Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 1451–1455, February 1995
Biochemistry

Bacteriophage T4 MotA and AsiA proteins suffice to direct *Escherichia coli* RNA polymerase to initiate transcription at T4 middle promoters

(positive control/development/regulation/viruses)

MOHAMED OUHAMMOUCH*, KAREN ADELMAN*, SHASHIKUMAR R. HARVEY*, GILBERT ORSINI†, AND EDWARD N. BRODY*‡

*State University of New York at Buffalo, Department of Biological Sciences, 109 Cooke Hall, Buffalo, NY 14260; and †Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette, France

Communicated by E. Peter Geiduschek, University of California, San Diego, CA, November 18, 1994

ABSTRACT **Development of bacteriophage T4 in *Escherichia coli* requires the sequential recognition of three classes of promoters: early, middle, and late. Recognition of middle promoters is known to require the *motA* gene product, a protein that binds specifically to the "Mot box" located at the −30 region of these promoters. *In vivo*, the *asiA* gene product is as critical for middle mode RNA synthesis as is that of the *motA* gene. *In vitro*, AsiA protein is known to loosen the σ^{70}–core RNA polymerase interactions and to inhibit some σ^{70}-dependent transcription, presumably through binding to the σ^{70} subunit. Here we show that, *in vitro*, purified MotA and AsiA proteins are both necessary and sufficient to activate transcription initiation at T4 middle promoters by the *E. coli* RNA polymerase in a σ^{70}-dependent manner. AsiA is also shown to inhibit recognition of T4 early promoters and may play a pivotal role in the recognition of all three classes of phage promoters.**

In the course of phage T4 development in *Escherichia coli*, all phage gene transcripts are synthesized by the host RNA polymerase (RNAP), whose structure and functional properties are modified by phage-coded proteins, leading to the sequential recognition of three different classes of promoters: early, middle, and late. The early promoters are recognized immediately after infection by unmodified host RNAP even when phage protein synthesis is inhibited. They are also recognized *in vitro* on T4 DNA by purified *E. coli* RNAP holoenzyme ($\alpha_2\beta\beta'-\sigma^{70}$). During this early period, the host RNAP undergoes several T4-induced changes (1). Its α subunits are ADP-ribosylated (by the T4 *alt* and *mod* gene products), and it becomes tightly associated with at least two small T4 proteins, RpbA and AsiA. By 3 min after infection (at 30°C), some, if not all, of these early promoters are turned off (2, 3), but the mechanism of this transcriptional shutoff has not yet been elucidated. At about the same time, transcription from middle promoters is turned on. These promoters have a conserved "Mot box" sequence, 5′-(a/t)(a/t)TGCTT(t/c)A-3′, centered around bp −30, 11–13 bases upstream of a standard *E. coli* −10 consensus sequence (4–6). The T4 late promoters have simpler sequence determinants, consisting primarily of the octamer TATAAATA at the −10 region. Transcription from these late promoters requires, among other things, the phage σ factor gp55 (7). *In vitro*, the *E. coli* σ^{70} has been shown to be dominant over gp55 in competition for core RNAP and reduces late promoter recognition (8, 9). Yet, during the late period of T4 development, σ^{70} and gp55 coexist (and apparently cofunction) in the infected cell (10, 11).

Recognition of T4 middle promoters requires the early gene product MotA. MotA protein has been purified and is known to bind specifically to the −30 Mot box sequence of middle promoters (refs. 12 and 13; M.O. and E.N.B., unpublished results). *In vitro*, MotA was shown to activate transcription from cloned middle promoters by T4-modified RNAP (purified from T4-infected cells) but not by unmodified RNAP, suggesting a direct role of one (or some) of the modifications undergone by the host RNAP in MotA-dependent transcription activation (12, 13). Recently, we showed that MotA-dependent middle mode RNA synthesis was completely abolished during T4 *amS22 asiA* gene mutant infection, suggesting that AsiA is a positive regulator of middle mode RNA synthesis, as important as is MotA. The T4 *asiA* gene codes for a 10.6-kDa protein whose biochemical properties have been known for two decades. It copurifies with RNAP and can be detached from it, along with σ^{70}, on phosphocellulose chromatography (14, 15). It inhibits σ^{70}-dependent transcription initiation by unmodified or ADP-ribosylated RNAP at relatively high ionic strength (14, 16, 17). In this paper, we report the development of an *in vitro* middle-transcription system utilizing unmodified *E. coli* RNAP and purified MotA and AsiA proteins. We show that both proteins are necessary and sufficient to specify transcription initiation at T4 middle mode promoters by the *E. coli* RNAP holoenzyme. We also confirm that AsiA protein inhibits recognition of T4 early promoters.

MATERIALS AND METHODS

Materials. Purified *E. coli* core RNAP and σ^{70} were generously provided by E. P. Geiduschek. σ^{70} was also provided by K. Severinov. Avian myeloblastosis virus (AMV) reverse transcriptase was obtained from Promega. Other enzymes and chemicals were purchased from commercial sources.

Bacteria, Phages, and Plasmids. *E. coli* strains JM101 and JM109 were used in the overproduction of AsiA and MotA proteins, respectively. The bacteriophage λ derivative CE6 (18) was used for delivering T7 RNA polymerase. The plasmid pBAS-M1 carries a copy of the T4 *asiA* gene with an improved ribosome binding site, under the control of a T7 promoter (17). Plasmid pGEM-MOT1 was constructed by inserting a 768-bp PCR DNA fragment containing the T4 *motA* gene in the multiple cloning site of the pGEM-3Z cloning vector (Promega), under the control of the T7 promoter.

Protein Expression and Purification. *E. coli* JM101 bacteria carrying the plasmid pBAS-M1 were grown and were infected as described (17). After lysis, proteins were subjected to $(NH_4)_2SO_4$ fractionation. The bulk of AsiA was soluble in 50%

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RNAP, RNA polymerase; AMV, avian myeloblastosis virus.

‡To whom reprint requests should be addressed.

saturated $(NH_4)_2SO_4$. It was then fractionated on a Sephacryl S-100 column. AsiA-containing fractions were pooled and the KCl concentration was adjusted to 50 mM before loading on a Q-Sepharose Fast Flow anion-exchange column (Pharmacia), which had been equilibrated with TGED/50 mM KCl [TGED: 50 mM Tris·HCl, pH 7.9/0.1 mM EDTA/0.1 mM dithiothreitol (DTT)/5% glycerol]. A 50–500 mM KCl linear gradient in TGED was used to elute AsiA. For the overproduction of MotA, *E. coli* JM109 bacteria carrying the plasmid pGEM-MOT1 were grown and infected with phage λ CE6. Cell lysis and MotA purification from inclusion bodies were performed following the procedure described by Nguyen *et al.* (19). Briefly, MotA inclusion bodies were isolated and solubilized with 0.4% Sarkosyl. After refolding, MotA was separated from contaminating proteins by ion-exchange chromatography on a Q-Sepharose Fast Flow anion-exchange column (Pharmacia). A 0–1.5 M NaCl linear gradient was used to elute MotA. AsiA and MotA were judged to be at least 95% pure by polyacrylamide/SDS gel electrophoresis. Protein concentrations were measured with the BCA assay (Pierce) using bovine serum albumin (BSA) as the standard.

***In Vitro* Transcription Assays.** A 158-bp PCR DNA fragment containing the T4 MotA-dependent PrIIB2 middle promoter was used as a template for single-round transcription. One-tenth picomole of DNA and a given amount of MotA were combined on ice in 30 μl of transcription buffer (50 mM Tris·HCl, pH 7.9/100 mM KCl/10 mM $MgCl_2$/0.1 mM EDTA/1 mM DTT/100 μg of acetylated BSA per ml] and incubated at 37°C for 5 min. Reconstituted RNAP holoenzyme (0.5 pmol of core and 0.5 pmol of σ^{70}) that had been incubated for 5 min at 37°C with a given amount of AsiA (in 5 μl of transcription buffer) was then added to the DNA template-containing mixture. After formation of open promoter complexes at 37°C for 10 min, the substrate solution {15 μl of 0.4 mM ATP/0.4 mM CTP/0.4 mM GTP/0.1 mM UTP/5 μCi of [α-^{32}P]UTP (3000 Ci/mmol)} containing heparin (final concentration, 670 μg/ml) was added to initiate one round of RNA synthesis. After 5 min, reactions were stopped. The labeled transcripts were precipitated with ethanol and analyzed on an 8% polyacrylamide gel containing 8 M urea, in TBE buffer (89 mM Tris-borate/2 mM EDTA). Wild-type T4 DNA (0.05 pmol) was transcribed *in vitro* (in the transcription buffer described above) using 20 pmol of *E. coli* RNAP holoenzyme (20 pmol of core RNAP and 50 pmol of σ^{70}) in the presence or the absence of 21 pmol of MotA and/or 100 pmol of AsiA. Open promoter complexes were formed at 37°C for 15 min and multiple-round transcription was allowed for 10 min. The transcription products were then isolated as described by Hinton (20) and analyzed by primer extension, using the AMV reverse transcriptase and specific end-labeled primers (21).

RESULTS

To assess the direct role of AsiA in MotA-dependent transcription activation at T4 middle promoters, we have undertaken the development of a minimum *in vitro* middle transcription system that could account for our recently reported *in vivo* results (21) and also the *in vitro* results reported by Hinton (12) and by Schmidt and Kreuzer (13). We have overproduced and purified to homogeneity the T4 MotA and AsiA proteins (see *Materials and Methods*). In a gel-shift assay (M.O. and E.N.B., unpublished results), purified MotA binds specifically to a double-stranded oligonucleotide containing the −30 sequence of PrIIB2, the stronger MotA-dependent middle promoter of the T4 *rIIB* gene (5). We analyzed the ability of unmodified *E. coli* RNAP holoenzyme to form open promoter complexes at PrIIB2. Open promoter complex formation was allowed by preincubation of the reconstituted enzyme with a PCR DNA fragment containing PrIIB2 in the absence of the four ribonucleoside triphosphates (rNTPs). A single round of transcription was initiated by the simultaneous addition of rNTPs and heparin. As shown in Fig. 1*A*, neither RNAP core (lane 1) nor RNAP holoenzyme (lane 2) is able to form open promoter complexes at PrIIB2 efficiently. Even at a saturating concentration, MotA is unable to activate open complex formation by the RNAP holoenzyme (Fig. 1*A*, lane 5). However, addition of increasing amounts of AsiA to this MotA-containing transcription reaction results in increasing efficiencies of open promoter complex formation (Fig. 1*A*, lanes 6–11). This AsiA-dependent activation of PrIIB2 recognition by the RNAP holoenzyme also depends on the presence of MotA, since AsiA alone cannot activate open complex formation (Fig. 1*A*, lanes 3 and 4). Fig. 1*B* shows the quantitative analysis of these results. At saturating concentrations

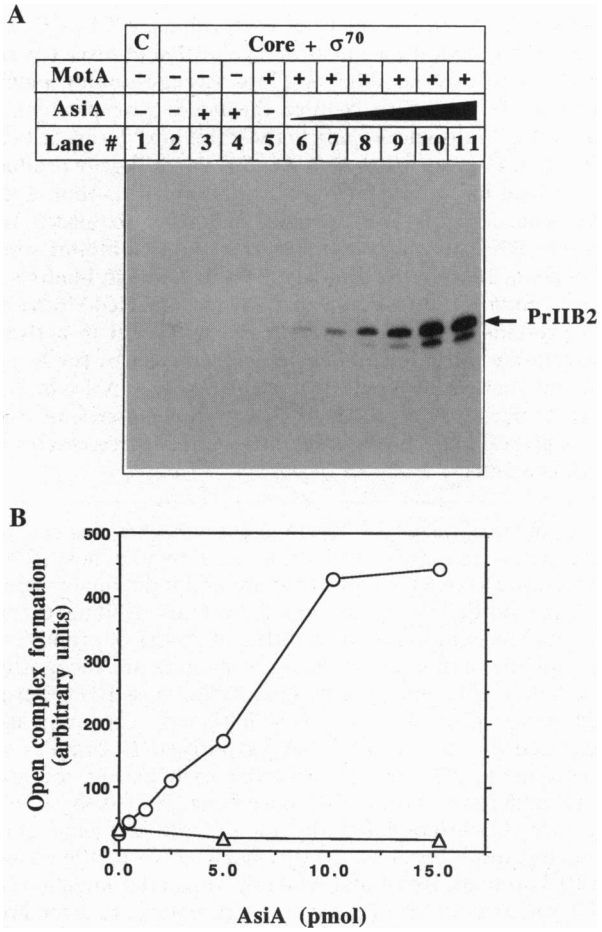

FIG. 1. *In vitro* transcription of the MotA-dependent PrIIB2 promoter by unmodified *E. coli* RNA polymerase. (*A*) For each reaction, 0.5 pmol of RNA polymerase core enzyme (C) was used, and the holoenzyme was reconstituted by the addition of 0.5 pmol of σ^{70}. Single round *in vitro* transcriptions were carried out as described in the text using core RNA polymerase (lane 1) or the holoenzyme (lanes 2–11) in the absence of MotA (lanes 2–4) or in the presence of 16 pmol of MotA (lanes 5–11); in the absence of AsiA (lanes 2 and 5) or in the presence of 0.5 pmol (lane 6), 1.3 pmol (lane 7), 2.5 pmol (lane 8), 5 pmol (lanes 3 and 9), 10.2 pmol (lane 10), or 15.3 pmol (lanes 4 and 11). PrIIB2 run-off transcripts are indicated by an arrow. Shorter PrIIB2 run-off transcripts probably initiated at position +3, relative to the major transcription initiation site, are also generated during these transcription reactions. (*B*) Quantitative analysis of the *in vitro* transcription results shown in *A*. Quantitation of PrIIB2 run-off transcripts was performed using a PhosphorImager, and transcription activity is presented as the efficiency of open promoter complex formation at PrIIB2 in the presence of various concentrations of AsiA, in the absence of MotA (△) or in its presence (○).

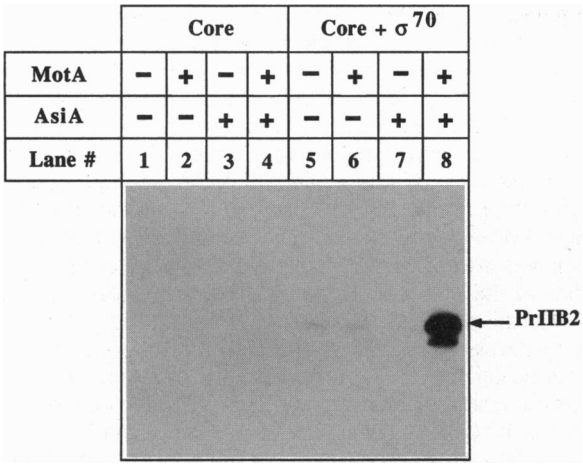

FIG. 2. Transcription activation by MotA at PrIIB2 requires σ^{70}. Single round *in vitro* transcriptions were carried out using 0.5 pmol of RNA polymerase core enzyme (lanes 1–4) or reconstituted holoenzyme (0.5 pmol of core and 0.5 pmol of σ^{70}) (lanes 5–8), in the presence of 16 pmol of MotA (lanes 2 and 6), 13.6 pmol of AsiA (lanes 3 and 7), or both proteins (lanes 4 and 8). PrIIB2 run-off transcripts are indicated by an arrow.

of MotA and AsiA, a 20- to 30-fold activation of open complex formation at the PrIIB2 promoter is seen, and the saturation curve is slightly, but reproducibly, sigmoidal. When the AsiA protein concentration is held constant and the MotA protein concentration is varied, we see the same sigmoidal activation curve (data not shown). The sigmoidal shape of these curves could reflect the cooperative nature of this activation, but that can only be assessed with further experiments.

Since the AsiA protein is known to loosen σ^{70}–core interactions (15, 17), we next asked whether the role of AsiA in MotA-dependent transcription activation is merely to dissociate σ^{70} from core RNAP. We thus compared *E. coli* core and RNAP holoenzyme with respect to their ability to promote open complex formation in the presence of AsiA, MotA, or a combination of the two proteins. Fig. 2 shows that σ^{70} is absolutely required for the stimulation of open complex formation at the PrIIB2 middle promoter and, again, that MotA and AsiA proteins are required.

The above experiments were performed with a cytosine-containing DNA template. In wild-type T4 DNA, however, cytosine is replaced with glucosylated 5-hydroxymethylcytosine; such modification could affect transcription activation at middle promoters. Furthermore, during the prereplicative period of phage development, 40–60 early promoters and perhaps 30–50 middle promoters are sequentially recognized by the host RNAP, with a transition from early to middle mode RNA synthesis at about 3 min after infection (at 30°C). To probe the role of AsiA and MotA in this transition, and to see whether transcription initiation at T4 DNA-borne middle promoters is activated by these two T4 proteins, we have transcribed purified T4 DNA, *in vitro*, using *E. coli* RNAP holoenzyme in the presence of either AsiA or MotA protein or the two together. Using specific ^{32}P-labeled oligonucleotides and the AMV reverse transcriptase, we analyzed transcripts initiated at five well-characterized MotA-dependent middle promoters: PrIIB1, PrIIB2, and P1 (5) as well as PuvsX (12) and PuvsY (13). As shown in Fig. 3, we saw very clearly that transcription activation at T4 PrIIB1 and PrIIB2 (Fig. 3*A*), P1 (Fig. 3*B*), and PuvsX (Fig. 3*C*) middle promoters depends on the simultaneous presence of the MotA and AsiA proteins. Similar results were obtained for PuvsY (data not shown).

Fig. 3 also shows that a different set of RNA molecules is synthesized in the absence of AsiA protein (lanes 1 and 3 of each series). These longer transcripts, which are initiated at early promoters located far upstream of these middle promoters, are absent when AsiA protein is present in the transcription reaction mixture, whether or not middle promoters are recognized (compare lanes 2 and 4 of each series). We therefore probed the same RNA samples for the presence of transcripts initiated at PmotA, a known and well-characterized T4 early promoter (2). The results in Fig. 4 show clearly that transcription from this early promoter continues unabated when the MotA protein is added to the reaction mixture but is almost completely inhibited in the presence of AsiA protein, whether or not MotA is present.

Thus, the AsiA protein alone, presumably by binding to σ^{70}, seems to suffice for the inhibition of early transcription and cooperates with MotA in simultaneously allowing recognition of middle promoters. Therefore, these two T4-coded proteins are sufficient to explain the major transcriptional switch that

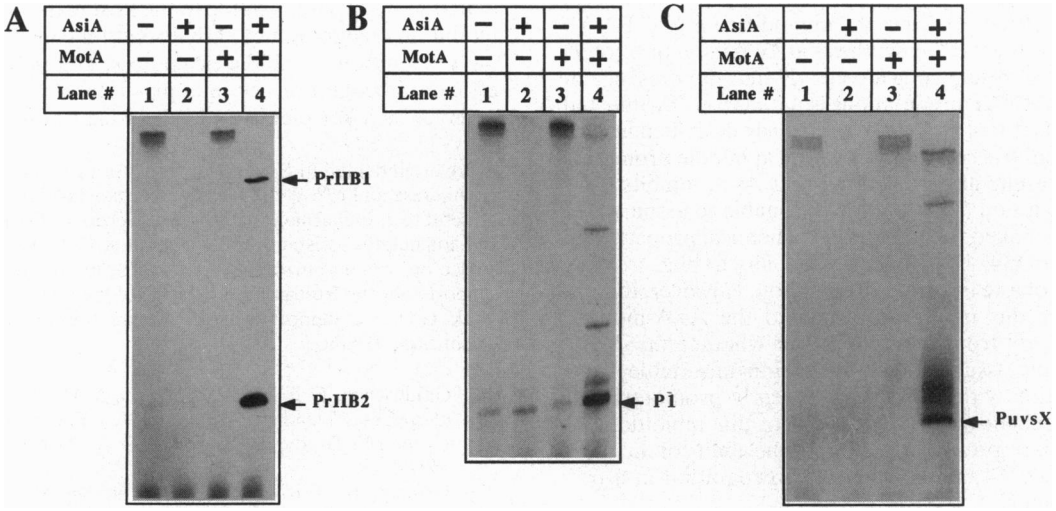

FIG. 3. Primer extension analyses of *in vitro rIIB (A)*, gene *1 (B)*, and *uvsX (C)* transcripts. Wild-type T4 DNA was transcribed *in vitro* using *E. coli* RNAP holoenzyme in the presence of AsiA (lanes 2), of MotA (lanes 3), or both proteins (lanes 4). The transcription products were isolated and analyzed by primer extension, using the AMV reverse transcriptase and specific end-labeled primers (12, 21). The labeled cDNA products were analyzed by electrophoresis on denaturing polyacrylamide gels. Reverse transcriptase stops corresponding to the 5′ ends of transcripts initiated at gene *rIIB* middle promoters PrIIB1 and PrIIB2, at gene *1* middle promoter P1, and those initiated at gene *uvsX* middle promoter PuvsX are indicated by arrows.

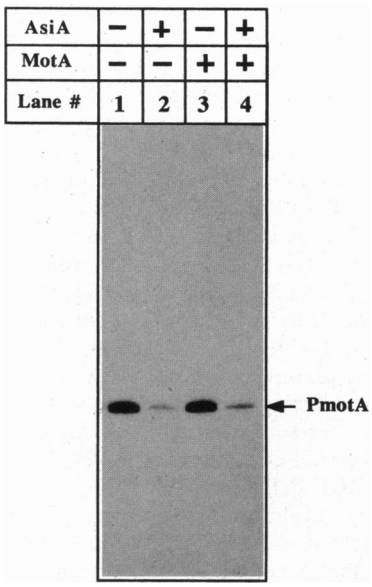

FIG. 4. Primer extension analysis of *in vitro motA* transcripts. The T4 DNA transcription products analyzed in Fig. 3 were hybridized with an end-labeled primer complementary to *motA* gene transcripts (2) and used as templates for AMV reverse transcriptase. Reverse transcriptase stops corresponding to the 5′ ends of transcripts initiated at gene *motA* early promoter PmotA are indicated by an arrow.

occurs during the prereplicative period of T4 development: the transition from early to middle mode RNA synthesis.

DISCUSSION

Bacteriophage T4 mutants in either *motA* or *asiA* genes exhibit identical phenotypes; most importantly, middle mode transcription is completely abolished during either mutant infection (21, 22). Previous work from Hinton's (12) and Kreuzer's (13) laboratories showed that the MotA protein binds to the highly conserved sequence 5′-(a/t)(a/t)TGCTT(c/t)A-3′ centered at the −30 region of T4 middle promoters. These studies also showed that, *in vitro*, MotA-dependent transcription activation at T4 middle promoters is achieved only when the transcribing RNAP is isolated from T4-infected *E. coli* cells. Here, we have shown that, *in vitro*, purified MotA and AsiA proteins are sufficient to direct the unmodified *E. coli* RNAP holoenzyme to initiate RNA synthesis at T4 middle promoters (Figs. 1–3). These results indicate clearly that the presence of AsiA protein in RNAP preparations isolated from T4-infected cells is the key factor allowing the previously described *in vitro* MotA-dependent transcription activation at middle promoters (12, 13). Our results also confirmed that AsiA inhibits early promoter recognition (Fig. 4). It is reasonable to assume that this inhibition is linked to the known biochemical properties of the AsiA protein (14–17)—namely, its ability to bind strongly to σ^{70} and to promote σ^{70}–core dissociation. However, it is not known whether this inhibition is due to the AsiA-induced dissociation of σ^{70} from core RNAP or whether the AsiA-bound σ^{70} simply assumes a conformation unsuitable for a proper interaction with *E. coli* and T4 early promoters. Although both mechanisms could account for that inhibition, we favor the latter interpretation because of the ability of the AsiA protein to induce T4 middle promoter recognition in a σ^{70}-dependent manner.

Genetic studies and sequence analysis have shown that σ^{70} has two specific subdomains, 2.4 and 4.2, which are responsible for −10 and −35 promoter recognition, respectively (23–27). T4 middle promoters have a conserved −10 recognition sequence for *E. coli* σ^{70}. The σ^{70} dependence of transcription activation at middle promoters by MotA and AsiA suggests that region 2.4 of σ^{70} interacts with this conserved −10 region during transcription initiation. How does MotA, bound to its recognition sequence, activate transcription initiation at T4 middle promoters? It is very likely that MotA interacts specifically with the RNAP at middle promoters. Like MotA, class II transcription activators bind to promoter DNA in a position overlapping the −35 region, and their corresponding promoters lack the *E. coli* −35 consensus, suggesting that these class II factors provide protein–protein interactions with the RNAP to replace the missing −35 DNA contacts. Recent *in vitro* studies of the two class II transcription activators PhoB and CRP (at the *pstS* and P1*gal* promoters, respectively) suggest that the necessary contact and/or activation sites for these two factors lie on the σ^{70} subunit, within a "contact site II" extending at least from conserved region 3.2 to the upstream end of region 4.2 (28). In the case of MotA, two scenarios come to mind. In one, MotA contacts σ^{70} within or near contact site II, and this interaction occurs only when AsiA is bound to σ^{70}. In the other, transcription activation at T4 middle promoters requires a direct interaction between MotA (bound to promoter DNA) and AsiA (bound to σ^{70}). Whatever the precise mechanism is, it is clear that AsiA is acting here as a positive control element and that the σ^{70}–AsiA complex must be interacting with core RNAP to exert its influence on open promoter complex formation.

AsiA has long been thought to play a third role in the T4 developmental program (8). Late promoter recognition requires, among other things, that the late σ factor, gp55, replace the host σ^{70} on core RNAP. *In vitro*, late RNA synthesis is attainable when gp55 is allowed to act in the absence of competing σ^{70}. Even small amounts of the latter effectively compete for core enzyme and poison *in vitro* late RNA synthesis (8, 9). During the late period of T4 infection, however, gp55-directed RNA synthesis is abundant despite the coexistence of gp55 and σ^{70}. This *in vitro* versus *in vivo* difference has been thought to be a result of the presence of AsiA protein *in vivo* and its absence in the *in vitro* system. By weakening σ^{70}–core interactions, σ^{70}-bound AsiA protein would allow the late σ factor gp55 to effectively compete for core RNAP. Although there is no direct proof for this third role of AsiA, it is quite plausible.

AsiA protein would thus play a pivotal role in the T4 transcriptional program, all elements of which would be mediated by its specific binding to the host major σ factor, σ^{70}. By inhibiting recognition of early promoters, by directly promoting recognition of middle promoters, and indirectly allowing efficient recognition of late promoters, the AsiA protein emerges as a key element in regulating phage development.

We are indebted to E. P. Geiduschek for providing pure core RNA polymerase and σ^{70}. We thank K. Severinov for donating σ^{70}. We are grateful to J. Huberman for providing access to the PhosphorImager and for helpful assistance. We also thank R. R. Burgess for helpful advice on protein purification from inclusion bodies. This work was supported by the Research Foundation of the State University of New York. G.O. was supported by the Centre National de la Recherche Scientifique (France).

1. Geiduschek, E. P. & Kassavetis, G. A. (1988) in *The Bacteriophages*, ed. Calendar, R. (Plenum, New York), Vol. 1, pp. 93–115.
2. Uzan, M., Brody, E. & Favre, R. (1990) *Mol. Microbiol.* **4**, 1487–1496.
3. Wilkens, K. & Rüger, W. (1994) in *The Molecular Biology of Bacteriophage T4*, ed. Karam, J. (Am. Soc. Microbiol., Washington, DC), pp. 132–141.
4. Brody, E. N., Rabussay, D. & Hall, D. H. (1983) in *Bacteriophage T4*, eds. Mathews, C. K., Kutter, E. M., Mosig, G. & Berget, P. B. (Am. Soc. Microbiol., Washington, DC), pp. 174–183.
5. Guild, N., Gayle, M., Sweeney, R., Hollingsworth, T., Modeer, T. & Gold, L. (1988) *J. Mol. Biol.* **199**, 241–258.

6. Stitt, B. & Hinton, D. (1994) in *The Molecular Biology of Bacteriophage T4*, ed. Karam, J. (Am. Soc. Microbiol., Washington, DC), pp. 142–160.
7. Williams, K. P., Kassavetis, G. A., Herendeen, D. R. & Geiduschek, E. P. (1994) in *The Molecular Biology of Bacteriophage T4*, ed. Karam, J. (Am. Soc. Microbiol., Washington, DC), pp. 161–175.
8. Williams, K. P., Kassavetis, G. A. & Geiduschek, E. P. (1987) *J. Biol. Chem.* **262,** 12365–12371.
9. Malik, S., Zalenskaya, K. & Goldfarb, A. (1987) *Nucleic Acids Res.* **15,** 8521–8530.
10. Engbaek, F., Gross, C. & Burgess, R. R. (1976) *Mol. Gen. Genet.* **143,** 291–295.
11. Zograff, Y. N. (1981) *Mol. Gen. Genet.* **183,** 557–558.
12. Hinton, D. M. (1991) *J. Biol. Chem.* **266,** 18034–18044.
13. Schmidt, R. P. & Kreuzer, K. N. (1992) *J. Biol. Chem.* **267,** 11399–11407.
14. Stevens, A. (1974) *Biochemistry* **13,** 493–503.
15. Stevens, A. (1976) in *RNA Polymerase*, eds. Losick, R. & Chamberlin, M. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 617–627.
16. Stevens, A. & Rhoton, J. (1975) *Biochemistry* **14,** 5074–5079.
17. Orsini, G., Ouhammouch, M., Le Caer, J.-P. & Brody, E. N. (1993) *J. Bacteriol.* **175,** 85–93.
18. Studier, F. W. & Moffatt, B. A. (1986) *J. Mol. Biol.* **189,** 113–130.
19. Nguyen, L. H., Jensen, D. B. & Burgess, R. R. (1993) *Protein Exp. Purif.* **4,** 425–433.
20. Hinton, D. M. (1989) *J. Biol. Chem.* **264,** 14432–14439.
21. Ouhammouch, M., Orsini, G. & Brody, E. N. (1994) *J. Bacteriol.* **176,** 3956–3965.
22. Mattson, T., van Houwe, B. & Epstein, R. H. (1978) *J. Mol. Biol.* **126,** 551–570.
23. Gardella, T., Moyle, H. & Susskind, M. M. (1989) *J. Mol. Biol.* **206,** 579–590.
24. Siegele, D. A., Hu, J. C., Walter, W. A. & Gross, C. A. (1989) *J. Mol. Biol.* **206,** 591–603.
25. Daniels, D., Zuber, R. & Losick, R. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8075–8079.
26. Waldburger, C., Gardella, T., Wong, R. & Susskind, M. M. (1990) *J. Mol. Biol.* **215,** 267–276.
27. Dombroski, A. J., Walter, W. A., Record, T. M., Jr., Siegele, D. A. & Gross, D. A. (1992) *Cell* **70,** 501–512.
28. Kumar, A., Grimes, B., Fujita, N., Makino, K., Malloch, R. A., Hayward, R. S. & Ishihama, A. (1994) *J. Mol. Biol.* **235,** 405–413.