Ammonium transport proteins (AMT/MEP) have been identified in bacteria, fungi, plants, and animals and constitute a conserved family of polytopic membrane proteins (54). AMT/MEP proteins are predicted to contain 11 transmembrane (TM) helices with an N_out-C_in topology (26, 48). Bioinformatic topology predictions indicate that bacterial AMT/MEP proteins generally contain an additional N-terminal TM domain but that this region acts as a signal peptide which is removed from the mature protein (9). Aside from the small cytoplasmic loop between TM domains 3 and 4 that displays sequence similarity to a major facilitator superfamily motif (34), the AMT/MEP sequences represent a unique group of transport membrane proteins. Although over 300 members of the AMT/MEP/Rh family have currently been assigned based on amino acid sequence similarity ( Pfam accession number PF00909), an ammonium transport function has not been confirmed for the vast majority of these sequences. Functional permeases have been described for bacteria, fungi, plants (reviewed in reference 54), and humans, where the rhesus blood group polypeptides, which display significant sequence identity to AMT/MEP proteins, have been shown to function as ammonium transporters in other systems (27, 53, 55, 59). The structure of Escherichia coli AmTb has been determined up to 1.35-Å resolution, and structural analysis revealed that the protein functions as a trimer that recruits ammonium which is then channeled as ammonia (18, 58). Genetic, molecular, and/or physiological evidence suggests that AMT/MEP proteins can function as homo- and/or heterocomplexes (6, 25, 29, 34).

The presence of multiple ammonium permeases with different kinetic properties within an organism is common (54). The yeast Saccharomyces cerevisiae has three AMT/MEP genes, MEP1, MEP2, and MEP3. MEP1 constitutes the majority of ammonium uptake for S. cerevisiae, with an affinity in the 5 to 10 μM range, MEP2 has the highest affinity for ammonium (1 to 2 μM), and MEP3 is a low-affinity (1 to 2 mM), high-capacity ammonium permease (28, 30). Certain fungal ammonium permeases, including S. cerevisiae Mep2, Candida albicans Mep2p, and Ustilago maydis UMP2, also function as ammonium sensors, generating a signal to regulate pseudohyphal or filamentous growth in response to nitrogen starvation (5, 23, 44). A MEP1 MEP2 MEP3 triple deletion mutant was unable to grow on media containing ≤5 mM ammonium as the sole nitrogen source, whereas single deletion strains displayed normal growth (28). Each of the MEP genes displays an expression profile typical of genes subjected to nitrogen catabolite repression. The expression of these genes is low on good nitrogen sources, such as asparagine, glutamine, and ammonium, that support optimal yeast growth, and levels are elevated on low-ammonium or sub-optimal nitrogen sources such as proline (28). The GATA factors Gln3p and Nil1p, which mediate nitrogen catabolite repression in S. cerevisiae, control the expression of all three MEP genes (28).

Two Aspergillus nidulans AMT/MEP genes, meaA and mepA, have been characterized (33). A meaAΔ mutant displays reduced growth on ammonium as the sole nitrogen source, whereas a mepAΔ mutant exhibits normal growth under these conditions. A mepAΔ meaAΔ mutant is unable to grow with low ammonium concentrations at pH 4.5, and the residual growth at pH 6.5 has been attributed to the passive diffusion of low ammonium concentrations at pH 4.5, and the residual growth at pH 6.5 has been attributed to the passive diffusion of ammonium (24, 33, 45). The MepA permease displays a higher affinity for methylammonium than does MeaA (K_m, 44.3 μM and 3.04 mM, respectively). MeaA serves as the main ammonium transporter, whereas the higher-affinity MepA permease is likely to have a scavenging role in ammonium uptake (33).

Here we present a further characterization of the A. nidulans ammonium transport system and its regulation.
identified two additional AMT/MEP-like sequences in the *A. nidulans* genome and present a functional analysis of these genes, designated mepB and mepC. We show that even though the four *A. nidulans* AMT/MEP genes are differentially regulated in response to the nitrogen status of the cell, their full expression requires the function of the GTA transcription factor AreA (3, 20, 31).

### MATERIALS AND METHODS

*A. nidulans* strains, media, and transformation. The *A. nidulans* strains used for this study are shown in Table 1. *A. nidulans* media (ANM) and growth conditions were as described by Cole (10), except that the pH of the media was adjusted to either pH 6.5 or pH 4.5. Genetic analysis was carried out using previously described techniques (8). Strains of *A. nidulans* were transformed according to the method of Andrianopoulos and Hynes (1).

Molecular techniques. Standard methods for the manipulation of *E. coli* cells and DNA were done as described by Sambrook and Russell (39). The *E. coli* strain used for this study was NM522. Restriction enzymes (Promega) were used according to the method of Andrianopoulos and Hynes (1).

**Cloning of mepB and mepC.** Based on the partial *A. nidulans* genome sequence available at the Monsanto Microbial Sequence Database (http://microbial.cereon.com), mep-specific primers were designed (Table 2). An 1,129-bp *mepB* product was amplified using primers mepB-F and mepB-R and 100 ng genomic DNA at an annealing temperature of 58°C with 1.5 mM MgCl₂. Hybridization of the *mepB* PCR product to an *A. nidulans* bacterial artificial chromosome (BAC) library (kindly provided by Ralph Dean, Department of Plant Pathology and Physiology, Clemson University) identified seven positive clones (3O2, 11P5, 15F8, 19Cl1, 27E10, 31H17, and 7P14). A 5.3-kb BamHI-XbaI fragment from BAC 7P14 was subcloned into pBluescript SK(+) (Stratagene) to create *pBJS536* and was sequenced. Based on the *A. nidulans* genome sequence available at the time via the Aspergillus Sequencing Project, Whitehead Institute/MIT Center for Genome Research (http://www.genome.wi.mit.edu), the mepC-specific primers mepC-1 and mepC-2 were designed and used to amplify a 5-kb product containing the coding region of the gene. The PCR product was ligated into pGEM-T Easy (Promega), creating pMA574.

**Creation of mepB-Δ and mepC-Δ mutants by homologous gene replacement.** The *mepB* deletion construct, *pBJS539*, was made by inserting a 1,457-bp BamHI bloomycin resistance cassette (Bleo³) from *pAmPl250* (4) into the BglII sites of *pBJS537*. *pBJS539* was linearized by digestion with KpnI and NotI and then transformed into the *mepA Δ* mutant MH10328. Transformants were selected for resistance to 1 mg/ml bloomycin, and 66 transformants were

### TABLE 2. Oligonucleotides used for this study

| Primer name | Primer sequence (5'-3') |
|-------------|------------------------|
| Bub3        | AGTGGTTACCGCCGAGCACGACGAC |
| meaA2       | GACGCTTACGGAGACAAGTCAG |
| meaA-RT     | GGAAACTCAAGGGTGTTGAACAG |
| meaB-RT     | GCAATGTCAGTACGCTCGAGAG |
| meaC-F      | ACTCTGGTGTGTTCGAGATCTTC |
| meaC-R      | AACTGTTCTGTGTTAGGAGT |
| mepB1       | TTCTGGCGATCGTTCATGAGT |
| mepB-F      | GTATCTGATCTACTGCTGAG |
| mepB-R      | CAGTCGTCCGCTCACACTCATC |
| mepB-RT     | CTTCACTGCTTGGTTAGT |
| mepC-1      | TACCCGTCATATCCGGAAC |
| mepC-2      | CGTTCAGAAGGAGAATAATGG |
| mepC-Δ      | TTGTCGAGAGGTCATCGGAG |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTGTCGAGAGGTCATCGGAG |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
| mepC-Δ      | TTCTGGCGATCGTTCATGAGT |
Aspergillus nidulans contains four AMT/MEP genes. Two A. nidulans AMT/MEP genes, meaA and mepA, have been described previously (33). An analysis of the draft A. nidulans genome sequence identified partial sequences of two additional AMT/MEP sequences, designated mepB and mepC. A mepB-specific PCR product was used to screen an A. nidulans genomic BAC library, and a mepC-specific PCR product was generated from genomic DNA (see Materials and Methods). Sequence analysis indicated that mepB is comprised of five exons and is predicted to encode a 472-amino-acid protein and that the mepC open reading frame of 1,452 bp contains three exons encoding a predicted product of 453 amino acids. Subsequent annotation of the A. nidulans genome (http://www.broad.mit.edu/annotation/fungi/aspergillus/) has confirmed the sequences for all four genes, i.e., meaA (AN7463.2), mepA (AN1181.2), mepB (AN0209.2), and mepC (AN0496.2).

Alignment of the four predicted A. nidulans AMT/MEP protein sequences reveals substantial amino acid conservation throughout the proteins (Fig. 1A). While MeaA and MepA share 55% amino acid sequence identity (75% similarity), MepB displays 46% identity (65% and 68% similarity, respectively) with MeaA and MepA. In contrast, MepC shares only 31% identity (53% similarity) with MeaA, 35% identity (56% similarity) with MepA, and 31% identity (53% similarity) with MepB. Like MeaA and MepA, MepB and MepC contain both ammonium transporter signature motifs (PF00909) (54), although MepC has a total of seven mismatches across both signature sequences (Fig. 1B). Eleven putative transmembrane helices with an N-out-C-in topology were identified for MepB, using the prediction program TMHMM (52), which is in agreement with predictions made for other AMT/MEP transporters, including MeaA and MepA (33). Although it is likely that the structure of MepC resembles that of other AMT/MEP transporters, topology predictions for MepC using the TMHMM and TMHMM (46) programs were not as convincing. Together, these comparisons suggest that the MepC protein is more divergent than MeaA, MepA, and MepB.

The A. nidulans mepB gene encodes a high-affinity ammonium permease. The mepDA gene (meaA and mepA) double mutant MH9828 is unable to grow on 1 mM ammonium at pH 4.5 and is resistant to methylammonium, a toxic analogue of ammonium (33). To assess whether mepB encoded a functional ammonium permease, multiple copies of mepB (pBJM5377) were cotransformed with the pyroA' carrying plasmid p4 into MH9828, and transformants were screened for suppression of the double mutant phenotype. Thirty-seven of the 83 PyroA' transformants tested displayed increased growth on 1 mM ammonium (pH 4.5), whereas no growth was observed for either p4-only
transformants on any ammonium concentration or the degree of methylammonium sensitivity never reached wild-type levels (Fig. 2A). Overall, these results indicate that mepB encodes a functional ammonium permease and suggest that the capacity of this permease is limiting.

The transformant with the highest mepB copy number (co-transformant D) was used in [14C]methylammonium uptake studies (Fig. 2B). The apparent \( K_m \) (methylammonium) for MepB was determined to be 73.5 (±12.14) \( \mu M \), with an apparent \( V_{max} \) of 4.87 (±0.176) nmoles/min/mg dry weight. Since mepB was overexpressed in cotransformant D, the MepB \( V_{max} \) value is not comparable to that for a wild-type strain, whereas the difference in the actual relative amounts of active MepB does not affect \( K_m \) determination. These results indicate that mepB encodes a high-affinity (methyl)ammonium permease. The \( K_m \) calculated for MepB was very similar to that calculated previously for MepA (44.3 \( \mu M \)), which was determined for a meaA\( \Delta \) mepB\( ^+ \) strain (33). A mepB\( ^- \) mutant was created by homologous gene replacement (see Materials and Methods), and the MepA kinetic parameters were reassessed in the mepB\( ^- \) meaA\( ^{+} \) strain MH10323 (Fig. 2C). This analysis yielded an apparent \( K_m \) (methylammonium) for MepA of 69.11 (±9.46) \( \mu M \) and a \( V_{max} \) value of 1.28 (±0.052) nmoles/min/mg (Fig. 2C). The \( K_m \) value for MepA presented here is within the 95% confidence intervals for the value estimated previously and confirms that A. nidulans contains two high-affinity ammonium permease genes, mepA and mepB.

**Analysis of A. nidulans mepB\( ^{+} \) mutant.** Deletion of the two high-affinity permease genes, mepA and mepB, either singly or combined, had no effect on growth for all ammonium concentrations tested, at either pH 6.5 or 4.5 (Fig. 3A). Indeed, the [14C]methylammonium uptake rates of the mutants with 500 \( \mu M \) methylammonium showed that the presence of MeaA alone was sufficient for wild-type levels of methylammonium uptake under these conditions (Fig. 3B). The methylammonium transport activity of the meaA\( ^{+} \) mepB\( ^{+} \) double mutant was similar to that of the meaA\( ^{+} \) single mutant, indicating that transport in the absence of MeaA was mediated by MepA. However, a comparison of the transport rates of the meaA\( ^{+} \) mepA\( ^{+} \) double mutant and the meaA\( ^{+} \) mepA\( ^{+} \) mepB\( ^{+} \) triple mutant indicated that MepB is able to contribute about 27% of the wild-type transport activity under these conditions. The [14C]methylammonium uptake assays performed with 20 \( \mu M \) methylammonium showed that the transport rate for the mepB\( ^{+} \) mutant was slightly lower than that for the meaA or mepA single deletion mutant (Fig. 3C). The combined deletion of the high-affinity ammonium transport genes mepA and mepB only reduced the transport activity of the cell to 48% that of the wild type, compared to 37% and 21% for the meaA\( ^{+} \) mepA\( ^{+} \) and mepB\( ^{+} \) meaA\( ^{+} \) mutants, respectively (Fig. 3). This indicates that the MeaA permease has a greater capacity than either MepA or MepB, so cells relying on only MepA or MepB activity have low methylammonium transport rates.

Across all ammonium concentrations tested at pH 6.5, the meaA\( ^{+} \) mepA\( ^{+} \) mepB\( ^{+} \) mutant displayed reduced growth compared to the meaA\( ^{+} \) mepA\( ^{+} \) strain (Fig. 3). This was particularly notable at 1 mM ammonium, where no growth was observed for the meaA\( ^{+} \) mepA\( ^{+} \) mepB\( ^{+} \) mutant, indicating that the growth seen for the meaA\( ^{+} \) mepA\( ^{+} \) mutant on this medium was due to MepB activity. Interestingly, no growth of the
meaAΔ mepAΔ mepBΔ or meaAΔ mepAΔ mepBΔ mutant was observed at pH 4.5 on ammonium concentrations of 10 mM or less, indicating that MepB activity appeared to be absent on media at pH 4.5. As shown by the ammonium growth phenotypes of the multiple-copy mepB transformants (Fig. 2A), the MepB protein is able to function at pH 4.5, suggesting that differences in MepB activity at pH 4.5 and 6.5 may be regulated at the transcriptional level. Ambient pH regulation of genes in A. nidulans is controlled by the zinc finger transcription factor PacC, which activates the expression of alkaline-expressed genes and represses the transcription of acid-expressed genes (50). Two potential PacC binding sites (GCCARG) are present in the mepB promoter. MepC does not normally contribute to ammonium uptake.

To analyze the function of MepC, a mepC mutant was created by homologous gene replacement (see Materials and Methods). The phenotype of the mepCΔ mutant was indistinguishable from that of the wild type, and the deletion of mepC in all possible combinations with meaA, mepA, and mepB deletions did not alter the growth phenotypes of the various single, double, and triple mutants at any tested ammonium concentration or pH (data not shown). Furthermore, no significant [14C]methylammonium uptake activity was detected for the meaAΔ mepAΔ mepBΔ strain MH10325 (Fig. 3). For example, with 20 μM methylammonium, the meaAΔ mepAΔ mepBΔ mutant had an uptake rate of 0.0161 (±0.0023) nmol methylammonium/min/mg dry weight, which is only 2% that of the wild type and less than the standard error values for all of the other strains. Therefore, no methylammonium transport attributable to MepC activity was present under the conditions tested.

To assess whether the structurally different MepC protein could function as an ammonium transporter, the effect of overexpression was determined. Multiple copies of mepC (pMAS5741) were introduced into the mepAΔ meaAΔ meaBΔ MH11031 strain by cotransformation with the pyroA+ plasmid pI4. No PyroA+ transformants showed stronger growth than the recipient strain when tested on 1 mM or 10 mM ammonium (pH 6.5 or pH 4.5). The presence of additional copies of mepC (approximate range of 2 to 20 copies) was confirmed by Southern blot analysis (data not shown). Therefore, multiple copies of mepC were unable to suppress the ammonium growth phenotype of the triple mutant. Since the expression of mepC from its native promoter is low (see below), it was possible that even with multiple copies, the expression levels were not sufficiently high to allow suppression. To further elevate the levels of MepC expression, the mepC coding region was fused to the highly inducible xylP promoter (see Materials and Methods). The xylP::mepC construct pMA6382 was cotransformed into MH10325 with pI4, and PyroA+ transformants were screened on 1 mM and 10 mM ammonium at pH 4.5. In the presence of glucose, where the xylP promoter is
repressed, all transformants retained the phenotype of the recipient strain. On xylose medium, where xylP expression is induced, approximately 20% of the transformants showed partial restoration of growth on ammonium at pH 4.5. The copy numbers of the xylP::mepC construct in these complementing cotransformants (approximate range of 3 to 20 copies) were determined by Southern blot analysis (data not shown). The extent of growth of the complementing xylP::mepC cotransformants on any ammonium concentration or the degree of methylammonium sensitivity never reached wild-type levels and was comparable to that of transformants overexpressing mepB (Fig. 2A). These results indicate that MepC can function as an ammonium permease, but only when highly overexpressed from multiple copies of a xylP-driven gene.

**Differential expression of A. nidulans ammonium permease genes.** The expression of the A. nidulans ammonium permease genes under different nitrogen conditions was investigated by Northern blotting and RT-PCR analysis (Fig. 4A and B). The expression of meaA was readily detected for cells grown in ammonium and also glutamine, indicating that the expression
of meaA occurs under nitrogen-sufficient conditions. In contrast, the expression of mepA was repressed under these nitrogen-sufficient conditions but was readily observed for cells grown in nonrepressing nitrogen sources (glutamate, proline, alanine, or nitrate) or nitrogen-starved cultures (Fig. 4). The expression of meaA or mepA was not detected under carbon starvation conditions. The expression profile for mepB was unique, with a single transcript detected only under conditions of complete nitrogen starvation and not under conditions of nitrogen limitation (Fig. 4). This nitrogen starvation response of mepB expression was not detected in nitrogen-starved cultures that were simultaneously starved of carbon or in carbon-starved and ammonium-sufficient cultures. The expression of mepC was not detected by Northern analysis under any conditions tested. However, RT-PCR analysis showed low levels of mepC expression on ammonium, glutamine, and alanine that increased slightly in response to nitrogen starvation (Fig. 4).

These results show that each of the A. nidulans ammonium permease genes displays a distinct expression pattern.

**Full expression of A. nidulans AMT/MEP genes requires the global nitrogen activator AreA.** To investigate the role of AreA in the regulation of the A. nidulans AMT/MEP genes, areAΔ meaAΔ and areAΔ meaAΔ mepAΔ double and triple mutants were created by genetic crosses and assessed for growth on a range of ammonium concentrations (Fig. 5A). The areAΔ mutant MHS699 displayed poorer-than-wild-type growth for all ammonium concentrations tested. The growth of the areAΔ mepAΔ mutant MH10313 was indistinguishable from that of the areAΔ single mutant. At 1 mM ammonium, the areAΔ meaAΔ mutant MH10311 displayed no growth, indicating that the residual growth observed for the areAΔ mutant was due to MeaA function (Fig. 5A). The lack of growth of the areAΔ meaAΔ mutant at 1 mM ammonium (pH 6.5) also suggested that AreA is absolutely required for the expression of MepA and MepB activity. [14C]methylammonium uptake assays on nitrogen-starved mycelia were in agreement with the ammonium growth phenotypes (Fig. 5B). The areAΔ mutant and the areAΔ mepAΔ strain displayed an appreciably reduced methylammonium transport activity (35% of the wild-type activity). The areAΔ meaAΔ mepAΔ mutant MH10312 had a methylammonium uptake rate similar to that of the meaAΔ mepAΔ

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**FIG. 4.** Expression analysis of A. nidulans ammonium permease genes in wild-type background. (A) Northern analysis of ammonium permease gene expression from the wild-type (MH1) strain. RNAs were isolated from mycelia grown in 1% glucose-ANM medium, pH 6.5, with 20 mM ammonium (NH4+) at 37°C for 16 h and then transferred to fresh medium that was nitrogen- and/or carbon-free, as indicated. Alternatively, RNAs were isolated from mycelia grown for 16 h at 37°C in 1% glucose medium, pH 6.5, with 10 mM glutamine (gln), glutamate (glu), proline (pro), alanine (ala), or nitrate (NO3−), as indicated. Northern blots were hybridized with probes specific for meaA, mepA, mepB, and mepC (see Materials and Methods) or A. nidulans histone H3 as a loading control (13), as indicated. Sizes to the right (in kb) represent the approximate sizes of the respective transcripts. (B) Multiplex RT-PCR analysis of meaA, mepA, mepB, and mepC gene expression. The sizes of the AMT/MEP products (upper bands) and the benA loading control (lower bands) are indicated, and the name of the respective AMT/MEP gene is shown at the top of each gel picture. The growth conditions were the same as those for panel A. RT-PCR conditions and primer details are described in Materials and Methods.

**FIG. 5.** Functional analysis of A. nidulans ammonium permeases in areAΔ mutant backgrounds. (A) Growth on a range of ammonium concentrations of areAΔ (MH5699), areAΔ mepAΔ (MH10313), areAΔ meaAΔ (MH10311), and areAΔ meaAΔ mepAΔ (MH10314) mutant and wild-type (MH1) strains. For each ammonium concentration, the pH of the medium was either 4.5 or 6.5 (normal growth pH), as indicated. Growth on nitrogen-free (−), 10 mM alanine (ala), GABA, nitrate (NO3−), and proline (pro) media is also shown. (B) [14C]methylammonium transport rates for the wild-type strain and mepAΔ, meaAΔ, meaAΔ mepAΔ, areAΔ, areAΔ mepAΔ, areAΔ meaAΔ, areAΔ meaAΔ mepAΔ, and meaAΔ mepAΔ mepBΔ mutant strains. Assays were performed with nitrogen-starved mycelia at a final methylammonium concentration of 200 μM. Error bars represent standard errors calculated for the results from at least two independent experiments.
mepBΔ mutant MH10321 (approximately 3% that of the wild type). These results showed the AreA is required for MepA, MepB, and MeaA transport activity, although there is a level of AreA-independent MeaA function.

Consistent with the established function of AreA as an activator of catabolic gene expression, the single areAΔ mutant showed very poor growth on nonpreferred nitrogen sources (Fig. 5A). The slight level of growth observed is presumed to be due to basal levels of catabolic gene transcription. Surprisingly, the growth observed for the areAΔ and areAΔ mepAΔ strains on nitrogen sources such as alanine and nitrate was absent for the areAΔ meaA Δ mutant. This is likely to reflect a requirement for retention and/or uptake of trace amounts of ammonium released by the catabolism of alternative nitrogen sources (34).

Northern blot analysis and RT-PCR were performed to assess the steady-state transcript levels of the AMT/MEP genes for the areAΔ mutant and the loss-of-function areA217 mutant, which contains a mutation within the DNA-binding domain (20). The expression of meaA was detected by RT-PCR but not by Northern blot analysis for the areAΔ or areA217 strain from either ammonium-grown or nitrogen-starved mycelia (Fig. 6). The significant but not total reduction of meaA expression in the areAΔ mutant is consistent with the growth phenotypes and [14C]methylammonium uptake data and indicates that the full expression of meaA under nitrogen-sufficient conditions requires AreA function.

AreA-dependent expression of mepA was shown by Northern blotting and RT-PCR analysis, as no mepA mRNA was detected for the areAΔ or areA217 strain for all growth conditions tested (Fig. 6). Furthermore, the nitrogen starvation-specific expression of mepB was shown to be absolutely dependent on AreA. The increase of mepC expression in response to nitrogen starvation was reduced in the areAΔ mutant, but expression levels on ammonium and glutamine were unaffected (Fig. 6). Since Northern analysis showed identical results for the areA217 point mutant and the areAΔ mutant, AreA regulation of the AMT/MEP genes is at the level of DNA binding.

Effects of TamA on AMT/MEP activity and gene expression. TamA has been shown to be a coactivator for the optimal expression of certain AreA-regulated genes (42, 43). The tamAΔ mutation had very little effect on [14C]methylammonium transport, other than a slight reduction attributable to a reduction in MecaA-mediated transport (Fig. 7A). Consistent with this, RT-PCR results for meaA, mepA, mepB, and mepC expression in the tamAΔ strain under ammonium growth or nitrogen-starved conditions indicated no effect of the tamAΔ mutation other than a slight reduction in meaA expression compared to the wild type (Fig. 7B). However, clear reductions in mepA and mepC expression were noted for the tamAΔ strain grown in glutamine or alanine, and [14C]methylammonium uptake analysis performed on alanine-grown mycelia confirmed that the tamAΔ mutant had reduced methylammonium transport activity under these conditions (Fig. 7B and C). Such a reduction is consistent with the methylammonium-resistant phenotype observed for tamA mutants which were originally identified as methylammonium resistant on media containing alanine as the nitrogen source (12, 19).

Northern analysis of meaA, mepA, and mepB expression on a range of nitrogen sources (ammonium, glutamine, glutamate, alanine, proline, and nitrate) indicated a clear reduction of meaA and mepA expression in a tamAΔ strain compared to the wild-type level for all nonammonium nitrogen sources tested (data not shown). These results show that the full expression of meaA and mepA on nonammonium nitrogen sources requires the combined activities of AreA and the transcriptional coactivator TamA.

FIG. 6. Expression of A. nidulans AMT/MEP genes in areA mutant background. (A) Northern analysis of meaA, mepA, and mepB gene expression in the wild-type (MH1) and areAΔ (MH5699) and areA217 (MH341) mutant strains. RNAs were isolated from mycelia grown for 16 h at 37°C in 1% glucose medium, pH 6.5, with 20 mM ammonium (NH₄⁺) and then transferred to nitrogen-free (NF) medium at pH 6.5 for 4 hours. Northern blots were hybridized with probes specific for meaA, mepA, mepB (see Materials and Methods), and A. nidulans histone H3 as a loading control (13), as indicated. (B) Multiplex RT-PCR analysis of meaA, mepA, mepB, and mepC expression in wild-type and areAΔ mutant strains. The name of the respective gene is shown at the top of each picture, and in all cases, the lower band is the internal loading control benA. RT-PCR conditions and primer details are described in Materials and Methods.
DISCUSSION

The *A. nidulans* genome contains a family of four AMT/MEP genes, namely, *meaA*, *mepA*, *mepB*, and *mepC*. These genes are differentially expressed in response to nitrogen availability, and the full expression of each AMT/MEP gene requires the global nitrogen regulator *AreA*. *AreA* functions as a transcriptional activator in response to nitrogen limitation, and its known targets are GATA sites in the promoters of genes involved in nitrogen acquisition (3, 17, 40, 56). The transcription of genes subjected to nitrogen metabolite repression is reduced under nitrogen-sufficient conditions by changes in the level and activity of *AreA*. Regulated *areA* mRNA degradation (35, 36) and the interaction of *AreA* with the negative regulator NmrA (2, 21) reduce or prevent *AreA*-dependent activation of these genes. Nitrogen limitation leads to stabilization of *areA* mRNA and a loss of NmrA-associated inhibition of activity. Together, these factors account for the influence of the quality of nitrogen source availability on *AreA* activity and levels of catabolic gene expression. Recent studies have shown that the complete absence of a nitrogen source results in enhanced *AreA* activity and increased expression of *AreA*-regulated genes through additional mechanisms that are independent of mRNA stability and NmrA and are correlated with modification of AreA and its hyperaccumulation inside the nucleus (51).

*AreA*-mediated regulation of *mepA* represents the pattern observed for the *amdS*, *gmdA*, and *fmdS* genes, which are regulated by nitrogen metabolite repression but do not require induction (11, 14–16). *mepA* is repressed under conditions of nitrogen sufficiency, activated in response to nitrogen limitation, and increased further in response to nitrogen starvation. *AreA* is absolutely required for these responses of *mepA* expression. Like *gmdA* and *fmdS*, *mepA* is not expressed under carbon starvation conditions, where *AreA* is thought to be inactive (14). MepA is a high-affinity ammonium transporter and is likely to serve an ammonium-scavenging function when nitrogen availability is limiting to growth.

The *mepB* gene has been shown to encode a second high-affinity ammonium permease. *mepB* expression has a novel...
expression profile whereby this gene is not expressed in the presence of any nitrogen source tested, whether it is repressing or limiting. This is thought to be the first example of an AreA-regulated gene in *A. nidulans* that is expressed specifically in response to complete nitrogen starvation. This highlights the observation that nitrogen starvation conditions result in enhanced AreA activity above that observed for nitrogen limitation. The *mepB* gene appears to be exquisitely sensitive to AreA, such that its expression is observed only under conditions where AreA is highly active. This pattern of regulation is consistent with MepB having a scavenging role under extreme nitrogen deprivation conditions.

A fourth potential member of the *A. nidulans* AMT/MEP family has been identified. However, we suggest that *mepC*, which is more poorly expressed than the other AMT/MEP genes, does not normally contribute to ammonium acquisition in *A. nidulans*. Deletion of *mepC* resulted in no detectable phenotypic effect, and furthermore, no significant methylammonium uptake activity was detected in a *meaAΔ mepAΔ mepBΔ* triple mutant retaining only MepC function. In a recent study, *Candida albicans* was shown to contain two MEP genes, *mep1* and *mep2*, that are similar in structure and function to the *S. cerevisiae* MEP1/MEP3 and MEP2 genes (5). A third, more divergent MEP-like gene identified in *C. albicans* was considered nonfunctional based on the phenotype of the Δ*mep1Δ2* double mutant (5). The predicted MepC product is also the most divergent of the *A. nidulans* family of AMT/MEP permeases, including differences in the ammonium signature sequences. Despite these differences, MepC expressed at high levels can partially compensate for the loss of the other three AMT/MEP permeases. Database searches revealed that orthologs of the *A. nidulans* AMT/MEP genes *meaA*, *mepA*, and *mepB* are present in the genomes of *A. fumigatus*, *A. oryzae*, *Neurospora crassa*, *Magnaporthe grisea*, and *Fusarium graminearum* (Fig. 8). In contrast, orthologs of *mepC* can be identified in the *N. crassa* genome but not in the related *M. grisea* genome, and among the *Aspergillus* spp., *mepC* is present in *A. oryzae* but not in *A. fumigatus*. The additional amino acid sequences apparent in the MepC amino acid sequence compared to the MeaA, MepA, and MepB sequences (Fig. 1) are present in all MepC orthologs. Therefore, *mepC* may be the result of an early duplication that has been independently lost.

![FIG. 8. Relatedness of AMT/MEP protein sequences from *A. nidulans*, *A. fumigatus*, *A. oryzae*, *N. crassa*, *F. graminearum*, and *M. grisea*. The dendrogram was constructed by the neighbor-joining method, using ClustalX (49) with default settings. Organisms and gene names or sequence notations are indicated. Each cluster is numbered with a roman numeral.]
in several fungal lineages. This further argues that mepC does not have a critical physiological or regulatory function in *A. nidulans*, although a subtle function in nitrogen signaling cannot be excluded.

The expression of meaA, encoding the major ammonium transporter, is both AreA dependent and AreA independent. The factors that promote AreA-independent expression are unknown, but it is clear that AreA plays an active role in the expression of meaA under all nitrogen conditions. It is paradoxical that AreA activity is required under ammonium-sufficient conditions, in which it has been assumed to be inactive. It is apparent that AreA does retain the capacity to activate gene expression from certain promoters under repressed conditions. The *gdhA* gene, encoding NADP-linked glutamate dehydrogenase, the major enzyme of ammonium assimilation in *A. nidulans*, is also regulated by AreA under nitrogen-sufficient conditions (7, 38, 41). However, the mechanisms that underlie the expression of meaA and *gdhA* on ammonium appear to be different. The full expression of *gdhA* under ammonium-sufficient conditions is dependent on TamA acting as a coactivator of AreA and an additional transcriptional activator, LeuB (38). In contrast, TamA has a relatively minor role in the activation of meaA expression on ammonium. Instead, TamA appears to act with AreA to activate meaA (and mepA) expression on nonammonium nitrogen sources, similar to the role that it plays in contributing to the expression of other nitrogen-regulated genes (12, 42, 43). Furthermore, LeuB is not required for meaA expression, consistent with the lack of predicted LeuB binding sites in the meaA promoter (B. J. Monahan, unpublished results). There is no indication that NmrA, which acts to inhibit AreA function at the promoters of genes subject to nitrogen metabolite repression, has a role in modulating meaA expression (Monahan, unpublished data). The factors that facilitate AreA function at the meaA promoter under nitrogen-sufficient conditions are unknown but are of considerable interest. By analogy with the complex interactions of AreA, TamA, and LeuB at the *gdhA* promoter, it is possible that interactions between AreA and other transcription factors at the meaA promoter are involved.

The contrasting expression profiles of the four AMT/MEP genes in *A. nidulans* have revealed that nitrogen sufficiency, limitation, and starvation can be differentiated as distinct physiological states by the organism. Furthermore, this study has highlighted the finding that promoter-specific contexts must be an important factor in determining the activation capacity of AreA under different nitrogen conditions. The AMT/MEP genes in *A. nidulans* provide an excellent system with which to further investigate these complexities of AreA function.

**ACKNOWLEDGMENTS**

This work was supported by the Australian Research Council and the award of Melbourne Research Scholarships to B.J.M. and M.C.A.

**REFERENCES**

1. Andrianopoulos, A., and M. J. Hynes. 1988. Cloning and analysis of the positively regulatory gene *amdR* from *Aspergillus nidulans*. Mol. Cell. Biol. 8:3532–3541.

2. Andrianopoulos, A., S. Kourambas, J. A. Sharp, M. A. Davis, and M. J. Hynes. 1996. Characterization of the *Aspergillus nidulans* meaA gene involved in nitrogen metabolite repression. J. Bacteriol. 180:1973–1977.

3. Arst, H. N., Jr., and D. J. Cove. 1973. Nitrogen metabolite repression in *Aspergillus nidulans*. Mol. Gen. Genet. 126:111–141.

4. Austin, B., R. M. Hall, and B. M. Tyler. 1990. Optimized vectors and selection for transformation of *Neurospora crassa* and *Aspergillus nidulans* to bleomycin and phleomycin resistance. Gene 93:157–162.

5. Biswas, K., and J. Morschhauser. 2005. The Mept2 ammonium permease contains a nitrogen starvation-induced filamentous growth in *Candida albicans*. Mol. Microbiol. 56:649–669.

6. Blakey, D., A. Leech, G. H. Thomas, G. Coutts, K. Findlay, and M. Merrick. 2002. Purification of the *Escherichia coli* ammonium transporterAmtB reveals a trimeric stoichiometry. Biochem. J. 364:45–55.

7. Christensen, T. M. J. Hynes, and M. A. Davis. 1996. Role of the regulatory gene *areA* of *Aspergillus oryzae* in nitrogen metabolism. Appl. Environ. Microbiol. 64:3232–3237.

8. Chuter, A. J. 1974. *Aspergillus nidulans* genetics, p. 447–510. In R. C. King (ed.), Handbook of genetics, vol. 1. Plenum Publishing Corp., New York, N.Y.

9. Conroy, M. J., S. J. Jamieson, D. Blakey, T. Kaufmann, A. Engel, D. Fotiadis, M. Merrick, and P. A. Bullough. 2004. Electron and atomic force microscopy of the trimeric ammonium transporter AmtB. EMBO Rep. 5:1153–1158.

10. Cove, D. J. 1966. The induction and repression of nitrate reductase in the fungus *Aspergillus nidulans*. Biochim. Biophys. Acta 113:51–56.

11. Davis, M. A., J. M. Kelly, and M. J. Hynes. 1993. Fungal catabolic gene regulation: molecular genetic analysis of the *amdS* gene of *Aspergillus nidulans*. Mol. Gen. Genet. 240:90:133–138.

12. Davis, M. A., A. J. Small, S. Kourambas, and M. J. Hynes. 1996. The *tamA* gene of *Aspergillus nidulans* contains a putative zinc cluster motif which is not required for gene function. J. Bacteriol. 178:3406–3409.

13. Ehinger, A., S. H. Denison, and G. S. May. 1990. Sequence, organization and expression of the core histone genes of *Aspergillus nidulans*. Mol. Gen. Genet. 222:416–424.

14. Fraser, J. A., M. A. Davis, and M. J. Hynes. 2001. The formamidase gene of *Aspergillus nidulans*: regulation by nitrogen metabolite repression and transcriptional interference by an overlapping upstream gene. Genetics 157:119–131.

15. Fraser, J. A., M. A. Davis, and M. J. Hynes. 2002. The genes *gmdA*, encoding an amidas, and *badA*, encoding a cytochrome P450, are required for benzamide utilization in *Aspergillus nidulans*. Fungal Genet. Biol. 35:135–146.

16. Hynes, M. J. 1994. Regulatory circuits of the *amdS* gene of *Aspergillus nidulans*. Antonie Leeuwenhoek 65:179–182.

17. Hynes, M. J. 1975. Studies on the role of the *areA* gene in the regulation of nitrogen catabolism in *Aspergillus nidulans*. Aust. J. Biol. Sci. 28:301–313.

18. Khademini, S., J. O’Connell III, J. Remis, Y. Robles-Colmenares, L. J. Mierecke, and R. M. Stroud. 2004. Mechanism of ammonia transport by Amt/MEP/Rh: structure of AmtB at 1.35 A. Science 305:1587–1594.

19. Kinghorn, J. R., and J. A. Pateman. 1975. Studies of partially repressed mutants at the *tama* and *areA* loci in *Aspergillus nidulans*. Mol. Gen. Genet. 140:137–147.

20. Kodula, B., M. X. Caddick, T. Langdon, N. M. Martinez-Rossi, C. F. Bennett, S. Sibley, R. W. Davies, and H. N. Arst, Jr. 1990. The regulatory gene *areA* mediating nitrogen metabolite repression in *Aspergillus nidulans*. Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger. EMBO J. 9:1335–1346.

21. Lamb, H. K., J. Ren, A. Park, C. Johnson, K. Leslie, S. Cocklin, P. Thompson, C. Cee, A. Cooper, D. K. Stammers, and A. R. Hawkins. 2004. Modulation of the ligand binding properties of the transcription repressor NmrA by GATA-containing DNA and site-directed mutagenesis. Protein Sci. 13:3127–3138.

22. Lee, S., and J. Taylor. 1990. Isolation of DNA from fungal mycelia and single spores, p. 282–287. In M. A. Innis, D. H. Gelfand, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, San Diego, Calif.

23. Lorenz, M. C., and J. Heitman. 1998. The MEP2 ammonium permease regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. EMBO J. 17:2326–1247.

24. Ludewig, U., N. von Wiren, and W. B. Frommer. 2002. Uniprot of NH₄⁺ by the root hair plasma membrane ammonium transporter LeAMT1;1. J. Biol. Chem. 277:13548–13555.

25. Ludewig, U., S. Wilken, B. Wu, W. Jost, P. Oredik, M. El Bakkour, A. M. Mattei, B. Andre, T. Hamacher, E. Boles, N. von Wiren, and W. B. Frommer. 2003. Homo- and hetero-oligomerization of ammonium transporter-1 NH₄⁺ uniprotos. J. Biol. Chem. 278:45603–45610.

26. Marini, A. M., and B. Andre. 2000. In vivo N-glycosylation of the Mep2 high-affinity ammonium transporter of *Saccharomyces cerevisiae* reveals an extracytosolic N-terminus. Mol. Microbiol. 38:552–564.

27. Marini, A. M., G. Matassi, V. Raynal, B. Andre, J. P. Cartron, and B. Cherif-Zahar. 2000. The human rhesus-associated RhAG protein and a kidney homologue promote ammonium transport in yeast. Nat. Genet. 26:341–344.

28. Marini, A. M., S. Soussi-Roudeout, S. Vissers, and B. Andre. 1997. A family of ammonium transporters in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 17:4282–4293.
31. Marzluf, G. A. 1997. Genetic regulation of nitrogen metabolism in the fungi. Microbiol. Mol. Biol. Rev. 61:17–32.
32. May, G. S., J. Gambino, J. A. Weatherbee, and N. R. Morris. 1999. The fungal GATA factors. Curr. Opin. Microbiol. 2:435–442.
33. Monahan, B. J., J. A. Fraser, M. J. Hynes, and M. A. Davis. 2001. Functional analysis of TamA, a coactivator of nitrogen-regulated genes by ambient pH. EMBO J. 19:1743–1750.
34. Morozov, I. Y., M. G. Martinez, M. G. Jones, and M. X. Caddick. 2001. Characterization of nitrogen metabolite signalling in Aspergillus nidulans via the regulated degradation of areA mRNA. Mol. Microbiol. 42:269–277.
35. Monahan, B. J., S. E. Unkles, I. T. Tsing, J. R. Kinghorn, M. J. Hynes, A. J. Small, R. B. Todd, M. C. Zanker, S. Delimitrou, M. J. Hynes, and M. A. Davis. 2002. Isolation and characterization of two ammonium permease genes, meaA and mepA, from Aspergillus nidulans. Eukaryot. Cell 1:85–94.
36. Monahan, B. J., S. E. Unkles, I. T. Tsing, J. R. Kinghorn, M. J. Hynes, and M. A. Davis. 2002. Mutation and functional analysis of the Aspergillus nidulans ammonium permease MeaA and evidence for interaction with itself and MepA. Fungal Genet. Biol. 36:35–46.
37. Morozov, I. Y., M. Galbis-Martinez, M. G. Jones, and M. X. Caddick. 2001. Characterization of nitrogen metabolite signalling in Aspergillus nidulans via the regulated degradation of areA mRNA. Mol. Microbiol. 42:269–277.
38. Morozov, I. Y., M. G. Martinez, M. G. Jones, and M. X. Caddick. 2000. A defined sequence within the 3’ UTR of the areA transcript is sufficient to mediate nitrogen metabolite signalling via accelerated deadenylation. Mol. Microbiol. 37:1249–1257.
39. Osmani, A. H., G. S. May, and S. A. Osmani. 1999. The extremely conserved pyroA gene of Aspergillus nidulans is required for pyridoxine synthesis and is required indirectly for resistance to photosensitizers. J. Biol. Chem. 274:23565–23569.
40. Polotniukia, R., B. J. Monahan, M. J. Hynes, and M. A. Davis. 2004. TamA interacts with LeuB, the homologue of Saccharomyces cerevisiae Leu3p, to regulate gdhA expression in Aspergillus nidulans. Mol. Genet. Genomics 272:452–459.
41. Sambrook, J., and D. W. Russell. 2000. The molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
42. Scacciozzi, C. 2001. The fungal GATA factors. Curr. Opin. Microbiol. 4:126–131.
43. Small, A. J. 2000. Characterisation of the tamA gene of Aspergillus nidulans. Ph.D. thesis. University of Melbourne, Melbourne, Australia.
44. Small, A. J., M. J. Hynes, and M. A. Davis. 1999. The TamA protein fused to a DNA-binding domain can recruit AreA, the major nitrogen regulatory protein, to activate gene expression in Aspergillus nidulans. Genetics 153:95–105.
45. Small, A. J., R. B. Todd, M. C. Zanker, S. Delimitrou, M. J. Hynes, and M. A. Davis. 2001. Functional analysis of TamA, a coactivator of nitrogen-regulated gene expression in Aspergillus nidulans. Mol. Genet. Genomics 265:636–646.
46. Smith, D. G., M. D. García-Pedragas, S. E. Gold, and M. H. Perlman. 2003. Isolation and characterization from pathogenic fungi of genes encoding ammonium permeases and their roles in dimorphism. Mol. Microbiol. 50:259–275.
47. Sonnhammer, E. L., G. von Heijne, and A. Krogh. 1998. A hidden Markov model for predicting transmembrane helices in protein sequences. Proc. Natl. Conf. Intell. Syst. Mol. Biol. 6:175–182.
48. Straubinger, B., E. Straubinger, S. Wirsel, G. Turgeon, and O. Yoder. 1992. Versatile fungal transformation vectors carrying the selectable bar gene of Streptomyces hygroscopicus. Fungal Genet. News. 39:82–83.
49. Thomas, G. H., J. G. Multinos, and M. A. Merrick. 2000. Membrane topology of the Mep/Amt family of ammonium transporters. Mol. Microbiol. 37:331–344.
50. Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876–4882.
51. Tiiburn, J., S. Sarkar, D. A. Widdick, E. A. Espeso, M. Orejas, J. Munngroo, M. A. Penalva, and H. N. Arst, Jr. 1995. The Aspergillus PacC zinc finger transcription factor mediates regulation of both acid- and alkaline-expressed genes by ambient pH. EMBO J. 14:779–786.
52. Todd, R. B., J. A. Fraser, K. H. Wong, M. A. Davis, and M. J. Hynes. 2005. Nuclear accumulation of the GATA factor AreA in response to complete nitrogen starvation by regulation of nuclear export. Eukaryot. Cell 4:1646–1653.
53. Tusnady, G. E., and I. Simon. 1998. Principles governing amino acid composition of integral membrane proteins: application to topology prediction. J. Mol. Biol. 283:489–506.
54. Van Kim, C. L., Y. Colin, and J. P. Cartron. 2005. Rh proteins: key structural and functional components of the red cell membrane. Blood Rev. [Epub ahead of print] doi:10.1016/j.blrd.2005.04.002.
55. von Weren, N., and M. Merrick. 2004. Regulation and function of ammonium carriers in bacteria, fungi, and plants. Top. Curr. Genet. 9:95–120.
56. Westhoff, C. M., D. L. Siegel, C. G. Burd, and J. K. Fossett. 2004. Mechanism of genetic complementation of ammonium transport in yeast by human erythrocyte Rh-associated glycoprotein. J. Biol. Chem. 279:17443–17448.
57. Wilson, R. A., and H. N. Arst, Jr. 1998. Mutational analysis of AreA, a transcriptional activator mediating nitrogen metabolite repression in Aspergillus nidulans and a member of the “streetwise” GATA family of transcription factors. Microbiol. Mol. Biol. Rev. 62:586–596.
58. Zadra, I., B. Abt, W. Parson, and H. Haas. 2000. yip promoter-based expression system and its use for antisense downregulation of the Penicillium chrysogenum nitrogen regulator NRE. Appl. Environ. Microbiol. 66:4810–4816.
59. Zheng, L., D. Kostrewa, S. Bernet, F. K. Winkler, and X. D. Li. 2004. The mechanism of ammonia transport based on the crystal structure of AmtB of Escherichia coli. Proc. Natl. Acad. Sci. USA 101:17090–17095.
60. Zidi-Yahiaoui, N., I. Monro-Chanteloup, A. M. D’Ambrosio, C. Lopez, P. Gane, C. le van Kim, J. P. Cartron, Y. Colin, and P. Ripoche. 2005. Human rhesus B and rhesus C glycoproteins: properties of facilitated ammonium transport in recombinant kidney cells. Biochem. J. 391:33–40.
61. Morozov, I. Y., M. G. Martinez, M. G. Jones, and M. X. Caddick. 2000. Membrane topology of the Mep/Amt family of ammonium transporters. Mol. Microbiol. 37:331–344.