, the co-expression of wild-type transporter and its specific mutation in yeast or *Xenopus* oocytes inhibited ammonium transport^[10, 19]. These observations suggest that in plant AMT proteins, conformational change or coupling between monomers may play a role either in the normal activity of the protein and/or in the regulation of ammonium uptake^[10, 19, 42].

Expressions and regulations of *OsAMT* genes

So far, studies on expressions and regulations of AMT genes in rice have been focused on the three genes of OsAMT1 family, which displayed different spatio-temporal expression patterns in response to changes in N levels or daily irradiance^[48-49]. The *OsAMT1.1* gene (AF289477) shows constitutive

expression in shoots and roots and is almost unaffected by N starvation, the form of N supplied or N levels^[48, 50]. *OsAMT1.2* (AF289479) and *OsAMT1.3* (AF289478) show root-specific expression^[48], but the expressions of them were also detected in shoots^[51-52]. The expression level of *OsAMT1.2* is up-regulated by the supply or resupply of ammonium^[48, 50]. Moreover, the mRNA expression of *OsAMT1.2* was located in the cortical cells and vascular cylinder of rice roots, which indicates a functional role of OsAMT1.2 not only in ammonium uptake from soil but also in transport and retrieval in the vascular system. By comparison, *OsAMT1.3* shows faint expression under N deprivation and is strongly up-regulated by resupplied ammonium, nitrate or both for 2 h^[50, 53]. However, two rice varieties (Nipponbare and an indica variety) showed diverse expression pattern for *OsAMT1* genes. Kumar et al^[49] found differential expressions of *OsAMT1* genes in *Oryza sativa* subsp. *indica*. The three genes of OsAMT1 were strongly expressed under the supply of 10 μmol/L ammonium. Additionally, the transcript expression levels of *OsAMT1.1* and *OsAMT1.2* were mainly regulated by the plant N status, being down-regulated when moved from 10 μmol/L ammonium to 10 mmol/L ammonium, whereas *OsAMT1.3* appeared to be regulated by diurnal variation, being more strongly expressed during the day than the night^[49].

By a cDNA microarray analysis, we found the expression levels of some AMT genes in *Oryza sativa* subsp. *japonica* cv. Wuyunjing 7 to be up-regulated or down-regulated by N-starvation or resupplied nitrate or ammonium as the sole nitrogen source, respectively, in contrast to ammonium nitrate nutrition^[54] (<http://www.ncbi.nlm.nih.gov/projects/geo/>). *OsAMT2.1* is constitutively expressed^[22] and shows enhanced expression in roots following N starvation treatments (Table 2). By contrast, the expression of *OsAMT3.1* is significantly decreased about four-fold in shoots. The expressions of both *OsAMT3.2* and *OsAMT3.3* are strongly up-regulated by resupplied ammonium or nitrate and N starvation in roots, while in shoots, only *OsAMT3.3* was markedly up-regulated either by ammonium or nitrate.

In physiological studies, feedback regulation of N uptake in plants has been determined by N assimilation, for example, glutamine or other amino acids depending

Table 2. Regulation of the expression of AMT genes in rice by cDNA microarray analysis^[54].

Gene name	Root			Shoot		
	NH ₄ ⁺	NO ₃ ⁻	N-free	NH ₄ ⁺	NO ₃ ⁻	N-free
<i>OsAMT2.1</i>	1.50	1.38	2.08	-1.01	1.02	1.03
<i>OsAMT3.1</i>	1.00	-1.03	-1.05	-1.03	-1.05	-4.55
<i>OsAMT3.2</i>	3.03	3.90	3.15	1.83	1.81	-1.52
<i>OsAMT3.3</i>	3.47	7.78	3.51	3.18	2.40	-1.02

The entire data can be searched in Gene Expression Omnibus (GEO accession No. GSE4409).

on crops^[55-57]. Molecular evidence also suggests that glutamine is a potential regulator controlling OsAMT1 transporters to affect N uptake in rice roots. The expression levels of *OsAMT1.1* and *OsAMT1.2* in rice roots were positively correlated with the content of glutamine in root tissue, whereas the expression patterns of *OsAMT1.3* were negatively correlated with the glutamine pool^[58]. Exogenous glutamine had the same regulatory effect on the expression of OsAMT1 as ammonium. Transcript expression levels were up-regulated by the supply of ammonium and glutamine, and down-regulated by adding methionine sulfoximine, an inhibitor of glutamine synthetase. The expression pattern of *OsAMT1.3* in response to treatments with ammonium, glutamine or methionine sulfoximine was opposite to those of *OsAMT1.1* and *OsAMT1.2*^[58]. These data indicate that glutamine is an essential regulator of the expression of OsAMT1 genes and consequently it has a clear feedback effect on ammonium uptake and transport in rice.

Future prospects for OsAMT genes study

Rice plants grow in submerged conditions and take up ammonium as their preferred N source. The OsAMT proteins play a key role in ammonium uptake. However, their molecular mechanisms, kinetic and structural properties are not well understood. The molecular mechanisms and structure functional characteristics of the AmtB/Mep family have been well studied and elucidated in the model plant *Arabidopsis* and the bacterium *E. coli*^[59]. These studies offer some insights into the molecular characteristics of the AMT family in rice, but the details of these systems are unclear, and with potentially up to 12 genes encoding AMT proteins, ammonium uptake and transport in rice may be very complex.

In the future, mRNA localization for each of the OsAMT genes and the ammonium transport properties of each of their products need to be studied in detail. One useful tool for such studies will be the construction of AMT mutant lines, but this approach is very difficult and time-consuming. In addition, heterologous expression of the rice genes in yeast and *Xenopus* oocytes^[60] together with the construction of point mutants *in vitro* are convenient and effective technologies that will assist the identification of rice AMT protein structure/function relationships. Such approaches can potentially make a very important contribution to our understanding of the mechanisms of ammonium transport in rice, in order to seek advisable measures to improve N use efficiency in agriculture and reduce the losses of N fertilizer.

ACKNOWLEDGEMENTS

This work was supported by the China Post-doctoral Science Foundation (Grant No. 20070421031), the National Basic Research Program of China (Grant No. 2007CB109303), Knowledge Innovation Project of the Chinese Academy of Sciences (Grant No. KSCX2-YW-N-002), and MM acknowledges support from the Biotechnology and Biological Sciences Research Council (UK).

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