Characterization by Deletion and Localized Mutagenesis In Vitro of the Promoter Region of the Escherichia coli ompC Gene and Importance of the Upstream DNA Domain in Positive Regulation by the OmpR Protein

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The ompC gene codes for a major outer membrane protein whose expression is regulated by the ompR and envZ genes. Two sets of promoter deletion mutants, with upstream and downstream deletions, were constructed on a plasmid in vitro, and their promoter activity was studied by connecting them with the lacZ gene. The DNA sequence for the ompC promoter, including the –35 and –10 regions and the mRNA start site, was determined at the region about 100 base pairs upstream from the ATG initiation codon for the pro-OmpC protein. An additional 61-base-pair sequence extending upstream from the –35 region was required for the ompC promoter to function fully. After targeting the upstream region of the ompC promoter fused to the lacZ gene on a plasmid, in vitro-localized mutagenesis was performed to isolate cis-dominant mutations that affect ompC transcription. Four mutant groups, each of which had common phenotypes for expression and regulation of the gene, were identified. The individual groups also had common base substitutions. In two of the groups, the common base substitutions were localized in the upstream region of the ompC promoter, whereas in the other two they were localized in the –35 region. From these results, the upstream region of the ompC promoter was considered to be the domain responsible for activation by the ompR gene product.

The major outer membrane proteins of Escherichia coli, OmpF and OmpC, are encoded by the ompF and ompC genes, respectively (26). Expression of these genes is regulated by the osmolarity of the culture medium (13, 32). The osmoregulation of the ompF and ompC genes is controlled by two genes, ompR and envZ (4–7). The nucleotide sequences of these two genes have also been determined (19, 25, 33), and their gene products have been characterized (18, 19, 25, 29). Many ompR mutants exhibiting different osmoregulation phenotypes have been isolated (4–7, 24, 25, 30) and characterized at the molecular level (25). The results of these studies strongly indicated that the OmpR protein is directly involved in osmoregulation.

The ompC and ompF genes have been cloned, and their total nucleotide sequences have been determined (11, 20, 21). The extensive homology between them in the coding DNA sequences and in the primary amino acid sequences is obvious (21). In spite of the high homology in their coding regions, their 5′-end noncoding sequences, including the possible promoter regions, seemed to differ to some extent. It is not yet fully understood how the expression of these two genes is regulated in a reciprocal manner by their common regulatory factors, the OmpR and EnvZ proteins. Recently, the promoter region for the ompF gene has been characterized in detail by means of mutagenesis in vivo and in vitro (3, 9, 31). On the other hand, detailed studies on the ompC promoter region have not yet been performed.

In the present work, to define and characterize the functional region for the ompC promoter in comparison with that of the ompF gene, deletions upstream and downstream of the ompC promoter were generated. It was found that about 60 base pairs (bp) upstream from the –35 region was required for the ompC promoter to function fully. In vitro-localized mutagenesis of the upstream regulatory region of the ompC promoter was also carried out by using the ompC-lacZ fusion gene. In the mutants thus isolated, most of the base substitutions were in the regulatory region. Based on the results, the structure and function of the ompC promoter are discussed in relation to regulation by the ompR and envZ genes.

MATERIALS AND METHODS

Bacterial strains and plasmids. The following strains derived from E. coli K-12 were used: MC4100 [F– ΔargF-lac]U169 araD139 rpsL150 fliB5301 relA1 ptsF25], MH1160 (ompR101; a mutant of MC4100), MH760 (ompR472; a mutant of MC4100), MH1461 (envZ11; a mutant of MC4100) (2, 5), SB4288 [F– recA thi-7 relA mal-24 spo-12 supE20 Δ(proB-lac)] (22, 23), and K58 (ung; a uracil-N-glycosidase mutant; kindly provided by M. Takanami, Kyoto University). The last strain was used as a host for localized mutagenesis (1). Plasmid pMY150, carrying the entire ompC gene (22, 23), and pKM005, carrying the promoter-deleted lacZ gene (15), were used.

Media, enzymes, and chemicals. Unless otherwise specified, bacteria were grown in L-broth. For the β-galactosidase assay on plates, lactose-MacConkey solid medium (Difco Laboratories) was used. When required, ampicillin was added at a concentration of 100 μg/ml. Restriction endonucleases, bacteriophage T4 ligase, exonuclease Bal31, the Klenow fragment of DNA polymerase I, synthetic XbaI linker (dCTCCTAGG), HindIII linker (dCAAGCCGG), and a dideoxy DNA sequencing kit were obtained from Takara

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same volume of 250 mM sodium phosphate buffer (pH 7.2) to accurately determine the cell density in the absence of sucrose. A portion of the cell suspension was subjected to the β-galactosidase activity assay. Cells were permeabilized with toluene before the assay.

Localized mutagenesis of plasmid DNA with sodium bisulfite. Localized mutagenesis of single-stranded DNA on a plasmid was carried out as described by Kaldon et al. (12) and Shortle et al. (28) with slight modifications. Plasmids pOMPC-X338 and pOMPC-X14 were linearized with EcoRI and SalI, respectively. Each linear plasmid (5 µg) was mixed together in 23 µl of H2O. Then 1 M NaOH (4.8 µl) was added, and the mixture was left at room temperature for 10 min. Renaturation was accomplished by sequential additions of 280 µl of H2O, 40 µl of 0.5 M Tris hydrochloride (pH 8.0), and 48 µl of 0.1 N HCl prior to incubation at 63°C for 2 h. The annealed products were precipitated in ethanol, suspended in a small volume of H2O, and then incubated in freshly prepared 3 M sodium bisulfite (pH 6.0)–2 mM hydroquinone at 37°C for 4 h in the dark under liquid paraffin. Sequential dialyses of the sample against buffers were carried out as described previously (28). The mutagenized plasmids were finally precipitated in ethanol and then transferred into E. coli K58 (ung).

RESULTS

Construction of the ompC-lacZ fusion gene on a plasmid. For the rapid and quantitative characterization of the ompC promoter function, the ompC promoter region was fused to the lacZ coding sequence on a promoter-proving vector, pKM005 (15). The resultant plasmid (pOMPC-A5) carries the 501-bp fragment encompassing the putative ompC promoter region and the translational initiation codon (ATG) of the ompC gene (Fig. 1A). Another plasmid (pOMPC-ASH) was also constructed, by converting the XbaI site of pOMPC-A5 to an HindIII site (Fig. 1B). The nucleotide sequence coding for the four amino acids at the amino terminus is derived from the ompC gene. The sequence is followed by a short linker sequence providing unique restriction sites (HindIII and BamHI) and the lacZ coding sequence in the correct reading frame. The nucleotide sequence of the junction region was confirmed by DNA sequencing (data not shown).

Construction of plasmids with deletions upstream and downstream of the ompC promoter region. The strategy for construction of plasmids with deletions upstream and downstream of the ompC promoter region fused to the lacZ gene is shown in Fig. 1A and B, respectively. For construction of plasmids with upstream deletions, plasmid pOMPC-A5 linearized with XbaI was digested with exonuclease Bal31 to produce the ompC promoter region deleted to various extents. After treatment with Klenow fragment, the resultant blunt-ended fragments were ligated with XbaI linker. The ligated products were digested with XbaI and SalI. The XbaI-SalI fragments, which were assumed to include the ompC promoter region with different upstream deletions, were ligated back into the XbaI-SalI sites of pOMPC-A5. The strategy for generation of the plasmids with downstream deletions was similar. Plasmid pOMPC-ASH was linearized with BamHI and then digested with Bal31. After treatment with Klenow fragment, the resultant blunt-ended fragments were ligated with XbaI linker. The ligated products were digested with XbaI and SalI, and then the XbaI-SalI fragments, which were assumed to include the ompC promoter region with different downstream deletions, were ligated into

![Diagram](https://example.com/diagram.png)

**FIG. 1.** Structure of plasmid pOMPC-A5, carrying the ompC-lacZ fusion gene, and construction of a series of plasmids with upstream deletions (A) and downstream deletions (B) of the ompC promoter region. Solid bars represent ompC DNA, and the approximate location of the ompC promoter region (Cp) is also indicated. The series of plasmids with upstream deletions and downstream deletions are designated the pOMPC-X series and pOMPC-B series, respectively.

Shuzo Co. ONTG (O-nitrophenyl-β-D-galactopyranoside) was obtained from Sigma Chemical Co. [α-32P]dCTP (3,000 Ci/mmol) was purchased from Amersham International. Sodium bisulfite and hydroquinone were obtained from Wako Chemical Co.

DNA techniques. The conditions used for restriction endonuclease reactions, exonuclease Bal31 digestion, and Klenow fragment treatment were those suggested by the suppliers. Other recombinant DNA techniques were carried out by the conventional methods described by Maniatis et al. (14). DNA sequences were determined by the dyeoxy chain termination method of Sanger et al. (27), using phages M13mp10 and M13mp11 and strain JM103 [Δ(lac-pro thi rpsL supE endA sbeB15 hsdR4 F′::traD36 proAB lacF2 lacZM15] as the host (16).

Assay of β-galactosidase activity. β-Galactosidase was assayed as described by Miller (17) with slight modifications. Cells were cultured until the mid-logarithmic phase in medium A (13) in the absence or presence of 15% (wt/vol) sucrose. The cells were then collected and suspended in the

![Diagram](https://example.com/diagram.png)
FIG. 2. Series of plasmids with upstream deletions and downstream deletions of the ompC-lacZ fusion gene, and β-galactosidase activity expressed by the series of deletion plasmids in wild-type cells. (A and B) Solid lines represent the promoter region remaining in the upstream deletions (A, pOMPC-X series) and the downstream deletions (B, pOMPC-B series). For the base pair scale, the zero point is the initiation codon (ATG) of the ompC-lacZ gene. The location of the ompC mRNA start site (●) is also indicated. The letters a through w represent the series of deletion plasmids: (a) pOMPC-A5 (original plasmid); pOMPC-X series plasmids (b) X79, (c) X41, (d) X153, (e) X322, (f) X105, (g) X500.
FIG. 3. β-Galactosidase activity expressed by upstream deletion plasmids in ompR and envZ mutant cells. The upstream deletion plasmids (a through o, see Fig. 2) were transferred into (A) MH1160 (ompR101), (B) MH760 (ompR472), and (C) MH1641 (envZ111). The cells were grown in medium A in the presence of 15% sucrose and then subjected to the β-galactosidase assay. The activity expressed by each plasmid is indicated at the position reached by the deletion in each ompC promoter (see Fig. 2). The two critical points for β-galactosidase activity are indicated by arrows A and B.

the 6.3-kilobase (kb) XbaI-SalI fragment of pKM005. Plasmid pKM005 carries a promoter-deleted lacZ gene (15). Over 100 deletion plasmids were thus constructed. As shown in Fig. 2A and B, 15 upstream deletion plasmids (pOMPC-X series) and 8 downstream deletion plasmids (pOMPC-B series) were selected and subjected to further characterization.

Characterization of a series of upstream and downstream deletions to define the functional ompC promoter region. Deletion plasmids were transferred into MC4100 (ompR + envZ + ), and then the β-galactosidase activities were examined. As shown in Fig. 2C, there were two critical points (indicated by arrows A and B) in the upstream deletions with respect to the level of expression. One was about 170 bp upstream from the ATG initiation codon (point A), and the other was about 110 bp upstream from the ATG initiation codon (point B). When the deletion proceeded beyond point A, the level of expression was significantly decreased. β-Galactosidase activity was completely abolished by deletion beyond point B. The results of β-galactosidase assays for the downstream deletions revealed the presence of a critical point (indicated by arrow C in Fig. 2D), which resided about 80 bp upstream of the ATG initiation codon. The expression of β-galactosidase was completely lost by further deletion upstream from point C. These results indicate that the ompC promoter resides between 110 and 80 bp.

X338, (h) X215, (i) X49, (j) X250, (k) X293, (l) X101, (m) X14, (n) X57, and (o) X251; pOMPC-B series plasmids (p) B71, (q) B98, (r) B83, (s) B110, (t) B76, (u) B7, (v) B64, and (w) B114. (C and D) β-Galactosidase activity of upstream deletion plasmids (C, a through o) and downstream deletion plasmids (D, p through w) were measured in MC1000 (ompR + envZ + ). The cells were grown in medium A in the presence of 15% sucrose and then subjected to the β-galactosidase assay as described in the text. The activity expressed by each plasmid is indicated at the position reached by the deletion in each ompC promoter. The three critical points for β-galactosidase activity are indicated by arrows A, B, and C.
upstream from the ATG initiation codon (i.e., the region between points B and C). It was also revealed that a region consisting of about 60 bp (i.e., the region between points A and B) was required for full expression of the ompC promoter.

To investigate the roles of the upstream region, the deletion plasmids were further studied in the ompR background (Fig. 3A and B). A critical point, corresponding to point B in the ompR<sup>+</sup> background, was observed. However, enhancement of expression by the upstream region was not observed. These results indicate that the nucleotide sequence between points A and B is responsible for activation of the ompC promoter by the ompR gene product. The profile of β-galactosidase activity in the envZ<sup>II</sup> mutant was essentially the same as that in the wild-type strain (Fig. 3C).

The results of β-galactosidase assay (Fig. 2 and 3) were obtained with a series of promoter deletion mutants carried on a multicopy plasmid (pBR322). Some of the deletion mutation genes were transferred onto a low-copy number plasmid, pSC101. The profile of β-galactosidase activity of the low-copy-number plasmids was essentially the same as that of the multicopy plasmids (data not shown).

**Localization of the ompC promoter on the nucleotide sequences.** To determine the exact location of the functional ompC promoter, the extents of deletions were determined by DNA sequencing (Fig. 4). The mRNA start site of the ompC gene has been determined to be the TT residue, 82 and 81 bp upstream from the ATG initiation codon (23). Based on this, the −35 and −10 regions have been tentatively shown as TTGGAT and GAGAAT, respectively, in Fig. 4. The results of nucleotide sequencing analyses of the deletion mutants clearly showed that the region between points B and C contained the putative −35 and −10 regions and the ompC mRNA start site, confirming that the region indeed contains the promoter region for the ompC gene. It is also clear that at least 61 bp upstream from the −35 region are essential for full expression of the ompC promoter.

**Localized mutagenesis of the ompC promoter region.** Two

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**TABLE 1. β-Galactosidase activity of mutant plasmids carrying the ompC-lacZ fusion gene**

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasmid or mutant</th>
<th>β-Galactosidase activity (U)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MC4100 (ompR&lt;sup&gt;+&lt;/sup&gt; envZ&lt;sup&gt;+&lt;/sup&gt;)</td>
</tr>
<tr>
<td>pOMPC-X338</td>
<td>3,854</td>
<td>472</td>
</tr>
<tr>
<td>pOMPC-X14</td>
<td>60</td>
<td>64</td>
</tr>
<tr>
<td>Down-I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>116W</td>
<td>255</td>
<td>230</td>
</tr>
<tr>
<td>109W</td>
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<td>120W</td>
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<td>149</td>
</tr>
<tr>
<td>231W</td>
<td>306</td>
<td>378</td>
</tr>
<tr>
<td>Down-II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>161W</td>
<td>711</td>
<td>167</td>
</tr>
<tr>
<td>169W</td>
<td>828</td>
<td>178</td>
</tr>
<tr>
<td>178W</td>
<td>766</td>
<td>219</td>
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<td>179W</td>
<td>721</td>
<td>146</td>
</tr>
<tr>
<td>Down-III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125W</td>
<td>327</td>
<td>156</td>
</tr>
<tr>
<td>185W</td>
<td>235</td>
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<td>483</td>
</tr>
<tr>
<td>213W</td>
<td>262</td>
<td>686</td>
</tr>
<tr>
<td>Up-I</td>
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</tr>
<tr>
<td>127R</td>
<td>4,669</td>
<td>2,782</td>
</tr>
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<td>102R</td>
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<td>3,250</td>
</tr>
<tr>
<td>109R</td>
<td>4,417</td>
<td>2,652</td>
</tr>
<tr>
<td>105R</td>
<td>3,965</td>
<td>2,228</td>
</tr>
</tbody>
</table>

<sup>a</sup> β-Galactosidase activity was measured as described in the text.
FIG. 5. Base substitutions observed in the mutant plasmids. The original DNA sequence of the ompC promoter region is shown at the top. The sequence that is indispensable for the ompC promoter function is boxed. The consensus sequences of the −35 to −10 regions of E. coli are indicated beneath the corresponding sequence of the ompC promoter. The ompC mRNA start site is numbered +1. The base substitutions in each mutant plasmid are indicated, and the common base substitutions in each group are boxed.

of the upstream deletion mutants were used. One (pOMPC-X338) has 121 nucleotides upstream of the mRNA start site, and the other (pOMPC-X14) has only 23 nucleotides from the mRNA start site, as shown in Fig. 4. Thus, pOMPC-X338 has a 98-bp-longer sequence that encompasses the −35 region and its upstream region. Plasmids pOMPC-X338 and pOMPC-X14 were linearized with EcoRI and SalI, respectively. Heteroduplex molecules formed after denaturation and annealing of the linearized plasmids contained the 98-bp single-stranded region, which was susceptible to the single-strand-specific chemical mutagen sodium bisulfite. The modified heteroduplexes were then transferred directly into E. coli K58, which lacks the uracil repair enzyme uracil-N-glycosidase (1). We thus isolated about 10^7 transformants which were assumed to contain the mutant ompC promoter.

Selection of mutants that exhibit decreased expression of the ompC-lacZ fusion gene. Transformants isolated as described above were combined by sixties, and plasmid mixtures were prepared from more than 50 such combined transformants. Plasmids were transferred into strain SB4288 [Δ(proB-lac)] and plated on lactose-MacConkey indicator agar. Cells harboring pOMPC-X338 were expected to be deep red, while cells harboring pOMPC-X14 were white. We looked for pink colonies, which were expected to harbor plasmids carrying the mutant ompC-lacZ gene showing a decreased expression level. We picked up only one colony from any one plate to avoid selecting the same mutant plasmid.

Selection of mutants that exhibit OmpR-independent expression of the ompC-lacZ fusion gene. The expression of the ompC-lacZ gene on pOMPC-X338 was OmpR-dependent (Fig. 2 and 3). To isolate mutant plasmids exhibiting OmpR-independent expression of the ompC-lacZ fusion gene, the mixtures of mutagenized plasmids described above were transferred into strain MH1160 (ompR101). This host cell appeared pale pink on lactose-MacConkey indicator agar when it was transformed with pOMPC-X338, the original plasmid. Deep red colonies, which were expected to carry OmpR-independent promoter mutations, were selected.

Classification and characterization of the mutant plasmids. We measured the β-galactosidase activity of strains MC4100 (ompR+ envZ+), MH1160 (ompR101), and MH1461 (envZ11), which carried the mutant plasmids. The plasmids carrying the ompC promoter mutation and exhibiting a low level of β-galactosidase activity (down mutants) were classified into three groups based on the enzyme level in the three strains (Table 1). Mutant plasmids classified as Down-I exhibited a significant reduction of β-galactosidase activity in strain MC4100 (ompR+ envZ+). In this group, however, about 70% of the original activity was maintained in the envZ mutant, which means that the activity was much higher in the envZ11 mutant than in the envZ+ background. Mutant plasmids classified as Down-II exhibited about 20% of the original β-galactosidase activity in both the wild type and the envZ mutant. The mutant plasmids classified as Down-III exhibited the same degree of reduction of β-galactosidase activity in MC4100 (ompR+ envZ+) as in the Down-I group. This group, however, was clearly distinguishable from the Down-I group with respect to the activity in the envZ11 background. Although the β-galactosidase activity expressed by these mutant plasmids was significantly low in the ompR101 background, it was comparable to that expressed by the wild-type plasmid. Mutant plasmids exhibiting OmpR-independent expression of the ompC-lacZ fusion gene exhibited increased levels of β-galactosidase activity in the ompR101 background, and the enzyme levels were comparable to that in the wild type plasmid in the wild-type background (Table 1). These mutants were classified as Up-I. We isolated four independent mutant plasmids for each group and subjected them to further characterization.

Sequence alterations in individual mutant ompC promoters. To confirm that the mutations on the plasmids resided only in the ompC promoter region, the XbaI-HindIII fragments encompassing the ompC promoter region were isolated from
the mutant plasmids and ligated back into the same sites of nonmutagenized parental plasmid pOMP-X338 (see Fig. 1). These reconstructed plasmids exhibited the phenotypes typical of each mutant group. We concluded, therefore, that the mutations resided only in the ompC promoter region. The DNA sequences of the XbaI-HindIII region were determined for all 16 mutant plasmids (Fig. 5). Base substitutions were found only within the region targeted by sodium bisulfite. In all cases, the G:C to A:T substitution, which is typical of sodium bisulfite mutagenesis, was the main event. Although

FIG. 6. DNA structures of the ompC and ompF promoter regions, and DNA sequence comparison between the ompC and ompF genes. (A) The upper and lower sequences represent the ompC promoter region and the ompF promoter region, respectively. The positions of the common base substitutions in the ompC promoter mutants are indicated by vertical arrows (short arrows for Down-I and long arrows for Down-II; see Fig. 5). The three horizontal arrows with lowercase letters (a through c) represent 10-bp repetitive sequences. Heavy horizontal arrows represent 21-bp highly complementary sequences found in the ompC and ompF promoter regions. Matched bases are indicated by dots beneath the ompF DNA sequence. (B) Structure of the ompF promoter region depicted on a cylindrical projection of B form DNA with 10.5 bp per turn. T. Template strand; TN, nontemplate strand. The topological positions of the three 10-bp repetitive sequences are indicated by arrows with lowercase letters (a through c). The positions of the common base substitutions observed in the Down-I (□) and Down-II (△) groups are also indicated. (C) DNA sequences of the ompF and ompC genes. The DNA sequences are numbered with the ompF and ompC mRNA start sites taken as +1. The Shine-Dalgarno (SD) sequences and the initiation codons (ATG) are underlined. The −35 and −10 regions of the ompF and ompC genes are boxed separately. Homologous nucleotides in regions showing significant homology are boxed together. The horizontal inverted arrows above the ompF sequence indicate inverted sequences. Matched nucleotides are dotted.
base substitutions were found at multiple sites and some of them differed from one mutant to another, mutations in each group had common base substitutions (Fig. 5). It was found that two of the mutant groups (Down-I and Down-II) had base substitutions only in the upstream region of the ompC promoter, whereas the other two (Down-III and Up-I) had base substitutions in the −35 region.

**DISCUSSION**

Mizuno et al. determined the total nucleotide sequence of the ompC gene (21) and tentatively assigned the promoter region of the gene (23). In the present work, the region that stretches from positions −33 to +1 was found to contain a canonical promoter of E. coli, including the mRNA start site (position +1), the −10 region (GAGAAT) at position −10 to −5, and the −35 region (TTGGAAT) at position −33 to −28 (Fig. 4). The 34-bp sequence encompassing the −10 and −35 regions, however, was insufficient for full expression of ompC transcription. At least an additional 61-bp sequence residing upstream from the −35 region (positions −94 to −34) was required for full activation of the ompC promoter. This upstream region could enhance ompC expression by one order of magnitude in the ompR+ background. Enhancement was not observed in the ompR101 and ompR472 mutants, which are phenotypically OmpC−. From these results, we conclude that the 61-bp region is responsible for activation by the ompR gene product of the ompC promoter. Although the ompC gene is one of the most efficiently expressed in E. coli cells, the promoter sequence appeared to show a low level of homology to the consensus sequences for E. coli promoters (8). The OmpR protein most likely interacts with the upstream 61-bp region, which facilitates the binding of RNA polymerase to the poorly conserved ompC promoter.

Although many trans-dominant mutations that affect the transcription of the ompC gene have been isolated and characterized (7, 25, 31), the cis-dominant mutations have not yet been characterized. We demonstrated here that the 61-bp upstream region extending to −94 from the −35 region of the ompC promoter is responsible for the OmpR-mediated activation of ompC transcription. Therefore, some of the mutations in this region are expected to be cis-dominant for ompC expression. After targeting the upstream region, we could isolate such mutants by localized mutagenesis of the ompC-lacZ fusion gene. In two mutant groups (Down-I and Down-II) that exhibited decreased levels of lacZ expression, base substitutions were localized in the region upstream from the −35 region. The base substitutions that were common to the Down-I group were at positions −88, −67, and −47 with the A-to-G transition, whereas those of the Down-II group were at positions −101, −63, and −43 with the C-to-T transition. It should be emphasized in this respect that mutant 116W in the Down-I group did not have any additional base substitutions, indicating that these common base substitutions are sufficient for exhibition of the mutant phenotype. Therefore, we suspect that these base substitutions hinder the functional recognition of the ompC promoter by an activator protein, the OmpR protein. It is interesting that the Down-I mutation was phenotypically suppressed by a mutation in another regulatory factor, the EnvZ protein. This may suggest either that the EnvZ protein also recognizes the upstream region of the ompC promoter or that the EnvZ protein functionally interacts with OmpR protein.

The common base substitution in the Up-I group, which rendered the ompC promoter independent of the OmpR protein, was localized at position −30 in the putative −35 region with the G-to-A transition. Comparison of E. coli promoter DNA sequences has revealed that the −35 consensus sequence is TTGACa (the bases that are strongly conserved are capitalized) (8). Considering these facts, the Up-I group can be regarded as carrying a mutation that makes the fourth base of the −35 region match that of the consensus sequence. In previous studies (3), we showed that the A-to-T substitutions at the first position of ompF allow the promoter to function independently of the OmpR protein. The substitution also increases the degree of homology with the consensus sequence of the −10 region of the ompF gene. In the Down-III group, common base substitutions were found at positions −31 and −30 with the GG-to-AA transition. Although the G-to-A substitution at position −30 was an up-promoter mutation, the Down-III mutation drastically lowered promoter function. It is highly probable that the G-to-A substitution at the third position of the −35 region almost completely abolishes ompC promoter activity, being dominant over the G-to-A substitution at the fourth position. Consistent with this view, G at the third position of the −35 region is one of the most strongly conserved bases in the E. coli promoter sequences (8). The results discussed here confirmed our assignment of a TTGGAAT sequence to the −35 region of the ompC promoter.

To further characterize the upstream region of the −35 region of the ompC promoter (positions −94 to −34), we plotted the base substitutions common to the Down-I and Down-II groups on the nucleotide sequence (Fig. 6A). In this region, two perfectly matched 10-bp sequences (TGAAAACATCT, at positions −89 to −80 and −68 to −59) and another similar sequence (aGATACAATaT, at positions −48 to −39; the bases matching those in the former sequences are capitalized) were found with an 11- or 10-bp interval. The three G-to-A substitutions of the Down-I group took place at the common second G’s of the three repetitive sequences. The two C-to-T transitions of the Down-II group were localized at the common sixth C’s in the two downstream repetitive sequences. These characteristic features became clearer when the data were depicted on a cylindrical projection of B form DNA with 10.5 bp per turn (Fig. 6B). The three repetitive sequences constitute three topologically corresponding DNA helices. Each base substitution of the Down-I and Down-II groups is lined up on one face of the DNA duplex. It is also noteworthy that the 21-bp sequence of ompC, which contains one of the repetitive sequences, was found to be highly complementary to a 21-bp sequence residing upstream of the −35 region of ompF, as shown in Fig. 6A. These features of the DNA sequences in the upstream regions of the ompC and ompF promoters may be important for reciprocal recognition by the common regulatory factor.

Finally, we compared the nucleotide sequences of the ompF and ompC genes. Several characteristic features were found (Fig. 6C). (i) Significant homology was found in the regions upstream from the −35 regions of the ompF and ompC promoters. Since these regions are suggested to be responsible for OmpR-mediated activation of both the ompF and ompC genes, the homology may be important for recognition by the common activator, the OmpR protein. (ii) The DNA sequences encompassing the canonical promoter regions for the two genes showed no significant homology. (iii) Homology was also found in the region between the mRNA start site and the initiation ATG codon. The ompF genes, however, had an extra 50-bp sequence. This extra sequence was found to be a part of the long inverted repeats,
as indicated in Fig. 6C. The ompF mRNA is therefore able to form a possible stable stem and loop structure at its 5’ end (ΔG was calculated to be −14.7 kcal). This particular feature, which is not seen in the ompC gene, might be involved in the regulation of expression of the ompF gene in a specific manner, although the importance of the region in regulation is not clear yet (10). The high degree of homology in the coding sequences for the mature OmpF and OmpC proteins and a part of the signal sequences was discussed previously (21). The homology and divergence found in the regulatory regions should provide us with information that will allow better understanding of the complex mechanism of osmoregulation.

Here we have provided genetic evidence for the importance of the upstream region of the ompC promoter in positive regulation by the ompR promoter. This should be further proved by biochemical evidence, for example, by a footprinting analysis and a direct DNA-binding assay with the purified OmpR protein. Recently, we purified the OmpR protein (Y. Jo, H. Nara, S. Ichihara, T. Mizuno, and S. Mizushima, J. Biochem., in press), and these experiments are now in progress in our laboratory.

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