The *Bacillus subtilis* Competence Transcription Factor, ComK, Overrides LexA-imposed Transcriptional Inhibition without Physically Displacing LexA*

Received for publication, May 15, 2001, and in revised form, September 5, 2001
Published, JBC Papers in Press, September 12, 2001, DOI 10.1074/jbc.M104407200

Leendert W. Hamoen‡‡, Bertjan Haitjema‡, Jetta J. Bijlsma, Gerard Venema‡, and Charles M. Lovett*‡‡‡

*From the ‡Department of Genetics, University of Groningen, NL-9751 NN Haren, The Netherlands, the †Institute of Virology, University of Utrecht, NL-3584 CL Utrecht, The Netherlands, the ‡Department of Medical Microbiology, Vrije Universiteit, Amsterdam, The Netherlands, and the *‡Department of Chemistry, Williams College, Williamstown, Massachusetts 01267

During the development of competence in *Bacillus subtilis* the *recA* gene is activated by the competence transcription factor, ComK, which is presumably required to alleviate the transcriptional repression of *recA* by LexA. To investigate the mechanism by which ComK activates *recA* transcription we examined the binding of ComK and LexA to the *recA* promoter in *vitro*. Using hydroxyl radical protection analyses to establish the location of ComK dimer-binding sites within the *recA* promoter, we identified four AT-boxes in a configuration unique for ComK-regulated promoters. Gel mobility shift experiments showed that all four ComK dimer-binding sites were occupied at ComK concentrations in the physiological range. In addition, occupation of all ComK-binding sites did not prevent LexA from binding to the *recA* promoter, despite the fact that the ComK and LexA recognition motifs partially overlap. Although ComK did not replace LexA from the *recA* promoter, *in vitro* transcription analyses indicated that the presence of ComK is sufficient to alleviate LexA repression of *recA*.

*Bacillus subtilis* differentiates into cells competent for genetic transformation by synthesizing a complex DNA binding and uptake system and by activating recombination genes. Paramount among the recombination gene products that enable cells to incorporate newly acquired genetic material is the RecA protein, which plays a crucial role in recombination by promoting homologous pairing and DNA strand exchange (1, 2). RecA is also essential for the regulation of the SOS DNA repair system, which is activated when DNA is damaged or when *B. subtilis* cells differentiate to a competent state (3).

The SOS system operates in many bacteria and has been the subject of extensive studies in *Escherichia coli* (for review, see Ref. 4). Following exposure to DNA damaging treatments, a set of damage inducible (*din*) genes becomes transcriptionally activated. Expression of *B. subtilis* *din* genes is regulated by the products of the *lexA* (formerly called *dinR*) and *recA* genes (5, 6). LexA acts as the repressor of all *B. subtilis* din genes, including *recA* and *lexA*, by binding specifically to DNA sequences located within the putative promoter regions (5, 7–10). Comparison of the DNA sequences of more than 20 din promoter regions that bind LexA revealed a consensus sequence for binding of a LexA dimer, CGAAGCATGTGTC. Analogous to the situation in *E. coli*, RecA is required for SOS induction in *B. subtilis* (6, 11, 12). *B. subtilis* RecA is activated to promote the auto cleavage of LexA repressor in *vitro* when it binds single-stranded DNA and nucleoside triphosphate (10). Correspondingly, *B. subtilis* RecA is activated in *vivo* by binding single-stranded DNA exposed by discontinuous replication past UV-induced lesions and to a lesser extent by the processing of gaps formed during excision repair (13).

Although the SOS system is induced during competence development by a similar RecA/LexA-dependent mechanism, *B. subtilis* recA expression is additionally stimulated by a competence-specific mechanism (8, 14). Competence is a starvation-induced differentiation process that develops optimally at sufficiently high cell densities and in minimal growth medium with glucose as the main carbon source (for review, see Ref. 15). The various environmental signals are interpreted by a complex signal transduction cascade and ultimately lead to the activation of comK, which encodes the competence transcription factor (16, 17). ComK is essential for: (i) the expression of all competence-dependent genes that assemble the DNA-binding and -uptake system; (ii) the competence-related expression of the recombination genes *recA* and *addAB* (the homologue of *E. coli* recBCD); and (iii) its own expression (8, 16, 18). Purified ComK has been shown to bind to the promoter regions of all these genes, and its transcription stimulating activity has been demonstrated in *vitro* with the late competence gene, *comG* (19). ComK footprinting analyses with a number of ComK-regulated genes established a conserved AT-rich palindromic sequence (called the AT-box) as the ComK-recognition sequence (19).

Since competence-dependent recA induction occurs in RecA minus cells, deficient in LexA cleavage, and before LexA is cleaved in wild-type cells, the LexA-imposed transcriptional repression of *recA* is presumably alleviated by the activity of ComK (14). To examine whether ComK is able to prevent the association of LexA with the *recA* promoter region, we analyzed...
the binding of purified ComK in the absence and presence of LexA. Hydroxyl radical footprinting analysis revealed four possible ComK dimer-binding sites, which was substantiated by the results of gel mobility shift experiments. Surprisingly, the occupation of all ComK-binding sites did not interfere with LexA binding, yet our in vitro transcription analysis showed that ComK is sufficient to overcome LexA repression.

EXPERIMENTAL PROCEDURES

General Methods and Materials—All molecular cloning and PCR procedures were carried out using standard techniques (20, 21). Labeled nucleotides were from Amersham Pharmacia Biotech. Media for growing E. coli have been described by Sambrook et al. (21) and Venema et al. (22). B. subtilis strain SGS chromosomal DNA used as template for PCR, was purified as described by Venema et al. (22).

Purification of ComK—ComK was purified as an MBP-ComK fusion protein on an amylose resin (New England Biolabs) column and separated from MBP by cleavage with protease Factor Xa, as previously described (16). After cleavage was complete, Factor Xa was inactivated by the addition of 1 mM phenylmethylsulfonyl fluoride. To separate ComK from MBP and DNA, the protein mixture was loaded onto a DEAE column (Amersham Pharmacia Biotech) equilibrated with 20 mM Tris-HCl, pH 8, 1 mM EDTA, and 0.5 mM dithioerytrol. MBP and ComK were sequentially eluted with a 0 to 50 mM Na2SO4 gradient and a 0 to 1 M KCl gradient (containing 50 mM Na2SO4). Fractions were collected and the Na2SO4 concentration increased to 100 mM to prevent precipitation of ComK. The ComK containing fractions were checked for the absence of contaminating DNA by ethidium bromide-stained agarose gel electrophoresis, aliquote, and stored at -70°C. Purification and cleavage of MBP-ComK were followed by SDS-polyacrylamide gel electrophoresis.

Purification of LexA—B. subtilis LexA was purified as described previously (10). E. coli strain BL21(DE3) containing pET21a-dinR was grown in 1 liter of LB broth containing carbenicillin (50 μg/ml) with shaking until an A600 of 0.6. The culture was inactivated with 10 mM isopropyl-1-thio-β-D-galactopyranoside and grown for an additional 3 h. Cells were harvested by centrifugation at 4°C, 5000 × g for 20 min, and resuspended in 5 ml of 20 mM Tris, pH 7.5, 10% (v/v) sucrose, 1 mM EDTA. Cells were lysed with lysozyme (0.2 mg/ml) by incubation on ice for 30 min followed by a 15-min incubation at 37°C. After 3 times freeze-thawing and sonication, debris was removed by centrifugation at 18,000 × g for 15 min, and the supernatant was used for further purification. The supernatant (0.5–2 ml) was filtered and applied to a 5-ml heparin-agarose column, equilibrated in 20 mM Tris, pH 7.5, 10 mM NaCl, and subjected to fast protein liquid chromatography. Following elution with a 40-ml linear NaCl gradient (10–500 mM NaCl), the fractions containing LexA protein were pooled, concentrated 10-fold by centrifugation and saved in 0.05% bromophenol blue and 0.05% xylene blue. After heat-etching at 70°C for 10 min and subsequent digestion with HindIII (position +49 relative to the transcriptional start of recA) and XbaI (underlined in R3). The HindIII site was converted to a blunt-end by using a Klenow fill-in reaction, prior to XbaI digestion. The transcription reactions were performed in the binding buffer described for the gel mobility shift experiments (poly(dI-dC) included). DNA templates, purified B. subtilis RNA polymerase, and purified ComK were incubated for 15 min at 37°C in a final volume of 20 μl, before the addition of 3 μl of a nucleotide mixture (1 mM ATP, 1 mM UTP, 1 mM GTP, 0.5 μl of [γ-32P]CTP). After 1 min incubation at room temperature, 2 μl of 0.3% heparin was added to the mixture and incubation resumed for another 10 min at 37°C. After the addition of 2 μl of 1 mM CTP, incubation was continued for 10 min before terminating the reaction by the addition of 18 μl of formamide containing 0.05% bromphenol blue and 0.05% xylene blue. After heating for 3 min at 90°C, the samples were loaded on a 8% polyacrylamide-urea gel and run at 300 V. Gels were subjected to autoradiography immediately after electrophoresis without prior drying.

RESULTS

The recA Promoter Contains Four ComK Dimer-binding Sites—In a previous study Hajema et al. (8) determined the ComK-binding site at the recA promoter using DNase I footprinting analysis (8). They obtained a clear footprint extending from approximately positions −150 to −50, which is the longest ComK-protected region identified thus far. By analyzing ComK

The abbreviations used are: PCR, polymerase chain reaction; MBP, maltose-binding protein; cCTD, a COOH-terminal domain.
footprints of several ComK-dependent promoters, Hamoen et al. (19) concluded that ComK binds as a tetramer composed of two dimers, where each dimer recognizes the dyad symmetrical sequence AAAAN₅TTTT, the so called AT-box (19). The two putative AT-boxes in the recA promoter appeared to be located at the center of the DNase I protections, yet the region confined by these AT-boxes covers only half the sequence protected by ComK (19).

To examine which nucleotide sequences could be responsible for this peculiarly extended ComK-binding region, we improved the resolution of the ComK footprint analyses by using a hydroxyl radical protection assay (Fig. 1). As shown in Fig. 2, the hydroxyl radical footprint fits well within the borders of the DNase I footprint. The central hydroxyl radical protections mark the two previously assigned AT-boxes in a pattern comparable with that found in a hydroxyl radical ComK footprint of the addAB promoter (19). A closer inspection of the hydroxyl radical protections at the extremities of the ComK-binding region revealed two additional AT-boxes: a perfect AT-box around position −140, and one AT-box, with two replacements in the thymine tract, around position −55.

The presence of four ComK-protected AT-boxes in the recA promoter suggests that this promoter is able to accommodate a total of four ComK dimers. Since it has been shown that ComK binds as a single tetramer to the promoter regions of the ComK-regulated genes studied so far (19), we were interested in determining the oligomerization state of ComK on the recA promoter with its two additional dimer-binding sites. Specifically, we wondered if the detection of partial saturation of the recA promoter at low ComK concentrations might provide clues regarding the nature of ComK binding. Although previous gel mobility shift assays with the recA promoter and purified ComK yielded a single retarded band, the retarded band displayed in the autoradiograms was diffuse and may have obscured the presence of multiple bands (8).

To increase the resolution of the gel mobility shifts we omitted the use of intensifying screens, reduced the thickness of the nondenaturing polyacrylamide gels, and applied a voltage gradient using buffers with various ionic strengths (for details, see “Experimental Procedures”). These alterations resolved the single diffuse band into three separate bands (Fig. 3). Repeating these high resolution gel mobility shift assays with other ComK-regulated promoters, such as addAB and comK, yielded only a single retarded band (data not shown) consistent with the binding of a single ComK tetramer (19). Gel mobility shift experiments at low ComK concentrations with these ComK-regulated promoter fragments never revealed intermediate retarded bands that would correspond to the binding of discrete ComK dimers. Based on its relative mobility, we assume that the least retarded recA fragment is bound by a single ComK tetramer, and that the two further retarded bands are due to successive binding of two additional ComK dimers.

**ComK Binding Does Not Interfere with LexA Binding**—The LexA-binding site of the recA promoter partially overlaps with the downstream AT-box (AT-box 4) suggesting that ComK binding to this AT-box could reduce the affinity of LexA for the recA SOS-box. Thus, a plausible mechanism for competence-dependent recA induction could be that ComK precludes stable binding of LexA to its site. Arguing against this simple mechanism, Haijema et al. (8) showed that both proteins can bind simultaneously to the recA promoter. However, it is possible that in the presence of LexA the downstream AT-box was not occupied (i.e. only three ComK dimers bound) since the unresolved mobility shift experiment could not distinguish between partial and complete occupancy of all ComK-binding sites.

To examine whether ComK is capable of displacing LexA from the recA promoter, we repeated the high-resolution gel mobility shift assays with ComK in the presence of purified LexA. Prior to testing retardation with both proteins, we tested the binding of LexA alone to determine the saturating LexA

![Fig. 1. Hydroxyl radical footprinting analysis of the recA promoter region in the absence (−) and presence of ComK (+). The left and right panels represents the footprint of the upper and lower strands, respectively. Footprints are flanked by G + A sequence ladders. Strongly protected regions are marked by solid lines and weaker protected regions by dots. The position of the −35 promoter sequences are marked.](image-url)
Concentration. The recA promoter contains a single SOS-box, and only a single shifted band was observed in a gel mobility shift assay (Fig. 4). Graphical analysis of these data gave a $K_d$ of 5 nM for LexA binding to the recA SOS-box (data not shown). Using a LexA concentration (13 nM) sufficient to ensure binding to all recA promoter molecules, we incubated the recA promoter with increasing concentrations of ComK in the absence and presence of LexA. As indicated in Fig. 5, the presence of LexA added to the electrophoretic mobility shift of all three ComK retarded bands. Apparently, despite partially overlapping recognition sites, ComK does not exclude LexA from binding to the recA promoter. The alternative possibility, that LexA-induced supershift resulted from a specific interaction between ComK and LexA, could be refuted. In a gel mobility shift assay with a ComK-dependent promoter which does not contain a SOS-box, the presence of LexA did not result in an additional retardation in electrophoretic mobility of ComK-bound promoter fragments (data not shown).

**LexA Does Not Affect the Affinity of ComK for the recA Promoter**—We quantified our mobility shift data by densitometry and analyzed the data graphically to determine equilibrium binding constants. Our objective was to assess the extent to which LexA alters the ability of ComK to bind the recA promoter. Any changes in ComK binding constants at saturating LexA concentration would suggest that saturating concentrations of ComK could alter the binding of LexA to the recA SOS-box.

Fig. 6 shows graphical analyses of our ComK titration data in the absence and presence of LexA at a saturating concentration of 13 nM. Assuming the stoichiometry indicated by our footprinting data (i.e., a maximum occupancy of four ComK dimers per recA promoter), we analyzed the average number of ComK dimers bound per recA promoter as a function of free ComK concentration. The data for the binding of ComK in the presence and absence of LexA fit a binding isotherm with a dissociation constant, $K_d$, of 84 nM. The value of 84 nM was determined from a Scatchard plot of the same data (Fig. 6, middle graph). It is noteworthy that the Scatchard plot is linear suggesting that the thermodynamics of binding is not cooperative despite the existence of multiple contiguous binding sites. Consistent with the linear Scatchard plots, Hill plots of the data (Fig. 6, lower graph) had slopes of exactly 1.0. These results do not rule out the possibility that the kinetics of ComK binding is cooperative, which we suspect is the case for binding of the ComK tetramer since we never detect binding of a single dimer. It is also possible that the kinetics of dimer binding is cooperative. In any case, the significant result is that the presence of LexA does not appear to affect the affinity of ComK for the recA promoter.

Our ability to resolve different ligation states for ComK binding makes it possible to analyze individual binding constants to better assess cooperative interactions that might occur between sites. Seneur and Brenowitz (25) demonstrated the validity of this approach for the Lac, Gal, and acl repressors (25). As described above, our gel mobility shift results can best be explained by a three-site system where one site is bound by a ComK tetramer and the other two sites are bound by ComK dimers. The fraction of DNA molecules with exactly $i$ dimers bound, $\Phi_i$ (where $i = 0, 2, 3, or 4$), was calculated from digitized autoradiogram images and the data were curve fitted as described under “Experimental Procedures.” Fig. 7 shows a plot of the fraction of DNA with ComK bound to 0, 1, 2, or 3 sites, the first site bound (i.e. the site bound at low ComK concentration) by a tetramer, and the other sites by dimers. The curves in Fig. 7 result from fitting the data from gel mobility shift assays, in the presence and absence of LexA, according to Equations 1–4 to give $K_1 = 1.21 \times 10^{17}$, $K_2 = 8.94 \times 10^{14}$, and $K_3 = 7.25 \times 10^{20}$ for the respective equilibrium association constants. It is noteworthy that the dissociation constant corresponding to $K_1 = 1.21 \times 10^{17}$ (i.e. the reciprocal of this value) is 83 nM, is in very good agreement with the dissociation constant determined from the Scatchard plot in Fig. 6. Although the curve fit is not
Inverted triangles, promoters with 4 dimers bound. Titrations 1
moters with 2 dimers bound; binding to the recA plot. Data are from titrations in the presence (open squares) of unbound ComK; middle graph, average number of ComK dimers bound per recA promoter (r) plotted versus concentration of LexA, Scatchard plot; lower graph, Hill plot. Data are from titrations with LexA and "i
perfect for our 1- and 2-site data, there is no significant difference between the data in the presence or absence of LexA.
As described for the binding of the λI repressor to its operator, cooperative binding of the second ligand in a 3-site system can be inferred if $K_2 > K_1^{2/3}$ and cooperative binding of the third ligand can be inferred if $K_3 > K_1 K_2/3$ (25). For ComK binding to the recA promoter the ratio of $K_2/K_1^{2/3}$ is 1.8 and the ratio of $K_3 K_2/3$ is 2.0 suggesting moderate cooperativity in each case. Although the ComK-binding sites are not identical, Senear and Brenowitz (25) showed that the ratios are greater than one when cooperativity equals or exceeds site heterogeneity. A possible explanation for the apparent discrepancy between these results and our Hill analysis is that the cooperativity is about equal to the site heterogeneity. That is, the free energy corresponding to cooperative interactions between the tetramer and the adjacent dimers is about equal to the respective differences in the free energy of binding.

Figs. 6 and 7 provide strong evidence that the binding constants for ComK are unaffected by the presence of LexA. According to equilibrium laws, it follows that if the binding constants for ComK are unaffected by the presence of LexA, the binding constant for LexA is unaffected by the presence of ComK. Thus, binding of ComK alone does not contribute to the displacement of LexA from the recA promoter.

**ComK Is Sufficient to Activate recA Transcription**—Since ComK does not prevent LexA from binding to the recA promoter, is ComK alone capable of counteracting the LexA-imposed repression or are additional factors required? We performed in vitro transcription experiments with purified RNA polymerase to address this question. The recA promoter region was cloned into the multiple cloning site of pAN583, followed by the strong T7 terminator for use in an in vitro transcription assay (26). The resulting pAN-recA construct was incubated in a transcription buffer containing purified RNA polymerase and combinations of purified LexA and ComK. Fig. 8 shows that the inhibition of recA transcription by LexA is overridden by the addition of ComK, although the level of transcription is not as high as with ComK alone. We therefore conclude that the presence of ComK is sufficient for competence induced recA expression.

**DISCUSSION**

Using hydroxyl radical protection analysis we identified a total of four ComK dimer-binding sites, two more than are present in other ComK-regulated promoters. Our mobility shift results further suggest that the recA promoter is bound by a ComK tetramer at low ComK concentration, and then successively by two additional ComK dimers as ComK concentration increases. Complete retardation of most recA promoter molecules does not occur until the ComK concentration reaches about 1 μM. Assuming a cell volume of $1 \times 10^{-6}$ μL (calculated from the size of a typical cell), and about 90,000 ComK molecules per cell (27), the physiological concentration of ComK would be well over 100 μM, high enough to ensure that all four ComK-binding sites are occupied in vivo.

On the basis of the separation of the ComK dimer-binding sites (AT-boxes), three classes of ComK-regulated promoters are distinguished (19). In the first class (addAB, dinA, and nuca) the interval between dimer-binding sites is 21 nucleotides, placing both AT-boxes at the same side of the DNA helix, assuming 10.5 nucleotides per helical turn (28). The second

**Fig. 6.** Graphical analyses of the gel mobility shift titration of recA promoter fragment with ComK. Upper graph, average number of ComK dimers bound per recA promoter (r) plotted versus concentration of ComK; middle graph, Scatchard plot; lower graph, Hill plot. Data are from titrations in the presence (open squares) or absence of LexA (closed circles).

**Fig. 7.** Graphical analyses of the gel mobility shift titration of recA promoter fragment with ComK. Fraction of DNA molecules with i dimers bound was calculated from bands as described under “Experimental Procedures.” Circles, unbound promoters; squares, promoters with 2 dimers bound; triangles, promoters with 3 dimers bound; inverted triangles, promoters with 4 dimers bound. Open symbols are from titrations with LexA and closed symbols are from titrations without LexA. The solid lines are from fitting the data according to Equations 1–4 under “Experimental Procedures.”

**Fig. 8.** In vitro transcription assays, with the recA promoter as template, in the presence of LexA (0.1 μM) and/or ComK (1 μM). Both proteins were absent in the first lane (–).
class comprises the late competence genes (comC, -G, -E, and -F). These promoters contain AT-boxes separated by an interval of 31 nucleotides, corresponding to 3 complete helical turns. The third class includes a single representative, the comK promoter. In this case the repetition of the two AT-boxes occurs at an interval of 44 nucleotides, ~4 helical turns. Although the distances between the ComK dimer-binding sites show a remarkable variability, in all classes the two AT-boxes are located at the same face of the DNA helix. This enables the ComK dimers to interact to form a tetramer, which has been shown to be essential for efficient binding of ComK (19). In the recA promoter the two centrally located AT-boxes (AT-boxes 2 and 3) are separated by an interval of 21 nucleotides, corresponding to the first class of ComK-regulated promoters. The interval between AT-box 1 and AT-box 2 is 33 nucleotides, equivalent to the second class of promoters. The same interval separates AT-box 3 and AT-box 4. Thus, all of the AT-boxes face the same direction, relative to the AT-box centers, which is indicative of the necessary to distinguish between these possibilities.

Miller et al. (10) have shown that binding of B. subtilis LexA to the recA promoter inhibits both binding of RNA polymerase and transcription in vitro. The location of the recA SOS-box suggests that the nature of LexA repression is unusual among the known din genes. Unlike many of the B. subtilis SOS-boxes, which are centered between position ~10 and position ~35, the SOS-box of recA is centered at position ~52. This region corresponds to the distal subsite of E. coli UP elements thought to interact with the C-terminal domain of an α-subunit (αCTD) of RNA polymerase (30). Although not as extensively characterized as those in E. coli, B. subtilis UP elements are required for high level expression of certain genes and interact with the B. subtilis αCTD (31–33). Indeed, the sequence of the recA promoter between position ~46 and position ~59 is AT-rich and resembles the E. coli UP element consensus sequence. We suppose that high-level transcription of the recA gene requires the interaction of the αCTD with the SOS-box region, and that LexA binding blocks this interaction.

Our results suggest that ComK overrides LexA repression without directly displacing LexA. Thus, ComK either enables RNA polymerase to displace LexA and/or provides an alternative binding interaction such that RNA polymerase can bind the recA promoter in the presence of LexA. In either case, we propose that the binding of DNA by ComK is an essential feature. DNA bending is commonly observed with transcriptional activators and is thought to facilitate wrapping of DNA around RNA polymerase (34). For the recA promoter, binding of ComK to all four AT-boxes may provide the appropriate bending for such wrapping. In support of a requirement for ComK binding at the most upstream site, which would contribute to such bending, Cheo et al. (35) have shown that deleting the region upstream of position ~120, containing AT-box 1, causes a 70% reduction in competence-related recA expression. In addition to DNA wrapping, binding of RNA polymerase to the recA promoter could also be facilitated by direct interactions between ComK and αCTD. Considering evidence for the interactions of E. coli αCTDs with the proximal UP element subsite, centered at ~42, and the distal UP element subsite, centered at ~52 (30), it seems significant that the downstream AT-boxes of the comC, comE, comF, and comG promoters are all centered at about position ~48, whereas the downstream AT-boxes of the recA and addAB promoters are centered at position ~58 (19). The precise location of all these downstream AT-boxes relative to the αCTD-binding sites suggests a mechanism in which ComK affects the binding of αCTD. In the case of the recA promoter, ComK may provide an alternative binding site for the αCTD of RNA polymerase when the UP element is masked by LexA. On the other hand, ComK may affect αCTD binding indirectly by inducing a conformation change in the DNA. Indeed, recent biochemical evidence suggests that some class I transcription factors, thought to interact with αCTD, may stimulate the DNA binding activity of αCTD by inducing a conformation change in the DNA (36). Further research will be necessary to distinguish between these possibilities.

Acknowledgments—We are indebted to Issar Smith and the late Gopalani Nair for purified B. subtilis RNA polymerase and for valuable comments. We thank David Dubnau and Aske van Werkhoven for helpful discussions, and Henk Mulder for preparing the figures.

REFERENCES

1. Kowalczykowski, S. C., Dixon, A. A., Eggleston, A. K., Lauder, S. D., and Rehrer, W. M. (1994) Microbiol. Rev. 58, 401–465
2. Lovett, C. M., and Roberts, J. W. (1985) J. Biol. Chem. 260, 3305–3313
3. Yasbin, R. E., Cheo, D. L., and Bayles, K. W. (1992) Mol. Microbiol. 6, 1263–1270
4. Walker, G. C. (1992) in Escherichia coli and Salmonella (Neidhardt, F. C., ed) pp. 1400–1416, American Society for Microbiology, Washington, D.C.
5. Raymond-Denise, A., and Guillen, N. (1991) J. Bacteriol. 173, 7084–7091
6. de Vos, W. M., and Venema, G. (1982) Mol. Gen. Genet. 187, 439–445
7. Raymond-Denise, A., and Guillen, N. (1992) J. Bacteriol. 174, 3171–3176
8. Hajejma, B. J., van-Sinderen, D., Winterling, K., Koosstra, J., Venema, G., and Hamoen, L. W. (1996) Mol. Microbiol. 22, 75–85
9. Lovett, C. M., Cho, K. C., and O’Gara, T. M. (1993) J. Bacteriol. 175, 6842–6849
10. Miller, M. C., Rosnick, J. B., Smith, B. T., and Lovett, C. M. J. (1996) J. Biol. Chem. 271, 33592–33598
11. Love, P. E., Lyle, M. J., and Yasbin, R. E. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 6201–6205
12. Lovett, C. M., Love, P. E., Yasbin, R. E., and Roberts, J. W. (1988) J. Bacteriol. 170, 1467–1474
13. Lovett, C. M., O’Gara, T. M., and Woodruff, J. N. (1994) J. Bacteriol. 176, 4914–4923
14. Lovett, C. M., Love, P. E., and Yasbin, R. E. (1989) J. Bacteriol. 171, 2318–2322
15. Dubnau, D. (1993) in Bacillus subtilis and Other Gram-positive Bacteria (Sonnenhain, A. L., Hoch, J. A., and Losick, R., eds) American Society for Microbiology, Washington, D. C.
16. van-Sinderen, D., Luttinger, A., Keng, L., Dubnau, D., Venema, G., and Hamoen, L. (1995) Mol. Microbiol. 15, 455–462
17. Hahn, J., Luttinger, A., and Dubnau, D. (1996) Mol. Microbiol. 21, 763–775
18. Haijema, B. J., Hamoen, L. W., Kooistra, J., Venema, G., and van-Sinderen, D. (1995) Mol. Microbiol. 15, 203–211
19. Hamoen, L. W., Van-Werkhoven, A. F., Bijlsma, J. J., Dubnau, D., and Venema, G. (1998) Genes Dev. 12, 1539–1550
20. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidham, J. G., Smith, J. A., and Struhl, K. (1998) Current Protocols in Molecular Biology, John Wiley & Sons, New York
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
22. Venema, G., Pritchard, R. H., and Venema-Schroder, T. (1965) J. Bacteriol. 89, 1250–1255
23. Tullius, T. D., and Dombroski, B. A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 5469–5473
24. O’Halloran, T. V., Frantz, B., Shin, M. K., Ralston, D. M., and Wright, J. G. (1989) Cell 56, 119–129
25. Senea, D. F., and Brenowitz, M. (1991) J. Biol. Chem. 266, 13661–13671
26. Predich, M., Nair, G., and Smith, I. (1992) J. Bacteriol. 174, 2773–2778
27. Turgay, K., Hahn, J., Burghoorn, J., and Dubnau, D. (1998) EMBO J. 17, 6730–6738
28. Lane, D., Prentki, P., and Chandler, M. (1992) Microbiol. Rev. 56, 509–528
29. Winterling, K. W., Chafin, D., Hayes, J. J., Sun, J., Levine, A. S., Yashin, R. E., and Woodgate, R. (1998) J. Bacteriol. 180, 2201–2211
30. Estrem, S. T., Ross, W., Gaal, T., Chen, Z. W., Niu, W., Elbritcht, R. H., and Gourse, R. L. (1999) Genes Dev. 13, 2134–2147
31. Banner, C. D., Moran, C. P., and Losick, R. (1983) J. Mol. Biol. 168, 351–365
32. Frisby, D., and Zuber, P. (1991) J. Bacteriol. 173, 7557–7564
33. Fredrick, K., and Heilman, J. D. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 4982–4987
34. Coulombe, B., and Burton, Z. F. (1999) Microbiol. Mol. Biol. Rev. 63, 457–478
35. Cheo, D. L., Bayles, K. W., and Yashin, R. E. (1993) J. Bacteriol. 173, 5907–5915
36. Ozoline, O. N., Fujita, N., and Ishihama, A. (2000) J. Biol. Chem. 275, 1119–1127

ComK Overrides LexA-imposed Transcriptional Inhibition