Ammonium transport in *Escherichia coli*: localization and nucleotide sequence of the *amtA* gene

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(Received 9 October 1990; revised 6 December 1990; accepted 12 December 1990)

Escherichia coli expresses a concentrative ammonium (methylammonium) transport system which is strongly repressed under conditions of nitrogen excess. We have previously reported the cloning of a structural gene (*amtA*) for this transporter by complementation. In this study, a 3.4 kb *Hind*III–*Bam*HI fragment containing *amtA* was cloned into the pBluescript KS(+) vector, and unidirectional nested deletions from each end of this 3.4 kb fragment were generated by exonuclease III digestion. The deletions were analysed by complementation of the structural gene mutation produced by Tn10 insertion. This allowed *amtA* to be localized within a 1.4 kb region which spans the site of the mutation. By application of the Sanger dideoxy method, we sequenced the region containing *amtA*. The gene contains an open reading frame which encodes a protein with a predicted molecular mass of 27 kDa. The open reading frame is preceded by a putative Shine–Dalgarno sequence and followed by an inverted repeat which might function as a simple transcription terminator. Hydropathic analysis of the inferred amino acid sequence of the gene product predicts that *amtA* encodes a cytoplasmic component of the ammonium transport system.

Introduction

The active transport of ammonium ions has been studied in a variety of bacterial species (Kleiner, 1985) and fungi (Dubois & Grenson, 1979; Hackett *et al.*, 1970) using [¹⁴C]methylammonium as a model substrate. At low extracellular concentrations, utilization of ammonium as the sole nitrogen source requires a high-affinity ammonium transport system capable of generating large concentration gradients, whereas this system is dispensable at high external levels (Kleiner, 1985). Under nitrogen-limiting conditions, *Escherichia coli* expresses such a concentrative ammonium (methylammonium) transport system (Amt), which is strongly repressed during ammonium excess (Jayakumar *et al.*, 1985). Studies of the nitrogen regulation of Amt in *E. coli* have shown that expression of the transport system is activated by the *glnG* and *glnL* regulatory proteins during nitrogen limitation, and repressed during ammonium excess via the *glnL* product (Jayakumar *et al.*, 1986).

A mutant deficient in Amt has been isolated after Tn10 transposon mutagenesis, and the site of Tn10 insertion was demonstrated to lie within a structural gene of the transport system. The structural gene for this component of the system has been cloned by complementation of the mutant (Jayakumar *et al.*, 1989). This *amtA* gene, previously designated as *amt* (Jayakumar *et al.*, 1989), has recently been mapped to 95.8 min on the *E. coli* chromosome, adjacent to the *cpdB* gene for a periplasmic cyclic phosphodiesterase (Jayakumar *et al.*, 1991). In this communication, we report the nucleotide sequence for *amtA*. Analysis of this sequence predicts a gene product with molecular mass of 27 kDa. Analysis of the peptide sequence reveals no significant homologies to other protein sequences, and suggests that the AmtA protein may be a peripheral membrane protein localized on the inner face of the cytoplasmic membrane. A preliminary report of these findings has been presented (Fabiny *et al.*, 1990).

Methods

Chemicals. ¹⁴CH₃NH₃Cl [43.8 Ci mol⁻¹ (1.621 TBq mol⁻¹)] was obtained from Dupont-NEN. [α -³⁵S]dATP [1000 Ci mmol⁻¹ (37 TBq mmol⁻¹)] was obtained from Amersham. Restriction endonucleases,

Abbreviation: ORF, open reading frame.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number M55170.

DNA ligase, urea, and acrylamide were obtained from Boehringer-Mannheim. Ampicillin, tetracycline, ammonium acetate, sodium glutamate, bis-acrylamide, boric acid, EDTA and Tris were obtained from Sigma. Agarose was obtained from International Biotechnologies. pBluescript KS(+) vector and the exonuclease III/mung bean nuclease deletion kit were from Stratagene Cloning Systems. CsCl, the Sequenase sequencing kits and Sequenase 2.0 (genetically modified T7 DNA polymerase) were from the US Biochemical Corp.

Bacterial strains and growth conditions. Strain AJ2653 (*amtA1::Tn10* Tc^r) was grown in M9 minimal medium, with sodium glutamate (20 mM) as sole source of nitrogen, as described previously (Jayakumar *et al.*, 1986). In complementation assays, restoration of the Amt⁻ phenotype to AJ2653 was monitored on M9 plates containing 100 µM-ammonium acetate as sole nitrogen source, as previously described (Jayakumar *et al.*, 1989). Strain XL1-Blue [*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1 lac* (F'*proAB lacIq ΔM15 Tn10* (Tet^r))]; Bullock *et al.*, 1987] was grown on LB plates (containing, per litre, 10 g Bactotryptone, 5 g yeast extract and 10 g NaCl). Where appropriate, ampicillin or tetracycline were included at 100 µg ml⁻¹ and 25 µg ml⁻¹ respectively. Liquid cultures were grown at 37 °C and aerated by shaking; plates were incubated at 37 °C.

DNA isolation and manipulation. Plasmid DNA isolation, phenol/chloroform extractions, ethanol precipitation of DNA, restriction digests, DNA ligation, bacterial transformation and agarose gel electrophoresis were done according to standard protocols as described by Sambrook *et al.* (1989).

Construction of unidirectional deletions of the Amt-complementing subclone. The Amt-complementing insert of pJH1A (Jayakumar *et al.*, 1989) was subcloned into the pBluescript KS(+) vector as a 3.4 kb *HindIII*-*Bam*HI fragment. The resulting plasmid (pKSAMT) was digested with *Sac*I and *Bam*HI, or *Kpn*I and *Hind*III. Each linearized plasmid was then subjected to exonuclease III (ExoIII) digestion as follows. DNA (80 µg) was diluted in ExoIII reaction buffer (50 mM-Tris/HCl, pH 8.0, 5 mM-MgCl₂, 10 µg tRNA mg⁻¹ and 10 mM-2-mercaptoethanol) at a final concentration of 0.2 µg µl⁻¹. The reaction mixture was incubated at 30 °C for 5 min, and an aliquot containing 5 µg of DNA was removed for a zero time point. ExoIII [20 units (µg DNA)⁻¹] was added and the incubation continued at 30 °C, removing aliquots (5 µg) at 1 min intervals. The samples were diluted into mung bean nuclease reaction buffer [30 mM-sodium acetate, pH 5.0, 50 mM-NaCl, 1 mM-ZnCl₂ and 50% (w/v) glycerol], and placed on dry ice. All samples were then heated to 65 °C for 15 min and placed on ice. Mung bean nuclease [75 units (µg DNA)⁻¹] was added and incubated at 30 °C for 30 min. Each sample was then brought to 0.3% SDS, 40 mM-Tris/HCl, pH 9.5, and 640 mM-LiCl, extracted with an equal volume of TE (10 mM-Tris/HCl, pH 8.0, and 1 mM-EDTA)-equilibrated phenol/chloroform, and finally extracted with an equal volume of chloroform. The samples were then brought to 0.3 M-sodium acetate (pH 7.0) and 10 ng tRNA µl⁻¹, and precipitated with 2.5 vols ice-cold ethanol. The DNA in each sample was dissolved in TE to a final concentration of 10 ng µl⁻¹. DNA (10 ng) from each time point was then ligated to form recircularized plasmid. Ligated DNA (5 ng) was used to transform competent XL1-Blue cells to ampicillin resistance.

Localization of *amtA*. Deletion plasmids were isolated from Amp^r colonies representing each time point and analysed by *Bgl*I digest. Selected plasmids were used to transform strain AJ2653, and the transformants examined for the ability to grow on ammonium-limited (100 µM-ammonium acetate) minimal plates (Jayakumar *et al.*, 1989). Their ability to transport [¹⁴C]methylammonium was assayed as previously described (Jayakumar *et al.*, 1986). Loss of transport activity in deletions from each end of the subclone was used to define the approximate boundaries of *amtA*.

Sequencing of *amtA*. Plasmid DNA (5 µg) was denatured at 37 °C for 30 min in 200 mM-NaOH and 0.2 mM-EDTA, in a final volume of 20 µl.

The DNA was neutralized by the addition of sodium acetate (pH 5.5) to a final concentration of 0.3 M. The DNA was precipitated with 4 vols ethanol, pelleted, washed and dried briefly. The DNA and oligodeoxynucleotide primer (1 pmol) were dissolved in sequencing buffer (40 mM-Tris/HCl, pH 7.5, 20 mM-MgCl₂, 50 mM-NaCl), heated at 65 °C for 2 min, and allowed to cool slowly (over 30 min) to 30 °C. This annealed template/primer was then used in the standard labelling and termination reactions of the sequencing method (Sanger *et al.*, 1977). The sequencing reactions were heated to 75 °C for 2 min and then loaded directly onto a 0.4 mm thick 6% or 8% (w/v) polyacrylamide sequencing gel, with a shark-tooth comb forming the wells. The gel was electrophoresed at 60 W constant power until the bromophenol blue tracking dye reached the bottom. If additional information was to be read from sequencing reactions, an additional set of samples was loaded onto the same gel, and the electrophoresis continued until the bromophenol blue tracking dye of the second set of samples reached the bottom. The gel was washed and fixed in 10% (v/v) acetic acid, 12% (v/v) methanol for 15 min, placed on Whatman 3MM paper, and dried under vacuum at 80 °C for 30 min. The dried gel was exposed to X-ray film overnight.

DNA sequence assembly and analysis. Overlapping sequences, generated by sequencing the deletion plasmids using M13 reverse primer or by oligodeoxynucleotide primer extension of sequence from pKSAMT, were assembled using the Sequence Assembly Manager (SAM) from the Molecular Biology Information Resource (MBIR), Baylor College of Medicine. The resulting contiguous sequence was then analysed using EUGENE (MBIR), and PCGENE (IntelliGenetics, Mountainview, CA, USA).

Results

Localization of *amtA*

It has previously been demonstrated that sequences within the 3.4 kb insert of pJH1A (*amtA*) are necessary for restoring Amt activity to the structural gene mutant AJ2653 (Jayakumar *et al.*, 1989). To facilitate more precise localization of *amtA* within this fragment, it was subcloned into pBluescript KS(+) vector so that a set of unidirectional nested deletions could be generated from each end of the complementing fragment. After transformation of AJ2653 with deletion plasmids, the transformants were assayed for their ability to grow on M9 plates containing 100 µM-ammonium acetate as sole nitrogen source and for their ability to transport [¹⁴C]methylammonium (Fig. 1). Loss of Amt-complementing activity in deletions from each end of the 3.4 kb fragment defined the approximate boundaries of the gene. As shown in Fig. 1, *amtA* was localized to a 1.4 kb region which includes the *Pst*I and *Eco*RI sites, and spans the site of the *Tn10* insertion in the complemented mutant. This region was the focus of the sequencing efforts.

Primary sequence of *amtA* and sequence characteristics

Deletion plasmids corresponding to the region containing *amtA* were sequenced using M13 reverse primer. The sequence was extended and the other strand sequenced using synthetic oligodeoxynucleotides, based on the

Fig. 1. Localization of *amtA*. The *amtA* mutant AJ2653 was transformed with the deletion plasmids shown. Complementation of the mutation was determined by ability of the transformants to grow on 100 μ M-ammonium (not shown) and to transport [14 C]methylammonium (+, >95% of normal activity; -, <10% of normal activity). Loss of complementing activity defines the approximate boundaries of *amtA*.

Fig. 2. Sequencing strategy. Deletion plasmids corresponding to the region containing *amtA* were sequenced using M13 reverse primer (open arrows). The undeleted clone was sequenced using oligodeoxynucleotide 17mers as primers to confirm and extend the sequence (closed arrows). Of the sequenced region 1 kb was confirmed by its identity to the *cpdB* gene and its 5'-flanking region. A modification of the Sanger dideoxy sequencing method was used to sequence double-stranded templates.

sequence obtained using the reverse primer. This strategy and the region sequenced (2070 bp) is illustrated in Fig. 2. Sequences from both strands were obtained for the 1 kb region containing the *PstI* and *EcoRI* sites as

indicated by the open arrows in Fig. 2. The single-strand sequence derived from the remaining 1 kb was confirmed by comparison to that of the *cpdB* gene (see below).

1: CACACCAAGA TGATCGCCCC ACATGCCTGG CATTACCGCC GGAACACCAT TCAGC
 56: GTGCC TTTGGCGATA TCAGCCCCTT CGATATCAGC AAAATCTTTA CCTGGGAAAA
 111: CGGCGTGAGC ATGGCCAAAC ATAATGGCGT TAACGCCCGG AATTTCAGTG AGGT
 165: AATAAA CTGAGTTTTT CGCCATCACT TTATACGGAT CGGCAGATAG CCCGGAATG
 220: T GCCAGAACGA CAACAACATC GGCACCTTTC TCGCGCATTT CAGGCACGTA TTT
 274: GCGCACG GTTTCGGTAA TATCATTCAC CGTCACTTTC CCGGATAAAT TAGCTTTA
 329: TC CCAGCCCATG ATTTGTGGTG GCACGACGCC AATATAGCCA ATCTTCAGCG TC
 383: TGTTTTTT TCCGTCTTTA TCGACCACTT CGGTGTCTTT AATTAAATAC GGTGTAA
 438: ACA TTGGCTGTTT GGTCTGGCG TCAATGACGT TGGCATTTAC ATAAGGGAAT T
 492: TCGCTCCTG CCAGCGCATT TTTCAGGTAA TCCAGACCGT AGTTAAACTC GTGGTT
 547: GCCA AGCGTTCCGA CGGTATAGTC CAGCGTATTT AATGCCTTAT AGACCGGGTG
 601: AATATCACCT GCTTTTAATC CTTTCGCCGA CATGTAATCG GCCAGCGGAC TCCCC
 656: TGAAT CAAATCGCCG TTATCAACCA GTACGCTGTT TTTCAGTTCA TTGCGGGCAT
 711: CGTTAATCAG GCTTGCCGTA CGTACCAGTC CGAATTTTTT CGTGGCGGTG TCTT
 765: TGTAAT AATCGAAATC CATCATGTTG CTATGCAGAT CAGTGGTTTC CATGATACG
 820: C AGATCGACCG TCGCTGCATT CACACTGGCG GCAATCAGCG TGGCCAGGAG CGT
 874: TGCGCTA AACTTAATCA TCAGGGACAT CCTTTTATCA TCGGGAATAC GAAAGAAA
 929: AG GGAGAATAAA CGTCTTACTT ATAGAACAGT GAAGAATGCC ACAATTTTAC GC
 983: TTTGAAAA TGATGACACT ATCACAGTTG GCGCATTCAT TAACGATAGG GTATAAG

 1038: TAA AACAATAAGT TAACACCGCT CACAGAGAC^{SD}G AGGTGGAGAA ATG TTA GAT
 1: MET LEU ASP
 1090: CAA GTA TGC CAG CTT GCA CGG AAT GCA GGC GAT GCC ATT ATG CAG
 4: GLN VAL CYS GLN LEU ALA ARG ASN ALA GLY ASP ALA ILE MET GLN
 1135: GTC TAC GAC GGG ACG AAA CCG ATG GAC GTC GTC AGC AAA GCG GAC
 19: VAL TYR ASP GLY THR LYS PRO MET ASP VAL VAL SER LYS ALA ASP
 1180: AAT TCT CCG GTA ACG GCA GCG GAT ATT GCC GCT CAC ACC GTT ATC
 34: ASN SER PRO VAL THR ALA ALA ASP ILE ALA ALA HIS THR VAL ILE

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1225: ATG GAC GGT TTA CGT ACG CTG ACA CCG GAT GTT CCG GTC CTT TCT
    49: MET ASP GLY LEU ARG THR LEU THR PRO ASP VAL PRO VAL LEU SER

1270: GAA GAA GAT CCT CCC GGT TGG GAA GTC CGT CAG CAC TGG CAG CGT
    64: GLU GLU ASP PRO PRO GLY TRP GLU VAL ARG GLN HIS TRP GLN ARG

1315: TAC TGG CTG GTA GAC CCG CTG GAT GGT ACT AAA GAG TTT ATT AAA
    79: TYR TRP LEU VAL ASP PRO LEU ASP GLY THR LYS GLU PHE ILE LYS

1360: CGT AAT GGC GAA TTC ACC GTT AAC ATT GCG CTC ATT GAC CAT GGC
    94: ARG ASN GLY GLU PHE THR VAL ASN ILE ALA LEU ILE ASP HIS GLY

1405: AAA CCG ATT TTA GGC GTG GTG TAT GCG CCG GTA ATG AAC GTA ATG
   109: LYS PRO ILE LEU GLY VAL VAL TYR ALA PRO VAL MET ASN VAL MET

1450: TAC AGC GCG GCA GAA GGC AAA GCG TGG AAA GAA GAG TGC GGT GTG
   124: TYR SER ALA ALA GLU GLY LYS ALA TRP LYS GLU GLU CYS GLY VAL

1495: CGC AAG CAG ATT CAG GTC CGC GAT GCG CGC CCG CCG CTG GTG GTG
   139: ARG LYS GLN ILE GLN VAL ARG ASP ALA ARG PRO PRO LEU VAL VAL

1540: ATC AGC CGT TCC CAT GCG GAT GCG GAG CTG AAA GAG TAT CTG CAA
   154: ILE SER ARG SER HIS ALA ASP ALA GLU LEU LYS GLU TYR LEU GLN

1585: CAG CTT GGC GAA CAT CAG ACC ACG TCC ATC GGC TCT TCG CTG AAA
   169: GLN LEU GLY GLU HIS GLN THR THR SER ILE GLY SER SER LEU LYS

1630: TTC TGC CTG GTG GCG GAA GGA CAG GCG CAC GTG TAC CCG CGC TTC
   184: PHE CYS LEU VAL ALA GLU GLY GLN ALA HIS VAL TYR PRO ARG PHE

1675: GGA CCA ACG AAT ATT TGG GAC ACC GCC GCT GGA CAT GCT GTA GCT
   199: GLY PRO THR ASN ILE TRP ASP THR ALA ALA GLY HIS ALA VAL ALA

1720: GCA GCT GCC GGA GCG CAC GTT CAC GAC TGG CAG GGT AAA CCG CTG
   214: ALA ALA ALA GLY ALA HIS VAL HIS ASP TRP GLN GLY LYS PRO LEU

1765: GAT TAC ACT CCG CGT GAG TCG TTC CTG AAT CCG GGG TTC AGA GTG
   229: ASP TYR THR PRO ARG GLU SER PHE LEU ASN PRO GLY PHE ARG VAL

1810: TCT ATT TAC TAA ATTCAGATG GCAGAAACAG TGTATTCCT GATTCTGCCA T
   244: SER ILE TYR ***
                                     ───▶ ───▶ ───▶ ───▶

1862: CCTGATTTC TCCCAACCTA AAAAGTTATA AATAAAAAGA GATTGTATTT AAAGTG
    1917: CAAA AATTCAATTG CTAATAAGTT ACATTTTAAT AATGAGCGTT TTTTGATAGT

1971: TTAATTCTAT AGTGAGATAT TTAATGGCGA CATAAAGTAA CCAAATAAAA TAAGG

2026: TTGTC ATATGTTACC CAGGATCAGA CACAATAATT TTATTGGTGC GGTGGAGTTA

2081: TTTGTAAAGT CTTCGTATAC AAAAACACAT TCAAACAATT T

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Fig. 3. Sequence of *amtA*. The coding strand is oriented 5' (*SalI* end) to 3' (*HindIII* end). The sequence features a Shine-Dalgarno sequence (SD), a 246 amino acid ORF, and inverted repeats which may function as a simple transcription terminator (arrows). The GenBank accession number for this sequence is M55170.

As shown in Fig. 3, the sequence contains an open reading frame (ORF) encoding a 246 amino acid protein, a ribosome binding site, and an inverted repeat which may function as a simple transcription terminator. The ORF was examined for codon usage preference according to Gribskov *et al.* (1984) using *E. coli* codon usage tables (Maruyama *et al.*, 1986). A codon usage typical of *E. coli* was found. The average preference score for the ORF is 1.14, while that of a random sequence of the same composition is 0.81. Furthermore, the ORF contains a low number (12 of 247) of rare usage codons. Comparison of the sequence upstream of the *amtA* ORF to consensus sequences for nitrogen-regulatory elements (Ausubel, 1984; Magasanik, 1988; Morett & Buck, 1989; Reitzer & Magasanik, 1986) did not reveal any significant matches to a nitrogen regulator I binding site or to a σ^{54} -dependent promoter.

The sequence also contains a portion of the divergently transcribed *cpdB* gene (Fig. 2) which encodes a periplasmic cyclic phosphodiesterase (Liu *et al.*, 1986). This includes the 5'-flanking region (150 bp) and a portion of the coding region of *cpdB* corresponding to the first 297 amino acids of the CpdB protein (nucleotides 891–1 of Fig. 3). The fusion plasmids previously produced by random insertion of *TnphoA* into pJH1A (Jayakumar *et al.*, 1989) mapped to this region. The location of *cpdB* sequences adjacent to *amtA* also confirms the map position of *amtA* at 95.8 min on the *E. coli* chromosome (Jayakumar *et al.*, 1991).

Analysis of the *amtA* peptide sequence

The coding sequence for *amtA* and the corresponding peptide sequence were compared to the PIR and GenBank databases, and no homologies were found. Hydropathy analysis of the AmtA peptide according to Kyte & Doolittle (1982) shows a protein which is almost as hydrophobic as it is hydrophilic (Fig. 4). Only one hydrophobic segment (residues 102 to 122) appears to be long enough to span a lipid bilayer. Computer modelling was done to determine whether this segment of the protein could be membrane associated. Hydrophobic moment analysis was done according to the method of Eisenberg *et al.* (1984). The value for the average hydrophobicity ($\langle H \rangle$) of this segment was calculated to be 0.49. This is below the minimum value of $\langle H \rangle = 0.68$ which defines a helix as membrane associated. The complete sequence of AmtA was also analysed by the method of Klein *et al.* (1985), which classifies membrane proteins as either integral or peripheral. The peripheral: integral odds value was 2.21, supporting a peripheral localization. Also, the inferred sequence of AmtA does not contain a prokaryotic signal sequence.

Fig. 4. Hydropathy plot of the *amtA* gene product. The hydropathy profile of the inferred amino acid sequence of AmtA was generated according to Kyte & Doolittle (1982). Values were averaged over a window of nine amino acids.

Discussion

Within 2.0 kb of the complementing insert of pJH1A, we have identified a gene sequence which corresponds to *amtA*. This gene was localized by deletion analysis of the 3.4 kb Amt-complementing fragment, and it contains the site of *Tn10* insertion in the complemented structural gene mutant. The *amtA* sequence contains a typical *E. coli* ORF encoding a 27 kDa protein, a ribosome binding site and a simple transcription terminator. The start codon is ATG, which is the most commonly used bacterial start codon. The ribosome binding site consists of the sequence GAGGTG, which is complementary to the sequence 5'...GAUCACCUCCUUA_{OH} at the 3' end of the 16S rRNA, and is located six nucleotides upstream of the start codon. An extensive comparison of 124 *E. coli* translation initiation sites shows that the average complementarity to the 16S rRNA is 5 ± 1 bases, and the average spacing between the ribosome binding site and the start codon is 7 ± 2 (Stormo *et al.*, 1982). The simple inverted repeat after the coding region could form a stable stem-loop structure, serving as a simple transcription terminator. The sequence we have designated the *amtA* coding region fits the generally accepted criteria for *E. coli* genes.

Analysis of the inferred sequence of the AmtA peptide predicts a protein which is neither a periplasmic nor an integral membrane protein. The former is also unlikely considering the absence of a required shock protein in the Amt system (Jayakumar *et al.*, 1985). Furthermore, random insertion of *TnphoA* into pJH1A produced numerous fusions which mapped in the *cpdB* gene, but none in *amtA*. This suggests that the AmtA peptide does not contain domains which are exported from the cytoplasm. Therefore, we propose that the AmtA protein is a cytoplasmic component of the ammonium transport system. Ample precedent exists for peripheral membrane protein components of ion transport systems. For

example, B. P. Rosen and coworkers have identified an arsenite pump encoded by the *ars* operon of the *E. coli* R-factor R773 (Chen *et al.*, 1985). One essential component of this system, the ArsA protein, is an arsenite-stimulated cytoplasmic ATPase (Rosen *et al.*, 1988) and requires the ArsB integral membrane protein as a site for attachment (Tisa & Rosen, 1990). Bossemeyer *et al.* (1989) report that the K⁺-transport protein TrkA of *E. coli* is a cytoplasmic protein, which requires three other integral membrane proteins (TrkE, TrkG and TrkH) for binding to the cytoplasmic membrane. TrkA does not appear to be an ATPase, and the other *trk* genes are at different locations on the chromosome. Since the *AmtA* protein does not contain a nucleotide binding consensus, and does not appear to be part of an operon, we suspect that the *Amt* system might be organized much like the Trk system. This possibility is being explored by characterization of additional *Amt* mutants.

Since the expression of ammonium transport activity in *E. coli* is regulated by the nitrogen-regulatory system (Jayakumar *et al.*, 1986), it is reasonable to expect that *amtA* might encode a protein whose expression is under the control of a nitrogen-regulated promoter. However, we were unable to identify a nitrogen-regulatory control region upstream of the *amtA* ORF. It seems likely, therefore, that other component(s) of the *E. coli* ammonium transport system provide sensitivity to nitrogen availability.

This work was supported by grant DMB-8715825 from the National Science Foundation.

We thank Glenn Murray of the MBIR for expert assistance in performing databank searches, and for assistance in producing the *amtA* sequence and hydropathy graphics. We also thank Meredith Riddell and Mary Jane Perez for the production of the deletion and sequencing graphics.

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