JOURNAL OF BACTERIOLOGY, Jan. 1992, p. 315–319
0021-9193/92/010315-05$02.00/0

Vol. 174, No. 1

Sequence and Expression of the *Escherichia coli* K1 *neuC* Gene Product

GERARDO ZAPATA, JANE M. CROWLEY, AND WILLIE F. VANN*

Laboratory of Bacterial Polysaccharides, Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, Maryland 20892

Received 1 July 1991/Accepted 26 October 1991

The nucleotide sequence of the *neuC* gene of the *Escherichia coli* K1 capsule gene cluster encodes a protein with a predicted molecular weight of 44,210 containing 391 amino acids. A chimeric protein with β-galactosidase fused to the carboxy terminus of the *neuC* gene product (P7) was constructed and purified. Its amino-terminal sequence confirmed the prediction from the nucleotide sequence that the *neuC* gene overlaps the distal end of the *neuA* gene by a single base pair. Both the *neuA* and *neuC* genes are coexpressed under the control of a single upstream T7 or *tac* promoter, suggesting that *neuA* and *neuC* are part of an operon.

Capsular polysaccharides (K antigens) are virulence factors for *Escherichia coli* strains that cause urinary tract infections and neonatal meningitis (11, 16, 17). Of the 50 chemically different capsular K antigens, only a few are associated with disease isolates. These few polysaccharides associated with disease isolates belong to the group II antigens characterized by acidic components such as 2-keto-3-deoxy-D-manno-octulonic acid, *N*-acetylneuraminic acid (neuAc), and *N*-acetylmannosaminuronic acid (10). Capsules protect bacteria against the host immune system. The α-2,8-linked neuAc polysaccharides of *E. coli* K1 and *Neisseria meningitidis* group B resemble host glycoconjugates such as cell adhesion protein N-CAM (9, 19).

The proteins necessary for synthesis, activation, and polymerization of neuAc are encoded by the 17-kb *kps* gene cluster of *E. coli* K1 (6, 22–24, 29). Three distinct regions have been identified in this gene cluster (18). The central region (region 2) is involved in polysaccharide polymerization and synthesis. Two genes important in neuAc utilization and synthesis, *neuA* and *neuC*, have been localized to a 2.7-kb *Eco*RI-*Hin*dIII fragment in region 2 of the *E. coli* K1 gene cluster (24, 28). *neuA* encodes CMP-neuAC synthetase, and *neuC* encodes a 45,000-molecular-weight protein (P7) necessary for the formation of neuAc (24). The *neuA* gene has been sequenced, and the product, CMP-neuAc synthetase, has been purified (33).

Silver et al. (24) have demonstrated that cells harboring mutations in *neuC* become sensitive to capsular polysaccharide-specific phage only when supplied with exogenous sialic acid, suggesting involvement of the *neuC* gene product in neuAc biosynthesis. Here we present the sequence of the *neuC* gene and analysis of its expression.

neuAc is synthesized in eucaryotes and procaryotes from *N*-acetylmannosamine (manNAc) derivatives as follows: manNAc + phosphoenolpyruvate → neuAc (*N. meningitidis*) (1a) and manNAc-6-P + phosphoenolpyruvate → neuAc-9-P (rat liver) (32).

Complementation of *neuC* mutants by neuAc. To confirm the role of the *neuC* gene product in neuAc metabolism, we used a complementation system that employed unencapsulated neuAc aldolase-negative strain EV80 of Vimr and Troy (30) as a background to prevent metabolism of exogenously added neuAc. EV80 was transformed (4) with plasmids pSR23 (24), containing all of the K1 genes; pSR50 (28), containing a Tn*5* insertion mutation in the *neuC* gene of pSR23; and pWA1 (33), containing only *neuA* and *neuC* as a negative control. Transformants were plated separately on antiserum agar plates containing either 250 μg of neuAc or manNAc per ml. Capsular polysaccharide production was detected as colonies with halos on agarose plates containing either tryptic soy broth or Davis minimal medium and a 1/10 dilution of horse 46 anti-K1 polysaccharide antisera as previously described (15, 16). When strain EV80 was transformed (4) with plasmid pSR50, halos were observed only when plates were supplemented with neuAc. The same plasmid, pSR50, was unable to complement *E. coli* EV80 when antiserum agar plates were supplemented with manNAc. This suggests that P7 is involved in the biosynthesis of neuAc at a biosynthetic step different from manNAc formation.

Strategy for sequencing. The *neuC* gene that codes protein P7 was isolated from plasmid pSR35 (24, 28) in a 2.7-kb *Hin*dIII-*Eco*RI fragment by using published methods (5, 13). This fragment was subcloned into sequencing vectors M13mp18 and M13mp19 to create pWG4 and pWG5 (33). These constructions permitted the generation of sequential deletions in both directions of the *neuC* gene by using Henikoff's method (8) of digestion with exonuclease III as described in Promega's Erase-A-Base kit. Enzymes *Kpn*I and *Bam*HI were used sequentially to prepare pWG4 for exonuclease digestion, and *Pst*I and *Hin*cII were used for pWG5. The sequencing strategy illustrated in Fig. 1 shows the positions of the sequences obtained from the different exonuclease deletions and the positions of the sequences obtained from synthetic oligonucleotide primers. Sequencing was performed by the method of Sanger et al. (21) with Sequenase (United States Biochemical Corp.) and the polyacrylamide-urea gel electrophoresis system described by Biggin et al. (1). Double-stranded sequencing was performed by using the alkaline-denaturation procedure (12, 27). The DNA analysis programs of Staden were used to analyze the sequence data. The sequence overlap is excellent in the 600-bp join region of the *neuA* and *neuC* genes. The quality of the sequence derived from regions where few exonuclease deletion clones overlapped was improved by using synthetic oligonucleotides to prime closer to these regions. Primers were synthesized to adjacent regions in which several over-

* Corresponding author.

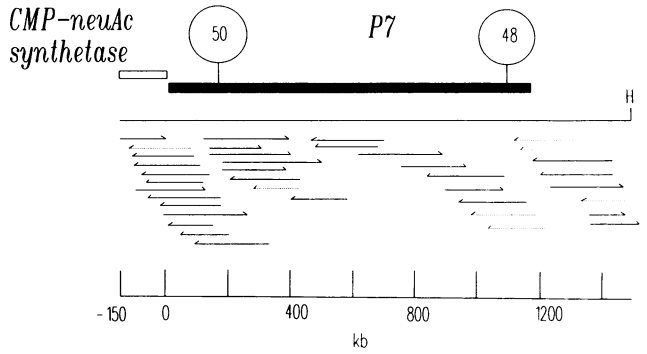

FIG. 1. Sequencing strategy. Sequences obtained from nested deletions are represented by solid lines, and those from sequence-derived oligonucleotide primers are shown as dashed lines. The locations of insert mutations by transposons Tn*5* (pSR50) and γδ (pSR48) are indicated by circles on the physical map. H, *Hin*dIII.

lapping sequences had been obtained by the exonuclease deletion clones. A total of 1,508 nucleotides cover the region from the end of the *neuA* gene to the *Hin*dIII insertion site in pSR35, thus including the *neuC* gene.

Analysis of the sequence. The sequence of the fragment containing the *neuC* gene is given in Fig. 2. The map positions of Tn*5* *neuC*-inactivating insertion mutations *kps-2* (Tn*5*-48), *kps-4* (Tn*5*-50) (Fig. 1), γδ42, and γδ24 (described by Silver et al. [24, 28]) are, as predicted, contained within the open reading frames of *neuC*. Two possible open reading frames were found upon analysis of the *neuC* gene (Fig. 2). The first open reading frame, found between nucleotides 1 and 1173, starts with an AUG preceded by a Shine-Dalgarno consensus sequence 7 bases upstream of the start codon. The second open reading frame is between nucleotides 67 and 1173 and is preceded by the same Shine-Dalgano sequence 9 bases upstream from the AUG start codon. The longer open reading frame of the *neuC* gene overlaps one nucleotide of the preceding *neuA* gene, suggesting that both genes *neuA* and *neuC* are transcribed as a single message. This possibility is also suggested by the lack of consensus for a promoter sequence upstream of the *neuC* gene and the presence of a possible transcription terminator at bp 1215 near the end of the *neuC* gene with a predicted free energy of formation of 1.8 kcal (1 cal = 4.184 J).

Purification and Edman sequencing of P7–β-galactosidase chimera 1. A chimeric protein (chimera 1) was constructed to facilitate purification of P7 and to determine the beginning of the *neuC* gene. In plasmid pWG143, the *lacZ* gene was fused in frame to the carboxy-terminal end of the *neuC* gene. The resulting plasmid contained the complete *neuC* and *lacZ* genes and 65 nucleotides from the 3′ end of the *neuA* gene. The plasmid was constructed by amplifying a 1.238-kb fragment from plasmid pWA1 containing 65 nucleotides from the 3′ end of the *neuA* gene and the complete sequence of the *neuC* gene with the following primers using polymerase chain reaction methodology: 5′CGCGGAATTCCGATGG CTTACATTTTAATAGTAATGGGTAT3′ and 5′CGCGC TCGAGTAGGCCCCCCAGGGTCATAACTGGTGGTAC ATTC3′. Polymerase chain reaction experiments were performed by using the reagents and protocols supplied by the manufacturer (Perkin-Elmer Cetus). These primers contain either *Eco*RI or *Xho*I sites at the 5′ ends of the oligonucleotides. The amplified fragment was cloned into pTTQ8-derived, isopropyl-β-D-thiogalactopyranoside-

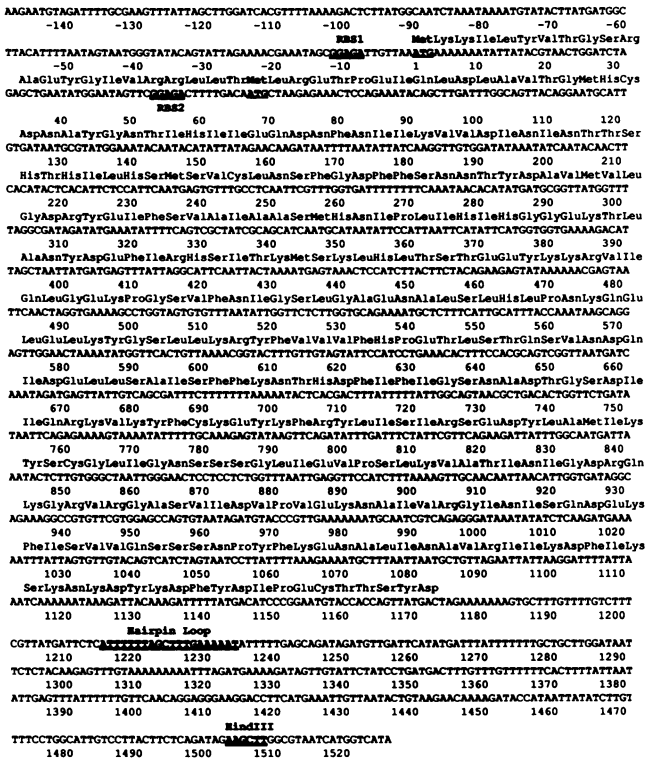

FIG. 2. Nucleotide sequence of the *neuC* gene and the translated amino acid sequence. The *neuC* gene coding sequence starts at bp 1 and ends at bp 1173. Underlined are the two possible Shine-Dalgarno sequences, the ATG start codons, the transcription termination sequence, and the *Hin*dIII restriction sequence. The sequence upstream of the methionine start codon belongs to the CMP-neuAc synthetase gene. RBS, ribosome-binding site.

inducible expression vector pSS1295 to form plasmid pWG142. The *lacZ* gene was excised from plasmid pSS1462 with the enzymes *Xho*I and *Hin*dIII and inserted into pWG142 to form plasmid pWG143. Plasmids pSS1462 and pSS1295 were obtained from Scott Stibitz, Laboratory of Bacterial Toxins, Food and Drug Administration.

Plasmid pWG143 was expressed in protease-deficient strain BL21, and the chimeric protein was purified by affinity chromatography on aminophenylthiogalactoside-agarose (3, 7, 25). The fractions possessing β-galactosidase (20, 25) activity were further purified by gel filtration on Superose 12 in 0.05 M Bicine–1 mM dithiothreitol, pH 7.6.

The purified protein was concentrated in a collodion bag and submitted for automatic Edman sequencing. P7–β-galactosidase chimera 1 migrated on sodium dodecyl sulfate (SDS)-gel electrophoresis with an apparent molecular size greater than that of β-galactosidase (Fig. 3). The apparent molecular weight of 150,000 is within experimental error of the expected 160,000. The amino-terminal sequence of the first 13 amino acids of chimera 1 was found to be in agreement with the predicted sequence of the longest open reading frame. Although the first amino acid in the sequence was not reliably detected, the cycle of the appearance of the amino acids in Edman sequencing was 1 less than that predicted by the nucleotide sequence, suggesting that lysine is the amino-terminal amino acid. The agreement of the protein sequence with the predicted amino N-terminal sequence confirms the single-base-pair overlap of the *neuA* and *neuC* genes.

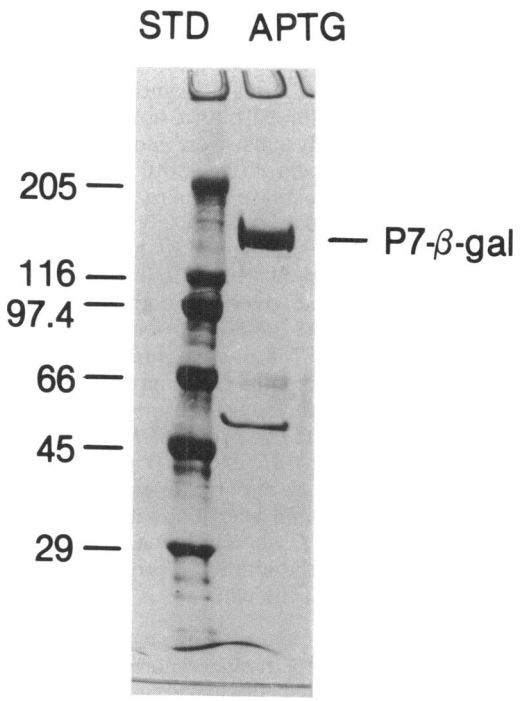

FIG. 3. SDS-polyacrylamide gel electrophoresis of P7–β-galactosidase chimera 1. The P7–β-galactosidase chimeric protein was purified by affinity chromatography on aminophenylthiogalactoside-agarose followed by gel filtration on Superose 12. Lanes: STD, molecular weight markers; APTG, partially purified P7–β-galactosidase chimeric protein (chimera 1). The numbers on the left indicate molecular sizes in kilodaltons.

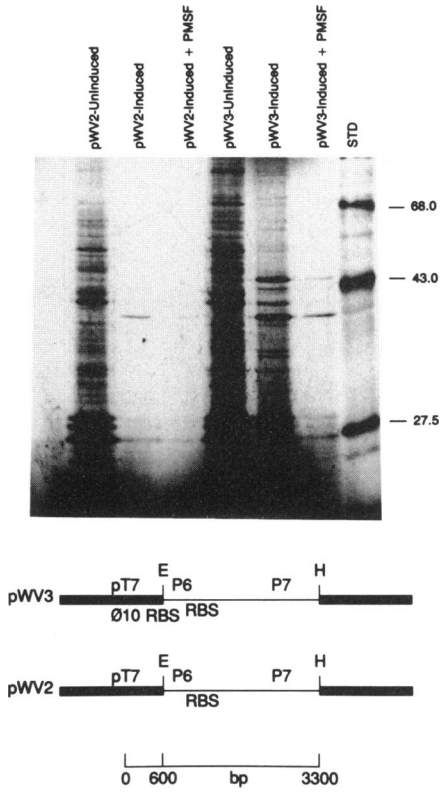

FIG. 4. SDS-polyacrylamide gel electrophoresis and autoradiography of expressed products of plasmids pWV2 and pWV3. *E. coli* K38(pGP1-2) containing plasmid pWV2 (lanes 1 to 3) or pWV3 (lanes 4 to 6) was grown in M9 medium supplemented with 18 amino acids minus cysteine and methionine. Uninduced cells (lanes 1 and 4) were grown at 30°C, and induction (lanes 2 and 5) was performed by shifting cell cultures to 42°C for 20 min. Rifampin was added to the induced cells, and after an additional 10 min at 42°C the cells were grown for 20 min at 30°C. Induced and uninduced cells were pulse-labeled with 10 μCi of [^{35}S]methionine, the cells were lysed and electrophoresed, and the gel was autoradiographed. Plasmid pWV3 differs from plasmid pWV2 by the presence of an additional ribosomal-binding site (RBS). H, *Hin*dIII; STD, molecular size marker proteins; PMSF, phenylmethylsulfonyl fluoride. The numbers on the right are molecular sizes in kilodaltons.

Translation of the nucleotide sequence based on the longest open reading frame and the above-described N-terminal amino acid sequence predicts that P7 contains 391 amino acids, has a molecular weight of 44,210, and has an isoelectric point of 7.38. This molecular weight is in agreement with that expected from previous results. The sequence predicts a hydrophilic protein. Interestingly, the protein lacks tryptophan and the carboxy terminus contains two serine tripeptides at residues 290 to 292 and 348 to 350. Significant homologous amino acid sequences were not detected against recent data base searches.

Expression of *neuA* and *neuC* under control of the T7 promoter. The overlap of the *neuA* and *neuC* genes (Fig. 4) suggests a polycistronic mRNA and, therefore, expression under the control of a single promoter. To study expression of the *neuA* and *neuC* gene products in more detail, the 2.7-kb DNA fragment containing both of these genes was cloned into the T7 expression vectors, pT7-5 and pT7-7, described by Tabor and Richardson (26). Two plasmids, pWV2 and pWV3, were constructed from the 2.7-kb fragment of plasmid pWA1 containing the *neuA* and *neuC* genes by digestion with *Eco*RI and *Hin*dIII and ligation overnight into pT7-5 and pT7-7. The resulting plasmids contained the genes *neuA* and *neuC*, with the *neuA* gene upstream and adjacent to the T7 promoter. These plasmids were transformed (4) into *E. coli* K38(pGP1-2), which contains the gene that encodes bacteriophage T7 RNA polymerase on a compatible plasmid under control of the temperature-inducible p_L promoter, and grown on Luria broth containing 75 μg of ampicillin per ml and 75 μg of kanamycin per ml at 30°C as described by Tabor and Richardson (26).

Proteins expressed under control of the T7 promoter were labeled with [^{35}S]methionine as follows. Cells were grown overnight in Luria broth plus ampicillin and kanamycin, diluted at 1:40, and grown to an A_{590} of 0.4. One milliliter of cells was centrifuged and washed with 5 ml of M9 medium. The cell pellet was suspended in 5 ml of M9 medium supplemented with a 0.02% concentration of 18 amino acids minus cysteine and methionine. The cells were grown at 30°C for 60 min and induced at 42°C for 20 min and then for an additional 10 min in the presence of rifampin (Sigma R-3501) (200 μg/ml). Induced cells were then transferred to 30°C for 20 min. A 0.5-ml portion of induced cells was incubated for 5 min at 30°C with 10 mCi of [^{35}S]methionine, centrifuged, and suspended in 150 μl of cracking buffer (60 mM Tris-HCl [pH 6.8], 1% 2-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue). Samples were heated to 95°C for 3 min and fractionated on an SDS-polyacrylamide gel, which was then dried and autoradiographed. Since T7 RNA polymerase is not inhibited by rifampin, specific labeling of P6 and P7 is enhanced. When induced, K38(pGP1-2)

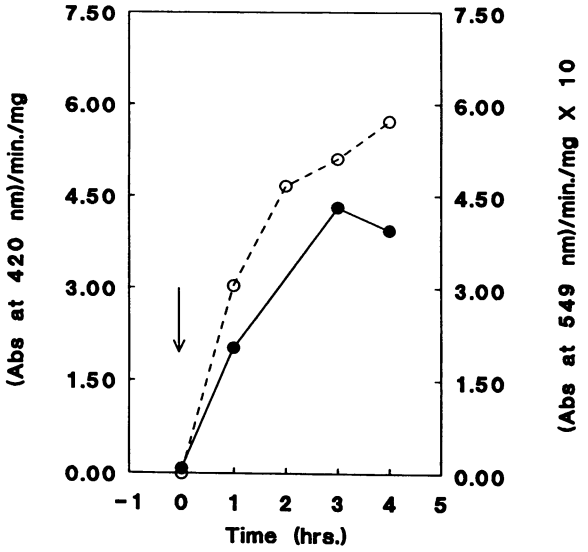

FIG. 5. Simultaneous expression of P7–β-galactosidase chimera 2 and CMP-neuAc synthetase. Strain DH5α harboring plasmid pWG106 was grown in the presence of ampicillin at 37°C and induced by addition of isopropyl-β-D-thiogalactopyranoside to 1 mM at time zero (arrow). Plasmid pWG106 contained the complete *neuA* gene and 259 nucleotides of the *neuC* gene ligated in frame to the *lacZ* gene. Aliquots were taken at different time points, β-galactosidase activity was measured at 420 nm (○), and CMP-neuAc synthetase activity was measured at 549 nm (●). The activities of the different lysates were normalized by using total-protein measurements.

cells harboring either plasmid pWV2 or pWV3 produced two proteins, CMP-neuAc synthetase and P7 (Fig. 4), suggesting that expression of both proteins is controlled by a single promoter.

Since protein P7 could not be assayed directly, expression of the *neuC* gene product was measured as a β-galactosidase chimeric protein (chimera 2) by using plasmid pWG106. Plasmid pWG106 contains the complete *neuA* gene and 259 nucleotides of the *neuC* gene that encode the amino-terminal 86 amino acids of P7, ligated to the *lacZ* gene. The isopropyl-β-D-thiogalactopyranoside-inducible *tac* promoter is located upstream of the complete *neuA* gene in pWG106. pWG106 was constructed as follows. A 1.537-kb fragment containing the *neuA* gene and 259 nucleotides of the *neuC* gene was amplified from pWA1 DNA by the polymerase chain reaction method using primers 5'CGCGAATTCAAAAATCAG GGGGAATAATGAGAA3' and 5'CGCGCTCGAGCAAAC GAATTGAGGCAAACAC3'. Recognition sequences for *Eco*RI and *Xho*I are contained at the 5' ends of the oligonucleotides. The amplified fragment was digested with *Eco*RI and *Xho*I and cloned into vector pSS1295 to form plasmid pWG105. The *lacZ* gene was excised from plasmid pSS1462 with enzymes *Xho*I and *Hin*dIII and cloned into plasmid pWG105 to form plasmid pWG106.

Expression of chimera 2 as β-galactosidase activity by induction of the upstream *tac* promoter was used to examine expression of the *neuA* and *neuC* genes. *E. coli* DH5α (pWG106) was grown to an A_{600} of 0.4 in 500 mg of ampicillin per ml at 37°C and then induced by addition of isopropyl-β-D-thiogalactopyranoside to 1 mM. Aliquots of 500 μl were taken at different time points, and chimera 2 β-galactosidase activity was measured as previously described (20), in a final volume of 1.2 ml. CMP-neuAc synthetase (*neuA*) activity was measured in 40-ml aliquots of the culture taken at different time points. The cells were centrifuged, suspended in 1 ml of 0.05 M Bicine–1 mM dithiothreitol (pH 7.6) and lysed by sonication. CMP-neuAc synthetase activity was measured by using the thiobarbituric acid method (28). The activities of different lysates were normalized by using total-protein measurements (2). Both CMP-neuAc synthetase and P7–β-galactosidase chimera 1 were expressed in parallel (Fig. 5). This confirms the above suggestion that both *neuA* and *neuC* are controlled by a single promoter.

The DNA sequence shows that the longest open reading frame of the *neuC* gene overlaps one nucleotide of the preceding *neuA* gene, and the protein sequence data confirm the longest open reading frame as the translated sequence for protein P7. Similar overlapping genes have been observed in the tryptophan operon and suggest translational coupling of expression of the *trpE* and *trpD* genes in *E. coli* (14). The two protein products of the *trpE* and *trpD* genes are found in equimolar amounts in a tetrameric functional enzyme complex. Perhaps the potential translational coupling between some of the genes in the *E. coli kps* locus represents a means to ensure equimolar production of protein products that function as a complex in the cell.

We thank John Ewell for performing automated Edman sequencing and Scott Stibitz for helpful discussions and supplying plasmids pSS1295 and pSS1462. We thank S. Tabor of Harvard Medical School for supplying the strains and plasmids for the T7 expression system and E. Vimr of the University of Illinois for strain EV80.

REFERENCES

1. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer gradient gels and ^{35}S-label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80**:3963–3965.
1a. **Blacklow, R. S., and L. Warren.** 1962. Biosynthesis of sialics by *Neisseria meningitidis*. J. Biol. Chem. **237**:3520–3526.
2. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
3. **Craven, G. R., E. Steers, Jr., and C. B. Anfinsen.** 1965. Purification, composition, and molecular weight of the β-galactosidase of *Escherichia coli* K12. J. Biol. Chem. **240**:2468–2477.
4. **Dagert, M., and S. D. Ehrlich.** 1979. Prolonged incubation in calcium chloride improves the competence of *Escherichia coli* cells. Gene (Amsterdam) **6**:23–28.
5. **Davis, L. G., M. D. Dibner, and J. F. Batty.** 1986. Basic methods in molecular biology, p. 234–255. Elsevier Science Publishing Co., Inc., New York.
6. **Echarti, C., B. Hirschel, G. J. Boulnois, J. M. Varly, F. Waldvogel, and K. N. Timmis.** 1983. Cloning and analysis of the K1 capsule biosynthesis genes of *Escherichia coli*: lack of homology with *Neisseria meningitidis* group B DNA sequences. Infect. Immun. **41**:54–60.
7. **Germino, J., J. G. Gray, H. Charbonneau, T. Vanaman, and D. Bastia.** 1983. Use of gene fusions and protein-protein interaction in the isolation of biologically active regulatory protein: the replication initiator protein of plasmid R6K. Proc. Natl. Acad. Sci. USA **80**:6848–6852.
8. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted break points for DNA sequencing. Gene (Amsterdam) **28**:351–359.
9. **Hoffman, S., B. C. Sorkin, P. C. White, R. Brackenbury, R. Mailhammer, U. Rutishauser, B. A. Cunningham, and G. M. Edelman.** 1982. Chemical characterization of a neural cell adhesion molecule purified from embryonic brain membranes. J. Biol. Chem. **257**:7720–7729.
10. **Jann, K., and B. Jann.** 1985. Cell surface components and virulence: *Escherichia coli* O and K antigens in relation to virulence and pathogenicity, p. 156–176. *In* M. Sussman (ed.),

The virulence of *Escherichia coli*. Academic Press, Inc., New York.

11. **Kaijser, B., L. A. Hanson, V. Jodal, G. Lindin-Janson, and J. B. Robbins.** 1977. Frequency of *E. coli* K antigens in urinary-tract infections in children. Lancet **i**:664–666.
12. **Lim, H. M., and J. J. Pene.** 1988. Optimal conditions for supercoil DNA sequencing with *Escherichia coli* DNA polymerase I large fragment. Gene Anal. Tech. **5**:32–39.
13. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 170. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Oppenheim, D. S., and C. Yanofsky.** 1980. Translational coupling during expression of the tryptophan operon of *Escherichia coli*. Genetics **95**:785–795.
15. **Orskov, F., I. Orskov, A. Sutton, R. Schneerson, W. Lin, W. Egan, G. E. Hoff, and J. B. Robbins.** 1979. Form variation in *Escherichia coli* K1: determined by O-acetylation of the capsular polysaccharide. J. Exp. Med. **149**:669–685.
16. **Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Orskov, I. Orskov, and L. A. Hanson.** 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. **290**:1216–1220.
17. **Robbins, J. B., R. Schneerson, W. B. Egan, W. Vann, and D. T. Liu.** 1980. Virulence properties of bacterial capsular polysaccharides—unanswered questions, p. 115–132. *In* H. Smith, J. J. Skehel, and M. J. Turner (ed.), The molecular basis of microbial pathogenicity. Dahlem Konferenzen 1980. Verlag Chemie GmbH, Weinheim/Bergst, Federal Republic of Germany.
18. **Roberts, I., R. Mountford, N. High, D. Bitter-Suermann, K. Jann, K. Timmis, and G. Boulnois.** 1986. Molecular cloning and analysis of genes for production of K5, K7, K12, and K92 capsular polysaccharides of *Escherichia coli*. J. Bacteriol. **168**: 1228–1233.
19. **Roth, J., C. Zuber, P. Wagner, D. J. Taatjes, C. Weisgerber, P. U. Heitz, C. Goridis, and D. Bitter-Suermann.** 1988. Re-expression of poly(sialic) acid units of neural cell adhesion molecules in Wilms tumor. Proc. Natl. Acad. Sci. USA **85**: 2999–3003.
20. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
22. **Silver, R. P., W. Aaronson, and W. F. Vann.** 1987. Translocation of capsular polysaccharides in pathogenic strains of *Escherichia coli* requires a 60-kilodalton periplasmic protein. J. Bacteriol. **169**:5489–5495.
23. **Silver, R. P., C. W. Finn, W. F. Vann, W. Aaronson, R. Schneerson, P. J. Kretschmer, and C. F. Garon.** 1981. Molecular cloning of the K1 capsular polysaccharide genes of *E. coli*. Nature (London) **289**:696–698.
24. **Silver, R. P., W. F. Vann, and W. Aaronson.** 1984. Genetic and molecular analyses of *Escherichia coli* K1 antigen genes. J. Bacteriol. **157**:568–575.
25. **Steers, E., Jr., P. Cuatrecasas, and H. Pollard.** 1971. Purification of β-galactosidase by affinity chromatography. J. Biol. Chem. **246**:196–200.
26. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82**:1074–1078.
27. **Tonneguzzo, F., S. Glynn, E. Levi, S. Mjolsness, and A. Hayday.** 1988. Use of a chemically modified T7 DNA polymerase for manual and automated sequencing of supercoiled DNA. Bio/Techniques **6**:460–469.
28. **Vann, W. F., R. P. Silver, C. Abeijon, K. Chang, W. Aaronson, A. Sutton, C. W. Finn, W. Lindner, and M. Kotsatos.** 1987. Purification, properties, and genetic location of *Escherichia coli* cytidine 5′-monophosphate N-acetylneuraminic acid synthetase. J. Biol. Chem. **262**:17556–17562.
29. **Vimr, E. R., W. Aaronson, and R. P. Silver.** 1989. Genetic analysis of chromosomal mutations in the polysialic gene cluster of *Escherichia coli* K1. J. Bacteriol. **171**:1106–1117.
30. **Vimr, E. R., and F. A. Troy.** 1985. Identification of an inducible catabolic system for sialic acids (*nan*) in *Escherichia coli*. J. Bacteriol. **168**:845–853.
32. **Watson, D. R., G. W. Jourdian, and S. Roseman.** 1966. The sialic acids. VIII. Sialic acid 9-phosphate synthetase. J. Biol. Chem. **241**:5627–5636.
33. **Zapata, G., W. F. Vann, W. Aaronson, M. S. Lewis, and M. Moos.** 1989. Sequence of the cloned *Escherichia coli* K1 CMP-N-acetylneuraminic acid synthetase. J. Biol. Chem. **264**:14769–14774.