

DNA Unwinding Mechanism for the Transcriptional Activation of *momP1* Promoter by the Transactivator Protein C of Bacteriophage Mu*

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Transcription factor-induced conformational changes in DNA are one of the mechanisms of transcription activation. C protein of bacteriophage Mu appears to transactivate the *mom* gene of the phage by this mode. DNA binding by C to its site leads to torsional changes that seem to compensate for a weak *momP1* promoter having a suboptimal spacing of 19 bp between the poor -35 and -10 elements. The C-mediated unwinding could realign the promoter elements for RNA polymerase recruitment to the reoriented promoter. In this study, the model has been tested by mutational analysis of the spacer region of *momP1* and by assessing the strength of the mutant promoters. The response to C-mediated transactivation was dependent on the spacer length of the promoters. Mutants with 17-bp spacing between the two promoter elements showed reduced activity in the presence of the transactivator as compared with their basal level. A synthetic promoter with near consensus promoter elements and optimal 17-bp spacing was also tested to evaluate the model. The results imply a role for C-mediated unwinding in *mom* transcription activation.

The central step in regulation of gene expression is the transcription process. In prokaryotes, the transcriptional apparatus consists of the RNA polymerase, the various regulators (activators and repressors), and the promoter region. The principal regulatory step in transcription occurs during initiation. The efficiency of transcription initiation process depends on various factors: 1) the promoter architecture, 2) the ability of RNAP to bind to the promoter, 3) isomerization of closed complex to open complex, 4) rapid clearance of the promoter to allow subsequent binding of more RNAP molecules, and 5) interaction of regulatory proteins with the promoter DNA and/or RNAP to facilitate any of the above mentioned steps.

A large number of genes having weak promoter elements are transcribed only when RNA polymerase is assisted by accessory factors called activators. Locations of activator binding sites are variable with respect to their distance from the transcription start site (1). Activators can either function by facilitating binding of RNA polymerase at the promoter or at any of the steps subsequent to the binding, namely, isomerization,

promoter clearance, or sometimes during elongation phase. There are two main mechanisms for activator-dependent transcription initiation process. In the first one, a direct communication via protein-protein contact between the activator and one or more subunits of RNAP results in productive RNAP-promoter interactions (2–4). In the second, activator-induced changes in the DNA structure lead to promoter activation (5). Binding of activators lead to DNA conformational changes such as DNA bending, looping, and unwinding (5, 6). Restructuring of the DNA allows favorable alignment of the *cis*-elements for productive RNAP-promoter and -activator interactions leading to transcription activation.

The extent of homology of the -10 and -35 elements to the consensus sequence and the length of the spacer region in between them determine the strength of a promoter. Accordingly RNA polymerase alone or in conjunction with both *cis*-elements and *trans*-acting factor(s) initiates the process of transcription. The bacteriophage Mu *mom* operon, which is responsible for a unique DNA modification function (7), contains two promoters, *momP1* and *momP2* (see Fig. 1A and Refs. 8 and 9). The promoter of the *mom* gene, *momP1*, is a typical example of a weak promoter with a poor -35 hexamer and suboptimal spacing of 19 bp between the -10 and -35 elements (see Fig. 1A). RNA polymerase is not able to bind to *momP1* on its own (8); instead RNAP binds to *momP2* (see Fig. 1A) directing leftward transcription (8, 9). Optimum transcription initiation at *momP1* requires the binding of the transactivator protein C to its recognition sequence, -28 to -57 (see Fig. 1A and Refs. 10 and 11) as a dimer (12).

Site-specific interaction of C protein results in high degree of distortion and localized unwinding of DNA (13–15). We had proposed a model for the C protein-mediated transcription activation of the *mom* gene (15). An additional twist of 34° caused by 19-bp spacing of the *momP1* promoter would result in positioning of the -10 and -35 hexamers out of phase with respect to each other. The C protein-mediated torsional changes (unwinding by ~30°) could reorient the promoter elements to a favorable conformation for RNAP recruitment to *momP1*. The above hypothesis could be tested by analyzing promoter spacing mutants. In an optimally spaced (17 bp) promoter one would expect constitutive expression. However, as a result of activator binding and unwinding, the optimally spaced promoter should show less activity, because the promoter elements will be reoriented in an unfavorable position for RNAP recognition and occupancy. In this study, a series of spacing mutants of *momP1* have been used to verify the unwinding model for transcription activation.

EXPERIMENTAL PROCEDURES

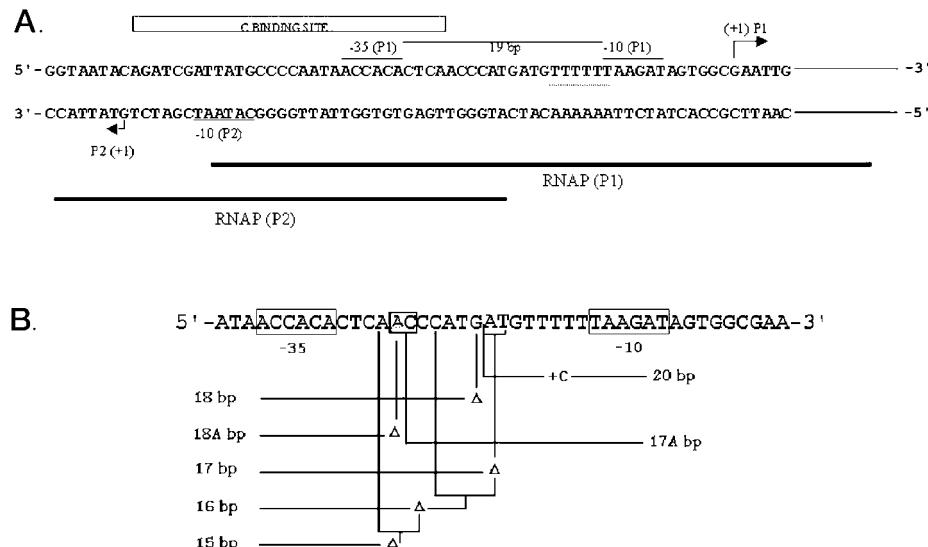
Strains, Plasmids, Primers, Enzymes, and Chemicals—Plasmids pVN184, pLW4, and pUW4 have been described earlier (8, 16). Esche-

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FIG. 1. A, sequence of the Mu *mom* regulatory region. The -10 and -35 elements of *momP1* promoter and the -10 box of *momP2* promoter are indicated. The *momP2* promoter does not have a recognizable -35 element. The C binding site is shown as an *open rectangle*. Transcription start sites of both the promoters are indicated with *arrows*. The stretch of six T residues is shown with a *dotted line*. The RNA polymerase binding regions at both the promoters are marked with *thick lines*. B, mutants of *momP1* promoter. Insertion and deletion mutations within the spacer region of *momP1* promoter are indicated. The -10 and -35 hexamers of *momP1* are shown with *open boxes*.



richia coli DH10B was used for all of the cloning experiments. The details about the primers used are available upon request. Restriction and modifying enzymes were purchased from Stratagene and Roche Molecular Biochemicals. *E. coli* DNA polymerase (Klenow fragment) was from New England Biolabs. Superscript reverse transcriptase was purchased from Life Technologies, Inc. Chemicals and other reagents were purchased from Life Technologies, Inc. and Sigma. [γ - 32 P]ATP (6000 Ci/mmol) was purchased from PerkinElmer Life Sciences. Routine DNA manipulations were carried out as described (17).

Construction of Spacer Mutants of *momP1*—Plasmid pUW4 (16) was used as template for the PCR-based mutagenesis methods. The mutants p18, p17, p16, and p15 (see Fig. 1B) were generated by using the Stratagene QuickChangeTM site-directed mutagenesis method involving a pair of mutagenic oligonucleotides and *Pfu*I DNA polymerase. A Promega Gene Editor site-directed mutagenesis kit was used to generate the mutants p20, p18A, and p17A. Mutants pWT-P2, p17A-P2, p17A-T2G, and p17P-1 were generated by using a modified mega primer method (18) as described earlier (19). All of the mutants generated in the pUW4 background were subcloned into pLW4 using *Eco*RI and *Bam*HI restriction enzymes to generate the promoter mutants as *lacZ* fusions. Sanger's dideoxy method of sequencing (17) was carried out to confirm the mutants.

In Vivo Promoter Strength Analysis—Promoter strength was analyzed by fusing the various mutant promoters of *momP1* with *LacZ* gene and assaying for the reporter gene activity. Isolated colonies harboring either a promoter mutant plasmid alone or with plasmid pVN184 (C protein-producing plasmid) were inoculated into LB broth containing 100 μ g/ml of ampicillin (for mutant promoter plasmids alone) or ampicillin and 25 μ g/ml chloramphenicol (both plasmids present). An overnight grown culture was used as preinoculum. The 3.0-ml cultures (in duplicate) were grown till they reached an A_{600} of 0.3–0.7. The samples were chilled on ice. The cells were treated with SDS-CHCl₃, and β -galactosidase activity was determined as described earlier (20). The values in Table I are the averages from three separate experiments, and the assays were done in duplicate for each culture. The variation was from 10 to 25% about the mean value.

RESULTS

In Vivo Activity of *momP1* Spacer Mutants—The spacing between the -10 and -35 elements of *momP1* was altered by either an insertion or a deletion of base(s) using appropriately designed mutagenic oligonucleotides (as described under “Experimental Procedures”; Fig. 1B). Thus, promoters with 20-, 18-, 17-, 16-, and 15-bp spacing were generated (Fig. 1B). Two different 18- and 17-bp spacing mutants were created to account for the effect of base-specific deletion(s) on promoter activity. Promoter strength was measured in terms of β -galactosidase activity of the wild type *mom* and spacer mutant promoters in the absence and presence of C protein (Table I). The response to C-mediated activation was different in the various mutants depending on the spacer length. Change in

TABLE I
Production of β -galactosidase activity in *E. coli* DH10B cells containing a pLW4-*momP1* promoter-*lacZ* fusion derivative with or without compatible plasmid pVN184

See “Experimental Procedures” for growth of cells and enzyme assays. Plasmid pVN184 produces C protein constitutively at a low level. *E. coli* DH10B cells alone and harboring pVN184 do not show any enzyme activity.

<i>momP1</i> mutant	LacZ		Fold activation ^a
	-C protein	+C protein	
Wild type	37 \pm 9	2083 \pm 525	56 inc.
20 bp	34 \pm 2	435 \pm 40	13 inc.
18 bp	39 \pm 9	646 \pm 156	17 inc.
18A bp	25 \pm 3	736 \pm 178	29 inc.
17 bp	14 \pm 1	1.4 \pm 0.2	10 dec.
17A bp	28 \pm 2	9 \pm 1	3 dec.
16 bp	22 \pm 2	911 \pm 198	41 inc.
15 bp	18 \pm 3	72 \pm 4	4 inc.

^a Fold activation is defined as the ratio of β -galactosidase activity produced by a *momP1* mutant plasmid in the presence or absence of the compatible C protein-producing plasmid, pVN184. Increases and decreases in activity are denoted as “inc.” and “dec.,” respectively.

spacer length by 1 bp (*i.e.* 20 and 18 bp) leads to a decrease in the transactivated levels by 3–5-fold as compared with the wild type promoter activity in the presence of C protein. In two different 17 bp spacing mutants, 3–10-fold decrease in β -galactosidase activity was observed in the presence of C protein as compared with their basal levels (Table I). These data support the unwinding model. C protein-mediated unwinding in a 17-bp spacer promoter mutant would result in reorientation of the promoter elements in an unfavorable position. This would result in less activity of the promoter as compared with its constitutive level. However, the 16- and 15-bp spacer length mutants showed an increase in C-mediated transactivation values by 41- and 4-fold respectively (Table I). This is due to the generation of a new -10 element (GATAGT) as a consequence of deletions made in the spacer region of the promoter. Formation of the new -10 hexamer was confirmed by mapping the $+1$ start site, which was found to be shifted by two bases downstream in the case of the 16-bp spacing mutant as compared with that of wild type *momP1* transcript (not shown). Thus, the actual spacing in the case of the 16- and 15-bp spacer mutants are 19 and 18 bp, respectively. The 15-bp spacer mutant (actual spacing 18 bp) shows less activity in the presence of C when compared with the transactivated level of the 16-bp spacing mutant (actual spacing 19 bp). These mutants essentially fol-

low a similar pattern as that of the wild type and 18-bp spacer deletion mutants (Table I). In all of the cases, however, the change in the spacer length by either an insertion of a base or by a deletion of base(s) did not alter their constitutive activities. The reason for this has been discussed in the section below.

Optimally Spaced -10 and -35 Elements Are Not Sufficient to Increase the Basal Activity of the *momP1* Promoter—Absence of high level of constitutive activity in the optimally spaced *momP1* promoter could be attributed to three possibilities: 1) RNA polymerase preferentially binds to *momP2* instead of *momP1* in the absence of C protein (8, 9) even when the promoter elements are optimally spaced; 2) the putative, weak -35 element of *momP1* (3 of 6 match at the least consensus positions) may not be functional; and 3) the negative element present in the form of T_6 run in the spacer region of *momP1* prevents transcription initiation at the promoter in the absence of the transactivator (19).

Effect of Divergent Promoter, *momP2*, on 17-bp Spacer Deletion Mutant of *momP1*—To test the effect of the divergent promoter (*momP2*) on the transcription of the 17-bp spacer mutant of *momP1*, the -10 element of *momP2* was disrupted by site-directed mutagenesis in the case of both the wild type and 17A spacer deletion mutant promoters (Fig. 2A). Disruption of *momP2* was confirmed by the lack of transcript formation from the *momP2* promoter (19). The β -galactosidase levels of the wild type and the 17A spacer deletion mutant were assayed in the background of *momP2* disruption and compared with the basal level of promoter activity obtained when *momP2* is intact (Fig. 2B). There was no increase in the basal activity of both the promoters in the *momP2* -10 hexamer mutated background. These results demonstrate that the nonfunctional *momP2* promoter does not enhance the constitutive level of *momP1*, and hence, the leftward promoter has a negligible role to play in the activity of *momP1* promoter.

Effect of Mutations in the -35 Element of *momP1*—To ascertain the presence of a functional -35 element with a spacing of 19 bp at *momP1* promoter, the -35 hexamer (ACCCACA) was mutated to obtain a perfect -35 box, TTGACA (Fig. 2C). Fig. 2D shows the result of the promoter strength analysis. Constitutive activity of the *momP1* promoter with a perfect -35 element was increased by ~ 18 -fold when compared with the wild type promoter activity. The transactivated level of this promoter in the presence of C protein could not be assessed as the C binding site gets disrupted because of the mutations made in the -35 box. Hence, in the 17-bp mutant, one of the possible reasons for the low constitutive activity could be attributed to having a weak -35 element.

Effect of Disruption of T_6 Run on the Basal Level Promoter Activity of 17-bp Spacer Deletion Mutant—The presence of a run of six T nucleotides next to the -10 element (Fig. 1A) imparts an unfavorable distortion to the *momP1* promoter (19). When the T stretch was disrupted by substitution with other bases, some of the mutants showed an increase in the basal level activity by 4–10-fold as compared with the wild type promoter. To assess the effect of the T stretch on the basal activity of the 17-bp spacer deletion mutant, $-16T$ was substituted with G to disrupt the T_6 run in the 17A mutant (Fig. 3A). As a control, T2G mutant having 19-bp spacing and T to G substitution at the -16 position (19) was used for the promoter strength analysis. There was an increase in the basal activity of the 17-bp spacer mutant with a disrupted T stretch (17A.T2G) by 10-fold as compared with the 17-bp promoter mutant with an intact T_6 run (i.e. 17A) (Fig. 3B and Table I). In the case of T2G mutant, there was an increase in the activity by 8-fold

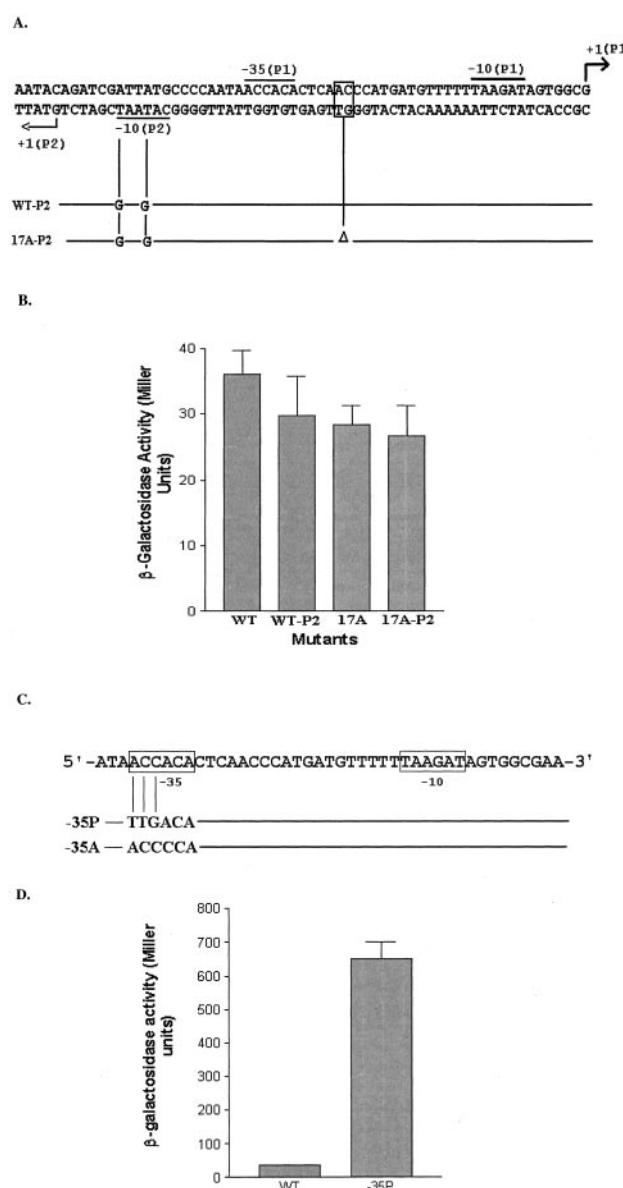


FIG. 2. Promoter activity of *momP1*. *A*, *momP2* -10 element mutation effect. The sequence of *mom* regulatory region is depicted with the mutation in the -10 box of *momP2* in WT and 17A spacer deletion mutant background. *B*, promoter strength of *momP1* mutants with disrupted -10 hexamer of *momP2*. *C*, effect of mutations in the -35 element of *momP1*. The sequence of the *mom* regulatory region is shown indicating the position of mutations in the -35 box of *momP1*. *D*, promoter strength of *momP1* with a perfect -35 element compared with the wild type *momP1* -35 box sequence. The values are the averages of at least three different experiments.

wherein the spacing between the promoter elements is 19 bp (Fig. 3B and Table I). Removal of the negative element increases the constitutive activity of the promoter irrespective of the spacer length between the promoter elements. Hence, the presence of the T_6 stretch within the spacer region of the 17-bp spacer mutant is the major factor responsible for its low level promoter strength.

However, the response of the promoters having different spacer length (19 and 17 bp) to transactivation by C produced contrasting results. The T2G mutant showed a 12-fold increase in the transactivated level as compared with its basal activity (Fig. 3B). In contrast to this, the 17A.T2G mutant promoter showed 2-fold less activity than its basal value in the presence of C protein (Fig. 3B), essentially supporting the proposed role for C protein.

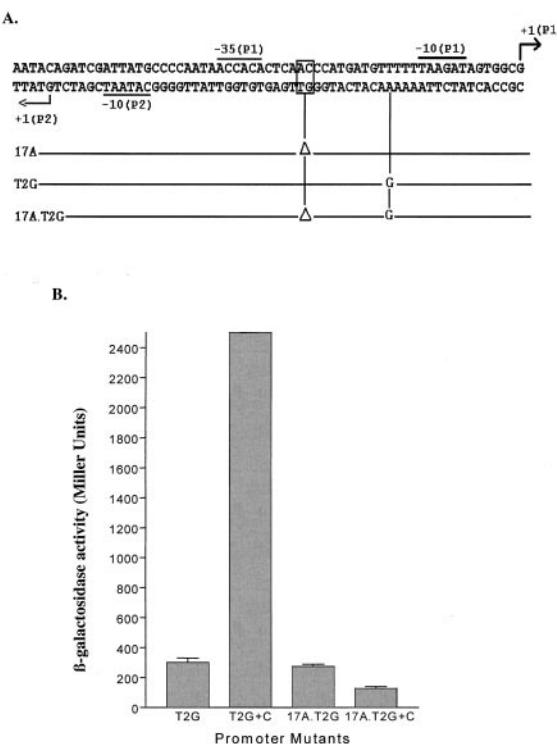


FIG. 3. Effect of disruption of the T_6 run on the activity of 17A spacer deletion mutant. *A*, sequence of *momP1* regulatory region with the mutation in the T_6 run indicated. *B*, β -galactosidase levels of the *momP1* mutants in the absence and presence of C protein.

Effect of C Protein on a Promoter Having a Strong -35 Element and Optimal Spacing—A test for the role of C protein-mediated unwinding in transactivation would be its ability to influence transcription by the same mechanism on synthetic promoters. Transcription from a promoter with optimally spaced (17 bp) elements should be alleviated in the presence of C protein bound to its recognition sequence positioned next to the -35 element. The design and construction of such a template is presented in Fig. 4A. Attempts to clone a synthetic promoter with a perfect -35 element (TTGACA) and 17-bp optimal spacing in pLW4 background were not successful. Different types of promoter down-mutations were encountered consistently while cloning such a promoter. Thus, the TTGACA sequence was replaced with TCGACA and 17-bp spacing in between the promoter elements with the C binding site located next to the -35 element (Fig. 4A). As expected, the promoter with better consensus and 17-bp spacing exhibits high constitutive activity (Fig. 4B). The 2-fold decrease of the promoter activity in the presence of C protein is a strong support for the C-mediated DNA unwinding mechanism.

DISCUSSION

In this paper, we have addressed the mechanism of C-mediated transactivation of the *mom* gene of bacteriophage Mu (15). C binding to its site leads to DNA unwinding possibly leading to reorientation of promoter elements, thus allowing RNA polymerase to bind to an erstwhile inaccessible promoter. Evaluation of this proposal was carried out by analyzing the effect of promoter element spacing of *momP1* on the constitutive as well as the transactivated levels of promoter activity. Change in the length of the spacer region between the -10 and -35 elements affected the activity of the mutant promoters in the presence of C protein. With an optimal spacing (17 bp), the promoter shows less activity in the presence of C protein as compared with its basal level (Table I and Figs. 3B and 4B).

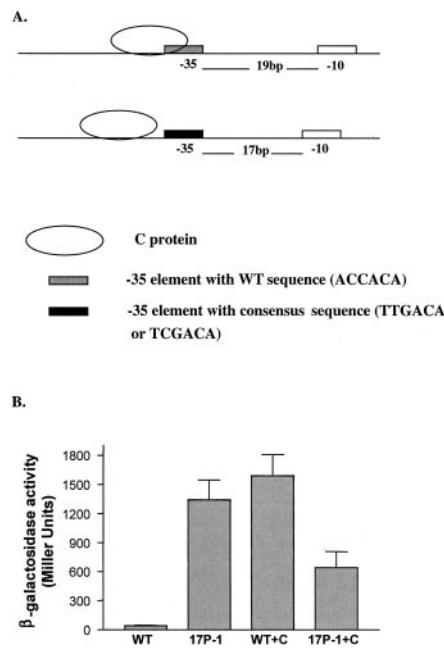


FIG. 4. *A*, schematic representation of the design of a synthetic promoter. The wild type -35 hexamer (ACCACA) is replaced with TCGACA, and the C protein binding site is placed next to the -35 element using appropriate oligonucleotides as described under “Experimental Procedures.” The spacing between the promoter elements is 17 bp. *B*, strength of the synthetic promoter (17P-1) and the wild type *momP1* promoter (WT) in the absence and presence of C protein.

These results lead us to conclude that C-mediated *momP1* transactivation requires a 19-bp spacing between the promoter elements.

The local DNA structure seems to influence the transcription process by providing proper conformation for the recognition and binding of RNA polymerase to promoter sequences. The spacer region of a promoter is believed to play an important role in the fine tuning of promoter activity both by virtue of its length and sequence (21–25). The role of the spacer DNA is postulated to position the -10 and -35 elements in a favorable orientation for RNA polymerase recognition without having any base-specific contacts with RNA polymerase. The spacer region of *momP1* has significant functions in the regulation of transcription initiation of *mom* gene. A lack of increase in the basal level activity of the optimally spaced promoter constructs implies the importance of the T_6 run as well as suboptimal spacing along with a weak -35 box in ensuring repression of *momP1* to prevent leaky expression of *mom* product. Complete silencing of *mom* is necessary until the late lytic phase of the phage because premature *mom* modification would be cytotoxic to the host cell. The role of C is, thus, to counter the negative regulation mediated by *cis*-elements in the promoter region.

A suboptimal spacing of 19 (and sometimes 20) bp between the -10 and -35 elements is found in some *E. coli* promoters. Many promoters that are dependent on transactivators responsive to metal ions have suboptimal spacing between their promoter elements. MerR is one such well characterized transcription activator that unwinds DNA in a symmetric fashion in response to Hg(II) leading to RNAP recruitment to *merT* promoter (26, 27). Proteins belonging to the MerR family like SoxR (28), ZntR (29), and CueR (30) are other examples that transactivate promoters having suboptimal spacing between their -10 and -35 elements. Some of these activator proteins like SoxR (28) and ZntR (29) have been shown to act via a DNA distortion/unwinding mechanism like that of MerR to activate transcription of their respective promoters. Based on the similarity in the pattern of transcription activation by C protein at

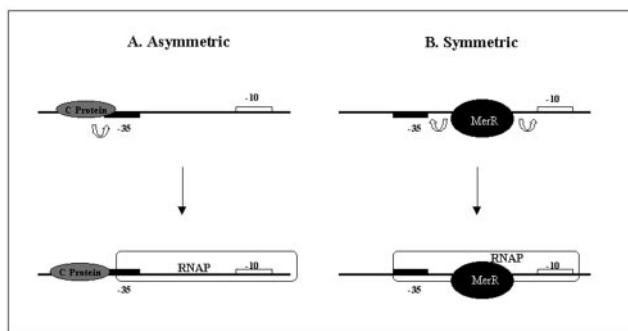


FIG. 5. *A*, C protein binds to its site overlapping the -35 element. Asymmetric unwinding by C protein realigns the unfavorably positioned promoter elements in proper orientation for recognition and binding by RNA polymerase. *B*, binding of MerR within the spacer region of its promoter results in symmetric unwinding and repositioning of its promoter elements for RNA polymerase occupancy.

momP1, it can also be grouped along with the MerR class of activators.

Although, mechanistically C protein appears to be similar to the MerR family of regulators, there are a number of differences in the interaction of C protein at its site as compared with the MerR class of proteins. Members of the MerR family bind to sites within the spacer region of the promoter between the -10 and -35 elements (Fig. 5). The binding site of C is upstream of the -35 element, with a partial overlap with the sequence (Figs. 1A and 5). This difference in the position of the activator-binding site probably accounts for the distinct features of C-mediated transactivation that is different from that of MerR and related transcription factors. In the presence of the metal inducer, all three proteins (MerR, SoxR, and ZntR) produce hypersensitive bands at the center of the palindromic binding site upon 5-phenyl-OP-Cu footprinting (29, 31, 32). (OP)₂Cu footprinting with both supercoiled and linear templates harboring the C binding site produced hypersensitive bands only in the 3' half-site of the C binding site (13, 15). Also, dimethyl sulfate protection and interference footprinting analysis reveal asymmetric interaction of C at the two half-sites of its recognition sequence (13, 15). Such an asymmetric interaction and unwinding of DNA by C protein seems to be necessary because of its location with respect to the -10 and -35 promoter elements in contrast to the MerR mode of interaction (Fig. 5). Upon recruitment of RNA polymerase, in the case of *merT* promoter, both MerR and RNAP would be bound facing each other on opposite faces of DNA, whereas at *momP1*, C and RNAP would perhaps bind on the same face of DNA alongside each other. In this context, MerR is shown to contact three different subunits of RNAP: α , β , and σ^{70} , in cross linking experiments (33). On the other hand, one would expect C to interact with α or σ^{70} subunits based on the location of its binding site. However, previous studies indicate that C does not interact with either one of them (34), and its interaction with other subunits, if any, is yet to be addressed.

In the case of MerR, the protein acts as a repressor of the *merT* promoter in the absence of the metal ion Hg(II), although MerR is still able to bind to its site without affecting the binding of RNA polymerase (31). On the other hand, the SoxR protein does not repress the *soxS* promoter under similar conditions. C protein in the presence of Mg(II) undergoes mandatory conformational changes for binding to its site specifically (35) to activate the *mom* gene. However, in the absence of Mg(II), it does not bind DNA, unlike MerR. Instead, the *momP1* promoter has a DNA negative element within the spacer region near the -10 element that keeps the promoter in the inactive state in the absence of C protein (19).

To summarize, in the absence of the transactivator protein, C, RNA polymerase engages in leftward transcription from *momP2* promoter. Inability of RNAP to bind to *momP1* is due to the presence of a weak -35 element, suboptimal spacing, and a negative element within the spacer region of *momP1*. Binding of C in the presence of Mg(II) to its specific site results in unwinding of DNA at the 3' half-site of its binding region. This alteration in the DNA structure appears to compensate for the presence of two extra bases within the spacer region and also probably for the intrinsic DNA distortion. These changes in the promoter lead to RNAP recruitment at *momP1*. From undetectable expression levels, *mom* gene is turned on completely by the binding of C. Thus, C acts as a decisive transactivation switch operating at the late phase of the phage lytic cycle. The Mu *mom* promoter has evolved in such a way that the architecture of the promoter assures a tight regulation of the gene by utilizing both phage and host-encoded proteins (36) in addition to the DNA structure (19). Such a tight and intricate regulation of the *mom* gene is essential for the survival of both the phage and the bacterial cell.

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REFERENCES

1. Busby, S., and Ebright, R. (1994) *Cell* **79**, 743–746
2. Ishihama, A. (1997) in *Nucleic Acids and Molecular Biology* (Eckstein, F., and Lilley, D., eds) Vol. II, pp. 53–70, Springer-Verlag, Berlin
3. Hochschild, A., and Dove, S. L. (1998) *Cell* **92**, 597–600
4. Rhodius, V. A., and Busby, S. J. (1998) *Curr. Opin. Microbiol.* **1**, 152–159
5. Dai, X., and Rothman-Denes, L. B. (1999) *Curr. Opin. Microbiol.* **2**, 126–130
6. Kolb, A., Busby, S., Buc, H., Garges, S., and Adhya, S. (1993) *Annu. Rev. Biochem.* **62**, 749–795
7. Kahmann, R., and Hattman, S. (1987) in *Phage Mu* (Symonds, N., Toussaint, A., Van de Putte, P., and Howe, M. M., eds) pp. 93–109, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
8. Balke, V., Nagaraja, V., Gindlesperger, T., and Hattman, S. (1992) *Nucleic Acids Res.* **20**, 2777–2784
9. Sun, W., and Hattman, S. (1998) *J. Mol. Biol.* **284**, 885–892
10. Bolker, M., Wulczyn, F. G., and Kahmann, R. (1989) *J. Bacteriol.* **171**, 2019–2027
11. Gindlesperger, T. L., and Hattman, S. (1994) *J. Bacteriol.* **176**, 2885–2891
12. De, A., Paul, B. D., Ramesh, V., and Nagaraja, V. (1997) *Protein Eng.* **10**, 935–941
13. Ramesh, V., and Nagaraja, V. (1996) *J. Mol. Biol.* **260**, 22–33
14. Sun, W., Hattman, S., and Kool, E. (1997) *J. Mol. Biol.* **273**, 765–774
15. Basak, S., and Nagaraja, V. (1998) *J. Mol. Biol.* **284**, 893–902
16. Ramesh, V., De, A., and Nagaraja, V. (1994) *Protein Eng.* **7**, 1053–1057
17. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Dutta, A. K. (1995) *Nucleic Acids Res.* **23**, 4530–4531
19. Basak, S., Lars, O., Hattman, S., and Nagaraja, V. (2001) *J. Biol. Chem.* **276**, 19836–19844
20. Miller, J. H. (1992) *A Short Course in Bacterial Genetics: A Laboratory Manual and Handbook for Escherichia coli and Related Bacteria*, pp. 72–74, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Mulligan, M. E., Brosius, J., and McClure, W. R. (1985) *J. Biol. Chem.* **260**, 3529–3538
22. Deuschle, U., Kammerer, W., Gentz, R., and Bujard, H. (1986) *EMBO J.* **5**, 2987–2994
23. Lozinski, T., Adrych-Rozek, K., Markiewicz, W. T., and Wierzchowski, K. (1991) *Nucleic Acids Res.* **19**, 2947–2953
24. Auble, D. T., Allen, T. L., and deHaseth, P. L. (1986) *J. Biol. Chem.* **261**, 11202–11206
25. Auble, D. T., and deHaseth, P. L. (1988) *J. Mol. Biol.* **202**, 471–482
26. Ansari, A. Z., Chael, M. L., and O'Halloran, T. V. (1992) *Nature* **355**, 87–89
27. Ansari, A. Z., Braden, J. E., and O'Halloran, T. V. (1995) *Nature* **374**, 371–375
28. Hidalgo, E., and Demple, B. (1997) *EMBO J.* **16**, 1056–1065
29. Outten, C. E., Outten, F. W., and O'Halloran, T. V. (1999) *J. Biol. Chem.* **274**, 37517–37524
30. Outten, F. W., Outten, C. E., Hale, J., and O'Halloran, T. V. (2000) *J. Biol. Chem.* **275**, 31024–31029
31. Frantz, V., and O'Halloran, T. V. (1990) *Biochemistry* **29**, 4747–4751
32. Hidalgo, E., Bollinger, J. M., Jr., Bradley, T. M., Walsh, C. T., and Demple, B. (1995) *J. Biol. Chem.* **270**, 20908–20914
33. Kulkarni, R. D., and Summers, A. O. (1999) *Biochemistry* **38**, 3362–3368
34. Sun, W., Hattman, S., Fujita, N., and Ishihama, A. (1998) *J. Bacteriol.* **180**, 3257–3259
35. De, A., Ramesh, V., Mahadevan, S., and Nagaraja, V. (1998) *Biochemistry* **37**, 3831–3838
36. Hattman, S. (1999) *Pharmacol. Ther.* **84**, 367–388