Distribution of a sub-class of bacterial ABC polar amino acid transporter and identification of an N-terminal region involved in solute specificity

David L. Walshaw[a], Shaun Lowthorpe[a], Alison East[b], Philip S. Poole[a,*]

[a]*School of Animal and Microbial Sciences, University of Reading, Whiteknights, P.O. Box 228, Reading RG6 6AJ, UK*
[b]*BBSRC Institute of Food Research, Whiteknights, Reading, UK*

Received 28 May 1997; revised version received 5 August 1997

Abstract **A new sub-class of binding protein-dependent transporter with specificity for a broad range of polar amino acids has been identified by sequence comparison, in *Rhizobium leguminosarum, Rhodobacter capsulatus, Escherichia coli* and *Pseudomonas fluorescens*. Southern blotting and PCR analysis has shown that transporters from this new sub-class are widely distributed in Gram-negative bacteria, including, in addition to the above, *Citrobacter freundii, Erwinia carotovorum* and *Rhizobium meliloti*. ABC transporters of polar amino acids can be divided into two groups: those with narrow solute specificity and the newly identified sub-class with broad solute specificity. The binding and inner membrane proteins from transporters with a broad solute specificity are larger by approximately 30% than those with a narrow solute specificity. Multiple alignment of the inner membrane proteins from all sequenced polar amino acid transporters indicates there is an N-terminal conserved region that may be involved in solute specificity. A conserved arginine or lysine at residue 30 of this region is changed to glutamate in arginine transporters. Residue 53 also has a strong correlation with the charge on the transported solute, with basic amino acid transporters replacing an aliphatic amino acid at this position with a negatively charged amino acid. The general amino acid permease from *R. leguminosarum*, which will transport aliphatic as well as basic and acidic amino acids, juxtaposes two prolines at residues 52 and 53 of the N-terminal conserved region.**

Key words: Transport; Amino acid; *Rhizobium*; *Escherichia coli*

1. Introduction

Members of the ATP-binding cassette (ABC) superfamily of transporters are one of the largest classes of systems that mediate solute uptake and export in prokaryotic and eukaryotic cells. ABC systems transport a wide variety of solutes including sugars, amino acids, ions, proteins and polysaccharides [1,2]. They possess a common organisation consisting of four domains, two of which are hydrophobic integral membrane proteins and two are hydrophilic ATP-binding proteins, which are thought to couple ATP hydrolysis to solute movement [1,2]. The various domains can be completely separate proteins or fused in a variety of ways, including fusion to form a single polypeptide as in cystic fibrosis transmembrane conductance regulator (CFTR) and in the multi-drug resistance P-glycoprotein [3–5]. Among the best studied ABC transporters are the periplasmic binding protein-dependent systems found in bacteria. Periplasmic binding proteins which are found in import but not export systems are solute specific and are present in addition to the four-domain structure described above.

While studying amino acid uptake in *Rhizobium leguminosarum* we recently cloned and characterised an ABC general amino acid permease, consisting of four genes (*aapJQMP*) organised in an operon [6]. This system will transport a wide range of L-amino acids including those with acid, base, amide and aliphatic side chains. Previously characterised ABC transport systems of amino acids are usually very specific for a single amino acid or a related group of amino acids, suggesting the general amino acid permease may represent a new sub-class of amino acid transporter. This is supported by the finding that a system with high identity to the *R. leguminosarum aap* genes has been identified in the *Escherichia coli* genome sequencing project (recently designated *yhdWXYZ*). We have cloned and attempted to express this system, however, it appears to be silent due to frame-shift mutations in the coding sequence [6]. The gene encoding the binding protein of *R. leguminosarum* (*aapJ*) also shows 57.6% identity with the translation of an incomplete open reading frame from *Pseudomonas fluorescens* [7]. Since binding proteins from different ABC transporters show little homology this suggests that a similar system may exist in *P. fluorescens*. Recently a glutamate/glutamine/aspartate/asparagine transporter (Bzt) was cloned and characterised in *Rhodobacter capsulatus* [8]. This transporter clearly belongs to the same class as the Aap since it consists of a four-gene operon *bztA,B,C,D* with identities of 58.2%, 42.4%, 46% and 83.6% to *aapJ,Q,M,P* respectively. The operon structure for the *aap*, *bzt* and *yhd* genes are also very similar. *aapJ* and *bztA* encode binding proteins, the next two (*aapQM* and *bztBC*) encode inner membrane proteins while *aapP* and *bztD* encode hydrophilic ATP-binding subunits. Sequence comparison suggests that *yhdWXYZ* would encode the same proteins if they were expressed. Strikingly the first three genes in each operon (e.g. *aapJQM*) are approximately 30% larger than the equivalent genes from other ABC polar amino acid transporters. The specificity of the Bzt for acids and amides is wider than seen in other polar amino acid transporters but it does not appear to have the full range of solute specificity of the Aap [8]. We therefore compared the sequences of these recently discovered ABC transport systems with each other and with ABC transporters of polar amino acids (acids, bases and amides). This allowed the identification of an N-terminal conserved region that is likely to be involved in solute specificity.

2. Materials and methods

2.1. Bacterial strains and media

The sources of bacterial strains are as follows: *R. leguminosarum* strain 3841 [9]. *P. fluorescens* strain Rhodes 1959, *Pseudomonas aeru-*

*Corresponding author. Fax: (44) (118) 9316671.
E-mail: p.s.poole@reading.ac.uk

PII S0014-5793(97)01023-5

ginosa, Erwinia carotovorum, Serratia marcescens and *E. coli* strain B were from the University of Reading stock culture collection. *Salmonella typhimurium* strain ATCC 10249, *Citrobacter freundii* strain ATCC 1433 and *E. coli* strain ATCC 26171 were from the American Type Culture Collection. *Rhodobacter sphaeroides* strain WS8 was from W.R. Sistrom while *Rhizobium meliloti* strain 1021 was from F.M. Ausubel.

2.2. Southern blotting and PCR analysis

Genomic DNA was prepared from several species of Gram-negative bacteria using Genosys DNA isolator reagent and then phenol extracted twice. Genomic DNA was digested with *Eco*RI, separated on an agarose gel (0.8%), and Southern transferred to positively charged nylon membrane (Boehringer Mannheim). This was tested for Southern hybridisation with a 0.75 kbp region of *E. coli yhdW* using an Amersham ECL kit according to the manufacturer's instructions. The most stringent wash used was 0.5×SSC at 60°C. The probe (0.75 kbp) was produced by PCR amplification of part of *E. coli yhdW*, cloned in pRU402 [6]. The primers used were P29 (GAACGCTTCACCGCKYTDCAGTC) and P30 (GTACTGAATACCGCCSTTRTTCCA), where D is G/A/T, K is G/T, R is A/G, S is G/C and Y is C/T. This *yhdW* probe was chosen because it encodes the binding protein which is a highly solute-specific component of an ABC transporter and has a GC ratio typical of enteric bacteria. PCR amplification reactions were performed with TAQ+ from Stratagene using 30 cycles at 94°C for 2 min, 55°C for 1.5 min and 72°C for 2 min.

3. Results and discussion

3.1. Multiple alignment of ABC transporters of polar amino acids

FASTA searches using AapQM as the test proteins always found the 'integral membrane component signature' in the C-terminus of all polar amino acid transporters. This is unremarkable since it is present in all periplasmic binding protein-dependent transport systems and polar amino acid transporters have been identified as a sub-family of ABC transporter [10]. However, BLAST searches were more revealing since besides the 'integral membrane component signature', a second region of homology was found between AapM, AapQ and the integral membrane components of ABC transporters of acidic or basic amino acids or glutamine. This second region was always towards the N-terminus of the proteins relative to the 'integral membrane component signature'. Sequence alignment of the N-terminal region of all the polar amino acid transporters, using ClustalV, revealed an N-terminal region of 63 amino acids that contains 19 well conserved residues (Fig. 1). This region is entirely distinct from the 'integral membrane component signature' sequence [10] and

```
                    10        20        30        40        50        60        70                  BLAST  Acc. No.
                                        ↓                   ↓
At  NocQ  LARGAMMTVV VAACSYFFGI IFGSLFAAAK LSRFWSLRLL GDVYTTVVRG VPELLIIFLV FFGGGTLLRT IAN  17-89 (236)  97   M77785
At  OccQ  MLRATAMTMA VAFSGFTIGL VFGCLGAAAS LSSSGALQAA ASGYTTALRG IPDLLVIYLF YFGSSSVISN VAS  18-90 (237) 131   M77784
Ec  ArtQ  LASAAGMTVG LAVCALIVGL ALAMFFAVWE SAKWRPVAWA GSALVTILRG LPEILVVLFI YFGSSQLLLT LSD   7-79 (238)  76   X86160
Hi  ArtQ  MFTAALMTLG LAVCSLLLGL FLSLIFAVLE ANRF--VGKP MTVFVALLRG LPEIIVVLLV YFGSTELVEM LTG   9-79 (221)  76   U17295
St  HisQ  ILQGAIVTLE LALSSVVLAV LIGLVGAGAK LSQNRVTGLI FEGYTTLIRG VPDLVLMLLI FYGLQIALNV VTD   9-81 (228)  92   V01373
At  NocM  LLAAVPTTLT LAFISLLIGF VVSVPVALMR LSKNRIVSSL AYGYVYIIRS TPLLVQMFLI YYGSAQFRGV LSE  13-85 (241) 133   M77785
At  OccM  LLSGIPLALQ LAVFSVALGT VLAFGLALMR VSRLWWLDLP ARFYIFAFRG TPLLVQIYII YYGLSQFPDV RHS  15-87 (246) 122   M77784
Ec  ArtM  LMKGLHTSLT LTVASLIVAL ILALIFTIIL TLKTPVLVWL VRGYITLFTG TPLLVRIFLI YYGPGQFPTL QEY   8-80 (222) 110   X86160
Hi  ArtM  IVKGIPTSLL LTVVSLLIAF FLALFLTFLL SMENKWIKSA VNLYLTLFTG TPLLVQFFLI YAGPGQFQWI IDS   9-81 (227)  92   U17295
St  HisM  RFTGVAITLW LLISSVVMGG LLAVILAVGR VSSNKFIRFP IWLFTYIFRG TPLYVQLLVF YSGMYTLEIV KGT  19-91 (235) 107   V01373

Bs  YckA  VIKGIGYTLL ISFVSMFAGT VIGLFISLAR MSKLALLRWP AKLYISFMRG VPILVILFIL YFGFPYIGIE FSA  23-95 (226) 119   D30762
Ec  GlnP  LIEGAKMTLW ISVLGLAGGL VIGLLAGFAR TFGGWIANHV ALVFIEVIRG TPIVVQVMFI YFALPMAFND LRI  15-87 (219) 106   X14180
Rp  GlnP  IIEGTLVTLK YSVIAVILGL VIGMLLAICK VNKNRVLRLF ANFYTSIFRG TPLLVQLSII YFAAPYIINI KFN  15-87 (218) 146   U02603
Bs  YckJ  LSGGIYYTIP LTILSFIFGM ILALITALAR MSKVRPLRWV FSVYVSAIRG TPLLVQLFII FYLFPAFNVT LDP  28-100(234) 131   X77636

Cg  GluC  LLPAFWVTIK LTIYSAIGAM IFGTILTTMR VSPVKILRTL STAYINTVRN TPLTLVVLFC SFGLYQNLGL TLA  12-84 (225)  91   X81191
Ec  GltJ  IWSGFQVTIA LSICAWIIAF LVGSFFGILR TVPNRFLSGL GTLYVELFRN VPLIVQFFTW YLVIPELLPE KIG  25-97 (246)  99   U10981
Cg  GluD  ILPGLWGTLK SAVFSVILAL VMGTALGLGR ISEIRILRWF CAVIIETFRA IPVLILMIFA YQMFAQYNIV PSS  26-98 (273) 104   X81191
Ec  GltK  LLDGLVITLK ITVTAVVIGI LWGTMLAVMR LSSFAPVAWF AKAYVNVFRS IPLVMVLLWF YLIVPGFLQN VLG  16-88 (224) 100   U10981

Ec  YHDX  FIVGLLNTLL VSALCIVFAS VLGFFIGLAR LSDNWLLRKL STIYIEIFRN IPPLLQIFFW YFAVLRNLPG PRQ  90-162(368) 120   U18997
Rl  AapQ  LLVGILNTLL VAVTGIFTAT IIGFLIGIGR LSRNWLIAKL CTVYVEVFRN IPPLLVIFFW YLGVLSVLPQ PRE  92-164(400) 110   X82596
Rc  BztB  LIEGLLNTLL VSVLGCILAT ILGTIIGVLR LSQNWLVARI MTVYVETFRN IPLLLWILLM GTILAETRPV PKD  88-160(426)  96   U37407
Ec  YHDY  QWGGLTLTLI IASVGIAGAL PWGILLALGR RSHMPIVRIL SVIFIEFWRG VPLITVLFMS SVMLPLFMAE GTS 156-228(368)  90   U18997
Rl  AapM  LWGGLMVTLV LSFVGIAVSL PVGILLALGR RSRMPVIRML CVTFIEVIRG VPLITVLFMA SVMLPLFLPT GWN 171-243(384)  93   X82596
Rc  BztC  QFGGFLLALV IGVTAIVVSL PLGILLALGR QSDMLIVKSL SVGIIEFVRG VPLITLLFTA SLLLQYFLPP GTN 223-295(434)  86   U37407

Consensus LLXGLXXTLX LAVXXXIXGL VLGXLLALAR LSXNRXLRXL AXXYIEXFRG TPLLVQLFXI YFG
          * *  **       *   *  * *  *   *     *   **  ***   *  * *
```

Fig. 1. Sequence alignment of a 73 amino acid region of the integral membrane components of 22 known prokaryotic ABC transporters of polar amino acids. The first 63 amino acids of this region contain a number of conserved residues. The location of the aligned region in each protein is indicated by residue numbers to the right of each sequence, with the total protein length given in parentheses. The EMBL accession number for each sequence is also given. The BLAST score for each sequence against the consensus is also given. Conserved residues (present in 11 or more proteins) are highlighted by *, with identical amino acids or conservative substitutions appearing in bold type. The consensus sequence consists of all conserved residues plus all residues present in 7–11 proteins. Residues that form part of putative membrane-spanning segments as determined with the Kyte-Doolittle method are indicated in italics. Residues 9–29 and 49–69 of the conserved sequence are overlined in bold to indicate the most common position for two transmembrane segments. From the top of the page sequences have been grouped as transport systems for bases, amides, acids and broad specificity respectively. Two residues that may be involved in charge specificity are arrowed. Deletion of the four underlined residues in HisM results in a change in solute specificity from L-histidine to L-histidinol. Species abbreviations are: *At, Agrobacterium tumefaciens; Bs, Bacillus subtilis; Cg, Corynebacterium glutamicum; Ec, Escherichia coli; Hi, Haemophilus influenzae; Rc, Rhodobacter capsulatus; Rl, Rhizobium leguminosarum; Rp, Rickettsia prowazekii; St, Salmonella typhimurium.*

Table 1
Identities obtained from pairwise alignment of the complete sequence of bacterial ABC transporters of polar amino acids

	AapQ	AapM	BztB	BztC	Orf2	Orf3	HisQ	HisM	OccQ	OccM	NocQ	NocM
AapQ	-	23	**42**	22	**49**	27	23	25	27	32	27	28
AapM		-	22	**46**	23	**51**	26	29	23	28	27	28
BztB			-	22	**42**	25	24	26	20	29	25	25
BztC				-	26	**46**	28	28	24	29	27	28
Orf2					-	24	23	25	23	29	30	29
Orf3						-	26	28	25	28	27	25
HisQ							-	**30**	**36**	**30**	**35**	**32**
HisM								-	**29**	**39**	27	**41**
OccQ									-	**31**	**48**	**33**
OccM										-	**29**	**50**
NocQ											-	**31**
NocM												-

Pairwise alignments of proteins from Fig. 1 were determined with the GAP program and are shown as percent identities. The two distinct sub-groups are shown shaded with high identities shown in bold. Proteins from Fig. 1 that show much lower identities relative to the values in the two shaded regions have been omitted for clarity.

confirms that these transporters constitute a sub-family of the ABC superfamily. When a consensus sequence is used in BLAST or FASTA searches the highest identities are found among the transporters of polar amino acids from which the sequence was derived.

Pairwise comparison of the entire sequence of the integral membrane proteins from all bacterial periplasmic polar amino acid transporters listed in Fig. 1 show that Aap, Bzt and Yhd form a tight sub-group of the polar amino acid transporters as do His, Occ and Noc (Table 1). None of the other proteins from Fig. 1 show similarly high identity to each other and they have been omitted from Table 1 for clarity. The cluster-

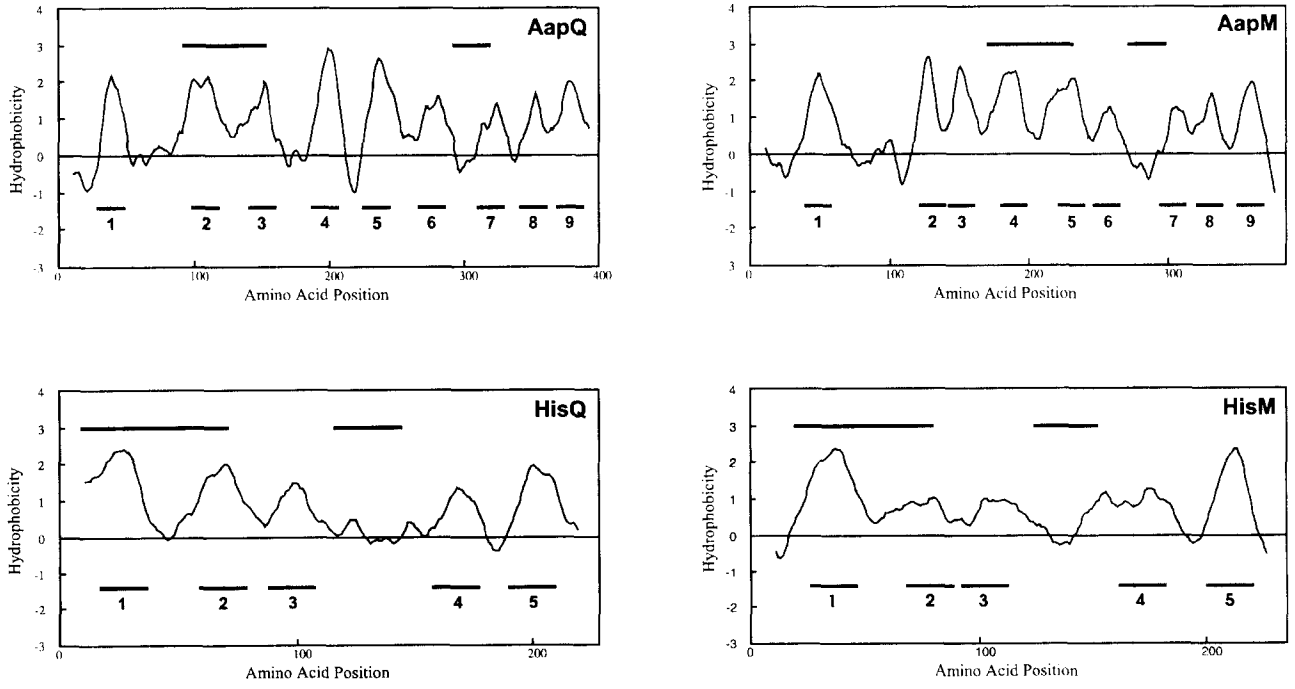

Fig. 2. Hydropathy plots (Kyte and Doolittle) of the integral membrane proteins of the *R. leguminosarum* Aap and the *S. typhimurium* histidine transporter. Predicted transmembrane domains are indicated by the numbered bars below each plot. The two bars above each plot indicate the positions of the N- and C-terminal conserved regions.

ing of the broad solute range transporters is particularly tight since they come from three very different bacteria, while the Occ and Noc proteins have the same levels of identity they transport similar opine solutes from the same organism.

3.2. Hydropathy analysis and localisation of the N-terminal conserved region

Hydropathy analysis of AapQ and AapM by the method of Kyte and Doolittle [11] taking account of the 'positive-inside rule' of von Heijne [12] predicts nine transmembrane segments (Fig. 2). Using the prediction method of Engelman et al. [13], instead of that of Kyte and Doolittle, removes transmembrane segment 7 from AapQ leaving eight transmembrane segments. Similar analysis of the polar amino acid transporters with a narrow solute range predicts five transmembrane segments in most cases. An increased number of transmembrane domains in AapQ and AapM is compatible with the increased size of these proteins relative to the corresponding proteins of the polar amino acid transporters with a narrow solute range.

Whichever of the predicted topologies is used, the N-terminal conserved residues in both AapM and AapQ lie within two membrane-spanning segments and the residues that connect them. In the case of AapM it is transmembrane segments 4 and 5 that are involved, while for AapQ segments 2 and 3 contain the conserved amino acids.

In all the proteins in Fig. 1 except those with broad solute range, the conserved region identified in this work is located between the first two transmembrane segments at the N-terminus. While computer prediction of protein topology is not a reliable method to assign the exact number and position of transmembrane segments, a five-transmembrane-segment topology has been demonstrated experimentally for HisM and HisQ [14,15]. This locates both the N-terminal conserved region and the C-terminal 'integral membrane component signature' sequence [10] in the cytoplasm in both cases (Fig. 2).

3.3. Role of the N-terminal conserved region in solute specificity

There are two residues in the N-terminal conserved region which may be particularly important in solute specificity (Fig. 1). Residue 30 contains a highly conserved arginine or lysine which is replaced with a glutamate in ArtQ and a leucine in ArtM from *E. coli* and *Haemophilus influenzae*. Replacing two positively charged amino acids with a neutral and an acidic residue in a transport system for arginine suggests this may be required for charge stabilisation of the solute.

In NocQ, OccQ, ArtQ and HisQ, each of which constitutes one half of the integral membrane complex of a transporter of (substituted) basic amino acid(s), the leucine at position 53 is replaced by an acidic amino acid, either glutamate or aspartate (Fig. 1). As for residue 30 such a dramatic change in charge suggests that D/E^{53} may be involved in charge stabilisation of basic amino acids during their transport. Similarly, in one of the two integral membrane components of the general amino acid permease of *R. leguminosarum*, this leucine is replaced by proline (Fig. 1). In both the integral membrane components (or the sole integral membrane component) of the remaining transporters, all of which transport either glutamate/aspartate or glutamine/asparagine, the leucine at position 53 is either conserved or replaced by a similar aliphatic amino acid. This analysis predicts that the *yhdWXYZ* operon from *E. coli* encodes a general amino acid permease, since

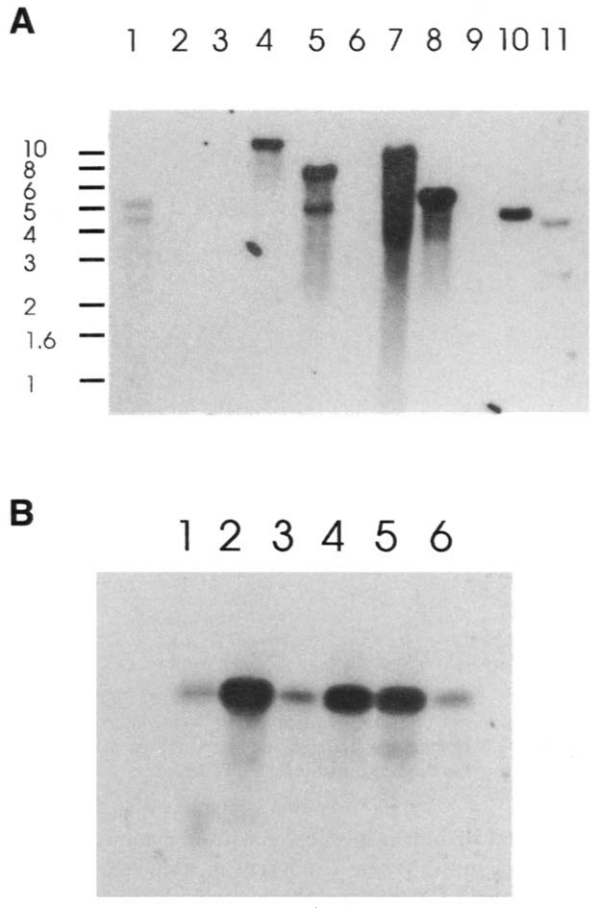

Fig. 3. Distribution of broad solute range transport systems in Gram-negative bacteria. A: Southern hybridisation of *E. coli yhdW* to *Eco*RI digested chromosomal DNA. Lane 1, *Pseudomonas fluorescens* strain Rhodes 1959; lane 2, *Pseudomonas aeruginosa;* lane 3, *Salmonella typhimurium* strain ATCC 10249; lane 4, *Citrobacter freundii* strain ATCC 1433; lane 5, *Erwinia carotovorum*; lane 6, *Serratia marcescens;* lane 7, *E. coli* strain B; lane 8, *E. coli* strain ATCC 26171; lane 9, *Rhodobacter sphaeroides* strain WS8; lane 10, *R. leguminosarum* strain 3841; lane 11, *Rhizobium meliloti* strain 1021. Sizes are given to the left in kbp. B: Southern hybridisation of *E. coli yhdW* to PCR amplification products from chromosomal DNA of various bacteria. Lane 1, *P. fluorescens* strain Rhodes 1959; lane 2, *C. freundii* strain ATCC 1433; lane 3, *E. carotovorum*; lane 4, *E. coli* strain ATCC 26171; lane 5, *R. leguminosarum* strain 3841; lane 6, *R. meliloti* strain 1021. The hybridising band is 0.75 kbp.

yhdX has a proline at position 53 of the N-terminal conserved region, although this cannot be tested due to frame-shift mutations in the coding sequence. Furthermore, while the sequence data suggest that the Bzt is similar to the Aap and Yhd, the nature of residue 53 in BztB and BztC suggests it should have specificity for acids and/or amides. This is in agreement with its measured ability to transport acids and amides but not other amino acids [8].

Position 53 is predicted to lie at the beginning of the second transmembrane segment of the conserved region in AapM and AapQ (Fig. 2), as well as the other integral membrane proteins shown in Fig. 1. Thus these data appear to be compatible with the amino acid at position 53 in (residue 144 in AapQ) being involved in specificity for the charge on the solute (Fig. 1). The positioning of two proline residues side by side, at the beginning of a putative transmembrane seg-

ment in the general amino acid permeases, is possibly highly significant for protein structure and α-helix formation in particular. Residue 30 of the conserved region may lie at the end of the first transmembrane segment or the beginning of the cytoplasmic segment. In the case of ArtQ where an acidic residue has been substituted for an aliphatic residue in transmembrane segment 2 and a second acidic residue replaces a positive residue close to the cytoplasmic loop it is tempting to speculate that a charge relay of the solute may occur.

Further evidence for the involvement of the N-terminal conserved region in solute specificity is provided by the observation that the in-frame deletion mutations of HisM from *S. typhimurium*, which alter its solute specificity from histidine to histidinol, delete positions 25–28 of the N-terminal conserved region [16]. The deletions remove a conserved alanine.

3.4. Distribution of broad solute range transporters

The sequence analysis presented here indicates that polar amino acid transporters with an extended solute range exist in *R. leguminosarum, E. coli, R. capsulatus* and *P. fluorescens*. To test how wide this distribution is among Gram-negative bacteria used as important experimental organisms we carried out genomic Southern blots on a range of organisms.

It can be seen that genes with homology to *yhdW* were found in several genera including *Rhizobium, Escherichia, Pseudomonas, Erwinia* and *Citrobacter* (Fig. 3A). Interestingly no hybridising band was detected in chromosomal Southern blots for either *S. typhimurium* or *R. sphaeroides*. This is even though closely related species do possess genes homologous to *yhdW*.

After aligning the sequences for the binding proteins from *R. leguminosarum, E. coli* and *P. fluorescens* two redundant PCR primers (P29 and P30, see above) were designed to the corresponding DNA sequence. These were used in PCR amplification of chromosomal DNA prepared for genomic Southern blotting, as described above. The expected amplification product (0.75 kbp) was detected in all strains of *E. coli* K12 and B, *R. leguminosarum* and *P. fluorescens* tested. It was also detected in *C. freundii, E. carotovorum,* and *R. meliloti*. The PCR amplification products were confirmed by Southern hybridisation with the *E. coli yhdW* probe as being homologous to the gene that encodes the putative binding protein of broad solute range transporters (Fig. 3B). PCR amplification products were found in all species in which the genomic Southern blot was positive, confirming that this new sub-class of ABC transporter is widely distributed.

In summary, sequence analysis has revealed the existence of a new sub-class of ABC polar amino acid transporter with a broad solute range. The evidence so far from BztA and AapJ is that they utilise a single binding protein of broad specificity [6,8]. These transporters are larger at the C-terminus of the binding protein and larger at the N-terminus of the inner membrane proteins by about 30% than other polar amino acid transporters, which are specific for one solute. This suggests that the additional residues present in the broad solute range transporters may be required for their extended solute range. However, a regulatory role cannot be ruled out. Alignment analysis of the inner membrane proteins of all currently sequenced polar amino acid transporters reveals the presence of an N-terminal conserved region that appears to be important in solute specificity. This is distinct from the well characterised C-terminal 'integral membrane component signature' [10]. Residue 30 is changed from a positively charged (Lys or Arg) to a negative charged residue (Glu) in the two arginine transporters. Such a dramatic change in charge of an otherwise conserved residue is likely to be highly significant for solute specificity. Similarly the charge on residue 53 of the N-terminal consensus region from one of the two inner membrane proteins of each transporter correlates with the charge on the solute being transported. The only general amino acid permease of the ABC family so far characterised juxtaposes two prolines at positions 52 and 53 of the N-terminal conserved sequence. Southern blot and PCR analyses suggest that transporters with a broad solute range for polar amino acids are widely distributed in Gram-negative bacteria.

Acknowledgements: We would like to thank the University of Reading Research Endowment Trust for supporting this research.

References

[1] Doige, C.A. and Ames, G.F.L. (1993) Annu. Rev. Microbiol. 47, 291–319.
[2] Higgins, C.F. (1992) Annu. Rev. Cell. Biol. 8, 67–113.
[3] Riordan, J.R., Rommens, J.M., Kerem, B.S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, N., Drum, M.L., Iannuzzi, M.C., Collins, F.S. and Tsui, L.C. (1989) Science 245, 1066–1072.
[4] Rommens, J.M., Iannuzzi, M.C., Kerem, B., Drumm, M.L., Melmer, G., Dean, M., Rozmahel, R., Cole, J.L., Kennedy, D., Hidako, N., Zsiga, M., Buchwald, M. and Riordan, R. (1989) Science 245, 1059–1065.
[5] Chen, C.-J., Chin, J.E., Ueda, K., Clark, D.P. and Pastan, I. (1986) Cell 47, 381–389.
[6] Walshaw, D.L. and Poole, P.S. (1996) Mol. Microbiol. 21, 1239–1252.
[7] Hong, K.H., Jang, W.H., Choi, K.D. and Yoo, O.J. (1991) Agric. Biol. Chem. 55, 2839–2845.
[8] Zheng, S. and Haselkorn, R. (1996) Mol. Microbiol. 20, 1001–1011.
[9] Johnston, A.W.B. and Beringer, J.E. (1975) J. Gen. Microbiol. 87, 343–350.
[10] Saurin, W., Köster, W. and Dassa, E. (1994) Mol. Microbiol. 12, 993–1004.
[11] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[12] von Heijne, G. (1992) J. Mol. Biol. 225, 487–494.
[13] Engelman, D.M., Steitz, T.A. and Goldman, A. (1986) Annu. Rev. Biophys. Biophys. Chem. 15, 321–353.
[14] Kerppola, R.E., Shyamala, U.K., Klebba, P. and Ames, G.F.-L. (1991) J. Biol. Chem. 266, 9857–9865.
[15] Kerppola, R.E. and Ames, G.F.-L. (1992) J. Biol. Chem. 267, 2329–2336.
[16] Payne, G., Spudich, E.N. and Ames, G.F.-L. (1985) Mol. Gen. Genet. 200, 493–496.