A Distant Upstream Site Involved in the Negative Regulation of the Escherichia coli ompF Gene

KE-JUNG HUANG, JAMES L. SCHIEBERL, AND MICHELE M. IGO*

Section of Microbiology, Division of Biological Sciences, University of California, Davis, Davis, California 95616

Received 20 September 1993/Accepted 23 December 1993

The two-component regulatory system, OmpR-EnvZ, of Escherichia coli K-12 regulates the expression of the major outer membrane porin protein, OmpF. OmpR is a DNA-binding protein which acts as both an activator and a repressor to control ompF transcription. In this article, we describe a new OmpR-binding site that is located between 384 and 351 bp upstream from the ompF start point of transcription. Inactivation of this site by insertion of a 22-bp fragment prevents the repression of ompF expression conferred by the dominant negative mutation, envZ473. On the basis of the location of this binding site, the presence of bent DNA in the ompF regulatory region (T. Mizuno, Gene 54:57-64, 1987), and the fact that mutations altering integration host factor result in constitutive ompF expression (P. Tsui, V. Helu, and M. Freundlich, J. Bacteriol. 170:4950-4953, 1988), we propose that the negative regulation of ompF involves a DNA loop structure.

The outer membrane of Escherichia coli K-12 contains two major porin proteins, OmpF and OmpC. The total amount of porin protein in the membrane remains fairly constant, but the relative amount of the individual porin species varies depending on the environmental conditions (for reviews, see references 10, 14, 24, and 48). Although the conditions that favor the production of OmpF are the opposite of the conditions that favor the production of OmpC, their synthesis is controlled by the same two regulatory proteins, EnvZ and OmpR (19). The question of how the system achieves its differential regulation therefore arises.

EnvZ and OmpR are related to a large number of bacterial regulatory proteins that control a variety of adaptive responses, known collectively as the two-component regulatory systems (37). In these systems, environmental information gathered by the sensory component is relayed to the effector component via phosphorylation (for reviews, see references 6, 40, and 48). In the porin regulon, EnvZ is an integral cytoplasmic membrane protein 

The biochemical properties of EnvZ suggest that its role in this signal transduction pathway is to control the level of OmpR phosphorylation in the cell and that it is the level of OmpR-P which determines whether ompF or ompC is expressed.

On the basis of genetic analysis of various ompR and envZ mutations and the biochemical properties of EnvZ, Slauch and Silhavy (46) proposed that the reciprocal osmoregulation of ompF and ompC is a result of the differential action of OmpR on the ompF and ompC promoters. In their model, OmpR-P is a transcriptional activator at the ompF promoter and can function as either an activator or a repressor at the ompC promoter. Inherent in this model is the prediction that a negative regulatory site exists upstream of ompF. In this article, we identify by DNase I footprinting analysis a new OmpR-binding site upstream of ompF and show by genetic analysis that this site is involved in the repression of ompF transcription in vivo.

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used in this study are listed in Table 1. The strains in this study were constructed by generalized transduction using bacteriophage P1 vir. Standard microbiological techniques were used for generalized transduction and bacterial growth (44).

Media, reagents, and enzymes. Most growth media were prepared as previously described (44). For studying the effects of osmolality on ompF expression, the cells were grown in A medium (27) with or without 20% (wt/vol) filter-sterilized sucrose (Pfanstiehl). When used, chloramphenicol was added to a final concentration of 10 μg/ml. Reagents for DNA sequence analysis were obtained from United States Biochemical Corp.

Small-scale cell envelope preparations. Cells were grown overnight in A medium containing 10 μg of chloramphenicol per ml, subcultured into 10 ml of the same medium, and grown to mid-log phase. For studying the effects of osmolality on ompF expression, the cells were grown in A medium containing 10 μg of chloramphenicol per ml with or without 20% sucrose. The cells were lysed by lysozyme treatment as previously described (36). The envelope fractions were collected and analyzed on an 11% polyacrylamide gel containing sodium dodecyl sulfate (SDS) and 4 M urea. The proteins were identified by staining with Coomassie brilliant blue R-250 (Kodak).

Construction of plasmids. (i) pIP275. To determine the effect of removing the region upstream of −238 on OmpF expression, we constructed two plasmids, pIP275 and pIP276. To construct pIP275, the 6-kb EcoRI-SalI fragment from pPR724 carrying ompF (32) was inserted between the EcoRI-SalI sites in pPR274 (32). Thus, pIP275 contains sequences from the EcoRI site located −1158 bp upstream of the ompF transcription start point (+1) and the entire ompF structural gene. In addition, since pPR274 has a mini-F replicon, pIP275 should be present in one or two copies per cell.

(ii) pIP276. The construction of pIP276 required two steps. First, pIP637 was constructed by replacing the 1,367-bp EcoRI-
BgelII fragment in pPR274 with the 470-bp EcoRI-BgelII fragment from pORF2 (53). Next, the EcoRI-SalI fragment from pP637, carrying ompF, was inserted between the EcoRI and SalI sites of pPR274. Thus, pIP276 contains sequences from the TaqI site at position −236 and the entire ompF structural gene.

(iii) pJLS4. To determine whether the OmpR-binding site located between −384 and −351 was required for normal regulation of ompF, we inserted a 22-bp fragment of DNA into the SspI site located at position −373. For this experiment, plasmid pIP41, which contains sequences from the EcoRI site located −1158 bp upstream of the ompF transcription start point to the BgelII site at +209 in the ompF structural gene, was partially digested with SspI. The linearized pIP41 was isolated and then ligated to the kanamycin cassette from pUC4K (51), which had been removed from the plasmid by digestion with EcoRI and treated with Klenow fragment of DNA polymerase I. The presence of the inserted cassette and the presence of the insertion was confirmed by DNA sequence analysis. To place the insertion upstream of the ompF structural gene, the 1,389-bp EcoRI-BgelII fragment from pJLS2 was used to replace the 470-bp EcoRI-BgelII fragment from pP637. The reconstructed ompF gene was then inserted into plasmid pPR274, creating plasmid pJLS4. Thus, pJLS4 contains sequences from the EcoRI site located −1158 bp upstream of the ompF transcription start point, a 22-bp insertion at position −371, and the entire ompF structural gene. Therefore, the only difference between pJLS4 and pPR275 is the presence of the 22-bp insertion at position −371.

(iv) pKJH2. Plasmid pKJH2, which contains the proposed OmpR-binding sites in the −384 to −351 region of ompF, was constructed by amplifying the DNA fragment spanning −524 to −337 bp upstream of ompF by PCR using oligonucleotide 5'-CGGATCCATCCGAGGCTG-3' as the nontemplate strand primer and oligonucleotide 5'-GACTGAGATTGTGTGGGGC-3' as the template strand primer. Following amplification, the DNA fragment was purified, digested with BamHI and PstI, and cloned into pUC19. The DNA sequence of the cloned fragment was confirmed by the dideoxy chain termination method using the Sequenase procedure (United States Biochemical Corp.).

DNase I footprinting experiments. DNase I footprinting experiments were performed with slight modification according to the method of Galas and Schmitz (17). The DNA fragments were labeled on either the template strand or the nontemplate strand. To label the template strand, plasmid pKJH2 was digested to completion with EcoRI and HaeIII. The digestion products were then labeled with [α-32P]dATP (3,000 Ci/mmol) by filling in the 3' end of the EcoRI site by using the Klenow fragment of DNA polymerase I. To label the nontemplate strand, pKJH2 was digested with EcoRI and treated with calf intestinal mucosa alkaline phosphatase. The digestion product was labeled with [γ-32P]ATP (3,000 Ci/mmol) by using T4 DNA polynucleotide kinase and then digested with HaeIII. The labeled DNAs were purified by phenol-chloroform extraction followed by ethanol precipitation. The OmpR used in the DNA footprinting analysis was purified as previously described (20).

DNase I footprinting experiments were then performed with DNA that had been labeled on either the nontemplate or the template strand. In these experiments, the DNA binding reaction mixture, in a volume of 15 μl, contained 0.011 μM labeled DNA, 4 mM Tris-HCl (pH 7.9), 1 mM EDTA, 1 mM 1,4-dithiothreitol, 20 mM KCl, 2 μg of sheared calf thymus DNA, and 12% (wt/vol) glycerol. Diluted OmpR was added to each reaction mixture at the concentrations indicated in the legend to Fig. 3, and the DNA binding reaction was allowed to proceed for 25 min at room temperature. (OmpR was diluted with OmpR storage buffer, which contains 20 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, 0.1 mM dithiothreitol, and 50% [wt/vol] glycerol.) Then, 0.0004 U of DNase I (EC 3.1.4.5; DPPF; Worthington Biochemical Corporation) in 15 μl of enzyme dilution buffer (4 mM Tris-HCl [pH 7.9], 10 μM MgSO4, 2 μM CaCl2, 20 mM KCl, 1 mM EDTA, and 12% glycerol) was added to the DNA binding reaction mixture. The DNase I digestion reaction was allowed to proceed at room temperature for exactly 2 min and stopped by phenol-chloroform extraction. The digestion products were subjected to electrophoresis on an 8% urea–8% polyacrylamide DNA sequencing gel and detected by autoradiography. The location of the protected region was determined by comparing the DNase I digestion patterns with a G+A sequencing ladder. The G+A reaction was performed by the method adapted from Maxam and Gilbert (31).

RESULTS

On the basis of previous results with ompF-lacZ+ fusions, Ostrow et al. (39) suggested that a cis-acting regulatory site lies somewhere in the region between −1158 and −238 bp upstream of the ompF start point of transcription (+1). The exact location of this site and its role in ompF regulation are the subject of this study.

The region upstream of −238 is required for the negative regulation of ompF. As a first step in our analysis, we examined the effect of removing the region upstream of −238 on the production of OmpF. Since asnS, which is thought to be an

TABLE 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC4100</td>
<td>F−araD139 Δ(ompF-lacZ')U169 rpsL50 relAI fabB301 deoC1 parC301 recA1II::Km</td>
<td>8</td>
</tr>
<tr>
<td>MHS13</td>
<td>MC4100 (ompF-lacZ')Δ16-13 araC*</td>
<td>19</td>
</tr>
<tr>
<td>M1760</td>
<td>MC4100 ompR472</td>
<td>18</td>
</tr>
<tr>
<td>M11471</td>
<td>MHS410 envZ473</td>
<td>18</td>
</tr>
<tr>
<td>JMS122</td>
<td>MHS13 ompR107</td>
<td>J. M. Slauch</td>
</tr>
<tr>
<td>JMS1160</td>
<td>MC4100 recA111::Km</td>
<td>46</td>
</tr>
<tr>
<td>IM200</td>
<td>MHS153 recA111::Km</td>
<td>This study</td>
</tr>
<tr>
<td>IM201</td>
<td>MHS153 recA111::Km ompR472</td>
<td>This study</td>
</tr>
<tr>
<td>IM202</td>
<td>MHS153 recA111::Km ompR107</td>
<td>This study</td>
</tr>
<tr>
<td>IM203</td>
<td>MHS153 recA111::Km envZ473</td>
<td>This study</td>
</tr>
<tr>
<td>KJH13</td>
<td>MC4100 (ompF-lacZ')Δ16-13</td>
<td>This study</td>
</tr>
<tr>
<td>Plasmids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pIP41</td>
<td>M. M. Igo</td>
<td>54</td>
</tr>
<tr>
<td>pORF2</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>pPR274</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>pUC4K</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>pKJH2</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>pJLS4</td>
<td>This study</td>
<td></td>
</tr>
</tbody>
</table>

To place the insertion upstream of the ompF structural gene, the 1,389-bp EcoRI-BgelII fragment from pJLS2 was used to replace the 470-bp EcoRI-BgelII fragment from pP637. The reconstructed ompF gene was then inserted into plasmid pPR274, creating plasmid pJLS4. Thus, pJLS4 contains sequences from the EcoRI site located −1158 bp upstream of the ompF transcription start point, a 22-bp insertion at position −371, and the entire ompF structural gene. The presence of the insertion was confirmed by DNA sequence analysis.

To place the insertion upstream of the ompF structural gene, the 1,389-bp EcoRI-BgelII fragment from pJLS2 was used to replace the 470-bp EcoRI-BgelII fragment from pP637. The reconstructed ompF gene was then inserted into plasmid pPR274, creating plasmid pJLS4. Thus, pJLS4 contains sequences from the EcoRI site located −1158 bp upstream of the ompF transcription start point, a 22-bp insertion at position −371, and the entire ompF structural gene. The presence of the insertion was confirmed by DNA sequence analysis.

To place the insertion upstream of the ompF structural gene, the 1,389-bp EcoRI-BgelII fragment from pJLS2 was used to replace the 470-bp EcoRI-BgelII fragment from pP637. The reconstructed ompF gene was then inserted into plasmid pPR274, creating plasmid pJLS4. Thus, pJLS4 contains sequences from the EcoRI site located −1158 bp upstream of the ompF transcription start point, a 22-bp insertion at position −371, and the entire ompF structural gene. Therefore, the only difference between pJLS4 and pPR275 is the presence of the 22-bp insertion at position −371.

The DNase I digestion reaction was allowed to proceed at room temperature for exactly 2 min and stopped by phenol-chloroform extraction. The digestion products were subjected to electrophoresis on an 8% urea–8% polyacrylamide DNA sequencing gel and detected by autoradiography. The location of the protected region was determined by comparing the DNase I digestion patterns with a G+A sequencing ladder. The G+A reaction was performed by the method adapted from Maxam and Gilbert (31).
essential gene (54), is located immediately upstream of ompF, it was not possible to delete the region between −1158 and −238 upstream from the chromosomal copy of ompF. Therefore, we constructed two plasmids, pIP275 and pIP276, which are derivatives of the mini-F plasmid, pPR274 (32) (see Materials and Methods). Plasmid pIP276 contains sequences from the Tσl site at position −238 and the entire ompF structural gene, whereas plasmid pIP275 contains the same sequences together with an additional 1 kb of upstream DNA. In order to determine the role of the upstream region in ompF regulation, we introduced these plasmids into strain IM200, which is defective in the production of OmpF. (IM200 contains an ompF∗-lacZ∗ transcriptional fusion at the normal chromosomal location of ompF). Therefore, the only intact copy of ompF in the cell is the copy that is located on the plasmid. We also introduced the two plasmids into derivatives of IM200 which contain mutations in either ompR or envZ. Using these strains, we investigated the effect of the upstream region on ompF expression by monitoring the levels of OmpF in the outer membrane.

The results of our analysis clearly indicate that the region upstream of −238 is involved in the negative regulation of the plasmid-borne ompF gene. As shown in Fig. 1, deletion of the region upstream of −238 had no detectable effect on OmpF production in an ompR∗ strain under low-osmolarity conditions. Similarly, the deletion had no effect on the phenotype caused by ompF472, a mutation in ompR that confers constitutive expression of ompF (18). Thus, loss of the upstream region does not affect positive activation of the ompF promoter. However, there was a dramatic effect on OmpF production in both an ompR107 and an envZ473 mutant. Both of these mutations are dominant and prevent the transcription of ompF (18). In the presence of these mutations, removal of the upstream region restored OmpF expression. These results support the conclusion of Ostrouw et al. that a cis-acting site is present in the region between −1158 and −238 and further indicate that this region is important for the negative regulation of ompF.

We also examined the effect of removing the region upstream of −238 on the ability of ompF to respond to changes in medium osmolarity. For this experiment, we introduced plasmids pIP275 and pIP276 into strain KH513, which is defective in the production of OmpF. Using these strains, we monitored the level of OmpF in the outer membrane when cells were grown under either low or high osmolarity conditions. As shown in Fig. 2, deletion of the region upstream of −238 had no detectable effect on OmpF production under low-osmolarity conditions. However, under high-osmolarity conditions, removal of the upstream region resulted in a fivefold increase in the level of OmpF compared with the level observed when the upstream region is present. This result suggests that the role of the cis-acting site in the region between −1158 and −238 is to repress ompF expression under high-osmolarity conditions.

Identification of a new OmpR-binding site upstream of ompF. The simplest explanation for the above results is that the deletion removes one or more OmpR-binding sites. To test this hypothesis, Igo and Silhavy (23) examined the sequences between −1158 and −238 for a region that was protected by OmpR from Dnase I digestion. Their preliminary DNA footprinting studies suggested that OmpR binds to a region from approximately −380 to −350 upstream of the ompF transcription start site.

To locate the region protected by OmpR more precisely, Dnase I footprinting experiments were performed with plasmid pKJH2, which was constructed as described in Materials and Methods. This plasmid contains the sequences from −524 to −337 bp upstream of ompF on a 440-bp EcoRI-HaeIII fragment. The Dnase I footprinting analysis revealed that OmpR protects a region from −384 to −354 (Fig. 3A) on the template strand and from −383 to −351 on the non-template strand (Fig. 3B). In order to obtain these results, micromolar concentrations of OmpR were required, and it was necessary to perform the footprinting reactions at low KCl concentrations (20 mM). These experiments suggest that OmpR has a low affinity for the distant upstream site. The genetic analysis reported in the next section demonstrates that this site is physiologically important in the negative regulation of ompF. The location of the region protected by OmpR relative to the DNA sequence is indicated in Fig. 3C.

A 22-bp insertion into the distant upstream OmpR-binding site prevents the negative regulation of ompF. To determine whether the OmpR-binding site between −384 to −351 is involved in the negative regulation of ompF in vivo, we constructed an insertion mutation that placed 22 bp into the SspI site located at position −373 (Fig. 3C). The insertion mutation was then placed upstream of the ompF gene in plasmid pIP275 as described in Materials and Methods. The resulting plasmid, pJLS4, was introduced into strain IM200, which is defective in the production of OmpF. We also introduced the plasmid into a derivative of IM200, which contains the envZ473 mutation. Using these strains, we investigated the effect of the 22-bp insertion mutation on ompF expression by monitoring the levels of OmpF in the outer membrane.

The results of this analysis indicate that the phenotype caused by the 22-bp insertion mutation is identical to the phenotype caused by deleting the region upstream of −238. As shown in Fig. 4, the 22-bp insertion had no effect on OmpF.
concentrations of Lanes 8% polyacrylamide gel DNA 3' end upstream of numbers extends from sequence of the ompF production in of the labeled 1312 HUANG, et al. FIG. 3. DNase I footprinting analysis of the OmpR-binding site upstream of \(-238\). A DNA fragment containing the region that extends from \(-524\) to \(-337\) bp upstream of ompF was labeled at its 3' end with \([\alpha^32P]ATP\) (A) or at its 5' end with \([\gamma^32P]ATP\) (B). The labeled fragments were then incubated with different amounts of OmpR. After treatment with DNase I, the samples were subjected to an 8% polyacrylamide gel and then analyzed by autoradiography. Lanes 1, G+A Maxam-Gilbert sequencing reaction. The micromolar concentrations of OmpR were as follows: lanes 2, 0; lanes 3, 38; lanes 4, 19; lanes 5, 9.5; lanes 6, 4.75; lanes 7, 2.38. In lane 8, the labeled DNA fragment was digested with the restriction enzyme SpI. The numbers on the left in each panel correspond to those of the DNA sequence of theompF regulatory region, with +1 being the transcription initiation site. (C) DNA sequence of the region protected by OmpR in the DNase I footprinting experiments. The sequences protected by OmpR with the labeled nontemplate (upper) strand and the labeled template (lower) strand are indicated by lines. The location of the SpI site is also indicated.

production in an ompR\(^*\) strain, indicating that the upstream site is not required for normal activation of ompF expression. However, there was a dramatic effect on OmpF production in an envZ473 mutant, in which ompF expression is normally inhibited. In this mutant, insertion of 22 bp into the distant upstream OmpR-binding site restored OmpF expression. This result supports the hypothesis that the OmpR-binding site located between \(-384\) to \(-351\) is involved in the negative regulation of ompF. This result also indicates that the form of OmpR found in an envZ473 mutant is still capable of activating ompF transcription.

**DISCUSSION**

The results presented here fulfill an important prediction of the model originally advanced by Slauch and Silhavy (46) and refined by work from a number of laboratories (2, 5, 13, 20, 42, 52). Slauch and Silhavy (46) proposed that the differential regulation of ompF and ompC is a direct result of the structure of the regulatory regions upstream of these genes and the nature of the interaction of OmpR-P with these sites. According to this model, under conditions of low osmolarity, the concentration of OmpR-P in the cell is very low. As a result, OmpR-P binds preferentially to the activating sites upstream of ompF, thereby resulting in the expression of ompF, but is unable to bind to the activating sites upstream of ompC. However, under conditions of high osmolarity, the concentration of OmpR-P in the cell increases. As a result, OmpR is now able to occupy the activating sites upstream of ompC, leading to the expression of ompC. The model also predicts that OmpR binds to additional sites upstream of ompF, causing ompF expression to be repressed. It is the concurrent binding of OmpR-P to the activating sites upstream of ompC and the repressing sites upstream of ompF that is responsible for the reciprocal regulation of ompF and ompC.

Inherent in this model is the prediction that there should be at least one positive site upstream of ompC and at least one positive and one negative site upstream of ompF. In this article, we describe a new OmpR-binding region upstream of the ompF start point of transcription. DNase I footprinting analysis indicates that this upstream site extends from \(-384\) to \(-351\) bp upstream of ompF. In order to obtain a reproducible footprint of this region, it was necessary to drop the KCl concentration in our footprinting experiments from 60 to 20 mM. This result would suggest that the region between \(-384\) to \(-351\) has a lower affinity for OmpR than those for previously characterized OmpR-binding sites. Nevertheless, genetic analysis clearly indicates that this site is physiologically important. A 22-bp insertion mutation into this OmpR-binding site suppresses the dominant negative phenotype conferred by envZ473. This result indicates that the OmpR-binding site located between \(-384\) to \(-351\) is required for the negative regulation of ompF.

On the basis of the location of this element and the phenotype of a strain in which this site has been inactivated, it seems likely that this distant upstream site exerts its influence on ompF transcription through a DNA loop. We would predict that this loop forms between OmpR bound at the site between \(-384\) to \(-351\) and OmpR bound at one of the sites located immediately upstream of the ompF promoter. OmpR has previously been shown to bind to a series of sites upstream of ompF (25, 26) that are located between \(-96\) and \(-41\) (Fig. 5). DNase I footprinting analysis indicates that the region between \(-96\) and \(-63\) exhibits high affinity for OmpR (34, 50). Deletions into this region abolish transcription from the ompF promoter (26), supporting the idea that the OmpR-binding sites in this region are required for the positive activation of ompF transcription. The region between \(-56\) and \(-41\) is also protected by OmpR, but the affinity of OmpR for this region is much weaker than that for the \(-96\) to \(-63\) region (34, 50). Moreover, the region between \(-56\) and \(-41\) is not protected by a mutant form of OmpR, OmpR472 (34). Because the ompR472 mutation results in the constitutive expression of

**FIG. 4.** Effect of the 22-bp insert on ompF expression. Cellular envelopes were prepared from cells that had been grown to mid-log phase in A medium containing 10 \(\mu\)g of chloramphenicol per ml. The samples were analyzed on an SDS–11% polyacrylamide gel containing 4 M urea. The positions of the outer membrane proteins OmpC, OmpF, and OmpA are indicated on the right. The strains used in this experiment were IM200 (ompR\(^-\) envZ\(^+\)) and IM203 (ompR\(^+\) envZ473), containing either plasmid pIP275 (+), plasmid pIP276 (A), or plasmid pJLS4 (+22).
ompF, these results suggest that the region between −56 and −41 is involved in the negative regulation of ompF. One interpretation of this result is that the OmpR bound at the site located between −56 and −41 interacts with OmpR bound at the site located between −384 to −351 to form a DNA loop, thereby repressing ompF transcription.

The idea that the repression of ompF transcription involves a DNA loop is supported by the existence of bent DNA and two integration host factor (IHF)-binding sites in the ompF regulatory region (Fig. 5). The region of bent DNA is located between −101 and −71 (33), thereby overlapping some of the OmpR-binding sites. This bend occurs in the absence of auxiliary proteins and involves two sets of T residues, which are separated by two turns of the DNA helix. Moreover, mutations that affect the three-dimensional structure of this region result in constitutive ompF expression (47). The ompF regulatory region also contains two recognition sites for IHF. IHF is a small histone-like protein that bends DNA, thereby facilitating the formation of DNA into higher-order structures (15, 16). One of the IHF-binding sites is located between −80 and −42, partially overlapping the OmpR-binding region and the region of bent DNA, while the second IHF-binding site is located between −199 and −159 (41). Studies of ompF expression in IHF mutants indicate that IHF is required for the decrease in ompF expression observed under high-osmolarity conditions (49). One interpretation of this result is that IHF facilitates the formation of a DNA loop between the OmpR-binding site located at −384 and −351 and the OmpR-binding site located at −56 and −41 and that this DNA loop is responsible for repressing ompF expression under conditions of high osmolarity.

Our analysis of ompF expression in mutant backgrounds allows us to refine the current model for the regulation of ompF expression in a wild-type strain. Figure 6 shows how ompF expression might respond to changes in medium osmolarity. Under low-osmolarity conditions, OmpR binds to the region between −96 and −63, thereby facilitating the binding of RNA polymerase to the ompF promoter (Fig. 6A). Under high-osmolarity conditions, OmpR binds to the region between −56 and −41 and to the region between −384 and −351 (Fig. 6B). The binding of OmpR to these regions may be facilitated by the presence of the bent DNA in the region between −101 and −71 and by IHF binding at one or both of

![Figure 5](http://jb.asm.org/)  
**FIG. 5.** Sequence of the nontemplate strand of the ompF regulatory region. The locations of the inverted repeats of the putative asnS terminators are indicated (horizontal arrows). The first putative terminator of asnS is located between −480 and −452, while the second terminator is located between −347 and −310 (7). OmpR-binding sites are boxed. The regions of bent DNA (wavy lines) and the IHF-binding sites (dashed lines) are indicated. Bent arrow at base +1, start site of ompF transcription.

![Figure 6](http://jb.asm.org/)  
**FIG. 6.** Model for the regulation of ompF by OmpR. According to this model, under low-osmolarity conditions (A), OmpR binds to the positive site between −96 and −63 upstream of ompF, thereby activating ompF expression. Under high-osmolarity conditions (B), OmpR binds to region between −384 and −351 and the region between −56 and −41. The concomitant binding of OmpR to these regions results in the formation of a DNA loop that is responsible for repressing ompF expression under high-osmolarity conditions. At present, it is not clear how many OmpR monomers bind to the different sites and whether or not OmpR bound at the positive site participates in the formation of the DNA loop.
its sites. Together, these interactions result in a loop of DNA that is responsible for repressing ompF expression under high-osmolarity conditions.

Although repression by the formation of a DNA loop has been identified in a large number of systems (for reviews, see references 1, 9, and 30), the exact mechanism by which a DNA loop results in the repression of transcription is still unclear. In addition, it is possible that there could be considerable variability in the mechanism from system to system. In the case of ompF, the existence of mutations in the alpha subunit of RNA polymerase that affect the negative regulation of ompF suggests that the interaction between OmpR and the alpha subunit is an important element in this regulation (29, 43, 47). Therefore, it seems likely that repression caused by the DNA loop is not the result of the simple steric hindrance of RNA polymerase from the ompF promoter.

ACKNOWLEDGMENTS

We thank T. Silhavy and the members of his laboratory for providing strains and assistance in the initial stages of this project and for many useful discussions. We are grateful to C. Price for his critical comments on the manuscript. This research was supported in part by Public Health Service grant GM48591 to M.M.I. from the National Institutes of Health.

REFERENCES


