

## Accelerated Publications

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### DNA Distortion Accompanies Transcriptional Activation by the Metal-Responsive Gene-Regulatory Protein MerR<sup>†</sup>

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**ABSTRACT:** Transcriptional regulation of the bacterial mercuric ion resistance operon (*mer*) in response to nanomolar concentrations of mercuric ion is achieved by the allosterically modulated transcriptional activator protein MerR. We now show that mercuric ion modification of MerR activates transcription, facilitating the conversion of an RNA polymerase complex with the *mer* promoter from the closed conformation to the strand-separated, transcriptionally competent open complex. An Hg–MerR-induced structural alteration at the center of the promoter has been detected in the presence or absence of RNA polymerase by use of chemical nucleases sensitive to variations in DNA secondary structure. This hypersensitivity correlates directly with transcriptional activation, lending further support to a previous proposal that a protein-induced distortion in local DNA structure can be the key step in an allosterically modulated transcription activation mechanism.

Signal responsive transcription is frequently mediated by a combination of protein–protein and protein–DNA interactions in nucleoprotein complexes. Inducible protein–protein interactions between a regulator and RNA polymerase are commonly invoked in models for transcriptional activation processes (Ptashne, 1988) whereas regulatory protein–DNA interactions that involve alterations in DNA helix geometry are generally attributed to protein–DNA recognition (Travers, 1989). An alternative role for protein–DNA interactions has been suggested for the transcriptional activator protein MerR (O'Halloran et al., 1989). Regulation of mercury resistance in bacteria is mediated by the *merR* gene product (Lund et al., 1986; Ni'Bhriain et al., 1983; Summers, 1986; Silver & Misra, 1988). MerR regulates transcription of the genes responsible for Hg(II) transport and reduction (*merTPAD* operon) and the *merR* gene in vitro (Helmann et al., 1989; O'Halloran, 1989) and in vivo (Lund & Brown, 1989a, 1989b) (Figure 1A). The Tn501 MerR protein used in the current

study has been overexpressed (O'Halloran & Walsh, 1987), purified to homogeneity (O'Halloran et al., 1989), and shown to be both a specific and an ultrasensitive sensor of mercuric ion (Ralston et al., 1989; Ralston & O'Halloran, 1990). The purified protein is an allosterically modulated transcription factor that directly mediates both repression and activation of the *merTPAD* operon in response to submicromolar levels of Hg(II) while bound at a single site close to the start of transcription (O'Halloran et al., 1989). Extensive mutagenesis studies indicate that different regions of the peptides are involved in Hg(II) and DNA binding (Ross et al., 1989; Shewchuk et al., 1989); however, no clearly defined "activation domain" or RNA polymerase binding region has yet been identified.

The recently established topology of Hg–MerR in the transcriptionally active complex with RNA polymerase (RNAP) and DNA suggests an unprecedented mechanism for transcriptional activation (O'Halloran et al., 1989). By characterizing intermediate transcription complexes, we have now identified specific Hg(II)-regulated steps in the activation mechanism and, furthermore, shown that the ability of Hg–MerR to distort the local DNA structure in the spacer region of the promoter directly correlates with its ability to facilitate transcriptional initiation by RNA polymerase. Our results

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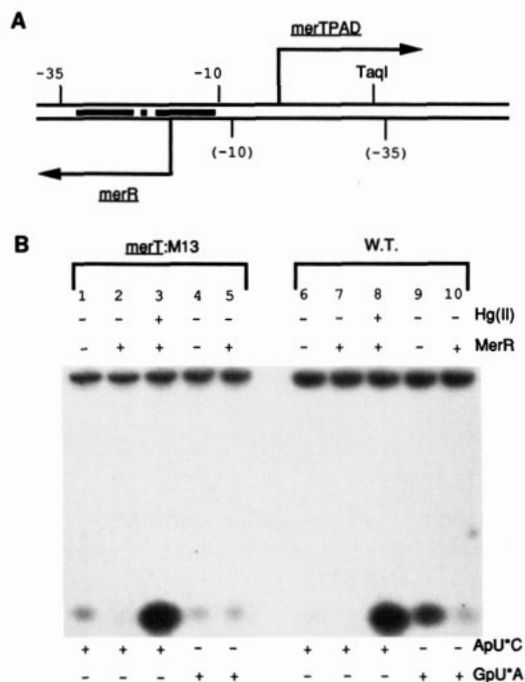


FIGURE 1: In vitro transcription in response to MerR and mercuric ion. (A) Orientation of bidirectionally transcribed *merR* and mercury-resistance promoter (*merTPAD*) or Tn501. Transcription initiation sites in vitro are separated by 17 base pairs; arrows indicate direction of transcription. MerR operator is designated by (---). (B) Comparison of abortive transcription products of the wild-type promoters and a *merR*-less promoter template (*merT:M13*) generated by insertion of the wild-type sequence in M13 at the *TaqI* site. Transcription initiates at *merTPAD* with an ApU dinucleoside (lanes 1-3 and 6-8) and at *merR* with GpU (lanes 9 and 10).

suggest that the role of protein-induced DNA distortion can extend beyond protein-DNA recognition and enzymatic catalysis to the allosteric modulation of RNA polymerase activity by a regulatory protein.

#### EXPERIMENTAL PROCEDURES

**Abortive Transcription.** Labeled template DNA (the uppermost band in each lane of Figure 1B) was incubated with RNAP in the presence or absence of MerR and Hg(II) as indicated for 30 min at 37 °C. Nucleoside initiators and limiting concentrations of labeled NTP were added in the presence of heparin, and the reaction was allowed to proceed for 15 min. Products were analyzed on 18% denaturing polyacrylamide gels.

**Chemical and Enzymatic Protection in Protein/DNA Complexes.** KMnO<sub>4</sub> reacts rapidly with unpaired thymidines in regions of non-base-paired DNA. Treatment of protein/DNA complexes with DNase I or KMnO<sub>4</sub> was performed as previously described (O'Halloran et al., 1989; Frantz & O'Halloran, 1989).

Phenylphenanthroline reactions (Spassky & Sigman, 1985) were modified as follows: Protein/DNA complexes were formed in 20- $\mu$ L incubation buffer containing 1 mM mercaptopropionic acid instead of DTT for 45 min at 37 °C. Ten microliters of 39 mM 5-phenyl-1,10-phenanthroline and 10  $\mu$ L of 9 mM CuSO<sub>4</sub> were mixed and immediately diluted to 400  $\mu$ L with H<sub>2</sub>O. One microliter of the diluted phenanthroline-Cu solution and 1  $\mu$ L of 100 mM mercaptopropionic acid were added to the complexes and incubated at room temperature for 2 min, followed by addition of 1  $\mu$ L of 56 mM 2,9-dimethyl-1,10-phenanthroline for 3 min. Treated complexes were separated by gel-shift assay, eluted, and analyzed on 8% denaturing polyacrylamide gels. Iron hydroxyl radical

footprinting analysis was performed as previously described (O'Halloran et al., 1989).

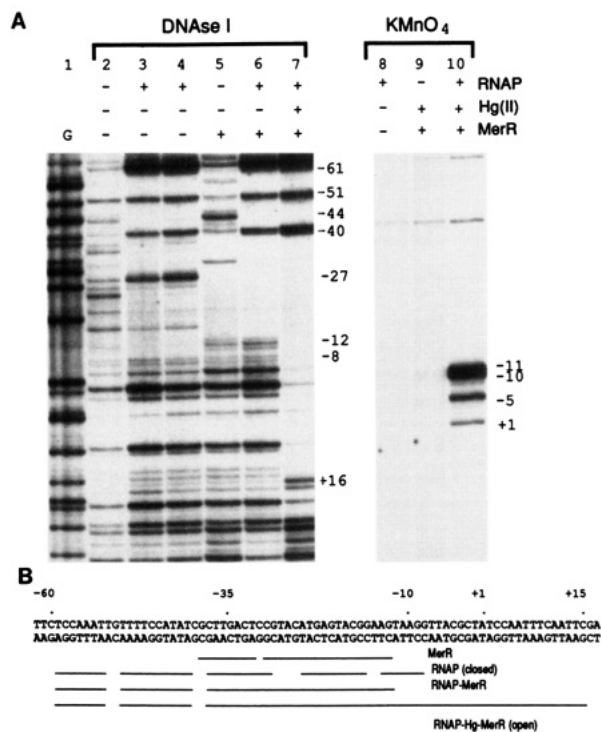
Methidiumpropyl-EDTA (generously provided by P. Derivan) was prepared as a 1.4 mM aqueous solution and stored at 4 °C in 20- $\mu$ L aliquots. Protein/DNA complexes were formed in 20  $\mu$ L of incubation buffer containing 100 mM potassium glutamate, 10 mM Tris, pH 7.7, 2 mM MgCl<sub>2</sub>, 100  $\mu$ g/mL BSA, 5% glycerol, 0.1 mM EDTA, and 1 mM DTT for 45 min at 37 °C. Four microliters of freshly prepared 4 mM Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> was mixed with 10  $\mu$ L of 1.4 mM MPE and immediately diluted to 400  $\mu$ L with H<sub>2</sub>O. One microliter was added to the DNA/protein mixture followed by the addition of 5 mM DTT. After 2 min at room temperature the reaction was terminated with 250  $\mu$ g of tRNA and 12 mM EDTA.

#### RESULTS

Attempts to elucidate the molecular events leading to the formation of transcriptionally competent RNAP complexes at the metal-responsive *merTPAD* promoter (P<sub>T</sub>) and the role of the mercuric ion effector are complicated by the presence of the overlapping MerR promoter P<sub>R</sub>. In order to examine events occurring at P<sub>T</sub> alone, the -35 region of the P<sub>R</sub> promoter was removed by excision at the *TaqI* site (+14 of *merT*, -31 of *merR*) and replaced with M13 mp18 DNA (Figure 1A). Figure 1B (lanes 4 and 5) demonstrates that transcription at the P<sub>R</sub> promoter is eliminated on the *merT:M13* hybrid template paralleled that of the wild type; low but detectable levels of basal transcription (lanes 1 and 6) are repressed by MerR in the absence of mercury (lanes 2 and 7) and are induced by MerR only in the presence of mercury (lanes 3 and 8). Hg-MerR does not activate P<sub>T</sub> transcription by selectively repressing P<sub>R</sub>. Competition between the overlapping promoters for RNAP is not, therefore, the key molecular event in the activation process as has been suggested for the mechanism of transcriptional activation at the P<sub>mnt</sub> promoter by the Mnt protein (Vershon et al., 1987). In order to identify the mercuric ion responsive step in the activation pathway, we have footprinted intermediate promoter complexes containing RNAP and MerR.

**RNAP/P<sub>T</sub> MerR Closed Complex.** The two-step model of transcription initiation (Chamberlain, 1974) requires the formation of an RNAP "closed" complex as the first step in the process. Borowiec and Gralla (1986) propose that this complex can not proceed to the strand-separated, transcriptionally active open complex unless the DNA is bent and untwisted, allowing simultaneous contacts with both the -10 and -35 consensus regions of the promoter. DNase I footprinting at both 4 and 37 °C (Figure 2A, lanes 3 and 4) demonstrates that RNAP forms a closed complex at P<sub>T</sub> similar to that reported (Kovacic, 1987) for the lacUV5 promoter at 4 °C. The RNAP/P<sub>T</sub> footprint exhibits characteristic 10-bp phasing from residue -9 to residue -60, suggesting protein contacts primarily along one face of the DNA helix, as summarized in Figure 2B. DNase I protection is eliminated by heparin (data not shown), and the complex is unstable to separation by gel-shift analysis. Potassium permanganate treatment failed to detect any non-base-paired residues (lane 8) in this complex, indicating that RNAP/P<sub>T</sub> progresses to the open, melted-out state necessary for transcription initiation at a slow rate ( $t_{1/2} > 45$  min) in the absence of the regulator protein.

Addition of MerR to RNAP/P<sub>T</sub> (lane 6) produced greater overall protection and some deprotection at the proximal end of the complex (bases -8 to -12). This footprint pattern

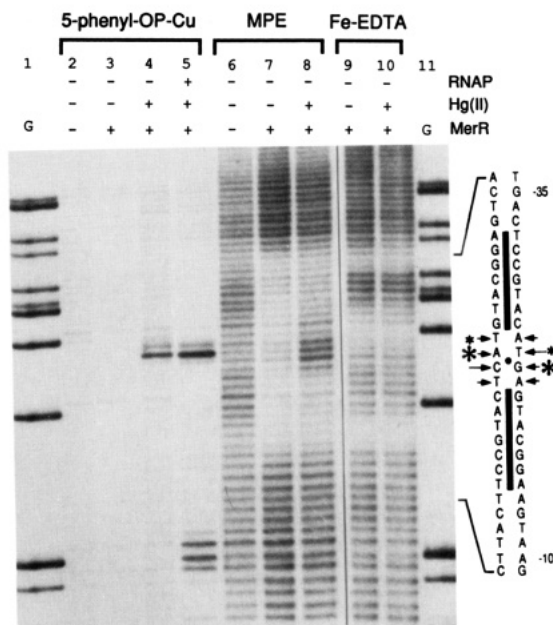


**FIGURE 2:** Enzymatic and chemical modification of protein/DNA complexes at the  $P_T'$  (*merT:M13*) promoter on the template strand. (A) Numbers indicate bases relative to *merT* transcription initiation. RNAP was incubated with DNA as described (O'Halloran et al., 1989) for 45 min at 37 °C (except for the reaction shown in lane 3, which was performed at 4 °C) in the presence or absence of 10 mM MerR and 1  $\mu$ M HgCl<sub>2</sub> (as indicated) followed by treatment for 1 min with DNase I (0.8  $\mu$ g/mL). Reactions were terminated with EDTA, and nucleolytic products were recovered by ethanol precipitation and analyzed on 18% denaturing polyacrylamide gels. Potassium permanganate reactions (lanes 8–10) were performed as described (O'Halloran et al., 1989). (B) Summary of DNase I protection on the template strand of protein/DNA complexes depicted in (A).

indicates a coincident binding of RNAP and MerR. MerR alone protects position -27 and *enhances* reactivity at position -31. RNAP alone *enhances* cleavage at -27 and protects position -31. As with the well-characterized open complex (O'Halloran et al., 1989), protection at these positions indicates the presence of both proteins on the DNA simultaneously. RNAP protection at base -44, upstream of the region protected by MerR (lane 5), can be used independently to measure the degree of RNAP occupancy at  $P_T$ .

In the absence of Hg(II) the RNAP/ $P_T$ /MerR complex is heparin sensitive, KMnO<sub>4</sub> resistant, and unstable to gel separation and is therefore analogous to a closed complex. In a manner similar to that in which the *lac* repressor acts as a transient activator of gene expression (Straney & Crothers, 1987), the MerR protein appears to "trap" polymerase in an intermediate complex at  $P_T$  ready for transcription when mercuric ion is encountered by the cell. Repression must arise from a mechanism other than steric occlusion of RNAP binding in both of these cases. Upon addition of Hg(II) (lane 7) the footprint extends to +15, and unpaired bases are detected by permanganate (lane 10), indicating isomerization of the closed RNAP/ $P_T$ /MerR complex to the previously characterized open RNAP/ $P_T$ /MerR-Hg complex (O'Halloran et al., 1989).

The *merT* promoter contains a 19-bp "spacer" region between the -10 and -35 regions instead of the consensus 17-bp distance. This divergence of the spacer region from consensus geometry may play a role in regulation of expression at this



**FIGURE 3:** Chemical reactivity of the MerR repressor and Hg-MerR activator forms of the protein/DNA complex. Complexes of the <sup>32</sup>P-labeled, kinased, wild-type  $P_T$  template strand were treated with 5-phenyl-CuOP nuclease in lanes 2–5, iron-methidiumpropyl-EDTA in lanes 6–8, and Fe-EDTA in lanes 9 and 10. The sequence of the spacer region that includes the operator is shown to the right of the autoradiograph, where the template strand sequence 5' → 3' is CTTACTTCC etc. Arrows indicate relative sensitivity to MPE, and asterisks indicate hypersensitivity to 5-phenyl CuOP cleavage in the presence of Hg-MerR on both the  $P_T$  template and nontemplate (data not shown) DNA strands in the absence of polymerase. The 5-phenyl-CuOP sensitivity to the melted-out region of the promoter at positions -6 to -4 (lane 5) on the template strand is characteristic of RNAP open complexes (Spassky et al., 1988).

and other promoters (Stefano & Gralla, 1982; Auble & de-Haseth, 1988; Martello et al., 1989; Lund & Brown, 1989). Footprinting results support the view that DNA in polymerase complexes of the  $P_T$  spacer deviates from the geometry found in strong promoters. First, the pattern of alternating DNase I hypersensitivity and protection typically displayed by RNAP complexes (Kovacic, 1987; Carpousis & Gralla, 1985) is displaced two to three bases upstream at  $P_T$  in comparison to the *lacUV5* promoter. Second, the intrinsic -10 region cleavage pattern generated by the nucleolytic activity of the complex between 1,10-phenanthroline and cuprous ions (Cu-OP) at strong promoters (Spassky et al., 1988) is not observed at the  $P_T$  promoter (data not shown).

**Signal-Induced MerR Distortion of DNA Structure.** Although footprint patterns of the repressor form of MerR/DNA were not distinguishable from those of the activator form (O'Halloran et al., 1989), additional chemical nuclease results suggest that the geometry of the  $P_T$  spacer region is modulated by Hg-MerR in the activation process. The reactions of a phenyl-substituted analogue of the 1,10-phenanthroline-copper nuclease indicate that Hg(II) binding to MerR alters the structure of the protein/DNA complex. Hypersensitive attack by this CuOP derivative on both strands of the DNA occurs at the center of the MerR operator at positions -24 on the template strand (Figure 3, lane 4) and -23 on the nontemplate strand only when Hg-MerR is present. These hypersensitive sites are detected in the activated RNAP/ $P_T$ /MerR-Hg open complex as well (lane 5).

CuOP cleavage of B-form DNA is proposed to occur in two steps: binding of the complex to the minor groove and subsequent generation of a metal-oxo complex which cleaves the DNA backbone (Sigman, 1986). DNA recognition by the

CuOP nuclease and its derivatives is sensitive to minor-groove geometry and apparently involves nonintercalative binding (Sigman, 1986; Thederahn et al., 1989) although partial intercalation coupled with minor-groove binding (Veal & Rill, 1989; Wilson et al., 1988) has also been proposed. In the MerR case, changes at the protein/DNA interface induced by mercuric ion are extremely hypersensitive to the 5-phenyl-CuOP complex but not to the 5-nitro-, 5-methyl-, or 5-bromo-substituted complexes (data not shown). Since complexes with other hydrophobic substituents (i.e., 5-methyl) did not exhibit hyperreactivity with the Hg-MerR/DNA complex, it is unlikely that DNA cleavage arises from a protein-bound CuOP reagent. The Hg-MerR-induced hyperreactivity apparently arises from the ability of the 5-phenyl-CuOP nuclease to bind and selectively cleave the sugar-phosphate backbone at a metalloprotein-induced alteration in local DNA secondary structure.

**MPE: Intercalating Nuclease Hypersensitivity.** To further test this proposal, we treated MerR/DNA complexes with the iron-methidiumpropyl-EDTA nuclease (MPE) which binds to duplex B-form DNA via intercalation from the minor groove (Dervan, 1986). Treatment of protein/DNA complexes with MPE produced cleavage products at bases in the center of the MerR operator only in the presence of the effector Hg(II) (Figure 3, lane 8). This region was protected from cleavage in the repressor form of the MerR/DNA complex (lane 7). Under these conditions, Hg(II) alone has no effect on the reactivity of MPE (data not shown). Comparable protein-induced changes in DNA structure have been correlated with enhanced MPE cutting at the center of the highly distorted *Resolvase/ResI* site (Hatfull et al., 1987). Enhanced cleavage of the *Resolvase/DNA* complex by MPE was attributed to a protein-induced bend or kink that makes the minor groove more accessible to the intercalating MPE reagent. In order to demonstrate that enhancement of cleavage by MPE at the center of the Hg-MerR/DNA complex was not due to a local increase in the sensitivity of the sugar-phosphate backbone to free-radical attack, we compared the cleavage patterns produced by the Fenton reaction using either the Fe-EDTA or the MPE reagents (Tullius et al., 1987). As reported previously, no Hg(II)-induced cleavage was observed with the Fe-EDTA protocol (Figure 3, lane 10).

## DISCUSSION

Unlike the Fe-EDTA reagent, both MPE and Cu-phenanthroline bind to the DNA helix prior to the cleavage event and are sensitive to variations in DNA conformation. The magnitude and direction of the offset in the cleavage pattern for the two strands of the Hg-MerR/DNA complex are not consistent with the binding of either reagent in a normal B-form minor groove. The MPE and 5-phenyl-CuOP cleavage patterns for the Hg-MerR/DNA complex are not separated by two base pairs, as expected if a single nuclease molecule was bound in the B-form minor groove, and the MPE pattern is offset to the 5' end of the DNA strand. MPE footprints, including those of *Resolvase*, typically show a 3' offset (Dervan, 1986; Hatfull et al., 1987). These unusual features, derived from two nucleases that use distinct recognition cleavage mechanisms, indicate that the hypersensitivity unique to the Hg-MerR/DNA complex is more than a simple deprotection and are consistent with an allosterically modulated protein distorting the local DNA structure. Two previous observations are also consistent with the proposed role of Hg-MerR in stabilizing a distorted DNA structure. When RNAP binds to the Hg-MerR/DNA complex to form the open RNAP/P<sub>T</sub>/MerR-Hg complex, differences are observed

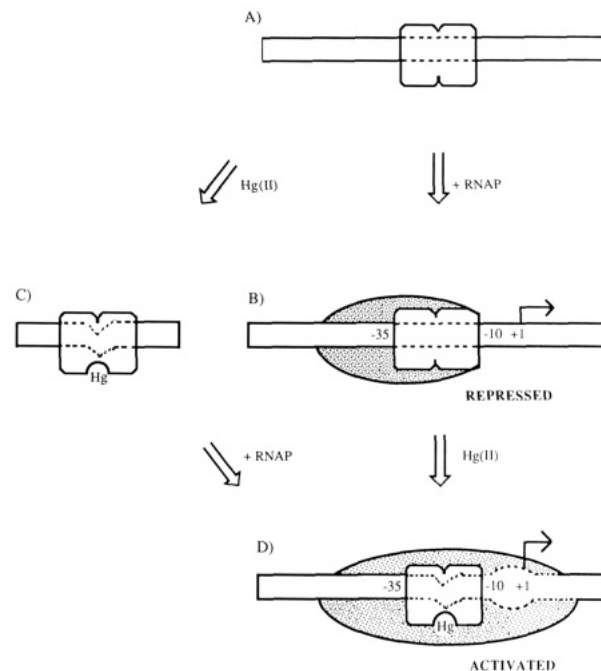


FIGURE 4: Scheme depicting characterized intermediates in the Hg(II)-induced transcriptional activation of the *merT* promoter (P<sub>T</sub>). In the absence of Hg(II) MerR binds to the center of the promoter (A); addition of RNAP results in coincident binding to form a closed RNAP/P<sub>T</sub>/MerR complex (B), which proceeds to a transcriptionally active strand-separated complex at a slow rate. This is the repressed state of the transcription complex. Addition of Hg(II) to (A) results in enhanced sensitivity to chemical nucleases, which is depicted with dashed lines at the center of the operator (C and D). Addition of RNAP to (C) results in rapid formation of the transcriptionally competent open RNAP/P<sub>T</sub>/Hg-MerR complex (D). The topology of (D), which represents the activated state of the transcription complex, has been established elsewhere (O'Halloran et al., 1989). Hg(II) apparently stimulates transcription by increasing the rate of conversion of (B) to (D).

in DMS protection at the nuclease-hypersensitivity site (O'Halloran et al., 1989). Additionally, Hg(II) induces a 3-fold increase in the  $K_D$  of the MerR/DNA complex, and this can be rationalized if the altered DNA structure is stabilized at the expense of the protein-DNA binding energy (O'Halloran et al., 1989).

**Proposed Mechanism for Transcriptional Activation.** A summary of the characterized intermediates in the transcriptional activation pathway is shown in Figure 4. In the repressor state MerR binds tightly to the operator (Figure 4A) and remains bound at the same site after conversion to the activator conformation by Hg(II) (Figure 4C). In the absence of Hg(II), RNA polymerase can bind to the MerR/DNA complex to form a closed complex (Figure 4B) but encounters a kinetic barrier in the transition to a strand-separated, open complex. Interaction of Hg(II) with MerR in the closed RNAP complex can conceivably lower this kinetic barrier by distorting the local structure of the spacer DNA and so lead to rapid formation of the open RNAP complex (Figure 4D). Hg(II) also induces a distortion in the MerR/DNA complex in the absence of RNAP (Figure 4C), and if RNAP is added to this complex, the only detected product is the previously characterized RNAP/P<sub>T</sub>/MerR-Hg open complex. Since the latter complex is transcriptionally active in the presence of NTPs, this distortion directly correlates with the stimulation of transcription. On the basis of analysis of these intermediates, we conclude that Hg-MerR facilitates the conversion of the closed complex to the open complex, and this has been corroborated in kinetic analysis of open complex formation

and abortive transcript formation (D. M. Ralston, M. K. Shin, M. T. Szatkowski, B. Frantz and T. V. O'Halloran, unpublished results). The existence of a similar closed complex has also been proposed on the basis of *in vivo* DMS footprints in the absence of Hg(II) (Heltzel et al., 1989).

The nature of the protein-induced distortion in DNA structure depicted in Figure 4C,D remains to be elucidated. On the basis of the enhanced affinity of the base pairs at the center of the Hg-MerR/DNA complex for the MPE nuclease, which underwinds the DNA upon intercalation (Dervan, 1986), we speculate that the distortion involves a local underwinding or kinking. Stabilization of an underwound region upstream of the transcription initiation site would be expected to lower the barrier to open complex formation by inducing favorable orientation between the -10 and -35 regions of the P<sub>T</sub> promoter. P<sub>T</sub> is a weak promoter in the absence of an activator protein because the spacer region is two base pairs longer than the 17-bp consensus promoter (Lund & Brown, 1988). Consequently, the orientation between the highly conserved -10 and -35 hexamer sequences of P<sub>T</sub> deviates from the consensus promoter by approximately 70° when the template is in the canonical B-form. Underwinding part of the spacer region would realign the relative positions of the conserved -10 and -35 regions of the P<sub>T</sub> promoter and provide a more favorable orientation for simultaneous contacts with RNAP. One precedent for this type of protein-induced DNA distortion, the type I neokink in the EcoRI-dodecamer cocrystal structure (McClarín et al., 1986), involves an abrupt disruption of double-helical structure and a highly localized underwinding of approximately 25°. Another example is the 434 repressor complex with its operator. The DNA at the center of the palindrome is overwound in a manner that is dependent on the DNA sequence (Koudelka et al., 1987). The CAP protein also distorts DNA structure; however, protein-protein interaction with RNAP apparently plays a more important role in transcriptional activation than protein-induced DNA bending (Straney et al., 1989). Physical studies of the Hg-MerR-induced DNA distortion are underway.

#### REFERENCES

- Auble, D. T., & deHaseth, P. L. (1988) *J. Mol. Biol.* 202, 471.
- Borowiec, J. A., & Gralla, J. D. (1986) *Biochemistry* 25, 5051.
- Carpousis, A. J., & Gralla, J. D. (1985) *J. Mol. Biol.* 183, 165.
- Chamberlain, M. E. (1974) *Annu. Rev. Biochem.* 43, 721.
- Dervan, P. B. (1986) *Science* 232, 464.
- Frantz, B., & O'Halloran, T. V. (1989) in *Metal-DNA Chemistry* (Tullius, T., Ed.) ACS Symposium Series 402, pp 97-105, American Chemical Society, Washington, DC.
- Hatfull, G. F., Noble, S. M., & Grindley, N. D. F. (1987) *Cell* 49, 103.
- Helmann, J. D., Wang, Y., Mahler, I., & Walsh, C. T. (1989) *J. Bacteriol.* 171, 222.
- Heltzel, A., Totis, P., & Summers, A. O. (1989) in *Metal Ion Homeostasis: Molecular Biology and Chemistry* (Winge, D., & Hamer, D., Eds.) UCLA Symposium on Molecular and Cellular Biology, New Series, Vol. 98, pp 427-438, Alan Liss, New York.
- Koudelka, G. B., Harrison, S. C., & Ptashne, M. (1987) *Nature* 326, 886.
- Kovacic, R. T. (1987) *J. Biol. Chem.* 262, 13654.
- Lund, P. A., & Brown, N. L. (1989a) *J. Mol. Biol.* 205, 343.
- Lund, P. A., & Brown, N. (1989b) *Nucleic Acids Res.* 17, 5517.
- Lund, P. A., Ford, S. J., & Brown, N. L. (1986) *J. Gen. Microbiol.* 132, 465.
- Martello, P. A., Bruzik, J. P., deHaseth, P., Youngquist, R. S., & Dervan, P. B. (1989) *Biochemistry* 28, 4455.
- McClarín, J. A., Frederick, C. A., Wang, B.-C., Greene, P., Boyer, H. W., Grable, J., & Rosenberg, J. M. (1986) *Science* 234, 1526.
- Ni'Bhriain, N. N., Silver, S., & Foster, T. J. (1983) *J. Bacteriol.* 155, 690.
- O'Halloran, T. V. (1989) in *Metal Ions in Biological Systems* (Sigel, H., Ed.) Vol. 25, pp 105-146, Marcel Dekker, New York.
- O'Halloran, T. V., & Walsh, C. (1987) *Science* 235, 211.
- O'Halloran, T. V., Frantz, B., Shin, M., Ralston, D. M., & Wright, J. G. (1989) *Cell* 56, 119.
- Ptashne, M. (1988) *Nature* 335, 683.
- Ralston, D. M., & O'Halloran, T. V. (1990) *Proc. Natl. Acad. Sci. U.S.A.* (in press).
- Ralston, D. M., Frantz, B., Shin, M. K., Wright, J. G., & O'Halloran, T. V. (1989) in *Metal Ion Homeostasis: Molecular Biology and Chemistry* (Winge, D., & Hamer, D., Eds.) UCLA Symposium on Molecular and Cellular Biology, New Series, Vol. 98, pp 407-416, Alan Liss, New York.
- Ross, W., Park, S. J., & Summers, A. O. (1989) *J. Bacteriol.* 171, 4009.
- Shewchuk, L. M., Helmann, J. D., Ross, W., Park, S. J., Summers, A. O., & Walsh, C. T. (1989) *Biochemistry* 28, 2340.
- Sigman, D. S. (1986) *Acc. Chem. Res.* 19, 180.
- Silver, S., & Misra, T. K. (1988) *Annu. Rev. Microbiol.* 49, 607.
- Spassky, A., & Sigman, D. S. (1985) *Biochemistry* 24, 8050.
- Spassky, A., Rimsky, S., Buc, H., & Busby, S. (1988) *EMBO J.* 7, 1871.
- Stefano, J. E., & Gralla, J. D. (1982) *Proc. Natl. Acad. Sci. U.S.A.* 79, 1069.
- Straney, S. B., & Crothers, D. M. (1987) *Cell* 51, 699.
- Straney, D. C., Straney, S. B., & Crothers, D. M. (1989) *J. Mol. Biol.* 206, 41.
- Summers, A. O. (1986) *Annu. Rev. Microbiol.* 40, 607.
- Theдераhn, T. B., Kuwabara, M. D., Larsen, T. A., & Sigman, D. S. (1989) *J. Am. Chem. Soc.* 111, 4941.
- Travers, A. A. (1989) *Annu. Rev. Biochem.* 58, 427.
- Tullius, T. D., Dombroski, B. A., Churchill, M. E. A., & Kam, L. (1987) *Methods Enzymol.* 155, 537.
- Veal, J. M., & Rill, R. L. (1989) *Biochemistry* 28, 3243.
- Vershon, A. K., Liao, S.-M., McClure, W. R., & Sauer, R. T. (1987) *J. Mol. Biol.* 195, 311.
- Wilson, W. D., Strekowski, L., Tanious, F. A., Watson, R. A., Mokrosz, J. L., Strekowska, A., Webster, G. D., & Neidle, S. (1988) *J. Am. Chem. Soc.* 110, 8292.