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Expression of the *Pseudomonas aeruginosa* PAK Pilin Gene in *Escherichia coli*

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***Pseudomonas aeruginosa* is a piliated opportunistic pathogen. We have recently reported the cloning of the structural gene for the pilus protein, pilin, from *P. aeruginosa* PAK (B. L. Pasloske, B. B. Finlay, and W. Paranchych, FEBS Lett. 183:408–412, 1985), and in this paper we present evidence that this chimera (pBP001) expresses *P. aeruginosa* PAK pilin in *Escherichia coli* independent of a vector promoter. The strength of the promoter for the PAK pilin gene was assayed, and the cellular location of the pilin protein within *E. coli* was examined. This protein was present mainly in the inner membrane fraction both with and without its six-amino-acid leader sequence, but it was not assembled into pili.**

Pseudomonas aeruginosa is an opportunistic pathogen that invades hosts weakened by cystic fibrosis, leukemia, and severe burns. Woods et al. (32, 33) have demonstrated that adherence to mucosal and epithelial surfaces is mediated by pili. These pili are chromosomally encoded polar filaments approximately 5.2 nm in diameter and 2.5 μm in length (4, 6, 13). They are retractable structures which promote infection by various pilus-specific bacteriophages (7) and a primitive form of movement called twitching motility (5).

Several other pathogenic gram-negative organisms also have chromosomally encoded adherence pili. These include *Neisseria gonorrhoeae* (20, 21), *Bacteroides nodosus* (11), *Moraxella nonliquefaciens* (14), *Moraxella bovis* (18), and *P. aeruginosa* PAK (23, 27). Pilin from all of these organisms has a highly conserved hydrophobic amino terminus (residues 1 to 22) and a short (6- to 7-amino-acid) positively charged leader sequence. The remaining residues in these pilus proteins appear to be unrelated. Pilin from *N. gonorrhoeae* (21), *M. bovis* (18), and *P. aeruginosa* (32) is known to vary antigenically. It has been demonstrated that several pilin genes are present within one *N. gonorrhoeae* strain, and at least one is a silent copy (20). In contrast, *P. aeruginosa* probably contains only one pilin gene copy (23), which varies between strains. Since these pathogenic organisms all share leader peptide and amino terminus homology, it is important to examine the expression and transport mechanisms of *P. aeruginosa* PAK pilin, because these diverse pilus proteins may share similar transport pathways.

With this goal in mind, we present evidence of expression of the *P. aeruginosa* PAK pilin gene in *Escherichia coli*. Experiments presented here include an assay of pilin promoter strength and studies on the compartmentalization and processing (or lack thereof) of the pilin gene product.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used were wild-type *P. aeruginosa* PAK (a gift from D. E. Bradley, Memorial University of Newfoundland, Saint John's, Newfoundland), *E. coli* JM83 (30), *E. coli* P67854 (1), *E. coli* ED5363 (19), *E. coli* HB101 (3), and *E. coli* JE2571 (Fla$^-$). The plasmids used were pUC8 (30) and pBP001 (23), a chimera of a 1.2-kilobase *Hin*dIII fragment containing the *P. aeruginosa* PAK pilin gene in pUC8. pBP010 is the *Hin*dIII-*Sau*3A 482-base-pair fragment from pBP001 upstream of the PAK pilin gene.

Isolation and labeling of minicells. A culture of strain P67854 (1) containing the desired plasmid was grown to stationary phase, and minicells were purified essentially as described by Roozen et al. (25), except that whole cells were removed by pelleting for 4 min at 1,500 × *g* before being loaded onto sucrose gradients. [^{35}S]methionine (50 μCi) was used to label minicells.

SDS-PAGE. Sodium dodecyl-sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE) was done with 15% SDS-polyacrylamide gels which were prepared as described by Laemmli (16). The gels were soaked in water (1 h) and then in 1 M sodium salicylate (1 h) before vacuum drying and autoradiography were performed.

Minicell fractionation. Labeled minicells were washed in 20% sucrose–30 mM Tris hydrochloride (pH 8) and suspended in 1 ml of 50 mM Tris hydrochloride (pH 7.5)–100 μg of gelatin per ml. Next, 40 μl of 0.25 M EDTA–5 mg of lysozyme per ml was added and incubated at 37°C for 1 h. Spheroplasts were removed by centrifuging the mixture for 3 min in an Eppendorf centrifuge. The supernatant solution (periplasmic fraction) was precipitated with 10% trichloroacetic acid, while the pellet (spheroplasts) was suspended in 50 mM potassium phosphate buffer (pH 6.6) containing 10 μg each of DNase and RNase per ml and sonicated with five 1-min pulses at 0°C. Unsonicated cells were removed after the initial 3-min centrifugation, and the membranes were pelleted (165,000 × *g* at 0°C for 1 h). The supernatant solution (cytoplasmic fraction) was precipitated in 10% trichloroacetic acid. The pellet (membrane fraction) was suspended in cold potassium phosphate buffer. All fractions were then counted in a Beckman LS6800 scintillation spectrometer and subjected to SDS-PAGE and autoradiography.

Whole-cell fractionation. The inner and outer membrane fractions of *E. coli* HB101(pBP001) were separated based on the procedure of Yamato et al. (34), except that the dialysis step was omitted and the inner membrane fraction was purified twice by layering on 44% (wt/wt) sucrose–3 mM EDTA (pH 7.2) and centrifuging at 176,000 × *g* for 60 min. After each centrifugation through the sucrose, the inner membrane fraction was diluted threefold in 3 mM EDTA (pH 7.2), pelleted, and suspended in 10% (wt/vol) sucrose–3 mM

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EDTA (pH 7.2). Periplasmic proteins were purified by the cold-shock procedure described by Dougan et al. (10).

To obtain the cytoplasmic proteins, we prepared spheroplasts from 500 ml of cells by the cold-shock technique (10) and then suspended them in 8 ml of cold water. The cells were passed through a French pressure cell three times at 14,000 lb/in^2. The unlysed cells were removed by centrifugation at 8,000 × *g* for 15 min. The supernatant was decanted into a Beckman SW41 centrifuge tube, topped with cold distilled water, and centrifuged for 3 h at 39,000 rpm to remove the crude membrane fraction. The supernatant was dried in a rotoevaporator and then subjected to SDS-PAGE.

Preadsorption of antiserum. A 40-ml culture of *E. coli* HB101 was grown to late exponential phase and suspended in 25 ml of the buffer used in the immunoblot procedure. To inhibit protease activity, 62 μl of 40 mM phenylmethylsulfonyl fluoride in 95% ethanol was added to the cell suspension. The cells were sonicated with five 1.5-min pulses of the Braunsonic 1510 sonicator at 4°C with a small probe. After lysis, 80 μl of the anti-PAK pilin antiserum was added, and the mixture was incubated at 4°C for 24 h with gentle rotation and then used in the immunoblot procedure.

Galactokinase K assay. The galactokinase K assay was done exactly as described previously (19), except that the specific activity of undiluted [^{14}C]galactose was 59.6 mCi/mmol. Units are expressed as nanomoles of galactose phosphorylated per minute at an optical density of 650 nm.

RESULTS

Expression of PAK pilin in *E. coli* minicells. A chimera containing the *P. aeruginosa* PAK pilin gene in vector pUC8, called pBP001 (23), was transformed into *E. coli* minicell strain P67854. ^{35}S-labeled minicells were harvested and subjected to SDS-PAGE followed by autoradiography. The results of such an experiment with pBP001 and pUC8 are shown in Fig. 1A. pBP001 makes only one novel protein, unlike pUC8. This heavily expressed protein has a molecular mass of 15,600 daltons and migrates slightly more slowly than *P. aeruginosa* PAK pilin (15,000 daltons). We hypothesized that this protein was unprocessed PAK pilin with a six-amino-acid (660-dalton) leader sequence (23). (The addition of 2-phenylethyl alcohol, a known inhibitor of signal peptide cleavage, had no effect on the mobility of this gene product [data not shown].) To test our hypothesis, unlabeled minicells were harvested, electrophoresed, and subjected to immunoblotting with unpreadsorbed anti-PAK pili polyclonal rabbit antiserum (Fig. 1B). The 15,600-dalton protein and PAK pilin were both recognized by anti-pilus antiserum, suggesting that this protein is probably unprocessed PAK pilin. Several fainter bands were also visible with both pBP001 and pUC8, suggesting that antibodies to several *E. coli* antigens were present in the antiserum.

Cellular location of PAK pilin in *E. coli* minicells. Besides being present in assembled pili, *P. aeruginosa* pilin is present in both the inner and outer membranes (31). To determine the cellular location within *E. coli* minicells, labeled minicells containing pBP001 were fractionated as described above, and 30,000-cpm fractions were separated by SDS-PAGE and autoradiographed (Fig. 2). Unprocessed PAK pilin was distributed in the cytoplasm and the membrane fraction. Only a small amount of unprocessed pilin was visible in the periplasmic fraction, but comparison of the amounts of pilin found in each fraction was not possible, as an equal amount of radioactivity, not equivalent amounts of each cellular fraction was loaded for each fraction. There was no evidence of processed pilin in any fraction, suggesting that transport of pilin within minicells to the membrane of *E. coli* is independent of leader sequence removal. Moreover, attempts to detect pilin in culture media after the labeling of cells with [^{35}S]methionine, by trichloroacetic acid precipitation of the culture medium and SDS-PAGE examination of the acid precipitate, showed that pilin is not secreted extracellularly (data not shown).

Cellular location of PAK pilin in *E. coli*. To determine the cellular location and distribution of PAK pilin in whole cells, *E. coli* HB101 (pBP001) was fractionated as described above. Approximately equivalent amounts of each cellular fraction were separated on a 15% polyacrylamide gel, and PAK pilin was detected by an immunoblot procedure with anti-PAK pilin antiserum preadsorbed against HB101 (Fig. 3). This analysis revealed that most of the pilin antigen was found in the inner membrane fraction (lane 5). *M. bovis* pilin, which has a similar leader sequence and amino terminus, was also found to be associated with the inner membrane fraction of HB101 (18).

The inner membrane fraction of HB101(pBP001) also contained a fainter band which migrated slightly above the pilin band. We suggest that this band was propilin, as it had

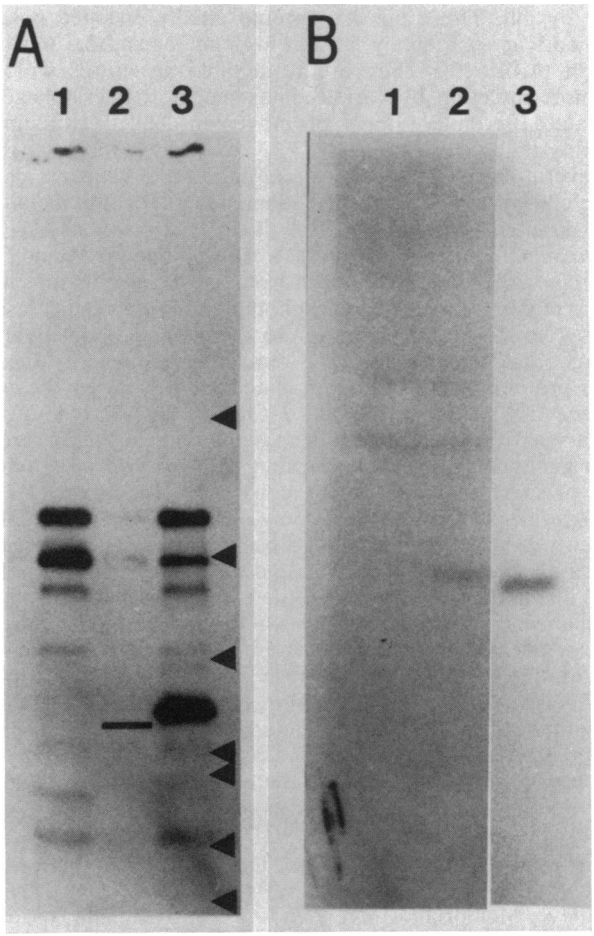

FIG. 1. (A) Autoradiograph of a 15% SDS-polyacrylamide gel containing ^{35}S-labeled minicells of pUC8 (lane 1) and pBP001 (lane 3). The bar (lane 2) represents the mobility of purified unlabeled *P. aeruginosa* PAK pilin. The arrows represent the mobility of size standards of (from the top) 43, 25.7, 18.4, 14.3, 12.3, 6.2, and 3 kilodaltons. (B) Immunoblot of unlabeled minicells from pUC8 (lane 1) and pBP001 (lane 2). Lane 3 contains 2 μg of purified PAK pilin.

the predicted molecular mass and reacted with anti-pilus antiserum. The presence of propilin in the membrane fraction from minicells and in the inner membrane fraction from HB101 suggests that proteolytic removal of the six-residue leader peptide is not required for transport of pilin to the inner membrane.

Although pBP001 encodes only one complete polypeptide, PAK pilin (23), tests were performed to determine whether an intact pilus was present and functional. Bacteriophage spot tests with the pilus-specific bacteriophage PO4 were negative. When pBP001 was transformed into bald *E. coli* JE2571 (JM83 expresses type 1 pili) and examined in the electron microscope, no pili were visible (data not shown).

Collectively, these data demonstrate that the chimera containing the *P. aeruginosa* PAK pilin gene expresses unprocessed and processed pilin which is located predominantly in the inner membrane of *E. coli* and not assembled into pili.

Promoter studies. Sequencing data of pBP001 demonstrated that the pilin gene was located in the opposite orientation of the pUC8 *lac* promoter (unpublished data). This suggested that pBP001 contained its own promoter which was being utilized in *E. coli*. To test for the presence of a promoter upstream of the pilin gene, the 484-nucleotide *Hin*dIII-*Sau*3A fragment was inserted into promoter assessment vector pKO4 (19). This fragment contains the entire region upstream of the pilin gene, as well as DNA encoding the first nine amino acids of unprocessed pilin. pKO4 contains the structural gene for galactokinase K (*galK*) but is constructed such that there is no promoter upstream from the *galK* gene. Unique *Hin*dIII and *Bam*HI sites upstream of the *galK* gene allow insertion of DNA fragments and measurement of promoter strength therein. Chimeras containing the 484-base-pair fragment within pKO4 demonstrated *galK* activity on MacConkey agar-galactose plates. The results of a *galK* assay (19) of this chimera indicate that, compared with the background of pKO4 (1.63 U [nmol of galactose phosphorylated per min at A_{650}]), the pBP010 fragment with the PAK pilin promoter contained about 1.5 times as much promoter activity (2.79 U). Both of these activities were very low as compared with that of the *lac* promoter assayed in pKL200 (56.8 U); nonetheless, they suggest that a weak promoter does exist within the first 484 nucleotides of pBP001. It is of interest that the pilin gene is expressed efficiently despite the weak promoter activity. This suggests that the pilin, the mRNA, or both may be exceptionally stable within the *E. coli* cells.

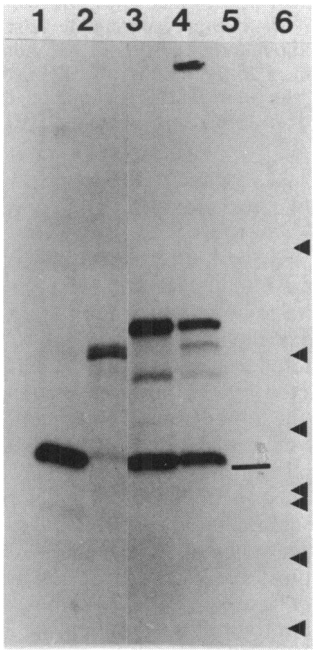

FIG. 2. Autoradiograph of a 15% SDS-polyacrylamide gel containing cell fractions from ^{35}S-labeled minicells containing pBP001. For each fraction, 30,000 cpm was loaded. Lanes: 1, cytoplasm; 2, periplasm; 3, total membrane; 4, unfractionated minicells. The bar (lane 5) represents the mobility of PAK pilin. The arrows (lane 6, from the top) represent the mobility of the size standards described in Fig. 1.

DISCUSSION

Several *P. aeruginosa* genes are expressed in *E. coli*. Phospholipase C (hemolysin), normally a secreted protein in *P. aeruginosa*, was reported to be in the cytoplasm (9, 29) and the outer membrane (17) when expressed in *E. coli*. A vector promoter was required for production of this protein within *E. coli* (29). Exotoxin A, another secreted protein of *P. aeruginosa*, was also expressed in *E. coli* (15). This protein was unprocessed, was found mainly in the cytoplasm, and required a vector promoter for expression in *E. coli*. Recently, phosphomannose isomerase, a cytoplasmic protein from *P. aeruginosa*, was reported to be expressed in *E. coli* when placed under vector promoter control (A. Darzins, R. I. Vanags, and A. M. Chakrabarty, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, D94, p. 70). Other examples of *Pseudomonas* species with promoters that are not recognized in *E. coli* include *P. fluorescens* (8) and *P. putida* (22). Thus, it seems that *Pseudomonas* promoters are not readily utilized in *E. coli*. We have reported here that a putative promoter for the *P. aeruginosa* PAK pilin gene appears to be recognized in *E. coli*. Examination of the first 450 bases of pBP001 revealed several sequences that partially resemble the consensus sequence of the *E. coli* promoter (26); however, we were unable to determine which of these may be serving as the promoter for the pilin gene.

The *P. aeruginosa* phospholipase C and exotoxin A gene products are unable to undergo signal peptide processing or secretion into the medium when expressed in *E. coli* (9, 17, 29). Normal processing of PAK pilin signal peptides involves the cleavage of a six-residue leader peptide and transport to the inner and outer membranes (23, 31). We have shown that this leader is not always removed in *E. coli* but that both pilin and propilin are transported to the inner membrane.

The observed interactions of PAK pilin with *E. coli* membranes may be considered in terms of the "helical hairpin hypothesis" proposed by Engelman and Steitz (12). These workers suggested that during synthesis of a membrane protein approximately 40 to 50 amino acid residues of the nascent polypeptide chain fold in an aqueous environment to form an antiparallel pair of helices consisting of a hydrophobic leader peptide containing 15 to 20 amino acid residues and a similar length of the polypeptide immediately distal to the hydrophobic leader region. The helical hairpin thus formed inserts spontaneously into the hydrophobic region of the bilayer principally on the basis of hydrophobic interactions. The amino-terminal helix formed by the leader sequence is oriented with its amino terminus at the cytoplasmic side. The second helix is connected to the first through a short peptide loop on the periplasmic side.

Secondary structure calculations (data not shown) indi-

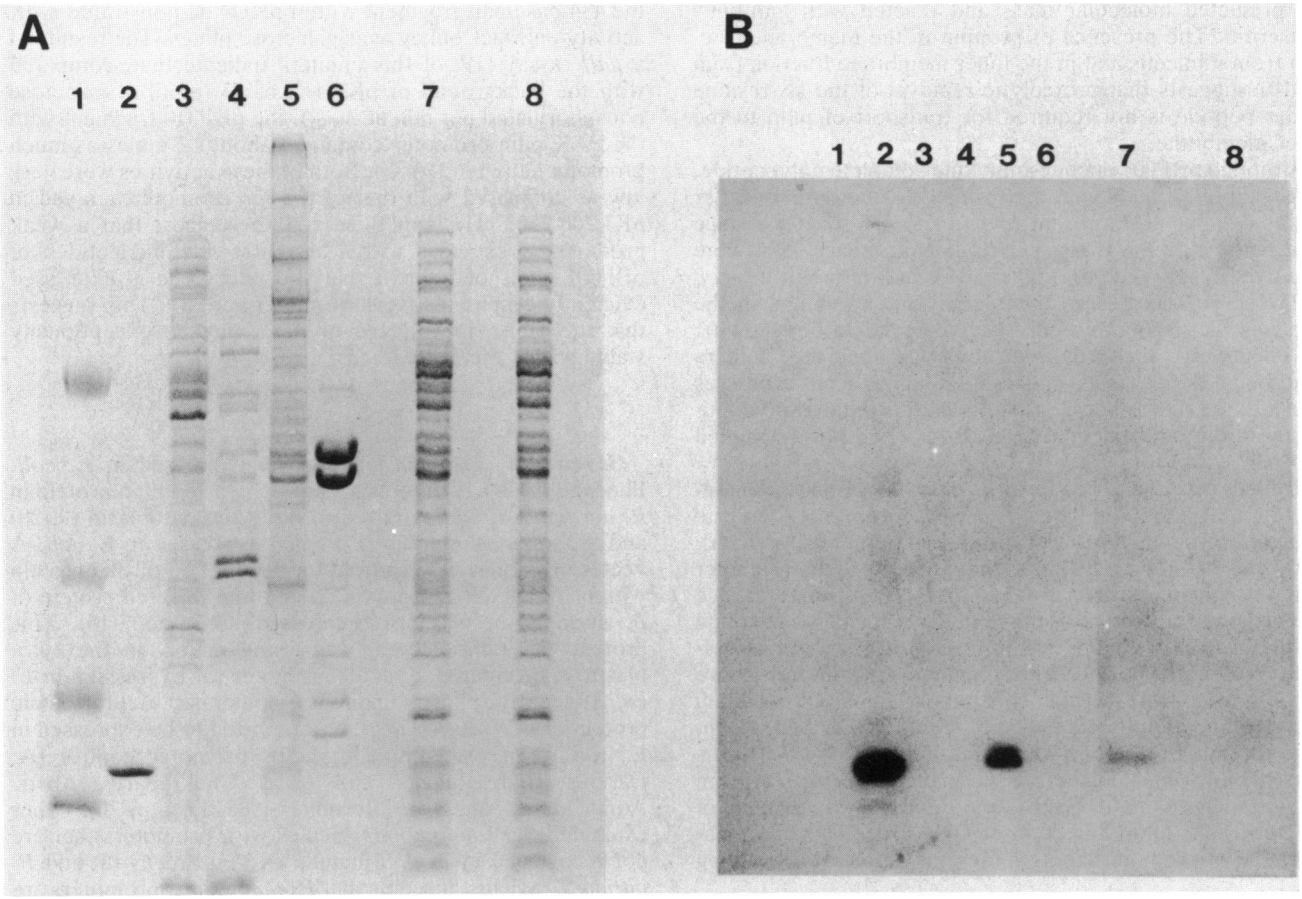

FIG. 3. Subcellular localization of *P. aeruginosa* propilin and pilin proteins in *E. coli* HB101 transformed with pBP001. Approximately equivalent amounts (on a weight basis) of the various cell fractions were electrophoresed on 15% SDS-polyacrylamide gels. (A) Coomassie blue-stained gel. Lanes: 1, molecular size standards of (from the top) 43, 25.7, 18.4, and 14.3 kilodaltons; 2, 4 μg of PAK pilin; 3, cytoplasm; 4, periplasm; 5, inner membrane; 6, outer membrane; 7, unfractionated cells. Lane 7 contains 1/10 the number of cells used to obtain the fractions shown in lanes 3 to 6. Lane 8 contains unfractionated HB101 cells. (B) Immunoblot of the gel in panel A, containing identical samples as used for panel A except that 0.2 μg of PAK pilin was used. The anti-PAK antiserum used was preadsorbed against HB101.

cate that both the N-terminal hydrophobic region (residues 1 to 28) and the domain encompassing residues 34 to 54 have high alpha helix potentials. The latter region appeared to be relatively polar, since it contained one Lys, one Arg, two Glu, two Thr, and three Ser residues. However, based on free-energy calculations, Engelman and Steitz (12) have suggested that the energy requirement of burying serines and threonines in a nonaqueous environment is not significant. Moreover, when acidic and basic amino acids occur three to four residues apart, a salt link can form between them which would greatly lower the energy barrier to entry of these charged residues into a nonaqueous environment. Such salt bridges could form between Lys-44 and Glu-48 and between Glu-49 and Arg-53. Therefore, it is possible that the region encompassing residues 34 to 54 could form the second required alpha helix and that this helix would have a favorable free energy for partitioning into the lipid bilayer.

Based on the foregoing considerations, the amino-terminal region of PAK pilin (residues 1 to 54) is an excellent candidate for a helical hairpin. The positively charged six-residue leader, which is predicted to be located on the cytoplasmic side of the inner membrane, would presumably be cleaved in *P. aeruginosa* cells before pilus assembly. However, the present studies with *E. coli* suggest that leader cleavage is not obligatory for pilin transport to the inner membrane.

pBP001 contains two palindromic sequences distal to the pilin gene. The first is capable of forming a 12-nucleotide inverted repeat with a 4-nucleotide loop starting 143 nucleotides downstream of the stop codon of pilin. This structure would have a predicted free energy of −30.4 kilocalories (ca. −127 kJ) as predicted by the program of Queen and Korn (24). The end of the downstream stem consists of seven thymines, five of which may be involved in stem formation. The other potential hairpin region starts 152 nucleotides downstream of the stop codon and has a predicted free energy of −24 kilocalories (ca. −100 kJ).

mRNA stability is believed to be enhanced by repetitive extragenic palindromic sequences in *E. coli* and *Salmonella typhimurium* (28). These stable palindromes are thought to form hairpin loops in the mRNA, preventing RNA degradation (28). The palindromic regions from *P. aeruginosa* could prevent mRNA degradation within *E. coli* in a manner similar to the repetitive extragenic palindromic sequence mechanism. This would explain the apparent high level of unprocessed pilin being produced and is analogous to the expression of the *B. nodosus* pilin gene in *E. coli* (2), which contains a 10-nucleotide inverted repeat followed by three

thymines distal to its pilin gene. However, preliminary data suggest that these inverted repeats are not required for high levels of pilin expression within *E. coli* (unpublished data). Stem and loop structures followed by several thymines are also characteristic of [*rho*]-independent terminators (26). Therefore, it is possible that the region starting 142 residues downstream of the pilin stop codon also functions as a transcription termination signal.

Pilin genes from the virulent organisms *B. nodosus* (2), *N. gonorrhoeae* (21), *M. bovis* (18), and *P. aeruginosa* PAK (23) have all been cloned and shown to be expressed in *E. coli*. These pilin genes all share a very similar amino acid sequence within the first 22 amino acids of processed pilin. In addition, they all have a similar, short (6- to 7-residue), positively charged leader sequence. Cloned pilin genes from *B. nodosus*, *N. gonorrhoeae*, and *P. aeruginosa* were all expressed efficiently in *E. coli* independent of a vector promoter. In addition, *B. nodosus* pilin was found in the membrane fraction of *E. coli* (2). Since the leader sequences and hydrophobic regions are conserved in these diverse gram-negative organisms, they may follow similar transport pathways.

We have presented here a unique system in which a membrane protein from *P. aeruginosa* was shown to be expressed within *E. coli* without a vector promoter. Its promoter strength was assayed, and its cellular location was examined. This information should facilitate studies of gram-negative protein transport and expression of virulence factors within *E. coli*.

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LITERATURE CITED

1. **Adler, H. I., W. D. Fisher, A. Cohen, and A. A. Hardigree.** 1967. Miniature *Escherichia coli* cells deficient in DNA. Proc. Natl. Acad. Sci. USA **57**:321–326.
2. **Anderson, B. J., M. M. Bills, J. R. Egerton, and J. S. Mattick.** 1984. Cloning and expression in *Escherichia coli* of the gene encoding the structural subunit of *Bacteroides nodosus* fimbriae. J. Bacteriol. **160**:748–754.
3. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
4. **Bradley, D.** 1972. A study on *Pseudomonas aeruginosa*. Genet. Res. **19**:39–51.
5. **Bradley, D. E.** 1980. A function of *Pseudomonas aeruginosa* PAO polar pili: twitching motility. Can. J. Microbiol. **26**: 146–154.
6. **Bradley, D. E.** 1980. Mobilization of chromosomal determinants for the polar pili of *Pseudomonas aeruginosa* PAO by FP plasmids. Can. J. Microbiol. **26**:155–160.
7. **Bradley, D. E., and T. L. Pitt.** 1974. Pilus dependence of four *Pseudomonas aeruginosa* bacteriophages with non-contractile tails. J. Gen. Virol. **24**:1–15.
8. **Buckel, P., and E. Zehelein.** 1981. Expression of *Pseudomonas fluorescens* D-galactose dehydrogenase in *Escherichia coli*. Gene **16**:149–159.
9. **Coleman, K., G. Dougan, and J. P. Arbuthnott.** 1983. Cloning, and expression in *Escherichia coli* K-12, of the chromosomal hemolysin (phospholipase C) determinant of *Pseudomonas aeruginosa*. J. Bacteriol. **153**:909–915.
10. **Dougan, G., G. Dowd, and M. Kehoe.** 1983. Organization of K88ac-encoded polypeptides in the *Escherichia coli* cell envelope: use of minicells and outer membrane protein mutants for studying assembly of pili. J. Bacteriol. **153**:364–370.
11. **Elleman, T. C., and P. A. Hoyne.** 1984. Nucleotide sequence of the gene encoding pilin of *Bacteriodes nodosus*, the causal organism of ovine footrot. J. Bacteriol. **160**:1184–1187.
12. **Engelman, D. M., and T. A. Steitz.** 1981. The spontaneous insertion of proteins into and across membranes: the helical hairpin hypothesis. Cell **23**:411–422.
13. **Folkhard, W., D. A. Marvin, T. H. Watts, and W. Paranchych.** 1981. Structure of polar pili from *Pseudomonas aeruginosa* strains K and O. J. Mol. Biol. **149**:79–93.
14. **Froholm, L. O., and K. Sletten.** 1977. Purification and N-terminal sequence of a fimbrial protein from *Moraxella nonliquefaciens*. FEBS Lett. **73**:29–32.
15. **Gray, G. L., D. H. Smith, J. S. Baldridge, R. N. Harkins, M. L. Vasil, E. Y. Chen, and H. L. Heyneker.** Cloning, nucleotide sequence, and expression in *Escherichia coli* of the exotoxin A structural gene of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. USA **81**:2645–2649.
16. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
17. **Lory, S., and P. C. Tai.** 1983. Characterization of the phospholipase C gene of *Pseudomonas aeruginosa* cloned in *Escherichia coli*. Gene **22**:95–101.
18. **Marrs, C. F., G. Schoolnik, J. M. Koomey, J. Hardy, J. Rothbard, and S. Falkow.** 1985. Cloning and sequencing of a *Moraxella bovis* pilin gene. J. Bacteriol. **163**:132–139.
19. **McKenney, K., H. Shimatake, D. Court, U. Schmeissner, and M. Rosenberg.** 1981. A system to study promoter and terminator signals recognized by *E. coli* RNA polymerase, p. 383–415. *In* J. Chirikjian and T. Papas (ed.), Gene amplification and analysis, vol. 2. Elsevier/North-Holland Publishing Co., New York.
20. **Meyer, T. F., E. Billyard, R. Haas, S. Storzbach, and M. So.** 1984. Pilus genes of *Neisseria gonorrheae*: chromosomal organization and DNA sequence. Proc. Natl. Acad. Sci. USA **81**:6110–6114.
21. **Meyer, T. F., N. Mlawer, and M. So.** 1982. Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. Cell **30**:45–52.
22. **Minton, N. P., T. Atkinson, and R. F. Sherwood.** 1983. Molecular cloning of the *Pseudomonas* carboxypeptidase G_2 gene and its expression in *Escherichia coli* and *Pseudomonas putida*. J. Bacteriol. **156**:1222–1227.
23. **Pasloske, B. P., B. B. Finlay, and W. Paranchych.** 1985. Cloning and sequencing of the *Pseudomonas aeruginosa* PAK pilin gene. FEBS Lett. **183**:408–412.
24. **Queen, C., and L. J. Korn.** 1984. A comprehensive sequence analysis program for the IBM personal computer. Nucleic Acids Res. **12**:581–599.
25. **Roozen, K. J., R. G. Fenwick Jr., and R. Curtiss III.** 1971. Synthesis of ribonucleic acid and protein in plasmid-containing minicells of *Escherichia coli* K-12. J. Bacteriol. **107**:21–33.
26. **Rosenburgh, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13**:319–353.
27. **Sastry, P. A., J. R. Pearlstone, L. B. Smillie, and W. Paranchych.** 1985. Studies on the primary structure and antigenic determinants of pilin isolated from *Pseudomonas aeruginosa* K. Can. J. Biochem. Cell Biol. **63**:284–291.
28. **Stern, M. J., G. F. Ames, N. H. Smith, E. C. Robinson, and C. Higgins.** 1984. Repetitive extragenic palindromic sequences: a major component of the bacterial genome. Cell **37**:1015–1026.
29. **Vasil, M. L., R. M. Berka, G. L. Gray, and H. Nakai.** 1982. Cloning of a phosphate-regulated hemolysin gene (phospholipase C) from *Pseudomonas aeruginosa*. J. Bacteriol. **152**: 431–440.
30. **Viera, J., and J. Messing.** 1982. The pUC plasmids, an M13 mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
31. **Watts, T. H., E. A. Worobec, and W. Paranchych.** 1982. Identification of pilin pools in the membranes of *Pseudomonas aeruginosa*. J. Bacteriol. **152**:687–691.
32. **Woods, D. E., B. H. Iglewski, and W. G. Johanson, Jr.** 1982.

Variable surface factors in bacterial adhesion: host and bacterial factors in the colonization of the respiratory tract, p. 348–352. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.

33. **Woods, D. E., D. C. Straus, W. G. Johanson, Jr., V. K. Berry, and J. A. Bass.** 1980. Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. Infect. Immun. **29**:1146–1151.
34. **Yamato, I., Y. Anraku, and K. Hirosawa.** 1975. Cytoplasmic membrane vesicles of *Escherichia coli*. I. A simple method for preparing the cytoplasmic and outer membranes. J. Biochem. **77**:705–718.