Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 1669–1673, March 1989
Microbiology

Molecular characterization and expression in *Escherichia coli* of the gene complex encoding the polysaccharide capsule of *Neisseria meningitidis* group B

(capsular polysaccharide/polysaccharide transport/phage sensitivity/interspecies DNA homology)

MATTHIAS FROSCH*†, CHRISTOPH WEISGERBER*, AND THOMAS F. MEYER†

*Institut für Medizinische Mikrobiologie, Medizinische Hochschule Hannover, Konstanty Gutschow Strasse 8, 3000 Hannover 61, Federal Republic of Germany; and †Max Planck Institut für Biologie, Infektgenetik, Spemannstrasse 34, 7400 Tübingen, Federal Republic of Germany

Communicated by Emil C. Gotschlich, October 27, 1988

ABSTRACT **The gene complex encoding all determinants of the biosynthesis pathway of the capsule of group B meningococci (*cps*) has been cloned in *Escherichia coli*. A 24-kilobase large chromosomal fragment is necessary for capsule expression on the *E. coli* surface. By transposon and deletion mutagenesis, two separate steps in transport of the polysaccharide from the cytoplasm to the periplasm and further to the cell surface became evident. Mutants were also isolated that accumulate soluble poly(sialic acid) in the cytoplasm. The cloned *cps* complex conferred to *E. coli* strain GC6 sensitivity for *E. coli* K1-specific phages; phage sensitivity was enhanced in two distinct classes of *cps* mutants. Southern blot experiments revealed homology to some or all other *Neisseria meningitidis* capsular types and other *Neisseria* species, depending on the fragment of the *cps* complex used as probe.**

Neisseria meningitidis is a major etiological agent of bacterial septicemia and meningitis. One of the most important pathogenicity factors of meningococci was shown to be the capsule, which is composed of acidic polysaccharides. Nine distinct capsular types are known, which differ in chemical composition. The capsule of group B meningococci consists of poly(sialic acid) with α-2,8-linked *N*-acetylneuraminic acid (NeuNAc) molecules and mediates resistance to important host defense mechanisms. The NeuNAc-containing capsule inhibits the activation of the alternative pathway of the complement system. Consequently, the meningococcus evades lysis by complement as well as opsonization and phagocytosis (1). Furthermore, due to the weak immunogenicity of the group B capsular polysaccharide, complement activation and opsonization by means of the classical pathway, normally mediated by the action of IgM or IgG antibodies, fails because of the missing humoral immune response. Recent studies suggest that the weak immunogenicity is likely to be attributed to an immunotolerance phenomenon. α-2,8-Linked poly(sialic acid) is expressed on the neural cell adhesion molecule (N-CAM), a cell surface glycoprotein involved in development (2, 3).

To gain insight into the molecular mechanisms of the biosynthesis of the capsular polysaccharide of group B meningococci and the mechanisms involved in transport of the capsular polysaccharide, we have cloned the gene complex for the capsule biosynthesis pathway of meningococci group B in *Escherichia coli*. Here we present evidence for the existence of distinct genetic functions that are essential for synthesis and transport of the capsule.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIAL AND METHODS

Bacterial Strains, Phages, and Plasmids. *N. meningitidis* strains A1493, B1940, and C1701, *Neisseria gonorrhoeae* strain 114, *Neisseria lactamica, Neisseria flava, Neisseria perflava, Neisseria mucosa, Neisseria sicca*, and *Neisseria elongata* were from U. Berger (Institute for Hygiene, Heidelberg). *N. gonorrhoeae* strains MS11 and FA1035 were from E. C. Gotschlich (New York) and P. F. Sparling (Department of Microbiology and Immunology, Chapel Hill, NC); *N. meningitidis* strains 29E, W135, Y, and Z were obtained from the American Type Culture Collection. *E. coli* strain GC6 ($K12_{r^-m^+}$, MDU, *recA*::Tn*10*), from M. So (Scripp's Research Institute, La Jolla, CA), was derived from GC1 (4) and used to establish the genomic library of *N. meningitidis* B1940. Cosmid vector pcos2EMBL (5) and *E. coli* AZ1069 (6) were from H. Lehrach (European Molecular Biology Laboratory, Heidelberg); plasmid pRU669 (7) carrying the transposon Tn*1725* was from R. Schmitt (Institute for Genetics, Regensburg, F.R.G.); *E. coli* K1 phages A, B, C, D, and E were from B. Rowe (Central Public Health Laboratory, London) (8).

Recombinant DNA Techniques. The isolation of chromosomal *Neisseria* DNA (9), the mapping of cosmids (10), and transposon mutagenesis (7) have been described. Southern blotting was performed under stringent conditions.

Detection of Poly(sialic acid). The ELISA procedure using monoclonal antibody 735 against meningococcal B capsular polysaccharide (11) was performed as described elsewhere (12).

Phage Sensitivity. *E. coli* cells (10^6) were incubated with an excess of bacteriophages, mixed with soft agar, and plated. Lysis was monitored after a 12-hr incubation at 37°C. For quantitative analysis, 10^2–10^3 *E. coli* cells were mixed with excess phages, incubated for 30 min at room temperature, and plated. Colony-forming units were determined and compared with the colony-forming units of the same cell suspension without phage.

RESULTS

Molecular Cloning of the Gene Complex for Capsule Synthesis. To isolate the genes responsible for capsule production in *N. meningitidis* group B, we established a cosmid library in *E. coli* GC6. This strain is a *recA* derivative of strain GC1, which has previously proven to be competent in accepting highly modified DNA, such as *Neisseria* and fish lymphocystis disease virus DNA (4, 13). This property might be effected by the *rglB* restriction system as suggested by recent

Abbreviation: NeuNAc, *N*-acetylneuraminic acid.

work (14). Two thousand five hundred clones of the cosmid library were screened by colony immunoblotting with IgG2a monoclonal antibody 735, which is specific for the capsular polysaccharide of group B meningococci (11). Eight clones reacted strongly with this antibody.

When encapsulated gram-negative bacteria grow in the logarithmic phase, they shed capsular polysaccharide from the cell surface into the surrounding medium. Detection of capsular polysaccharide in the cell-free growth medium hence serves as an indicator for the functional expression of the capsule on the surface of the *E. coli* host. Therefore, we tested the growth media of all positive cosmid clones by ELISA; only in three cases, however, could cell-free capsular polysaccharide be detected, indicating that these clones have the complete genetic information for both synthesis and transport of the capsular polysaccharide. The remaining five clones gave positive reactions in ELISA only after lysis by sonication.

Physical Analysis of the *cps* Gene Cluster of Group B Meningococci. One of the *E. coli* clones producing detectable extracellular capsular polysaccharide, pMF32, was used for further analysis. The physical map of cleavage sites for *Eco*RI, *Eco*RV, *Hin*dIII, and *Pst* I of this clone was determined. To test the physical integrity of the insert in pMF32 in comparison with the arrangement of the *cps* gene cluster in the genome of *N. meningitidis* B1940, we used pMF32 as a labeled probe in Southern hybridizations with genomic DNA (data not shown). It was evident that all fragments of pMF32, except for the two flanking fragments of the insert, had counterparts of identical sizes in the digested genomic DNA (compare with Fig. 4).

To obtain a minimal segment sufficient for extracellular capsular polysaccharide production, cosmid DNA of clone pMF32 was partially digested with *Eco*RI, religated, and transferred into *E. coli* GC6. The smallest subclone that still elicited the functions of pMF32, termed pMF32.35, was 24 kilobases in size (Fig. 1).

Localization of Functional Determinants in the *cps* Cluster. To define more precisely the location and genetic organization of the genes involved in capsule biosynthesis, we prepared a collection of deletion mutants of pMF32.35 (Fig. 1).

All mutant clones were tested in the ELISA for their potential to encode the entire capsular polysaccharide synthesis pathway. In this initial experiment, we distinguished extracellular, periplasmic, and crude cytoplasmic fractions. Five distinct groups of deletion mutants could be assigned (Fig. 1).

Mutations in region A (mutants ΔE1, ΔE3, and ΔE7) led to an absolute defect in capsular polysaccharide antigen synthesis: monoclonal antibody 735 did not detect capsular polysaccharide in the culture supernatant or in the lysate of sonicated bacteria. This antibody requires oligomers of more than eight α-2,8-linked NeuNAc molecules for recognition (3). Therefore, this collection of mutants may have defects in any step of the biosynthesis pathway of poly(sialic acid).

The poly(sialic acid) biosynthesis pathway in meningococci includes three major steps: (*i*) synthesis of monomeric NeuNAc, (*ii*) activation of NeuNAc to CMP-NeuNAc by the enzyme CMP-NeuNAc synthetase, and (*iii*) polymerization of activated NeuNAc by the cytoplasmic membrane-associated sialyltransferase complex, which links NeuNAc to an endogenous acceptor and catalyzes chain elongation (16). To specify defects of mutants in region A in this regard, a series of transposon mutants with insertions in region A were generated (T1–T5, Fig. 1). Mutagenesis was performed with transposon Tn*1725* (7). To analyze defects in biosynthetic steps, the mutants were grown in LB broth to the late logarithmic phase in the presence of neuraminic acid at a concentration of 150 μg/ml. Supernatants and sonicated bacteria of these cultures were tested by ELISA. Mutants T2,

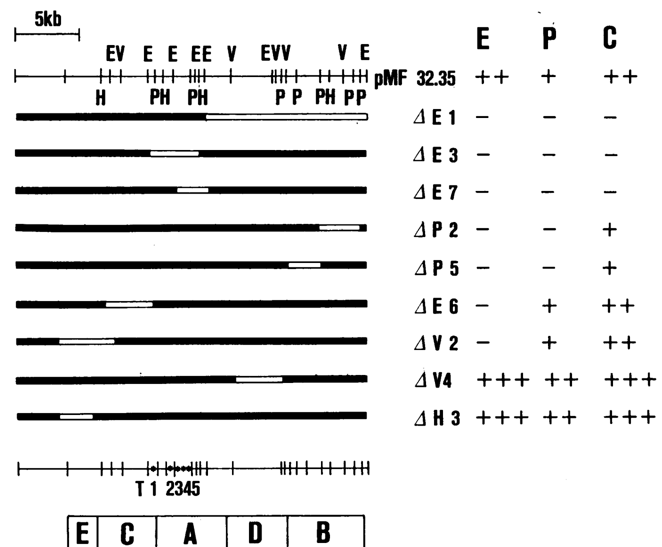

FIG. 1. Physical map of clone pMF32.35, transposon insertions, and deletion mutants of the *cps* gene complex. (*Left*) The top line shows the restriction map of pMF32.35. E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; V, *Eco*RV. Deletion mutants are indicated below the restriction map; open boxes indicate the deleted fragments. Deletion mutants derived from pMF32.35 were prepared by partial digestion with *Eco*RI, *Hin*dIII, *Pst* I, or *Eco*RV. Large fragments (18–25 kilobases) were isolated by sucrose density centrifugation and were religated in the same vector. Insertions of transposon Tn*1725* (T1–T5) are given in the line below the deletion mutants. Boxes at the bottom specify the functional regions A–E of the *cps* cluster. (*Right*) Deletion mutants were tested for localization of capsular polysaccharide in ELISA using monoclonal antibody 735. E, polysaccharide in culture supernatant; P, polysaccharide in the periplasm; supernatants of osmotically shocked cells. The shocked cells were sonicated, centrifuged (17,000 × *g*), and analyzed as a crude cytoplasmic fraction for poly(sialic acid) content, designated C. Osmotic shock experiments were performed as described, including the appropriate controls (15). Purified meningococcal B capsular polysaccharide (Connaught Laboratories, Swiftwater, PA) was used as standard for determination of the polysaccharide concentration in the cell fractions. +++, 150–300 ng/ml; ++, 50–150 ng/ml; +, 10–50 ng/ml. For construction of clone pMF32, vector pcos2EMBL was digested with *Bam*HI and *Pvu* II. Fragments (40–50 kilobases) of meningococcal chromosomal DNA from strain B1940, prepared by partial restriction with *Mbo* I and separation by sucrose gradients, were ligated into the *Bam*HI site of the vector arms. Ligated DNA was packaged *in vitro* into infective λ particles. The particles were used to infect *E. coli* GC6 cells. Transfectants were scored by colony blotting (9) with monoclonal antibody 735 and protein A-phosphatase.

T3, and T4 produced intra- and extracellular poly(sialic acid), indicating that these mutants are defective in the biosynthesis of monomeric NeuNAc (Table 1).

To investigate sialyltransferase activity in transposon and deletion mutants, we determined chain elongation of exogenously added colominic acid, an oligomer of α-2,8-linked NeuNAc, in comparison to the activity promoted by pMF32.35. The polymeric nature of incorporated radioactivity was demonstrated by its sensitivity to α-2,8-linked NeuNAc-specific endoneuraminidase (18). As shown in Table 1, mutant T5 and all mutants with deletions in the area of the T5 insertion did not incorporate CMP-NeuNAc. All other mutants incorporated CMP-NeuNAc in amounts comparable with clone pMF32.35. These results allowed the assignment of the sialyltransferase gene(s) to region A.

Mutants with deletions in region B (ΔP2 and ΔP5) did not elicit detectable amounts of capsular polysaccharide in either supernatants of the culture or of osmotically shocked cells. Only after complete disruption by sonication of spheroblasts

Table 1. Effect of transposon and deletion mutagenesis on NeuNAc biosynthesis and sialyltransferase activity

Strain	Poly(sialic acid) synthesis enabled by NeuNAc supplementation	NeuNAc incorporation, nmol/hr per mg of protein	NeuNAc incorporation, % pMF32.35
GC6	−	0.005	0
pMF32.35	ND	6.23	100
Mutant T1	−	3.01	48
Mutant T2	+	5.92	95
Mutant T3	+	ND	ND
Mutant T4	+	3.12	50
Mutant T5	−	0.005	0
ΔE1	ND	0.005	0
ΔE3	ND	0.004	0
ΔE7	ND	0.008	0
ΔP2	ND	3.50	56
ΔE6	ND	3.02	48
ΔV4	ND	4.82	77
ΔH3	ND	7.17	115

Sialyltransferase activity was performed as described (17). Proof of α-2,8-NeuNAc linkage of the polymer synthesized *in vitro* was achieved by demonstrating its sensitivity to an α-2,8-NeuNAc-specific endoneuraminidase. The endoneuraminidase was purified according to a published procedure (18); the DEAE-Sephadex chromatography step was replaced by FPLC on Mono Q. Sensitivity of the polymer to endoneuraminidase was tested as described (19). ND, not determined.

did region B mutants release soluble capsular antigen. To localize more precisely the polysaccharide produced in mutants ΔP2 and ΔP5 and clone pMF32.35, we performed additional cell fractionation experiments and determined the polysaccharide content of the fractionated inner and outer membranes and cytoplasm by ELISA. Previous observations suggest that the inner membrane-associated sialyltransferase directs polymerization of poly(sialic acid) to a membrane-bound acceptor (16) and that capsular polysaccharide is attached on the outer membrane by linkage to a phospholipid (20). As expected, poly(sialic acid) was associated with the inner and outer membrane of pMF32.35 (Fig. 2). In the cytoplasmic fraction, only a minor portion [less than 5% of the total poly(sialic acid) content] was detectable. In contrast, no membrane-associated polysaccharide was found in mutants ΔP2 and ΔP5. Quantitative analysis revealed that almost 100% of the total polysaccharide content of these mutants was in the cytoplasm. These data suggest that polysaccharide is synthesized in the cytoplasm (probably at the cytoplasmic site of the inner membrane) and, therefore, a mechanism has to be postulated for translocation of the polysaccharide through the cytoplasmic membrane to the periplasm.

A further step in capsule formation must direct translocation of polysaccharide from periplasm to the cell surface. The genetic function for this process is located in region C. Mutations in region C (ΔE6 and ΔV2) allow capsular polysaccharide to be transported from the cytoplasm to the periplasm but not to the cell surface, as determined by the absence of polysaccharide in culture supernatants and its presence in osmotic shock preparations.

Mutations in regions D and E affected neither capsular polysaccharide biosynthesis nor polysaccharide transport functions. However, in the ELISA assay, higher levels of capsular polysaccharide were detectable, as compared with the intact clone pMF32.35.

Immunofluorescence and Phage Sensitivity of Cosmid Clone pMF32.35 and Mutants ΔH3 and ΔV4. Immunofluorescence of *E. coli* GC6 containing plasmid pMF32.35 revealed that only part of the cells reacted with monoclonal antibody 735; about 50% of the cells of the total population expressed

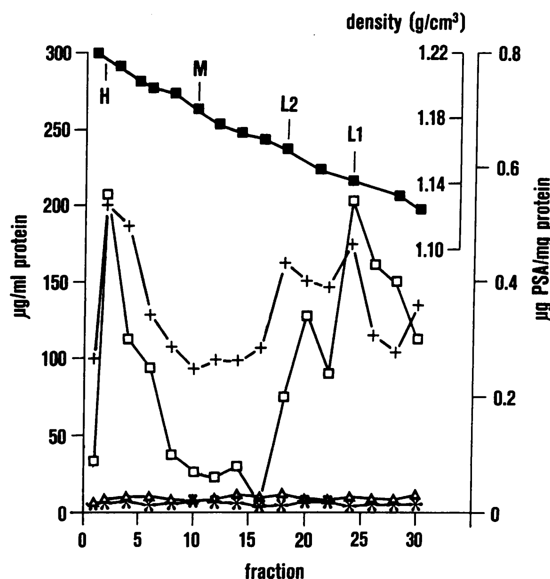

FIG. 2. Sedimentation density gradient centrifugation of membrane fractions of GC6(pMF32.35) and mutants ΔP2 and ΔP5. Sucrose gradients were collected and the protein concentration of the fractions (+) was determined by a modified method of Lowry, which is commercially available (Pierce). Polysaccharide concentration was determined in ELISA and is given as micrograms of poly(sialic acid) (PSA) per milligram of protein for pMF32.35 (□), ΔP2 (△), and ΔP5 (*). ■, Density. Four different protein bands (H, M, L_2, and L_1) were obtained in accordance with Osborn *et al.* (21). Outer membrane sedimented in band H; the cytoplasmic membrane sedimented in bands L_1 and L_2. The cytoplasmic fraction was prepared by collecting the supernatant of sonicated and subsequently high-speed-centrifuged (360,000 × *g*) spheroblasts.

capsular antigen on their surface (Fig. 3 *A* and *B*). The control, GC6 without plasmid, did not react with the antibody. In contrast, mutants ΔH3 and ΔV4 reveal surface labeling of >95% of the bacteria (Fig. 3 *C* and *D*). This phenomenon might be associated with the increased level of capsular antigen production in these mutants (Fig. 1).

For a more detailed analysis of this phenomenon, we have used the *E. coli* bacteriophages A, B, C, D, and E. These phages have been used to identify *E. coli* strains with capsular type K1 (8, 12), which is chemically identical to the capsular polysaccharide of group B meningococci (22). These phages require the K1 capsular antigen for adsorption. Nevertheless, meningococci group B are not sensitive to these phages, probably due to the lack of a second receptor. Here, we can demonstrate that capsule expression in *E. coli* directed by pMF32.35 confers sensitivity to *E. coli* GC6 for the K1-specific phages. Interestingly, in correlation with the immunofluorescence data, *E. coli* GC6 (pMF32.35) exhibited only partial phage susceptibility. Quantitative analysis suggested about 50% of the *E. coli* cells to be sensitive. In contrast, mutants ΔH3 and ΔV4 conferred complete sensitivity to *E. coli* GC6; phage-resistant colonies of mutants ΔH3 and ΔV4 arose at a frequency of 0.001%. Phage-resistant colonies of pMF32.35 were isolated and rescreened for phage sensitivity. Surprisingly, a 1:1 ratio with regard to phage sensitivity and resistance was observed again.

DNA Homologies Between Capsule Genes of *N. meningitidis* B and Other *Neisseria* Species. To determine the presence of sequences related to the cloned *cps* gene cluster of group B meningococci in other *Neisseria* species, chromosomal DNA from all meningococcal capsular types, three different *N. gonorrhoeae* strains and several apathogenic *Neisseria* species were assayed by Southern blotting. The chromosomal DNAs were digested with *Eco*RI or *Eco*RV and hybridized

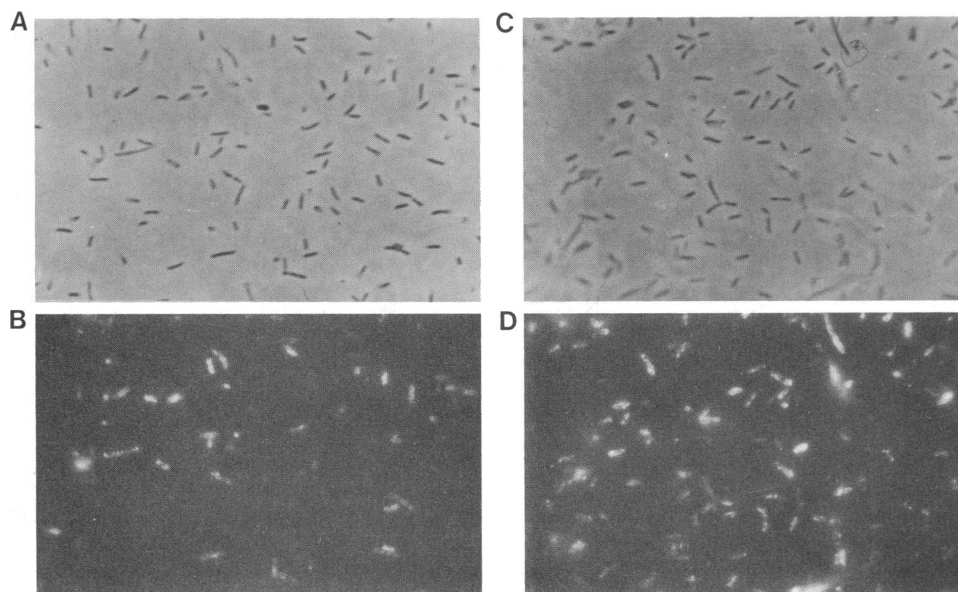

FIG. 3. Light microscopy (*A* and *C*) and immunofluorescence (*B* and *D*) with monoclonal antibody and fluorescein isothiocyanate-labeled protein A of GC6(pMF32.35) (*A* and *B*) and deletion mutant ΔH3 (*C* and *D*). Fluorescence of mutant ΔV4 was comparable to the fluorescence of mutant ΔH3 (data not shown).

with labeled fragments of plasmid pMF32.35. Two different fragments (A_1 and A_2) were derived from region A; each of the other regions (except E) were represented by single fragments. Hybridization of fragment A_1, thought to be involved in NeuNAc polymerization to α-2,8-linked poly(sialic acid), was specific for group B meningococci (Fig. 4A_1). In contrast, fragment A_2, believed to direct NeuNAc synthesis, hybridized to an identical fragment in all meningococcal serogroups with capsules composed of NeuNAc (groups B, C, W135, and Y). Hybridization was absent in the other meningococcal serogroups and *N. gonorrhoeae* strains (Fig. 4A_2). Fragments involved in capsular polysaccharide transport function (Fig. 4 *B* and *C*) gave strong hybridizations with different chromosomal DNA fragments of all meningococcal capsular serotypes, indicating a general transport mechanism, regardless of the chemical composition of the capsular polysaccharide. No hybridization was found with DNA of *N. gonorrhoeae*. An *Eco*RV fragment isolated from region D, possibly involved in regulatory processes, hybridized with DNA from all *N. meningitidis* serogroups and *N. gonorrhoeae* strains tested (Fig. 4*D*). Interestingly, this fragment, which hybridizes to two different bands in Fig. 4*D*, seems to contain a repetitive element that is present at least three times in the cloned *cps* cluster (data not shown).

DISCUSSION

In this report, we describe the molecular cloning of the determinants (*cps*) for the capsular polysaccharide biosynthesis pathway of *N. meningitidis* group B. By transposon and deletion mutagenesis, functional regions involved in the biosynthesis and transport of poly(sialic acid) were located within a chromosome segment of about 24 kilobases. Region A seems to be essential for NeuNAc biosynthesis since mutants in this region do not produce capsular material that is detectable by monoclonal antibody 735. Within region A, areas responsible for the synthesis of NeuNAc and polymerization of NeuNAc to α-2,8-linked poly(sialic acid) can be distinguished. Furthermore, there exist two distinct steps for the translocation of polysaccharide from the cytoplasm to the periplasm and from the periplasm to the cell surface. Region B is thought to code for functions involved in the translocation of polymerized capsular polysaccharide from the cytoplasm to the periplasm. Cell fractionation revealed that region B mutants accumulate polysaccharide in the cytoplasm. Such mutants might be defective in an inner membrane molecule acting as acceptor for polymerized sialic acids (16). Alternatively, the acceptor-bound polysaccharide is shed into the cytoplasm after polymerization by the sialyltransferase, due to a possible defect in translocation through the inner membrane. Our analysis of region C provides evidence for its involvement in transport of capsular polysaccharide to the outer membrane. Mutations in region D and E do not affect polysaccharide synthesis or transport in the *E. coli* host, but rather possess regulatory functions.

The capsule gene complex of *Haemophilus influenzae* (23) and of *E. coli* K1, the capsule of which is chemically and immunologically identical to the capsule of group B meningococci (22), has been cloned previously (24, 25). A mechanism for translocation of capsular polysaccharide from the periplasm to the cell surface has been described for several *E. coli* capsular types (26, 27). However, a process for capsular polysaccharide transport through the cytoplasmic membrane as proposed here has not been previously detected. Whether the principles of polysaccharide transport for *E. coli* and meningococci are related is questionable, since DNA homologies between the *E. coli* K1 genes and meningococcal B DNA have not been detected (25).

A variable degree of homology between the *cps* gene cluster of group B *N. meniningitidis* and other *Neisseria* species has been observed in Southern blot experiments. One *Eco*RV fragment of pMF32.35, derived from region D and presumably involved in regulation of the *cps* gene cluster, hybridizes with chromosomal DNA of all tested *Neisseria* species.

In the *Neisseria* species producing capsules composed of NeuNAc (meningococci of group B, C, W135, and Y), homology with a fragment involved in NeuNAc synthesis was observed. The linkage of NeuNAc is different in these four serogroups; no homology is consistently seen between the fragment likely to be responsible for α-2,8 polymerization of NeuNAc and any other serogroup. In contrast, all encapsulated *Neisseria* species, including all *N. meningitidis* serogroups, share homology in the transport determinants.

In terms of vaccine application, two aspects might be worth mentioning. (*i*) The polysaccharide capsule of menin-

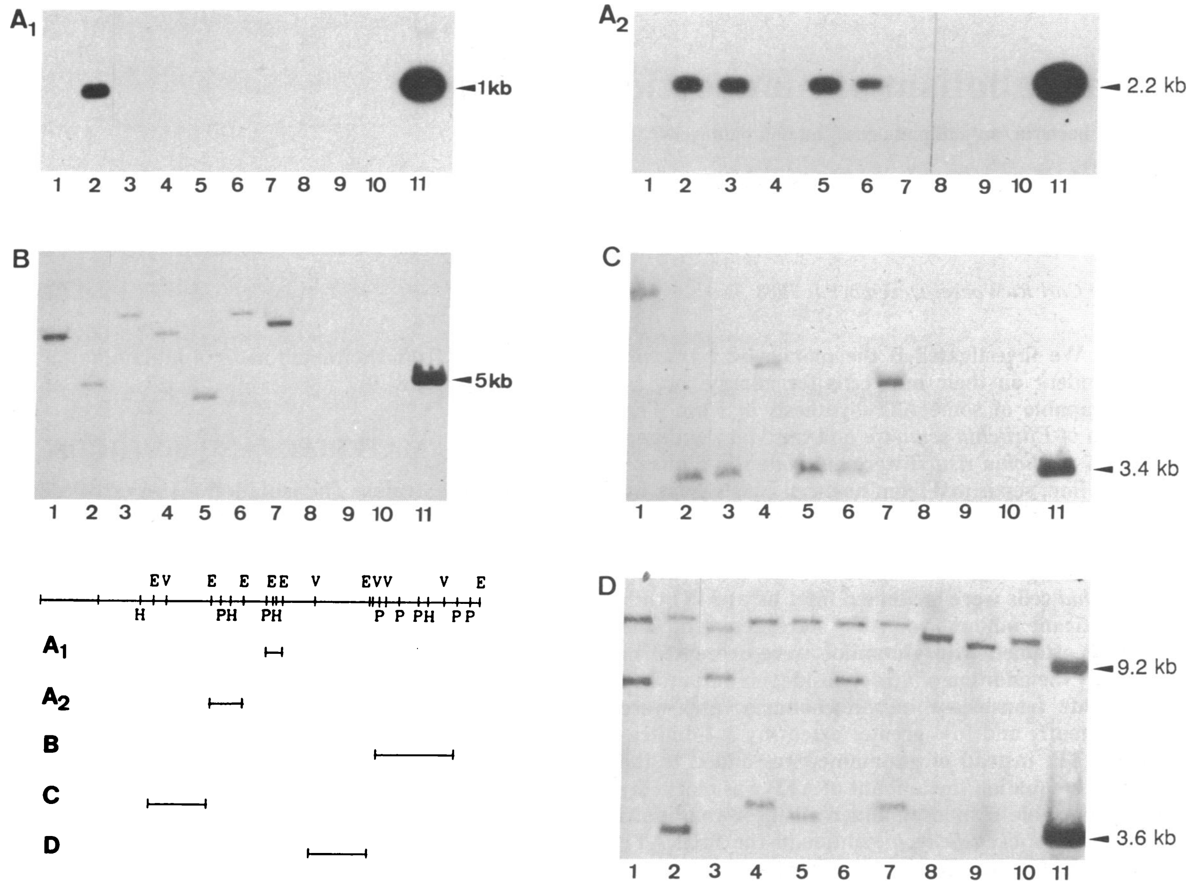

FIG. 4. DNA homologies between the *cps* cluster of *N. meningitidis* B and other *Neisseria* isolates. DNA fragments of pMF32.35 used for hybridization are indicated below the physical map; these fragments (A_1–D) were used as probes in the respective panels showing Southern blots. Hybridizations A_1 and A_2 were performed with ^{32}P-labeled DNA fragments, hybridizations B, C, and D were performed with biotinylated fragments. In all panels the same order of chromosomal DNAs was used. Lanes: 1, *N. meningitidis* A; 2, *N. meningitidis* B; 3, *N. meningitidis* C; 4, *N. meningitidis* 29E; 5, *N. meningitidis* W135; 6, *N. meningitidis* Y; 7, *N. meningitidis* Z; 8, *N. gonorrhoeae* MS11; 9, *N. gonorrhoeae* FA1035; 10, *N. gonorrhoeae* clinical isolate 114; 11, pMF32.35. Chromosomal DNA was digested with *Eco*RI (*A_1–C*) and *Eco*RV (*D*), respectively. kb, Kilobase(s).

gococci B, the most important etiological agent of meningococcal meningitis, is poorly immunogenic (2, 3). In this context, the proteins involved in capsular polysaccharide transport might be of particular interest as vaccine candidates. (*ii*) The group B meningococcal capsule can be expressed on the surface of another gram-negative bacterium. It seems feasible to clone the genes coding for other meningococcal capsular types and to utilize them in suitable live bacterial vaccine strains.

We thank Dr. Paul Manning for critical comments during the course of this work and Dr. C. Gibbs for reviewing the manuscript. M.F. gratefully acknowledges receipt of a postdoctoral fellowship and a grant (Fr689/2-1) from the Deutsche Forschungsgemeinschaft.

1. Jarvis, G. A. & Vedros, N. A. (1987) *Infect. Immun.* **55,** 174–180.
2. Finne, J., Leinonen, M. & Mäkelä, P. H. (1983) *Lancet* **ii,** 355–357.
3. Finne, J., Bitter-Suermann, D., Goridis, C. & Finne, U. (1987) *J. Immunol.* **138,** 4402–4407.
4. Meyer, T. F., Mlawer, N. & So, M. (1982) *Cell* **30,** 45–52.
5. Poustka, A., Rackwitz, H. R., Frischauf, A.-M., Hohn, B. & Lehrach, H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4129–4133.
6. Rackwitz, H.-R., Zehetner, G., Murialdo, H., Delius, H., Chai, J. J. H., Poustka, A., Frischauf, A. & Lehrach, H. (1985) *Gene* **40,** 259–266.
7. Ubben, D. & Schmitt, R. (1986) *Gene* **41,** 145–152.
8. Gross, R. J., Cheasty, T. & Rowe, B. (1977) *Infect. Immun.* **6,** 548–550.
9. Stern, A., Nickel, P., Meyer, T. F. & So, M. (1984) *Cell* **37,** 447–456.
10. Rackwitz, H.-R., Zehetner, G., Frischauf, A. M. & Lehrach, H. (1984) *Gene* **30,** 195–200.
11. Frosch, M., Görgen, I., Boulnois, G. J., Timmis, K. N. & Bitter-Suermann, D. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1194–1198.
12. Frosch, M., Roberts, I., Görgen, I., Metzger, S., Boulnois, G. J. & Bitter-Suermann, D. (1987) *Microb. Pathogen.* **2,** 319–326.
13. Darai, G. & Apfel, H. (1985) *Dev. Biol. Stand.* **59,** 3–9.
14. Noyer-Weidner, M., Diaz, R. & Reiners, L. (1986) *Mol. Gen. Genet.* **205,** 469–475.
15. Neu, H. C. & Heppel, L. A. (1965) *J. Biol. Chem.* **240,** 3685–3692.
16. Masson, L. & Holbein, B. E. (1983) *J. Bacteriol.* **154,** 728–736.
17. Vimr, E. R. & Troy, F. A. (1985) *J. Bacteriol.* **164,** 854–860.
18. Tommlinson, S. & Taylor, P. W. (1985) *J. Virol.* **55,** 374–378.
19. Hallenbeck, P. C., Vimr, E. R., Yu, F., Bassler, B. & Troy, F. A. (1987) *J. Biol. Chem.* **262,** 3553–3561.
20. Gotschlich, E. C., Fraser, B. A., Osamu, N., Robbins, J. B. & Liu, T.-Y. (1981) *J. Biol. Chem.* **256,** 8915–8921.
21. Osborn, M. J., Gander, J. E., Parisi, E. & Carson, J. (1972) *J. Biol. Chem.* **247,** 3962–3972.
22. Kasper, D. L., Winkelhake, J. L., Zollinger, W. D., Brandt, B. L. & Artenstein, M. S. (1973) *J. Immunol.* **110,** 262–268.
23. Kroll, J. S., Hopkins, I. & Moxon, E. R. (1988) *Cell* **53,** 347–356.
24. Silver, R. P., Finn, C. W., Vann, W. F., Aaronson, W., Schneerson, R., Kretschmer, P. J. & Garon, C. F. (1981) *Nature (London)* **289,** 696–698.
25. Echarti, C., Hirschel, B., Boulnois, G. J., Varley, J. M., Waldvogel, F. & Timmis, K. N. (1983) *Infect. Immun.* **41,** 54–60.
26. Boulnois, G. J., Roberts, I. S., Hodge, R., Hardy, K. R., Jann, K. B. & Timmis, K. N. (1987) *Mol. Gen. Genet.* **208,** 242–246.
27. Roberts, I. S., Mountford, R., Hodge, R., Jann, K. B. & Boulnois, G. J. (1988) *J. Bacteriol.* **170,** 1305–1310.