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Identification and Characterization of the *Treponema pallidum tpn50* Gene, an *ompA* Homolog†

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***Treponema pallidum* is a pathogenic spirochete that has no known genetic exchange mechanisms. In order to identify treponemal genes encoding surface and secreted proteins, we carried out Tn*phoA* mutagenesis of a *T. pallidum* genomic DNA library in *Escherichia coli*. Several of the resulting clones expressed enzymatically active *T. pallidum*-alkaline phosphatase fusion proteins. The DNA sequence of the 5′ portion of a number of the treponemal genes was obtained and analyzed. A recombinant clone harboring plasmid p4A2 that encoded a treponemal protein with an approximate molecular mass of 50,000 Da was identified. Plasmid p4A2 contained an open reading frame of 1,251 nucleotides that resulted in a predicted protein of 417 amino acids with a calculated molecular mass of 47,582 Da. We have named this gene *tpn50* in accordance with the current nomenclature for *T. pallidum* genes. A 1.9-kb *Hin*cII-*Cla*I fragment from p4A2 that contained the *tpn50* gene was subcloned to produce p4A2HC2. Comparison of the predicted amino acid sequence of TpN50 with protein sequences in the National Center for Biotechnology Information data base indicated statistically significant homology to the *Pseudomonas* sp. OprF, *E. coli* OmpA, *Bordetella avium* OmpA, *Neisseria meningitidis* RmpM, *Neisseria gonorrhoeae* PIII, *Haemophilus influenzae* P6, *E. coli* PAL, and *Legionella pneumophila* PAL proteins. These proteins are all members of a family of outer membrane proteins that are present in gram-negative bacteria. The *tpn50* gene complemented *E. coli ompA* mutations on the basis of two separate criteria. First, morphometry and electron microscopy data showed that *E. coli* C386 (*ompA lpp*) cells harboring plasmid vector pEBH21 were rounded while cells of the same strain harboring p4A2HC2 (TpN50$^+$), pWW2200 (OprF$^+$), or pRD87 (OmpA$^+$) were rod shaped. Second, *E. coli* BRE51 (MC4100 Δ*sulA-ompA*) cells harboring pEBH21 grew poorly at 42°C in minimal medium, while the growth of BRE51 cells harboring p4A2HC2 was similar to that of the parental MC4100 cells. These results demonstrate that the TpN50 protein is functionally equivalent to the *E. coli* OmpA protein. If TpN50 functions in a similar fashion in *T. pallidum*, then it may be localized to the treponemal outer membrane.**

The identification and localization of membrane proteins of *Treponema pallidum*, the causative agent of syphilis, are major focuses of treponemal research. Although *T. pallidum* possesses a cellular architecture similar to that of the gram-negative bacteria (37, 56, 58), the outer membrane of this organism is unique (60, 75, 76). The intramembranous protein particle content of the *T. pallidum* outer membrane is approximately 100-fold less than that of *Escherichia coli* (60, 76). It has been proposed that the paucity of surface proteins contributes to the resistance of *T. pallidum* to clearing by the immune system (13, 60). Recent studies have revealed the presence of treponemal rare outer membrane protein (TROMP) molecules that are surface exposed and antigenic (60, 76). Studies involving the kinetics of complement activation and outer membrane particle aggregation have indicated that TROMPs may be a target of treponemicidal antibodies (6). Since outer membrane proteins are often important virulence factors, the identification and further characterization of TROMPs should increase our understanding of the pathogenesis of syphilis (55).

Cellular localization of native treponemal proteins has been difficult because of the inability to continuously cultivate *T. pallidum* in vitro and the fragility of the treponemal outer membrane. In addition, the lack of any identified genetic exchange mechanisms precludes the use of standard genetic manipulations in the native organism. To circumvent these obstacles, several genes have been cloned and expressed in *E. coli* (56, 72). However, only in the case of the flagallar proteins has a treponemal gene product been definitively localized to a specific extracytoplasmic compartment in *T. pallidum* (4, 10). Most of the genes that have been identified by immunological techniques are highly antigenic lipoproteins (8, 9, 34, 59, 67, 73). The cellular location of these proteins is thought to be subsurface (13). In addition, several laboratories initially claimed to have identified surface proteins, yet these claims have not withstood further investigation (56, 72). To date, none of the currently identified gene products appear to be TROMPs.

To facilitate the identification of *T. pallidum* surface and secreted gene products, we have taken an approach that utilizes the transposon Tn*phoA* to screen a *T. pallidum* genomic DNA library in *E. coli* (44). Alkaline phosphatase fusions have been used by a wide variety of investigators to identify genes encoding translocated proteins (45). Tn*phoA* contains a signalless, promoterless alkaline phosphatase gene in the IS*50*$_L$ element of Tn*5*. By transposition into a gene, Tn*phoA* can randomly generate gene fusions to *phoA*. The fusion protein expressed can display alkaline phosphatase activity only if its amino (N)-terminal portion contains an appropriate export signal that facilitates the extracytoplasmic localization of the PhoA moiety. *E. coli* colonies expressing

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† This work is dedicated to the memory of Philip J. Bassford, Jr. His knowledge, friendship, and enthusiasm are deeply missed.

TABLE 1. Bacterial strains, plasmids, and bacteriophage

Strain, plasmid, or bacteriophage	Genotype or characteristic	Reference or source
E. coli K-12 derivatives		
BRE51	MC4100 Δ(*sulA-ompA*)*51*	7
C386	*ompA lpp*	69
CC118	araD139 Δ(*ara leu*)*7697* Δ*lacX74 phoA*Δ*20 galE galK thi rpsE rpoB arg*(Am)*recA1*	44
DH5α(MCR)	F$^-$ *mcrA* Δ(*mrr-hsd* RMS-*mcrBC*) ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 deoR recA1 endA1* λ$^-$ *thi-1 gyrA96 relA1*	GIBCO-BRL
JM109	e14$^-$ (*mcrA*) *recA1 endA1 gyrA96 thi-1 hsdR17* (r_K^- m_K^+) *supE44 relA1* Δ(*lac-proAB*) [F′ *traD36 proAB lacI*^{q}ZΔM15]	Stratagene
KS330	F$^-$ Δ*lacX74 galE galD rpsL* (Str^r) Δ*phoA* (*Pvu*II) *lpp-5508 degP4*::Tn*5*	26
MC4100	*araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*	68
SE5000	MC4100 *recA56*	68
UH203	*lac supF ompA recA proA* or *proB rpsL* [F′ *lacI*^{q} *lacZ*ΔM15 *proAB*]	21
Plasmids		
pBC-SK$^+$	ColE1 replicon, Cm^r	Stratagene
pEBH21	pBC-SK$^+$ derivative	This study
p4A2	pEBH21 with an 8.0-kb *Sau*3A fragment insert including the *tpn50* gene	This study
p4A2HC2	pEBH21 with a 1.9-kb *Hin*cII-*Cla*I-fragment insert including the *tpn50* gene	This study
p4A2(T21)	p4A2 with a Tn*phoA* insertion into the *tpn50* gene at base 1156	This study
p4A2HC2(T7)	p4A2HC2 with a Tn*phoA* insertion into the *tpn50* gene at base 676	This study
p4A2HC2(T26)	p4A2HC2 with a Tn*phoA* insertion into the *tpn50* gene at base 937	This study
pJMH242a	pBC-SK$^+$ with a Tn*phoA* insertion into the remnant *bla* gene	This study
pRD87	pUC8 with a 1.28-kb *Pst*I fragment including the *E. coli ompA* gene	23
pWW2200	pRK404 with a 2.5-kb *Pst*I fragment including the *P. aeruginosa oprF* gene	78
Bacteriophage		
λTn*phoA*	Tn*5*::*phoA′*	44

enzymatically active alkaline phosphatase can be visualized as blue colonies on medium containing 5-bromo-4-chloro-3-indolyl phosphate (XP). A partial DNA sequence of the *T. pallidum* gene into which Tn*phoA* inserted can be obtained by using primers complementary to the 5′ portion of *phoA*. The DNA and predicted amino acid sequences can then be used to search available DNA and protein data bases for homologous sequences.

We have utilized the Tn*phoA* fusion methodology to identify and sequence a cloned gene that encodes a 417-amino-acid protein. Using the nomenclature of Norris et al. (56), we have named this gene *tpn50*. The TpN50 protein has amino acid sequence homology to a family of outer membrane proteins that includes *Pseudomonas* sp. OprF (17) and *E. coli* OmpA (12). The *tpn50* gene complemented *E. coli ompA* mutations. Since members of the OmpA family of proteins localize to the outer membrane and TpN50 has been shown to be functionally analogous to the OmpA protein, TpN50 may also localize to the outer membrane. This study demonstrates the utility of the Tn*phoA* fusion protein analysis approach for a bacterium for which the lack of genetic exchange mechanisms precludes standard molecular techniques.

(A preliminary report of this work [33] was presented at the 1993 93rd General Meeting of the American Society for Microbiology in Atlanta, Ga.)

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophage. *T. pallidum* subsp. *pallidum* (Nichols strain) was maintained and cultivated in the testicles of New Zealand White rabbits as previously described (70, 71). The *E. coli* strains, plasmids, and bacteriophage used in this study are listed in Table 1. *E. coli* C386 (*ompA lpp*) (69) was obtained from R. Hancock, University of British Columbia, Vancouver, British Columbia, Canada. *E. coli* UH203 (21) was obtained from J. Weiser, Children's Hospital of Philadelphia, Philadelphia, Pa. *E. coli* SE5000 (68) was used for maxicell analysis as described elsewhere. *E. coli* DH5α(MCR) was used as the host strain in the preparation of plasmid DNA for DNA sequence determination. *E. coli* KS330 was obtained from Kathryn Strauch, Biogen, Boston, Mass., and used in the "blue halo" assay of Giladi et al. (26). Strains containing plasmid DNA were grown at 37°C in Luria (L) broth or Terrific broth containing ampicillin (50 μg/ml), chloramphenicol (100 μg/ml), kanamycin (300 μg/ml), or tetracycline (20 μg/ml) for positive selection of plasmids.

Plasmid pEBH21 is a derivative of pBC-SK$^+$ (Stratagene, La Jolla, Calif.). Two modifications to pBC-SK$^+$ were made to improve the vector for Tn*phoA* mutagenesis. First, the *lac* promoter was changed to a *lacUV5* promoter (61) by site-directed mutagenesis (82). Second, the remnant *bla* gene (the *bla* promoter, the *bla* signal sequence, and the first third of the *bla* coding sequence) was removed since alkaline phosphatase fusions to β-lactamase generate false positives. Therefore, an *Xmn*I restriction site was engineered into pBC-SK$^+$ at position 3376. A 296-bp *Xmn*I fragment was then removed from the plasmid, creating the 3.1-kb vector designated pEBH21.

Plasmid pWW2200 (78) encoding the *Pseudomonas aeruginosa* OprF protein was obtained from R. Hancock. Plasmid pRD87 (23) encoding the *E. coli* OmpA protein was obtained from J. Weiser. In this plasmid, the *ompA* gene is transcribed from either its own promoter or the *lac* promoter. *E. coli* UH203 was used to propagate plasmid pRD87. Plasmids pWW2200 and pRD87 were used as positive controls in complementation studies.

DNA manipulations. Plasmid DNA was isolated by using the Magic Minipreps DNA purification system (Promega Corp., Madison, Wis.). DNA restriction enzymes were purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.), GIBCO-BRL (Gaithersburg, Md.), or Promega Corp. All other DNA manipulations were performed according to the methods of Sambrook et al. (65).

Library construction. A *T. pallidum* genomic DNA library was constructed by using the plasmid vector pEBH21. *T. pallidum* cells were gradient purified (3), and genomic DNA was prepared as previously described (64). Genomic DNA was partially digested with *Sau*3A (GIBCO-BRL), electrophoresed in a 0.4% low-gelling-temperature agarose gel (FMC Corp., Rockland, Maine), and DNA fragments of from 3 to 15 kb were isolated with the Qiaex gel extraction kit (Qiagen, Chatsworth, Calif.). Size-fractionated genomic DNA was then ligated into the *Bam*HI site of plasmid pEBH21 that had been dephosphorylated with calf intestine alkaline phosphatase (Boehringer Mannheim). The ligation products were transformed into competent *E. coli* JM109 cells by the Hanahan protocol (29) and plated on tryptone-yeast extract (TYE) medium containing chloramphenicol (100 μg/ml).

Tn*phoA* transposition and identification of *E. coli* clone 4A2. Clones from the *T. pallidum* genomic DNA library were infected with λTn*phoA* according to the protocol of Manoil and Beckwith (44). *E. coli* JM109 cells harboring recombinant plasmid DNA were grown to early stationary phase in L broth containing chloramphenicol (100 μg/ml) and 15 mM $MgSO_4$. The cells were infected with λTn*phoA* at a multiplicity of infection of 3:1, plated on TYE medium containing chloramphenicol (100 μg/ml) and kanamycin (300 μg/ml), and incubated at 30°C for 48 h. Plasmid DNA was prepared from the resulting colonies and transformed into *E. coli* CC118 (*phoAΔ20*). The cells were plated on TYE medium containing chloramphenicol (100 μg/ml), kanamycin (300 μg/ml), and XP (40 μg/ml). Colonies expressing secreted alkaline phosphatase appear blue on this medium. Plasmid DNA was prepared from blue colonies, and the location of the Tn*phoA* insertion was determined by restriction endonuclease analysis. A partial DNA sequence of the treponemal genes was obtained as described below. The DNA sequences and the predicted amino acid sequences were analyzed in order to identify clones that warranted further study. When an *E. coli* clone harboring plasmid p4A2 was infected with λTn*phoA*, several alkaline phosphatase-positive recombinant clones were obtained. On the basis of the analysis of preliminary DNA sequence obtained from p4A2(T21), the recombinant *E. coli* clone 4A2 was chosen for further study.

Subcloning of *tpn50*. The *tpn50* gene was subcloned by digestion of plasmid p4A2 with *Hin*cII and *Cla*I. The digest was electrophoresed in a 0.4% low-gelling-temperature agarose gel. A 1.9-kb *Hin*cII-*Cla*I fragment was isolated with the Qiaex gel extraction kit and ligated into plasmid pEBH21 that had been previously digested with *Hin*cII and *Cla*I. The resulting plasmid was designated p4A2HC2.

Maxicell analysis. Maxicell extracts of *E. coli* SE5000 (68) cells containing various plasmid DNAs were prepared according to the method of Stamm et al. (71). The solubilized, radiolabeled extracts were analyzed by electrophoresis on 15% polyacrylamide slab gels according to the procedure of Laemmli (39). The gels were stained with Coomassie brilliant blue, destained, and processed for fluorography (71). Approximate molecular masses of proteins were based on their migration in relation to protein standards of known molecular mass (Bio-Rad Laboratories, Hercules, Calif.).

DNA sequence determination and analysis. The DNA sequence of the *tpn50* gene was determined by the dideoxy chain termination method of Sanger et al. (66) with the Sequenase version 2.0 kit from U.S. Biochemicals Corp. (Cleveland, Ohio). DNA sequence analysis was carried out with [^{35}S]dATP (1,000 to 1,500 Ci/mmol) obtained from Du Pont NEN Research Products (Boston, Mass.). The initial DNA sequence was obtained from plasmid p4A2(T21) with the primer 5′-TGCAGTAATATCGCCCTGAGCACCC-3′. This sequence is complementary to the 5′ portion of the *phoA* gene in the Tn*phoA* construct. The remaining DNA sequence of the *tpn50* gene was determined with primers complementary to *tpn50* DNA sequences in plasmid p4A2 or p4A2HC2. Both DNA strands were sequenced a minimum of three times.

DNA and amino acid sequence analysis was done using the following computer software: Genetics Computer Group software package (University of Wisconsin Biotechnology Center, Madison), MacMolly Tetra version 1.2.1 (Europa Scientific Software Corp., Hollis, N.H.), and HelixScan version 2.1.5 (Helix, San Diego, Calif.). DNA and amino acid sequences were used to search the DNA and protein data bases at the National Center for Biotechnology Information (National Library of Medicine, Washington, D.C.) by using the BLAST algorithm (1). For multiple sequence alignments, the following amino acid sequences were used: *Pseudomonas syringae* OprF (74), *Pseudomonas fluorescens* OprF (16), *P. aeruginosa* OprF (17), *E. coli* OmpA (51), *Bordetella avium* OmpA (25), *Neisseria meningitidis* RmpM (38), *Neisseria gonorrhoeae* PIII (28), *Haemophilus influenzae* P6 (15, 54), *E. coli* PAL (11), and *Legionella pneumophila* PAL (19, 41).

Complementation of *E. coli ompA* mutations. Plasmids pEBH21, p4A2HC2 (TpN50$^+$), pWW2200 (OprF$^+$), and pRD87 (OmpA$^+$) were transformed into competent *E. coli* C386 (69) cells. Overnight cultures of C386 cells harboring plasmid DNAs were grown at 37°C in L broth containing 15 mM $MgSO_4$ and 50 μg of ampicillin per ml, 100 μg of chloramphenicol per ml, or 20 μg of tetracycline per ml. The overnight cultures were subcultured at a dilution of 1:100 and incubated at 37°C in an orbital shaker at 300 rpm until an optical density at 600 nm of 0.3 to 0.5 was reached on an Ultrospec III spectrophotometer (Pharmacia LKB Biochrom Ltd., Cambridge, England).

For morphometry studies, cells were fixed to glass slides and stained with safranin. The cells were visualized at a ×1,000 magnification with an FXA research light microscope (Nikon, Garden City, N.Y.). Images were captured with a Panasonic WV-CD50 CCD television camera and analyzed by using the Image Measure/IDIM 2500 morphometry system software from Phoenix Technology (Seattle, Wash.). The cell length, width, perimeter, and area were measured. The roundness coefficient (RC) was calculated by using the following formula: $RC = 4 \times \text{area}/\text{perimeter}^2$. Data sets were statistically analyzed by using the StatWorks version 1.2 program from Cricket Software (Philadelphia, Pa.).

For growth curve determination, *E. coli* BRE51 (7) cells harboring pEBH21 or p4A2HC2 and *E. coli* MC4100 (68) cells harboring pEBH21 were grown overnight in M9a medium (65) containing 50 μg of chloramphenicol per ml. The cultures were washed twice in M9a medium, resuspended in M9a medium, and subcultured into M9a medium containing 50 μg of chloramphenicol per ml to an optical density of 0.03 to 0.045 at 600 nm. Duplicate cultures were grown with aeration at 30 and 42°C. At various time points, aliquots were removed and the optical density at 600 nm was recorded.

Electron microscopy. *E. coli* C386 cells harboring pEBH21, p4A2HC2, pWW2200, and pRD87 were grown at 37°C with aeration overnight in L broth containing 15 mM $MgSO_4$ and 50 μg of ampicillin per ml, 100 μg of chloramphenicol per ml, or 20 μg of tetracycline per ml. The cultures were diluted 1:100 and allowed to grow to mid-log phase (optical density at 600 nm of 0.3 to 0.5). The cells were centrifuged for 5 min at 4°C at 3,000 × *g* and washed three times with 500 μl of 0.1 M sodium cacodylate buffer (0.1 M sodium cacodylate, 0.1 M sucrose, 0.01% $CaCl_2$, pH 7.2 to 7.3) each time. The cells were

then prefixed with 1 ml of a 2% glutaraldehyde–0.5% paraformaldehyde solution for 1 h at room temperature, processed for electron microscopy by the procedure of Wyrick et al. (80), and embedded in an Epon-araldite resin (standard Mollenhauer mixtures according to the instructions of Polysciences, Inc., Warrington, Pa.). The samples were cured at 60°C for a minimum of 48 h. The blocks were trimmed, and then ultrathin sections of silver to gold interference colors were obtained with a Reichert Ultracut S ultramicrotome (Leica Inc., Deerfield, Ill.). The sections were placed on copper grids and poststained with a 3% solution of aqueous uranyl acetate followed by lead citrate. The cells were visualized with a Zeiss-10 transmission electron microscope (Carl Zeiss, Thornwood, N.Y.) with an accelerating voltage of 80 kV and an objective aperature of 30 μm.

For scanning electron microscopy, the cells were grown as described above. All buffers and fixatives were prewarmed to 37°C to minimize temperature shock. Mid-log-phase cultures were diluted to approximately 10^8 cells per ml with 0.1 M Sorensen's sodium phosphate buffer. Aliquots containing 3×10^8 cells were filtered onto a 0.2-μm-pore-size polycarbonate filter (Poretics Corp., Livermore, Calif.) and washed by filtration with 10 ml of 0.1 M Sorensen's buffer. The cells were fixed with 10 ml of 2% glutaraldehyde–2% paraformaldehyde in 0.1 M Sorensen's buffer, and washed with 10 ml of 0.1 M Sorensen's buffer. The cells were then dehydrated by a series of increasing-concentration ethanol washes (30, 50, 75, and 100%). The samples were dried in a critical point dryer (Balzers Corp., Hudson, N.H.) with liquid CO_2 as the transition solvent. The samples were then trimmed, mounted on aluminum planchettes, and sputter coated with 10 nm of gold-palladium (60:40) with a Hummer X sputter coater (Anatech Ltd., Alexandria, Va.). The cells were visualized with a Cambridge stereoscan S-200 scanning electron microscope (Leica Inc.).

Blue halo assay. The blue halo assay was performed as described by Giladi et al. (26) to determine the cellular location of PhoA fusion proteins. *E. coli* KS330 cells harboring plasmids pEBH21, p4A2HC2(T7), and pJMH242a (a Tn*phoA* insertion into the remnant *bla* gene of the plasmid vector pBC-SK$^+$) were grown in L broth containing chloramphenicol (100 μg/ml) at 30°C for approximately 8 h. The optical density at 600 nm was determined. The cells were pelleted and resuspended in L broth to yield similar optical densities. Aliquots of 5 μl were spotted onto TYE medium containing chloramphenicol (100 μg/ml) and XP (200 μg/ml). The plates were incubated at 30°C for 15 to 20 h and then visually inspected for halo formation.

Nucleotide sequence accession number. The GenBank accession number for the *tpn50* gene sequence is U02628.

RESULTS

Identification and subcloning of *tpn50*. The plasmid vector pEBH21 was used to prepare a *T. pallidum* genomic DNA library in *E. coli* JM109. Clones from the library were infected with λTn*phoA*. Clone 4A2, which yielded alkaline phosphatase-positive colonies after Tn*phoA* mutagenesis, was chosen for further study on the basis of preliminary DNA sequence obtained from p4A2(T21). Plasmid p4A2 contained an approximately 8-kb DNA insert of *T. pallidum* origin. A 1.9-kb *Hin*cII-*Cla*I fragment of p4A2 was ligated into pEBH21 to create p4A2HC2. Tn*phoA* insertions into the p4A2HC2 insert DNA also yielded functional alkaline phosphatase fusion proteins. Figure 1 shows the results of maxicell analysis of *E. coli* SE5000 cells harboring pEBH21, p4A2, p4A2HC2, and three Tn*phoA* insertions into the *tpn50* gene of either p4A2 or p4A2HC2 [p4A2(T21), p4A2HC2(T7), and p4A2HC2(T26)]. Plasmid pEBH21 encoded the chloramphenicol acetyltransferase protein (Fig. 1, lane A). Plasmid p4A2 encoded four *T. pallidum* proteins of 50, 35, 31, and 20 kDa (Fig. 1, lane B). Plasmid p4A2HC2 encoded only the 50- and 20-kDa treponemal proteins indicated by the arrows (Fig. 1, lane D). Both the 50- and 20-kDa proteins were immunoprecipitated with rabbit syphilitic serum (data not shown). *E. coli* SE5000 cells harboring plasmids p4A2(T21) and p4A2HC2(T26) no longer expressed the 50- and 20-kDa proteins (Fig. 1, lanes C and F). In contrast, *E. coli* SE5000 cells harboring p4A2HC2(T7) still expressed the 20-kDa protein while the 50-kDa protein was not expressed (Fig. 1, lane E). The PhoA-TpN50 fusion proteins that are indicated by the dots (Fig. 1, lanes C, E, and F) were identified by immunoblotting with anti-PhoA serum (data not shown). These fusion proteins are unstable and are readily broken down to yield the mature alkaline phosphatase moiety of 45 kDa (Fig. 1 lanes C, E, and F). Three additional proteins of 30, 48, and 51 kDa are encoded by Tn*phoA* and represent the kanamycin resistance protein, the bleomycin resistance protein, and the Tn*5* transposase (Fig. 1, lanes C, E, and F).

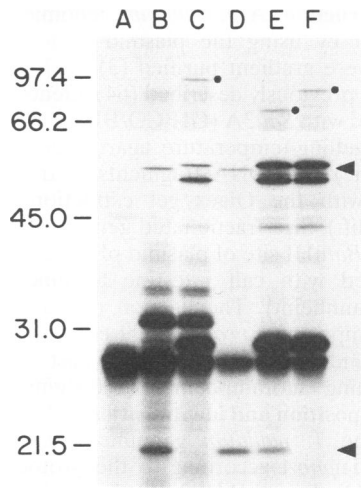

FIG. 1. Maxicell analysis of *E. coli* SE5000 cells harboring various recombinant plasmids by SDS-PAGE and fluorography. Plasmid vector pEBH21 and five recombinant plasmids were transformed into *E. coli* SE5000. Radiolabeled maxicell extracts were prepared as described in the text and electrophoresed in a 15% polyacrylamide gel. Lane A, pEBH21; lane B, p4A2; lane C, p4A2(T21); lane D, p4A2HC2; lane E, p4A2HC2(T7); lane F, p4A2HC2(T26). Plasmid p4A2(T21) represents a Tn*phoA* insertion into the *tpn50* gene of p4A2. Plasmids p4A2HC2(T7) and p4A2HC2(T26) represent Tn*phoA* insertions into the *tpn50* gene of p4A2HC2. The dots indicate the positions of the TpN50-alkaline phosphatase fusion proteins. The arrowheads indicate the positions of TpN50 and the 20-kDa protein. The upper portion of the fluorograph was exposed for a longer period of time to show the presence of the unstable PhoA-TpN50 fusion proteins.

DNA sequence analysis. The initial DNA sequence of the gene encoding the 50-kDa protein was obtained with plasmid p4A2(T21) and sequencing primers complementary to the 5′ portion of the mature *phoA* gene. The DNA sequence of the remainder of the gene was obtained from plasmid p4A2 or p4A2HC2 by using specifically designed oligonucleotide primers. We have named the gene encoding the 50-kDa protein *tpn50*. Figure 2 shows a partial restriction map of the p4A2 and p4A2HC2 plasmid insert DNA. The locations of the Tn*phoA* insertions into either p4A2 or p4A2HC2 are indicated by the

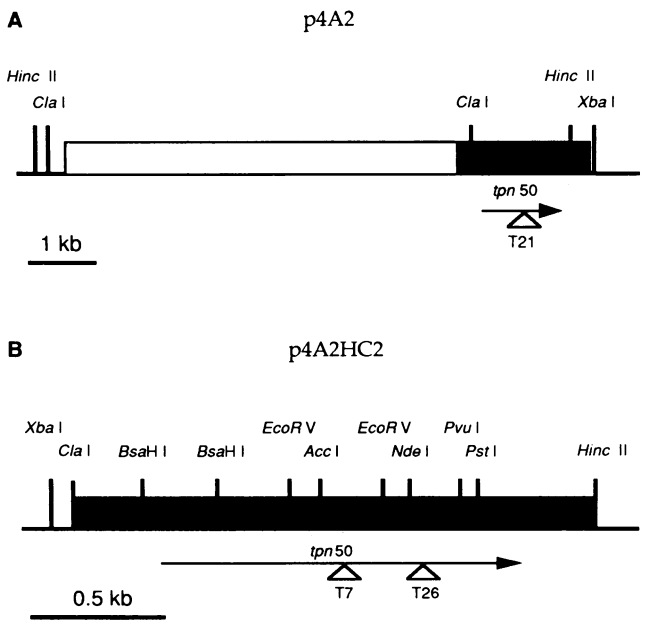

FIG. 2. Restriction maps for the p4A2 (A) and p4A2HC2 (B) plasmids. Plasmid pEBH21 DNA is shown as the solid line, while the treponemal insert DNA is indicated by the box. The DNA sequence of the shaded region of the insert DNA has been determined. The arrows indicate the location and directionality of the *tpn50* gene. Open triangles indicate the locations of in-frame Tn*phoA* insertions.

open triangles. The complete DNA sequence and the deduced amino acid sequence of the *tpn50* gene are shown in Fig. 3. The *tpn50* gene is 1,251 nucleotides long and encodes a 417-amino-acid protein with a calculated molecular mass of 47,582 Da. This is in close agreement with the apparent molecular mass of 50 kDa as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). An apparent Shine-Dalgarno ribosome binding site (AGGT) precedes a putative GTG initiation codon by 8 nucleotides. The two ATG codons or the GTG codon located at bases 7, 10, and 19 relative to the first GTG codon could also serve as initiation codons. The GTG codon at base 19 is preceded by a possible Shine-Dalgarno ribosome binding site (GGA). The initiation codon used by *T. pallidum* is unknown. The spacing of the putative initiation codons and the possible ribosome binding sites is within the observed limits for *E. coli* genes (49). The TpN50 protein contains a putative signal peptide of 18 to 26 amino acids. There are three possible signal peptidase I consensus cleavage sites in the signal peptide (Ala-Ala-Ser, Ala-Ser-Ala, and Ser-Ala-Ala). The putative signal peptide contains the three regions characteristic of signal peptides (57). It contains a positively charged N terminus, a hydrophobic central core, and a signal peptidase I consensus cleavage site. The two positively charged amino acids (RK) are located 9 and 10 amino acids downstream of the initial valine. This distance is unusually long. If the GTG start codon at base 19 is utilized, the positively charged amino acids are only 3 and 4 amino acids downstream of the initial valine. Computer predictions indicate that the TpN50 protein is hydrophilic, contains a high proportion of β-sheets (data not shown), and has a pI of 8.1. The overall G+C content of the *tpn50* gene is 50.81%, which agrees with the reported G+C content (53%) for the *T. pallidum* genome (48).

The 20-kDa protein was not expressed in *E. coli* SE5000 cells harboring p4A2(T21) and p4A2HC2(T26) (Fig. 1, lanes C and F) yet was expressed in cells harboring p4A2HC2(T7) (Fig. 1, lane E). Therefore, we speculate that there is a secondary initiation site located in frame of *tpn50* but downstream of the insertion site of Tn*phoA* in p4A2HC2(T7). Analysis of the DNA sequence of *tpn50* in this region revealed two possible start codons at bases 682 and 691 of *tpn50* that could serve as the start codon for the 20-kDa protein. A possible ribosome binding site (AGGA) that could serve as the translation initiation signal for either of these start codons is located at bases 678 to 681. Although the Tn*phoA* insertion in p4A2HC2(T7) would terminate the *tpn50* mRNA, the 20-kDa protein could still be produced from transcripts initiating within Tn*phoA*.

Comparison of the deduced amino acid sequence of the *tpn50* gene with the amino acid sequence data bases. The deduced amino acid sequence of the *tpn50* gene was used to

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                                (S/D)       V  C  M  M  G  S  V  F  S  R  K  G  M  L  C  V  L  A  V  W  C  A  A
   1 TTCTCGTGCGGATATGACGGATAGGTCCGTTCGGTGTGTATGATGGGAAGTGTGTTTTCTAGGAAGGGAATGCTGTGTGTTCTTGCAGTCTGGTGCGCGG   100
       S  A  A  Y  P  L  R  L  R  Y  K  F  R  K  G  D  T  H  R  I  N  S  L  I  R  E  D  V  F  V  N  D  T
 101 CAAGTGCGGCGTACCCTCTGCGCTTGCGCTACAAGTTTCGTAAGGGGGATACGCACCGTATCAACTCGCTGATACGCGAGGATGTGTTTGTGAACGATAC   200
       L  A  H  T  A  E  I  T  N  R  I  T  V  H  V  S  E  V  R  V  A  H  G  S  A  P  D  A  A  R  Y  V  C
 201 GCTTGCTCACACGGCGGAGATTACGAATCGAATCACGGTGCATGTTTCCGAAGTACGTGTTGCACACGGGTCGGCGCCTGATGCGGCGCGGTATGTGTGT   300
       H  F  M  T  S  E  K  S  P  N  N  T  F  R  W  G  R  H  Y  E  S  I  F  W  R  D  A  F  G  V  Y  D  I  D
 301 CACTTCATGACATCCGAGAAAAGTCCCAATAATACGTTTCGTTGGGGGAGGCACTACGAAAGTATTTTCTGGCGCGACGCATTTGGTGTGTATGATATTG   400
         R  S  F  F  M  P  V  V  R  N  V  P  V  F  P  D  Y  D  I  E  V  G  D  T  W  E  H  E  A  E  E  A  H
 401 ATCGTTCATTCTTCATGCCGGTAGTACGGAATGTGCCGGTGTTTCCTGATTATGATATCGAAGTTGGAGATACCTGGGAGCATGAAGCAGAAGAAGCGCA   500
       D  L  R  D  G  F  G  I  Q  T  P  F  I  V  P  F  T  V  S  Y  T  Y  R  G  E  V  Q  R  G  S  R  R  Y
 501 TGATCTGCGTGACGGCTTTGGTATCCAAACGCCCTTCATTGTGCCGTTTACCGTGTCGTATACCTACCGTGGTGAAGTTCAGCGTGGTAGTCGGCGCTAT   600
       H  H  I  T  A  A  Y  S  M  S  Y  E  S  P  K  R  T  H  G  V  Q  R  N  A  K  E  G  M  Y  P  V  R  T  T
 601 CATCACATTACTGCGGCGTATTCTATGAGTTATGAGTCGCCCAAACGGACACATGGCGTGCAGCGCAATGCAAAAGAAGGAATGTATCCGGTGCGTACTA   700
         G  V  S  K  Q  N  L  Y  W  D  N  E  L  G  N  I  A  E  Y  D  D  E  F  R  I  L  L  Y  L  S  G  G  T
 701 CGGGCGTTTCTAAGCAAAATTTGTATTGGGATAACGAACTTGGGAACATAGCTGAGTACGACGATGAGTTTCGCATTCTCTTATACCTGAGTGGAGGAAC   800
       V  L  R  Y  Q  G  T  A  T  A  K  N  F  A  P  E  R  F  D  P  A  R  T  V  V  E  L  Q  E  T  L  K  D
 801 GGTTTTGAGATATCAGGGTACTGCGACGGCAAAGAATTTTGCACCTGAGCGCTTTGATCCTGCACGCACGGTGGTGGAACTGCAAGAAACGCTCAAGGAC   900
       L  H  M  P  D  A  K  V  R  E  T  E  E  G  V  T  I  S  I  E  N  V  Q  F  D  A  D  S  A  S  L  A  P  S
 901 TTGCATATGCCTGATGCGAAGGTGCGTGAAACAGAAGAGGGAGTAACCATCAGTATAGAAAATGTTCAATTTGACGCAGACTCTGCATCCCTTGCACCTT  1000
         E  Y  E  N  L  R  K  I  A  E  L  L  R  A  F  P  D  R  E  L  L  V  S  G  H  A  A  R  R  G  S  V  Q
1001 CTGAGTACGAGAACCTGCGCAAAATAGCAGAACTTTTGCGCGCGTTTCCCGATCGAGAGTTGTTGGTGTCTGGACATGCGGCACGACGGGGGTCTGTGCA  1100
       D  Q  Q  R  I  S  E  E  R  A  D  V  V  A  R  Y  L  Q  E  L  G  V  V  D  A  A  H  V  Y  T  R  G  C
1101 GGATCAACAGAGGATTTCTGAAGAGCGTGCTGATGTGGTTGCACGTTACCTGCAGGAATTAGGTGTTGTGGATGCAGCGCATGTATATACGCGCGGTTGT  1200
       G  A  Q  Q  S  I  A  P  N  D  S  E  D  G  R  R  K  N  R  R  V  E  I  T  I  I  S  K  *
1201 GGTGCGCAGCAGTCTATTGCGCCAAATGATTCAGAGGATGGTCGTAGAAAAAACCGACGGGTAGAAATCACTATCATAAGTAAGTAAAGTGTTGCCCTGT  1300
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FIG. 3. Nucleotide sequence and the deduced amino acid sequence of the *T. pallidum tpn50* gene. The putative Shine-Dalgarno ribosome binding site is underlined. The putative GTG start and TAA stop codons are boxed. The putative signal peptidase I consensus cleavage site(s) is double underlined. The calculated molecular mass of the full-length TpN50 protein is 47,582 Da.

	T. pallidum TpN50	*P. syringae* OprF	*P. fluorescens* OprF	*P. aeruginosa* OprF	*E. coli* OmpA	*B. avium* OmpA	*N. meningitidis* RmpM	*N. gonorrhoeae* PIII	*H. influenzae* P6	*E. coli* PAL	*L. pneumophila* PAL
T. pallidum TpN50	- -										
P. syringae OprF	47.2 20.6	- -									
P. fluorescens OprF	45.3 23.3	78.4 64.3	- -								
P. aeruginosa OprF	47.4 22.2	84.2 68.2	75.6 56.5	- -							
E. coli OmpA	43.2 23.5	50.6 26.6	52.7 27.8	49.1 26.9	- -						
B. avium OmpA	47.9 26.6	54.2 34.9	50.0 35.1	48.5 32.0	54.2 36.3	- -					
N. meningitidis RmpM	44.9 25.6	47.8 31.7	54.1 32.4	51.9 32.2	52.2 33.0	51.0 26.7	- -				
N. gonorrhoeae PIII	44.3 25.1	47.1 30.9	53.7 31.5	52.9 31.4	56.1 35.0	51.8 30.4	97.9 95.8	- -			
H. influenzae P6	45.8 26.1	60.0 33.3	47.1 30.1	52.9 32.0	50.3 29.8	50.7 30.9	49.7 30.7	52.6 34.2	- -		
E. coli PAL	45.8 24.1	46.2 26.0	46.5 26.7	50.9 28.7	44.4 26.0	52.7 29.0	47.4 26.6	45.9 29.1	76.5 60.1	- -	
L. pneumophila PAL	45.6 24.0	47.7 26.7	46.6 31.9	48.3 27.3	45.1 27.4	41.8 25.9	47.0 28.6	44.5 30.6	60.8 41.2	59.6 36.3	- -

FIG. 4. Homology matrix of the amino acid sequence alignment scores for selected members of the OmpA family of proteins. The alignment scores are for comparisons of the full-length proteins. The percent similarity is shown above the percent identity for each comparison (dashes indicate 100%). The percentages were obtained by using the Gap program from the Wisconson Genetics Computer Group sequence analysis software package and the Dayhoff PAM-250 similarity matrix.

conduct a BLAST search of the National Center for Biotechnology Information protein data bases (1). Statistically significant matches to several members of a family of outer membrane proteins that includes *P. aeruginosa* OprF and *E. coli* OmpA were found. Figure 4 shows the percent similarity and percent identity derived from optimal alignments of selected members of the OmpA family of proteins. The observed homology of the TpN50 protein with members of the OmpA family is in the same range as the observed homology between family members (excluding the homologous proteins within a genus). The TpN50 protein is 47.4 and 43.2% similar to the *P. aeruginosa* OprF and the *E. coli* OmpA proteins, respectively. Members of the OmpA family exhibit greater homology in their carboxy (C)-terminal regions than in their N-terminal regions (79). Large gaps were introduced in sequence alignments in the N-terminal regions to allow for optimal alignment. Conversely, small or no gaps were introduced in the sequence alignments in the C-terminal regions. A multiple sequence alignment of the C-terminal regions of several members of the OmpA family to the C terminus of TpN50 is shown in Fig. 5. When the C-terminal region of TpN50 was compared with the C termini of *P. aeruginosa* OprF and *E. coli* OmpA, the percent similarities were 48.6 and 50.0%, respectively. The multiple sequence alignment reveals two regions of higher conservation. These two regions, which correspond to amino acids 359 to 367 and 407 to 414 of TpN50, contain di-arginine residues in the majority of the OmpA like proteins. The di-arginine residues have been implicated in gram-negative organism-mediated arthritis (81). However, the function of these conserved regions is unknown.

Complementation of *E. coli ompA* mutations with *tpn50*. The *P. aeruginosa* OprF protein, the *E. coli* PAL protein, and the *E. coli* OmpA protein have previously been shown to play a role in the structural integrity of the outer membrane (27, 40, 69, 79). The C terminus of the OprF protein has been implicated in the structural role of the protein (30). Since the C termini of TpN50 and members of the OmpA family of proteins exhibit similarity, we sought to determine whether the TpN50 protein could functionally replace the OmpA protein.

E. coli C386 (*ompA lpp*) displays an almost spherical morphology due to the absence of OmpA (69). *E. coli* C386 cells were transformed with plasmids pEBH21, p4A2HC2 ($TpN50^+$), pWW2200 ($OprF^+$), and pRD87 ($OmpA^+$). These constructs were then assayed for the ability of TpN50 to restore a rod-shaped phenotype to *E. coli* C386 cells. Table 2 shows the results of the complementation experiments. *E. coli* C386 cells harboring plasmid vector pEBH21 were 33.7, 36.4, and 32.4% shorter than C386 cells harboring p4A2HC2, pWW2200, or pRD87, respectively. The RC is an indicator of the cell shape. Values closer to one correspond to more-spherical cells. The RCs were calculated to be 0.94, 0.85, 0.84, and 0.85 for *E. coli* C386 cells harboring plasmids pEBH21, p4A2HC2, pWW2200, and pRD87, respectively. The differences in the cell lengths and RCs between *E. coli* C386 cells harboring pEBH21 and the same strain harboring either p4A2HC2, pWW2200, or pRD87 are statistically significant on

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T. pallidum TpN50      311  VQF.DADSAS LAPSEYENLR KIAELLRAF. .PDRELLVSG HAARRGSVQD QQRISEERAD  367
P. syringae OprF       236  VKF.DFDKSV VKPNSYGDIK NLADFMQQY. .PQTTTTVEG HTDSVGPDAY NQKLSERRAN  292
P. fluorescens OprF    219  VKF.DFDKSV VKPNSYGDVK NLADFMAQY. .PATNVEVAG HTDSIGPDAY NQKLSQRRAD  275
P. aeruginosa OprF     242  VKF.DFDKSK VKENSYADIK NLADFMKQY. .PSTSTTVEG HTDSVGTDAY NQKLSERRAN  298
E. coli OmpA           221  VLF.NFNKAT LKPEGQAALD QLYSQLSNLD PKDGSVVVLG YTDRIGSDAY NQGLSERRAQ  279
B. avium OmpA           87  TFF.DFDKST LKPEGRQLLD QVAQQARAID ..LETIIAVG NTDSIGTEAY NMKLSERRAA  143
N. meningitidis RmpM   101  TLF.GFDKDS LRAEAQDNLK VLAQRLGQTN ..IQSVRVEG HTDFMGSDKY NQALSERRAY  157
N. gonorrhoeae PIII     97  TLF.GFDKDS LRAEAQDNLK VLAQRLSRTN ..VQSVRVEG HTDFMGSEKY NQALSERRAY  153
H. influenzae P6        65  VQILDAH... .......... ..AAYLNAT. .PAAKVLVEG NTDERGTPEY NIALGQRRAD  107
E. coli PAL             71  VYF.DLDKYD IRSDFAQMLD AHANFLRSN. .PSYKVTVEG HADERGTPEY NISLGERRAN  127
L. pneumophila PAL      70  YLF.AYDDST LASKYLPSVN AQAEYLKTH. .PGARVMIAG HTDERGSREY NVALGERRAD  126

T. pallidum TpN50      368  VVARYLQEL. GV.VDAAHVY TRGCGAQQSI APNDSEDGRR K.NRRVEITI ISK  417
P. syringae OprF       293  AVKQVLVNQY GV .GASRVN SVGYGESKPV ADNATEAGRA V.NRRVEAEV EAQA K  344
P. fluorescens OprF    276  RVKQVLVKD. GV..APSRIT AVGYGESRPV ADNATEAGRA V.NRRVEASV EAQA Q  326
P. aeruginosa OprF     299  AVRDVLVNEY GV..EGGRVN ANGYGESRPV ADNATAEGRA I.NRRVEAEV EAEA K  350
E. coli OmpA           280  SVVDYLISK. GI..PADKIS ARGMGESNPV TGNTCDNVKQ R*DRRVEIEV KGIK DVVTQPQA  346
B. avium OmpA          144  SVKAYLVSK. GI..DPNRIY TEGKGKLNPI ASNKTAEGRA R.NRRVEIEI VGSR K  194
N. meningitidis RmpM   158  VVANNLVSN. GV*GESQAQM TQVCEAEVAK LGAKVSKAKK R*DRRVDVKI RSIV TRQVVPAHNH HQH  240
N. gonorrhoeae PIII    154  VVANNLVSN. GV*GESQAQM TQVCQAEVAK LGAKASKAKK R*DRRVDVKI RSIV TRQVVPARNH HQH  236
H. influenzae P6       108  AVKGYLAGK. GV..DAGKLG TVSYGEEKPA VLGHDEAAYS K.NRRAVLAY  153
E. coli PAL            128  AVKMYLQGK. GV..SADQIS IVSYGKEKPA VLGHDEAAYS K.NRRAVLVY  173
L. pneumophila PAL     127  TVAEILRMA. GV..SRQQIR VVSYGKERPA NYGHDEASHA Q.NRRVEFIY EATR  176
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FIG. 5. Multiple sequence alignment of the TpN50 amino acid sequence and the amino acid sequences of the indicated proteins. The region shown is in the C-terminal portion of all the proteins. Amino acid sequence identity with the *T. pallidum* sequence is denoted by bars above the amino acid sequence. Amino acid sequence similarity with the *T. pallidum* sequence is denoted by colons (based on the Dayhoff PAM-250 similarity matrix). The asterisks represent amino acid insertions. The insertion in the *E. coli* OmpA sequence is AALIDCLAP. The two insertion sequences in *N. meningitidis* RmpM and *N. gonorrhoeae* PIII are PVSRISAVGL and EALIACIEP. In *N. gonorrhoeae* PIII there is an alanine at position 170 instead of the valine present in *N. meningitidis* RmpM. The amino acid sequences were aligned by using the PileUp program from the Wisconson Genetics Computer Group sequence analysis software package.

the basis of Student's *t* test (Table 2). Transmission and scanning electron micrographs of the C386 cells harboring the pEBH21, p4A2HC2, pWW2200, and pRD87 are shown in Fig. 6. *E. coli* C386 cells harboring pEBH21 were rounded, whereas C386 cells harboring p4A2HC2, pWW2200, or pRD87 were rod shaped. The size and shape of the cells in the scanning electron micrographs correlated well with the data obtained in the morphometry studies.

Additionally, Manning et al. (43) have shown that *E. coli* cells deficient in OmpA grow poorly at 42°C in minimal medium. Figure 7 shows the growth curves of *E. coli* BRE51 (MC4100:*ompA*) (7) harboring pEBH21 or p4A2HC2 and *E. coli* MC4100 harboring pEBH21 in minimal medium. *E. coli* BRE51 cells harboring pEBH21 grew poorly at 42°C. In contrast, *E. coli* BRE51 cells harboring p4A2HC2 grew as well at 42°C as the wild-type *E. coli* MC4100 cells harboring pEBH21. The growth rates of all three strains at 30°C were virtually identical.

Blue halo assay. The blue halo assay has been used to localize PhoA fusion proteins to either the inner membrane or the periplasmic-outer membrane compartments (26). *E. coli* KS330 cells expressing alkaline phosphatase fusions to β-lactamase and to TpN50 yielded blue halos when spotted on medium containing XP. The halos observed for KS330 harboring pJMH242a (*bla-phoA*) were much larger than the halos for KS330 harboring p4A2HC2(T7) (*tpn50-phoA*). The presence of a halo in *E. coli* KS330 cells harboring p4A2HC2 indicates that the TpN50-PhoA fusion protein is localized to either the periplasm or the outer membrane. The sizes of the halos resulting from *bla-phoA* fusions were similar to those observed by Giladi et al. (26). Interestingly, the sizes of the halos resulting from *tpn50-phoA* fusions were analogous to those from the *ompA-phoA* fusions seen by Giladi et al. (26).

DISCUSSION

The purpose of this study was to use genetic techniques in *E. coli* to clone treponemal genes that encode cell surface pro-

TABLE 2. Effect of the *T. pallidum* TpN50 protein on the cellular morphology of *E. coli* C386 (*ompA lpp*)[a]

Strain	Mean cell length (μm) ± SD	Mean cell area (μm²) ± SD	RC[b]
C386/pEBH21	1.385 ± 0.222	1.191 ± 0.336	0.936 ± 0.033
C386/p4A2HC2[c] (*T. pallidum* TpN50⁺)	2.082 ± 0.353	1.751 ± 0.585	0.850 ± 0.056
C386/pWW2200[c] (*P. aeruginosa* OprF⁺)	2.166 ± 0.350	2.070 ± 0.512	0.844 ± 0.057
C386/pRD87[c] (*E. coli* OmpA⁺)	2.036 ± 0.348	1.838 ± 0.515	0.849 ± 0.058

[a] Cells were fixed to glass slides, stained with safranin, and viewed at a magnification of ×1,000 with a Nikon FXA research light microscope. Images were captured with a Panasonic WV-CD 50 CCD television camera and analyzed by using the Image Measure/IPIM 2500 morphometry system software from Phoenix Technology. Approximately 200 cells were measured for each sample. Cell width measurements were not significantly different. The average cell width was 1.14 μm.

[b] An RC of 1 represents a spherical cell. RC = 4 × area/perimeter².

[c] The measurements for the cell length, cell area, and RC were significantly different from those for C386/pEBH21 on the basis of Student's *t* test ($P < 0.001$).

teins. We utilized Tn*phoA* to screen a *T. pallidum* genomic DNA library in *E. coli* for treponemal genes encoding secreted proteins, some of which are likely to be outer membrane proteins. Blanco et al. (5) and Giladi et al. (26) have described a similar approach to identifying spirochetal genes encoding secreted proteins. In their approach, an alkaline phosphatase-containing plasmid vector, pMG, was utilized to construct a genomic DNA library. An advantage imparted by using λTn*phoA* to infect individual clones from a library in *E. coli* is that the parental gene to which Tn*phoA* has fused is already isolated. This allows for rapid identification of the treponemal protein that has been identified by Tn*phoA*-mediated DNA sequence analysis.

In this paper we report the cloning and sequencing of a treponemal gene that encodes a 50-kDa protein. This gene has been designated *tpn50* according to the nomenclature of Norris et al. (56). A BLAST search of the available protein data bases indicated that TpN50 is homologous to members of the OmpA family of outer membrane proteins. Members of this family include the *Pseudomonas* sp. OprF, *E. coli* OmpA, *B. avium* OmpA, *N. meningitidis* RmpM, *N. gonorrhoeae* PIII, *H. influenzae* P6, *E. coli* PAL, and *L. pneumophila* PAL proteins. These proteins have amino acid sequence homology mainly in their C termini. For example, the C termini of the *E. coli* OmpA and the *P. aeruginosa* OprF proteins are 57% similar. When the C termini of the *E. coli* OmpA and *T. pallidum* TpN50 proteins are compared, a 50% similarity is observed. Large gaps were introduced in the N-terminal regions of amino acid sequence alignments of TpN50 and members of the OmpA family, while few gaps were introduced in the C-terminal regions. This has also been observed with the amino acid sequence alignments of *P. aeruginosa* OprF and *E. coli* OmpA (79). TpN50 and the OmpA family of proteins also share other similarities. All contain substantial β-sheet structure as predicted by computer analysis, are predominantly hydrophilic, and have acidic pIs. In addition, extracts of *E. coli* cells expressing the recombinant *B. avium*, *N. gonorrhoeae*, or *E. coli* OmpA protein contain faster-migrating protein species (24, 25, 28). These protein species arise from processing, incomplete translation, or association with the membrane (24). *E. coli* maxicells expressing the TpN50 protein also contained a faster-migrating protein species of approximately 20 kDa. However, because of the expression pattern of the 20-kDa protein in maxicell extracts of *E. coli* SE5000 cells harboring p4A2HC2(T7) or p4A2HC2(T26) (Fig. 1, lanes E and F), we speculate that the 20-kDa protein is the product of a secondary translational initiation site located within the *tpn50* gene downstream of base 675. Whether the 20-kDa protein is expressed in *T. pallidum* is unknown. A similar result was observed with the recombinant 39-kDa basic membrane protein of *T. pallidum* (14). In this instance, two proteins (35 and

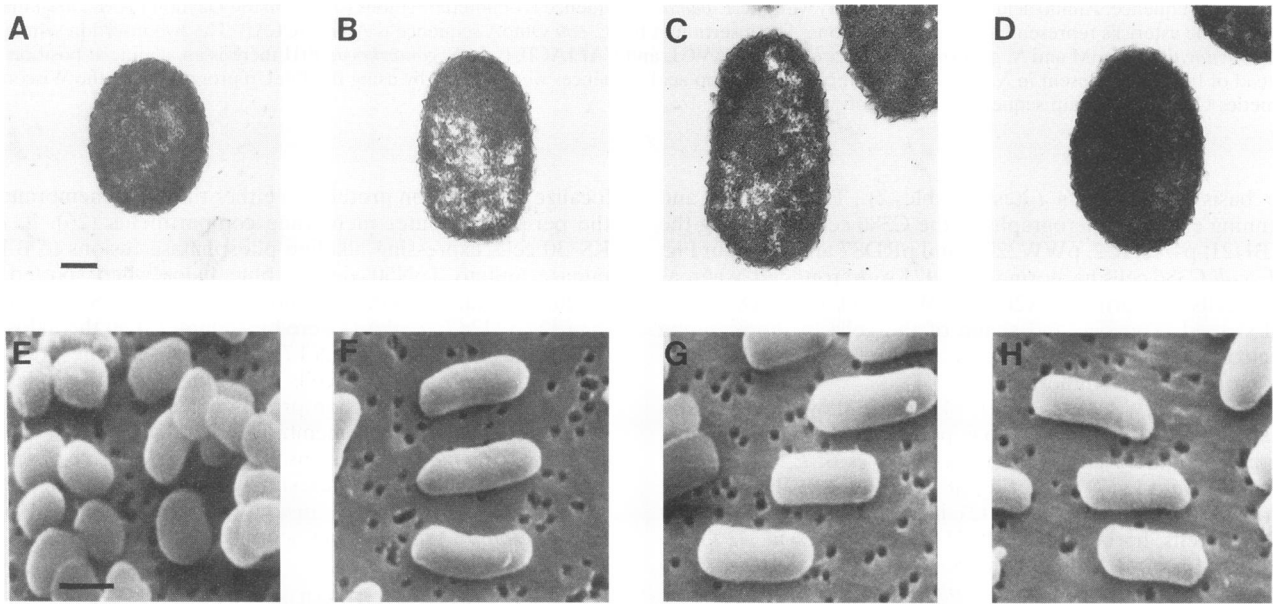

FIG. 6. Transmission (A to D) and scanning (E to H) electron micrographs of *E. coli* C386 (*ompA lpp*) cells harboring various recombinant plasmids. Experimental details are given in the text. (A and E) pEBH21; (B and F) p4A2HC2 (TpN50⁺); (C and G) pWW2200 (OprF⁺); (D and H) pRD87 (OmpA⁺). (A to D) Bar = 0.25 μm; (E to H) bar = 1.0 μm.

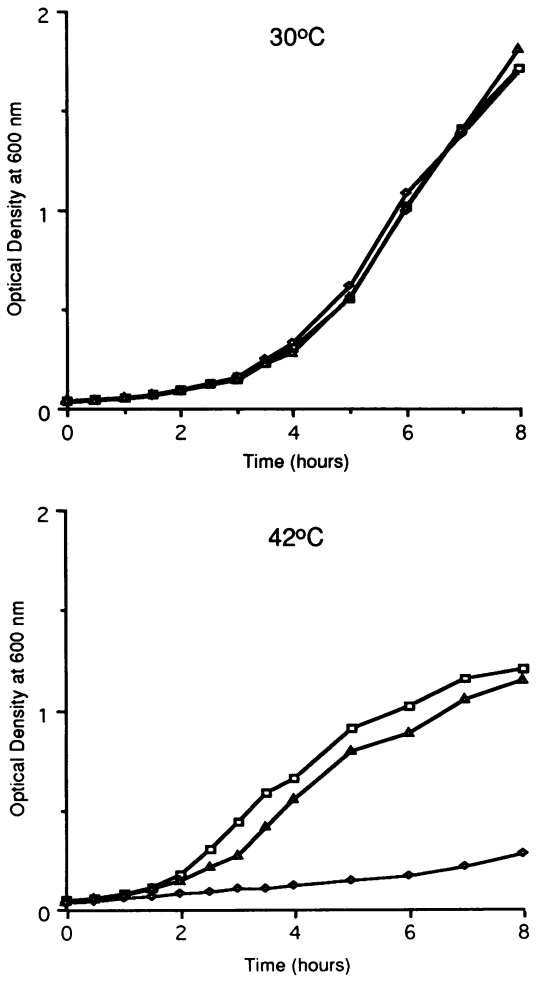

FIG. 7. Complementation of an *ompA* mutation with *tpn50*. Growth of wild-type *E. coli* MC4100 cells harboring pEBH21 (△), *E. coli* BRE51 (*ompA*) cells harboring pEBH21 (◇), and *E. coli* BRE51 cells harboring p4A2HC2 (□) in minimal medium at 30 and 42°C. Experimental details are given in the text.

25 kDa) were observed in addition to the 39-kDa protein only in *E. coli* maxicells and not in *T. pallidum* cells.

Proteins in the OmpA family have several functions, including the following: (i) receptors for bacteriophages, (ii) mediators of colicin uptake, (iii) mediators of F-dependent conjugation, (iv) maintenance of cell structure and outer membrane integrity, and (v) pore formation (32, 40, 42, 46, 69). The homology of TpN50 to the OmpA family of proteins led us to investigate whether the *tpn50* gene could complement mutations in the *ompA* gene. The *E. coli* C386 (*ompA lpp*) cell morphology mutant has been used to show complementation of an *ompA* mutation with the *P. aeruginosa oprF* gene (79). Expression of OmpA, OprF, or TpN50 in *E. coli* C386 cells restored the rod-shaped phenotype to the cells. Expression of the 39-kDa treponemal basic membrane protein (14) or the treponemal 35.5-kDa lipoprotein (34) had no effect on the morphology of the C386 cells (data not shown).

In addition to the effect of OmpA on cellular morphology, Manning et al. (43) have shown that *E. coli ompA* mutants grow poorly in minimal medium at 42°C (43). This may be due to the putative effect of OmpA on the stability of the outer membrane. The expression of TpN50 in *E. coli* BRE51 restored normal cell growth at 42°C. The results for *E. coli* MC4100 and BRE51 harboring pEBH21 correlated well with the results of Manning et al. (43), who utilized *E. coli* P400 and P460 (P400 *ompA1*).

Since *tpn50* complemented *ompA* mutations on the basis of two separate criteria, it is conceivable that TpN50 functions to stabilize the treponemal outer membrane. It is therefore of interest to speculate as to the location of TpN50 in *T. pallidum*. Members of the OmpA family of proteins are surface exposed (20, 22, 50, 53), and antibodies directed against OmpA (77), OprF (35, 47), PAL (36), and P6 (52) are bactericidal, opsonic, or protective. However, antibodies against the *N. gonorrhoeae* PIII protein can be either protective or harmful (because of a blocking activity) (62). Additionally, two homologs of TpN50, OmpA and PIII, have been shown to play a role in pathogenesis (62, 77). It is plausible that TpN50 is located in the outer membrane of *T. pallidum* and plays a role in pathogenesis. Indirect evidence using the blue halo assay (26) suggests that TpN50 is localized to either the periplasm or outer membrane of *E. coli* (data not shown). It is intriguing that TpN50 is homologous to OmpA, since the OmpA protein does not require the presence of lipopolysaccharide (LPS) for proper outer membrane insertion (63) and *T. pallidum* lacks LPS (2). The synthesis and membrane incorporation of the *E. coli* OmpC and OmpF porin proteins have been shown to be dependent on the presence of LPS (63). When *E. coli* cells were exposed to cerulenin, which blocks LPS synthesis, the OmpC and OmpF proteins were no longer incorporated into the outer membrane whereas the OmpA protein was normally localized (63).

The OprF protein (and possibly the OmpA protein) has been shown to function as a porin in addition to its structural role. Outer membrane porins are generally in the range of 28 to 48 kDa (31). There are exceptions to this, however. For example, *Treponema denticola*, an organism that is related to *T. pallidum*, has recently been shown to have an outer sheath protein with a molecular mass of 53 kDa that functions as a porin (18). The TpN50 protein and the *T. denticola* 53-kDa protein are not homologous (30). It would be reasonable to assume that *T. pallidum* contains a porin in its outer membrane that facilitates the uptake of nutrients. On the basis of the homology of TpN50 with OprF and OmpA, it is conceivable that TpN50 also functions as a porin. The TpN50 protein is an intriguing protein that warrants further investigation. Experiments are currently under way in our laboratory to study the N terminus, processing, localization, potential pore-forming ability, and vaccinogenic potential of TpN50.

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