TABLE 1. Abbreviations, specificities, sizes, and sources of members of the MFP family included in this study

MFP abbreviation	Cofunctioning permease family	Species transported	Organism	No. of residues	Accession no.	Reference(s)
LktD	ABC	Leukotoxin	*Pasteurella haemolytica*	478	LKTD_PASHA (SP)	39
LktD	ABC	Leukotoxin	*Actinobacillus actinomycetemcomitans*	477	LKTD_ACTAC (SP)	16
HlyD	ABC	Hemolysin	*Actinobacillus pleuropneumoniae*	477	HLYD_ACTPL (SP)	5
HlyD	ABC	Hemolysin	*E. coli*	478	HLYD_ECOLI (SP)	37
CyaD	ABC	Cyclolysin	*Bordetella pertussis*	440	CYAD_BORPE (SP)	15
PrtE	ABC	Proteases	*Erwinia chrysanthemi*	448	PRTE_ERWCH (SP)	21
AprE	ABC	Alkaline proteases	*Pseudomonas aeruginosa*	432	S26697 (PIR)	10
CvaA	ABC	Colicin V	*E. coli*	413	CVAA_ECOLI (SP)	14
EmrA	MFS	Multiple drugs	*E. coli*	390	EMRA_ECOLI (SP)	24
YibH	?	Unknown	*E. coli*	378	YIBH_ECOLI (SP)	43
YjcR	?	Unknown	*E. coli*	343	YJCR_ECOLI (SP)	2
FusE[a]	?	Fusaric acid	*Pseudomonas cepacia*	168	FUSE_PSECE (SP)	40
NolF	RND	Lipooligo-saccharide?	*Rhizobium meliloti*	367	NOLF_RHIME (SP)	1
CzcB	RND	Cadmium, zinc, cobalt	*Alcaligenes eutrophus*	520	CZCB_ALCEU (SP)	27
CnrB	RND	Cobalt, nickel	*A. eutrophus*	395	F47056 (PIR)	23
EnvC[b]	RND	Acriflavine	*E. coli*	385	ENVC_ECOLI (SP)	19
AcrA	RND	Acriflavine	*E. coli*	397	ECOACRAB_1 (GP)	25
MexA	RND	Multiple antibiotics, pyoverdine[c]	*P. aeruginosa*	383	PSEENVCD_1 (GP)	31, 32
MtrC[d]	?	Hydrophobic compounds, drugs	*Neisseria gonorrhoeae*	271	NGMTRRC_2 (GP)	29a

[a] This sequence is incomplete and therefore was not included in the phylogenetic analysis reported in this communication. Statistical analyses showed that it clusters with EmrA. Possible errors in the published sequence of FusE have been detected (unpublished observations). On the basis of DNA sequence analyses, a putative N-terminal, transmembrane spanner is probable for this protein, as for the other members of the MFP family.

[b] This protein has been implicated in septum formation, but it was also shown to transport drugs, including acriflavine. Its natural substrates are not known.

[c] Pyoverdine is a siderophore concerned with iron uptake.

[d] This sequence is incomplete and therefore was not included in the phylogenetic analysis reported in this communication. Statistical analyses showed that it clusters with EnvC.

conjunction with an MFS permease (26) to export drugs, or (iii) in conjunction with an RND permease for the expulsion of various drugs or, in the case of one protein (NolF), probably a lipooligosaccharide (36). They are of fairly uniform size. One MFP family protein, the CzcB protein, is substantially larger (520 residues) because of an N-terminal extension not present in the other proteins. The CzcB N-terminal extension proved to be unique and was not homologous to any other protein sequence in the database.

Table 2 presents the binary comparison scores and percent identity values that establish that the 19 proteins of the MFP family are homologous. With one exception, all comparison scores presented are equal to or greater than nine standard deviations, and the data are sufficient to establish that these proteins are homologous. No other protein in the database proved to be homologous to any one of the currently recognized members of the MFP family. Thus, no member of the MFP family has yet been identified in a gram-positive bacterium, an archaebacterium, or a eukaryote. As noted below, a single animal viral protein, the simian virus 5 F protein, which is implicated in membrane fusion, exhibits significant sequence similarity to representative MFP family proteins.

TABLE 2. Binary comparison scores establishing homology for members of the MFP family

Protein 1[a]	Protein 2[a]	No. of residues compared	% Identity	Comparison score[b]
HlyD Apl	EmrA Eco	210	18	10
NolF Rme	EmrA Eco	107	24	9
EnvC Eco	EmrA Eco	132	20	7
FusE Pce	EmrA Eco	79	27	19
YjcR Eco	EmrA Eco	335	26	24
YibH Eco	EmrA Eco	299	20	15
AcrA Eco	EnvC Eco	380	64	97
MtrC Ngo	EnvC Eco	227	46	44
MexA Pae	AcrA Eco	383	45	77
CnrB Aeu	MexA Eco	318	19	9
CzcB Aeu	CnrB Aeu	351	29	26
LktD Pha	HlyD Apl	477	64	93
CyaD Bpe	HlyD Apl	201	33	29
PrtE Ech	HlyD Apl	357	24	15
AprE Pae	HlyD Apl	417	24	21
CvaA Eco	HlyD Apl	248	20	9
HlyD Eco	HlyD Apl	477	64	82
LktD Aac	HlyD Apl	477	64	98

[a] The three-letter abbreviation following the protein designation refers to the bacterial species of origin (see Table 1).

[b] The Los Alamos program with 100 random shuffles (18) was used for calculation of the comparison scores. Values are standard deviations.

JOURNAL OF BACTERIOLOGY, July 1994, p. 3825–3831
0021-9193/94/$04.00+0

Vol. 176, No. 13

MINIREVIEW

A Family of Extracytoplasmic Proteins That Allow Transport of Large Molecules across the Outer Membranes of Gram-Negative Bacteria

THIEN DINH,[1] IAN T. PAULSEN,[2] AND MILTON H. SAIER, JR.[1]*

Department of Biology, University of California at San Diego, La Jolla, California 92093-0116,[1] and School of Biological Sciences, University of Sydney, Sydney NSW 2006, Australia[2]

Seventeen fully sequenced and two partially sequenced extracytoplasmic proteins of purple, gram-negative bacteria constitute a homologous family termed the putative membrane fusion protein (MFP) family. Each such protein apparently functions in conjunction with a cytoplasmic membrane transporter of the ATP-binding cassette family, major facilitator superfamily, or heavy metal resistance/nodulation/cell division family to facilitate transport of proteins, peptides, drugs, or carbohydrates across the two membranes of the gram-negative bacterial cell envelope. Evidence suggests that at least some of these transport systems also function in conjunction with a distinct outer membrane protein. We report here that the phylogenies of these proteins correlate with the types of transport systems with which they function as well as with the natures of the substrates transported. Characterization of the MFPs with respect to secondary structure, average hydropathy, and average similarity provides circumstantial evidence as to how they may allow localized fusion of the two gram-negative bacterial cell membranes. The membrane fusion protein of simian virus 5 is shown to exhibit significant sequence similarity to representative bacterial MFPs.

The envelopes of purple gram-negative bacteria possess two membranes: the inner, cytoplasmic membrane and an outer, lipopolysaccharide-containing membrane. These bacteria contain stereospecific solute transport systems in their inner membranes and nonstereoselective porins in their outer membranes that allow the passage of small ions, nutrients, and metabolic products across these lipid bilayer structures (28, 29, 34, 35). Hydrophobic and large hydrophilic molecules cannot pass through the outer membrane porin channels, yet numerous proteins, peptides, carbohydrates, and hydrophobic drugs are actively exported directly from the cytoplasm of these bacteria to the external medium. Three types of cytoplasmic membrane transporters appear to allow passage of such molecules across the bimembranous envelope: drug resistance porters of the major facilitator superfamily (MFS) (24, 26), protein or peptide exporters of the ATP-binding cassette (ABC) superfamily (11, 17, 33), and drug, metallic cation, and oligosaccharide exporters of the heavy metal resistance/nodulation/cell division (RND) family (1, 19, 23, 27, 35, 36, 42). Exactly how these three distinct types of permease systems allow transport of their substrates across the two membranes of the cell envelope in a single step is not known.

The transport systems of the types noted above function together with a second protein that is embedded in the cytoplasmic membrane, sometimes as a lipoprotein, and that extends into the periplasm where it possibly interacts with the outer membrane (22, 36, 37, 41). Its mechanism of action is poorly defined, but the available evidence suggests that it must bind to its cognate transport protein(s) in the cytoplasmic membrane and somehow create a channel from the periplasmic mouth of the transport system to the external surface of the outer membrane (22, 24, 36, 37). In a few cases, an outer membrane protein has been implicated in the transport process (31, 32; see reference 22 for a current and insightful overview). The phylogenetic relationships of the sequenced members of this newly discovered class of proteins have not yet been defined, and their sequences have not been analyzed for clues regarding their potential functional significance.

In this minireview, we identify 19 sequenced members of this novel family of transport proteins, which we designate the putative membrane fusion protein (MFP) family (22, 36). Size evaluation reveals that all but one of the fully sequenced proteins of the MFP family are of about the same size (422 residues ± 13%). Average hydropathy and average similarity plots reveal that no single residue within these proteins is fully conserved but that two regions of intermediate hydrophobicity, a 60-residue segment just following the N-terminal cytoplasmic membrane spanner and a 100-residue segment near the C termini of these proteins, are most strongly conserved. These two regions are postulated to interact with inner membrane and outer membrane constituents, respectively. The phylogenetic relationships of the MFP family proteins are shown to correlate with the type of cytoplasmic transport system with which they function as well as with their substrate specificities.

MEMBERS OF THE MFP FAMILY

Table 1 lists members of the MFP family and provides information concerning their substrates, sources, and sizes. All of the MFPs are derived from purple gram-negative bacteria, and no protein from a gram-positive bacterium proved to be homologous to one of these proteins. They function (i) in conjunction with ATP-binding cassette (ABC)-type permeases (17) for the export of protein toxins (leukotoxins, hemolysins, and cyclolysins), proteases, and a bacteriocin (colicin V), (ii) in

* Corresponding author. Phone: (619) 534-4084. Fax: (619) 534-7108. Electronic mail address: msaier@ucsd.edu.

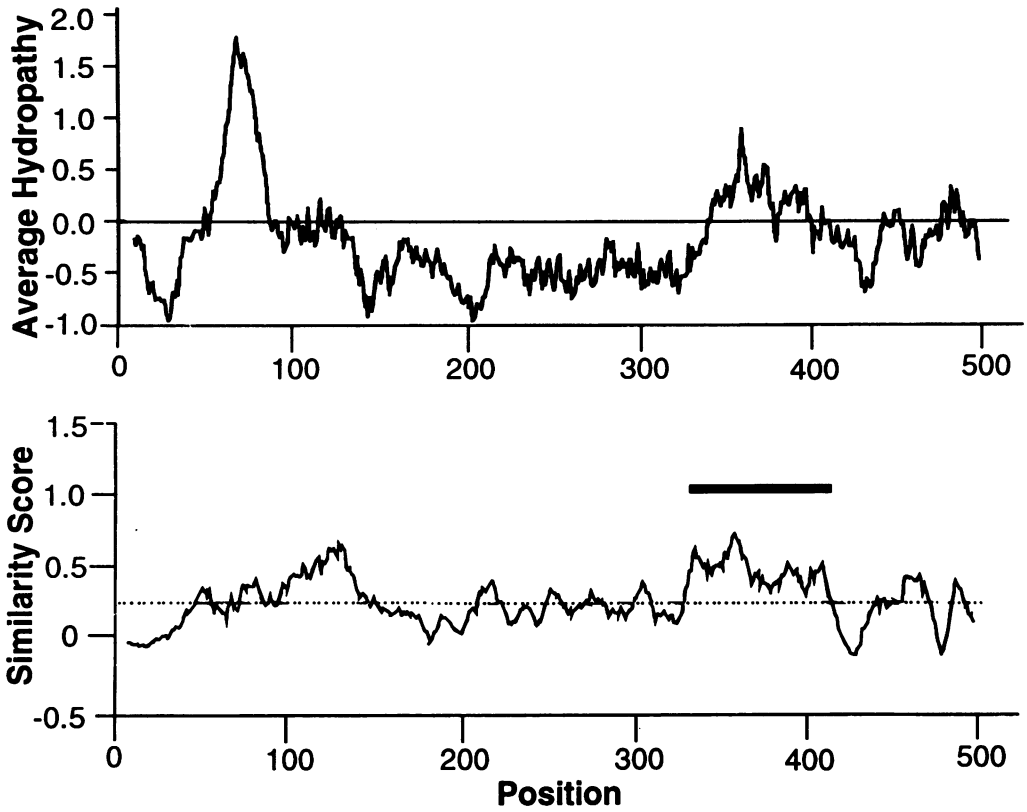

FIG. 1. Average hydropathy plot (20) (top) and average similarity score (bottom) for the fully sequenced MFPs included in this study (see Table 1). A sliding window of 20 residues was used to generate both plots. The bar indicates the portion of the multiple alignment that is relatively conserved and is predicted to consist of β-structure. Most of this region is presented in Fig. 2. Position (at bottom) corresponds to the alignment position in the multiple alignment of these proteins. The dotted line in the bottom figure reveals the average similarity score for the entire plot. The N-terminal extension of CzcB (see text) was deleted for this analysis.

UNIFORM TOPOLOGY FOR MFPs SUGGESTIVE OF FUNCTION

Figure 1 presents the average hydropathy (top) and average similarity scores (bottom) for the common portions of the fully sequenced proteins listed in Table 1. The hydropathy plot reveals that each of these proteins exhibits a short N-terminal hydrophilic region followed by a segment of about 20 residues which is strikingly hydrophobic. The N-terminal hydrophilic region usually, but not always, contains an excess of basic over acidic residues, while the hydrophobic stretch is of 14 to 23 residues in length, exhibits poor sequence conservation (Fig. 1), and lacks prolyl and strongly hydrophilic residues altogether (data not shown). Minimal sequence conservation and the absence of prolyl residues in these putative spanners provide evidence that they function solely in a structural capacity, anchoring the proteins to the cytoplasmic membrane (3, 4, 7, 8, 35). The presence of the lipoprotein signal peptidase cleavage site motif in the N-terminal regions of AcrA, EnvC, and MexA (unpublished observation), the published evidence showing that EnvC is, in fact, a lipoprotein (38), and the fact that remaining proteins in the family lack this motif as well as the invariant cysteine which is modified during lipoprotein processing support the above-mentioned conclusion that the N-terminal hydrophobic domains in MFPs play only a structural role.

Immediately adjacent to the transmembrane hydrophobic stretch (37, 41) is a region (alignment positions 88 to 150 in Fig. 1) of moderate hydrophobicity that exhibits a striking degree of sequence conservation. No single position in the entire multiple alignment of these proteins exhibits full residue conservation, but several residues within this region are largely conserved. Most of these conserved residues are structural (G and P) or hydrophobic (L, V, I, and A), although a few are hydrophilic (data not shown). Neither the pattern of hydrophobicity nor that of conserved amphipathicity within this region is clearly suggestive of the presence of specific membrane-associated secondary structural elements. The programs of Chou and Fasman (6) and Garnier et al. (13) predicted comparable amounts of α and β structure for this region.

Alignment positions 150 to 318 in the complete multiple alignment exhibit strong hydrophilicity but low sequence conservation (Fig. 1). Secondary structural predictions (6, 13) indicated that this region is largely α-helical. Then, beginning with alignment position 319, and continuing through position 420, the most strikingly conserved region within the sequenced MFPs occurs. The multiple alignment of most of this region, which is markedly hydrophobic (Fig. 1), is presented in Fig. 2. Examination of the consensus sequence in Fig. 2 reveals an unusual distribution of conserved residues, with well-conserved aliphatic residues (V, I, L, and A) predominating. Gs and Ts are also prevalent, while strongly hydrophilic residues (E and Q) represent a minority of the residues included in the consensus sequence. Both secondary structural predictive programs clearly suggested that this region consists largely of β strands (6, 13), as would be expected for an outer membrane-associated domain (28, 29). The sequence alignment does not

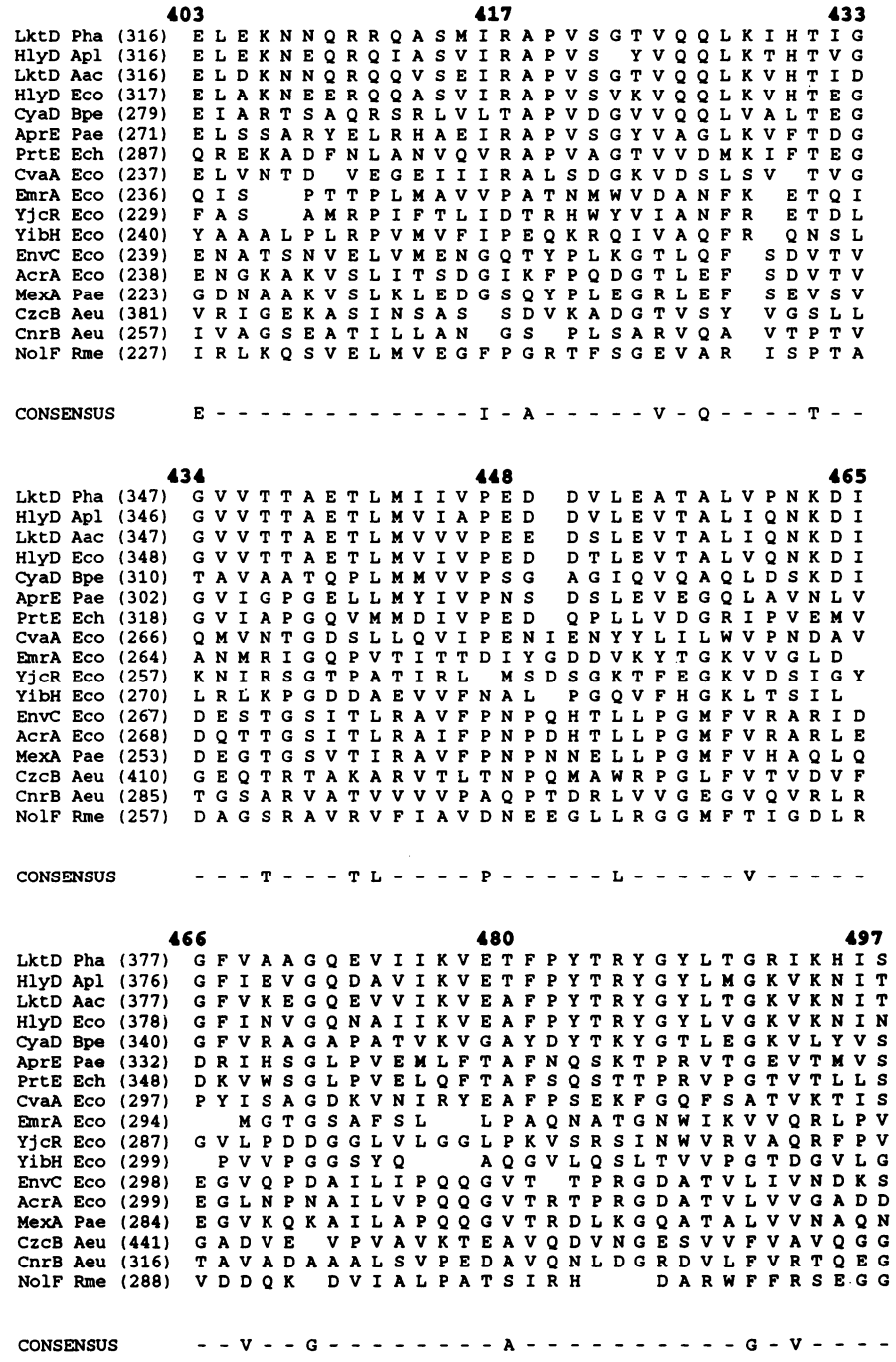

FIG. 2. Multiple alignment of the region of the 17 fully sequenced MFPs indicated by the bar in Fig. 1. Abbreviations are as indicated in Tables 1 and 2. The residue numbers in each protein are provided at the beginning of each line. Alignment positions in the complete multiple alignment of the seventeen proteins are provided above the alignment, while the consensus sequence (CONSENSUS; eight or more residues conserved) is provided below it. The complete multiple alignment of the MFPs generated with the PREALIGN and TREE programs (12) (data not shown) will be provided to any interested reader upon request to M.H.S., Jr.

reveal the consistent pattern of alternating hydrophobic and hydrophilic residues which is suggestive of amphipathic β-structure.

PHYLOGENETIC RELATIONSHIPS OF MFPs

The phylogenetic tree for the 17 fully sequenced MFPs is presented in Fig. 3. All of the MFPs that transport proteins in conjunction with ABC-type permeases (17) cluster together (also see Table 1). The toxin-transporting MFPs (two LktDs, two HlyDs, and CyaD) constitute one coherent subcluster, while the two protease-specific MFPs (PrtE and AprE) constitute another. Finally, the MFP concerned with colicin V transport (CvaA) forms a distinct subbranch of the protein-transporting cluster. The EmrA protein of *Escherichia coli*,

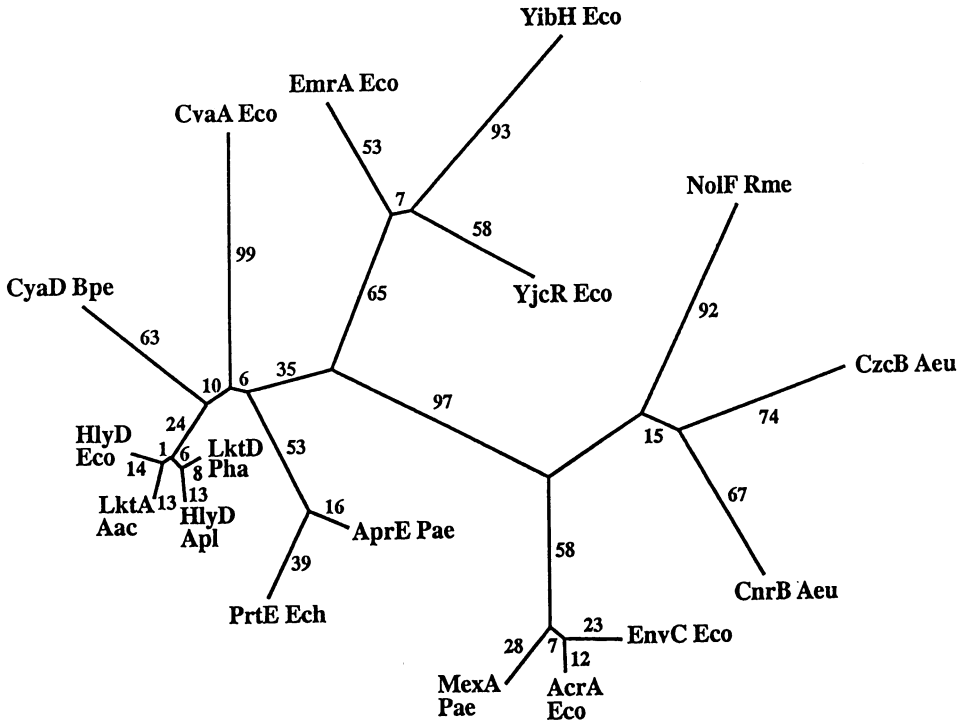

FIG. 3. Phylogenetic tree for members of the MFP family. Abbreviations are as specified in Tables 1 and 2. Relative evolutionary distance is proportional to branch length. Numerical values in arbitrary units are provided adjacent to the branches. Tree construction was performed as detailed by Feng and Doolittle (12).

which transports drugs in conjunction with an MFS-type permease (26), is off on a distant branch together with two *E. coli* proteins of unknown function, the sequence of one of which has recently been revealed as a result of *E. coli* genome sequencing (Table 1). The six remaining proteins transport drugs (and probably lipooligosaccharides) in conjunction with RND-type permeases (36), and they similarly cluster together. Phylogenetic grouping of the MFPs thus correlates with the types of cytoplasmic membrane transport systems with which they function as well as with the substrate specificities of the transport systems. The observation that phylogenetic grouping correlates with substrate specificity might suggest that these proteins interact with their substrates as do substrate-specific porins.

SEQUENCE SIMILARITY OF MFP FAMILY PROTEINS WITH THE MEMBRANE FUSION PROTEIN OF SIMIAN VIRUS 5

Every member of the MFP family was screened against the protein sequence databases for sequence similarity. In addition to the recognized members of the MFP family (Table 1), only one protein was found to clearly exhibit statistically significant sequence similarity. This protein is the membrane fusion protein (F protein) of the paramyxovirus simian virus 5 (30). The F protein mediates virus penetration, hemolysis, and cell fusion and is required for the intracellular spread of the virus. When analyzed by the Los Alamos program with 100 random shuffles (18) this protein, of 529 amino acyl residues, gave a

```
          260       270       280       290             300
EmrA Eco    ETQIANMRIGQPVTITTDIYGDDVKYTGKVVGLDMGT-----GSAFSLLPAQNA
            .:::..  .  .  .:..: : :..:. .:. ... :      ....  :.. .:
VglF Sv5    NTQISAAELLSSGLLTGQIVGLDLTYMQMVIKIELPTLTVQPATQIIDLATISA
          241       250       260       270       280       290.

          309       319       329       339       349       359
EmrA Eco    TGNWIKVVQRLPVRIELDQKQLEQYPLRIGLSTLVSVNTTNRDGQVLANKVRS
              :  .:...:.:. .. . ...:: .    :  .:    .:.:::.....
VglF Sv5    FINNQEVMAQLPTRVMVTGSLIQAYPASQCTITPNTVYCRYNDAQVLSDDTMA
          295       305       315       325       335       345
```

FIG. 4. Binary alignment of a portion of the EmrA protein from *E. coli* (EmrA Eco) and the membrane fusion protein of simian virus 5 (VglF Sv5). The alignment reveals 24% identity and 68% similarity in a segment of 107 amino acyl residue overlap. Residue numbers in the two proteins are provided. A double dot indicates an identity, while a single dot indicates a conservative substitution. The LFASTA program (30a) was used for optimization of the alignment and assignment of similarity.

comparison score with *E. coli* EmrA of 6 S.D. when their entire sequences were aligned. The probability that this comparison score could have arisen by chance is one in one billion (9). Figure 4 shows a portion of the binary alignment of these two proteins revealing 24% identity and 68% similarity in a 107-amino-acid overlap. Two secondary structural predictive programs (6, 13) for the simian virus fusion protein suggested that β-structure may predominate. Two strongly hydrophobic regions near the N and C termini of the F protein were observed, with the remainder of the protein exhibiting hydrophobic indices greater than those of most water-soluble proteins and comparable to those of the proposed outer membrane interaction domains of the bacterial membrane fusion proteins (unpublished observations; see Fig. 1A).

CONCLUSIONS

In this minireview, we have characterized a novel family of bacterial transport accessory proteins that we have termed the MFP family. All of these proteins probably function in conjunction with an export permease, localized to the inner membrane, to allow transport across both membranes of the gram-negative bacterial cell envelope rather than across a single membrane. The important observations are as follows. (i) These proteins form a loose but coherent family of proteins having similar sizes, sequences, and hydropathy profiles. (ii) These proteins are all probably embedded at their N termini in the cytoplasmic membrane which they span. (iii) These proteins exhibit a short (60-residue) region, adjacent to the transmembrane segment, of elevated sequence similarity and hydrophobicity which we propose serves as a region of interaction with cytoplasmic membrane constituents, presumably the permeases with which they function. (iv) These proteins then exhibit an extended α-helical region of high hydrophilicity but low sequence similarity that presumably allows them to cross the periplasmic space. (v) This region is followed by a largely β-structural region of about 100 residues of elevated sequence similarity and hydrophobicity which we propose serves as a region of interaction with outer membrane constituents. (vi) The short C-terminal regions of the MFPs are of various lengths, exhibit low sequence similarity and average hydrophobicities, and may possess little or no functional significance. (vii) These proteins exhibit phylogenies that correlate with their substrate specificities as well as with the types of transport system with which they interact.

The detailed molecular mechanism by which the MFPs allow export of solutes across the outer membrane has yet to be determined. Do they interact with the two membranes in a highly specific and ordered fashion to effectively allow fusion of the inner and outer membranes, or to form an oligomeric protein pore in the outer membrane as we and others have postulated, or do they function by a very different mechanism? Do these proteins function in conjunction with other outer membrane proteins, or can they create transmembranous pores by themselves? Do they share a common mechanism of action with viral membrane fusion proteins such as that of simian virus 5? The sequence and phylogenetic analyses reported here provide clues which should serve as guides for future study.

ACKNOWLEDGMENTS

We thank Kim Lewis for valuable discussions and Mary Beth Hiller for excellent assistance in the preparation of this manuscript.

This work was supported by USPHS grants 5RO1AI 21702 and 2RO1AI 14176 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. **Baev, N., G. Endre, G. Petrovics, Z. Banfalvi, and A. Kondorosi.** 1991. Six nodulation genes of *nod* box locus 4 in *Rhizobium meliloti* are involved in nodulation signal production: *nodM* codes for D-glucosamine synthetase. Mol. Gen. Genet. **228:**113–124.
2. **Blattner, F. R., V. Burland, G. Plunkett III, H. J. Sofia, and D. L. Daniels.** 1993. Analysis of the *Escherichia coli* genome. 4. DNA sequence of the region from 89.2 to 92.8 minutes. Nucleic Acids Res. **21:**5408–5417.
3. **Brandl, C. J., and C. M. Deber.** 1986. Hypothesis about the function of membrane-buried proline residues in transport proteins. Proc. Natl. Acad. Sci. USA **83:**917–921.
4. **Brandl, C. J., R. B. Deber, L. C. Hsu, G. A. Woolley, X. K. Young, and C. M. Deber.** 1988. Evidence for similar function of transmembrane segments in receptor and membrane-anchored proteins. Biopolymers **27:**1171–1182.
5. **Chang, Y.-F., R. Young, and D. K. Struck.** 1991. The *Actinobacillus pleuropneumoniae* hemolysin determinant: unlinked *appCA* and *appBD* loci flanked by pseudogenes. J. Bacteriol. **173:**5151–5158.
6. **Chou, P. Y., and G. D. Fasman.** 1978. Empirical prediction of protein conformation. Annu. Rev. Biochem. **47:**251–276.
7. **Deber, C. M., C. J. Brandl, R. B. Deber, L. C. Hsu, and X. K. Young.** 1986. Amino acid composition of the membrane and aqueous domains of integral membrane proteins. Arch. Biochem. Biophys. **251:**68–76.
8. **Deber, C. M., M. Glibowicka, and G. A. Woolley.** 1990. Conformations of proline residues in membrane environments. Biopolymers. **29:**149–157.
9. **Doolittle, R. F.** 1986. Of urfs and orfs. A primer on how to analyze derived amino acid sequences. University Science Books, Mill Valley, Calif.
10. **Duong, F., A. Lazdunski, B. Cami, and M. Murgier.** 1992. Sequence of a cluster of genes controlling synthesis and secretion of alkaline protease in *Pseudomonas aeruginosa*: relationships to other secretory pathways. Gene **121:**47–54.
11. **Fath, M. J., R. C. Skvirsky, and R. Kolter.** 1991. Functional complementation between bacterial multidrug resistance-like export systems: colicin V, alpha-hemolysin, and *Erwinia* protease. J. Bacteriol. **173:**7549–7556.
12. **Feng, D.-F., and R. F. Doolittle.** 1990. Progressive alignment and phylogenetic tree construction of protein sequences. Methods Enzymol. **183:**375–387.
13. **Garnier, J., D. J. Osguthorpe, and B. Robson.** 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. **120:**97–120.
14. **Gilson, L., H. K. Mahanty, and R. Kolter.** 1990. Genetic analysis of an MDR-like export system: the secretion of colicin V. EMBO J. **9:**3875–3884.
15. **Glaser, P., H. Sakamoto, J. Bellalou, A. Ullmann, and A. Danchin.** 1988. Secretion of cyclolysin, the calmodulin-sensitive adenylate cyclase-haemolysin bifunctional protein of *Bordetella pertussis*. EMBO J. **7:**3887–4004.
16. **Guthmiller, J. M., E. Kraig, M. P. Cagle, and D. Kolodrubetz.** 1990. Sequence of the *lktD* gene from *Actinobacillus actinomycetemcomitans*. Nucleic Acids Res. **18:**5292.
17. **Higgins, C. F.** 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. **8:**67–113.
18. **Kanehisa, M.** 1982. Los Alamos sequence analysis package for nucleic acids and proteins. Nucleic Acids Res. **10:**183–196.
19. **Klein, J. R., B. Henrich, and R. Plapp.** 1991. Molecular analysis and nucleotide sequence of the *envCD* operon of *Escherichia coli*. Mol. Gen. Genet. **230:**230–240.
20. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157:**105–132.
21. **Letoffe, S., P. Delepelaire, and C. Wandersman.** 1990. Protease secretion by *Erwinia chrysanthemi*: the specific secretion functions are analogous to those of *Escherichia coli* α-haemolysin. EMBO J. **9:**1375–1382.
22. **Lewis, K.** 1994. Multidrug resistance pumps in bacteria. Trends Biochem. Sci. **19:**119–123.
23. **Liesegang, H., K. Lemke, R. A. Siddiqui, and H.-G. Schlegel.** 1993. Characterization of the inducible nickel and cobalt resistance determinant *cnr* from pMOL28 of *Alcaligenes eutrophus* CH34. J.

Bacteriol. **175:**767–778.
24. **Lomovskaya, O., and K. Lewis.** 1992. Emr, an *Escherichia coli* locus for multidrug resistance. Proc. Natl. Acad. Sci. USA **89:** 8938–8942.
25. **Ma, D., D. N. Cook, M. Alberti, N. G. Pon, H. Nikaido, and J. E. Hearst.** 1993. Molecular cloning and characterization of *acrA* and *acrE* genes of *Escherichia coli*. J. Bacteriol. **175:**6299–6313.
26. **Marger, M. D., and M. H. Saier, Jr.** 1993. A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trends Biochem. Sci. **18:**13–20.
27. **Nies, D. H., A. Nies, L. Chu, and S. Silver.** 1989. Expression and nucleotide sequence of a plasmid-determined divalent cation efflux system from *Alcaligenes eutrophus*. Proc. Natl. Acad. Sci. USA **86:**7351–7355.
28. **Nikaido, H.** 1992. Porins and specific channels of bacterial outer membranes. Mol. Microbiol. **6:**435–442.
29. **Nikaido, H., and M. H. Saier, Jr.** 1992. Transport proteins in bacteria: common themes in their design. Science **258:**936–942.
29a. **Pan, W., and B. G. Spratt.** Unpublished sequence.
30. **Paterson, R. G., T. J. R. Harris, and R. A. Lamb.** 1984. Fusion protein of the paramyxovirus simian virus 5: nucleotide sequence of mRNA predicts a highly hydrophobic glycoprotein. Proc. Natl. Acad. Sci. USA **81:**6706–6710.
30a. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
31. **Poole, K., D. E. Heinrichs, and S. Neshat.** 1993. Cloning and sequence analysis of an EnvCD homologue in *Pseudomonas aeruginosa*: regulation by iron and possible involvement in the secretion of the siderophore pyoverdine. Mol. Microbiol. **10:**529–544.
32. **Poole, K., K. Krebes, C. McNally, and S. Neshat.** 1993. Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. J. Bacteriol. **175:**7363–7372.
33. **Reizer, J., A. Reizer, and M. H. Saier, Jr.** 1992. A new subfamily of bacterial ABC-type transport systems catalyzing export of drugs and carbohydrates. Protein Sci. **1:**1326–1332.
34. **Saier, M. H., Jr.** 1991. Mechanisms of carbohydrate transport in bacteria and comparisons with those in eukaryotes, p. 833–890. *In* P. L. Yeagle (ed.), The structure of biological membranes. CRC Press, Boca Raton, Fla.
35. **Saier, M. H., Jr.** 1994. Computer-aided analyses of transport protein sequences: gleaning evidence concerning function, structure, biogenesis, and evolution. Microbiol. Rev. **58:**71–93.
36. **Saier, M. H., Jr., R. Tam, A. Reizer, and J. Reizer.** 1994. Two novel families of bacterial membrane proteins concerned with nodulation, cell division and transport. Mol. Microbiol. **11:**841–847.
37. **Schulein, R., I. Gentschev, H. J. Mollenkopf, and W. Goebel.** 1992. A topological model for the haemolysin translocator protein HlyD. Mol. Gen. Genet. **234:**155–163.
38. **Seiffer, D., J. R. Klein, and R. Plapp.** 1993. EnvC, a new lipoprotein of the cytoplasmic membrane of *Escherichia coli*. FEMS Microbiol. Lett. **107:**175–178.
39. **Strathdee, C. A., and R. Y. C. Lo.** 1989. Cloning, nucleotide sequence, and characterization of genes encoding the secretion function of the *Pasteurella haemolytica* leukotoxin determinant. J. Bacteriol. **171:**916–928.
40. **Utsumi, R., T. Yagi, S. Katayama, K. Katsuragi, K. Tachibana, H. Toyoda, S. Ouchi, K. Obata, Y. Shibano, and M. Noda.** 1991. Molecular cloning and characterization of the fusaric acid-resistance gene from *Pseudomonas cepacia*. Agric. Biol. Chem. **55:** 1913–1918.
41. **Wang, R. C., S. J. Seror, M. Blight, J. M. Pratt, J. K. Broome-Smith, and I. B. Holland.** 1991. Analysis of the membrane organization of an *Escherichia coli* protein translocator, HlyB, a member of a large family of prokaryote and eukaryote surface transport proteins. J. Mol. Biol. **217:**441–454.
42. **Xu, J., M. L. Nilles, and K. P. Bertrand.** 1993. The *Escherichia coli acrAB* and *acrEF* genes: a new family of multidrug efflux pumps, K-169, p. 290. Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993. American Society for Microbiology, Washington, D.C.
43. **Zhao, S., C. H. Sandt, G. Feulner, D. A. Vlazny, J. A. Gray, and C. W. Hill.** 1993. Rhs elements of Escherichia coli K-12: complex composites of shared and unique components that have different evolutionary histories. J. Bacteriol. **175:**2799–2808.