The Bacteriophage T4 Transcription Activator MotA Interacts with the Far-C-Terminal Region of the $\sigma^{70}$ Subunit of Escherichia coli RNA Polymerase

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Transcription from bacteriophage T4 middle promoters uses Escherichia coli RNA polymerase together with the T4 transcriptional activator MotA and the T4 coactivator AsiA. AsiA binds tightly within the C-terminal portion of the $\sigma^{70}$ subunit of RNA polymerase, while MotA binds to the 9-bp MotA box motif, which is centered at $-30$, and also interacts with $\sigma^{70}$. We show here that the N-terminal half of MotA (MotA NT), which is thought to include the activation domain, interacts with the C-terminal region of $\sigma^{70}$ in an Escherichia coli two-hybrid assay. Replacement of the C-terminal 17 residues of $\sigma^{70}$ with comparable $\sigma^{35}$ residues abolishes the interaction with MotA NT in this assay, as does the introduction of the amino acid substitution R608C. Furthermore, in vitro transcription experiments indicate that a polymerase reconstituted with a $\sigma^{70}$ that lacks C-terminal amino acids 604 to 613 or 608 to 613 is defective for MotA-dependent activation. We also show that a proteolyzed fragment of MotA that contains the C-terminal half (MotACTD) binds DNA with a $K_{D(app)}$ that is similar to that of full-length MotA. Our results support a model for MotA-dependent activation in which protein-protein contact between DNA-bound MotA and the far-C-terminal region of $\sigma^{70}$ helps to substitute functionally for an interaction between $\sigma^{70}$ and a promoter $-35$ element.

A programmed cascade of transcriptional events is initiated when bacteriophage T4 infects its host Escherichia coli (reviewed in reference 57). T4 early genes are transcribed immediately after infection by using the existing host RNA polymerase holoenzyme comprising the core $(\alpha_2\beta\beta')$ and the $\sigma^{70}$ subunit. Early T4 promoters do not require T4-encoded transcription factors, since they contain excellent matches to the T4 early promoter consensus sequence (5, 9, 17, 29, 54). In the absence of MotA, AsiA binding to $\sigma^{70}$ inhibits transcription by RNA polymerase from promoters that require recognition of the $\sigma^{70}$ canonical sequences (8, 45, 51), suggesting that the presence of AsiA inhibits the $\sigma^{70}$ region 4.2-DNA interaction.

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In this paper we show that the interaction of a MotA N-terminal peptide (amino acids 1 to 97) with $\sigma^{70}$, like the interaction of AsiA with $\sigma^{70}$, involves the C-terminal region of $\sigma^{70}$. In addition, deletions of the amino acids within the far-C-terminal region of $\sigma^{70}$ (amino acids 604 to 613) impair the ability of RNA polymerase to perform MotA-dependent activation in vitro. We also show that a MotA C-terminal peptide, beginning at amino acid 102, binds DNA with an apparent dissociation constant like that of wild-type MotA. Thus, MotA-dependent activation in which the interaction between DNA-bound MotA and the C-terminal region of $\sigma^{70}$ helps to substitute functionally for an interaction between $\sigma^{70}$ and a promoter $-35$ element.

**MATERIALS AND METHODS**

Strains. Escherichia coli KS1 (12) contains a chromosomal lacZ reporter gene under the control of a derivative of the lac promoter $P_{lac}$ that carries a lambda operator (O$_{\lambda}$(2)) centered at position $-62$ in place of the binding site for the catabolite receptor protein normally associated with $P_{lac}$. KS1 also contains an F$^+$ episome

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bearing lac\(^{p}\) and a gene for kanamycin resistance. *E. coli* XLI-Blue (Stratagene) was used for plasmid propagation and plasmid constructions.

DNA. Oligonucleotide primers were obtained from Gene Probe Technologies and Cruachem Inc. ( Primer sequences are available upon request.) The 5′-terminal labeled 74-bp pAC\(^{p}\) DNA containing the P\(_{\text{mal}}\) promoter is present in five to +18, was obtained as described previously (23), pDKT90, which contains the T4 middle promoter P\(_{\text{mal}}\), has been described elsewhere (37). Linear templates for transcription, obtained by BsaAI restriction of pDKT90, were purified by phenol extraction followed by ethanol precipitation.

The pBR\(^{78}\) plasmid isoschimera plasmid (11) contains a ColE1 replication origin, confers carbenicillin resistance, and directs transcription of an α-σ\(^{78}\) plasmid gene under the control of the tandem promoters P\(_{\text{pp}}\) and P\(_{\text{mal}}\). The resulting α-σ\(^{78}\) plasmid is a composite of the NTD of an amino acid (1 to 248) fused in frame to the C-terminal region of α-σ\(^{78}\) (amino acids 302 to 613). The pBR\(^{78}\) derivative encoding α-σ\(^{78}\) (R596H) has been described previously (11), and the pBR\(^{78}\) derivatives encoding α-σ\(^{78}\) carbenicillin-bearing substrates H606X, H606X+R, and R606C were constructed similarly. All of these derivatives are identical to pBR\(^{78}\) except for the indicated changes. The α-σ\(^{78}\) plasmid isoschimera pBR\(^{78}\) (11), is identical to pBR\(^{78}\) except that all of the α-σ\(^{78}\) sequences have been replaced with the comparable α-σ\(^{78}\) sequences (encoding amino acids 243 to 330). The α-σ\(^{78}\) hybrid plasmid, pBR\(^{78}\)α-σ\(^{78}\), was constructed by replacing the α-σ\(^{78}\) sequences encoding amino acids 597 to 613 with the corresponding α-σ\(^{78}\) sequences (encoding amino acids 312 to 330) by PCR and standard cloning techniques.

The pAChC322 plasmid (27), which was used to construct chimeric fusion proteins, contains a chloramphenicol resistance marker, a p15A replication origin, and a short polymerase (Stratagene) and cloned into pAC\(^{ Not II sites to allow ligation with pAC\(^{III}\) and pAChC322. Primers contained the necessary standard cloning techniques.

Mot21NTD) (18), or pAsiA (to construct pAsiA/AsiA) (22). The 70 chimera protein is composed of the NTD of an amino acid (1 to 248) fused in frame to the C-terminal region of α-σ\(^{78}\) (amino acids 302 to 613). The pAChC322 plasmid was obtained from the resulting plasmid. This fragment was used to replace the corre-
where the control was the units obtained with KS1/pc-MotA170/pBRa-s70.

Native protein gels. Protein complexes were assayed by electrophoresis on native polyacrylamide gels as described previously (18).

In vitro transcriptions. Incubation buffer I contained 44 mM Tris-Cl (pH 8), 50 mM NaCl, 42% glycerol, 1 mM EDTA, 0.18 mM dithiothreitol, 0.008% Triton X-100, and 0.2 mM 2-mercaptoethanol. Incubation buffer II contained 5 mM Tris-acetate (pH 7.5), 69 mM NaCl, 5% glycerol, 0.18 mM EDTA, 0.27 mM dithiothreitol, 260 mM potassium glutamate, 6.9 mM magnesium acetate, and 173 $\mu$g of bovine serum albumin/mL. DNA buffer contained 7.4 mM Tris-Cl (pH 7.9), 51 mM NaCl-acetate (pH 7.9), 57 mM NaCl, 2.1% glycerol, 0.66 mM EDTA, 0.13 mM dithiothreitol, 0.21 mM 2-mercaptoethanol, 190 mM potassium glutamate, 5.1 mM magnesium acetate, 130 $\mu$g of bovine serum albumin/mL, 220 $\mu$M ATP, 220 $\mu$M GTP, 220 $\mu$M CTP, and 11 $\mu$M [32P]UTP (6.5 x 10^7 dpm/umol). Finally, 1x Tris-borate-EDTA (TBE) contained 2.5 mM EDTA and 89 mM Tris-borate (pH 8.3).

Proteins were preincubated and mixed with the DNA template and other transcription components as indicated in the figure legend. Reaction mixtures were then placed at 37°C for 20 s before the addition of 0.5 $\mu$M rifampin at 300 pg/mL. After an additional 7.5 min at 37°C, reaction mixtures were collected on 17 mM Tris-Cl (pH 7.9), 280 mM NaCl, 22% glycerol, 0.7 mM EDTA, 0.07 mM 2-mercaptoethanol, 0.04 mM dithiothreitol, and (as indicated by $\beta$ or $\alpha$) 80 pmol of AsIA, 50 pmol of MotA, 14 pmol of $\sigma^{70}$ and 14 pmol of $\sigma^{70}$-570 was incubated at 37°C for 5 min. Samples were then subjected to electrophoresis in a 6% polyacrylamide native protein gel, and proteins were detected after staining with colloidal Coomassie blue (Invitrogen). The positions of free $\sigma^{70}$ and free $\sigma^{70}$-570 are indicated. (Both the $\sigma^{70}$ and the $\sigma^{70}$-570 preparations contain a slow-moving band that is consistent with the presence of $\sigma$ dimer.) The locations of the AsIA-70 complex, the MotA-70 complex, and the AsIA-70-MotA complex are indicated by arrowheads.

RESULTS

A $\sigma^{70}$ lacking the last 43 amino acids ($\sigma^{70}$-570) fails to make a discrete complex with MotA. The T4 MotA and AsIA proteins each form a complex with $\sigma^{70}$ that can be distinguished from the free proteins on native gel electrophoresis (18) (Fig. 1, lanes 4 and 5). $\sigma^{70}$-570 contains 613 amino acids, which are divided into regions 1 through 4 based on sequence similarity among the various members of the sigma protein family (35). Binding sites for AsIA have been found within the C-terminal region of $\sigma^{70}$ in both region 4.1 and region 4.2 (amino acids 567 to 600) (8, 49, 50, 53, 59). To test whether the last 43 residues of $\sigma^{70}$, which include region 4.2, were also important for MotA-$\sigma^{70}$ complex formation, we assayed the formation of complexes by using $\sigma^{70}$-570, a truncated $\sigma^{70}$ that contains amino acids 1 through 570. As expected, AsIA did not form a complex with $\sigma^{70}$-570 (Fig. 1, lane 8). MotA also did not form the discrete complex with $\sigma^{70}$-570 that was seen with $\sigma^{70}$ (Fig. 1, compare lanes 9 and 5). Instead, a very diffuse band migrating behind the position of free AsIA was observed. These results indicate that a $\sigma^{70}$ missing region 4.2 is not capable of forming a discrete complex with MotA that is stable under the conditions of electrophoresis and thus implicate region 4.2 in the MotA-$\sigma^{70}$ interaction.

The C-terminal region of $\sigma^{70}$ interacts with the N-terminal domain of MotA in an E. coli two-hybrid assay. To investigate further the possibility of an interaction between MotA and the C-terminal region of $\sigma^{70}$, we used an E. coli-based two-hybrid system (11, 27). In this system (Fig. 2), a chromosomal reporter lacZ gene lies downstream of a promoter that has a binding site for $\lambda$ cI protein at position -62. The bait is created by the fusion of a protein or domain of interest to the 3' end of cI. The prey consists of $\sigma^{70}$ amino acids 528 to 613, which start within region 3.2 and then include all of region 4 plus the far-C-terminal region (35). This prey is fused in frame to the NTD of the $\alpha$ subunit of RNA polymerase. The addition of IPTG induces the synthesis of both the $\alpha$-70 chimera and the cI-bait protein. An interaction between the bait protein positioned at -62 and the C-terminal region of $\sigma^{70}$, which is available in the pool of RNA polymerase that contains the $\alpha$-70 chimera, then increases lacZ transcription.

As has been previously reported (10), there was a large increase in $\beta$-galactosidase activity upon the addition of IPTG to cells containing pC1-AsIA and pBRa-s70 (Fig. 3). To assay an interaction between MotA and the $\sigma^{70}$ prey, we tested a bait consisting of cI fused to the entire MotA gene (cI-MotA) and a bait in which the motA gene was positioned out of frame with cI (cI-MotA). The addition of IPTG resulted in an increase in

![FIG. 1. Formation of discrete complexes of AsIA-$\sigma^{70}$ and MotA-$\sigma^{70}$ requires the last 43 amino acids of $\sigma^{70}$. A 7.25:1 mixture containing 17 mM Tris-Cl (pH 7.9), 280 mM NaCl, 22% glycerol, 0.7 mM EDTA, 0.07 mM 2-mercaptoethanol, 0.04 mM dithiothreitol, and (as indicated by $\beta$ or $\alpha$) 80 pmol of AsIA, 50 pmol of MotA, 14 pmol of $\sigma^{70}$ and 14 pmol of $\sigma^{70}$-570 was incubated at 37°C for 5 min. Samples were then subjected to electrophoresis in a 6% polyacrylamide native protein gel, and proteins were detected after staining with colloidal Coomassie blue (Invitrogen). The positions of free $\sigma^{70}$ and free $\sigma^{70}$-570 are indicated. (Both the $\sigma^{70}$ and the $\sigma^{70}$-570 preparations contain a slow-moving band that is consistent with the presence of $\sigma$ dimer.) The locations of the AsIA-$\sigma^{70}$ complex, the MotA-$\sigma^{70}$ complex, and the AsIA-70-MotA complex are indicated by arrowheads.)](http://jb.asm.org/)

![FIG. 2. E. coli two-hybrid system for detecting interactions between the C-terminal region of $\sigma^{70}$ and other proteins. The cartoon depicts the positions of RNA polymerase subunits $\beta$, $\beta'$, and $\sigma^{70}$ and the $\alpha$-$\sigma^{70}$ chimera, which consists of the N-terminal domain of $\alpha$ fused to the C terminus of $\sigma^{70}$, at a promoter upstream of the reporter lacZ gene. The cI-bait fusion protein is located at position -62. (See text for details.)](http://jb.asm.org/)

![FIG. 3. Formation of discrete complexes of AsIA-$\sigma^{70}$ and MotA-$\sigma^{70}$ requires the last 43 amino acids of $\sigma^{70}$. A 7.25:1 mixture containing 17 mM Tris-Cl (pH 7.9), 280 mM NaCl, 22% glycerol, 0.7 mM EDTA, 0.07 mM 2-mercaptoethanol, 0.04 mM dithiothreitol, and (as indicated by $\beta$ or $\alpha$) 80 pmol of AsIA, 50 pmol of MotA, 14 pmol of $\sigma^{70}$ and 14 pmol of $\sigma^{70}$-570 was incubated at 37°C for 5 min. Samples were then subjected to electrophoresis in a 6% polyacrylamide native protein gel, and proteins were detected after staining with colloidal Coomassie blue (Invitrogen). The positions of free $\sigma^{70}$ and free $\sigma^{70}$-570 are indicated. (Both the $\sigma^{70}$ and the $\sigma^{70}$-570 preparations contain a slow-moving band that is consistent with the presence of $\sigma$ dimer.) The locations of the AsIA-$\sigma^{70}$ complex, the MotA-$\sigma^{70}$ complex, and the AsIA-70-MotA complex are indicated by arrowheads.)](http://jb.asm.org/)

![FIG. 4. E. coli two-hybrid system for detecting interactions between the C-terminal region of $\sigma^{70}$ and other proteins. The cartoon depicts the positions of RNA polymerase subunits $\beta$, $\beta'$, and $\sigma^{70}$ and the $\alpha$-$\sigma^{70}$ chimera, which consists of the N-terminal domain of $\alpha$ fused to the C terminus of $\sigma^{70}$, at a promoter upstream of the reporter lacZ gene. The cI-bait fusion protein is located at position -62. (See text for details.)](http://jb.asm.org/)

![FIG. 5. E. coli two-hybrid system for detecting interactions between the C-terminal region of $\sigma^{70}$ and other proteins. The cartoon depicts the positions of RNA polymerase subunits $\beta$, $\beta'$, and $\sigma^{70}$ and the $\alpha$-$\sigma^{70}$ chimera, which consists of the N-terminal domain of $\alpha$ fused to the C terminus of $\sigma^{70}$, at a promoter upstream of the reporter lacZ gene. The cI-bait fusion protein is located at position -62. (See text for details.)](http://jb.asm.org/)
the level of β-galactosidase activity in cells containing the cI-MotA fusion compared to the activity seen in the presence of cI-MotA or cI alone (Fig. 3). Although this was only a twofold increase over background, it was highly reproducible. In addition, in control assays, cells containing pC-MotA but lacking pBrα-σ70 expressed background levels of lacZ (data not shown), indicating that by itself pC-MotA was not responsible for the increase in β-galactosidase activity.

Residues within the NTD of MotA are required for MotA activation (14, 18). Induced synthesis of a cI-MotA fusion, which contained MotA amino acids 1 to 97, in cells containing the α-σ70 chimera resulted in a significant increase in β-galactosidase activity (Fig. 3). As a control, we constructed a plasmid containing a fusion of cI with the comparable NTD of Mot21, a mutant of MotA in which the first 8 amino acids of MotA have been replaced with 11 different amino acids. Mot21 is a positive control mutant of MotA (18). Full-length Mot21 binds DNA like wild-type MotA but fails to activate transcription or form a complex with σ70 in a native protein gel (18). Production of cI-Mot21NTD in cells containing the α-σ70 chimera resulted in a β-galactosidase activity curve that was coincident with that observed with cI alone (Fig. 3). In addition, the presence of pC-Mot21NTD alone (in the absence of pBrα-σ70) resulted only in background levels of β-galactosidase activity (data not shown). Taken together, these results suggest that MotA interacts with the C-terminal region of σ70 and that the NTD of MotA is sufficient for this interaction.

The MotA-σ70 Interaction in the Two-hybrid Assay Involves the Far-C-terminal Region of σ70. σ70, an alternative sigma factor for E. coli RNA polymerase that is used during stationary phase and under certain conditions of stress (58; reviewed in references 20 and 21), has a region 4 which is similar in amino acid sequence to that of σ38 (35). Replacement of the σ70 residues in the α-σ70 chimera with the comparable region of σ38 (amino acids 243 to 330) resulted in background levels of β-galactosidase activity when tested with either cI-MotA or cI-MotA but resulted in nearly 600 Miller units of β-galactosidase activity when tested with cI-AsiA (Fig. 4). In addition, the R608C substitution had no significant effect on the level of β-galactosidase activity observed with cI-AsiA (Fig. 4). Furthermore, an α-σ38 hybrid chimera, in which only the last 17 amino acids of σ70 (597 to 613) were replaced with comparable σ38 residues (amino acids 312 to 330), also gave background levels of β-galactosidase activity with cI-MotANTD (Fig. 5). In contrast, this chimera gave only twofold less activity with the cI-AsiA fusion than did the α-σ70 chimera (Fig. 5). These results suggest that the far-C-terminal region of σ70 is involved in the σ70-MotA interaction.

To investigate the need for specific σ70 C-terminal residues, we tested α-σ70 chimeras containing the single amino acid substitutions R596H, H600A, H600R, or R608C in the σ70 moiety (Fig. 5). The R596H, H600A, and H600R substitutions had little effect on the level of β-galactosidase activity observed with either cI-MotANTD or cI-AsiA. In addition, the R608C substitution had no significant effect on the level of β-galactosidase activity observed with cI-AsiA. However, this substitution caused a significant defect when the mutant chimera was tested with cI-MotANTD. Varying the IPTG concentration from 5 μM to 1 mM gave results similar to those obtained with 100 mM IPTG (data not shown), indicating that the low level of β-galactosidase activity observed with cI-MotANTD and α-σ38 could not be improved by increasing the levels of these proteins. In summary, these two-hybrid assays suggested that a residue(s) within the far-C-terminal region of σ70 is important for an interaction with MotA but is relatively unimportant for the σ70-AsiA interaction.
Polymerase reconstituted with a σ70 lacking either amino acids 608 to 613 or amino acids 604 to 613 is defective for MotA-dependent transcription in vitro. To investigate the involvement of the far-C-terminal amino acids of σ70 in MotA-dependent transcription, we tested σ70 mutant proteins in single-round in vitro transcription reactions by using a DNA template containing the T4 middle promoter P\textit{uvX}\textsubscript{R596H}. Control reactions contained either wild-type σ70 (σ\textsuperscript{R596H}), α-σ70 (H600A), α-σ70 (H600R), or α-σ70 (R608C). (See Materials and Methods for the determination of relative β-galactosidase activity.) Points and standard deviations (indicated by error bars) represent the averages of the results of two to eight assays.

FIG. 5. The MotA-σ70 interaction in the E. coli two-hybrid assay requires the last 17 amino acids of σ70. Relative β-galactosidase activity is shown for assays with cl-AsiA (top panel) or cl-MotANTD (bottom panel) with α-σ70 (σ35), α-σ70 (R596H), α-σ70 (H600A), α-σ70 (H600R), or α-σ70 (R608C). (See Materials and Methods for the determination of relative β-galactosidase activity.) Points and standard deviations (indicated by error bars) represent the averages of the results of two to eight assays.

result suggested either that the MotA-σ70 interaction inferred from the two-hybrid assay was not relevant or that in the context of the transcription complex, this single mutation was not sufficient to impair MotA activity. Thus, we investigated whether σ70 C-terminal deletions of 6 (σ\textsuperscript{Δ608-613}) or 10 (σ\textsuperscript{Δ604-613}) amino acids affected transcription from P\textit{uvX}. Although the level of transcription was lower than that of the wild type, polymerase containing either deletion was able to transcribe from P\textit{uvX} in the absence of MotA/AsiA. Thus, the deletions did not destroy polymerase activity (Fig. 6A). When polymerase with σ\textsuperscript{Δ608-613} or σ\textsuperscript{Δ604-613} was used, AsiA alone inhibited transcription significantly or partially, respectively (Fig. 6). Thus, polymerases with these deletions were still susceptible to AsiA inhibition, although the larger deletion was more resistant to AsiA action. In contrast, polymerase with either σ70 deletion was significantly impaired in MotA/AsiA activation at P\textit{uvX} (Fig. 6). The transcription results support the idea that the far-C-terminal region of σ70 is important for MotA to function effectively as an activator. We speculate that the single R608C substitution was not sufficient to interfere with MotA activation in vitro because other stabilizing contacts within the transcription complex compensated for this mutation.

The CTD of MotA binds DNA. We tested MotA\textsuperscript{cloned CTD}, a protein containing amino acids 105 to 211, and MotA\textsuperscript{proteolyzed CTD}, a proteolyzed fraction of MotA starting at amino acid 102, for their abilities to bind a DNA fragment containing the T4 middle promoter P\textit{uvX} in a gel retardation assay. Both peptides bound P\textit{uvX} DNA (Fig. 7 and data not shown). The K\textsubscript{D(app)} determined for MotA\textsuperscript{proteolyzed CTD} was 400 nM, a value that is similar to the previously reported values of 220 nM (6) and 130 nM (52) for wild-type MotA. For MotA\textsuperscript{cloned CTD}, the determined K\textsubscript{D(app)} was fourfold higher (2,000 nM). These results suggest that the CTD of MotA is sufficient to bind DNA. As expected, MotA\textsuperscript{proteolyzed CTD}, which lacks the MotA\textsuperscript{NTD} that is required for activation, did not support activated transcription from P\textit{uvX} in vitro (data not shown).

DISCUSSION

Bacteriophage T4 middle promoters represent a hybrid of host and phage promoter sequences, having an excellent match to the σ70 − 10 recognition sequence but lacking a good match to σ70 recognition sequences at −35 (3, 19, 38). It is common for a promoter that lacks canonical −35 sequences to require an activator(s) for transcription initiation by RNA polymerase. Such activators fall into two general categories (reviewed in references 4, 26, and 46). Class I activators bind to sites located upstream of the promoter sequences (at −61.5 or farther), while class II activators bind to sites centered near position −41.5, immediately adjacent to core promoter sequences. In both cases, however, these proteins appear to work by contacting polymerase directly and stabilizing the interaction of σ70 region 4.2 with noncanonical sequences within the −35 region of the promoter (2, 11, 30; reviewed in references 4, 26, and 46).

Evidence indicates that MotA/AsiA-dependent activation does not fit either class I or class II. The MotA binding site lies within the core promoter sequence rather than adjacent to it or
farther upstream (3, 19, 38). In addition, within the MotA-AsiA-RNA polymerase-middle promoter complex, σ70 retains its contacts with the region of the DNA, but the upstream protein-promoter contacts are significantly rearranged (1, 24).

We have previously proposed a model to explain this middle promoter architecture (52). In this model (Fig. 8), AsiA interacts with residues within region 4 of σ70 (8, 49, 50, 53, 59), MotA binds to the MotA box (23, 48) and interacts with σ70 (18), and σ70 region 2.4 retains its contacts with the -10 element of the promoter DNA (24). We speculated that positioning an interaction between MotA and the C-terminal region of σ70 would be reasonable, given that residues within region 4.2 of σ70 normally interact with the -35 sequences of the DNA and that the MotA binding site is centered at -30 (52). The work here demonstrates that the NTD of MotA, which contains residues needed for activation (14, 18), can

![Retarded DNA](image1.png)

**FIG. 7.** A C-terminal peptide of MotA binds DNA. Gel retardation assays, which contained 0.5 pmol of the 32P-labeled 74-bp PuvX DNA, 26 ng of poly(dI-dC) competitor DNA, and buffer (lane 1), protein fraction from uninduced BL21(DE3) cells containing the plasmid with MotA cloned CTD (lane 2), or 16 pmol of MotA cloned CTD protein in a purified fraction from induced BL21(DE3) cells containing the plasmid with MotA cloned CTD (lane 3), are shown. The fraction used in lane 2 was purified in a manner similar to that of the fraction used in lane 3.

![Model](image2.png)

**FIG. 8.** Model of MotA/AsiA activation at a T4 middle promoter. The cartoon depicts the positions of σ70, AsiA, and MotA at a T4 middle promoter. MotANTD interacts with the MotA box motif (5'-T/A)[T/A]TGCTT[T/C]A 3') centered at -30. Both AsiA and MotANTD interact with the C-terminal region of σ70. The positions of σ70 regions 2.4 and 4.2 are shown. (See text for details.)
interact with the C-terminal region of $\sigma^{70}$. In addition, we have shown that a C-terminal peptide of MotA starting at amino acid 102 can bind DNA with a binding constant similar to that of the full-length protein. These results are consistent with the idea that the two physical domains of MotA (15), an NTD that is formed by five $\alpha$-helices and a short $\beta$-ribbon (34) and a CTD that is composed of three $\alpha$-helices interspersed with six $\beta$-strands (33), represent two functionally distinct domains. We suggest that MotA belongs to a class of activators that is distinct from both class I and class II. Instead of stabilizing the typical contacts between region 4 of $\sigma^{70}$ and the DNA, an activator in this third class functionally replaces such contacts by serving as a molecular bridge between $\sigma^{70}$ and the DNA.

The amino acid sequence of region 4 of $\sigma^{38}$ is similar to that of region 4 of $\sigma^{70}$ (35) and recognizes the same $\sim 35$ canonical promoter element (13, 16). We found that AsIA interacted with the C-terminal region of $\sigma^{38}$ in the two-hybrid assay. AsIA binds to a broad surface of $\sigma^{70}$ region 4.2 (8, and AsIA can tolerate amino acid changes throughout this binding surface (41). In addition, in the two-hybrid assay, the AsIA-$\sigma^{70}$ interaction was not significantly affected by mutations at residues R596, H597, or R608 (reference 10 and this paper). Thus, the simple explanation for an AsIA-$\sigma^{38}$ interaction is that this interaction occurs because region 4.2 of $\sigma^{38}$ is very similar to that of $\sigma^{70}$. However, this result is surprising, since AsIA neither inhibits transcription by polymerase containing $\sigma^{38}$ nor forms a complex with full-length $\sigma^{38}$ in a native protein gel (8). Our results suggest that AsIA is indeed able to interact with the C-terminal region of $\sigma^{38}$ but that some feature of the full-length protein prevents this interaction.

In contrast to the results seen with AsIA, replacement of even the last 17 amino acids of $\sigma^{70}$ with comparable $\sigma^{38}$ residues eliminated the stimulation of $\beta$-galactosidase activity observed with c1-MotA$^{NTD}$. This effect is specific for MotA, since the $\sigma^{70}/\sigma^{38}$ chimera worked both with c1-AsIA (this paper) and with a c1 that had been fused to the E. coli anti-sigma protein Rsd (S. L. Dove and A. Hochschild, unpublished data). In addition, a substitution of R608C in $\alpha$-$\sigma^{70}$ also reduced the interaction with c1-MotA$^{NTD}$ but had no effect on the interaction with c1-AsIA. However, not all substitutions within the C-terminal region of $\sigma^{70}$ weakened the interaction of $\sigma^{70}$ with c1-MotA$^{NTD}$. In particular, a substitution at R596 or H600 had no significant effects. In contrast, the substitution R596H significantly reduced the interaction between the fused $\sigma^{70}$ moiety and either Rsd from E. coli or the related regulator, AlgQ, from Pseudomonas aeruginosa (10). The specific effects of these various substitutions argue that the defects seen with c1-MotA$^{NTD}$ and the mutant $\alpha$-$\sigma^{70}$ chimeras arise from a loss of the MotA-$\sigma^{70}$ interaction rather than a misfolding of the mutant chimeras. Furthermore, our in vitro transcription experiments indicated that a $\sigma^{70}$ lacking 6 or 10 C-terminal amino acids is defective for MotA activation of transcription. Taken together, our results are consistent with the idea that the far-C-terminal region of $\sigma^{70}$ contacts MotA and that this contact is necessary for MotA to work as an activator. Previous work has indicated that an amino acid substitution at position 604 can partially suppress the growth defect of a T4 motA-positive control mutant in vivo (7), a result that is compatible with this conclusion.

The far-C-terminal region of $\sigma^{70}$ lies within an alpha helix (amino acids 603 to 613) (5, 36) at the very end of the protein. This region is just C-terminal of residues that interact with the $\sim 35$ region of DNA (residues 584, 585, and 588) (5, 9, 17, 29, 54) and of residues that have been implicated in the interactions of $\sigma^{70}$ with E. coli class II activators (residues 590 to 603) (32, 36). Thus, the C terminus of $\sigma^{70}$ appears to be involved both in class II activation and in the architecturally different activation achieved by MotA/AsIA. Further studies will be needed to determine exactly how the region is configured in class II- versus MotA/AsIA-dependent activation.

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