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The *Escherichia coli tppB* (*ydgR*) Gene Represents a New Class of OmpR-Regulated Genes

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The EnvZ/OmpR two-component regulatory system plays a critical role in the *Escherichia coli* stress response. In this study, we examined the expression of a new OmpR-regulated gene, *ydgR*. Our results indicate that *ydgR* is equivalent to the *Salmonella enterica* serovar Typhimurium *tppB* gene and represents a new class of OmpR-regulated genes.

The EnvZ/OmpR two-component regulatory system plays a critical role in the response of *Escherichia coli* to environmental stress (for reviews, see references 2, 3, 5, 20, 23, 33 and 35). The transmembrane protein EnvZ is a histidine kinase that is thought to monitor changes in environmental osmolarity. Following autophosphorylation, EnvZ transfers its high-energy phosphoryl group to its cognate response regulator, OmpR. Phosphorylation changes the conformation of OmpR and stimulates the ability of OmpR to interact with specific sites upstream of its target genes. Some of these target genes include *ompF*, *ompC*, *tppB*, *fadL*, *flhDC*, and *csgD* (6, 8, 11, 38, 44). Interestingly, EnvZ also possesses a phosphatase activity that removes the phosphoryl group from the phosphorylated form of OmpR (OmpR-P). The interplay between the kinase and phosphatase activities of EnvZ in response to environmental changes is responsible for controlling the cellular level of OmpR-P (40).

In this study, we examined the regulation of *ydgR*, a gene that we identified as part of a large-scale DNA microarray project aimed at discovering new members of the OmpR regulon. This gene was also identified as an OmpR-regulated gene in a recent transcriptome study of *E. coli* two-component regulatory systems (29). Based on its putative amino acid sequence, the *ydgR* gene is predicted to encode a 500-amino-acid oligopeptide permease that exhibits sequence similarity to proteins belonging to the POT (proton-dependent oligopeptide transport) family of peptide transporters (30, 31). Early studies by Higgins and Gibson identified three different peptide permease systems in *E. coli* and *Salmonella enterica* serovar Typhimurium (12). One of the systems, the tripeptide permease encoded by the *tppB* gene, was found to be transcriptionally regulated by the EnvZ/OmpR regulatory system in serovar Typhimurium (6, 7). The *tppB* gene is located at 27 min on the serovar Typhimurium linkage map (7). However, its precise location on the serovar Typhimurium chromosome is not known because the *tppB* gene has not been sequenced. The functional similarity between *tppB* and *ydgR*, as well as the dependence of *ydgR* transcription on OmpR observed in our DNA microarray experiments, led us to hypothesize that *ydgR* is equivalent to the serovar Typhimurium gene *tppB*.

To test this hypothesis, we isolated chromosomal DNA from the serovar Typhimurium strain CH695, which carries the *tppB16*::Tn*10* mutation (7). The region containing the Tn*10* insertion was amplified by inverse PCR using the two sets of primers described by Nichols et al. (27), and the nucleotide sequence of the junction was determined as described by Ochman et al. (28). DNA sequence analysis revealed that the *tppB16*::Tn*10* insertion mapped between nucleotide positions 1527921 and 1527922 of the serovar Typhimurium LT2 genome. This places the Tn*10* insertion within the opening reading frame designated *ydgR* in the serovar Typhimurium genome. From this result, we established that the serovar Typhimurium *ydgR* gene is *tppB.* Since the serovar Typhimurium *ydgR* amino acid sequence exhibits 89% identity and 93% similarity to the *E. coli ydgR* amino acid sequence, we conclude that the *E. coli ydgR* gene is also *tppB*.

We next carried out an in-depth characterization of *tppB* and compared its transcriptional regulation to that of *ompF* and *ompC*. The *ompF* and *ompC* genes encode the outer membrane porin proteins OmpF and OmpC, respectively, and are two of the most studied targets of the EnvZ/OmpR regulatory system (20, 35). OmpF predominates in the outer membrane at low osmolarity, whereas OmpC predominates at high osmolarity. Genetic, molecular, and biochemical experiments have established that the differential expression of OmpF and OmpC is a direct consequence of the cellular OmpR-P concentration and is dependent upon the way in which OmpR-P interacts with regulatory elements located upstream of these genes. Using similar techniques, we investigated how OmpR and EnvZ regulate the transcription of *tppB*. To carry out this analysis, we constructed a transcriptional fusion between the *tppB* regulatory region and a *lacZ* reporter gene. This was accomplished by cloning a 0.7-kb fragment that corresponds to nucleotide positions 1710073 to 1710769 of the *E. coli* K-12 genome upstream of the promoterless *lacZ* gene in the chloramphenicol-resistant integration vector pTTW1 (T. T. Wei and M. M. Igo, unpublished data). The resulting plasmid, pDS3, was then integrated as a single copy into the *E. coli* chromosome at the λ *attB* site by using the method described by Platt et al. (32). This integration resulted in the *tppB′-lacZ* fusion strain EG107, which served as the parental strain for all subsequent strain

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constructions. EG107 is a derivative of MG1655, which contains a single copy of the plasmid pDS3 at λ *attB*, the *lacIpo*ΔZ (Mlu) mutation (36), and two markers that flank the *ompR* and *envZ* genes, *aroB*$^-$ and *malPQ*::Tn*10*. P1-mediated generalized transductions were performed to introduce various *ompR* and *envZ* alleles into the *tppB′-lacZ* fusion strain EG107 (45), and the resulting strains were subjected to β-galactosidase assays (25).

We began this analysis by examining the effect of three different *envZ* mutations on the regulation pattern of *tppB*. These mutations were chosen because they alter EnvZ functions that are predicted to result in different cellular levels of OmpR-P (13, 14, 40). We first investigated the effect of the *envZ473* mutation on *tppB′-lacZ* expression (8). This mutation results in the production of a mutant protein that retains its kinase activity but loses its phosphatase activity (K^+ P^-) and is predicted to cause high cellular levels of OmpR-P. As shown in Fig. 1A, the presence of the *envZ473* mutation results in levels of *tppB′-lacZ* fusion expression that are similar to wild-type levels. This result suggests that increasing the cellular concentration of OmpR-P does not result in an increase in *tppB* transcriptional activation. We next studied the effect of the *envZ*::Tn*10* mutation on *tppB* transcription (46). Cells containing this null mutation do not contain any functional EnvZ protein. As a result, OmpR will not be phosphorylated or dephosphorylated by EnvZ. As shown in Fig. 1A, *tppB′-lacZ* fusion expression is approximately threefold lower in the *envZ* null strain than in the wild-type strain. This result supports the idea that EnvZ plays an important role in stimulating the level of *tppB* transcription. However, there is still significant expression of the *tppB′-lacZ* fusion in the absence of EnvZ. This expression could be due to the activation of *tppB* transcription by the unphosphorylated form of OmpR. Alternatively, this expression could be due to the low levels of OmpR-P produced by nonpartner kinases or small molecule phosphodonors, such as acetyl phosphate (14, 24). To distinguish between these possibilities, we examined the effect of the *envZ343* mutation on *tppB′-lacZ* expression (14). This mutation results in the production of a mutant protein that retains its phosphatase activity but loses its kinase activity (K^- P^+) and is predicted to eliminate any residual OmpR-P in the cell. As shown in Fig. 1A, the *tppB′-lacZ* fusion expression is extremely low in the strain containing the *envZ343* mutation. In fact, the level of *tppB′-lacZ* fusion expression is similar to that of the strain containing the *ompR101* null mutation (Fig. 1B). Therefore, based on our genetic analysis of *tppB* transcription, we conclude that the activation of *tppB* transcription is dependent on OmpR-P and that low cellular levels of OmpR-P are sufficient to activate *tppB* transcription.

We next examined the effect of the three different classes of *ompR* mutations on *tppB* transcription. These mutations are genetically defined based on the phenotypes that they confer on OmpF and OmpC (35, 46). The first mutation, *ompR101* (9), belongs to the OmpR1 class. This class of mutations, which includes all null mutations of *ompR*, results in no expression of OmpF or OmpC. As shown in Fig. 1B, *tppB′-lacZ* fusion expression is extremely low in the strain containing the *ompR101* mutation, confirming that the activation of *tppB* transcription is dependent on OmpR. The second mutation, *ompR472* (8), belongs to the OmpR2 class. This class of mutations results in

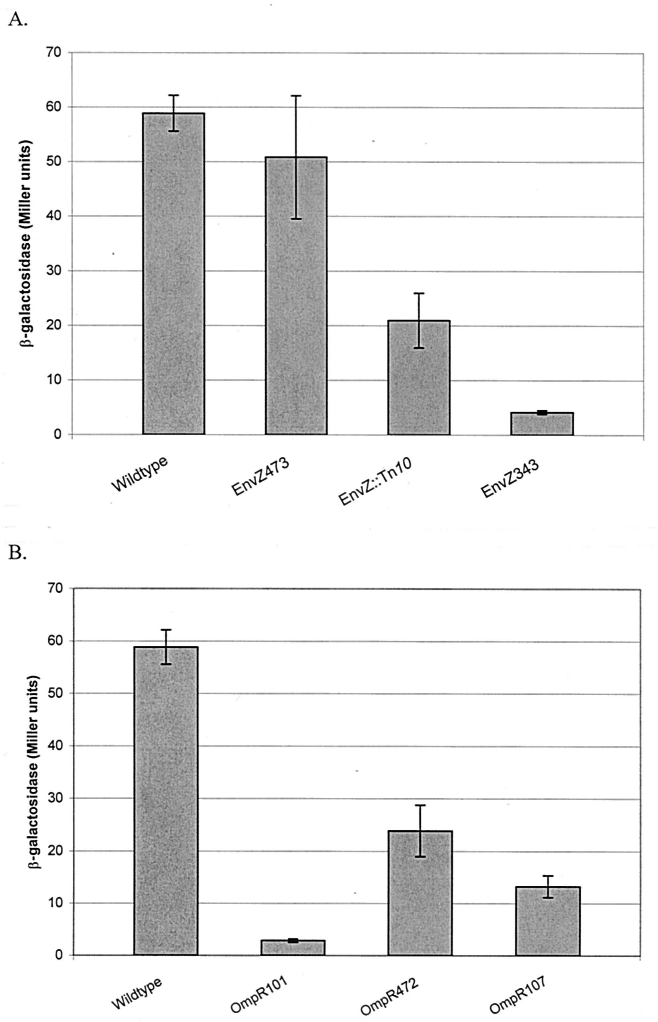

FIG. 1. Effects of various *envZ* and *ompR* mutations on *tppB* transcription. β-Galactosidase assays were performed on cells grown to mid-exponential phase in Luria-Bertani broth, and the β-galactosidase activity is expressed in Miller units for strains containing the indicated *envZ* (A) and *ompR* (B) alleles. All strains were assayed in duplicate in at least three independent experiments. The values represent averages of results for three independent cultures, with the error bars corresponding to ±1 standard deviation from the average result for the three independent cultures.

constitutive expression of OmpF but little expression of OmpC. In the strain containing the *ompR472* mutation, *tppB′-lacZ* expression is approximately two- to threefold lower than that of the wild-type strain (Fig. 1B). The *ompR472* mutation resulted in a Val-to-Met conversion at the 203rd amino acid of the OmpR polypeptide chain (26). DNase I footprinting studies indicate that this change affects the binding of OmpR at specific sites within the *ompF* and *ompC* regulatory regions (26) and may also cause a defect in OmpR phosphorylation (49). Either of these properties could account for the observed decrease in *tppB* transcription. The third mutation, *ompR107* (46), belongs to the OmpR3 class. This class of mutations results in little expression of OmpF but constitutive expression of OmpC. In the strain containing the *ompR107* mutation, *tppB′-lacZ* fusion expression is approximately fourfold lower

TABLE 1. Effects of different environmental stimuli on *tppB′-lacZ* production

Allele	*tppB′-lacZ* production (Miller units)			
	Osmolarity		Anaerobiosis	
	Glycerol-MOPS (50 mM NaCl)	Glycerol-MOPS (300 mM NaCl)	Glucose-MOPS with O_2	Glucose-MOPS without O_2
ompR$^+$	53 ± 4	43 ± 2	33 ± 11	84 ± 15
ompR101	4 ± 1	3 ± 1	3 ± 1	5 ± 2

than in the wild-type strain (Fig. 1B). The *ompR107* mutation resulted in an Arg-to-Cys change at the 15th amino acid of the OmpR polypeptide chain (41). DNase I footprinting studies with the OmpR36 mutant protein, which contains the same amino acid substitution, suggest that this change does not affect the DNA-binding pattern within the *ompF* and *ompC* regulatory region (26). Instead, like the *envZ473* mutation, this amino acid change in OmpR results in a defect in the dephosphorylation of OmpR-P and leads to an accumulation of OmpR-P in the cell (1). Therefore, we predicted that the level of *tppB′-lacZ* production in the strain containing the *ompR107* mutation would be similar to that of the strain containing the *envZ473* (K^+ P^-) mutation. However, this interpretation is not consistent with our results. The level of production of the *tppB′-lacZ* fusion in the strain containing the *ompR107* mutation is significantly lower than the levels observed in either the wild type or the strain containing the *envZ473* mutation (Fig. 1A). The simplest explanation for our results is that the OmpR107 mutant protein is not just defective in the dephosphorylation of OmpR-P but is also defective in either *tppB* transcriptional activation or DNA binding at the *tppB* regulatory region. Thus, the biochemical properties of the OmpR3 mutant proteins may be more complex than originally thought.

The results presented in Fig. 1 indicate that the transcriptional regulation of *tppB* is dependent on OmpR and EnvZ. According to the current model, the EnvZ/OmpR two-component system regulates the expression of *ompF* and *ompC* in response to varying osmolarity (20, 35). Changes in osmolarity alter the cellular OmpR-P concentration, which in turn affects the transcription of these target genes. Interestingly, not all genes regulated by OmpR and EnvZ seem to be sensitive to osmotic change. One notable example is the serovar Typhimurium *tppB* gene. Gibson et al. conducted studies on the environmental regulation of this gene and showed that changes in medium osmolarity do not significantly affect *tppB* transcription (6). Our analysis of the *E. coli tppB* gene revealed a similar pattern of regulation. In this experiment, we examined the effect of osmolarity on *tppB* transcription by growing the *tppB′-lacZ* fusion strain in glycerol-MOPS (morpholinepropanesulfonic acid) medium at either low (50 mM NaCl) or high (300 mM NaCl) osmolarity as previously described (43) and then harvesting these cells for β-galactosidase analysis. As shown in Table 1, cells grown at low osmolarity and high osmolarity exhibit similar levels of *tppB′-lacZ* fusion expression. Moreover, this expression is dependent on the presence of OmpR. The fact that *tppB* is not osmoregulated suggests that *tppB* transcription is not sensitive to changes in the cellular OmpR-P concentration. Therefore, the low cellular levels of OmpR-P present at low osmolarity are sufficient to activate *tppB* transcription, and the elevated levels of OmpR-P present at high osmolarity have little effect on the *tppB* transcription level. This interpretation is supported by our β-galactosidase results with the various *envZ* mutations (Fig. 1A), which implied that once it reaches a certain threshold, further increases in the cellular OmpR-P concentration do not affect *tppB* transcription.

In addition to the observation that *tppB* is not osmoregulated, Gibson et al. (6) and Jamieson and Higgins (18) reported that *tppB* transcription is strongly stimulated under anaerobic conditions. Jamieson and Higgins also reported that the anaerobic regulation of *tppB* occurs through an OmpR-independent mechanism (19) and that the magnitude of the change in *tppB* transcription was dependent on the growth medium (6, 18). To determine if the transcription of the *E. coli tppB* gene shows a similar pattern of regulation under anaerobic conditions, the strain containing the *tppB′-lacZ* fusion was grown aerobically and anaerobically in glucose-MOPS medium as described by Stewart and Parales (47) and subjected to β-galactosidase analysis. As shown in Table 1, our results differ from the results reported for the serovar Typhimurium *tppB* gene in two ways. First, our results indicate that the regulation of *tppB* under aerobic and anaerobic conditions is dependent on OmpR (Table 1). Second, we observe only a modest two- to threefold increase in *tppB′-lacZ* fusion expression under anaerobic conditions compared to expression under aerobic conditions (Table 1). It is possible that the modest effect of anaerobiosis on *tppB′-lacZ* expression observed in our studies is due to the medium conditions that we used. However, in a recent *E. coli* DNA microarray study examining the effect of anaerobiosis on global gene expression (42), *tppB* was not identified as an anaerobically induced gene. Therefore, anaerobiosis may not play as important a role in the transcription of *E. coli tppB* as previously predicted based on the studies of the serovar Typhimurium *tppB* gene.

A major conclusion of our studies with the *tppB′-lacZ* fusion construct is that the transcriptional activation of *tppB* is dependent on OmpR. To gain further insights into the mechanism involved in the OmpR-dependent regulation of *tppB*, we examined the interaction between OmpR and the *E. coli tppB* regulatory region. To accomplish this, we carried out DNase I footprinting experiments using the plasmid pDS1. This plasmid contains the sequences between 12 and 715 bp upstream of the predicted *tppB* translational start codon. The plasmid DNA was linearized with XbaI and radiolabeled on either the template or nontemplate strand. The labeled fragment was then incubated with different concentrations of purified OmpR, and the OmpR-DNA complexes were subjected to DNase I footprinting analysis as previously described (15). The digested products were then separated and analyzed on a standard 8% DNA sequencing gel. Figure 2 shows the results of the DNase I footprinting analysis for the *tppB* template strand. As shown in Fig. 2A, OmpR protects a region within the *tppB* regulatory region that is approximately 30 bp in length. Based on the size of the protected region, it is likely that the *tppB* regulatory region contains only a single OmpR-binding site (4, 10, 15, 22). Next, to examine the effect of OmpR phosphorylation on the binding pattern, we included acetyl phosphate in the DNase I reaction mixtures. As shown in Fig. 2B, the binding of OmpR

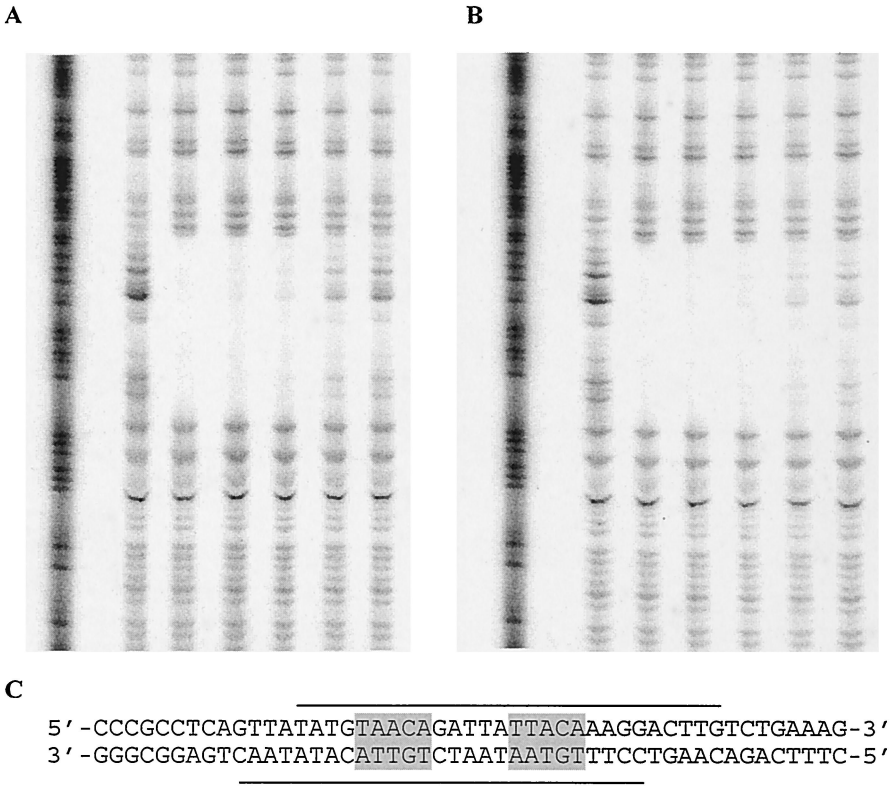

FIG. 2. Identification of the OmpR-protected region at the *tppB* regulatory region. A DNase I footprinting analysis was performed on the template strand of the *tppB* regulatory region in the absence of acetyl phosphate (A) or in the presence of 25 mM acetyl phosphate (B). The precise positions of the nucleotides protected by OmpR were determined by comparison with the results of the Maxam-Gilbert G+A cleavage reaction shown in lane 1. OmpR was purified as previously described (15) and used in the binding reactions at the following concentrations: 0 μM (lane 2), 6.5 μM (lane 3), 3.3 μM (lane 4), 1.6 μM (lane 5), 0.8 μM (lane 6), and 0.4 μM (lane 7). (C) The nucleotides protected by OmpR during DNase I footprinting analysis of the nontemplate (upper) strand and the template (lower) strand of the *tppB* regulatory region are indicated by the lines. Sequences conserved among strong OmpR-binding sites are shaded in gray.

to the DNA is stimulated approximately twofold in the presence of acetyl phosphate. This result, together with our genetic analysis of *tppB* transcription, supports the idea that the phosphorylation of OmpR plays an important role in the transcriptional activation of *tppB*. The sequences protected by OmpR on both the template and nontemplate strands are presented in Fig. 2C.

Our DNase I footprinting analysis provides strong evidence that OmpR binds to a specific site in the *tppB* regulatory region. Examination of the region protected by OmpR revealed that it contains a T-N-A-C-A sequence at the beginning of each half-site (Fig. 2C). Variations of this sequence are present in many strong OmpR-binding sites (10, 15, 20, 23, 34). Interestingly, computational approaches aimed at predicting OmpR-regulated genes failed to identify any potential OmpR-binding sites upstream of the *tppB* gene (37, 48). Such computational approaches use the known binding sites of a DNA-binding protein to establish a position-weight matrix. Since only a small number of OmpR-binding sites were available when these matrices were built, it is not surprising that the *tppB* gene was missed in these early analyses. Therefore, it is important to identify more OmpR-regulated genes and to define their OmpR-binding sites. By including these additional sites when building the OmpR consensus matrix, the reliability of these computational approaches can be improved dramatically. This would allow more accurate predictions of potential OmpR-regulated genes.

Finally, to determine the location of the OmpR-binding site relative to the *tppB* promoter, we needed to identify the transcriptional start site of *tppB*. To accomplish this, a primer extension analysis was performed as described by Kingston (21). A γ-^{32}P-labeled primer that mapped between bases 30 and 49 downstream of the predicted *tppB* start codon was hybridized to total RNA extracted from the *E. coli* strain MG1655. The resulting products of the primer extension reaction were analyzed on a standard 8% DNA sequencing gel. As shown in Fig. 3, a major band mapped to the G residue located 102 bp upstream from the *tppB* start codon. Two minor bands were also present on the gel, and the locations of these bands are indicated in the figure. Based on the results shown in Fig. 3, we predict that the G residue at nucleotide 1710695 in the *E. coli* K-12 chromosome is the major start site of *tppB* transcription.

Examination of the region upstream of the transcription start site revealed −35 and −10 elements that resemble a sigma-70 promoter (Fig. 4). This places the OmpR-binding site immediately upstream of the −35 element of the *tppB* promoter. At *ompF* and *ompC*, the OmpR-binding sites required

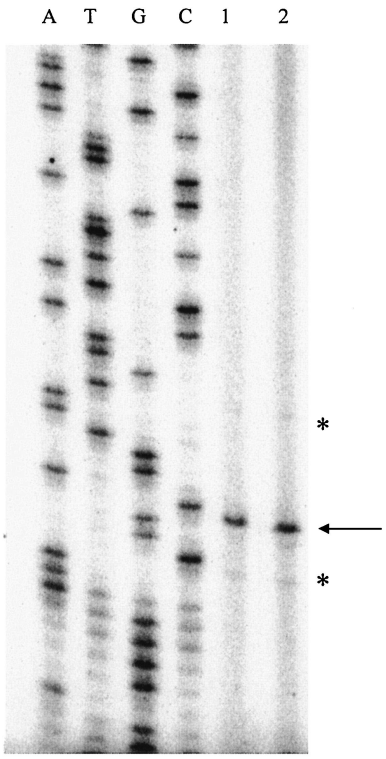

FIG. 3. Identification of the *tppB* transcriptional start site. For this analysis, total RNA was extracted and purified from the *E. coli* strain MG1655 using the protocol described at the URL http://www.microarrays.org/pdfs/Total_RNA_from_Ecoli.pdf. Primer extension analysis of *tppB* was then performed on the extracted RNA with the primer (ydgRprimer3) that mapped between bases 30 and 49 downstream of the predicted *tppB* start codon. The arrow indicates the position of the major primer extension product in the presence of 10 μg (lane 1) and 15 μg (lane 2) of total RNA. The asterisks indicate the positions of two minor products. Another experiment using a second primer that mapped between 27 and 47 bases upstream of the predicted *tppB* start codon also gave the same three products. However, the two minor products were more visible in this experiment (data not shown). Lanes A, T, G, and C show the results of the dideoxy sequencing reaction of *tppB* with the same primer (ydgRprimer3).

FIG. 4. Key regulatory elements of the *tppB* gene. The figure shows sequences of the *tppB* nontemplate strand. The arrow labeled +1 indicates the location of the major start site of transcription, and the asterisks indicate the locations of the two minor products that were present in our primer extension experiments. Based on the major start site of transcription, a putative *tppB* promoter was mapped, and the −35 and −10 elements of this promoter are highlighted with gray shading. The squiggly line indicates the OmpR-protected region, which is located approximately 30 to 60 nucleotides upstream of the transcriptional start site. The box around the three nucleotides (GTG) indicates the putative start codon of the *tppB* gene.

for transcriptional activation are also located upstream of the −35 promoter element. The binding of OmpR to these sites facilitates the interaction between OmpR and the C-terminal domain of the α-subunit of RNA polymerase and results in transcriptional activation (39). Based on these properties, OmpR has been classified as a class I transcription factor (16, 17). Given the position of the OmpR-binding site relative to the identified transcription start site of *tppB*, it is likely that OmpR activates *tppB* transcription through a similar mechanism.

The distinctive binding pattern of OmpR at the *tppB* regulatory region provides further insights into why the transcriptional regulation of *tppB* is not osmoregulated like that of *ompF* and *ompC*. Our DNase I footprinting studies revealed that the *tppB* regulatory region contains a single OmpR-binding site. In contrast, the *ompC* regulatory region contains three OmpR-binding sites and the *ompF* regulatory region contains four OmpR-binding sites (20, 35). According to the current model, the presence of multiple sites with different affinities for OmpR-P is responsible for the osmoregulation of *ompF* and *ompC*. At low osmolarity, low cellular concentrations of OmpR-P are present. These levels are sufficient for OmpR-P to bind to the strong sites of *ompF* that are responsible for activating *ompF* transcription. At high osmolarity, higher cellular levels of OmpR-P are present. These higher levels result in the occupancy of both strong and weak sites, which allows the activation of *ompC* transcription and the repression of *ompF* transcription. In the case of *tppB*, only a single OmpR-binding site is present. Our experiments suggest that this site is occupied at low cellular OmpR-P concentrations and remains occupied at high cellular OmpR-P levels. As a result, the transcription of *tppB* is not sensitive to changes in medium osmolarity.

Based on these distinctive characteristics, *tppB* may represent a new class of OmpR-regulated gene. We are currently conducting a series of DNA microarrays aimed at identifying new OmpR regulon members. We predict that some of the identified genes will be regulated through a single OmpR-binding site and that some of these genes will not respond to changes in osmolarity. The identification and characterization of new OmpR-regulated genes will be valuable for uncovering the general mechanism by which OmpR controls the transcription of its regulon members and for discovering the global role of OmpR in the *E. coli* stress response.

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REFERENCES

1. **Aiba, H., F. Nakasai, S. Mizushima, and T. Mizuno.** 1989. Evidence for the physiological importance of the phosphotransfer between the two regulatory components, EnvZ and OmpR, in osmoregulation in *Escherichia coli.* J. Biol. Chem. **264:**14090–14094.
2. **Dutta, R., L. Qin, and M. Inouye.** 1999. Histidine kinases: diversity of domain organization. Mol. Microbiol. **34:**633–640.
3. **Egger, L. A., H. Park, and M. Inouye.** 1997. Signal transduction via the histidyl-aspartyl phosphorelay. Genes Cells **2:**167–184.
4. **Forst, S., I. Kalve, and W. Durski.** 1995. Molecular analysis of OmpR

binding sequences involved in the regulation of *ompF* in *Escherichia coli.* FEMS Microbiol. Lett. **131:**147–151.
5. **Forst, S. A., and D. L. Roberts.** 1994. Signal transduction by the EnvZ-OmpR phosphotransfer system in bacteria. Res. Microbiol. **145:**363–373.
6. **Gibson, M. M., E. M. Ellis, C. K. Graeme, and C. F. Higgins.** 1987. OmpR and EnvZ are pleiotropic regulatory proteins: positive regulation of the tripeptide permease (*tppB*) of *Salmonella typhimurium.* Mol. Gen. Genet. **207:**120–129.
7. **Gibson, M. M., M. Price, and C. F. Higgins.** 1984. Genetic characterization and molecular cloning of the tripeptide permease (*tpp*) genes of *Salmonella typhimurium.* J. Bacteriol. **160:**122–130.
8. **Hall, M., and T. J. Silhavy.** 1981. Genetic analysis of the *ompB* locus in *Escherichia coli* K-12. J. Mol. Biol. **151:**1–15.
9. **Hall, M. N., and T. J. Silhavy.** 1979. Transcriptional regulation of *Escherichia coli* K-12 major outer membrane protein 1b. J. Bacteriol. **140:**342–350.
10. **Harlocker, S. L., L. Bergstrom, and M. Inouye.** 1995. Tandem binding of six OmpR proteins to the *ompF* upstream regulatory sequence of *Escherichia coli.* J. Biol. Chem. **270:**26849–26856.
11. **Higashitani, A., Y. Nishimura, H. Hara, H. Aiba, T. Mizuno, and K. Horiuchi.** 1993. Osmoregulation of the fatty acid receptor gene *fadL* in *Escherichia coli.* Mol. Gen. Genet. **240:**339–347.
12. **Higgins, C. F., and M. M. Gibson.** 1986. Peptide transport in bacteria. Methods Enzymol. **125:**365–377.
13. **Hsing, W., F. D. Russo, K. K. Bernd, and T. J. Silhavy.** 1998. Mutations that alter the kinase and phosphatase activities of the two-component sensor EnvZ. J. Bacteriol. **180:**4538–4546.
14. **Hsing, W., and T. J. Silhavy.** 1997. Function of conserved histidine-243 in phosphatase activity of EnvZ, the sensor for porin osmoregulation in *Escherichia coli.* J. Bacteriol. **179:**3729–3735.
15. **Huang, K. J., and M. M. Igo.** 1996. Identification of the bases in the *ompF* regulatory region, which interact with the transcription factor OmpR. J. Mol. Biol. **262:**615–628.
16. **Igarashi, K., A. Hanamura, D. Makino, H. Aiba, H. Aiba, T. Mizuno, A. Nakata, and A. Ishihama.** 1991. Functional map of the α subunit of *Escherichia coli* RNA polymerase: two modes of transcription activation by positive factors. Proc. Natl. Acad. Sci. USA **88:**8958–8962.
17. **Ishihama, A.** 1993. Protein-protein communication within the transcription apparatus. J. Bacteriol. **175:**2483–2489.
18. **Jamieson, D. J., and C. F. Higgins.** 1984. Anaerobic and leucine-dependent expression of a peptide transport gene in *Salmonella typhimurium.* J. Bacteriol. **160:**131–136.
19. **Jamieson, D. J., and C. F. Higgins.** 1986. Two genetically distinct pathways for transcriptional regulation of anaerobic gene expression in *Salmonella typhimurium.* J. Bacteriol. **168:**389–397.
20. **Kenney, L. J.** 2002. Structure/function relationships in OmpR and other winged-helix transcription factors. Curr. Opin. Microbiol. **5:**135–141.
21. **Kingston, R. E.** 1987. Primer extension, p. 4.8.1–4.8.3. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology, vol. 1. Wiley Interscience, New York, N.Y.
22. **Maeda, S., and T. Mizuno.** 1990. Evidence for multiple OmpR-binding sites in the upstream activation sequence of the *ompC* promoter in *Escherichia coli*: a single OmpR-binding site is capable of activating the promoter. J. Bacteriol. **172:**501–503.
23. **Martinez-Hackert, E., and A. M. Stock.** 1997. The DNA-binding domain of OmpR: crystal structure of a winged helix transcription factor. Structure **5:**109–124.
24. **McCleary, W. R., and J. B. Stock.** 1994. Acetyl phosphate and the activation of two-component response regulators. J. Biol. Chem. **269:**31567–31572.
25. **Miller, J. H.** 1992. A short course in bacterial genetics: a laboratory manual and handbook for *Escherichia coli* and related bacteria. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
26. **Mizuno, T., M. Kato, Y. L. Jo, and S. Mizushima.** 1988. Interaction of OmpR, a positive regulator, with the osmoregulated *ompC* and *ompF* genes of *Escherichia coli.* Studies with wild-type and mutant OmpR proteins. J. Biol. Chem. **263:**1008–1012.
27. **Nichols, B. P., O. Shafiq, and V. Meiners.** 1998. Sequence analysis of Tn*10* insertion sites in a collection of *Escherichia coli* strains used for genetic mapping and strain construction. J. Bacteriol. **180:**6408–6411.
28. **Ochman, H., M. M. Meetha, D. Garza, and D. L. Hartl.** 1990. Amplification of flanking sequences by inverse PCR, p. 219–227. *In* M. A. Innis (ed.), PCR protocols: a guide to methods and application. Academic Press, San Diego, Calif.
29. **Oshima, T., H. Aiba, Y. Masuda, S. Kanaya, M. Sugiura, B. L. Wanner, H. Mori, and T. Mizuno.** 2002. Transcriptome analysis of all two-component regulatory system mutants of *Escherichia coli* K-12. Mol. Microbiol. **46:**281–291.
30. **Paulsen, I. T., L. Nguyen, M. K. Sliwinski, R. Rabus, and M. H. Saier, Jr.** 2000. Microbial genome analyses: comparative transport capabilities in eighteen prokaryotes. J. Mol. Biol. **301:**75–100.
31. **Paulsen, I. T., M. K. Sliwinski, and M. H. Saier, Jr.** 1998. Microbial genome analyses: global comparisons of transport capabilities based on phylogenies, bioenergetics and substrate specificities. J. Mol. Biol. **277:**573–592.
32. **Platt, R., C. Drescher, S. K. Park, and G. J. Phillips.** 2000. Genetic system for reversible integration of DNA constructs and *lacZ* gene fusions into the *Escherichia coli* chromosome. Plasmid **43:**12–23.
33. **Pratt, L. A., W. Hsing, K. E. Gibson, and T. J. Silhavy.** 1996. From acids to *osmZ*: multiple factors influence synthesis of the OmpF and OmpC porins in *Escherichia coli.* Mol. Microbiol. **20:**911–917.
34. **Pratt, L. A., and T. J. Silhavy.** 1995. Identification of base pairs important for OmpR-DNA interaction. Mol. Microbiol. **17:**565–573.
35. **Pratt, L. A., and T. J. Silhavy.** 1995. Porin regulon of *Escherichia coli*, p. 105–127. *In* J. A. Hoch and T. J. Silhavy (ed.), Two-component signal transduction. ASM Press, Washington, D.C.
36. **Rasmussen, L. J., P. L. Møller, and T. Atlung.** 1991. Carbon metabolism regulates expression of the *pfl* (pyruvate formate-lyase) gene in *Escherichia coli.* J. Bacteriol. **173:**6390–6397.
37. **Robison, K., A. M. McGuire, and G. M. Church.** 1998. A comprehensive library of DNA-binding site matrices for 55 proteins applied to the complete *Escherichia coli* K-12 genome. J. Mol. Biol. **284:**241–254.
38. **Römling, U., Z. Bian, M. Hammar, W. D. Sierralta, and S. Normark.** 1998. Curli fibers are highly conserved between *Salmonella typhimurium* and *Escherichia coli* with respect to operon structure and regulation. J. Bacteriol. **180:**722–731.
39. **Russo, F. D., and T. J. Silhavy.** 1992. Alpha: the Cinderella subunit of RNA polymerase. J. Biol. Chem. **267:**14515–14518.
40. **Russo, F. D., and T. J. Silhavy.** 1991. EnvZ controls the concentration of phosphorylated OmpR to mediate osmoregulation of the porin genes. J. Mol. Biol. **222:**567–580.
41. **Russo, F. R.** 1992. Ph.D. thesis. Princeton University, Princeton, N.J.
42. **Salmon, K., S. P. Hung, K. Mekjian, P. Baldi, G. W. Hatfield, and R. P. Gunsalus.** 2003. Global gene expression profiling in *Escherichia coli* K12. The effects of oxygen availability and FNR. J. Biol. Chem. **278:**29837–29855.
43. **Sharif, T. R., and M. M. Igo.** 1993. Mutations in the alpha subunit of RNA polymerase that affect the regulation of porin gene transcription in *Escherichia coli* K-12. J. Bacteriol. **175:**5460–5468.
44. **Shin, S., and C. Park.** 1995. Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. J. Bacteriol. **177:**4696–4702.
45. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
46. **Slauch, J. M., and T. J. Silhavy.** 1989. Genetic analysis of the switch that controls porin gene expression in *Escherichia coli* K-12. J. Mol. Biol. **210:**281–292. (Erratum, **212:**429, 1990.)
47. **Stewart, V., and J. Parales, Jr.** 1988. Identification and expression of genes *narL* and *narX* of the *nar* (nitrate reductase) locus in *Escherichia coli* K-12. J. Bacteriol. **170:**1589–1597.
48. **Thieffry, D., H. Salgado, A. M. Huerta, and J. Collado-Vides.** 1998. Prediction of transcriptional regulatory sites in the complete genome sequence of *Escherichia coli* K-12. Bioinformatics **14:**391–400.
49. **Tran, V. K., R. Oropeza, and L. J. Kenney.** 2000. A single amino acid substitution in the C terminus of OmpR alters DNA recognition and phosphorylation. J. Mol. Biol. **299:**1257–1270.