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Molecular Characterization of FrpB, the 70-Kilodalton Iron-Regulated Outer Membrane Protein of *Neisseria meningitidis*

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The structural gene encoding the 70-kDa outer membrane protein FrpB of *Neisseria meningitidis* was cloned and sequenced. A mutant lacking FrpB was constructed. No difference in iron utilization between the mutant and the parental strain was observed. A minor effect of the mutation on serum resistance was observed. A topology model for FrpB in the outer membrane is proposed.

Iron is an essential nutrient for almost all bacteria, but the concentration of free iron in the human host is too low to support growth. In the extracellular compartments of the human body, iron is bound mainly to transferrin (6) and lactoferrin (20). Many bacteria deal with iron limitation by the secretion of siderophores, which are small organic compounds that bind iron very efficiently. The Fe^{3+}-siderophore complex is bound to specific receptors on the bacterial cell surface and internalized. The transport across the outer membrane requires energy, which is provided in *Escherichia coli* by the inner membrane protein TonB (27). The pathogenic neisseriae have a different mechanism of iron utilization. They are able to acquire iron directly from transferrin (22) or lactoferrin (21). Other iron sources are hemin, hemoglobin, citrate, and the siderophore aerobactin (1, 36). The uptake of iron from transferrin or lactoferrin is initiated by binding of these proteins to specific outer membrane receptors (29, 30). Recently, the structural genes for these receptors have been characterized (8, 17, 25, 26). Homology to the TonB-dependent siderophore receptors of *E. coli* was found. FrpB is another iron-limitation-inducible outer membrane protein of *Neisseria meningitidis*. Its function is unknown. Previously, we have raised monoclonal antibodies against this protein, most of which appeared to be bactericidal in an in vitro assay with human complement (24). Furthermore, since the protein is expressed in large amounts in all strains under iron limitation (11), it could be an interesting vaccine candidate for meningococcal disease.

Cloning and sequencing of the *frpB* gene. Initial attempts to clone the *frpB* gene by screening of phage or plasmid libraries with the monoclonal antibodies directed against the protein (24) were unsuccessful. Therefore, another strategy was chosen. First, we tried to determine the N-terminal amino acid sequence of the FrpB protein. Outer membrane proteins isolated as described previously (24) from strain H44/76 were separated on a sodium dodecyl sulfate (SDS)-polyacrylamide gel and blotted onto a ProBlot polyvinylidene difluoride membrane (Applied Biosystems) with a semidry multigel electroblotter (Ancos). The 70-kDa band was cut out and placed in an Applied Biosystems gas phase protein sequencer, model 475, with on-line PTH analysis using the ABI PTH analyzer model 120. However, no signals were obtained, possibly because the N terminus was blocked. Finally, to obtain an internal sequence, the protein was digested with cyanogen bromide (CNBr) as described previously (15). After SDS-polyacrylamide gel electrophoresis (PAGE) and blotting, two peptide fragments of 35 and 18 kDa, respectively, could be detected (data not shown). Sequencing of the 18-kDa fragment revealed two signals in each Edman degradation step, indicating that it consisted of two different peptides. The following sequence was obtained: Gly/Lys-Asp/Gln-Asn/Gln-His/Ser-Arg/Val-Asp/Gly-Ile-Arg/Lys-Thr/Val-Asp/Val-Asn/Arg-Glu/Ala-Glu/Tyr-Ser/Phe-Asp/Thr-Ser/Val-Gln/Gly-Asp/Ile. The 35-kDa band gave the following sequence: Lys-Asp-Arg-Ala-Asp-Glu-Asp-Thr-Val-Xaa-Ala-Tyr-Lys-Leu-Ser. By using the additional knowledge that CNBr cleaves after a Met residue, a degenerate oligonucleotide probe was synthesized on a Biosearch 8600 DNA synthesizer. The probe, with the sequence 5′-AC(G/A/T/C)GT(G/A)TC(C/T)TC(G/A)TC(G/A/T/C)GC(G/A/T/C)C(G/T)(G/A)TC(C/T)TTCAT-3′, was labeled with the digoxigenin oligonucleotide tailing kit (Boehringer Mannheim) and purified by a spun column procedure (19). To prepare an enriched gene library, chromosomal DNA of strain H44/76 was isolated (2), digested with restriction enzymes, and hybridized with the probe on a Southern blot. Southern blotting was performed as before (26) except that the hybridization temperature was 55°C. A 2-kb *Ssp*I fragment hybridized with the probe (data not shown). Subsequently, *Ssp*I-digested chromosomal DNA was separated on an agarose gel and fragments of 1.8 to 2.2 kb were excised and cloned in *Sma*I-digested pUC19. The colonies obtained after transformation of *E. coli* PC2494 (26) were screened by colony blotting with the probe. Among 100 clones screened, three positive ones were detected. Restriction analysis showed that they all contained the same 1.3-kb insert. The insert of one of them, pAM19 (Fig. 1), was recloned in pEMBL18, resulting in pAM20. Deletions in pAM20 were prepared with the double-stranded nested deletion kit (Pharmacia). The *Pst*I and *Bam*HI sites of pAM20 were used as nuclease-resistant and -sensitive ends, respectively. Single-stranded DNA of appropriate plasmids was propagated by using the helper phage VCSM13 (Stratagene). Plasmid DNA was purified after alkaline lysis (28) on Qiagen columns (Diagen). DNA was sequenced manually with the deaza G/A T7 sequencing mixes (Pharmacia) by using the forward and reverse universal sequencing primers and specific primers hybridizing

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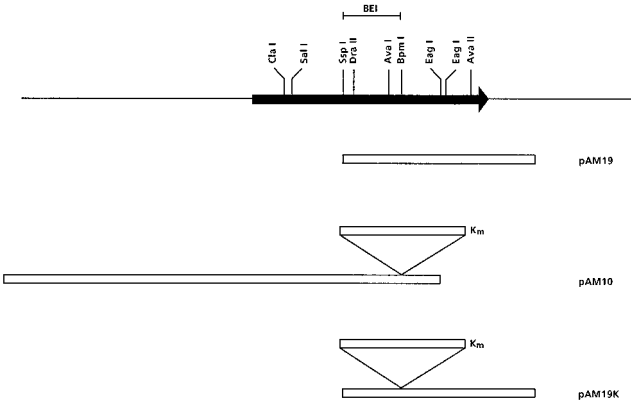

FIG. 1. Restriction map of the DNA fragment encoding the *frpB* gene of strain H44/76. The inserts in the recombinant plasmids are shown as open boxes. The open reading frame is marked with a thick arrow. Probe BE1 used for Southern and colony blotting is indicated above the reading frame. The kanamycin resistance box derived from pUC4K (K_m) is shown above the open boxes for pAM10 and pAM19K.

with *frpB* sequences. The nucleotide sequence revealed the presence of the end of an open reading frame.

To clone the promoter-proximal part of the gene, chromosomal DNA was digested with various enzymes and hybridized with probe BE1 (Fig. 1), which was labeled with the digoxigenin labeling kit (Boehringer Mannheim), in a Southern blot. After the detection of hybridizing bands, fragments of the appropriate length were ligated into pUC19. However, no colonies reacting with probe BE1 were detected on colony blots. Therefore, another strategy had to be used. First, mutant strain CE1415, in which the *frpB* gene is interrupted by a kanamycin resistance gene, was constructed (see below). Chromosomal DNA from this mutant was digested with *Eag*I. A DNA fragment of 6.5 kb reacted on a Southern blot with probe BE1. DNA fragments of this size were excised from the gel, ligated into *Eag*I-digested pBluescript II SK+ (Stratagene), and electroporated (10) into strain DH5α. Of approximately 4,500 colonies obtained, a single one was kanamycin resistant and thus contained the desired DNA fragment. The plasmid was designated pAM10 (Fig. 1). Subcloning of the part of the insert corresponding to the N terminus of the protein failed. The nucleotide sequence was finally obtained by gene walking using pAM10 as a template. The nucleotide sequence of the *frpB* gene and the deduced amino acid sequence are shown in Fig. 2.

Features of the sequence. In the nucleotide sequence, an open reading frame encoding a protein of 720 amino acid residues was detected. Upstream of the putative start codon, a typical Shine-Dalgarno sequence and a sequence reminiscent of a Fur-binding site of *E. coli* could be discerned (Fig. 2). The Fur protein acts as a repressor in conjunction with Fe^{2+} by binding to a 19-bp sequence of iron-regulated promoters (3). In the putative Fur box of *frpB*, 12 of 19 bp are conserved with respect to the consensus sequence in *E. coli*, which is GATA ATGATAATCATTATC. The putative *fur* box in *frpB* is part of a perfect inverted repeat, consisting entirely of A and T base pairs. Another perfect inverted repeat, which could possibly function as a transcriptional terminator, was found downstream of the open reading frame (Fig. 2).

In the deduced amino acid sequence of the FrpB protein, the amino acid sequences of the CNBr fragments could be discerned (Fig. 2), and this confirms the identity of the cloned gene. The protein consists of a putative signal sequence and a

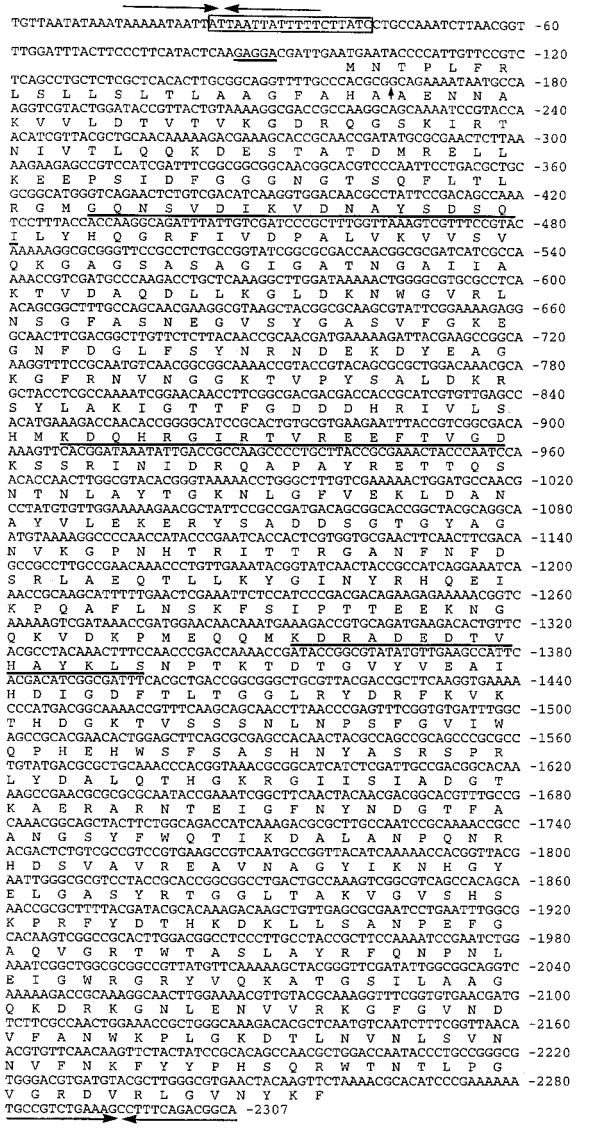

FIG. 2. Nucleotide sequence of the *frpB* gene and the deduced amino acid sequence. The numbers on the right indicate the positions of the nucleotides sequenced. The amino acid sequences determined by protein sequencing are underlined. The first two underlined sequences correspond to the double sequence obtained from the 18-kDa CNBr fragments. The most downstream underlined sequence corresponds to the unambiguously determined sequence of the 35-kDa CNBr fragment. Also the putative Shine-Dalgarno sequence is underlined. The signal sequence cleavage site is marked with an arrow. A possible Fur-binding site is boxed. A putative transcriptional terminator is marked with arrows underneath. An inverted repeat in the promoter region is marked with arrows above. The region upstream of the coding region was sequenced only in one direction.

mature domain of 22 and 698 amino acid residues, respectively. The calculated molecular mass of the mature FrpB protein is 77.3 kDa, which is somewhat higher than its apparent molecular mass of 70 kDa as determined by SDS-PAGE. The isoelectric point is 9.87. Screening of the Swiss Prot database revealed homology to HemR, the hemin-binding outer membrane protein of *Yersinia enterocolitica* (31). The overall identity was 23%. Furthermore, a higher level of homology was observed with CopB, an outer membrane protein of *Moraxella catarrhalis* (13) that is involved in serum resistance (14). The overall identity was 49%. Moreover, sequence stretches with

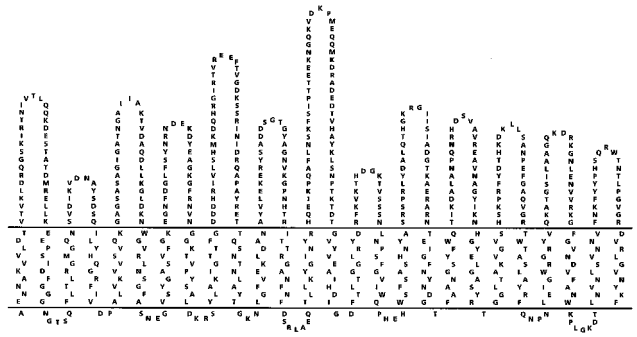

FIG. 3. Topology model of FrpB in the outer membrane. The top part shows the surface-exposed regions, the central part shows the membrane-spanning segments, and the lower part shows the periplasmic loops.

similarity to the four homology boxes found in TonB-dependent siderophore receptors of *E. coli* (18) are present in the FrpB sequence (data not shown).

Topology model for FrpB. Outer membrane proteins of gram-negative bacteria lack hydrophobic stretches long enough to span the membrane in an α-helical conformation. Instead, they consist of antiparallel amphipathic β-strands (9, 35). The cell surface-exposed domains are the most hydrophilic regions, and when related proteins are compared, they show the most variability. The β-strands, which are embedded in the membrane, and the periplasmic domains are more conserved. Furthermore, by applying secondary structure predictions, β-turns are found in most of the periplasmic and exposed loops. Thus, by applying these criteria, the topology of outer membrane proteins can be predicted (for a review, see reference 33). We constructed a topology model for FrpB by applying these general rules. We started at the conserved C terminus, where the last 10 amino acid residues constitute the consensus sequence for bacterial outer membrane proteins (32). In the porins, these last 10 amino acid residues form a membrane-spanning segment with the C terminus directed towards the periplasm (9). We assumed that this fact also holds for other outer membrane proteins. A total of 26 putative membrane-spanning amphipathic β-strands could be discerned in the sequence of FrpB (Fig. 3). Interestingly, PupA, the pseudobactin receptor of *Pseudomonas putida* (5), and LbpA, the lactoferrin-binding protein of *N. meningitidis* (23), are also proposed to contain 26 membrane-spanning segments. This structure might be a common feature for the family of TonB-dependent outer membrane receptors. Of course, one has to keep in mind that these models are very preliminary and need to be confirmed experimentally.

Construction of a mutant lacking the 70-kDa outer membrane protein. To determine the function of FrpB, a mutant lacking this protein was constructed by marker exchange. The kanamycin resistance (Km^r) cassette from pUC4K (Pharmacia) was excised with *Hinc*II. Plasmid pAM19 (Fig. 1) was partially digested with *Bpm*I, and the linearized plasmid was isolated from gel by using β-agarase (New England Biolabs). After T4 DNA polymerase treatment to obtain blunt ends, the plasmid was ligated to the Km^r cassette. The ligation mixture was used to transform *E. coli* DH5α, selecting for ampicillin- and kanamycin-resistant colonies. One of the resulting plasmids, designated pAM19K (Fig. 1), contained the Km^r cassette in the *Bpm*I site in *frpB*. Plasmid pAM19K was linearized with *Eco*RI and used to transform meningococcal strains H44/76 and HB^-1 (a capsule-negative derivative of H44/76) as described previously (34). Transformants were selected on GC plates (Difco) with kanamycin. Correct gene replacement was verified by Southern blot analysis (results not shown). The mutants obtained, CE1431 (from H44/76) and CE1415 (from HB^-1), contained the Km^r cassette inserted in the chromosomal *frpB*. The *frpB* mutant strains did not produce the FrpB protein under iron limitation as determined with the FrpB-specific monoclonal antibodies by Western blotting (immunoblotting) (results not shown). The ability of the mutant strain CE1415 to utilize different iron sources was investigated by testing for reversal of iron-limited growth (25). Both the *frpB* mutant strain CE1415 and the parental strain HB^-1 were able to utilize human transferrin and lactoferrin. As a control, *lbpA* mutant strain CE1402 (25) was tested, and it was able to grow on transferrin but not on lactoferrin. Furthermore, bovine hemin (300 μM) (Sigma), ferrated citrate (100 mM), and ferrated aerobactin (0.1 mM) were tested. All these iron sources were able to stimulate the growth of the parental strain as well as that of the *frpB* mutant (data not shown). As controls, *E. coli* UT5600, which is unable to synthesize the siderophore enterobactin (12), and a derivative containing the *iutA* gene encoding the aerobactin receptor on pLO1 (16) were tested. Iron utilization assays in *E. coli* were performed as for the meningococci (25) except that Luria-Bertani plates were used and iron limitation was imposed with 400 μg of 2,2′-bipyridyl (BDH Laboratory Supplies) per ml. Both *E. coli* strains were able to utilize citrate as an iron source, whereas only the pLO1-containing strain grew on aerobactin. Apparently, the FrpB protein is not essential for the acquisition of iron from any of the iron sources tested.

Recently, the CopB protein of *M. catarrhalis* was shown to play a role in serum resistance (14). To determine whether FrpB contributed to meningococcal serum resistance, strain H44/74 and *frpB* mutant CE1431 were tested in an in vitro serum resistance assay. Meningococci were grown overnight in the presence of 5 μg of ethylenediamine di-*o*-hydroxyphenylacetic acid (EDDA) per ml. A 1:100 dilution in fresh medium with the same EDDA concentration was grown to an optical density at 600 nm of 0.20 to 0.25. The bacteria were diluted in 50 mM phosphate buffer, pH 7.2, containing 10 mM $MgCl_2$, 10 mM $CaCl_2$, and 0.5% bovine serum albumin, and incubated with various concentrations of baby rabbit complement for 1 h at 37°C (7). Dilutions of the suspension were spotted on GC plates to determine the percentage of surviving bacteria. There was a slight difference in serum resistance between the mutant and the parental strain (Table 1), but whether this observation is of physiological importance is uncertain.

After the completion of this report, the cloning and sequencing of the gonococcal *frpB* gene were described (4). The gonococcal and meningococcal proteins appear to be very similar, with 92% of the amino acids being conserved (data not shown). The vast majority of the differences between the two proteins are located in the predicted cell surface-exposed loops 5, 6, and

TABLE 1. Serum resistance assay[a]

Strain	Survival (%) after incubation in the following concn of complement:						
	50%	40%	30%	20%	15%	10%	0%
H44/76	9.0 ± 3.4	33 ± 6.2	+[b]	+	+	+	+
CE1431	0.75 ± 1.5	13 ± 3.4	67 ± 9.9	92 ± 9.0	+	+	+

[a] After incubation with complement, 10-μl portions of cell suspensions were plated out, and colonies were counted after an overnight incubation. Percentages of survival (means ± standard deviations) relative to that of the samples in cultures without complement are indicated.

[b] +, 100% survival compared with that of the sample without complement.

7. Furthermore, like the meningococcal *frpB* gene, the gonococcal *frpB* appeared to be difficult to clone, probably because of harmful expression in *E. coli*, and an *frpB* mutant was not impaired in the utilization of transferrin, lactoferrin, citrate, hemin, and hemoglobin as iron sources.

Nucleotide sequence accession number. The nucleotide sequence data for the *frpB* gene will appear in the EMBL, GenBank, and DDBJ nucleotide sequence databases under accession number X89755.

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REFERENCES

1. **Archibald, F. S., and I. W. DeVoe.** 1980. Iron acquisition by *Neisseria meningitidis* in vitro. Infect. Immun. **27:**322–334.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. M. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. Greene Publishing Associates, New York.
3. **Bagg, A., and J. B. Neilands.** 1987. Ferric uptake regulation protein acts as a repressor, employing iron (II) as a cofactor to bind the operator of an iron transport operon in *Escherichia coli*. Biochemistry **26:**5471–5477.
4. **Beucher, M., and P. F. Sparling.** 1995. Cloning, sequencing, and characterization of the gene encoding FrpB, a major iron-regulated, outer membrane protein of *Neisseria gonorrhoeae*. J. Bacteriol. **177:**2041–2049.
5. **Bitter, W., H. Zomer, P. J. Weisbeek, and J. Tommassen.** Unpublished data.
6. **Bullen, J. J., H. J. Rogers, and E. Griffiths.** 1974. Bacterial iron metabolism in infection and immunity, p. 518–551. *In* J. B. Neilands (ed.), Microbial iron metabolism: a comprehensive treatise. Academic Press, Inc., New York.
7. **Claassen, I., J. Meylis, P. van der Ley, C. Peeters, H. Brons, J. Robert, D. Borsboom, A. van der Ark, I. van Straaten, P. Roholl, B. Kuipers, and J. Poolman.** Production, characterization and control of a hexavalent class 1 outer membrane protein containing vesicle vaccine manufactured from tailor-made *Neisseria meningitidis* strains. Submitted for publication.
8. **Cornelissen, C. N., G. D. Biswas, J. Tsai, D. K. Paruchuri, S. A. Thompson, and P. F. Sparling.** 1992. Gonococcal transferrin-binding protein 1 is required for transferrin utilization and is homologous to TonB-dependent outer membrane receptors. J. Bacteriol. **174:**5788–5797.
9. **Cowan, S. W., T. Schirmer, G. Rummel, M. Steiert, R. Ghosh, R. A. Pauptit, J. N. Jansonius, and J. P. Rosenbusch.** 1992. Crystal structures explain functional properties of two *E. coli* porins. Nature (London) **358:**727–733.
10. **Dower, W. J., J. F. Miller, and C. W. Ragsdale.** 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucleic Acids Res. **16:**6127–6145.
11. **Dyer, D. W., E. P. West, W. McKenna, S. A. Thompson, and P. F. Sparling.** 1988. A pleiotropic iron-uptake mutant of *Neisseria meningitidis* lacks a 70-kilodalton iron-regulated protein. Infect. Immun. **56:**977–983.
12. **Elish, M. E., J. R. Pierce, and C. F. Earhart.** 1988. Biochemical analysis of spontaneous *fepA* mutants of *Escherichia coli*. J. Gen. Microbiol. **134:**1355–1364.
13. **Helminen, M. E., I. Maciver, J. L. Latimer, L. D. Cope, G. H. McCracken, and E. J. Hansen.** 1993. A major outer membrane protein of *Moraxella catarrhalis* is a target for antibodies that enhance pulmonary clearance of the pathogen in an animal model. Infect. Immun. **61:**2003–2010.
14. **Helminen, M. E., I. Maciver, M. Paris, J. L. Latimer, S. L. Lumbley, L. D. Cope, G. H. McCracken, and E. J. Hansen.** 1993. A mutation affecting expression of a major outer membrane protein of *Moraxella catarrhalis* alters serum resistance and survival in vivo. J. Infect. Dis. **168:**1194–1201.
15. **Jahnen-Dechent, W., and R. Simpson.** 1990. A method for preparing proteins and peptides for microsequencing. Plant Mol. Biol. Reporter **8:**92–103.
16. **Krone, W. J. A., J. Luirink, G. Koningstein, B. Oudega, and F. K. de Graaf.** 1983. Subcloning of the cloacin DF13/aerobactin receptor protein and identification of a pColV-K30-determined polypeptide involved in ferric-aerobactin uptake. J. Bacteriol. **156:**945–948.
17. **Legrain, M., V. Mazarin, S. W. Irwin, B. Bouchon, M.-J. Quentin-Millet, E. Jacobs, and A. B. Schryvers.** 1993. Cloning and characterization of *Neisseria meningitidis* genes encoding the transferrin-binding proteins Tbp1 and Tbp2. Gene **130:**73–80.
18. **Lundrigan, M. D., and R. J. Kadner.** 1986. Nucleotide sequence of the gene for the ferrienterochelin receptor FepA in *Escherichia coli*. J. Biol. Chem. **261:**10797–10801.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Masson, P. L., J. F. Heremans, and C. H. Dive.** 1966. An iron-binding protein common to many external secretions. Clin. Chim. Acta **14:**735–739.
21. **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.
22. **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.
23. **Pettersson, A., V. Klarenbeek, J. van Deurzen, J. T. Poolman, and J. Tommassen.** 1994. Molecular characterization of the structural gene for the lactoferrin receptor of the meningococcal strain H44/76. Microb. Pathogen. **17:**395–408.
24. **Pettersson, A., B. Kuipers, M. Pelzer, E. Verhagen, R. H. Tiesjema, J. Tommassen, and J. T. Poolman.** 1990. Monoclonal antibodies against the 70-kilodalton iron-regulated protein of *Neisseria meningitidis* are bactericidal and strain specific. Infect. Immun. **58:**3036–3041.
25. **Pettersson, A., A. Maas, and J. Tommassen.** 1994. Identification of the *iroA* gene product of *Neisseria meningitidis* as a lactoferrin receptor. J. Bacteriol. **176:**1764–1766.
26. **Pettersson, A., P. van der Ley, J. T. Poolman, and J. Tommassen.** 1993. Molecular characterization of the 98-kilodalton iron-regulated outer membrane protein of *Neisseria meningitidis*. Infect. Immun. **61:**4724–4733.
27. **Postle, K.** 1990. TonB and the gram-negative dilemma. Mol. Microbiol. **4:**2019–2025.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **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.
30. **Schryvers, A. B., and L. J. Morris.** 1988. Identification and characterization of the human lactoferrin-binding protein from *Neisseria meningitidis*. Infect. Immun. **56:**1144–1149.
31. **Stojiljkovic, I., and K. Hantke.** 1992. Hemin uptake system of *Yersinia enterocolitica*: similarities with other TonB-dependent systems in gram-negative bacteria. EMBO J. **11:**4359–4367.
32. **Struyvé, M., M. Moons, and J. Tommassen.** 1991. Carboxy-terminal phenylalanine is essential for the correct assembly of a bacterial outer membrane protein. J. Mol. Biol. **218:**141–148.
33. **Tommassen, J.** 1988. Biogenesis and membrane topology of outer membrane proteins in *Escherichia coli*, p. 351–373. *In* J. A. F. Op Den Kamp (ed.), Membrane biogenesis. Nato ASI series, vol. H16. Springer-Verlag KG, Berlin.
34. **van der Ley, P., and J. T. Poolman.** 1992. Construction of a multivalent meningococcal vaccine strain based on the class 1 outer membrane protein. Infect. Immun. **60:**3156–3161.
35. **Weiss, M. S., A. Kreusch, E. Schilz, U. Nestel, W. Welte, J. Weckesser, and G. E. Schultz.** 1991. The structure of porin from *Rhodobacter capsulatus* at 1.8 Å resolution. FEBS Lett. **280:**379–382.
36. **Yancey, R. J., and R. A. Finkelstein.** 1981. Assimilation of iron by pathogenic *Neisseria* spp. Infect. Immun. **32:**592–599.