JOURNAL OF BACTERIOLOGY, Apr. 1985, p. 256–262
0021-9193/85/040256-07$02.00/0

Vol. 162, No. 1

Evidence that the Outer Membrane Protein Gene *nmpC* of *Escherichia coli* K-12 Lies within the Defective *qsr'* Prophage

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Received 17 September 1984/Accepted 21 January 1985

Recombinants between phage λ and the defective *qsr'* prophage of *Escherichia coli* K-12 were made in an *nmpC* (p^+) mutant strain and in the *nmpC*$^+$ parent. The outer membrane of strains lysogenic for recombinant *qsr'* phage derived from the *nmpC* (p^+) strain contained a new protein identical in electrophoretic mobility to the NmpC porin and to the Lc porin encoded by phage PA-2. Lysogens of *qsr'* recombinants from the *nmpC*$^+$ strain and lysogens of λ *p4*, which carries the *qsr'* region, did not produce this protein. When observed by electron microscopy, the DNA acquired from the *qsr'* prophage showed homology with the region of the DNA molecule of phage PA-2 which contains the *lc* gene. Relative to that of the recombinant from the *nmpC* (p^+) mutant, the DNA molecule of the recombinant from the *nmpC*$^+$ parent contained an insertion near the *lc* gene. These results were supported by blot hybridization analysis of the *E. coli* chromosome with probes derived from the *lc* gene of phage PA-2. A sequence homologous to the *lc* gene was found at the *nmpC* locus, and the parental strains contained an insertion, tentatively identified as IS*5B*, located near the 3' end of the porin coding sequence. We conclude that the structural gene for the NmpC porin protein is located within the defective *qsr'* prophage at 12.5 min on the *E. coli* K-12 map and that this gene can be activated by loss of an insertion element.

Strains of *Escherichia coli* K-12 which carry mutations resulting in the loss of the OmpC and OmpF porin proteins are deficient in the transport of small hydrophilic compounds and are multiply colicin resistant. Upon prolonged culture, such mutant strains accumulate extragenic pseudorevertants which produce new porin proteins which restore transport of solutes through the outer membrane and partial colicin sensitivity. One of these extragenic pseudorevertants produced a new protein which was nearly identical to the Lc protein (formerly called protein 2) which is produced in cells lysogenic for phage PA-2 (23). The locus of the mutation resulting in the production of this new protein was mapped to 12.5 min on the *E. coli* chromosome and was termed *nmpC*; hence, the protein is termed the NmpC protein (23). Because of the similarity between the Lc and NmpC proteins, Lee et al. (17) have proposed that they arose from the same ancestral gene and that an ancestor of phage PA-2 acquired the gene by recombination with the *E. coli* chromosome.

The chromosome of *E. coli* K-12 is known to contain several defective lambdoid prophages (1, 15). An equally plausible hypothesis is that a number of lambdoid phages carry porin genes related to the *lc* porin gene of phage PA-2 and that the *nmpC* locus is part of a defective prophage. We set out to investigate this possibility.

MATERIALS AND METHODS

Bacteria and bacteriophages. Bacteria and bacteriophages are listed in Tables 1 and 2. *E. coli* K-12 strains containing *supF* were constructed by making NM1166 (λ *att* c1857 *supF*) lysogens and then selecting for cells which subsequently lost the prophage but retained *supF*. Lysogens were expected to form by recombination between the *supF* regions of the phage and the *E. coli* chromosome. Prophages were expected to be lost by the reverse process. Putative lysogens were tested by spotting phages λ clear and λ *imm*^{434} on them. The cells were then streaked out on L agar and grown at 42°C. Only cells which had lost the prophage, which has a temperature-sensitive repressor, were expected to grow at this temperature. Several hundred individual colonies were picked and tested for the presence of *supF* by spotting on to L agar spread with phage λ *N*am7 am53, which requires *supF* for growth. Colonies which were lysed by phage λ *N*am7 am53 were grown up and tested for lysis by phage NM698 (λ *Q*73 *S*7).

Culture media. L broth (18) without glucose was used for all liquid cultures. L agar was made from the same medium with the addition of Difco agar (15 g liter^{-1}). BBL agar was made from BBL medium (21) with the addition of Difco agar (bottom layer, 10 g liter^{-1}; top layer, 6.5 g liter^{-1}).

Phage buffer. One liter contained the following: KH_2PO_4, 3 g; Na_2HPO_4 (anhydrous), 7 g; NaCl, 5 g; 0.1 M $MgSO_4$, 10 ml; 0.01 M $CaCl_2$, 10 ml; 1% gelatin, 1 ml.

Formation of lysogens. Lysogens were formed by spotting phage lysates of *E. coli* K-12 C600 grown in L broth onto L agar seeded with *E. coli* K-12. After incubation overnight, cells from the center of the spot were grown up in L broth and then streaked out on L agar spread with 10^9 homoimmune clear plaque forming phages to kill nonlysogens. After 24 h of incubation, individual colonies were grown up, plated out, and tested by spotting appropriate phages on them.

Phage λ *p4* has the *b2* region deleted, which means that part of the chromosomal attachment site is missing. We looked for λ *p4* lysogens on the assumption that integration might occur at low frequency by recombination between the regions of homology with the *E. coli* chromosome, known to exist in the *p4* region (15). To form λ *p4* lysogens, the phages were first allowed to adsorb to the cells, which were then spread on L agar previously spread with 10^9 NM507 (*h* λ *b2* *imm*^{21} c1) phages to destroy nonlysogens and 10^9 NM848 (*h*^{82} *imm*^{21} c1) phages to destroy λ-resistant cells.

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TABLE 1. *Escherichia coli* K-12 strains

Strain	Relevant features	Source (reference)
C600	*omp*$^+$ *nmp*$^+$	(2)
C600 (PA-2)	lysogenic for phage PA-2	This work
C600 (λ *p4*)	lysogenic for phage λ *p4*	This work
C600 [λ *qsr'* (*p*$^+$)]	lysogenic for phage λ *qsr'*(*p*$^+$)	This work
C600 (λ *qsr'*)	lysogenic for phage λ *qsr'*	This work
CS180 (=P1700)	*omp*$^+$ *nmp*$^+$	P. Reeves (23)
CS327	*ompR156* of CS180	C. Schnaitman (23)
CS384	*nmpC* (*p*$^+$) of CS327	C. Schnaitman (23)
CS457	Δ(*cbr-nmpC*) of CS384	C. Schnaitman (23)

Surviving colonies were grown up and tested as described above. To produce cells lysogenic for phages containing temperature-sensitive repressors, all plates were incubated at 30°C instead of 37°C. Putative PA-2 lysogens of C600 were tested for PA-2 immunity and sensitivity to PA-2 by spotting phage PA-2 and phage 82, which uses the same receptor as PA-2 (P. J. Highton and Y. Chang, unpublished data), onto them. Putative λ *p4* lysogens of C600 were similarly tested with phages λ *p4* and NM848 (h^{82} imm^{21} cI). Tests for other lysogens are given below.

Formation of *qsr'* recombinants. To form *qsr'* recombinants, the phage λ mutant NM698, which is *QS* and has two deletions totaling about 15% of the length of the λ DNA molecule, was plated on strains CS180 and CS384. The deletions were necessary to accommodate the extra length of the *qsr'* DNA (27). Only *qsr'* recombinants were expected to form plaques, but to avoid selecting recombinants already in the phage stock, it was necessary to use *supF* strains.

Single plaques formed by phage NM698 on *supF*-containing derivatives of CS180 and CS384 were used to make lysates of these strains on BBL agar. (Since all of the phages in the NM698 stock could grow on the *supF* strains, the chance of having picked a recombinant already in the stock was low.) Each plate lysate was then plated with the appropriate original (non-*supF*) strains of CS180 and CS384 to look for *qsr'* recombinants that had occurred during growth of the lysates. Only one plaque was selected from each lysate so that each was produced by a separate recombinational event. Lysogens of C600 were made with these recombinant phages and tested by spotting phages λ and λ imm^{434} on them.

Isolation of outer membranes. Outer membrane proteins were isolated and analyzed on Tris-glycine-sodium dodecyl sulfate gels as described by Schnaitman and McDonald (25).

DNA isolation. Concentrated phage suspensions were prepared from liquid lysates of *E. coli* K-12 C600 grown in L broth. Chloroform was added to a concentration of 2 μl ml^{-1} to lyse any intact cells, and then DNase was added to a concentration of 1 μl ml^{-1} to digest the bacterial DNA. After 2 h at 37°C, the cell debris was removed by centrifugation at 1,500 × *g* for 6 min. Polyethylene glycol 6000 and NaCl were then added to concentrations of 10% (wt/vol) and 40 g liter^{-1}, respectively, to precipitate the phage (28). After standing at 4°C overnight, the lysate was centrifuged again at 1,500 × *g* for 5 min, and then the phage pellet was resuspended in phage buffer. Protein was removed from the phage solutions by extracting three times with equal volumes of phenol at room temperature. The resulting solutions were dialyzed against 0.01 M Tris-hydrochloride (pH 8.5)–0.001 M EDTA.

Heteroduplexes. Heteroduplexes were made and spread for electron microscopy by the method of Davis et al. (4). The hyperphase was 0.1 M Tris-hydrochloride (pH 8.5)–0.01 M EDTA–50% formamide, and the hypophase was 0.01 M Tris-hydrochloride (pH 8.5)–0.001 M EDTA–15% formamide. The molecules were picked up on parlodion films mounted on electron microscope grids, stained in uranyl acetate, and then rotary shadowed with Pt. The specimens were then coated with carbon to give stability on irradiation in the electron microscope, and the parlodion was removed by immersion in absolute ethanol for 60 s.

Electron microscopy and measurement. Specimens were viewed with a Siemens Elmiskop 101 and photographed at ×10,000 to ×20,000 magnification. Negatives were projected with a photographic enlarger at a further ×5 magnification and were measured with a Ferranti Cetec tablet digitizer linked to an Olivetti P6040 calculator.

So that it would be possible to relate the measurements of one heteroduplex to those of another, circular double-stranded pAT153 DNA molecules (3.657 kilobases [kb]) and circular single-stranded M13 DNA molecules (6.407 kb) were mixed with the heteroduplexes before spreading so that they appeared in each heteroduplex photograph.

Recombinant DNA techniques. *E. coli* chromosomal DNA was isolated as described by Marmur (19), and plasmid isolation, Southern blot hybridization, and cleavage, ligation, and electrophoresis of DNA fragments were as described by Silhavy et al. (26). Plasmid probes were labeled by nick translation, using a kit supplied by Bethesda Research Laboratories, Bethesda, Md., and labeling of M13mp8 carrying the cloned *lc* Msp fragment from PA-2 was done with an M13 sequencing kit supplied by Amersham Corp.,

TABLE 2. Phage strains

Strain	Relevant features	Source (reference)
λ cI857 *S*am7		K. Murray (8)
λ *c*I	Clear	N. Murray
λ imm^{434}		N. Murray (14)
λNM1166	*att* cI857 *supF*	N. Murray
λNM698	Δ(*srI* 1-2) cI857 *nin*5 *Q*am7 *S*am7	N. Murray
λ *N*am7 am53	Requires *supF*	N. Murray
λ *p4*	*b2* imm^{21} *p4*	I. Herskowitz
λ *qsr'* (p$^+$)	NM698 *qsr'* recombinant from CS384	This work
λ *qsr'*	NM698 *qsr'* recombinant from CS180	This work
PA-2	Wild type	C. Schnaitman (24)
PA-2h^{λ}*1*cam3	Selected as colicin E3^r Ag^{++r} mutant of an *ompR* strain lysogenic for Hy7 (23)	C. Schnaitman
PA-2h^{λ}*1c*4	As above, except not am	C. Schnaitman
PA-2h^{λ}*1c*5	As above, except not am	C. Schnaitman
PA-2*C*ts9		C. Schnaitman (22)
λNM507	h^{λ} *b2* imm^{21} cI	N. Murray
λNM848	h^{82} imm^{21} cI	N. Murray
λ*	Wild type	C. Schnaitman
λ *p4* imm^{434}*	*b2*(?) imm^{434} *p4*	I. Herskowitz
λ *p4* *F*am*	*b2*(?) cI *int6 red3 p4* imm^{21} *F*am204	I. Herskowitz
λ *p4**	*b2*(?) cI *int6 red3 p4* imm^{21}	I. Herskowitz
λ imm^{21} *F*am*	cI *int6 red3 b2* *F*am204 imm^{21}	I. Herskowitz
λ imm^{21}*	cI *int6 red3* imm^{21}	I. Herskowitz

[a] * indicates strains used only in the transactivation experiment described in Table 4.

Arlington Heights, Ill., with [α-^{32}P]dCTP as the source of label. After labeling, the polymerase reaction mixture was incubated with appropriate restriction enzymes, and the fragments to be used as probes were purified by polyacrylamide gel electrophoresis.

Phage complementation. Transinduction infection experiments were carried out as described by Herskowitz and Signer (10) with the following modifications: infecting phage were diluted to 5 × 10^6 PFU/ml, and infected cells were incubated with shaking at 37°C for 120 min before assay of progeny. The efficiency of adsorption was assayed in all experiments by lysing a portion of the infected cells with chloroform 20 min after infection and titering unadsorbed phage; in all experiments, the efficiency of adsorption was greater than 95%.

RESULTS

Heteroduplexes between the DNA molecules of λ and PA-2 visualized by electron microscopy exhibit several large regions of nonhomology (R. J. Meyers, Ph.D. thesis, University of Edinburgh, Edinburgh, United Kingdom, 1981). One of these regions of nonhomology lies in the right-arm region of the phage genome which corresponds, in λ, to genes *Q*, *S*, and *R*. This region, which is shown schematically in Fig. 1A, is flanked by regions which are homologous; to the left of the region of nonhomology is a homologous region including genes *O* and *P*, and to the right is a homologous region including all of gene *Rz* and extending to the rightward *cos* site.

Table 3 shows the results of three genetic crosses used to map the *lc* porin function of phage PA-2. These data indicate

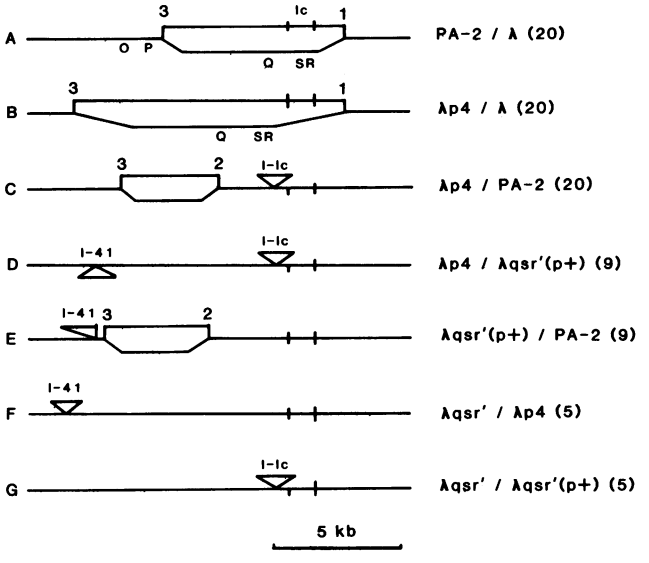

FIG. 1. Schematic drawing of the right ends of heteroduplexes formed between the DNA molecules of phages λ, PA-2, λ *p4*, λ *qsr'* (a recombinant from CS180), and λ *qsr'* (p^+) (a recombinant from CS384). The span of the right ends shown is ca. 15 kb, and regions of nonhomology due to different immunity regions at the left ends are not shown. The number of heteroduplexes measured and averaged for each drawing is shown in brackets on the right. Only horizontal lines represent distances in the molecules; the sloping and vertical lines are simply for connection. The upper molecule is the one given first in the heteroduplex name. The heteroduplexes are aligned on their right termini. The numbers 1 through 3 indicate corresponding points in the heteroduplexes.

TABLE 3. Mapping of the *lc* locus of phage PA-2

Cross[a]	Total h^λ selected	Frequency (%) of unselected markers[b]		
		cts9	lc^+	c^+ lc+
PA-2h^λ *lcam3* × PA-2cts9	88	27	34	7
PA-2h^λ *lc4* × PA-2cts9	300	25	35	10
PA-2h^λ *lc5* × PA-2cts9	1,128	21	34	9
Total	1,516	22	34	9

[a] A lysogen of PA-2cts9 was infected with the phage to be crossed at a multiplicity of ca. 0.1. After incubation at 42°C for 90 min, h^λ were selected by plating on an *ompR* host.

[b] cts9 was scored as clear plaques on an omp^+ strain at 42°C. lc^+ was scored as porin$^+$ plaques at 33 and 42°C on an *ompR* host.

that *lc* mapped to the right of the immunity region, placing it within the region of nonhomology noted above. As a more direct proof that this region of nonhomology carried the *lc* porin function, a λ-PA-2 hybrid phage carrying this region was constructed.

This hybrid phage, which is designated as λ lc-1, was constructed by crossing λ *c*I857 *P*am3 with wild-type PA-2 and selecting for imm^λ h^λ P^+. The resulting phage were screened for porin$^+$ plaques (25) on an *ompR* indicator strain. λ lc-1 was chosen as a phage which produced porin$^+$ plaques on a variety of porin-deficient indicator strains. Heteroduplexes between λ and λ lc-1 DNA molecules showed a single region of nonhomology located between the *OP* region and the *Rz-cosR* region identical to that shown in Fig. 1A (data not shown).

Restriction site data indicate that λ lc-1 resulted from a recombination between λ and PA-2 near the junction between genes *O* and *P*. The *Eco*RI site within *O* (λ coordinate 39,168) is retained in λ lc-1, and the restriction map to the left of this site is identical to that of λ for the enzymes *Eco*RI, *Bam*HI, *Hin*dIII, and *Bgl*II. The *Ava*I site located within gene *P* of λ (coordinate 39,888) is absent in λ lc-1, and from this point to *cosR* the restriction map is identical to that of PA-2. A portion of this restriction map is shown below (see Fig. 5).

E. coli K-12 strains lysogenic for λ lc-1 produce a porin which is identical in its migration on sodium dodecyl sulfate gels to that of the Lc porin produced by PA-2 lysogens, and the porin protein of λ lc-1 lysogens is regulated by temperature and catabolite repression in exactly the same manner as the porin protein produced by PA-2 lysogens (7). Thus, all of the information necessary for expression of the *lc* function lies in the *lc* region to the right of gene *P*. Gregg (unpublished data) constructed derivatives of λ lc-1 which carry hybrid *ompC-lc* porin genes. Lysogens of these derivatives produce hybrid porin proteins, indicating that the structural gene for the Lc porin lies in this region. The putative location of the *lc* gene is shown in Fig. 1 and is based on the molecular weight of the protein (5) and restriction mapping of DNA from phages carrying *ompC-lc* hybrid porin protein genes.

The region of nonhomology corresponding to the *lc* region of phage PA-2 and the *QSR* region of λ is flanked by regions which are also homologous to portions of a defective prophage located in the 12-min region of the *E. coli* K-12 chromosome (1, 15). In this defective prophage, an alternative set of *QSR* genes called *qsr'* lies between these homologous regions. In certain λ mutants (termed *qin* mutants) the *QSR* genes of λ have been exchanged for the *qsr'* genes of this defective prophage, presumably by recombination between the flanking homologous regions, as suggested by

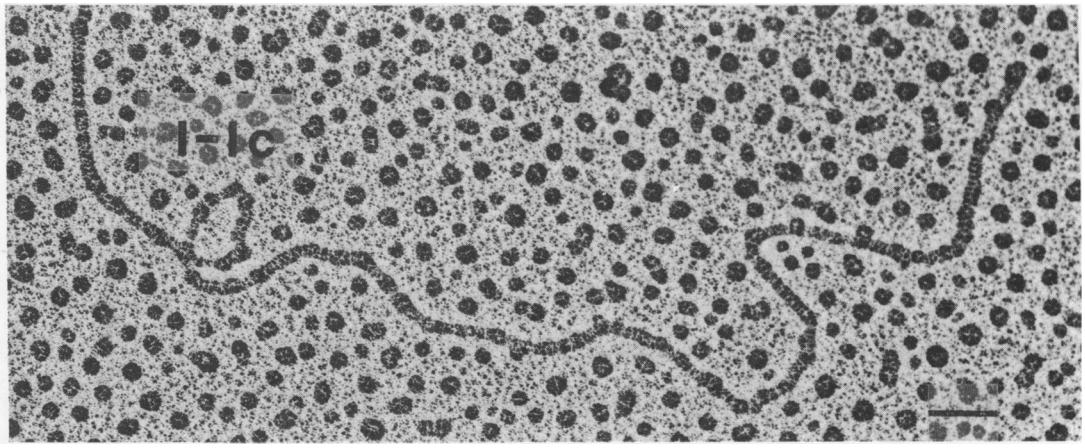

FIG. 2. The right end of a λ *qsr'*-λ *qsr'* (p^+) heteroduplex showing the insertion I-*lc* (see Fig. 1G). The scale marker is 100 nm.

Fiandt et al. (6). The oldest of these *qin* mutants is λ *p4*, which was isolated as a UV-induced small plaque mutant by Jacob and Wollman (13). Since the *qsr'* substitution from the defective prophage (termed the *p4* region) is much longer than the corresponding *QSR* region of λ (Fig. 1B), λ *p4* also carries a compensating deletion in the *B2* region (6).

Since the NmpC porin is nearly identical to the Lc porin encoded by a gene lying near or within the *QSR* region of phage PA-2, and since the *nmpC* locus which determines production of this porin maps to the same 12-min region of the chromosome as the *qsr'* defective prophage (23), it was reasoned that the *nmpC* locus might lie within the *p4* region of the *qsr'* defective prophage. If this were true, a portion of the *p4* region of λ *p4* might be expected to be homologous with the *lc* region of the DNA molecule of phage PA-2. Figure 1C shows that this is indeed the case, since about half of the *p4* region is homologous with the *lc* region of PA-2, including the putative site of the *lc* structural gene. Comparison of the stability of heteroduplexes and homoduplexes spread in higher concentrations of formamide indicate that there was not more than 2 to 3% mismatch in the *lc* gene region. This was based on values for the lowering of melting temperature produced by formamide and mismatch given by Hyman et al. (12).

Thus, there appears to be a gene in the *qsr'* prophage which is largely homologous with the *lc* gene in PA-2. This could well be the structural gene for the NmpC porin protein. However, unlike the *lc* gene of PA-2, that in λ *p4* did not appear to be expressed in lysogens. There was no new outer membrane protein visible in sodium dodecyl sulfate gels of λ *p4* lysogens (see Fig. 3G). λ *p4* did not yield porin$^+$ plaques on porin-deficient indicator strains (25), which is a very sensitive assay for porin functions carried on λ phages.

Figure 1C also shows that relative to PA-2, λ *p4* contains an insertion (I-*lc*) just to the left of the *lc* gene 2.4 kilobases (kb) from the right end of the *p4* region. This insertion could inhibit expression of the porin gene in lysogens. If the gene in λ *p4* is indeed *nmpC*, then loss of this insertion could be the process by which the gene was activated in the *nmpC* (p^+) mutant. To test this, we constructed *qsr'* recombinants in an *nmpC* (p^+) strain, CS384, and in the parental strain CS180. Figure 1D and E shows that the DNA molecule of a recombinant from CS384 did not contain the I-*lc* insertion, whereas Fig. 1F and G shows that a recombinant from the parental strain CS180 did contain an insertion homologous to the I-*lc* insertion of λ *p4*. Both recombinant molecules contained another insertion relative to λ (called I-41) which was assumed to be the same as that found in the *qsr'* recombinant λ *p41* (10). An electron micrograph of the I-*lc* insertion is shown in Fig. 2.

Figure 4 shows that an extra protein with the mobility of the Lc and NmpC proteins was present in the outer membrane of strain C600 lysogenic for a λ *qsr* (p^+) recombinant. The amount of this protein present was found to be regulated by temperature, as are those of the Lc and NmpC proteins (7). The *c*I857 allele present in these lysogens prevented the testing of these strains at temperatures which were expected to lead to the maximum expression of the protein.

In an independent experiment, we constructed a recombinant phage by propagating λ *p4* on strain CS384 and screening the progeny phage for porin$^+$ plaques on an *ompR* indicator strain (CS457). The recombinant phage was strongly porin$^+$ on this indicator strain, and lysogens produced a protein with mobility identical to that of the Lc and NmpC strains. Presumably, λ *p4* lost the I-*lc* insertion by recombination with the *qsr'* (p^+) prophage is CS384. In Fig. 1, the location of *lc* in the PA-2 DNA molecule is based on unpublished data, but the above results indicate that *nmpC* lies within the *p4* region, no matter where *lc* may be located. The constitutions of the four phages shown in Fig. 1 are given in Table 4.

During the course of sequencing the *lc* region of phage PA-2, we obtained several restriction fragments from the *lc* region which have been used to examine the structure of chromosomal DNA from *E. coli* K-12. The fragments which

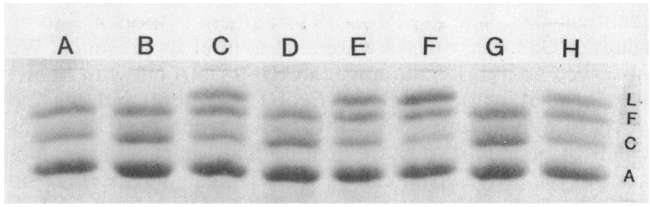

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel of the major outer membrane proteins produced by various λ *qsr'* lysogens. Lane A, nonlysogenic strain C600; lanes B and D, two C600 lysogens of a λ *qsr'* recombinant phage from CS180; lanes C, E, and F, three C600 lysogens of a λ *qsr'* (p^+) recombinant phage from CS384; lane G, C600 lysogenic for λ *p4*; and lane H, C600 lysogenic for PA-2. The letters at the right show the migration of Lc-NmpC protein (L), OmpF protein (F), OmpC protein (C), and OmpA protein (A). The cultures were grown at 34°C.

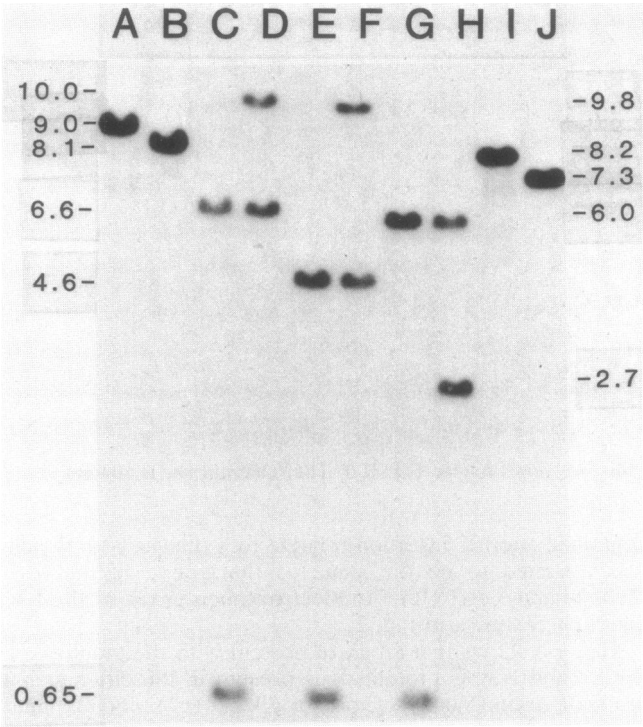

FIG. 4. Blot of chromosomal DNA from CS384[*nmpC* (p^+)] and CS180 (*nmp*$^+$) cut with various restriction enzymes and probed with the *Msp*I-*Bgl*II fragment from the *lc* region of PA-2 (Fig. 5). Lanes A, C, E, G, and I are DNA from CS180, and lanes B, D, F, H, and J are DNA from CS384. The restriction enzymes were as follows: lanes A and B, *Hin*dIII; lanes C and D, *Eco*RI; lanes E and F, *Eco*RI plus *Bam*HI; lanes G and H, *Eco*RI plus *Hin*dIII; lanes I and J, *Hin*dIII plus *Bam*HI. Numbers indicate approximate fragment sizes in kb.

we have used as probes and the restriction map of the right arm of phage PA-2 are shown in the top portion of Fig. 5. The 155-base-pair *Eco*RI-*Eco*RI fragment and the left half of 385-base pair *Eco*RI-*Bal*II fragment lie entirely within the *lc* coding region, as indicated by our most recent sequence data and the structure of hybrid porin genes constructed in PA-2 by Gregg (unpublished data). When these fragments were used to probe chromosomal DNA from the parental strain CS180, we determined that there is both sequence homology and apparent restriction site identity between the *lc* region and the region containing the *nmpC* gene. When the *Eco*RI-*Bgl*II fragment was used to probe CS180 DNA cut with *Eco*RI and *Bgl*II, the probe hybridized to a fragment of identical size. The *Eco*RI-*Eco*RI fragment is too small to be retained on blots, but we were able to infer its presence. We observed no hybridization when CS180 DNA cut with *Eco*RI was probed with the *Eco*RI-*Eco*RI fragment, but we did observe hybridization with this probe when the DNA was cut with other enzymes, indicating that the region homologous to the probe is flanked by *Eco*RI sites. We observed no hybridization with any of the probes to DNA from CS457, which carries a deletion of the *nmpC* locus (23). As a control, we reprobed the filter containing CS457 DNA with a cloned fragment from the *ompC* locus (25) and observed appropriate restriction fragments, indicating that the DNA was cut and transferred properly.

The size and arrangement of the larger restriction fragments in the physical map of the *nmpC* region of CS180 is in agreement with the data presented by Kaiser (15) on the basis of homology to λ and λ *p4* DNA, and with the extended restriction map of the *proA-purE* region presented by Hadley et al. (9). A comparison of our data with that of Hadley et al. indicates that *nmpC* is located ca. 212 kb to the right of *lacZ* and ca. 22 kb to the right of *purE*, in excellent agreement with the current assignment of *nmpC* to the *E. coli* K-12 linkage map (3).

When we examined DNA from the *nmpC* (p^+) strain CS384, we found, in agreement with the results from the recombinant *qsr'* phage, that this DNA carried a deletion of ca. 900 base pairs lying to the left of the *nmpC* locus. This is shown by the Southern blot in Fig. 4, which also shows that the deletion removes an *Eco*RI site lying to the left of the *Msp*I-*Bgl*II probe. Other blots (not shown) indicated that the deletion also removed as adjacent *Bgl*II site. The bottom two lines of Fig. 5 show a summary of the differences in restriction maps in the *nmpC* regions of CS180 and CS384. It is highly probable that the deletion has removed the IS*5B* element known to be located at this site (9), since this element contains both *Bgl*II and *Eco*RI sites. This is also consistent with the observation by Kaiser (15) that λ *p4* contains a transposable element which is present in multiple copies in the *E. coli* K-12 chromosome. To confirm that the *Bgl*II and *Eco*RI sites lie within an IS*5* element, we cloned the 2-kb *Bgl*II fragment from the *nmpC* region of CS180 and partially sequenced the leftward *Bgl*II-*Eco*RI and *Eco*RI-*Eco*RI fragments from this *Bgl*II fragment (Fig. 5). Both fragments contained a sequence identical to the published sequence of IS*5* (data not shown).

The heteroduplex molecules shown in Fig. 1C and E indicate a region of nonhomology between the PA-2 DNA molecule and the half of the *p4* region lying to the left of the *lc* gene and I-*lc*. Since this region may carry phage genes which function in the lytic cycle, we examined the ability of PA-2 to functionally complement the *Q* functions of λ and λ *p4* by using the transinduction assay of Herskowitz and Signer (11). This assay depends upon the ability of the *Q* function of a heteroimmune infecting phage to antiterminate transcription from the P_R' late promoter of a resident prophage. The results of such an experiment are shown in Table 5 and indicate that neither the *Q* function of λ nor the corresponding function of λ *p4* are capable of efficiently activating the late genes of PA-2, although in both cases the number of phage produced upon superinfection of a PA-2 lysogen was significantly greater than that produced upon infection of a nonlysogen. The results also show that the *Q* function of λ does not activate the late genes of λ *p4* or vice versa, as shown by Herskowitz and Signer (11). We repeated this experiment several times with similar results. We interpret this to indicate that there may be weak complementation between PA-2 and both λ and λ *p4* but that the *Q* analog of PA-2 is not completely functionally homologous to either λ*Q* or the *qsr'* analog present in λ *p4*. This lack of complementation should not be due to an inability of the *F* product of PA-2 to complement the *F*am mutation of the superin-

TABLE 4. Constitutions of four phages[a]

Phage	*lc*/*nmpC*	I-lc	I-41	Lc/NmpC expression
PA-2	+	−	−	+
λ *p4*	+	+	−	−
λ *qsr'*	+	+	+	−
λ *qsr'*(p^+)	+	−	+	+

[a] The four phages are shown in Fig. 1.

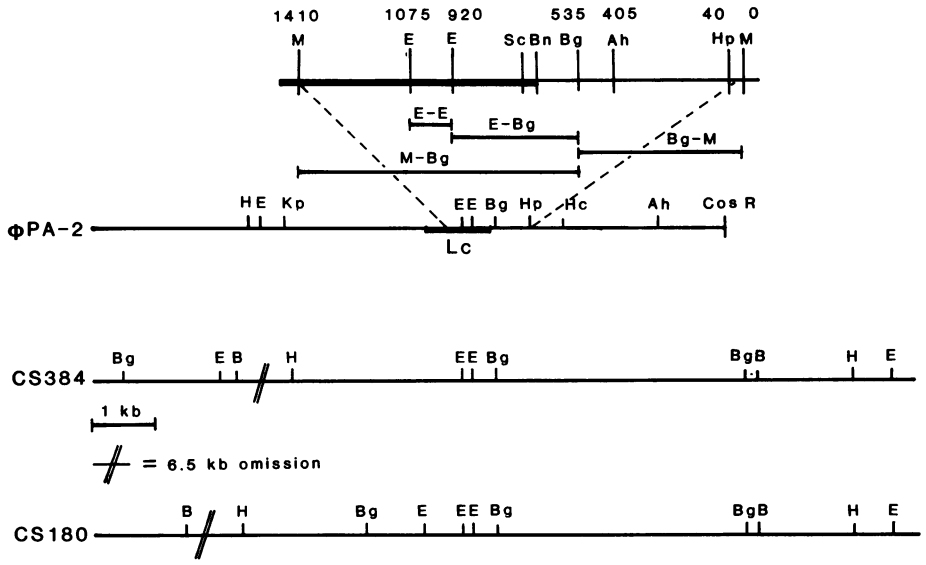

FIG. 5. Physical maps of the right arm of phage PA-2, and the *nmpC* region of CS384 [*nmpC* (p^+)] and CS180 (*nmp*$^+$). The top insert shows a more detailed restriction map of the 1,410-base pair *Msp*I fragment from the *lc* region of PA-2. The numbers at the top indicate the locations of the restriction sites in base pairs numbered from the rightward *Msp*I site and determined by dideoxy sequencing of this fragment. The location of the coding region of the *lc* protein structural gene is shown by the double-heavy line. The signal sequence begins at base 653, and the start of the mature protein sequence is at base 722 (A. Blasband, unpublished data). Below this are shown the four probes: the *Eco*RI-*Eco*RI fragment was cloned into the *Eco*RI site of pBR325, and the *Eco*RI-*Bgl*II fragment was cloned into the *Eco*RI and *Bgl*II sites of the cosmid pHC79. Both of the resulting plasmids were labeled by nick translation for blot hybridization. The *Msp*I-*Bgl*II and *Bgl*II-*Msp*I probes were made by labeling the complementary strand of a phage M13 template carrying the cloned *Msp*I fragment and excising the appropriate probe fragments with restriction enzymes. As noted in the two bottom lines of the figure, 6.5 kb of DNA from the 7.5-kb *Bam*HI-*Hin*dIII fragment have been omitted to allow the figures to be drawn to a reasonable scale. Abbreviations: Ah, *Aha*III; B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; Hp, *Hpa*I; Kp, *Kpn*I; M, *Msp*I; Sc, *Sca*I.

fecting phages, since the head genes of λ and PA-2 appear to be both physically and functionally homologous (T. Gregg, unpublished data).

DISCUSSION

These results show that the *p*4 region of the defective *qsr'* prophage contains the structural gene for the NmpC porin. The right half of this region is strongly homologous to the region of phage PA-2 thought to contain the *lc* gene. In the mutant strain which produces the porin protein, a region just to the left of the gene is deleted, and the sequence which is deleted is almost certainly the IS5B insertion element which is known to lie at this location in the chromosome (9). Studies by Gregg on *ompC-lc* hybrid genes and their products, and more recent sequence studies by Blasband (which will be published elsewhere) indicate that the *lc* gene is transcribed from right to left, from the DNA strand complementary to the strand encoding the phage *Q* and lysis genes. Thus, the question arises as to how deletion of an insertion element located 3' to the porin gene can cause it to become active. This question cannot be answered at present; it is hoped that studies on the structure and regulation of the *lc* gene and its relationship to nearby phage late genes which are presently underway will provide a plausible answer. Recent studies on the *ompC* locus (20, 25) indicate that porin loci may be quite complicated and include regulatory elements distinct from the protein coding sequence.

The results presented here allow speculation on the origin of phages such as PA-2 which carry a porin gene. The fact that the *Q* analog of the *p*4 region of the *qsr'* prophage and the *Q* gene of λ appear unable to efficiently activate transcription of late genes from PA-2, and the presence of a region to the left of the *lc* locus of PA-2 which is not homologous to either λ or the λ *qsr'* phages (Fig. 1) indicate that the *Q-lc* region of PA-2 did not arise simply by recombination between an ancestor of PA-2 and the *qsr'* prophage, within the flanking regions of homology, as λ *p*4 and the λ *qsr'* phages are assumed to have done.

One of us (Y.C.) has recently isolated six new lambdoid phages, the DNA molecules of which are homologous with that of PA-2 in the *lc* region, although they have extensive regions of nonhomology elsewhere. Three of these which were tested encode a protein similar to the Lc porin (Y. C. Chang, Ph.D. thesis, University of Edinburgh, Edinburgh, United Kingdom, 1984). *Salmonella typhimurium* LT-2 encodes a porin very similar to the Lc porin, and mutants lacking this porin map at a locus, *ompD*, which is distinct from either the *nmpC* locus or any of the known lambdoid phage attachment sites on the *E. coli* K-12 chromosome (3, 16, 22). Taken together, these observations suggest that the

TABLE 5. Transactivation of prophage late genes (*Q* complementation)

Prophage	Yield of infecting phage (PFU/ml per infected cell)[a]					
	λ *p*4 (A)	λ *p*4 *Fam* (B)	B/A ratio	λ (C)	λ *Fam* (D)	D/C ratio
None	130	0.12	9.4×10^{-4}	121	0.21	1.7×10^{-3}
λ	115	0.23	2.0×10^{-3}	129	18.9	0.14
PA-2	104	2.58	2.5×10^{-2}	104	1.14	1.1×10^{-2}
λ *p*4 *imm*^{434}	4.1	0.76	0.18	11.6	0.0064	5.6×10^{-4}

[a] All infecting phage were *imm*^{21}.

presence of a porin gene on the chromosome of lambdoid phages may not be an uncommon occurrence. In the case of PA-2, the presence of the active porin gene leads to repression of the OmpC protein which is the phage receptor (24), and this is true of the three new lambdoid phages mentioned above as well. This may be of survival value in facilitating the escape of phage particles from induced lysogens without neutralization by cell debris or nearby uninduced lysogenic cells.

ACKNOWLEDGMENTS

We thank N. Murray and I. Herskowitz for supplying bacteria and phages, A. Blasband and G. McDonald for supplying DNA probes, K. Kaiser, N. Murray, and M. Bownes for advice, and P. Beattie for assistance with electron microscopy.

Portions of this work were supported by Public Health Service grant GM18006 from the National Institutes of Health and grant PCM-82-159-12 from the National Science Foundation.

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