The *fbpABC* Locus of *Neisseria gonorrhoeae* Functions in the Periplasm-to-Cytosol Transport of Iron

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We have determined that the DNA sequence downstream of the well-characterized gonococcal *fbp* gene contains two open reading frames: one designated *fbpB*, which encodes a protein proposed to function as a cytoplasmic permease, and one designated *fbpC*, which encodes a protein proposed to function as a nucleotidebinding protein. The *fpbABC* operon composes an iron transport system that is homologous to the *sfu* and *hit* operons previously reported for *Serratia marcescens* and *Haemophilus influenzae*, respectively, and displays elements characteristic of ATP binding cassette transporters. The *fpbABC* operon differs from these loci in that it is lethal when overexpressed in *Escherichia coli*.

A prerequisite for infection by Neisseria gonorrhoeae is the ability to multiply within the human host. Many factors contribute to this, including the capacity to compete for hostsequestered iron (13, 14, 16). For N. gonorrhoeae, the process of iron acquisition begins in vivo with the binding of an iron source (e.g., human transferrin) by outer membrane protein receptors (12, 16, 21). Previous studies have determined that upon entry into the periplasm, free iron is chelated by the iron-binding protein Fbp (ferric-iron-binding protein), which initiates the transport of iron from the periplasm to the cytosol (9, 16, 17). Molecular details of the transport of Fbp-bound iron from the periplasm to the cytosol have not been previously described. An emerging theme in the biology of import and export processes across membranes is facilitation by the general class of ATP binding cassette (ABC) transporters (the ATP-binding cassette is a hallmark of transport systems of this type) (11). In gram-negative bacteria, active transport of molecules from the periplasm to the cytosol often involves a periplasmic binding protein in conjunction with an ABC transporter (11). We have previously suggested that periplasm-tocytosol transport of iron by N. gonorrhoeae proceeds by an analogous mechanism (9). Therefore, at least two further activities (that have not been previously described), that of a cytoplasmic permease and that of a nucleotide-binding protein, would logically be implicated in gonococcal periplasm-to-cytosol iron transport. This report describes the genetic loci and physical existence of these activities.

Sequence and genetic organization of the neisserial fbp operon. The major focus of this study was to demonstrate the existence of the fbpABC operon. The fbp gene sequence has been well characterized (5, 6, 22) and has been designated fbpA for the purpose of this study. Since fbpA had already been sequenced (5, 6, 22), only partial sequencing of this gene near its 3' end was performed. Sequencing downstream of fbpA was accomplished by digestion of gonococcal chromosomal DNA with RsaI followed by ligation into the RsaI site of pUC18. This ligation mixture was used as a template for a standard PCR

with the oligonucleotides FbpTaa and For, as described previously by Berish et al. (5, 6) (Table 1). This generated a PCR fragment of approximately 1,350 bp that contained one-half of the *fbpB* gene sequence. Similarly, primer WLK1 (Table 1) was used with ClaI-digested genomic DNA after ligation into the ClaI site of pUC18. This consistently amplified a 2,500-bp fragment that encoded regions overlapping the *fbpB* gene locus to 3' of the *fbpC* locus. Primers based upon known sequences were developed and used in the preparation of various PCR products for sequencing (Fig. 1), as previously described (7). The accuracy of the sequences obtained was ensured by multiple rounds of sequencing on different PCR templates with a wide panel of different overlapping primers for both strands (Fig. 1). The sequence of the complete *fbpABC* operon obtained from N. gonorrhoeae F62 is reported in Fig. 1. Sequence analysis of the *fbpA* locus has been reported previously by Berish et al. (4, 5) and Zhou and Spratt (22). The latter reported the presence of an additional codon that would result in an insertion of Ala at position 231 of the mature Fbp amino acid sequence. Subsequent analysis of our sequencing results has confirmed the presence of this codon. A 60-bp intragenic region follows the *fbpA* stop codon and includes a strong stemloop structure with the potential to form 38 hydrogen bonds (8). Analogous structures are predicted to be in similar positions for the hit and sfu operons (data not shown). Since stemloop structures can function in message stability (19), the presence of this structure may allow Fbp expression at levels much higher than those of the fbpB and fbpC gene products. This is consistent with our inability to detect proteins corresponding to FbpB and FbpC in iron-stressed gonococcal membranes or soluble extracts or in Escherichia coli constructs expressing the fbpABC operon, as described below (data not shown).

The open reading frames (ORFs) of the *fbpB* and *fbpC* gene sequences were deduced on the basis of homology to those previously reported for *hitBC* (20) and *sfuBC* (2). The predicted product of *fbpB* is compared with the proposed cytoplasmic permeases (HitB and SfuB) in Fig. 2A, and the predicted product of *fbpC* is compared with the nucleotidebinding protein (HitC and SfuC) components of the previously described operons in Fig. 2B.

The predicted FbpB protein is a 511-amino-acid polypeptide

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Strain, plasmid, or primer	Relevant characteristics				
Strains					
N. gonorrhoeae F62	Genomic DNA for PCR sequencing and amplification of <i>fbpABC</i> fragment	S. Morse			
E. coli DH5aMCR	Host for cloning <i>fbpABC</i> operon	BRL			
E. coli H-1443	aroB mutant; no growth on nutrient agar containing 200, 2,2'-dipyridyl	V. Braun			
Plasmids					
pREP4	3.7-kb plasmid derived from a p15A replicon which expresses large quantities of <i>lac</i> repressor; Kan ^r	Qiagen			
pBSKS ⁻	3.1-kb plasmid used as a vector for expression of the $fbpABC$ operon in these studies; Amp ^r	Stratagene			
pUC18	2.6-kb plasmid used as a ligation vector for site-specific PCR in these studies	BRL^a			
pAFbpO	FbpO 3.6-kb PCR fragment of the <i>fbpABC</i> operon ligated into the <i>Eco</i> RV and <i>Bam</i> HI sites of pBSKS ⁻ . This plasmid puts the Fbp operon under the control of the <i>lac</i> repressor and is selected for by Amp ^r .				
Primers					
FBPTaa	Oligonucleotide used in site-specific PCR for RsaI ligation; 5'GAAAAAGAACACGCCACCCGGCTG3'	This study			
For	Oligonucleotide used in site-specific PCR for both <i>RsaI</i> and <i>ClaI</i> ligations; 5'CCCAGTCACGACGTTGTAA AACG3'	BRL			
WLK1	Oligonucleotide used in site-specific PCR for ClaI ligation; 5'CGGACACTTCTTTATTTTCAGGAC3'	This study			
FbpO-3'BamHI	3' oligonucleotide used for the amplification of the <i>fbpABC</i> operon; 5'CGGGATCCAAGATAAATATCCCG CAGGCATTGTGG3'	This study			
F4-ScaI	5' oligonucleotide used for the amplification of the <i>fbpABC</i> operon; 5'AAAAAGTACTCGATATGAAAACA TCTATCCGA3'	This study			

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^a BRL, Bethesda Research Laboratories, Inc.

with an estimated molecular weight of 56,320. Comparison of FbpB with the predicted HitB sequence by BESTFIT analysis indicates an identity of 64.4% and a similarity of 77.5%. A similar comparison between the *fbpB* and *sfuB* gene products indicates an identity of 34.9% and a similarity of 58.2%. These values are similar to the homologies reported for the FbpA, SfuA, and HitA protein homologs (1, 4). FbpB is proposed to function as a cytoplasmic membrane permease. Optimal alignment of FbpB, HitB, and SfuB proteins identifies 11 regions of primary sequence that, on the basis of the algorithm of Persson and Argos (18), are predictive of transmembrane segments. These segments are commonly associated with membrane permeases (11). In addition, two sequences that match the consensus permease motif EAA---G-----I-LP can be identified (Fig. 2Å). These regions are thought to be located on cytoplasmic loops that interact with the ATP-binding protein component (11). This sequence and location are analogous to those previously reported for MalF, MalG, HisQ, HisM, and OppC, all of which are well-characterized cytoplasmic permeases (10, 11).

The ORF corresponding to *fbpC* encodes a 357-residue peptide with a predicted molecular weight of 38,173. A comparison of FbpC with the *hitC* and *sfuC* gene products indicates 51 and 40% identity and 68 and 58% similarity, respectively. In contrast to FbpB, which is composed of 62% hydrophobic amino acids (indicative of an integral membrane protein), FbpC is composed of 50.5% hydrophobic residues. FbpC is proposed to interact with FbpB to supply the energy for the transport of iron across the cytoplasmic membrane through the binding and hydrolysis of a nucleotide triphosphate. A common ATP binding domain is characteristic of the nucleotidebinding protein components of ABC transporters. These domains are about 200 residues in length and have considerable sequence identity. Short consensus sequences designated the Walker A and B motifs are specifically positioned within this 200-residue region (11). Comparison of the fbpC, hitC, and sfuC gene products demonstrates highly conserved Walker A and B motifs (Fig. 2B).

Comparison of the ORFs derived from *fbpBC* with those

from hitBC and sfuBC illustrates sequence similarities conserved among the general class of ABC transporters (11). This observation, coupled with the homology across this operon, argues that the *fbp*, *hit*, and *sfu* operons have evolved separately but function similarly in the periplasm-to-cytosol transport of free iron.

Cloning of the intact fbpABC operon. The cloning of the fbpABC operon was initially attempted with high- and medium-copy-number vectors such as pUC19 and pBR322. These cloning attempts led to constructs in which all or portions of the *fbpABC* operon were spontaneously deleted (data not shown), suggesting that expression of the complete operon was lethal in E. coli. To overcome this problem, expression of the *fbpABC* operon was placed under the control of the isopropyl- β -D-thiogalactopyranoside (IPTG)-inducible *lac* promoter on pBSKS⁻. In order to clone the operon into this vector, primers with engineered restriction sites were synthesized on the basis of the *fbpABC* operon DNA sequence (Table 1). PCR amplification with these primers yielded a 3.6-kb fragment that was subsequently ligated into the EcoRV and BamHI sites of pB-SKS⁻, creating a fusion with *lacZ*. The ligation mixture was then used to transform E. coli DH5αMCR carrying pREP4 (a p15A replicon derived from pACYC184) (3). The plasmid pREP4 overexpresses LacI, and in the absence of IPTG, genes driven from the lac promoter are not transcribed. From this ligation mixture, 86 transformants were obtained; they were transferred to Luria-Bertani (LB) agar with ampicillin and kanamycin, with or without 32 mg of IPTG per ml. Nine of 86 transformants grew poorly on LB agar containing IPTG whereas all grew well on medium containing no IPTG, suggesting that upregulation of this operon is inhibitory. Plasmid isolation and restriction enzyme analysis indicated that of the nine transformants that grew poorly in the presence of IPTG, six contained pREP4 and pBSKS- with an intact fbpABC operon (data not shown).

Toxicity associated with expression of the intact *fbpABC* operon in *E. coli*. *E. coli* DH5 α MCR(pREP4) transformed with either pAFbpO or pBSKS⁻ was inoculated into LB broth and grown to mid-log phase, and the *fbpABC* operon was

T V Y N G Q H K E A A Q A V A D A F T R A T G I K V K CTC AAC AGT GCC AAA GGC GAC CAG CTT GCC GGC CAA ATC AAA GAA GAA GGC AGC CGA AGC CCG GCC GAC GTA TTC TAT TCC L N S A K G D Q L A G Q I K E E G S R S P A D V F Y S GAA CAA ATC CCG GCA CTC GCC ACC CTT TCC GCA GCC AGC CTC CTA GAG CCC CTG CCC GCC TCC ACC ATC AAC GAA ACA CGC E Q I P A L A T L S A A N L L E P L P A S T I N E T R GGC AAA GGC GTG CCG GTT GCC GCC AAA AAA GAC TGG GTG GCA CTG AGC GGA CGT TCG CGC GTC GTC TAC GAC ACC CGC G K G V P V A A K K D W V A L S G R S R V V V Y D T R AAA CTG TCT GAA AAA GAT TTG GAA AAA TCC GTC CTG AAT TAC GCC ACG CCG AAA TGG AAA AAC CGC ATC GGT TAC GTC CCC K L S E K D L E K S V L N Y A T P K W K N K L G T V P ACT TCC GGC GCG TTC TTG GAA CAG ATT GTC GCC ATC GTC AAA CTG AAA GGC GAA GCG GCC GCA TTG AAA TGG CTC AAA GGC T S G A F L E Q I V A I V K L K G E A A A L K W L K G CTG AAA GAA TAC GGC AAG CCT TAC GCT AAA AAC TCC GTC GCC CTT CAA GCG GTT GAA AAC GGC GAA ATC GAT GCC GCC CTC LKEYGKPYAKNSVALQAVENGEIDAA ATC AAC AAC TAC TGG CAC GCT TTC GCG CGT GAA AAA GGC GTA CAA AAT GTC CAC ACC CGC CTG AAT TTC GTC CGC CAC T R L L E Q A G M K ACT ATG TCT CCT AAA AAA ATA CCC ATT TGG CTT ACC GGC CTC ATC CTA CTG ATC GCC CTG CCG CTT ACC CTG CCT TTT TTA T M S P K K I P I W L T G L I L L I A L P L T L P F L TAT GTC GCT ATG CGT TGG TGG TGG GTC GGC ATC AAC CGC GCC GTC GAA CTG TTG TTC CGC CCG GGT ATG TGG GGG GAT TTG CTC Y V A M R S W Q V G I N R A V E L L F R P R M W D L TCC AAC ACC TTG ACG ATG ATG GGG GGC GTT ACC CTG ATT TCC ATT GTT TTG GGC ATT GCT GGG CCC TTT TGT TCC AAC GTT <u>S N T L T M M A G V T L I S I V L G</u> I A A P F C S N V ACC GCT TCT TCG GGC AAA ACC TTT TTT CÁG AGG GCA ATC ACC CTG CCT TTG TGC ATC CCC GCA TTT GTC AGC TGT TTC ACC TASSGKTFFQTAITLPLCIPAFVSCFT TGGATCAGCCTGACCTTCCGTGTCGAAGGATTTTGGGGGACAGTGATGATGAGCCTGTCCTCGCTTCCGCTCGCCTAC W I S L T F R V E G F W G T V M I M S L S S F P L A Y CTG CCC GTC GAG GCG GCA CTC AAA CGC ATC AGC CTG TCT TAC GAA GAA GTC AGC CTG TCT TAC GAA AGC CGC CTG CAA L P V E A A L K R I S L S Y E E V S L S L S L G K S R L Q ACC TIT TIT TCC GCC ATC CTC CCA CAG TIC AAA CCC GTC ATC GGT AGC AGC GTG TTA CTG ATC GCC CTG CAT ATG CTG GTC T F F S A I L <u>P Q F K P V I C</u> S S V L L I A L H M L V GAA ATT TGT CGC GGT ATC CAT TTT GGA ACT ACC CCA CTT TTA CCA CTG GCA TTT TCC AAG AAT ACG AAA TGT CCT ACA ACA ACA ATT CCC CGC CTG CCC TGG TTT TCC GTT GTG TGG ACG GCG GTG TGC GGA ATC GTC GTA TTT GGA GAA AGC ATA TTT CGC T I P R L P W F S V V W T A V C G I V V F G E S I F R GGA AAA GCC AAG ATT TAC CAC AGC GGA AAA GGG GTT GCC CGT CCT TAT CCC GTC AAA ACC CTC AAA CTG CCC GGT CAG ATC GC GCG ATT GTT TTT AGC AGC TTG TTG ATT TTG GGC ATT ATT ATC CCC TTT GGC GTA TTG ATA CAT TGG ATG ATG GTC G A I V F L S S L L I L G I I I P F G V L I H W M M V GGC ACT TCC GGC ACA TTC GCG CTC GTA TCC GTA TTC GAT GCC TTT ATC CGT TCC TTA AGC GTA TCG GCT TTA GGT GCG ATT G T S G T F A L V S V F D A F I R S L S V S A L G A I TTG ACT ATA TTA TGT GCC TTG CCC CTT GTT TGG GCA TCG GTT CGC TAT CGC AAT TTT TTA ACC GTT TGG ATA GAC AGG CTG LT I L C A L P L V W A S V R Y R N F L T V W I D R L CCG TTT TTA CTG CAC GCC GTC CCC GGT TTG GTT ATC GCC CTA TCC TTG GTT TAT TTC AGC ATC AAC TAC ACC CCT GCC GTT P F L L H A V P G L V I A L S L V Y F S I N Y T P A V TAC CAA ACC TIT AIC GIC GIC ATC CTT GCC TAT TIC ATG CTT TAC CTG CCG ATG GCG CAA ACC ACC CTG AGG ACT TCC TTG <u>Y Q T F I V V I L A</u> Y F M L Y L P M A Q T T L R T S L GAA CAA CTC CCC AAA GGG ATG GAA CAG GTC GGC GCA ACA TTG GGG CGC GGA CAC TTC TTT ATT TTC AGG ACG TTG GTA CTG E Q L P K G M E Q V G A T L G R G <u>eH F F I F R T</u> L V L CCG TCC ATC CTG CCC GGC ATT ACC GCC GCA TTC GCA CTC GTC TTC CTC AAG <u>CTG ATG AAA GAG TTG ACC GCC ACC CTG CTG</u> PSILPGITAAFALVFLKLMKELIA<u>ILL</u> CTG ACC GCC GAC GAT GTC CACA GG CTC TCC ACC GCC GTT TGG GAA TAC ACA TCG GAC GAA TAC GCC GCC GCC GCC CCT L T A D D V H T L S T A V W E Y T S D A Q Y A A A T P TAC GCG CTG ATG CTG GTG TTA TTT TCC GGC ATA CCC GTA TTC CTG CTG AAG AAA TAC GCC TTC AAA TAA CAGCTTGAGGAAGCACCGCT ATG ACC GCC GCC CTG CAC ATC GGA CAC CTG TCC AAA AGT TTT CAA AAC ACC CCG GTT TTA AAC GAC ATT TCG CTC AGC CTC M T A A A L H I G H L S K S F Q N T P V L N D I S L S L GAC CCG GGA GAA ATT CTC TTT ATC ATC GGG GCG TCC GGC TGC GGT AAA ACC ACC CTT TTA CGC TGC CTT GCC GGT TTC GAA <u>DPGEILFIIGASG</u> CKIILLRCLAGFE <u>CAA CCC GAT TEC GGG</u> GAA ATT TCG CTT TCC GGC AAA ACC ATC TTC TCG AAA AAT ACC AAC CTT CCC GTC CGA GAA ACG ACG Q P D S G E I S L S G K T I F S K N T N L P V, R E T T TTI GGG TTA CCT CGT ACA GGA AGG TGT TCT GTT CCC CAC CTG ACC GTT TAC CGC AAT ATC GCC TAC GGT CTC GGC AAC GGC F G L P R T G R C S V P H L T V Y R N T A Y G L G N G F G <u>L P R T G R C S V P H L T V Y R N I A Y G L G N G</u> AAA GGC AGG ACG GCG CAA GAG CGA CAG CGC ATC GAA GCC ATG TTG GAA TTG ACC GGC ATT TCC GAA CTT GCC GGA CGC TAT K G R T A Q E R Q R I E A M L E L T G I S E L A G R Y CCG CAC GAA CTT TCG GGC GGA CAA CAA CAG CGC GTC GCC CTC GCC CCC GCC CCC GAC CCC GAA CTG ATT TTG TTG P H E L S G G Q Q Q R V A L A R A L A R D P E L I L L GAC GAA CCC TTC AGC GCG CTG GAC GAA CAG TTG CGC CGC CAG ATT GCC GAA GAC ATG ATT GCC GCC CTG CGC GCC AAC GGA D E P F S A L D E Q L R R Q I R E D M I A A L R A N G AAA TEC GEC GTT TTT GTE AGE CAE GAE CGE GAA GAA GEE CTG CATA TAE GEE GAE CGG ATT GEE GTG ATG AAA CAG GEG CGE K S A V F V S H D R E E A L Q Y A D <mark>R I A V M K Q</mark> G R ATC CTC CAA ACC GCA AGC CCT CAC GAA TTG TAC CGA CAA CCT GCC GAC CTT GAT GCC GTC CTG TTT ATC GGA GAA GGC ATC I L Q T A S P H E L Y R Q P A D L D A V L F I G E G I GTG TTC CCC GCC GCG CTC AAC GCC GAC GGC ACC GCC GAT TGC AGA TTG GGC GGC CTG CCC GTC CAA AGC GGC GCA CCC GCA V F P A A L N A D G T A D C R L G R L P V Q S G A P A GGC ACG CGC GGT ACA CTG CTC CATC CGT CGC GAA CAG TTC AGC CTT CAC CCC CAT TCC GCA CCC GTC GTC TCC ATT CAC GCC GT R G T L L I R P E Q F S L H P H S A P V V S I H A GTG GTT CTC AAA ACC ACG CCC AAA GCG CGG TAT ACC GAA ATC AGC CTC AGG GCC GGA CAA ACC GTC CTC AGG CTC AAC CTC

FIG. 1. Sequence of the gonococcal *fbpABC* operon. DNA sequence of the 3,862-bp *fbpABC* operon is depicted with predicted ORFs. Primers used in the sequencing of the operon are shown as arrows above the appropriate sites. —, primer corresponding to complementary strand; —, primer corresponding to coding strand. A predicted stem-loop structure within the intragenic region between *fbpA* and *fbpB* is indicated by black boxes. An Ala at position 231 has been added to the mature FbpA sequence previously reported (6) and is denoted by an open box.

induced by the addition of 1 mM IPTG. Upon the addition of IPTG, the growth of *E. coli* DH5 α MCR(pREP4/pAFbpO) was rapidly inhibited, whereas the growth of *E. coli* DH5 α MCR (pREP4/pBSKS⁻) was not (Fig. 3). Comparison of viable counts of IPTG-induced organisms on LB agar indicated that in-

creased expression of the *fbpABC* operon in *E. coli* was bactericidal rather than bacteriostatic (data not shown). This observation may explain the general difficulty previously encountered in attempts to clone *fbp* and linked sequences (6).

Overexpression of either FbpA (4) or FbpC (unpublished

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A	(
	Fl H S	bpB, itB fuB	MSNLSTHAP	Q TARRYSVVP	M SPKKIPIWL . LPRRPPFWL R HPRPGAIVV [F GLILLIALPL F LLIILIGLPL / SAVLLSLLAL TM	TLPFLYVAMR CLPFLYVILR -LPLGFVIGV (1	SWQVGINRAV ATEVGLTRSV AFETGWQTVK]	ELLFRPRMWD ELLFRPRMAE ALVFRPRVAE	LLSNTLTMMA LLSNTMLLMV LLLNTLLLVV [65 60 79
			GVTLISIVI CVTIGAISI LTLPICAVI TM 2	G IAAPFCSNV G TLCAFLLER G VALAWLTER	T ASSGKTFFQ Y RFFGKAFFE T TLPGRRLWA [F AITLPLCIPA / AMTLPLCIPA / LATAPLAVPA TM 3	FVSCFTWISL FVSCFTWISL FVQSYAWISL]	TFRVEGFWGT TFRVEGFWGT VPSMHGLGAG	VMIMSLSSFP IGIMTLSSFP VFISVLAYFP	LAYLPVEAAL LAYLPVSAIL FIYLPAAAVL	145 140 159
			E KRISLSYEE KRLDRSLEE RRLDPGIEI	ZA AG ZV SLSLGKSRL ZV SLSLGKSPV ZV ATSLGSRPP	I-LP Q TFFSAILPOI Y TFWYAIFPOI A VFFRVVLPOI	F KPVIGSSVLL L KPAIGSSILL L KLAVCGGSLL [IALHMLVEIC IALHMLVEFG IALHLLAEYG	RGIHFGTTPL AVSILNYQTF LYAMIRFDTF]	LPLAFSKNTK TTAIFQEYEM TTAIFDQFQS	CPTTTIPRLP SFNNSTAAL- TFNGPAANM- [225 219 238
			WFSVVWTAU -LSAVLMAI -LAGVLVLC	/C GIVVFGESI C ILIVFGEIF C LGLLLLEAI TM 5-	F RGKAKIYHS F RGKQTLYHS S RGRARYARV	G KGVARPYPVK G KGVMRPYLVK G SGSARSQTPR]	TLKLPGQIGA STLSFGKQCLT RLSPPLAALA [IVFLSSLLIL FGFFSSIFIL LLLPIALTAL	GIIIPFGVLI SIGVPVIMLI ALGVPFITLA M 6	HWMMVGTSGT YWLIVGTSLE RWLWLGGFEV]	305 298 317
			FALVSVF SAGDFSEFI WRNAELW	PD AFIRSLSVS. LS AFSNSFIIS NP ALWQTLSLS. [-	A LGAILTILC G LGALLTVMC A AGALLITLC TM	A LPLVWASVRY A LPLVWAAVRY A IPMAWLSVRY 7]	RNFLTVWIDR RSYLTIWIDR PARLYRVLEG [LPFLLHAVPG LPYLLHAVPG CNYVTSSLPG TM	LVIALSLVYF LVIALSLVYF IVVALALVTI 8]	SINYTPAVYQ SIHYANDLYQ TIHSFRPIYQ [383 378 395
			TFIVVILAY TFFVIIIAY TEITLLLAY	YF MLYLPMAQT YF MLYLPMAQT YL LMFMPRALI 19]	T LRTSLEQLPI T LRASLEQLSI N LRAGIAQAP	EAAG K GMEQVGATLG O QIEKVGQSLG / ELENVARSL <mark>G</mark>	I RGHFFIFRTL RNPFYIFRTL KSPAQALWST [-LP VLPSILPGIT TLPAILPGVA TLRLAAPGVA TM	AAFALVFLKL AAFALVFLNL AGAALVFLAI 10	MKELTATLLL MKELTATLLL ANELTATLLL]	463 458 475
			TADDVHTLS TSNDIKTLS APNGTRTLA	T AVWEYTSDA I AVWEHTSDA T GFWALTSEI	Q YAAATPYALI Q YAAATPYALI D YVAAAPYAL [M LVLFSGIPVF M LVLFSGIPVF I MVALSLPLTW TM 11	LLKKYA LLKKYA LLYSQSKRTA]	FK 511 FK 507 GL 528			
	FbpC HitC SfuC	2 C MR C	.GSTAMTA LNKMINNP MS	ALHIGHLSKS LLTVKNLNKF TLELHGIGKS	FONTPVLNDI FNEQQVLHDI YNAIRVLEHI	SLSLDPGEIL SFSLQRGEIL DLQVAAGSRT	FII <mark>GASG</mark> CGK FLLGASGCGK AIVGPSGSGK GPSGSGK Walke	TTLLRCLAGF TTLLRAIAGF TTLLRIAGF STLLR or A	EQPDSGEISL EQPSNGEIWL EIPDGGQILL	SGKTIFSKNT KERLIFGENF QGQAMGNGSG	77 80 72
		NL NL WV	PVRETTFG PTQQRHLG PAHLRGIG	LPRTGRCSVP YVVQEGILFP FVPQDGALFP	HLTVYRNIAY HLNVYRNIAY HFTVAGNIGF	GLGNGKGRTA GLGNGKGKNS GLKG <mark>GK</mark> R	QERQRIEAML EEKTRIEQIM EKQRRIEALM	ELTGI-SELA QLTGI-FELA EMVALDRRLA	GRYPHELSGG DRFPHQLSGG ALWPHELSGG	QQQRVALARA QQQRVALARA QQQRVALARA	150 159 149
		LA LA LS	PDPELILL PNPELILL QQPRLMLL VLLL W	DEPFSALDEQ DEPFSALDEH DEPFSALDTG DEPTSALD alker B	LRRQIREDMI LRQQIRQEML LRAATRKAVA	AALRANGKSA QALRQSGASA ELLTEAKVAS	VFVSHDREEA IFVTHDRDEA ILVTHDQSEA	LQYADRIAVM LRYADKIAII LSFADQVAVM	KQGRILQTAS QQGKILQIDT RSGRLAQVGA	PHELYRQPAD PRTLYWSPNH PQDLYLRPVD	236 239 229
		LD LE EP	AVLFIGEG TAKFMGES TASFLGET	IVFPAALNAD IVLPANLLDE LVLTAEL-AH	GTADCRLGRL NTAQCQLGNI GWADCALGRI	PVQSGAPAGT PIKNKSISQN AVDDRQRSG-	RGTLLIRPEQ QGRILLRPEQ PARIMLRPEQ	FSL-HPHSAP FSLFKTSENP IQIGLSDP	VVSIHAVVLK TALFNGQIKQ AQRGQAVITG	TTPKARYTEI IEFKGKITSI IDFAGFVSTL	315 319 305

SLRAGQTV-- LTLNLPSAPT LSDGISAVLH LDGPALFFPG NTL* 357 QIEINGYA-- IWIENVISPD LSIGDNLPVY LHKKGLFYA* 357 NLQMAATGAQ LEIKTVSREG LRPGAQVTLN VMGQAHIFAG * 346

FIG. 2. Comparison of the predicted amino acid sequences of the proposed ABC transporters encoded by the *fbp*, *hit*, and *sfu* operons. (A) Comparison of the predicted amino acid sequences of FbpB, HitB, and SfuB demonstrates the predicted transmembrane regions which are characteristic of cytoplasmic permeases. The predicted transmembrane regions are indicated as TM 1 through TM 11. A sequence motif common to cytoplasmic permeases can be found twice within this sequence and is indicated by text beginning "EAA" above the amino acid sequences. (B) Comparison of predicted amino acid sequences of FbpC, HitC, and SfuC, the proposed nucleotide-binding components. The Walker motifs conserved across the predicted FbpC, HitC, and SfuC protein sequences are indicated with boxes. For both panels, gaps in the alignment are designated with a dash.



FIG. 3. Toxicity of the *fbpABC* operon for *E. coli* DH5 α MCR(pREP4). To demonstrate lethality due to increased expression of the gonococcal *fbpABC* operon in *E. coli*, *E. coli* DH5 α MCR(pREP4/pAFbpO) (closed circles) and DH5 α MCR(pREP4/pBSKS⁻) (closed squares) were grown as described in the text. At mid-log phase the cultures were divided and IPTG was added to the culture (indicated by the arrow). Growth was monitored turbidometrically into stationary phase (>5 h).

data) has been readily achieved. Clones expressing even the 5' one-third of FbpB exhibited growth kinetics analogous to that of the intact *fbpABC* operon in *E. coli*, suggesting that increased expression of the hydrophobic *fbpB* gene product was specifically responsible for the lethality associated with this operon. This toxicity remained associated with *fbpB*, even when only a partial gene product was produced (data not shown).

The functionality of the *fbpABC* operon in iron transport was demonstrated in a fashion similar to that reported for the analogous operons in *Serratia marcescens* (23) and *Haemophilus influenzae* (1). Briefly, this entailed demonstrating that the presence of pAFbpO enabled an *aroB E. coli* strain to grow on nutrient agar containing inhibitory concentrations of the iron chelator 2,2'-dipyridyl. Similar to what has been previously described (1, 23), growth of single microcolonies could be observed (data not shown), indicating that like HitABC and SfuABC, FbpABC could complement the periplasm-to-cytosol transport of iron in an *E. coli* background.

The presence of *fbp* operon homologs in *H. influenzae* and *S. marcescens* suggests that the function of this operon is conserved across species boundaries. For *Neisseria* spp., the presence of *fbpABC* correlates with the ability of a strain to obtain iron from transferrin or lactoferrin (13, 14) and with the ability of these organisms to cause disease (15). These observations underscore the importance of an efficient iron acquisition system for the pathogenesis of bacterial infection.

Nucleotide sequence accession number. The sequence of *fbpABC* described in the report is listed in GenBank under the accession number U33937.

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REFERENCES

- Adhikari, P., S. D. Kirby, A. J. Nowalk, K. L. Veraldi, A. B. Schryvers, and T. A. Mietzner. 1995. Biochemical characterization of a *Haemophilus influenzae* periplasmic iron transport operon. J. Biol. Chem. 270:25142–25149.
- Angerer, A., S. Gaisser, and V. Braun. 1990. Nucleotide sequences of the sfuA, sfuB, and sfuC genes of Serratia marcescens suggest a periplasmicbinding-protein-dependent iron transport mechanism. J. Bacteriol. 172:572– 578.
- Anonymous. 1995. QIAexpress expression system, p. 69. Qiagen product guide. Qiagen, Chatsworth, Calif.
- Berish, S. A., C.-Y. Chen, T. A. Mietzner, and S. A. Morse. 1991. Expression of a functional neisserial Fbp gene in *Escherichia coli*. Mol. Microbiol. 6: 2607–2615.
- Berish, S. A., D. Kapczunski, and S. A. Morse. 1990. Sequence of the meningococcal Fbp gene. Nucleic Acids Res. 18:4596.
- Berish, S. A., T. A. Mietzner, L. W. Mayer, C. W. Genco, B. P. Holloway, and S. A. Morse. 1990. Molecular cloning and characterization of the structural gene for the major iron-regulated protein expressed by *Neisseria gonorrhoeae*. J. Exp. Med. 171:1535–1546.
- Berish, S. A., S. Subbarao, C.-Y. Chen, D. L. Trees, and S. A. Morse. 1993. Identification and cloning of a *fur* homolog from *Neisseria gonorrhoeae*. Infect. Immun. **61**:4599–4606.
- Brendel, V., and E. N. Trifonov. 1984. A computer algorithm for testing potential prokaryotic terminators. Nucleic Acids Res. 12:4411–4427.
- Chen, C.-Y., S. A. Berish, S. A. Morse, and T. A. Mietzner. 1993. The ferric iron-binding protein of pathogenic *Neisseria* spp. functions as a periplasmic transport protein in iron acquisition from human transferrin. Mol. Microbiol. 10:311–318.
- Dassa, E., and M. Hofnung. 1985. Sequence of gene malG in E. coli K12: homologies between integral membrane components from binding proteindependent transport systems. EMBO J. 4:2287–2293.
- Higgins, C. F. 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. 8:67–113.
- Lee, B., and A. B. Schryvers. 1988. Specificity of the lactoferrin and transferrin receptors in *Neisseria gonorrhoeae*. Mol. Microbiol. 2:827–829.
- Mickelsen, P. A., E. Blackman, and P. F. Sparling. 1982. Ability of Neisseria gonorrhoeae, Neisseria meningitidis, and commensal Neisseria species to obtain iron from lactoferrin. Infect. Immun. 35:915–920.
- Mickelsen, P. A., and P. F. Sparling. 1981. Ability of Neisseria gonorrhoeae, Neisseria meningitidis, and commensal Neisseria species to obtain iron from transferrin and iron compounds. Infect. Immun. 33:555–564.
- Mietzner, T. A., G. H. Luginbuhl, E. Sandstrom, and S. A. Morse. 1984. Identification of an iron-regulated 37,000-dalton protein in the cell envelope of *Neisseria gonorrhoeae*. Infect. Immun. 45:410–416.
- Mietzner, T. A., and S. A. Morse. 1994. The role of iron-binding proteins in the survival of pathogenic bacteria. Annu. Rev. Nutr. 14:471–493.
- Nowalk, A. J., S. B. Tencza, and T. A. Mietzner. 1994. Coordination of iron by the ferric iron-binding protein of pathogenic *Neisseria* is homologous to the transferrins. Biochemistry 33:12769–12775.
- Persson, B., and P. Argos. 1994. Prediction of transmembrane segments in proteins utilising multiple sequence alignments. J. Mol. Biol. 237:182–192.
- Petersen, C. 1991. Multiple determinants of functional mRNA stability: sequence alterations at either end of the *lacZ* gene affect the rate of mRNA inactivation. J. Bacteriol. **173**:2167–2172.
- Sanders, J. D., L. D. Cope, and E. J. Hansen. 1994. Identification of a locus involved in the utilization of iron by *Haemophilus influenzae*. Infect. Immun. 62:4515–4525.
- Schryvers, A. B., and B. C. Lee. 1989. Comparative analysis of the transferrin and lactoferrin binding proteins in the family *Neisseriaceae*. Can. J. Microbiol. 35:409–415.
- Zhou, J., and B. G. Spratt. 1992. Sequence diversity within the *argF*, *fbp* and *recA* genes of natural isolates of *Neisseria meningitidis*: interspecies recombination within the *argF* gene. Mol. Microbiol. 6:2135–2146.
- Zimmermann, L., A. Angerer, and V. Braun. 1989. Mechanistically novel iron(III) transport system in *Serratia marcescens*. J. Bacteriol. 171:238–243.