Signal-dependent Requirement for the Co-activator Protein RcsA in Transcription of the RcsB-regulated ugd Gene*

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The RcsC/YojN/RcsB phosphorelay system controls gene expression in response to a variety of signals, including changes in temperature, osmolarity, and overproduction of membrane proteins. Transcription of certain RcsB-activated genes, such as the capsule synthesis cps operon, requires the co-activator protein RcsA, whereas expression of other RcsB-activated genes is RcsA-independent. We have established previously that a tolB mutation induces transcription of the Salmonella UDP-glucose dehydrogenase ugd gene in an RcsA- and RcsB-dependent manner. This induction is independent of the two-component systems PhoP/PhoQ and PmrA/PmrB, which are required for ugd expression in response to low Mg2++. We now report that the RcsC/YojN/RcsB system is activated in a pmrA mutant experiencing Fe3+ and low Mg2+, resulting in expression of both cps and ugd genes. However, whereas cps transcription remained RcsA-dependent, ugd transcription became RcsA-independent but dependent on the PhoP protein. S1 mapping experiments demonstrated that RcsA-dependent and -independent transcription of the ugd gene use the same promoter. DNase footprinting analysis identified a PhoP-binding site in the ugd promoter. Yet, PhoP-mediated ugd transcription required either the RcsC/YojN/RcsB or the PmrA/PmrB systems.

The RcsC/YojN/RcsB phosphorelay system controls a variety of cellular functions including the synthesis of colanic acid capsule (1–4), the decoration of the lipopolysaccharide with 4-aminoarabinose (5) as well as motility and chemotaxis (6, 7) in several bacterial species. RcsC is a sensor protein, RcsB is its cognate response regulator, and YojN is a histidine-containing phosphotransfer protein that is apparently used as an intermediary in the phosphoryl transfer from RcsC to RcsB (7). Genes regulated by RcsB can be divided into two classes based on their requirement for the co-activator protein RcsA: RcsA-dependent (e.g. cps and rcsA; Refs. 2, 8–10) and RcsA-independent (e.g. ftsA, osmC, rprA, and trvA; Refs. 11–14). The RcsB and RcsA proteins bind to a sequence motif, termed RcsAB box, present in the cps genes of several species (15, 16), whereas the RcsB protein binds to a different sequence motif, termed RcsB box, found in the promoter of the RcsA-independent osmC gene (17). The RcsB protein fails to bind stably to DNA by itself (18), suggesting that RcsB may function with other co-factors at RcsA-independent promoters. For example, RcsB activation of the tvIA gene requires the TviA protein (11), and full activation of the osmC and ftsA genes needs an unidentified factor(s) (12, 14).

The Salmonella ugd gene encodes UDP-glucose dehydrogenase, an enzyme required for the production of both colanic acid (19) and 4-aminoarabinose (5). Transcription of the ugd gene is induced by three different signals by means of three different two-component regulatory systems (Fig. 1). Low Mg2+ promotes ugd transcription in a process that requires the Mg2+-responsive PhoP/PhoQ system, the PhoP-activated protein PmrD, and the PmrA/PmrB system (21). Fe3+ promotes ugd transcription in a process that is dependent on the Fe3+-responsive PmrA/PmrB system but independent of the PhoP/PhoQ system and the PmrD protein (22). A tolB mutation induces ugd transcription via the RcsC/YojN/RcsB system and its co-activator RcsA protein but independently of the PhoP/PhoQ and PmrA/PmrB systems (20).

The ugd promoter has putative binding sites for the PmrA, RcsB, and PhoP proteins (20). The PmrA protein binds to a region of the ugd promoter (23) that harbors a motif found in the promoter of other PmrA-activated genes, such as pbgP and pmrC (24). Although binding of the RcsB protein to the ugd promoter has not been reported, mutation of the putative RcsB-binding site abolished ugd transcription promoted by the tolB mutation (20). On the other hand, the presence of a putative PhoP-binding site in the ugd promoter is intriguing because (i) the low Mg2+ induction of the ugd gene, like that of other PmrA-activated genes, is dependent on the PmrB protein and the PmrA/PmrB system (21); and (ii) there is no clear 10 region near the putative PhoP-binding site, which is in contrast to genes that are directly regulated by PhoP, such as phoP, mgtA, and mgrB (25).

In this paper, we report a new condition that activates the RcsC/YojN/RcsB system and establish that the requirement for the RcsA protein in RcsB-mediated transcription of the ugd gene is dependent on the inducing condition, but that cps transcription is RcsA-dependent, regardless of the inducing condition. Our results uncover a potential new function for the PhoP protein in activating gene expression. The participation of several combinations of regulatory systems in the control of ugd expression may be due to the participation of UDP-glucuronic acid (i.e. the product of the reaction catalyzed by UDP-glucose dehydrogenase) in the biosynthesis of different cellular structures produced under different conditions.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, Recombinant Molecular Techniques, and Growth Conditions—Bacterial strains and plasmids used in this study are listed in Table I. Mutants were constructed by phage P22-mediated transductions as described (26). Recombinant DNA tech-
niques were performed according to standard protocols (27). The allele flbD5213::MudJ was a gift from Kelly T. Hughes (University of Washington). Bacteria were grown at 37 °C in LB medium or in modified N minimal medium (50 mM Tris base, 50 mM KCl, 75 mM (NH4)2 SO4, 8 mM K2SO4, and 10 mM KH2PO4) containing 0.1% casamino acids and 38 mM glycerol in which 100 mM Tris-HCl was replaced by a mixture of 50 mM bis-tris and 50 mM Tris adjusted to pH 7.7 or 5.8 with HCl. MgCl2 was added to a concentration of 10 μM FeSO4, and was used at a final concentration of 0.1 mM from a freshly prepared 0.1 M stock solution. Kanamycin was used at a final concentration of 50 μg/ml, chloramphenicol at 25 μg/ml, and tetracycline at 10 μg/ml.

Construction of Chromosomal Deletions and Chromosomal Mutants—A chromosomal deletion mutant of the PhoP-binding site was constructed as described (28), using primers 1744 (5'-GAAAGCCATTTCGATTTAAGGCTGCGTTTGAGTCTCGGCCG-3') and 2002 (5'-GGAGATCCAACTTAATTAGATTTGCGCCCGTAC-3'). The construction of base substitutions at the RcsB site was accomplished through a combination of multiple PCRs based on a described method (28). To retain an intact PhoP-binding site and to conserve the distance between the transcription factor-binding sites described in this study, (28). To retain an intact PhoP-binding site and to conserve the distance between the transcription factor-binding sites described in this study, (28). To retain an intact PhoP-binding site and to conserve the distance between the transcription factor-binding sites described in this study, (28).

mote chromosomal DNA as a template. Prior to the PCR, primers 2001 (5’-GACTAATGTTCGAGTACCCAG-3') and 1827 (5’-CGGATCCCTCAATGACAGAAGAG-3') were used to amplify the DNA sequence ladder generated with the appropriate primer. DNA fragments were used for DNase I footprinting were amplified by the PCR using Salmonella enterica serovar Typhimurium chromosomal DNA as a template. Prior to the PCR, primers 2001 (5’-GACTAATGTTCGAGTACCCAG-3') and 1827 (5’-CGGATCCCTCAATGACAGAAGAG-3'), which anneal to the coding and non-coding strand of ugd respectively, were labeled with T4 polynucleotide kinase (Invitrogen) as described (20).

RESULTS

**Fe**3+ Promotes ugd Transcription Independently of the PmrA/PmrB System—Transcription of PmrA-activated genes is coordinately induced by Fe**3+** in a process that requires the Fe**3+** sensor PmrB and the DNA-binding transcriptional regulator PmrA (22). Although this is true for the PmrA-activated phgP and pmrC genes, we determined that Fe**3+** could still promote ugd transcription in pmrA (Fig. 2A) and pmrB mutants (data not shown). This is in contrast to the induction of PmrA-activated genes that takes place in low Mg**2+**, where inactivation of the pmrA gene abolished transcription of both the ugd (Fig. 2B) and phgP (Fig. 2D) genes. As expected, expression of the PhoP-activated PmrA-independent mgtA gene was not affected by Fe**0** or by inactivation of the pmrA gene (Fig. 2, E and F). These data demonstrate that Fe**3+** can specifically activate ugd transcription independently of the PmrA/PmrB system.

**Fe**3+ Activates the RcsC(YojN)/RcsB System, which Mediates the PmrA-independent Transcription of the ugd Gene—We hypothesized that the RcsC(YojN)/RcsB system might be responsi-
RcsA-independent Transcription of RcsB-regulated ugd Gene

### Table 1: Bacterial strains and plasmids

| Strain or plasmid | Description | Reference or source |
|-------------------|-------------|---------------------|
| S. enterica serovar Typhimurium | 14028s wild-type | (41) |
| MS7593s | phoP7953::Tn10 | (41) |
| EG7139 | pmrA1::cat | (42) |
| EG9524 | ugd::Mu1-8 | This work |
| EG12882 | ugd::Mu1-8 pmrA1::cat | This work |
| EG12886 | ugd::Mu1-8 phoP7953::Tn10 | (20) |
| EG13290 | ugd::Mu1-8 rcsC::Tn10d-Tet | (20) |
| EG13291 | ugd::Mu1-8 yojN::Tn10d-Tet | (20) |
| EG13292 | ugd::Mu1-8 rcsA::Tn10d-Tet | (20) |
| EG12885 | ugd::Mu1-8 rcsB::Km | (20) |
| EG13294 | ugd::Mu1-8 pmrA | (20) |
| EG12883 | ugd::Mu1-8 pmrA1::cat phoP7953::Tn10 | This work |
| EG12906 | ugd::Mu1-8 pmrA1::cat rcsB::Km | This work |
| EG12905 | ugd::Mu1-8 pmrA1::cat phoP7953::Tn10 | (20) |
| EG13295 | ugd::Mu1-8 pmrA1::cat rcsC::Tn10d-Tet | This work |
| EG13297 | ugd::Mu1-8 pmrA1::cat rcsA::Tn10d-Tet | This work |
| EG12884 | ugd::Mu1-8 pmrA1::cat phoP7953::Tn10 rcsB::Km | (20) |
| EG13307 | ΔpmrA | (20) |
| EG13308 | rcsB::Km | (20) |
| EG12925 | ΔrcsB | (20) |
| EG13309 | ΔpmrA phoP7953::Tn10 | This work |
| EG13310 | ΔpmrA rcsB::Km | This work |
| EG13311 | ΔpmrA phoP7953::Tn10 rcsB::Km | This work |
| EG99241 | phgPl::MudI | (42) |
| EG13352 | phgPl::MudJ ΔpmrA | This work |
| EG95521 | mgtA9226::MudJ | (42) |
| EG13353 | mgtA9226::MudJ ΔpmrA | This work |
| EG13354 | tofB::Tn10d-Cm | (20) |
| EG13384 | cps::MudJ | (20) |
| EG13385 | cps::MudJ ΔpmrA | This work |
| EG13386 | cps::MudJ ΔpmrA rcsB::Km | This work |
| EG13390 | cps::MudJ ΔpmrA rcsA::Tn10 | This work |
| EG13391 | ugd::Mu1-8 ΔpmrA rcsC::Tn10 | This work |
| EG13392 | ugd::Mu1-8 ΔpmrA yojN::Tn10 | This work |
| EG13772 | flhDC5213::MudJ | This work |
| EG13773 | flhDC5213::MudJ ΔpmrA | This work |
| EG13774 | flhDC5213::MudJ ΔpmrA rcsB::Tn10 | This work |
| EG13775 | flhDC5213::MudJ ΔpmrA rcsA::Tn10 | This work |
| EG13863 | O1 ugd::Mu1-8 | This work |
| EG13864 | O1 ugd::Mu1-8 ΔpmrA | This work |
| EG13865 | O2 ugd::Mu1-8 | This work |
| EG13866 | O2 ugd::Mu1-8 ΔpmrA | This work |
| EG13867 | O3 ugd::Mu1-8 | This work |
| EG13868 | O3 ugd::Mu1-8 ΔpmrA | This work |
| Escherichia coli DH5α | supE44 ΔlacU169 (φ80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1 | (43) |

### Plasmids

- pΔC52: repA18C, Ap<sup>+</sup> promoter-probe vector
- pEG13393: Transcriptional fusion in pΔC52 of 283-bp upstream of start codon of the ugd
- pEG13394: Derivative of the pEG13393 without the PhoP-binding site
- pEG13396: Derivative of pEG13393 containing a GAA to ACC substitution at the RcsB-binding site
- pEG13398: Derivative of pEG13395 containing a CTG to GGC substitution at the RcsB-binding site
- pΔC52: repA18C, Ap<sup>+</sup> promoter-probe vector

Note: Gene designations are summarized by Ref 45. O1 indicates deletion of the PhoP-binding site in the ugd promoter, whereas O2 and O3 indicate GAA to ACC and GGC substitutions, respectively, at the putative RbsB-binding site in the ugd promoter.

**Possible** for the Fe<sup>3+</sup>-promoted PmrA-independent activation of the ugd gene because, like a TolB mutation (30, 31), Fe<sup>3+</sup> treatment of a pmrA mutant perturbs the outer membrane (32) and because a TolB mutation promotes ugd transcription in an RcsC/YojN/RcsB-dependent manner (20). Consistent with this notion, Fe<sup>3+</sup> could not promote ugd transcription in rcsB pmrA (Fig. 3A), rcsC pmrA, and yojN pmrA double mutants (data not shown). On the other hand, the rcsB mutant displayed wild-type levels of ugd expression in response to the Fe<sup>3+</sup> and low Mg<sup>2+</sup> signals (Fig. 3A), which were mediated by the PmrA/PmrB system and the PhoP/PhoQ-PmrD-PmrA/PmrB pathway, respectively (Fig. 1). Fe<sup>3+</sup> treatment of the pmrA mutant appears to activate the RcsC/YojN/RcsB system (as opposed to acting solely on the ugd promoter) because it also promoted transcription of the cps operon (Fig. 3B) and repressed expression of the flhDC genes (Fig. 3C). These results establish that Fe<sup>3+</sup> treatment of a pmrA mutant activates the RcsC/YojN/RcsB system, which is necessary for the pmrA-independent transcription of the ugd gene.

**The Fe<sup>3+</sup>-promoted PmrA-independent Activation of the ugd Gene Does Not Require the RcsA Protein**—Genes under transcriptional control of the RcsC/YojN/RcsB system can be divided into two classes based on their requirement for the RcsA protein: RcsA-dependent (e.g. cps and rcsA) and RcsA-independent (e.g. osmC, flsZ, and twiA). Although the TolB-promoted transcription of the ugd gene requires RcsA (20), the Fe<sup>3+</sup>-promoted PmrA-independent transcription of the ugd gene seems to be RcsA independent because ugd expression was similar in pmrA and pmrA rcsA strains (i.e. only 35% lower in the latter; Fig. 3A). This is in contrast to cps transcription, which was reduced nearly 8-fold by the rcsA mutation. These results indicate that the requirement for the RcsA protein in the RcsB-mediated transcription of the ugd gene is signal-dependent.
The Regulatory Protein PhoP Is Necessary for the Fe$^{3+}$-promoted PmrA-independent Transcription of the ugd Gene—We investigated the possibility that the PhoP protein might be involved in the Fe$^{3+}$-promoted PmrA-independent transcription of the ugd gene because (i) these experiments were carried out in low Mg$^{2+}$, which is the signal that activates the PhoP protein (29), and (ii) the ugd promoter harbors a putative PhoP-binding site (20). We established that Fe$^{3+}$-promoted ugd transcription in phoP, pmrA, and rcsB single mutants and in a phoP rcsB double mutant but not in phoP pmrA or rcsB pmrA double mutants (Fig. 4). This finding indicates that bacteria experiencing Fe$^{3+}$ and low Mg$^{2+}$ promote ugd transcription via two pathways: the PmrA/PmrB pathway (operating when the RcsB protein is not active) and a pathway requiring both the PhoP/PhoQ and RcsC/Toj/N/RcsB systems (operating when the PmrA protein is not active; Fig. 1).

**RcsA-independent and -dependent ugd Transcription Is Mediated by the Same Promoter**—To define the ugd promoters used by the various regulatory proteins, we performed S1 mapping experiments using RNA harvested from bacteria grown under different conditions. When grown in low Mg$^{2+}$, the wild-type strain produced an S1 product that originated from the PmrA-dependent promoter because it was absent from RNA prepared from the pmrA mutant (Fig. 5A). This S1 product was more intense in wild-type cells induced by low Mg$^{2+}$ and Fe$^{3+}$ (Fig. 5A) and of the same size as that detected in organisms grown in LB broth (Ref. 20; Fig. 5B), which is an environment of relatively low Mg$^{2+}$. On the other hand, a pmrA mutant exposed to Fe$^{3+}$ produced a smaller S1 product (Fig. 5A), which is of the same size as that induced by the tolB mutation in an RcsB-dependent fashion (Ref. 20; Fig. 5B). These experiments indicate that two promoters mediate ugd transcription: a distal promoter that is PmrA-dependent but RcsB-independent, and a proximal promoter that is RcsB-dependent but PmrA-independent (Fig. 5C). Moreover, they indicate that both RcsA-dependent and -independent transcription of the ugd gene use the same promoter.

Defining the cis-Acting Sequences Required for the Fe$^{3+}$-promoted PmrA-independent Transcription of the ugd Gene—Using plasmid-borne lac fusions to the ugd promoter, we have recently established that the 283-bp region upstream of the ugd start codon (Fig. 5C) has all the necessary cis-acting sequences necessary for ugd transcription promoted by the tolB mutation (20). This region includes a PmrA-binding site (21), the putative RcsB-binding site, as well as a bona fide PhoP-binding site, because the PhoP protein footprinted the region −223 to −198 from the ugd start codon (Fig. 5D). We have now determined that this region harbors the sequence information required for the low Mg$^{2+}$, PmrA-dependent, as well as for the Fe$^{3+}$-promoted PmrA-dependent and -independent transcription of the ugd gene (Fig. 6). Indeed deletion of the 79-bp sequence including the PhoP-binding site abolished the Fe$^{3+}$-promoted PmrA-independent transcription of the ugd gene (Fig. 6).

To rule out the possibility of potential phenotypes resulting from the lac fusion being in a multicopy number plasmid, we constructed strains harboring a lac fusion to the chromosomal copy of the ugd gene and mutations in the RcsB- and PhoP-binding sites. Deletion of the PhoP-binding site abolished ugd expression promoted by low Mg$^{2+}$ and Fe$^{3+}$ in a pmrA mutant (Fig. 7), but had no effect on ugd transcription promoted by Fe$^{3+}$ or by the tolB mutation in a pmrA$^{-}$ strain (Fig. 7). Mutation of the conserved GAA and CTG sequences in the RcsB-binding site abolished the Fe$^{3+}$-promoted activation that takes place in the pmrA mutant (Fig. 6B) but had no effect on the low Mg$^{2+}$ and Fe$^{3+}$ activation that is mediated by the PmrA protein (Fig. 7). Cumulatively, these results are in agreement with the data resulting from inactivation of the regulatory genes: (i) mutation of the phoP gene or the PhoP-binding site in the ugd promoter abolished the Fe$^{3+}$-promoted PmrA-independent activation of ugd but had no effect on the Fe$^{3+}$-promoted activation mediated by the PmrA protein or the RcsB-mediated activation promoted by a tolB mutation; and (ii) mutation of the rcsB gene or the RcsB site in the ugd promoter abolished both the tolB- and Fe$^{3+}$-promoted PmrA-independent activation of ugd transcription.
We have identified a new condition that activates the RcsC/YojN/RcsB system, established that the requirement for the RcsA protein in RcsB-promoted transcription depends on the particular promoter and inducing condition, and uncovered a novel interaction between two-component system proteins.

We have demonstrated that the RcsC/YojN/RcsB system is activated in a pmrA mutant experiencing Fe^{3+}/H_{11545} and low Mg^{2+}/H_{11545}, resulting in transcriptional activation of the ugd gene (Fig. 3A) and cps operon (Fig. 3B) and transcriptional repression of the flhDC operon (Fig. 3C). What this condition has in common with previously reported activating conditions of the Rcs system, such as those resulting from inactivation of the tolB gene (30, 31) and the overproduction of the DnaJ-like protein DjlA (33–37), is the perturbation of the outer membrane. Indeed, Fe^{3+}/H_{11545} disrupts the outer membrane of pmrA Salmonella, rendering it susceptible to killing by vancomycin and lysis by deoxycholate (32).

We have established that the two conditions that induce RcsB-dependent transcription of the ugd gene (i.e. a tolB mutation or Fe^{3+} and low Mg^{2+} in a pmrA mutant) use the same transcription start site (Fig. 5, A and B; Ref. 20) and require the same putative RcsB-binding site at the ugd promoter (Fig. 7; Ref. 20). This is despite the fact that these activation conditions differ in their requirement for the regulator protein PhoP and the co-activator protein RcsA (Fig. 4; Ref. 20). The PhoP protein had been implicated previously in the transcriptional induction of the ugd gene in response to low Mg^{2+} because of its role as transcriptional activator of the PmrD protein, which is a posttranscriptional activator of the PmrA/PmrB system (Ref. 21; Fig. 1). However, the PhoP protein plays a different role in ugd transcription in a pmrA mutant experiencing Fe^{3+} and low Mg^{2+} because (i) this activation requires the PhoP-binding site at the ugd promoter (Figs. 6 and 7), (ii) it is dependent upon the RcsB protein (Fig. 3A), (iii) it is independent of the PmrA protein (Fig. 3A), and (iv) different tran-

FIG. 3. Fe^{3+} and low Mg^{2+} activate the RcsC/YojN/RcsB system in a pmrA mutant, promoting ugd transcription in an RcsA-independent manner. β-galactosidase activity (Miller units) expressed by strains grown in N minimal medium, pH 7.7, 10 μM MgCl_{2}, and 100 μM FeSO_{4} (A, B, C) were determined for strains harboring a lac-transcriptional fusion to the ugd gene (A), cps operon (B), and flhDC operon (C). The transcriptional activity was investigated in six genetic backgrounds: wild type, pmrA, rcsB, pmrA rcsB, pmrA rcsA, and rcsA. Data correspond to mean values of four independent experiments performed in duplicate. Error bars correspond to the standard deviation (shown only if greater than the resolution of the figure).

FIG. 4. The PhoP/PhoQ and RcsC/YojN/RcsB systems are required for the Fe^{3+}-promoted PmrA-independent transcription of ugd gene. β-galactosidase activity (Miller units) expressed by strains grown in N minimal medium, pH 7.7, 10 μM MgCl_{2}, and 100 μM FeSO_{4} were determined in mutants harboring a lac-transcriptional fusion to the ugd gene. The transcriptional activity was investigated in seven genetic backgrounds: wild type, pmrA, phoP, rcsB, pmrA phoP, phoP rcsB, and pmrA rcsB. Data correspond to mean values of four independent experiments performed in duplicate. Error bars correspond to the standard deviation (shown only if greater than the resolution of the figure).
**FIG. 6.** The cis-acting sequences required for the Fe$^{3+}$-promoted PmrA-independent transcription of the *ugd* gene. β-galactosidase activity (Miller units) expressed by strains grown in N minimal medium, pH 7.7, 10 μM MgCl$_2$, and with or without 100 μM FeSO$_4$ were determined for mutants harboring a lac-transcriptional fusion to the wild-type *ugd* promoter and to the deleted PhoP binding site *ugd* promoter present in the multicopy number plasmid pIC552 in two genetic backgrounds, wild-type and pmrA. For comparison purposes, we reproduced the transcriptional activity of the *ugd* gene in a tolB mutant grown in LB broth (20). Representative results of three independent experiments are shown. Note that deletion of the PhoP-binding site eliminates the Fe$^{3+}$ and low Mg$^{2+}$ activation that takes place in a pmrA mutant but had no effect on that promoted by the tolB mutation.

**FIG. 5.** Molecular analysis of the *ugd* promoter. (A) S1 mapping of *ugd* transcripts produced by wild type, pmrA, and pmrA rcsB bacteria grown in minimal medium, pH 7.7, containing 10 μM MgCl$_2$ or 10 μM MgCl$_2$ and 100 μM FeSO$_4$ and harvested during the logarithmic phase. The S1 protection assay was performed as described under “Experimental Procedures.” Lanes G, A, T, and C correspond to dideoxy chain-termination reactions for this region. The transcription start sites are marked with arrows. The PmrA-dependent and RcsB-dependent transcriptional start sites are indicated by +1A and +1B, respectively. (B) S1 mapping of *ugd* transcripts produced by wild type, tolB, and tolB rcsB bacteria grown in LB broth and harvested during the logarithmic phase (reproduced from Ref. 20). Lanes G, A, T, and C correspond to dideoxy chain-termination reactions for this region. The transcription start sites are marked with arrows. The PmrA-dependent and RcsB-dependent transcriptional start sites are indicated by +1A and +1B, respectively. Note that the RcsB-dependent transcription start site is the same in a tolB mutant grown in LB broth and in a pmrA mutant exposed to Fe$^{3+}$ and low Mg$^{2+}$. (C) DNA sequence corresponding to the 283-bp region upstream of the start codon of *ugd* containing the cis-acting elements required for transcription promoted by the PmrA/PmrB, PhoP/PhoQ, and RcsC/YojN/RcsB systems. The PhoP-binding site is indicated by a red box, the direct repeat in the PmrA-binding site by a blue box, and the putative RcsB-binding site by a green box. Underlined sequence represents DNA region footprinted by the PhoP protein. The transcription start sites are indicated by arrows. (D) Footprinting analysis of the *ugd* promoter region was performed with the end-labeled coding, non-coding strand, and the PhoP-H6 protein added at 0, 10, 12.5, and 50 pmol. Solid bars represent the PhoP-binding regions. The position of the binding was determined by comparison with sequence ladder, obtained by using the same labeled primers as those utilized for the probe.
A pattern that seems to be emerging about RcsB-regulated genes is that all RcsA-dependent genes are involved in the production of capsule, whereas those that are RcsA-independent participate in a variety of different cellular functions. Thus, the dual status of the ugd gene is likely caused by its participation in both capsule and lipopolysaccharide biosynthesis.

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