Transcription Activation by the Bacteriophage Mu Mor Protein Requires the C-terminal Regions of Both α and σ^{70} Subunits of Escherichia coli RNA Polymerase*

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Bacteriophage Mu is a temperate phage of Escherichia coli K-12 and several other enteric bacteria (1). Mu uses the host RNA polymerase (RNAP)\(^1\) throughout its lytic development (2) to transcribe three sets of genes: early, middle, and late (3). Middle operon transcription requires phage DNA replication and the early gene product Mor (3, 4) and results in expression of C, which in turn activates transcription of late genes encoding phage morphogenesis, cell lysis, and DNA modification functions (5, 6). Transcription from the middle promoter, P\(_m\), requires activation by Mor and is carried out by the E. coli RNAP holoenzyme containing σ^{70} (4, 7). The detailed mechanism by which this activation occurs remains unknown; for example, it might involve protein-protein interactions between Mor and RNA polymerase, conformational changes in the promoter DNA, or a combination of both.

Previous in vivo and in vitro footprinting analysis of P\(_m\) revealed single-stranded bases resulting from distortion in the −33 region, close to the predicted interface between Mor and RNAP (8). The distortion was dependent on the presence of both Mor and RNAP in vitro and involved strand separation confined to positions −35 through −31, as inferred from sensitivity to KMnO\(_4\) modification and Mung bean or S1 nuclease cleavage following modification with dimethyl sulfate. This unwinding was enhanced or abolished in Up or Down spacer-region mutants, respectively, indicating that it may play a role in the activation of transcription.

The middle promoter possesses characteristic features of a promoter under positive control (9)(Fig. 1); it has a recognizable −10 hexamer but lacks similarity to the canonical −35 hexamer (at most, a 2-base pair match to consensus at 16–18-base pair spacing). Previous analyses demonstrated that Mor forms dimers in solution and recognizes an imperfect dyad-symmetry element centered at −43.5 (10). The position of the Mor binding site (11), which overlaps the region normally recognized by σ^{70} region 4.2 (12), as well as the absence of the “extended −10” sequence (13) and −35 hexamer, lead to the hypothesis that Mor, similar to class II transcriptional activators such as PhoB, λ CI, and CRP, might use protein-protein interactions with the σ subunit to activate transcription (14, 15).

The CRP-dependent galP1 promoter is a particularly well-characterized class II promoter (16). The C-terminal part of σ^{70} is required for transcriptional activation of galP1 by CRP (17), with the critical σ residues located between amino acid positions 529 and 540. The α-C-terminal domain (αCTD) is dispensable for activator function at galP1 (18); however, it apparently interacts with promoter DNA upstream of bound CRP (16) and could be specifically cross-linked to CRP upon the formation of an initiation complex (19). In the absence of CRP, RNAP binds to galP1 and is capable of significant transcription, perhaps due to the presence of the extended −10 sequence (17, 18).

Protein-protein interactions are implicated in both positive and negative control of transcription in E. coli (14–20). Contact sites for a number of transcriptional activators are located in the C-terminal parts of the α and σ^{70} subunits of polymerase close to or overlapping their DNA-binding regions (17, 21–24). In this study, we analyzed interactions between Mor and the α and σ^{70} subunits of RNAP by in vitro transcription, DNase I footprinting, and a yeast interaction trap assay system (25). The results demonstrate that the C-terminal regions of both α

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\(^1\) The abbreviations used are: RNAP, E. coli RNA polymerase; P\(_m\), middle promoter; CRP, cyclic AMP receptor protein; αCTD, the C-terminal domain of the α subunit of RNAP; TEMED, N,N,N′,N′-tetramethylethylenediamine; PCR, polymerase chain reaction.
Mor-RNA Polymerase Interactions

EXPERIMENTAL PROCEDURES

Media, Strains, Chemicals, and Enzymes—E. coli strain JM109 (mcrA spo-locl thi gyrA96 endA1 hsdR17 relA1 supE44 recA1 F' traD36 lacY1 lacZM15 proAB') was used as a host in plasmid construction and preparation, was propagated in LB (26) supplemented with 75 μg/ml ampicillin for selection, and transformed with plasmid DNAs purchased from DuPont NEN.

Acrylamide, bisacrylamide, and TEMED were from Bio-Rad. Calf thymus DNA, TRNA, heparin, dimethyl sulfoxide, piperidine, and ammonium persulfate were purchased from Sigma. Bovine serum albumin (transcription grade) and T4 polymerase, and T4 DNA ligase were from Boehringer Mannheim; DNase I was obtained from Worthington, and nucleotide triphosphates were from Pharmacia Biotech Inc.

Proteins—Mor was purified as described previously (10). Wild-type and mutant α subunits were overexpressed and purified as described previously (27, 35); the mutant RNAP core enzymes containing mutant α subunits were reconstituted by mixing the core enzymes with a 4-fold molar excess of σ70 subunit. The C-terminal truncated σ70 subunit was purified as described previously (17). Wild-type RNAP used in DNase I footprinting was a gift from M. T. Record, Jr.

Plasmids and Plasmid Construction—Plasmids pEG202, pJG4-5, and pSH18-34 (28) were used for the interaction trap assay. Plasmids pH7 fls265A and pH7 fls258A (29) were used as templates for PCR amplification of the σ70 mutants. The “extended 10 promoter” (30) construct pIA51 was made as follows. Oligonucleotides IR185 (CCGG-AGCTTTCCTCGGCGATTAATCGTCCAGGAA) and IR186 (GGATCCCTTTAGAGAAAAATATTAACATAGCTGGCARRACA) were annealed to each other, filled in with T4 polymerase, digested with HindIII and BamHI, and cloned into HindIII and BamHI sites of the pUC19-spf vector (30). Plasmid pIA54 is a derivative of pKM90 (7) containing a NdeI-XhoI nor gene fragment from plA54, which was cloned between the EcoRI and XhoI sites of pG4-5, along with an EcoRI-NdeI linker (top strand, ATATGCTGGTGGTGGCTGGAC; bottom strand, TAGCTCCAGCAGCAACAGCCA) designed to retain the reading frame of the B4-2 Mor fusion. Plasmid pIA91 contains sequence encoding the C-terminal domain of the α subunit of RNAP (amino acid residues 323–536), which was PCR-amplified from genomic DNA of strain M5835 (11) with oligonucleotides IR1104 (ATGAAGAGCATGATGTCGAGCTGAAA) and IR1105 (GATCGGTAGGTCTGTCGCGATCA) in a standard amplification (25 cycles of 40 s at 94°C, 40 s at 55°C, 40 s at 72°C; then followed by 7 min at 72°C); PCR product was purified using a QiA spin PCR purification kit (Qiagen), digested with EcoRI and XhoI, and cloned into similarly digested pEG202. Plasmid pIA92 contains sequence encoding the C-terminal part of the σ70 subunit of RNAP (amino acid residues 530–613), which was amplified as above with oligonucleotides IR1106 (TAATGCAAGTCTTGAGGTCTGGAC) and IR1107 (ATCATCTCGGAGATATCTCAGGAA) and cloned into pEG202 using the EcoRI and XhoI restriction sites. The mutant αCTD containing Ala substitutions at positions 265 and 258 were PCR-amplified using pH7 fls265A and pH7 fls258A plasmids as templates and cloned into pEG202 as described above, resulting in pIA121 and pIA123, respectively. The sequences of amplified fragments were confirmed by dideoxy sequencing analysis of the plasmid clones.

Interaction Trap Assays—Saccharomyces cerevisiae strain EG48 (28) was transformed by standard methods (31) with plasmids expressing LexA-fusions and B42-fusions, together with the reporter plasmid pSH18-34; cells were grown on CM (complete minimal) triple dropout plates (Ura- His- Trp-) supplemented with 2% glucose (31). Liquid cultures for β-galactosidase assays were grown in CM triple dropout media supplemented with 2% total sugar (2% glucose or 1.5% raffinose + 0.5% galactose) to an OD600 of 0.5–1.0. Samples of 10 ml were added to 200 μl of 1% cycloheximide on ice, and cells were collected by centrifugation at 7000 × g for 10 min and resuspended in 1 ml of buffer Z (31). Cells were made permeable by the addition of 50 μl each of 0.1% SDS and chloroform and vortexing for 20 s; then cells were diluted 10-fold with buffer Z. Samples (1 ml) were preheated at 30°C; assays were initiated by the addition of 200 μl of α-nitrophenyl-β-D-galactopyranoside (4 mg/ml in H2O) and terminated by the addition of 500 μl of 1 M Na2CO3. Activities were determined using the standard procedure (31).

In Vitro Transcription—Linear templates for in vitro transcription were generated by PCR amplification of either pLA51 (Pmnew) or pKM43 (Pm) using primers annealing upstream of the HindIII site (IR78; TTCCAGTCAAGGCGTGT) and downstream of the EcoRI site (IR13; ATTTGGACCCGATAACAA) of pUC19-spf. The resulting constructs should generate transcripts of 120 nucleotides (Pm new) and 126 nucleotides (Pm) respectively. DNA template (75 fmol), Mor (10 pmol), and reconstituted RNA polymerase holoenzyme (1 pmol) were preincubated in 20 μl of 20 mM Tris-Cl, pH 7.5, 5 mM MgCl2, 0.1 mM EDTA, 50 mM NaCl, 0.2 mM dithiothreitol, and 20 μg/ml bovine serum albumin for 30 min at 30°C. Single-round reactions were initiated by the addition of unlabeled nucleotide triphosphates (ATP, UTP, and GTP to 200 μM; CTP to 20 μM), heparin (100 μg/ml), and 5 μCi of [α-32P]CTP (800 Ci/mmol), then incubated 15 min at 37°C, and terminated by the addition of an equal volume of loading buffer (98% formamide, 20 mM Tris-HCl, pH 8.0, 0.1% bromphenol blue, and 0.1% xylene cyanol). Fusions (2–3 μl) were analyzed on 6% sequencing gels (26). Gels were dried and exposed overnight at ~80°C with screens to X-Omat AR film (Eastman Kodak Co.).

DNase I Footprinting—Linear DNA fragments containing Pm sequences from -115 to +71 were PCR-amplified from pMK100 (11) in a standard reaction (10) using a combination of one unlabeled primer (either top: MLK12, -115 to -96; or bottom: MLK16, +71 to +52) and the second primer end-labeled with T4 polynucleotide kinase and [γ-32P]ATP (3000 Ci/mmol). Fragments containing Pm sequences from -62 to +10 were made analogously from pLA14 (10) using primers IR121 and IR122 (10). Complexes were formed for 30 min at 30°C using purified Mor (1 μg), RNAP (8 μg), and linear DNA fragment (20 ng) in buffer containing 25 mM Tris-HCl, pH 7.9, 50 mM NaCl, 6 mM MgCl2, 0.5 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol, 2% polyvinyl alcohol, and 10 ng/ml of carrier calf thymus DNA in a 50-μl volume. Then 10 ng of DNase I in 50 μl of 5 mM CaCl2 and 10 mM MgCl2 were added, followed by incubation for 45 s at room temperature. Reactions were terminated by the addition of an equal volume of stop solution (200 mM NaCl, 10 mM EDTA, 1% SDS, and 250 μg/ml tRNA), extracted with phenol-chloroform, and precipitated with ethanol. Pellets were washed with ethanol, dried, dissolved in loading buffer, and analyzed on 6% sequencing gels (26). Markers were generated by Maxam-Gilbert sequencing reactions (26) of the same DNA fragments.

RESULTS

In Vitro Transcription with Reconstituted RNAP Holoenzymes Containing C-terminal Deletions of α and σ70—To determine whether the C-terminal regions of the α and σ70 subunits of RNAP are involved in middle promoter function, we assayed the ability of reconstituted holoenzymes containing C-terminal deletions in the α or σ70 subunits (truncated at amino acids 235 and 529, respectively) to direct transcription from linear templates containing Pm or the control promoter P126. Because the P126 promoter contains the “extended –10” region (13), rendering it active in the absence of the C terminus of σ70, and lacks an UP element, making it insensitive to deletions of the
Fig. 2. In vitro transcription with reconstituted RNAP holoenzymes containing deletions in the α subunit (truncated at residue 235) or σ70 subunit (truncated at residue 529). Single-round transcription assays were carried out in the presence of Mor (5 pmol), RNAP (1 pmol), and linear DNA template(s) containing Pm and/or PRE# cloned upstream of the spf terminator. The lane containing the Δσ RNAP was loaded with four times as much reaction volume to compensate for the reduced activity of this enzyme (17).

αCTD, this promoter was used to determine the activity of the mutant enzymes and for normalization of activity at Pm. The σ70 deletion resulted in a dramatic reduction of Pm activity relative to Pm (Fig. 2); in fact, there was no transcript detectable. Deletion of the αCTD led to a less dramatic but still substantial (~20-fold) loss of Pm transcription. Because the C-terminal regions of α and σ70 contain not only activator contact sites but also the DNA-binding regions (17, 18, 21–24), this experiment did not distinguish whether the reduced activity of mutant holoenzymes to support transcriptional activation at Pm was due to the loss of DNA binding or contact with Mor.

DNase I Footprinting—To determine the relative positions of Mor and RNAP bound at Pm, we used DNase I footprinting with purified wild-type E. coli holoenzyme and purified Mor. Consistent with previous findings (11), Mor protected positions –56 to –33 from digestion by DNase I (Fig. 3). The addition of RNAP resulted in extension of the protected region downstream to position +14, a protection pattern characteristic of open and intermediate complexes (33). On the top strand, position –25 remained sensitive to cleavage; the hypersensitive sites detected previously from –29 to –31, using crude extracts of a Mor-overproducing strain (11), were not observed here, raising the possibility that subtle differences in the proteins, the footprinting conditions, or the presence of host factors caused their appearance. The addition of RNAP also resulted in extended protection of positions –59 to –62 on the top strand upstream of bound Mor, while the intervening positions –57 and –58 remained accessible to cleavage. This pattern of upstream protection remained the same when both short (Fig. 3A) and long (Fig. 3, B and C) promoter fragments were used, suggesting that it does not result from binding of polymerase to the ends of the linear template. On the bottom strand, the effect of RNAP addition on cleavage in the –59 to –62 region was more subtle and appeared to be an enhancement rather than reduction in cleavage. This polymerase-dependent upstream protection could be most easily explained by binding of the αCTD, which is known to interact with DNA in this region in some promoters, especially those containing UP elements (16, 24). Although single-point mutations in this region of Pm do not confer a “down” phenotype in vivo (10), the region is AT-rich, as are UP-like elements (24), and might allow specific or nonspecific binding of the αCTD.

When binding of RNAP to Pm was assayed in the absence of Mor, it did not result in a completely clear footprint, but there was weak protection in the region from –62 to –6, and several positions (–53, –51, –15, –12, +11 on the top strand, and –47 on the bottom strand) were notably hypersensitive (Fig. 3). We are inclined to believe that this pattern results from specific rather than nonspecific binding of RNAP because we used an excess of competitor DNA and the same amount of RNAP as used for footprinting with Mor, and there was no binding to flanking sequences in the template.

Comparison of the footprints with RNAP alone to those with RNAP and Mor reveals that RNAP interacts with the region to be bound by Mor as well as several bases upstream. The addition of Mor causes dramatic changes in the footprint, clearly altering the association of RNAP with the DNA. Furthermore, the addition of Mor appears to shift polymerase-dependent upstream protection, perhaps by displacing the αCTD to a position farther upstream. A similar displacement has been seen at galP1 and shown to require the αCTD (16).

Effects of Ala Substitutions in the αCTD on Pm Activation—

The αCTD is known to comprise an independently folded protein domain containing two groups of amino acid residues implicated in UP element utilization and, therefore, DNA binding: 262–269 and 296–299 (34, 35). To ascertain whether the role of the αCTD at Pm involves α-DNA interaction, α-Mor interaction, or both, we assayed Pm transcription using reconstituted holoenzymes containing single Ala substitutions at positions 258 through 275 and positions 297 and 298. Because the activities of the reconstituted holoenzymes could vary, we determined the specific effect of substitutions in the αCTD on Pm activation by comparison of the Pm activity to that observed with the control promoter Pm. The transcripts produced are shown in Fig. 4A, with the ratio of Pm to Pm promoter activity in Fig. 4B. Among the mutant enzymes tested, there were four
that had a significant effect on $P_m$ activation, reducing transcription to less than one-half of that observed with wild-type enzyme; they contained substitutions D258A, L262A, R265A, and N268A. The D258A substitution had the greatest effect, resulting in a decrease in promoter activity almost as large as that occurring with the enzyme deleted for the entire CTD. In previous experiments, the three other substitutions (L262A, R265A, and N268A) decreased transcription stimulation by the $rrnBP1$ UP element, suggesting that they may reduce $\alpha$-DNA binding, but the D258A change did not (34, 35). As a control, we tested our reconstituted holoenzymes with D258A, L262A, and R265A substitutions for transcription from an UP element-dependent but activator-independent form of $rrnBP1$, with results consistent with the previous findings (34, 35); both L262A and R265A substitutions resulted in a significant loss of transcription, whereas the activity of the D258A mutant enzyme was not distinguishable from that of the wild-type.²

Analysis of Mor-RNA Polymerase Interactions using the Interaction Trap Assay—To assay directly for Mor- and Mor-α interaction in the absence of α and σ DNA binding, we used the interaction trap assay in yeast (Fig. 5(25)). In this approach, one protein (X) is fused to the DNA-binding domain of LexA protein (pEG202 vector); this LexA-X fusion is expressed constitutively in yeast cells. A second protein (Y) is fused to an acidic activation domain B42; the B42-Y fusion is under the control of the galactose-inducible GAL1 promoter. Expression of both chimeric proteins in a yeast cell containing a lacZ-reporter cloned downstream of one or more LexA binding sites results in the activation of lacZ expression if the chimeric proteins associate. Because the LexA protein fusion might activate transcription by itself, the interaction potential of a chimeric pair is usually estimated from the ratio of galactose-induced levels to noninduced levels of β-galactosidase activity. In this study a B42-Mor fusion and several pEG202-derived LexA fusions containing the C-terminal regions of α (either wild-type or with Ala substitutions) or σ70 subunits of RNA polymerase (Fig. 5) were expressed and assayed for their ability to activate transcription of the lacZ gene cloned downstream of eight tandem LexA operators (pSH18-34). The β-galactosidase values measured for cells grown in galactose + raffinose (conditions inducing B42-Mor expression) or glucose (noninducing) supplemented media, as well as the ratio of those values are presented in Fig. 5. Three conclusions regarding Mor-α and Mor-σ interactions can be drawn from these experiments: (i) transcription increased above the LexA-σ70 fusion background upon induction of the B42-Mor fusion, indicating weak interaction between Mor and σ70; (ii) the combination of LexA-α and B42-Mor also resulted in a modest but reproducible enhancement of lacZ expression; (iii) the Ala substitution at position 258 of α abolished this effect, whereas the R265A substitution did not. In addition, we found that the LexA-σ70 fusion activated transcription in the absence of Mor (with and without induction of the pJG4-5 B42 vector plasmid). Transcriptional activity of the LexA-σ70 fusion and its apparent interaction with the acidic activation domain of B42 is consistent with the high degree of homology between σ70 and eukaryotic general transcription factors (36, 37) in regions required for their function. These factors were demonstrated to activate transcription when fused to LexA (38) and interact in vitro with a variety of acidic activation domains (39, 40).

**DISCUSSION**

In this study, we observed a reduction in $P_m$ activity in vitro with reconstituted mutant RNA polymerases containing deletions of the C-terminal regions of either the α or σ70 subunits. The C-terminal region of σ70 interacts with the ~35 hexamer

² I. Artsimovitch, unpublished observations.
DNA in typical activator-independent promoters (12); it also contains contact sites used by activators at class II activator-dependent promoters to facilitate open complex formation (14, 16, 17, 22, 23). Typically, the aCTD interacts with the activator at class I promoters, facilitating recruitment of RNAP to the promoter (14, 15).

The absence of both the −35 hexamer and “extended −10” sequence in Pm is consistent with the total dependence of Pm promoter activity on the presence of the activator protein Mor. One possible mechanism for Mor activation is that Mor could recruit RNAP to the promoter using protein-protein (Mor-α and/or Mor-σ) interactions (14, 15); the results of the interaction trap assay would be consistent with this hypothesis. An alternative possibility is that RNAP can bind to Pm to form a closed complex in the absence of Mor; Mor binding might then facilitate isomerization of this complex into a transcription-competent open complex. The altered pattern of DNase I digestion of Pm caused by the addition of RNAP alone would lend support to the second hypothesis; the weak protection and strong hypersensitive sites observed would be consistent with the formation of an unstable closed complex, which exists in rapid equilibrium with free RNAP (41). The properties of base substitutions in Pm suggest that a flexible spacer is needed to facilitate interactions between RNAP and Mor; they also indicate that the −35 hexamer is irrelevant to Pm promoter function; mutations increasing the fit to the −35 consensus did not result in increased promoter activity, and several decreased it (10). In contrast, mutations at positions −29 to −31 affected the DNA distortion observed at positions −32 to −34; mutations that caused Up or Down phenotypes showed increased or decreased distortion, respectively. Nevertheless, these findings do not rule out the possibility of a direct interaction between RNAP and bases in this region; the analysis of the effects of base substitutions on binding of RNAP to Pm should be helpful in distinguishing these possibilities.

The results of in vitro transcription assays with reconstituted RNAP containing Ala substitutions in the aCTD revealed that four residues, Asp-258, Leu-262, Arg-265, and Asn-268, are critical for Mor-dependent activation. These residues are located relatively close to each other on one side of the aCTD (Fig. 6) and could constitute a contact surface for Mor. The residue Asp-258, located in the turn preceding the 260–263 loop, is also involved in Fis-dependent activation at rnrBp1 (43). The other three, Leu-262, Arg-265, and Asn-268, are believed to be involved in DNA binding because they affect UP element utilization (34); these three residues are also essential for activation by OxyR (44) and CRP (35). Curiously, residue Cys-269, which is needed for UP element utilization (34, 35), is not needed for activation of either Pm or P1ac (35). One possible model, proposed to explain the results from analysis of P1ac activation by CRP and mutant RNAP holoenzymes (35), is that the same amino acid residues of the aCTD mediate mutually exclusive α-CRP and α-DNA binding. Although unusual, the existence of domains capable of both DNA binding and protein-protein interactions is not without precedent. It was recently demonstrated that the zinc-finger domain of the transcription factor GATA-1, in addition to its well documented role in DNA binding, mediates self-association as well as heterotypic interactions with other GATA proteins and Krüppel-type transcription factors (45, 46).

In the case of Pm activation, however, we prefer an alternative model in which Asp-258 serves as a specific Mor contact site, and the role of the remaining three residues is to stabilize Mor-α interaction by aCTD binding to DNA. Several arguments contribute to this preference: (i) the effect of the D258A substitution on activity was almost as large as the effect of deletion of the entire aCTD, whereas the other substitutions had lesser effects; (ii) because in galP1 the aCTD protects promoter sequences just upstream from CRP, despite the absence of an UP element (16), it appears that RNAP-CRP interactions are sufficient to position the aCTD close to the DNA. Thus, an RNAP-activator complex might be mutually stabilized by weak protein contacts and weak DNA binding. This model predicts that α residues involved in DNA binding could affect activation solely due to the loss of favorable DNA interactions and that changes in these residues would cause a less severe reduction in activation than changes in amino acid residues that interact directly with activator; (iii) in the interaction trap assay system, α-D258 also played a key role in aCTD-Mor interaction; the D258A substitution abolished interaction of reporter gene expression, whereas the R265A substitution, which dramatically reduces CRP-dependent activation of lacP1 UP element (35), had no effect. Nevertheless, since the interaction detected by this assay was weak, it is possible that stable association may require scaffolding by DNA, as suggested previously for CRP (19). Based on the calibration of the interaction trap assay with proteins of known affinities (28), our results suggest that Mor is capable of associating with α and α subunits in solution with affinities near the threshold of detection, $K_A \approx 10^{-6}$ M, a value similar to that reported for interaction between CRP and RNAP in solution in the absence of their DNA-binding sites (47).

It seems reasonable to think that residues Arg-265 and Asn-268 mediate base-specific or nonspecific interactions with the DNA backbone, because arginine and asparagine are known to participate in such interactions (48). Since Leu-262 may comprise part of the aCTD hydrophobic core, the Leu to Ala substitution may lead to an altered conformation in which the presentation of residues directly contacting DNA and/or Mor is affected, resulting in reduced transcription activation. The abstraction trap assay system, α-D258 also played a key role in aCTD-Mor interaction; the D258A substitution abolished interaction of reporter gene expression, whereas the R265A substitution, which dramatically reduces CRP-dependent activation of lacP1 UP element (35), had no effect. Nevertheless, since the interaction detected by this assay was weak, it is possible that stable association may require scaffolding by DNA, as suggested previously for CRP (19). Based on the calibration of the interaction trap assay with proteins of known affinities (28), our results suggest that Mor is capable of associating with α and α subunits in solution with affinities near the threshold of detection, $K_A \approx 10^{-6}$ M, a value similar to that reported for interaction between CRP and RNAP in solution in the absence of their DNA-binding sites (47).

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sence of a role at P_m for residue Cys-269 suggests that it is a specific determinant for UP element recognition.

When both Mor and RNAP were used in DNase I footprinting experiments, the presence of RNAP caused an upstream extension of the protected region. The simplest hypothesis, and one consistent with the high AT content of this region, is that this protection is due to binding of the αCTD; alternative explanations include: (i) the presence of a Mor+RNAP-induced distortion, rendering DNA resistant to cleavage (compression of the minor groove); and (ii) extension or repositioning of the Mor-DNA contact in response to protein-protein interaction. The DNase I protection experiments with RNAP alone indicate that the enzyme is capable of binding to P_m in the region from −62 to −6 in the absence of Mor. A similar pattern of protection was observed for meAda-dependent promoters, aidB and ada, where RNAP apparently recognizes UP element-like sequences in the −40 to −60 region, largely overlapping the meAda-binding site (49). At these promoters, binding of RNAP is not increased by meAda; instead, the activator seems to function by facilitating contacts of already bound polymerase with core promoter elements at −35 and −10. Because P_m does not have a −35 hexamer, it would be tempting to propose that the binding of polyoma to P_m upstream of −10 is mediated by the α subunit rather than the σ70 subunit of RNAP.

Our results lead to the following model for interaction of Mor with RNAP during activation of P_m transcription (Fig. 7). The central point of this model is that Mor bound as a dimer to a hexamer, it would be tempting to propose that the binding of Mor-RNA polymerase interactions

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