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Chromosomal Location of a Gene (*nmpA*) Involved in Expression of a Major Outer Membrane Protein in *Escherichia coli*

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The phenotypic expression of protein E, a recently described major outer membrane protein, is associated with a mutation at a locus on the *Escherichia coli* chromosome that we call *nmpA*. *nmpA* is located between *rbsK* and *uncA* at 82.7 min on the *E. coli* linkage map. The *nmpA* locus is also the site of the mutations which lead to the formation of major outer membrane proteins Ic or e. It is likely proteins E, Ic, and e are closely related or identical. The mutant *nmpA* allele is dominant.

The major outer membrane proteins of *Escherichia coli* usually include one or more peptidoglycan-associated proteins (29) such as proteins Ia, Ib (12, 16, 18, 21), 2 (31), and the *lamB* gene product (11). The nomenclatures used by various authors to describe the outer membrane proteins have been summarized by Lugtenburg et al. (22) and more recently by Bassford et al. (2). The peptidoglycan-associated proteins facilitate the diffusion of low-molecular-weight hydrophylic substances through the outer membrane (9, 24, 27), presumably by the formation of transmembrane channels or pores. The term "porin" has been used to describe these proteins (27). Evidence for the role of porin proteins in the diffusion of molecules through a membrane comes from in vivo (3, 24) and in vitro (26, 27) studies which demonstrate that small molecules more readily pass through membranes containing porin protein than through membranes that do not.

E. coli strains which as a result of mutation are missing proteins Ia and/or Ib have been described. At least three genetic loci are involved in the phenotypic expression of proteins Ia and Ib. *tolF* (6) and *cry* (4) strains are missing protein Ia, and it is likely these loci are allelic. *Par* (2), *meo* (36), or TuIb^r (18) strains are missing protein Ib, and it is likely that these loci are allelic. Some *ompB* mutants are missing both proteins Ia and Ib (18, 30).

An *E. coli* strain missing both proteins Ia and Ib, such as a *tolF par* double mutant, is at a disadvantage, since the uptake of sugars and amino acids is restricted by the outer membrane barrier. Faster-growing revertants appear in cultures of such strains. We have recently described one such revertant, strain JF694 (14). Strain JF694 is a *tolF par* strain that carries a third mutation which results in the appearance of a new major outer membrane protein we have called protein E (14). Other laboratories have reported strains that produce a similar new major outer membrane protein. These proteins have been designated protein Ic (18) and e (35).

Insertion of the protein E in the outer membrane restores the antibiotic susceptibility lost as a result of the *tolF* mutation (14) and markedly increases the uptake of amino acids (T.-J. Chai and J. Foulds, manuscript in preparation).

Protein E remains associated with the peptidoglycan layer of the cell envelope after heating at 60°C in 2% sodium dodecyl sulfate solutions as described by Rosenbusch (29; Chai and Foulds, manuscript in preparation). We have described a new bacteriophage that uses protein E as at least part of its receptor (14). Since protein E both serves as a bacteriophage receptor and interacts with the peptidoglycan layer of the cell envelope, it is likely that this protein spans the outer membrane. This is an appropriate location for a protein which we believe to be involved in the formation of transmembrane channels or pores.

In this report we describe a mutation at a single genetic locus that results in the appearance of protein E in the outer membrane. We will refer to this locus as *nmpA* (new membrane protein A). Strains which contain protein Ic or e also carry a mutation at the same or a closely linked locus. We previously reported that protein Ic migrated more rapidly than protein E (14). We have been unable to confirm this and now find that proteins E, Ic, and e are electro-

phoretically indistinguishable and are likely to be the same.

MATERIALS AND METHODS

The *E. coli* K-12 strains and most bacteriophage used in this study are described in Table 1. Bacteriophage P1*vir* was obtained from John Cronan, and bacteriophage f2 was obtained from Peter Model. Cells were grown in L broth (19) containing 2.5 mM $CaCl_2$ or minimal medium (37) supplemented with 0.2% glucose, 0.4% sodium succinate or 0.4% D-ribose, and appropriate growth requirements. These media were solidified with 1.5% agar and, where indicated, 100 μg of streptomycin sulfate per ml was added.

Nomenclature. We suggest the gene symbol *nmpA*$^+$ to refer to the wild-type allele present in strain JF568 and *nmpA* to refer to the allele present in strain JF694. Strains which carry the *nmpA*$^+$ allele do not contain detectable amounts of protein E in their outer membrane, whereas strains which carry the *nmpA* allele contain substantial amounts of protein E in their outer membrane. This gene notation is similar to that for the genes which result in the ability to metabolize β-glucosides where the wild type, *bgl*$^+$, cannot metabolize these sugars.

Genetic techniques. Mapping by gradient of transmission method (10) used cells grown in L broth at 37°C with vigorous aeration to a density of about 2 × 10^8 cells per ml. After a 10-fold dilution of the Hfr strain with prewarmed broth, 2.5 ml was mixed with 2.5 ml of the F$^-$ culture. After a 5-min incubation at 37°C, the mixture was diluted 100-fold into prewarmed L broth, and incubation was continued for 90 min. Next, the mating was interrupted, using a device described by Low (20), and plated on selective medium containing streptomycin.

An F′$_{111}$ merodiploid derivative of strain JF697 was

TABLE 1. *Microorganisms used*

E. coli K-12 strains	Characteristics[a]	References
JF568	*aroA357 ilv-277 metB65 his-53 purE41 proC24 cyc-1 xyl-14 lacY29 rpsL77 tsx-63* λ$^-$	12
JF694	JF703 *par nmpA1*	14
JF697	JF720 *thyA*$^+$ *recA*	This paper
JF699	*aroA*$^+$ *ompA* transductant of strain JF568, using strain JF404-2a as donor	[b]
JF703	*aroA*$^+$ *tolF4* transductant of strain JF568, using strain JF404-4a as donor	14
JF709	*nalA par*$^+$ transductant of strain JF694, using strain W1485 *nalA* as donor	This paper
JF720	JF709 *thyA*, spontaneous mutant	This paper
JF738	*ilv*$^+$ *rbsK* transductant of strain JF694, using strain AA100 as donor	This paper
JF739	*ilv*$^+$ *uncA* transductant of strain JF694, using strain AN120 as donor	This paper
JF743	*ilv*$^+$ *uncA bgl* transductant of strain RK1041, using strain AN120 as donor	This paper
JF404-2a	*thyA ompA* HfrH	13
JF404-4a	*thyA tolF* HfrH	13
AA100	CGSC[c] strain no. 5398 *thr-1 leu-6 thi-1 his-1 argH1 try-1 rbsK3 mtl-2 xyl-7 malA-1 ara-13 gal-6 lacY1 rpsL9 tonA2 supE44?* λ$^-$	B. Bachmann
AN120	CGSC[c] strain no. 5100 *uncA401 argE3 thi-1 mtl-1 xyl-5 galK2 rpsL704 tfr-3? supE44?* λ$^-$	B. Bachmann
RK1041	*argH ilv his metB pyrE60 cysE bgl mtl*	R. Kadner
KL14	*thi* Hfr, point of origin ca. 66 min, clockwise	B. Low
KL16-99	*thi recA* Hfr, point of origin ca. 61 min, counterclockwise	B. Low
KL228	CGSC[c] strain no. 4318 *thi-1 leu-6 gal-6 lacY1* or *Z2R supE44?* λ$^-$ Hfr, point of origin ca. 83 min, counterclockwise	B. Low
KLF′$_{111}$/JC1553	CGSC[c] strain no. 4258 *argG6 metB1 his-1 leu-6 recA mtl-2 xyl-7 malA1 gal6 lacY1* or *Z4 rpsL104 tonA2 tsx-1 supE44Z?* λ$^-$ F′111	M. Lipsett
CE1108	*thr leu thi pyrF cod thyA argG ilvA his lacY tonA tsx phx meo deoC rpsL*, protein e synthesis	B. Lugtenberg
W620	*thi pyrD gltA galK rpsL* TuIa^r, TuIB^r, protein Ic synthesis	U. Henning
PB103	*nalA*, derivative of strain W1485	2

[a] Genetic nomenclature is as described by Bachmann et al. (1), except as follows: *ompA* is listed as *tolG*, *ompB* is described by Sarma and Reeves (30), *par* is described by Bassford et al. (2), *meo* is described by Verhoef et al. (36), and *nmpA* is described in this paper.

[b] Foulds and Chai, submitted for publication.

[c] CGSC, Coli Genetic Stock Center, Yale University, New Haven, Conn.

constructed by the following procedure. First, starting with strain JF694 and a bacteriophage P1*vir* lysate prepared on strain PB103 (*nalA par*$^+$), *nalA* transductants were selected by the method of Hane and Wood (15). Several *nalA* transductants were screened for sensitivity to bacteriophage TuIb. Cell envelope materials prepared from one of the *nalA* transductants sensitive to this bacteriophage, strain JF709, were shown to contain outer membrane protein Ib by sodium dodecyl sulfate-gel electrophoresis. Next a *thyA* derivative of strain JF709, JF720, was isolated using trimethoprim (33). Then a *thyA*$^+$ *recA* recombinant (JF697) was isolated after conjugation of JF720 with Hfr strain KL16-99. Freshly grown cells of JF697 were mixed with freshly grown cells of KLF'$_{111}$/JC1553. The mixture was incubated at 37°C for 25 min. After appropriate dilution, the mixture was plated on selective minimal agar lacking leucine, isoleucine, and valine. Presumptive JF697F'$_{111}$ merodiploid strains were picked and purified by two successive single colony isolations on the selective minimal agar. The JF697F'$_{111}$ isolate selected for further study was shown to be a merodiploid by demonstrating both its sensitivity to bacteriophage f2 and its ability to donate the episome to an *ilv recA* strain.

Preparation of bacteriophage P1*vir* lysates and transduction techniques were accomplished as described by Signer (32). *uncA*$^+$ transductants were selected on minimal agar containing 0.4% sodium succinate plus appropriate supplements. *bgl* transductants were selected on minimal agar containing 0.4% salicin plus appropriate supplements.

Bacteriophage TC45 lysates were prepared by plate lysis as described previously (7), using JF694 as the host.

Polyacrylamide gel electrophoresis. Preparation and solubilization of cell envelope materials, electrophoresis of samples in polyacrylamide gels containing sodium dodecyl sulfate, and staining of gels after electrophoresis were done as previously described (5). In this gel electrophoresis system, protein E migrates more slowly than protein Ia (14).

RESULTS

Location of *nmpA* by conjugation. The gradient of transfer conjugation experiments summarized in Table 2 place the *nmpA* locus close to *ilv*. The relative frequency of the *nmpA* and recombinants in the first cross (Table 2) indicates that this locus was either close to the origin of Hfr strain KL228 or between *xyl* and *rpsL*. The second cross demonstrated that *nmpA* was located between *xyl* and *ilv*, close to the origin of Hfr strain KL228.

Location of *nmpA* by three-factor transduction. The transduction experiments summarized in Table 3 place the *nmpA* locus between *rbsK* and *uncA*. We were unable to select either *rbsK*$^+$ or *nmpA*$^+$ transductants directly. The *rbsK* strain that we used gave relatively few *rbsK*$^+$ transductants, which appeared after 2 to 3 days of incubation on a lawn of slow-growing *rbsK* cells. The *nmpA*$^+$ locus, which determines resistance to bacteriophage TC45, requires at least 6 h for expression. This presumably allows sufficient dilution of protein E in the outer membrane. However, *nmpA* and *nmpA*$^+$ strains grow at different rates, a difference that depends, in part, on the genetic background (data not shown). Therefore, it was not possible to estimate the frequency of unselected markers after 6 h of growth when *nmpA*$^+$ was used to select transductants. The data in Table 3 are summarized in Fig. 1. *nmpA* is located close to the origin of replication, *ori* (38, 39), of the *E. coli* chromosome.

TABLE 2. *Location of nmpA on the E. coli chromosome by gradient of transmission*

Donor	Relative frequency of unselected markers (%)				
	thyA$^+$	*rpsL*$^+$	*xyl*$^+$	*nmpA*$^+$[a]	*ilv*$^+$
KL228	2.1	24	100[b]	78	
KL14		58	100	83	80

[a] *nmpA*$^+$ was scored as resistant to bacteriophage TC45.

[b] The selected marker in both crosses was *xyl*$^+$ and was given a value of 100%. Strain JF709 was the recipient, and 480 recombinants were tested per cross.

Location of the mutation in strains containing protein Ic or e. Outer membrane proteins Ic and e, like outer membrane protein E, were described in *E. coli* mutant strains missing protein Ia (also called protein b) and Ib (protein c). It is likely that proteins E, Ic, and e are identical, for they are all associated with the peptidoglycan, are involved in the formation of pores in the outer membrane, and serve as at least part of the receptor for bacteriophages TC45 and TC23 (7, 18, 35). The transduction experiments summarized in Table 4 show that the mutations which lead to the phenotypic expression of proteins Ic and e are located close to *uncA*. Both mutations cotransduce with the *ilv* and *uncA* loci with the same frequency as the *nmpA1* mutation of JF694. Complementation tests to demonstrate that these mutations are allelic were not possible because the mutations are dominant (see below).

Expression of *nmpA*. Strain JF694, containing the *nmpA* allele, does not contain the major outer membrane proteins Ia and Ib. We have transduced the *nmpA* allele into a number of strains that contain two or three of the major outer membrane proteins, Ia, Ib, and II*. These strains were constructed by cotransduction of the *nmpA1* allele with *ilv*$^+$, using JF738 as the donor. *ilv*$^+$ transductants were purified by two single colony isolations and tested for cotransduction of the *nmpA* allele by demonstrating

TABLE 3. *Three-factor cotransduction of nmpA*

Bacterial strains and relevant loci		Selected marker	No. tested	Distribution of unselected markers
Donor	Recipient			
AA100 *rbsK nmpA*$^+$	JF694 *ilv nmpA*	*ilv*$^+$	288	*rbsK*$^+$*nmpA*$^+$ (9) *rbsK*$^+$*nmpA* (30) *rbsK nmpA*$^+$ (126) *rbsK nmpA* (123)
AN120 *nmpA*$^+$*uncA*	JF694 *ilv nmpA*	*ilv*$^+$	288	*nmpA*$^+$*uncA*$^+$ (34) *nmpA*$^+$*uncA* (137) *nmpA uncA*$^+$ (105) *nmpA uncA* (12)
AA100 *rbsK nmpA*$^+$	JF739 *nmpA uncA*	*uncA*$^+$	288	*rbsK*$^+$*nmpA*$^+$ (105) *rbsK*$^+$*nmpA* (24) *rbsK nmpA*$^+$ (147) *rbsK nmpA* (12)
JF694 *ilv nmpA*	AN120 *nmpA*$^+$ *uncA*	*uncA*$^+$	192	*ilv*$^+$*nmpA*$^+$ (14) *ilv*$^+$*nmpA* (82) *ilv nmpA*$^+$ (5) *ilv nmpA* (91)
JF738 *rbsK nmpA*	JF568 *ilv nmpA*$^+$	*ilv*$^+$	192	*rbsK*$^+$*nmpA*$^+$ (30) *rbsK*$^+$*nmpsA* (10) *rbsK nmpA*$^+$ (42) *rbsK nmpA* (110)
AN120 *uncA bgl*$^+$	RK1041 *ilv bgl*	*ilv*$^+$	288	*uncA*$^+$*bgl*$^+$ (15) *uncA*$^+$*bgl* (128) *uncA bgl*$^+$ (59) *uncA bgl* (86)
JF743 *uncA bgl*	JF694 *nmpA*	*bgl*	288	*nmpA*$^+$*uncA*$^+$ (4) *nmpA*$^+$*uncA* (146) *nmpA uncA*$^+$ (19) *nmpA uncA* (119)

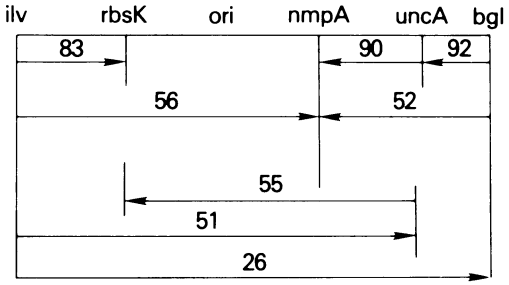

FIG. 1. *Position of nmpA on the genetic map of E. coli. The figure corresponds with the portion of the map of Bachmann et al. (1) near min 82 to 83. The numbers above the arrows represent cotransduction frequencies rounded to the nearest percent, and the arrowheads indicate the unselected marker. The data were taken from Table 3.*

sensitivity of the transductants to bacteriophage TC45. Although only 16 *ilv*$^+$ transductants were tested, the cotransduction frequency of *nmpA* with *ilv*$^+$ was within the range expected (7 to 11 of 16). This showed that the frequency of cotransduction of the *nmpA* allele was not depressed by the presence of *tolF* or *par* alleles.

Cell envelope materials prepared from two or four independently isolated *ilv*$^+$ *nmpA* transductants of JF568, JF699, JF703, and JF709 were analyzed by polyacrylamide gel electrophoresis, and, in every instance, protein E was found to be one of the major proteins, along with polypeptides Ia, Ib, and II* (JF568), Ia and Ib (JF699), Ib and II* (JF703), Ia and II* (JF709), or Ib and II* (JF697).

Dominance of *nmpA*. An F′$_{111}$ merodiploid strain was prepared which contained the *nmpA* allele on the chromosome and presumably contained the silent *nmpA*$^+$ allele on the episome. Cell envelope materials prepared from this strain, JF697F′$_{111}$, contained similar amounts of protein E when compared with the F$^-$ parent JF697.

DISCUSSION

We have previously referred to the mutation in strain JF694 as *ompE* (14). We wish to change this to *nmpA* (new membrane protein) and reserve the *omp* designation for proven structural genes coding for synthesis of outer membrane proteins. Although it is possible that *nmpA* is the structural gene for protein E, we have presented no evidence here to support this view.

The *nmpA* locus is a gene involved in the phenotypic expression of protein E. Isolation of *nmpA* mutations should be easily accomplished in any strain of *E. coli*. These mutants may be useful both for the study of new major outer membrane proteins and for the characterization and amplification of nearby loci. For example, the *nmpA* gene is located close to both the origin of transfer of Hfr strain KL228 and the origin of replication, *oriC*, of the bacterial chromosome. The *nmpA*$^+$ (wild-type) allele is silent. These strains produce no protein E detectable by polyacrylamide gel electrophoresis and are completely resistant to bacteriophage TC45. We have shown that bacteriophage TC45 is inactivated by a mixture of protein E and lipopolysaccharide, supporting our previous conclusion that bacteriophage TC45 uses protein E as at least part of its receptor (7). The presence of a normally silent gene on the *E. coli* chromosome might be a means whereby *E. coli* cells sensitive to porin-specific bacteriophages like TuIa or TuIb (8) survive by a mutation resulting in the appearance of a new porin, such as protein E, which does not serve as a receptor to these bacteriophages (7) but does facilitate the diffusion of small hydrophylic molecules. It should be noted that the frequency of bacteriophages such as TC45, found in raw sewage, which use protein E as at least part of their receptor, is much lower than those that use proteins Ia or Ib (14).

The control of the level of peptidoglycan-as-

TABLE 4. *Cotransduction of nmpA locus in strains producing protein Ic or e*

Bacterial strains and relevant markers		Selected marker	Transductants with unselected donor marker/ total transductants tested	Cotransduction frequency (%)
Donor	Recipient			
CE1108 *nmpA uncA*$^+$	AN120 *nmpA*$^+$ *uncA*	*uncA*$^+$	84/96 *nmpA*[a]	88
AA100 *ilv*$^+$ *rbsK nmpA*$^+$	CE1108 *ilvA nmpA*	*ilvA*$^+$	81/192 *nmpA*$^+$[b]	42
W620 *nmpA uncA*$^+$	AN120 *nmpA*$^+$ *uncA*	*uncA*$^+$	80/96 *nmpA*	83

[a] Unselected marker.

[b] Analysis of three-factor cross suggests the order of loci to be *ilv rbsK nmpA*.

sociated proteins is not simply a reflection of the total protein in the outer membrane. An *E. coli* strain unable to synthesize phospholipid continues to synthesize substantial amounts of outer membrane protein and incorporates these proteins into its outer membrane (25). This indicates that normally the outer membrane is not "saturated" with protein. Although the outer membrane may be able to accommodate additional protein, it is not clear that this can be accomplished without an alteration in functional integrity.

Outer membrane materials prepared from all strains that carry the *nmpA1* mutation contain substantial amounts of protein E. In JF694, the amount of protein E approximately equals the amounts of proteins Ia plus Ib in JF568 (Foulds and Chai, unpublished observation). We are currently trying to estimate the individual amounts of protein E, Ia, and Ib in strains like JF568 *ilv*$^+$ *nmpA*. Although we have not yet sufficiently resolved proteins E and Ia electrophoretically, we can say that the sum of proteins E plus Ia plus Ib in JF568 *ilv*$^+$ *nmpA* approximates the sum of proteins Ia plus Ib in JF568.

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