DNA Unwinding Mechanism for the Transcriptional Activation of \textit{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 \textit{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 \textit{momP1} promoter having a suboptimal spacing of 19 bp between the poor \textendash\textendash10 and \textendash35 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 \textit{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 \textit{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 RNA polymerase molecules, and 5) interaction of regulatory proteins with the promoter DNA and/or RNA 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\textendash4). 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 \textendashactivator interactions leading to transcription activation.

The extent of homology of the \textendash10 and \textendash35 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 \textit{mom} operon, which is responsible for a unique DNA modification function (7), contains two promoters, \textit{momP1} and \textit{momP2} (see Fig. 1A and Refs. 8 and 9). The promoter of the \textit{mom} gene, \textit{momP1}, is a typical example of a weak promoter with a poor \textendash35 hexamer and suboptimal spacing of 19 bp between the \textendash10 and \textendash35 elements (see Fig. 1A). RNA polymerase is not able to bind to \textit{momP1} on its own (8); instead RNAP binds to \textit{momP2} (see Fig. 1A) directing leftward transcription (8, 9). Optimum transcription initiation at \textit{momP1} requires the binding of the transactivator protein C to its recognition sequence, \textendash28 to \textendash57 (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\textendash15). We had proposed a model for the C protein-mediated transcription activation of the \textit{mom} gene (15). An additional twist of 34° caused by 19-bp spacing of the \textit{momP1} promoter would result in positioning of the \textendash10 and \textendash35 hexamers out of phase with respect to each other. The C protein-mediated torsional changes (unwinding by \textendash30°) could reorient the promoter elements to a favorable conformation for RNAP recruitment to \textit{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 \textit{momP1} have been used to verify the unwinding model for transcription activation.

**EXPERIMENTAL PROCEDURES**

\textit{Strains, Plasmids, Primers, Enzymes, and Chemicals—Plasmids
pVN184, pLW4, and pUW4 have been described earlier (8, 16). Esche-
Construction of Spacer Mutants of momP1—Plasmid pUW4 (16) was used as a template for the PCR-based mutagenesis methods. The mutants p18, p17, p16, and p15 (see Fig. 1B) were generated by using the Stratagene QuickChange™ site-directed mutagenesis method involving a pair of mutagenic oligonucleotides and Pfu 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 megaprimer method (18) as described earlier (19). All of the mutants generated in the pUW4 background were subcloned into pLW4 using primer method (18) as described earlier (19). The samples were chilled on ice. The cells were treated with SDS-CHCl₃, and DNA manipulations were carried out as described (17).

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

<table>
<thead>
<tr>
<th>momP1 mutant</th>
<th>LacZ</th>
<th>+C protein</th>
<th>Fold activation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>37 ± 9</td>
<td>2083 ± 525</td>
<td>56 inc.</td>
</tr>
<tr>
<td>20 bp</td>
<td>34 ± 2</td>
<td>435 ± 40</td>
<td>13 inc.</td>
</tr>
<tr>
<td>18 bp</td>
<td>39 ± 9</td>
<td>646 ± 156</td>
<td>17 inc.</td>
</tr>
<tr>
<td>18A bp</td>
<td>25 ± 3</td>
<td>736 ± 178</td>
<td>29 inc.</td>
</tr>
<tr>
<td>17 bp</td>
<td>14 ± 1</td>
<td>1.4 ± 0.2</td>
<td>10 dec.</td>
</tr>
<tr>
<td>17A bp</td>
<td>28 ± 2</td>
<td>9 ± 1</td>
<td>3 dec.</td>
</tr>
<tr>
<td>16 bp</td>
<td>22 ± 2</td>
<td>911 ± 198</td>
<td>41 inc.</td>
</tr>
<tr>
<td>15 bp</td>
<td>18 ± 3</td>
<td>72 ± 4</td>
<td>4 inc.</td>
</tr>
</tbody>
</table>

* Fold activation is defined as the ratio of α-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.

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 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 T6 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 (ACCACAA) 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 T6 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 T6 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 T6 run (i.e. 17A) (Table I). In the case of T2G mutant, there was an increase in the activity by 8-fold 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 T6 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. 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.](image-url)
C Protein-mediated Transactivation of mom Gene

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 in 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).

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 Tp 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
protein does not repress the binding of RNA polymerase (31). On the other hand, the SoxR MerR is still able to bind to its site without affecting the site. However, previous studies indicate that C does not interact in the absence of C protein (19).

The asymmetric unwinding by RNA polymerase at the center of the palindromic binding site upon 5-phenylpyridyl-OP-Cu footprinting (29, 31, 32). (OP)2Cu footprinting with both supercoiled and linear templates harboring the C binding site at the two half-sites of its recognition sequence (13, 15). Such an interaction (Fig. 5). Upon recruitment of RNA polymerase, in the interaction of C protein at its site as compared with the 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


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 MgII) 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.