Interference of P$_R$-bound RNA Polymerase with Open Complex Formation at P$_{RM}$ Is Relieved by a 10-Base Pair Deletion between the Two Promoters

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Bacteriophage $\lambda$ promoters P$_R$ and P$_{RM}$ direct RNA synthesis in divergent orientations from start sites 82 base pairs apart. We had previously determined that the presence on the same DNA fragment of a wild type P$_R$ promoter interfered with the utilization of the P$_{RM}$ promoter. The results reported here concern the effects of changing the distance between the start sites by insertion or deletion of 5 or 10 base pairs. Three different techniques (run-off transcription, gel mobility shift, and permanganate probing) were employed to monitor complex formation at P$_{RM}$. Unexpectedly we find that deletion of 10 base pairs between the start sites abolishes the interference, whereas insertion of 10 base pairs does not. Deletion of 5 base pairs, however, essentially prevents joint complex formation at P$_R$ and P$_{RM}$. These findings suggest several ways in which for the wild type separation of the two promoters the utilization of P$_{RM}$ could be affected by an RNA polymerase at P$_R$. In addition to direct steric interference, these include the obstruction of access to DNA sites necessary for optimal contact with the RNA polymerase.

Promoters P$_R$ and P$_{RM}$ of bacteriophage $\lambda$ direct the synthesis of nonoverlapping, divergent transcripts originating from start sites separated by 82 base pairs. We (1, 2) as well as Gussin and co-workers (3, 4) have shown that the presence of the P$_R$ promoter has a negative effect on the ability of RNA polymerase to form open complexes at P$_{RM}$. The use of P$_R$ mutants allowed Gussin's group to demonstrate that conformational transitions in RNA polymerase and/or DNA. The interference slows down but does not prevent open complex formation at P$_{RM}$: ultimately open complexes at both promoters do co-exist on the same DNA fragment (Refs. 1 and 9 and this work).

Reduction of the distance between P$_R$ and P$_{RM}$ by one base pair further slows open complex formation at P$_{RM}$ (4). The effects of considerably shorter separation between the P$_R$ and P$_{RM}$ promoters can be assessed from studies on other lambdoid phages. For the 434 phage where the distance between the start sites of P$_R$ and P$_{RM}$ is about 65 base pairs, the 35 regions of the two promoters overlap and concurrent binding of RNA polymerases at the two promoters is not observed (10). For P22 the interpromoter distance is even shorter, making it quite unlikely that concurrent binding of two polymerases could occur (11). To establish how the P$_R$ promoter affects P$_{RM}$ function over a range of distances not covered in the above studies, we have investigated open complex formation at P$_{RM}$ using constructs with 5- and 10-base pair insertions or deletions between the 35 regions of the phage $\lambda$ P$_R$ and P$_{RM}$ promoters. Surprisingly, we found that the variant with the 10-base pair deletion in the region between the two promoters, leaving just two base pairs between their 35 regions, was essentially impervious to the presence or the absence of a functional P$_R$ promoter on the same DNA fragment.

EXPERIMENTAL PROCEDURES

Materials—Escherichia coli RNA polymerase holoenzyme prepared by the method of Burgess and Jendrisak (12) was further purified as described (13). DNA constructs were made by annealing and ligation of oligonucleotides essentially as described previously (14) and then cloned into the BamHI and HindIII sites of PKK232-8 and sequenced. All experiments were carried out on DNA fragments that had been generated by polymerase chain reaction using oligodeoxynucleotide primers complimentary to sequences in the vector. For the wild type spacing between the two promoters, the resulting fragment was 261 base pairs. Singly 5'-end-labeled DNA fragments necessary for footprinting or permanganate probing and also used in the gel mobility shift experiments were generated by polymerase chain reaction using two oligodeoxynucleotides of which one had been labeled at the 5'-end by 32P with polynucleotide kinase and [γ-32P]ATP. On these fragments the lengths of the run-off transcripts from P$_R$ and P$_{RM}$ were 101 and 85 nucleotides, respectively, regardless of the actual distance between the start sites.

Determination of Open Complex Formation by Run-off Transcription—The reactions (20 μl) contained DNA (5 nM) in transcription buffer (30 mM Tris-HCl (pH 8.2), 100 mM KCl, 3 mM MgCl$_2$, 0.1 mM EDTA, 10 mM dithiothreitol, and 45 μg of bovine serum albumin/ml). Complex formation was initiated by the addition of RNA polymerase to 50 nM. After various amounts of time, heparin (to 50 μg/ml) and an ATP mix (providing final concentrations of 2 mM GTP, ATP, and CTP, and 2 μM UTP (containing 15 μCi of [32P]UTP)) were added simultaneously to initiate single round run-off RNA synthesis from open complexes extant at that time. Following incubation for another 10 min at 37 °C, the RNA products were separated on a 6% polyacrylamide/6M urea gel. Autoradiographs of the gels were scanned to allow quantitation of the radioactivity incorporated in the RNA bands running at 101 and 85 nucleotides to assess complex formation at P$_R$ and P$_{RM}$, respectively.

Permanganate Probing—These experiments followed a modification...
of the protocol described by Borowiec and Gralla (15). Each 20-μl reaction mix contained DNA (30,000 cpm) to a final concentration of 1 nM in transcription buffer (as above, but the dithiothreitol concentration was reduced to 0.5 mM for these experiments). Binding reactions were initiated by the addition of RNA polymerase to a final concentration of 200 nM, as described above. After various amounts of time, heparin was added to 50 μg/ml followed after 1 min by K[152]3 MnO4 to 7.5 mM. After another 2 min, the reaction was quenched with 20% (v/v) ethanol. The samples were precipitated twice with ethanol, treated at 90°C for 30 min with 1 M piperidine, dried and loaded onto a 6% polyacrylamide gel containing 6 M urea. For quantitation of the extent of RNA polymerase-induced sensitivity of thymines to permanganate, the amount of radioactivity in each of several bands was taken as a measure of open complex formation in each of several bands.

Gel Mobility Shift—RNA polymerase gel mobility shift experiments were carried out essentially as described (1). About 1 nM radiolabeled DNA fragments were incubated with 200 nM RNA polymerase at 37°C in 20 μl of transcription buffer for various amounts of time. Heparin was added for 1 min, and the entire reaction mixture was loaded onto a prerunning 1% agarose gel in TAE (0.04 M Tris acetate and 0.001 M EDTA) buffer. The gel was run at 6 V/cm for 2.5 h and then exposed to X-ray film for detection and quantitation of bands. PRM complexes were quantified by exposing the gel to radioautography and then quantitating bands by planar densitometry. The amount of DNA in each complex band was expressed as the fraction of the total radioactivity on the gel. The sum of the fractions of several bands was taken as a measure of open complex formation at each promoter.

Analysis of Kinetic Data—RNA polymerase binding to the mutated PR promoter. DNase I footprinting of species with particular electrophoretic mobilities was carried out by pretreatment of complexes with the nuclease after the addition of the heparin, in transcription buffer containing 10 mM MgCl2 (instead of 3 mM) to increase the cutting efficiency of the DNase. Upon electrophoretic separation of the nuclease treated complexes, the DNA was isolated from individual bands and analyzed on sequencing gels as described (1). In some experiments the binding of RNA polymerase to an uncharacterized DNA region resulted in the appearance of an extra complex band in mobility shift assays. The relative fast formation of this complex necessitated the footprinting of the equivalent of a 3R Po complex in lane 3 (but no other lanes) of Fig. 4. As the results are identical to our other results on 2R Po complexes (1), the presence of the uncharacterized extra RNA polymerase apparently does not affect the results in any way.

Analysis of Kinetic Data—RNA polymerase was in large (>100-fold) molar excess over the DNA fragment, allowing the kinetics to be adequately described by a (pseudo) first order formalism. Thus all data have been fit with Sigma Plot (Jandel Scientific) to the equation: $y = a(1 - \exp(-kt_{obs}^{-1}) + c)$, where $y$ is the experimentally observed quantity, $t$ is the time after mixing RNA polymerase and promoter DNA, $k_{obs}$ is the pseudo first order rate constant, and $(a + c)$ are the limiting and starting values of $y$, respectively. The fit was to the optimal values of the latter 3 parameters. The parameter $k_{obs}$, the RNA polymerase concentration-dependent rate constant, is the reciprocal of $t_{obs}$ (representing an average reaction time), the parameter that has usually been used to describe the rate of open complex formation (1, 3, 16).

RESULTS

The DNA sequences between the start sites of PR and PRM are shown in Fig. 1 for the constructs used in this work, aligned with respect to the start site at the PRM promoter. The actual ligated fragments cloned into pKK 232-8 extended some 6 base pairs beyond the start site of each promoter. In addition to the wild type spacing between PR and PRM, we constructed templates with deletions (D5 and D10) or insertions (I5 and I10).
between the −35 regions of \( P_R \) and \( P_{RM} \). Assuming the DNA is B-form with 10.5 base pairs/turn, for the Wt, D10, and I10 constructs, the centers of the −35 regions of \( P_R \) and \( P_{RM} \) are under-rotated by about 100° with respect to each other, but for D5 and I5 they are over-rotated by about 80°. Thus the −35 regions are separated by similar angles in both cases but approach each other from opposite sides. To address the effect of RNA polymerase binding at \( P_R \) on open complex formation at \( P_{RM} \) for the fragment with wild type, I10, and D10 spacings, we also made variants for which \( P_R \) had been inactivated by a point mutation in the −10 region. All constructs are identical to each other with respect to the \( P_{RM} \) promoter from the −35 region and downstream.

To determine the extent to which the \( P_{RM} \) and \( P_R \) promoters are utilized on each of the DNA constructs, single round run-off transcription reactions were carried out using the wild type template and those with the 10-base pair insertion or deletion (I10 and D10, respectively). The experimental protocol involves the addition of heparin to inactivate free RNA polymerase and that in closed promoter complexes (5 or 60 min after mixing the DNA and RNA polymerase, followed by the addition of the nucleoside triphosphates. Thus the RNA synthesized would be derived from open complexes formed in the time interval ending with the addition of heparin and be a measure of the extent to which open complexes had formed during the interval. The results are shown in Fig. 2. The sizes of the run-off products from \( P_{RM} \) and \( P_R \) are not affected by the presence of the promoter, indicating that the same start sites are used on the variants D10 and I10 (and I5; no RNA product was obtained with D5 (data not shown)) as with the wild type DNA sequence. As expected from previous work, inactivation of the \( P_R \) promoter (evident from the disappearance of the corresponding bands in Fig. 2 (lanes marked \( P_R^{-} \)) facilitated utilization of the \( P_{RM} \) promoter on the fragment with wild type (82-base pair) spacing between \( P_R \) and \( P_{RM} \) (Fig. 2, compare lanes marked Wt). Surprisingly however, the intensity of the \( P_{RM} \)-derived band was greatly enhanced on D10 as compared with the wild type or I10 constructs. Inactivation of the \( P_R \) promoter served as an equalizer, as is evident from the last six columns of Fig. 2; the mutation lead to greatly enhanced intensities of the \( P_{RM} \) bands on the wild type and I10 templates to levels more akin to those on D10 with or without the \( P_R \) mutation.

Even though the interpromoter distance on the D10 fragment is 10 base pairs shorter than that on the wild type one, no interference from the \( P_R \) promoter on \( P_{RM} \) function was observed in the above experiments. In view of the fact that \( P_R \) and \( P_{RM} \) could concurrently be occupied for the wild type separation between them (1,9), it was of interest to determine whether on the D10 template this would be the case as well. We addressed this question using gel shift analysis (Fig. 3A) and found that the two more slowly moving complexes that could be detected for both the wild type and the D10 DNAs had similar mobilities. Previously we had established that the slowest moving complex contained RNA polymerases at both \( P_R \) and \( P_{RM} \) (1). We therefore interpret these results as indicative that on the D10 construct, joint occupancy of the two promoters occurs as well. This was confirmed by DNase I footprinting of the more slowly moving complexes for the Wt, D10, and I10 fragments (see Fig. 4). The footprint pattern determined for the Wt DNA is similar to that previously reported for this DNA (1), demonstrating the concurrent protection of the \( P_R \) and \( P_{RM} \) promoters. Such a pattern is also observed for the two-promoter complexes at D10 and I10, providing further evidence of their ability to concurrently bind a polymerase at each promoter. In addition some subtle differences in the footprints of complexes on the three templates are seen that may be related to template-dependent differences in the extents to which the regions covered by RNA polymerase at the two promoters overlap. With complexes protected at just \( P_R \), two similarly positioned bands of enhanced nuclease sensitivity are observed for all three DNAs, whereas I10 has an additional third hypersensitive site further upstream from \( P_R \) (compare lanes 2, 5, and 8 of Fig. 4). With complexes that show protection at \( P_{RM} \) as well, differences among the three templates are observed in the extent to which the hypersensitivity of the bands is suppressed (see lanes 3, 6, and 9 of Fig. 4).

The gel mobility shift technique also enabled us to follow the kinetics of open complex formation at the \( P_{RM} \) promoter. Visual inspection of the data in Fig. 3A suggests that formation of
two-promoter complexes (i.e. RNA polymerase binding concurrently at PR and PRM) is much faster on the D10 template than on the one with wild type spacing. As in both cases complex formation at PR is instantaneous on the time scale of these experiments; this difference reflects the different rates of complex formation at PRM. Quantitative analysis of the results is presented in Fig. 3B, which clearly shows the much faster rate of complex formation at PRM on the D10 template as compared with the wild type template. Conversely, the two templates show very similar curves for complex formation at PRM in the context of an inactivated PR promoter. The rate constants determined by these and other gel mobility shift experiments are presented in Table I (first column).

In view of the surprising result that shortening the interpromoter distance between PR and PRM can lead to an enhanced rate of complex formation at the PRM promoter, we have investigated the strand separation process by a direct technique, that of probing with permanganate (15). T residues in single stranded DNA are much more sensitive to the oxidizing agent than those in double helical DNA. Thus the RNA polymerase-induced melting of the DNA spanning the start site and the −10 element can be detected by the increase in sensitivity of these residues. In Fig. 5 the patterns of reactivity are displayed for all eight promoter variants shown in Fig. 1 after incubation of each DNA with RNA polymerase for 5 or 60 min. The almost complete inactivation of the PR promoter by the single base substitution at position −7 is again readily apparent; only very weak bands indicative of RNA polymerase-induced enhanced cutting can be seen in the lanes containing this mutated DNA. It is clear that RNA polymerase leads to similar patterns of enhanced DNA breakage at PRM in the context of the five different spacings investigated, regardless of whether PR is inactivated or not. From sequencing reactions using the same primer as was used here to label the bottom strands (Fig. 1) in polymerase chain reaction reactions, we assigned the four most prominent bands in the PRM (lower) region of the gel from top to bottom to T residues at positions −11, −9, −5, and −4, respectively (see Fig. 5). The similarity of the patterns indicates that the region of strand separation must be similar for all the constructs and that RNA polymerase must be positioned similarly on the PRM promoters of each. The data displayed in Fig. 5 also show that “half-turn” insertion or deletion as in I5 and D5 have entirely different effects. With I5, open complex formation at PRM is clearly detectable, but the deletion of 5 base pairs (as in D5) essentially renders open complex formation at PRM unable to be achieved, presumably due to interference from the RNA polymerase bound to PR.

Table I

| Promoter variant | k_{gel} | k_{perm} | k_{run} |
|------------------|---------|----------|---------|
| Wt PR            | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 |
| Wt PRM           | 0.04 ± 0.02 | 0.01 ± 0.01 | 0.00 ± 0.01 |
| I5 0.05 ± 0.01   | 0.01 ± 0.01 | 0.00 ± 0.01 | 0.00 ± 0.01 |
| D10 0.07 ± 0.02 | 0.01 ± 0.01 | 0.00 ± 0.01 | 0.00 ± 0.01 |
| I10 0.10 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.01 |
| D5 0.07 ± 0.01  | 0.01 ± 0.01 | 0.00 ± 0.01 | 0.00 ± 0.01 |

* No meaningful fit was obtained, likely due to the very small extent of complex formation observed for this DNA variant.

* Not done.

2 Y. Tang, unpublished observations.
Fig. 6. Kinetics of open complex formation at the P\textsubscript{RM} promoter as determined by the permanganate assay. The aggregate intensity (expressed as the percentage of the total amount of radioactivity on the gel) of the four bands resulting from modification at positions –11, –9, –5, and –4 was used as a measure of complex formation. The curves were drawn for the best fit values of $k_{\text{obs}}$, as indicated below. a, the P\textsubscript{R} promoter is wild type. Open triangles, D10, $k_{\text{obs}} = 0.19$ min$^{-1}$; open circles, Wt, $k_{\text{obs}} = 0.05$ min$^{-1}$; filled circles, I10, $k_{\text{obs}} = 0.02$ min$^{-1}$. b, the P\textsubscript{R} promoter has been inactivated by mutation. Open triangles, D10 P\textsubscript{R} , $k_{\text{obs}} = 0.15$ min$^{-1}$; open circles, Wt P\textsubscript{R} , $k_{\text{obs}} = 0.09$ min$^{-1}$; filled circles, I10 P\textsubscript{R} , $k_{\text{obs}} = 0.04$ min$^{-1}$.

Wt, D10, and I10 templates are shown in Fig. 6a, those for these same templates but with an inactivated P\textsubscript{R} promoter in Fig. 6b. Again the faster formation of an open complex at P\textsubscript{RM} on the D10 template is readily apparent, as is the acceleration of the rates on I10 and Wt DNA by the inactivation of P\textsubscript{R}. The rate constants obtained by computer fitting of the results are shown in Table I (second column). The reasonable agreement between the values obtained here and those from the gel shift experiments indicates that both methods must be monitoring the same process, namely formation of open complexes.

We have also performed kinetic studies by the single round run-off assay, following the rate of appearance of the transcripts originating at P\textsubscript{R} and P\textsubscript{RM} as a function of the time of incubation of the DNA with RNA polymerase prior to termination of the reaction by the addition of heparin. As is apparent from Table I (third column), in general the rates measured by the run-off assay are smaller than those observed by the gel shift and permanganate probing techniques, and the differences between the various templates are less pronounced. In prior studies (17) on other variants of the P\textsubscript{RM} promoter, we had observed a similar trend in comparing kinetic results from run-off experiments with those obtained by the abortive initiation assay. These findings suggest that in the run-off experiments a slower process not observed in the measurements by the other two methods is rate-limiting. We have not studied this phenomenon further, but likely candidates for such a process are initiation of RNA synthesis and promoter clearance. The fits to the run-off data did indicate a significant (2-3-fold) increase in the plateau values of the kinetic curves upon inactivation of P\textsubscript{R}, thus reconciling the similarity in $k_{\text{obs}}$ values evident in Table I with the large differences in band intensities shown in Fig. 2. Finally it should be pointed out that for the wild type template the values of $k_{\text{obs}}$ calculated from the reciprocals of the $k_{\text{obs}}$ values presented in Table I (a range of 25-50 min for the three techniques) are in good agreement with values obtained by others for this promoter (3).

**DISCUSSION**

We have shown by three independent assays that open complex formation at P\textsubscript{RM} benefits from a 10-base pair reduction in the distance between the P\textsubscript{R} and P\textsubscript{RM} promoters. This is a surprising result, as it had previously been demonstrated that the presence of a functional P\textsubscript{R} promoter interfered with P\textsubscript{RM} function even for the wild type 82-base pair separation between the start sites of the two promoters. Our results indicate that with a DNA construct for which the separation is reduced to 72 base pairs, open complex formation at P\textsubscript{RM} is not inhibited by the presence in cis of a functional P\textsubscript{R} promoter. Because open complex formation at the wild type P\textsubscript{R} sequence is very fast, this implies that on the D10 template the RNA polymerase engaging in open complex formation at P\textsubscript{RM} is essentially impervious to the presence of an RNA polymerase at P\textsubscript{R}. The open complexes formed at P\textsubscript{RM} on the D10 template and also the other variants shown in Fig. 1 are similar to those formed on the wild type template with respect to both the T residues at which base pairing is disrupted and the start site for RNA synthesis. Thus the creation of new promoters as a consequence of the sequence alterations cannot be invoked in explaining the observations reported here.

The observed relief of promoter interference by a reduction in the distance between two promoters suggests two ways that are not mutually exclusive by which an RNA polymerase at P\textsubscript{R} could affect an RNA polymerase in the process of formation of an open complex at the P\textsubscript{RM} promoter: steric interference and occlusion of DNA sites contacted by P\textsubscript{RM}-bound RNA polymerase. In the former case, possibly the 10-base pair deletion allows a RNA polymerases to bind at the adjacent promoters without unfavorable contacts between them, as shown schematically in Fig. 7. In order to conform to the kinetic results reviewed in the introduction (1, 3, 4), no interference would occur in the intermediate (closed) complex. There is indirect evidence (7) for the occurrence of a conformational change in RNA polymerase in the process of formation of an open promoter complex after the initial contact between the enzyme and promoter DNA. This process (shown as the straight arrows in Fig. 7) would be slowed down at P\textsubscript{RM} due to interference of the polymerase at P\textsubscript{R}. The occurrence of steric interference is a reasonable possibility in view of the size of RNA polymerase as deduced from the DNaseI footprints at the promoters. The individual footprints of RNA polymerase at P\textsubscript{R} and P\textsubscript{RM} each extend at least 50 base pairs upstream of the respective start sites (18). Thus for the wild type interpromoter distance of 82 base pairs a stretch of 10 base pairs or more is expected to be covered by RNA polymerases bound at P\textsubscript{R} as well as P\textsubscript{RM}, creating the possibility of unfavorable contacts between the two polymerases over this region.

For the D5 construct little if any joint occupancy of the P\textsubscript{R} and P\textsubscript{RM} promoters is observed. Apparently when the two promoters are rotated by half a turn with respect to each other by deletion of 5 base pairs between their –35 regions, increased physical interactions between the two polymerases prevent them from concurrently being accommodated on the same DNA fragment. For I10, the two RNA polymerases are oriented as they would be for D5, but the extra 10-base pair separation between them is evidently sufficient to allow for their concurrent binding; the I15 construct behaves very similarly to the I10 variant (see Table I). On I10 the separation of P\textsubscript{R} and P\textsubscript{RM} is still within the range for which the RNA polymerases bound at the two promoters might be expected to cover overlapping sites (18).3 Thus for the wild type interpromoter distance of 82 base pairs a stretch of 10 base pairs or more is expected to be covered by RNA polymerases bound at P\textsubscript{R} as well as P\textsubscript{RM}, creating the possibility of unfavorable contacts between the two polymerases over this region.

3 X.-Y. Li and W. R. McClure, unpublished observations.
stretches of DNA. Steric effects could then play a role here as well, leading to the observed favorable effect of PR inactivation on PRM function for I10, just as with the wild type template. The D10 spacing might be unique, resulting in a relative position of the two promoters where their surfaces fit to each other so that open complex formation at the two promoters occurs without interference (as shown in Fig. 7). An even shorter distance between the two promoters, as in the lambda-like phase 434, with a 65-base pair distance between the promoters PR and PRM, does not permit concurrent RNA polymerase binding to the two promoters (10).

For both 110 and the wild type spacer, the RNA polymerase at PR slows down but does not completely inhibit open complex formation at PRM. Such an effect could also be due to the polymerase at PR interfering with open complex formation at PRM by depriving the polymerase at PRM from favorable contacts with certain regions of DNA. In this second model, the protrusions on the PRM-bound RNA polymerase molecules shown in Fig. 7 would be able to contact upstream DNA regions on the D10 template but not the wild type template. Recent findings (19, 20) have indicated that the C-terminal domain (CTD) of the α subunit of RNA polymerase is flexible with respect to the rest of the enzyme, allowing it to contact AT-rich regions at various distances upstream of the −35 region. Inspection of the sequences in Fig. 1 shows the presence of a candidate AT-rich region for the PRM promoter, just upstream (with respect to the PRM start site) of the −35 region of PR. Possibly on the D10 template but not any of the others investigated, this region is accessible to the αCTD (protrusion in Fig. 7) of the polymerase at PRM, despite the presence of a polymerase at PR. In the case of the wild type 82-base pair spacing between PR and PRM (and also for the 92-base pair spacing of I10), the polymerase at PR would make it difficult for the αCTD of the polymerase at PRM to "reach" in far enough to contact the upstream sequence. However, with the shorter inter promoter distance of D10, the DNA-binding region of the CTD would be able to overcome steric barriers imposed by the polymerase at PR. Experiments with polymerases deleted in the αCTD as well as with templates harboring sequence alterations in the AT region downstream of the −35 region of PR indicate that such upstream interactions may be quite important for formation of an open complex at the PRM promoter.4

The data presented in Table I hint at a third way in which on the D10 template an RNA polymerase at PR could facilitate open complex formation at PRM. With each of the three techniques a slightly greater rate of open complex formation at PRM was observed for fragments with the D10 spacing in the context of a wild type PR promoter as compared with fragments with an inactivated PR. Thus for a 72-base pair distance between the start sites, the presence of an RNA polymerase at PR may actually create a favorable environment for open complex formation at PRM. Such an effect could be due to favorable interactions between the RNA polymerases at the two promoters or be transmitted through the DNA itself. In the latter case one might expect RNA polymerase-induced DNA distortions in the stretch of DNA between the −35 regions of PR and PRM. Using copper 5-phenyl-1,10-phenanthroline (21) we have searched for but failed to detect such distortions for RNA polymerase complexes on the templates investigated (WT, D10, and I10).

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