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# Regulation in *Escherichia coli* of the Porin Protein Gene Encoded by Lambdoid Bacteriophages

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**Specialized λ transducing phages carrying the cloned *lc* porin gene from the lambdoid bacteriophage PA-2, including various amounts of a sequence 5′ to the start of transcription, were used to study the regulation of the porin gene. It was found that a cyclic AMP receptor protein consensus binding site 65 base pairs 5′ to the start of transcription was required for catabolite repression of *lc* but was not sufficient for maximum expression under derepressing conditions. A sequence located more than 209 base pairs 5′ to the start of transcription was necessary for maximum expression. By manipulating the copy number of the *lc* gene and the temperature and by measuring both the rate of synthesis of mRNA and the amount of Lc protein in the outer membrane, it was determined that the expression of *lc* is regulated primarily at the level of transcription and that expression is not autoregulated. Evidence is also presented that the silent phage porin gene *nmpC* of *Escherichia coli* K-12 is transcribed to the same extent as *lc* even though it does not give rise to a stable pool of mRNA. The structure of the 5′ end of *lc* and *nmpC* is similar to that of *ompF*, and a model for transcriptional regulation is presented which may apply to all of these porin genes.**

The outer membrane porin proteins are among the most abundant proteins in gram-negative cells, and regulation of these proteins presents an interesting problem. The cell probably has no means of sensing the amount of any given protein in the outer membrane; thus, regulation must be performed concurrently with synthesis and translocation of the proteins. Since the outer membrane is nearly saturated with protein (2), failure to regulate these genes would have unfavorable consequences.

Studies of the regulation of porin genes have focused almost entirely on *ompC* and *ompF*. The sum of the OmpC and OmpF proteins remains reasonably constant, but the relative abundance of these two proteins is strongly influenced by culture conditions. The relative amounts of OmpC and OmpF are is controlled by the osmolarity of the culture medium (11, 22), the level of cyclic AMP (cAMP) (20), and the growth temperature (12). Of these, osmoregulation has received the most attention.

Transcription of the *ompC* and *ompF* genes requires the products of the *ompR* and *envZ* genes, which together constitute the *ompB* operon. Hall and Silhavy (5, 6) proposed that the *ompR* product is a positive regulator of transcription of *ompF* and *ompC* and that the *envZ* product acts both as an osmosensor and as a functional modifier of *ompR*, which mediates the differential expression of porins in response to osmolarity. Recent results have added complexity to this picture. The phenotype of mutants with chain termination mutations in *envZ* suggests that the EnvZ protein may play a more direct role in transcription regulation than envisioned in the Hall-Silhavy model (4). There is disagreement concerning the role of a sequence 5′ to the transcription start site of *ompF* in mediating the response of this gene to osmolarity. Ramakrishnan et al. (17) have shown that *ompF* expression from the *lpp* promoter still shows osmoregulation, indicating that the transcribed portion of the gene is sufficient for osmoregulation. A somewhat contradictory study by Inokuchi et al. (10) showed that a sequence immediately 5′ to the transcriptional start site, which included the putative OmpR binding site (9), was sufficient for osmoregulation of an *ompF-lacZ* fusion. A more recent study by Ostrow et al. (15) indicated that there were three separate sequence domains which were necessary for osmoregulation of single-copy *ompF-lacZ* fusions. One of these domains extended more than 240 bases 5′ to the start of transcription.

In the hope of finding a somewhat simpler system in which to study the general features of porin gene regulation, we investigated the regulation of the bacteriophage *lc* (lysogenic conversion) gene. This gene is present in the genome of phage PA-2 and some other lambdoid phages which use OmpC protein as their receptor, as well as in the defective *qsr′* prophage at 12 min on the *Escherichia coli* K-12 genome, which includes a silent porin gene designated *nmpC* (1, 8). Production of the Lc porin is regulated by catabolite repression (19), but it is independent of the osmolarity of the medium and does not depend on the *ompR* and *envZ* genes. The production of the Lc porin is regulated by growth temperature (3), but this modulation is independent of the *ompY* gene. Thus, the only known host regulatory protein is the cAMP receptor protein (CRP). In this report, we show that the regulation of *lc* expression occurs primarily at the level of transcription and that *lc* regulation is similar to that of *ompF* in that a sequence lying far upstream from the transcriptional start site is required for fully regulated transcription.

## MATERIALS AND METHODS

**Recombinant DNA techniques and strain construction.** Construction of plasmids carrying cloned restriction fragments from the *lc* region of phage PA-2 and DNA manipulation were done as previously described (1). In all of the constructions, the ends of restriction fragments were filled in with the Klenow polymerase fragment and blunt-end ligated to eight-base *Hin*dIII linkers. The restriction fragments were then inserted into the *Hin*dIII site of the cloning vector λ540 (14), and phages carrying porin genes were identified by the

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TABLE 1. Bacterial strains and bacteriophages

| Strain or phage | Relevant property(ies) | Source or reference |
|---|---|---|
| Strains | | |
| CS109 | W1485 $F^-$, prototrophic | Lab stock |
| CS137 | PA-2 lysogen of CS109 | Lab stock |
| CS457 | *ompR1* Δ(*nmpC-fep*) | 16 |
| CS1385 | As CS457, but *ompR*$^+$ | This study |
| CS1626 | CS109 transformed with multicopy *lc* plasmid pLc4 (1) | This study |
| CS1627 | CS109 lysogenic for λLc4H (monolysogen) | This study |
| CS1628 | As CS1627, except dilysogen | This study |
| CS1629 | PA-2 (Hy-7) lysogen of CS1628 (three copies of *lc*) | This study |
| CS1630 | CS109 lysogenic for λLc5H | This study |
| CS1631 | CS109 lysogenic for λLc6H | This study |
| Phages | | |
| Hy-7 | PA-2*h*$^\lambda$ | This study |
| λ540 | *att*$^+$ *int*$^+$ *imm*$^{21}$ *Hin*dIII cloning phage | N. Murray (14) |
| λLc4H | 1,900-bp *Hpa*I-*Eco*RV *lc* fragment subcloned into λ540 | This study |
| λLc5H | 1,600-bp *Dra*I-*Eco*RV *lc* fragment subcloned into λ540 | This study |
| λLc6H | 1,500-bp *Bgl*II-*Eco*RV *lc* fragment subcloned into λ540 | This study |

porin plaque assay (18). The copy number of all phage lysogens was verified by Southern blot hybridization as previously described (18). The phages and bacterial strains are listed in Table 1.

**Measurement of porin protein.** Outer membrane protein was isolated and analyzed on Tris-glycine-sodium dodecyl sulfate gels, and the Coomassie brilliant blue-stained gels were scanned with a laser densitometer as previously described (18). Only the central region of the gel containing the OmpC, OmpF, and Lc porins and the OmpA protein was scanned, and the relative amount of each protein was calculated as a percentage of the total protein in this region. All gels were loaded with a constant amount of protein per lane. To eliminate any minor variations due to gel staining, an identical sample of outer membrane protein from a wild-type PA-2 lysogen (CS137) grown at 36°C was included on each gel, and this was used to standardize the densitometer readings for each gel.

**Growth of cultures.** Cultures grown under catabolite-derepressing conditions were grown on 3-(*N*-morpholino)propanesulfonic acid (MOPS) minimal medium with 1% acid-hydrolyzed casein (AHC) as the carbon source. To elicit catabolite repression, glucose was added to MOPS-AHC medium at a final concentration of 0.4%. Cells were grown at various temperatures with vigorous shaking in a gyratory water bath, and growth was monitored with a Klett-Summerson photoelectric colorimeter. Cultures were grown to approximately $3 \times 10^8$ cells per ml (60 Klett units) for both protein and mRNA measurements.

**mRNA measurement.** The basic protocol followed for mRNA measurement was that of Stewart and Yanofsky (21). Cultures (10 ml) growing in MOPS-AHC medium or MOPS-AHC medium plus glucose were pulse-labeled with [$^3$H]uridine for 30 s followed by rapid killing and RNA isolation as described by Yanofsky and Soll (23). Ten microliters of the RNA extract along with carrier calf thymus DNA was precipitated with 5% trichloroacetic acid (20 min at 0°C) and passed through a Whatman GF/c filter. The filter was washed and dried, and radioactivity was counted in a liquid scintillation counter to determine the total amount of labeled uridine incorporated. The RNA was hybridized to 3 μg of linearized M13 replicative-form DNA containing the 2.5-kilobase-pair *Acc*I-*Hin*dIII fragment carrying the entire *lc* gene (1) or, as indicated below, linearized plasmids carrying cloned *ompC* and *ompF* sequences. The *ompF* plasmid pDME consisted of an insert of ca. 2,600 base pairs (bp) which was an *Eco*RI-*Hpa*I chromosomal fragment carrying the entire *ompF* gene and ca. 1,000 bp 5′ to the *ompF* coding sequence cloned into the *Eco*RI and *Ava*I sites of pBR322. This plasmid was obtained from S. Benson. The *ompC* plasmid pGMC65 consisted of an insert of ca. 1,700 bp extending from ca. 520 bp 5′ to the *ompC* gene through the entire gene to the *Hin*dIII site 3′ to *ompC*, which was cloned into the *Hin*dIII site of pAT153. This plasmid was obtained from G. McDonald. The DNA was immobilized on GeneScreen Plus (Du Pont Co.) as indicated by the manufacturer. Hybridization was performed with gentle agitation at 55°C in RNase-treated scintillation vials. The buffers used were those listed in Appendix A of the GeneScreen Plus manual. Samples (100 μl) of labeled RNA (usually $1 \times 10^6$ to $3 \times 10^6$ cpm) were boiled for 3 min and immediately added to a prewarmed vial containing the hybridization filter and 3 ml of hybridization buffer. Hybridization was performed for a minimum of 8 h. The filters were removed from the hybridization solution and washed as follows: (i) two 5-min room temperature washes with constant agitation in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate); (ii) one 60-min room temperature wash with constant agitation in 2× SSC containing 20 μg of RNase A (Sigma Chemical Co.) per ml; (iii) one 15-min wash at 50°C with constant agitation in 2× SSC with 1% sodium dodecyl sulfate. The filters were dried, and radioactivity was counted in a liquid scintillation spectrometer. The values reported are the averages for two separate labeling experiments.

## RESULTS

**5′ sequence required for regulation and expression.** A series of specialized transducing phages were constructed which carried the *lc* coding region and the untranslated leader region but which had different amounts of sequence 5′ to the start of transcription. This was done by inserting various restriction fragments into the *Hin*dIII site of vector λ540.

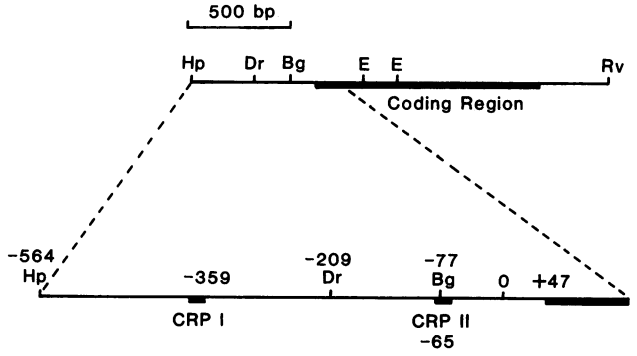


FIG. 1. Physical map of *lc* region from bacteriophage PA-2. The *lc* coding region is indicated by the thick line, and the lower, expanded portion of the map shows the location of pertinent sites in the 5′ end of the region. Numbering is in relation to the start of transcription of *lc*. Abbreviations: Bg, *Bgl*II; CRP, cAMP receptor protein binding sequences; Dr, *Dra*I; E, *Eco*RI; Hp, *Hpa*I; and Rv, *Eco*RV.

TABLE 2. Experimental calculations used to determine mRNA levels by filter hybridization

| Strain | Expt 1 | | | | Expt 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Counts[a] bound (10-min counts) | Counts[b] applied (cpm [$10^6$]) | % Bound | Net[c] % bound | Counts[a] bound (10-min counts) | Counts[b] applied (cpm [$10^6$]) | % Bound | Net[c] % bound |
| CS109 | 818 | 1.78 | 0.046 | 0.032 | 914 | 2.22 | 0.041 | 0.028 |
| CS137 | 1,895 | 1.86 | 0.102 | 0.088 | 1,427 | 2.00 | 0.071 | 0.058 |
| CS1627 | 2,065 | 2.24 | 0.091 | 0.077 | 1,586 | 2.27 | 0.70 | 0.057 |
| CS1630 | 1,113 | 1.99 | 0.056 | 0.042 | 1,020 | 2.11 | 0.048 | 0.035 |
| CS1631 | 1,295 | 2.34 | 0.055 | 0.042 | 1,066 | 2.33 | 0.046 | 0.033 |
| CS1385 | 345 | 2.47 | 0.014 | | 446 | 3.42 | 0.013 | |

[a] Average $^3H$ counts for duplicate filters. Not corrected for background radiation (ca. 300 counts per 10 min).
[b] Acid-precipitable $^3H$ counts added per filter.
[c] Corrected for percentage of RNA from CS1385 (Δ*nmpC*) bound to filters. This included both background radiation and nonspecific binding of labeled RNA.

The restriction sites in relation to the transcription and translation start sites and the two CRP consensus binding sites which were previously identified from the sequence (1) are shown in Fig. 1. The sequence is numbered with respect to *lc* mRNA; +1 is the first base of the transcript and +47 is the first base of the Lc protein initiation codon. In all of the phages the 3′ end of the *lc* insert was the *Eco*RV site 327 bp 3′ to the termination codon. Preliminary studies showed that additional 3′ sequences had no effect on the expression of Lc protein. In addition, the lengths of the two *lc* transcripts indicated that they terminate before this site (1). Phage λLc4H has an insert beginning at the *Hpa*I site at −564, phage λLc5H has an insert beginning at the *Dra*I site at −209, and phage λLc6H has an insert beginning at the *Bgl*II site at −77. Thus, λLc4H has both upstream CRP consensus binding sites, whereas λLc5H has only CRP II and λLc6H does not have an intact CRP site since the *Bgl*II site interrupts CRP II (1).

Strains monolysogenic for these phages were used to examine the production of both Lc protein in the outer membrane and *lc* mRNA. The procedure used to measure mRNA involved a brief pulse of [$^3H$]uridine followed by rapid lysis and RNA extraction. The actual counts of labeled mRNA hybridized to *lc* DNA and the experimental calculations for one set of experiments are shown in Table 2. It should be noted that the counts bound from CS1385 (Δ*nmpC*) were essentially background, whereas the counts bound from the nonlysogenic wild-type strain CS109 were significant and represented about half of the counts bound by the PA-2 lysogen CS137. The counts bound from CS109 were subject to catabolite repression and increased with temperature (see Tables 4 and 5). Since there is no sequence in the cell other than *nmpC* which cross hybridizes with *lc* (8), the hybridizable counts detected in CS109 must have represented transcripts from *nmpC*. We have previously shown that full-length *nmpC* transcripts cannot be detected in wild-type strains by Northern blot hybridization and have suggested that this is due to rapid mRNA degradation caused by the IS5 insertion in the 3′ end of *nmpC* (1). The observation that *nmpC* mRNA synthesis could be detected by using a rapid labeling and isolation technique supports this interpretation and shows that the assay provides a reasonable measure of mRNA synthesis under conditions in which the rate of degradation is too great to permit estimation of the steady-state mRNA pool size.

If the results shown in Table 3 are corrected for the contribution of *nmpC* by subtracting the mRNA value for strain CS109 from the other mRNA values, there is reasonable agreement between the amount of Lc protein in the outer membrane and the amount of *lc* mRNA. It was observed in several experiments similar to the one for which results are shown in Table 3 that there was no significant difference in the amounts of either mRNA or protein between the strain lysogenic for λLc4H and strain CS137. Thus, a sequence extending from the 5′ *Hpa*I site to the 3′ *Eco*RV site appears to contain all of the information necessary for fully regulated expression of the *lc* gene. In contrast, both λLc5H and λLc6H resulted in the production of less protein and mRNA. This indicates that the sequence between −209 and −564 is necessary for maximum *lc* expression.

The results in Table 3 show the expression of mRNA and protein under conditions of maximum catabolite derepression (MOPS-AHC medium). The results in Table 4 show a

TABLE 3. Requirement for 5′ untranscribed sequence for expression of *lc*

| Strain | Relevant genotype | Relative level[a] of: | |
|---|---|---|---|
| | | mRNA | Protein |
| CS109 | Wild-type, no *lc* | 0.42 | |
| CS137 | PA-2, wild-type *lc* | 1.00 | 1.00 |
| CS1627 | λLc4H (+612 bp)[b] | 0.92 | 0.82 |
| CS1630 | λLc5H (+254 bp) | 0.54 | 0.19 |
| CS1631 | λLc6H (+124 bp) | 0.52 | 0.11 |

[a] Values expressed relative to those for strain CS137. Protein refers to the amount of Lc protein as determined by densitometry of stained gels of outer membrane protein. mRNA values are the averages of data from separate labeled cultures. The values in this table are from the two experiments for which results are shown in Table 2.
[b] The numbers in parentheses indicate the 5′ ends of the inserts as shown in Fig. 1.

TABLE 4. Role of 5′ untranscribed sequence in regulation of *lc* by catabolite repression

| Strain | Relevant genotype | Relative level[a] of: | | | |
|---|---|---|---|---|---|
| | | mRNA[b] | | Protein | |
| | | −cAMP | +cAMP | −cAMP | +cAMP |
| CS109 | Wild-type, no *lc* | 0.15 | 0.51 | | |
| CS137 | PA-2, wild-type *lc* | 0.16 | 1.00 | 0.08 | 1.00 |
| CS1627 | λLc4H (+612 bp)[c] | 0.09 | 1.17 | 0.02 | 0.93 |
| CS1630 | λLc5H (+254 bp) | 0.12 | 0.97 | <0.01 | 0.29 |
| CS1631 | λLc6H (+124 bp) | 0.32 | 0.79 | 0.32 | 0.31 |

[a] The values reported are expressed relative to the values obtained for CS137 under derepressed conditions (+cAMP).
[b] The mRNA values are the averages of data from separate labeled cultures.
[c] See Table 3, footnote *b*.

TABLE 5. Effect of copy number and temperature on expression of *lc*

| Strain (no. of copies of *lc*) | Temp (°C) | Relative level[a] of: | |
|---|---|---|---|
| | | mRNA[b] | Protein |
| CS109 (0) | 33 | 0.51 | |
| | 36 | 0.48 | |
| | 39 | 1.09 | |
| CS137 (1) | 33 | 0.56 | 0.53 |
| | 36 | 1.00 | 1.00 |
| | 39 | 2.55 | 1.71 |
| CS1629 (3) | 33 | 1.23 | 0.85 |
| | 36 | 2.33 | 2.23 |
| | 39 | 2.38 | 2.92 |
| CS1626 (many) | 33 | 3.33 | 3.13 |
| | 36 | 7.04 | 4.16 |
| | 39 | 5.33 | 3.76 |

[a] Values expressed relative to those for CS137 grown at 36°C. Cultures were grown on MOPS-AHC medium.
[b] The mRNA values are the averages of data from separate labeled cultures.

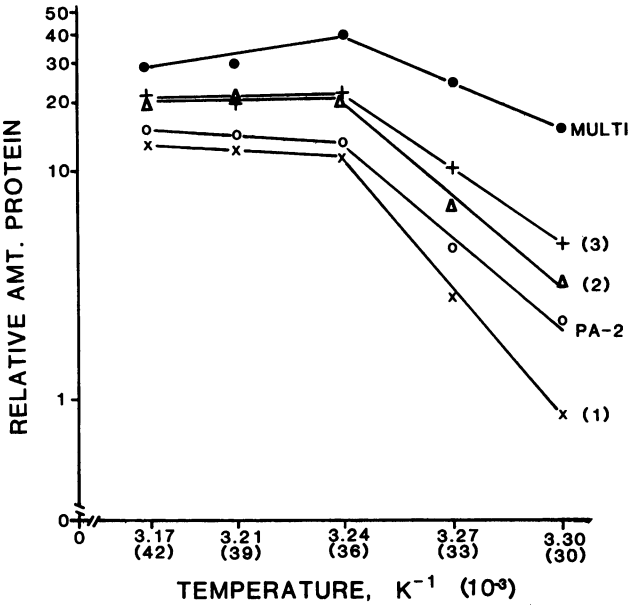


FIG. 2. Effect of temperature on expression of Lc protein. The relative area of protein peaks on a densitometer tracing of a gel of outer membrane proteins was plotted against the reciprocal of the temperature in kelvins. The temperature in degrees Celsius is given in parentheses, and the copy number is indicated for each line.

somewhat different picture, when expression was examined under repressing conditions (MOPS-AHC medium plus glucose) and under conditions of partial derepression induced by the addition of cAMP. Again, the transcription results must be corrected for the contribution of *nmpC* by subtracting the values for strain CS109. In contrast to the results in Table 3, under fully catabolite-repressing conditions, expression from λLc6H was greater than that from wild-type PA-2 or from the two Lc phages with longer inserts. Although expression from λLc5H was somewhat less than that from λLc4H or wild-type PA-2, it showed a similar strong stimulation by addition of cAMP. The expression of Lc protein by λLc6H was not stimulated by cAMP, and the apparent stimulation of mRNA was probably due to the contribution of *nmpC*.

These results demonstrate that CRP II is necessary for regulation of *lc* expression by catabolite repression. Although a sequence lying between the *Hpa*I and *Dra*I sites is required for maximum expression, we have no evidence to indicate that CRP I has a regulatory function.

**Effect of temperature and copy number.** During the screening of lysogens by Southern blot hybridization to verify copy number, we found that strain CS1628 was a tandem dilysogen of phage λLc4H. Since vector λ540 and PA-2 have different immunity and attachment sites, we were able to construct a strain (CS1629) which carries three copies of the *lc* gene by lysogenization of CS1628 with a derivative of PA-2. This allowed us to examine in more detail the effect of copy number and temperature on expression of *lc*. We also examined expression from a multicopy plasmid carrying *lc*, in which we assume that the copy number is about 20.

Both the amount of mRNA and the amount of Lc protein increased as a function of temperature and copy number (Table 5). If CS109 is considered to be haploid due to the contribution from *nmpC*, the amount of mRNA detected is roughly proportional to copy number. The amount of protein does not appear to be as proportional to copy number. If CS137 is considered to produce the haploid level of protein, the amount of Lc protein increased less than threefold in the triploid strain and only four- to sixfold in the strain carrying *lc* on a multicopy plasmid. This may have been due to competition for translation or secretion machinery, since at higher copy numbers we observed a significant decrease in the amount of OmpA protein in the outer membrane (data not shown).

To examine the effect of temperature in more detail, the expression of Lc protein from strains carrying various *lc* copy numbers was examined over a broader temperature range. The results are shown in Fig. 2, in which the data are shown as an Arrhenius plot. At all copy numbers, there was a proportional increase with temperature up to 36°C, at which point a sharp break was observed. To test whether this was a consequence of transcription or a limitation in translation or translocation of Lc protein, the relative amounts of mRNA were examined at some of the copy

TABLE 6. Effect of *lc* on expression of *ompC* and *ompF*

| Strain (no of copies of *lc*) | Temp (°C) | Relative level[a] of: | | | |
|---|---|---|---|---|---|
| | | *ompF* | | *OmpC* | |
| | | mRNA[b] | Protein | mRNA[b] | Protein |
| CS109 (0) | 33 | 1.03 | 0.79 | 0.87 | 1.22 |
| | 36 | 1.00 | 1.00 | 1.00 | 1.00 |
| | 39 | 1.25 | 1.02 | 1.09 | 1.42 |
| CS137 (1) | 33 | 0.63 | 0.74 | 0.49 | 0.38 |
| | 36 | 0.62 | 0.73 | 0.36 | 0.29 |
| | 39 | 0.70 | 0.79 | 0.65 | <0.01 |
| CS1629 (3) | 33 | 0.94 | 0.57 | 0.72 | 0.20 |
| | 36 | 0.99 | 0.33 | 0.59 | <0.01 |
| | 39 | 0.70 | 0.11 | 0.54 | <0.01 |
| CS1626 (many) | 33 | 0.63 | 0.06 | 4.49[c] | 0 |
| | 36 | 0.41 | 0 | 5.74[c] | 0 |
| | 39 | 0.72 | 0 | 8.41[c] | 0 |

[a] Values expressed relative to those for CS109 at 36°C.
[b] The mRNA values are the averages of data from separate labeled cultures.
[c] DNA bound to filters was purified 1,700-bp *ompC* restriction fragment from pGMC65.

numbers (data not shown). Although there was more scatter in the values, the trend of a proportional increase up to a breakpoint at about 36°C was also seen for relative amounts of mRNA. Thus, the primary response of *lc* expression to temperature appears to be at the level of transcription.

**Inhibition of *ompC* and *ompF* by *lc*.** The most dramatic effect of the expression of Lc protein is the almost total inhibition of OmpC protein expression and the partial inhibition of OmpF expression (3, 18, 19). Hall and Silhavy (7) found that expression of β-galactosidase from *ompC*- and *ompF-lacZ* operon fusions was not affected by expression of the *lc* gene. To confirm by a more direct method that the down regulation of the OmpF and OmpC proteins by *lc* does not involve inhibition of transcription, the relative amounts of *ompC* and *ompF* mRNA and the corresponding proteins in the outer membrane were examined in strains carrying various *lc* copy numbers (Table 6). Although there was some decrease in the amount of *ompC* mRNA in strains expressing *lc* and a somewhat less pronounced decrease in the amount of *ompF* mRNA, in almost all instances it was much less than the decrease in the amounts of the OmpC and OmpF proteins. Thus, the conclusion of Hall and Silhavy that the down regulation of the OmpC and OmpF proteins is not at the level of transcription appears to be correct. A rather surprising observation was that in the strain carrying *lc* on a multicopy plasmid, the transcription of *ompC* was increased severalfold at all temperatures. This observation was reproducible, and to rule out transcription of plasmid vector sequences as a source of error, the assay was performed with a purified restriction fragment bound to the filters which contained only DNA from the chromosomal *ompC* locus.

## DISCUSSION

One of the conclusions of this study is that the expression of the Lc protein appears to be regulated at the level of transcription. The rate of synthesis of *lc* mRNA after correction for the contribution of *nmpC* was roughly proportional to the copy number, whereas the amount of Lc protein in the outer membrane appeared to decrease somewhat with respect to copy number. Both the amount of Lc protein in the outer membrane and the amount of transcription increased in a linear fashion in response to temperature, and both showed a breakpoint at about 36°C. This indicates that the primary effect of temperature on porin synthesis is on the rate of transcription and not on the translation or translocation of the protein.

Autoregulation of the *lc* gene was suggested both by the results of our previous study with multicopy plasmids (1) and by the results of Fralick and Diedrich (3), who found that the amount of Lc protein in the outer membrane reached a maximum level independent of copy number. In the present study, in which we were able to manipulate copy number over a broader range, we saw no evidence for autoregulation. These studies also indicate the desirability of using single-copy or known-copy-number constructions to analyze porin gene regulation. This point has been well established in studies of the regulation of *ompC* (18) and *ompF* (15).

The sequence 5′ to the coding region is strongly conserved between the *lc* and *nmpC* genes, even though the defective *qsr′* prophage which includes *nmpC* is otherwise unrelated to PA-2 (8). Of the first 457 bp 5′ to the *nmpC* coding region, there were only 18 single-base-pair substitutions. The present work indicated that some of this conserved sequence is in fact necessary for expression of *lc*, since maximum expression under derepressing conditions required a sequence lying between bases −209 and −564 (Table 3).

With respect to regulation, there are similarities between the *lc* and *ompF* genes. First, both genes have positive activation sites which are similarly spaced with respect to the site of initiation of transcription. Based on deletion analysis, Ostrow et al. (15) have shown that a sequence lying between −240 and +2 of *ompF* contains the *ompR* activation site. A recent estimate based on homology with *ompC* narrowed the range for the *ompR* regulation site to between −45 and −95 (13). A sequence between −209 and −77 of *lc* is necessary for regulation of *lc* expression in response to cAMP level, and the likely candidate for this regulation is the CRP consensus binding site lying between −65 and −85 (Fig. 1). Second, Ostrow et al. (15) have shown that a sequence between −240 and −1400 of *ompF* is required for maximum expression of *ompF*, which occurs in media of low osmolarity. Thus, the positive activation site is necessary, but not sufficient, for full expression. Likewise, we found that a sequence lying between −209 and −564 of *lc* is necessary for maximum expression of *lc* under derepressed conditions and that the CRP II site is necessary, but not sufficient, for full expression of *lc*. Finally, it should be noted that 5′ deletions of both *ompF* (15) and *lc* (Table 4) can result in partially constitutive expression.

The results in Table 4 suggest a mechanism for positive regulation which may also be applicable to *ompF*. Although λLc6H, which lacks a portion of CRP II, exhibited much lower expression than did either λLc4H or λLc5H when grown on MOPS-AHC medium (Table 3), under conditions of catabolite repression (MOPS-glucose medium), the expression from λLc6H was more than that from the other two phages; even after partial derepression with added cAMP, the expression from λLc6H remained significant in comparison with that from the two longer phage constructions. This suggests that sequences lying between −77 and −209 may inhibit expression of the *lc* gene and that the function of CRP and cAMP acting at the CRP II site is to activate transcription by changing the structure of the DNA in this region to relieve the inhibition. Deletion of a sequence 5′ to the *Bgl*II site at −77 and its replacement by a novel joint and vector sequence appeared to alleviate this inhibition and allow transcription to take place in the absence of CRP (Table 4). It is tempting to propose that a similar model can be applied to *ompF*, namely that a sequence 5′ to the *ompR* binding site serves to inhibit transcription and that binding of *ompR* to the positive regulatory site releases the inhibition imposed by this sequence in this region. Such a model is formally equivalent to antitermination except that it occurs 5′ to the initiation of transcription. This model could be tested by constructing appropriate deletions which remove the *ompR* binding site but leave the 5′ sequence intact.

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