Activation of the Bacteriophage Mu *lys* Promoter by Mu C Protein Requires the σ^{70} Subunit of *Escherichia coli* RNA Polymerase

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Bacteriophage Mu C protein, a product of the middle operon, is required for activation of the four Mu late promoters. To address its mechanism of action, we overproduced the ~16.5-kilodalton C protein from a plasmid containing the C gene under the control of a phage T7 promoter and ribosome-binding site. A protein fraction highly enriched for *Escherichia coli* RNA polymerase ($E\sigma^{70}$) and made from the overproducing strain was able to activate transcription in vitro from both the *tac* promoter (P_{tac}) and a Mu late promoter, P_{lys} . The behavior of P_{lys} was similar in vivo and in vitro; under both conditions, transcription was C dependent and the RNA 5' ends were identical. When anti- σ^{70} antibody was added to C-dependent transcription reactions containing both P_{tac} and P_{lys} templates, transcription from both promoters was inhibited; transcription was restored by the addition of excess $E\sigma^{70}$. This result suggests that C-dependent activation of P_{lys} requires σ^{70} . Further supporting evidence was provided by a reconstitution experiment in which an $E\sigma^{70}$ -depleted fraction containing C was unable to activate transcription from P_{lys} unless both purified σ^{70} and core polymerase were added. These results strongly suggest that C is not a new sigma factor but acts as an activator for $E\sigma^{70}$ -dependent transcription.

Bacteriophage Mu, a temperate phage of several species of enteric bacteria (see reference 29 for a review), has a 37-kilobase-pair linear double-stranded DNA genome. Transcripts produced during lytic development fall into early, middle, and late classes, which are all transcribed rightward (1, 19, 26, 33; C. F. Marrs, Ph.D. thesis, University of Wisconsin, Madison, 1982). At early times after the induction of a Mu prophage, transcription from P_e results in the expression of replication functions (15, 33). Once replication is under way (19, 26, 33; Marrs, Ph.D. thesis), middle transcription initiating from P_m results in the expression of the C gene, which is required to activate late transcription (10, 11, 17, 25).

The late region (the rightmost ~27 kilobase pairs of the genome), which includes genes for phage morphogenesis and host range, cell lysis (*lys*), and DNA modification (*mom*), is transcribed from four major late promoters, P_{lys} , P_I , P_P , and P_{mom} (2, 9, 18, 26, 29). C is the only Mu protein required to initiate transcription in vivo from these promoters (10, 11, 17, 18). All four promoters contain an $E\sigma^{70}$ -10 region but lack a recognizable $E\sigma^{70}$ -35 region, consistent with their inactivity in the absence of C (18, 26). Instead, they share a conserved sequence centered at about -40 (18).

The mechanism by which C activates Mu late promoters is not yet known. It is unlikely that C is a new RNA polymerase, since C is a small protein, ~16.5 kilodaltons (11, 17). In addition, Mu development requires the host core RNA polymerase (31). The absence of detectable leader transcripts and the C dependence of β -galactosidase expression from late promoter-*lacZ* fusions containing only a few bases of the Mu RNA 5' end make it unlikely that C is an antiterminator (18, 26). C binds to P_{mom} DNA (20), and its footprint overlaps the late promoter consensus sequence in P_{lys} and P_{mom} (2), implicating C as an activator protein. In this paper, we provide further evidence that C is an activator

by using in vitro transcription to demonstrate the σ^{70} dependence of Mu late promoter activation by C.

MATERIALS AND METHODS

Media, chemicals, and enzymes. LB agar, LB broth, and SB broth (18) were supplemented with ampicillin and kanamycin at 50 μ g/ml when necessary. T7 overproduction medium was an equal mixture of LB and SB broths with 0.2% glycerol and both antibiotics. T7 labeling medium was M9 medium supplemented with 20 μ g of thiamine per ml and 0.01% of each amino acid except for Met and Cys (30). $E\sigma^{70}$ was from C. Gross; σ^{70} , core polymerase, and purified 2D4 and 3D3 monoclonal antibodies (27) were from S. Lesley and R. Burgess. Other enzymes were from New England BioLabs, Inc. Radiolabeled compounds were from New England Nuclear Corp. Protein size markers were from Bethesda Research Laboratories, Inc.

Bacterial strains and plasmids. All strains were derivatives of *Escherichia coli* K-12. Strain MH7407 is MH7295 (*gal lac rpsL recA F' lacI^{q1} lacZ::Tn5*) (18) containing pWM16. Strain K38 [HfrC (λ)] (22) was used as the host for both pGP1-2 and the compatible plasmids pWM18 (MH9028), pWM15 (MH9222), and pT7-7 (MH9029). Strain MH9037 is a derivative of MC1061 [*lacX74 galK galU Δ (ara-leu)7697 araD139 hsdR rpsL*] (5) containing Δ *lacZ*M15 (from J. Hu). Strain MH429 (F^- *thyA malt::Mu cts62*) (17) was the source of Mu cts62 RNA. Plasmid pGP1-2 expresses phage T7 RNA polymerase from the λ p_L promoter (30). Plasmid pT7-7 (from S. Tabor), a multistep derivative of pT7-1 (30) with an inverted *bla* gene, carries base pairs 22857 to 22972 of the T7 sequence (6) containing the T7 gene 10 promoter and ribosome-binding site (RBS) just upstream of an *NdeI* and a *BamHI* site (S. Tabor, personal communication). Plasmid pUC19spf' (from J. Erickson) contains a 130-base-pair (bp) *EcoRI*-*BamHI* fragment carrying base pairs 2941 to 3061 of the sequence flanking the strong Rho-independent *spf* terminator (14) inserted between the *EcoRI* and *BamHI* sites of pUC19.

Plasmid constructions and manipulations. Plasmid pWM15

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was made by cloning the *C*-containing *EcoRI-HindIII* fragment from plasmid pWM16 (18) into *EcoRI-HindIII* cleaved pGEM3Z (Promega Biotec), allowing transcription of *C* from the T7 promoter.

The *C*-overproducing plasmid pWM18 contains the *C* gene coding region downstream from the T7 promoter and RBS of pT7-7 such that the pT7-7 *NdeI* site ATG, with optimal spacing to the RBS, serves as the initiating ATG for a wild-type *C* gene coding sequence (11, 17). For construction of pWM18, complementary 83- and 84-base oligonucleotides (synthesized at the University of Tennessee—Memphis Molecular Resource Center) were annealed to create the *NdeI-HinfI* fragment containing the amino-terminal end of *C* (17). The resulting fragment was ligated to a 422-bp *HinfI-BamHI* fragment from pWM15 containing the remainder (carboxy-terminal end) of *C*. The 505-bp *NdeI-BamHI* ligation product was gel purified, ligated to *NdeI-BamHI*-cleaved pUC18, and transformed into MH9037. The desired transformants were detected by restriction analysis of plasmids from transformants exhibiting insertional inactivation of *lacZ*. One such plasmid, pWM17, was isolated, purified, and cleaved with *NdeI* and *BamHI* to yield a large amount of the 505-bp fragment, which was then gel purified and ligated to *NdeI-BamHI*-cleaved pT7-7 to create pWM18. Sequences within and flanking the *NdeI-HinfI* fragment were confirmed by DNA sequencing (Sequenase kit; United States Biochemical Corp.). MH9037 transformants containing pWM18 grew more slowly than did cells containing pT7-7, suggesting that *C* was produced even in the absence of T7 RNA polymerase and was detrimental to cell growth.

Plasmid pWM57 was made by filling in the termini of a 280-bp *BglII-BstNI* fragment from pWM6 containing P_{lys} (17, 18) with Klenow polymerase and deoxynucleoside triphosphates and then cloning the fragment into *HincII*-cleaved pUC19sp⁺. Plasmid pWM58 was made by cloning a *HindIII-BamHI tac* promoter (P_{tac}) fragment (Pharmacia, Inc.) into *HindIII-BamHI*-cleaved pUC19sp⁺. Transcripts originating from P_{lys} and P_{tac} and ending at the *spf* terminator should be ~249 and ~144 nucleotides (nt) long, respectively.

Protein radiolabeling and detection. Cells were grown at 28°C in LB broth plus antibiotics to a density of 2×10^8 cells per ml, washed, suspended to 4×10^7 cells per ml in 1 ml of T7 labeling medium, and grown at 28°C for 1 h. After heat induction at 42°C for 15 min, 200 μ g of rifampin (Sigma Chemical Co.) was added. Incubation was continued at 42°C for 10 min and then at 28°C for 20 min to allow mRNA turnover, and the cultures were pulsed for 5 min with 10 μ Ci of [³⁵S]methionine (1,200 Ci/mmol).

All samples were boiled for 2 to 4 min in cracking buffer (30) and subjected to electrophoresis on sodium dodecyl sulfate–12.5% polyacrylamide gels (6). [³⁵S]methionine-labeled proteins were detected by autoradiography with Kodak XAR-5 film and quantitated by scanning with an Ultrosan XL laser densitometer (LKB Instruments, Inc.). Proteins stained with Coomassie brilliant blue R were quantitated by scanning with a model 620 video densitometer (Bio-Rad Laboratories).

In vitro transcription and RNA analysis. Transcription reactions were done as described previously (8) with minor modifications. Reactions (40- μ l volume) contained 20 mM Tris hydrochloride (pH 7.5), 10 mM MgCl₂, 1 mM EDTA, 1 mM K₂HPO₄ (pH 7), 1 mM dithiothreitol, 0.5 mg of bovine serum albumin per ml, 100 mM NaCl, and either (i) 0.35 mM each ATP, UTP, GTP, and CTP for the synthesis of unlabeled RNA (to be S1 nuclease mapped) or (ii) 0.35 mM each ATP, UTP, and GTP, 14 μ M CTP, and 10 μ Ci of [α -³²P]CTP

(800 Ci/mmol) for the synthesis of labeled RNA. Approximately 0.3 pmol of each supercoiled plasmid DNA template was added per reaction. RNA polymerase components were added either as step iv extract (2 μ l; see below) or as purified σ^{70} , E, or E σ^{70} . All components except nucleotides were incubated at 0°C for 15 min; RNA synthesis was initiated by the addition of nucleotides and transfer to 37°C for 15 to 25 min. Reactions were stopped by adding 72 μ l of stop mix (4 M ammonium acetate [pH 5], 25 mM EDTA, 0.17 mg of phenol-extracted type XXI *E. coli* tRNA [Sigma] per ml), followed by phenol-CHCl₃ extraction, isopropanol precipitation, and a 70% ethanol wash. Radiolabeled samples were denatured at 95°C and subjected to electrophoresis on 6% polyacrylamide–7 M urea gels and autoradiography with Kodak XAR-5 film (21).

Protein overproduction and purification. Overproduction of *C* protein was accomplished by growth of MH9028 at 28°C in T7 overproduction medium to a density of 5×10^8 cells per ml, followed by incubation at 43°C for 30 min to induce T7 RNA polymerase and *C* production. After cells were harvested by centrifugation, step iv extracts enriched for E σ^{70} were made from a cell lysate by polyethylene glycol (PEG) 8000 precipitation and high-salt elution of the PEG pellet as described previously (7), except that 300 ml of cells was used to increase the protein concentration. An E σ^{70} -depleted, *C*-enriched fraction was obtained by fractionating the PEG supernatant from the above-described procedure through a 1-ml heparin-agarose column (2). One milliliter of PEG supernatant (containing 0.3 M NaCl) was diluted with 2 ml of buffer 1 (2) to achieve a 0.1 M NaCl concentration, gently mixed with 1.5 ml of heparin-agarose slurry at 0°C for 1 h, and poured into the column. About 20 ml of buffer 1 containing 0.1 M NaCl was used to wash the column, and *C* protein was eluted with successive 2-ml volumes of 0.15, 0.20, 0.25, and 0.30 M NaCl in buffer 1. Fractions containing the most *C* (as visualized on silver-stained gels [28]) were pooled, concentrated, and dialyzed against buffer 1 containing 0.2 M NaCl with a Centricon-10 microconcentrator (Amicon Corp.). Glycerol was added to 30%, and the preparations were stored at –70°C.

RESULTS

Detection of *C* protein. Our first objective in studying the mechanism by which *C* protein activates Mu late promoters was to overproduce the protein. When total stained or pulse-labeled cell proteins were visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, *C* protein was not detectable after isopropyl- β -D-thiogalactopyranoside induction of a P_{tac} -*C* fusion present on pWM16 (MH7407). In contrast, when pWM16 was used as the template in a DNA-directed in vitro protein synthesis system, radiolabeled *C* protein and β -lactamase were easily detected (Fig. 1B, lane 3). When full-length and truncated *C* RNAs were synthesized in vitro from a DNA template (pWM15) linearized at a site downstream from *C* (*BamHI*) or within *C* (*BglII*), translation of the full-length and truncated RNAs in a wheat germ extract resulted in the predicted ~16- and ~13-kilodalton proteins, respectively (Fig. 1A, lanes 4 and 3), identifying the ~16-kilodalton band as *C* protein.

Detection of *C* protein made in vivo was then accomplished with a phage T7 promoter expression system. Plasmid pWM15, containing *C* fused to the T7 promoter, was introduced into a strain harboring pGP1-2, a compatible plasmid carrying the gene for T7 RNA polymerase, which is not sensitive to rifampin, under the control of the λ P_L

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of [^{35} S]methionine-labeled (A and B) and Coomassie-stained (C) proteins. (A) Lane 1 contains protein markers (molecular masses in kilodaltons are given at the left). Lanes 2 to 4 contain radiolabeled products of *in vivo* translation reactions with a wheat germ lysate (Promega Biotec): lane 2, no RNA; lanes 3 and 4, translation products from 1- μ g samples of truncated and full-length RNAs made by T7 RNA polymerase from pWM15 DNA cleaved with *Bgl*II and *Bam*HI, respectively. (B) Lanes 1, 4, 5, and 6 contain radiolabeled proteins made *in vivo* after induction of the T7 promoter on pT7-7 (MH9029; lane 1), pWM18 (MH9028; lane 4), and pWM15 (MH9222; lanes 5 and 6). Lane 5 contains four times the amount of protein as lanes 1, 4, and 6. Lane 2 contains protein markers. Lane 3 contains radiolabeled protein synthesized *in vitro* in an S-30 extract (Amersham Corp.) with pWM16 as the template; the upper band is β -lactamase. (C) Strains MH9028 and MH9029 were grown at 28°C in T7 overproduction medium to 5×10^8 cells per ml, and aliquots were maintained at 28°C (uninduced) or shifted to 42°C for 30 min to induce T7 RNA polymerase production. Lanes: 1 and 2, extracts from induced (lane 1) and uninduced (lane 2) MH9029 (lacking C); 3 and 4, extracts from induced (lane 3) and uninduced (lane 4) MH9028 (containing C); 5, protein markers. To prevent overloading, lane 3 contains one-third the amount of protein present in the other lanes.

promoter and *cI857* repressor. Weak but clearly visible synthesis of [^{35} S]methionine-labeled C protein was observed only when the expression of other host proteins was inhibited by rifampin (Fig. 1B, lanes 5 and 6, and data not shown).

Overproduction of C protein. We postulated that low C protein production might result from inefficient translation initiation at the poor Shine-Dalgarno sequence in the putative C RBS (11, 17), as recently demonstrated by Bölker et al. (2). Therefore, we replaced the C gene RBS with the highly efficient RBS from the T7 major capsid gene (gene 10), using a synthetic oligonucleotide to fuse the wild-type C gene coding sequence downstream from the T7 promoter and RBS present on pT7-7. Upon induction of T7 polymerase, ~25-fold more labeled C protein was made from the resulting plasmid, pWM18, than from pWM15 (Fig. 1B, compare lane 4 to lanes 5 and 6). It was now possible to synthesize large quantities of unlabeled C protein from pWM18 by performing the T7 polymerase induction in rich medium without rifampin. C protein was easily visualized as a ~16-kilodalton band representing 10 to 20% of the protein detectable on a Coomassie-stained gel loaded with a whole-cell extract from induced MH9028 containing pWM18 (Fig. 1C, lane 3).

Activation of transcription from P_{lys} *in vitro* by C. A C-containing fraction enriched for $E\sigma^{70}$ (step iv extract from

FIG. 2. S1 nuclease mapping of P_{lys} -promoted transcripts synthesized *in vivo* and *in vitro*. The 280-bp *Bgl*II-*Bst*NI DNA probe, 5' end-labeled at the *Bst*NI site located 131 bp downstream from the P_{lys} transcription initiation site (18), was prepared as described previously (17). Total *in vivo* RNA was isolated from MH429 35 min after prophage induction (17); RNA was synthesized *in vitro* as described in Materials and Methods. After hybridization of the RNA to the DNA probe and S1 nuclease treatment (4), the samples were subjected to electrophoresis and autoradiography. Lanes 1 and 4 show A+G sequencing ladders made from the DNA probe; such DNA fragments migrate 1 to 2 nt farther than S1 nuclease-protected species of identical lengths (24). Lanes 2 and 5 show the S1 nuclease-resistant species after protection by unlabeled RNAs synthesized *in vivo* and *in vitro*, respectively. Lane 3 shows probe only (no S1 nuclease treatment).

the C-overproducing strain; see Materials and Methods) was used to activate transcription *in vitro* from P_{lys} on a supercoiled plasmid template (pWM57). S1 nuclease mapping of transcripts initiated from P_{lys} *in vitro* and *in vivo* revealed identical RNA 5' ends located 131 ± 2 and 120 ± 2 nt upstream of the *Bst*NI site (Fig. 2), confirming the origin of the *in vitro* transcript and demonstrating the accuracy of C-dependent initiation *in vitro*. The upstream 5' end is the actual transcription start site (18); the downstream 5' end probably results from S1 nuclease digestion in an incompletely annealed AT-rich region.

Transcripts made *in vitro* from supercoiled templates containing P_{lys} (pWM57) and P_{tac} (pWM58) by use of C-containing step iv extracts were detectable as ~249- and ~144-nt species, respectively (Fig. 3, lanes 2 to 4). When both P_{tac} and P_{lys} templates were incubated with a C-deficient extract or with purified $E\sigma^{70}$, only P_{tac} was active (Fig. 3, lanes 1 and 11), demonstrating that P_{lys} activity is C dependent. The lack of transcription from P_{lys} *in vitro* with $E\sigma^{70}$ alone is consistent with the absence of an $E\sigma^{70}$ -35 region in P_{lys} and the inactivity of P_{lys} *in vivo* in the absence of C (18).

Inhibition of transcription from both P_{tac} and P_{lys} by anti- σ^{70} antibody. To determine whether C is a new sigma factor or an activator that utilizes the host $E\sigma^{70}$ holoenzyme, we performed *in vitro* transcription reactions in the presence of a monoclonal antibody (2D4) directed against the putative DNA-binding domain of σ^{70} (region 4) (27). Increasing amounts of antibody resulted in decreased transcription from both P_{lys} and P_{tac} (Fig. 3, lanes 5 to 7); the activity of both promoters was restored by the addition of increasing amounts of purified $E\sigma^{70}$ (Fig. 3, lanes 8 to 10) or purified σ^{70} (data not shown), suggesting that the inhibition was due to a specific interaction between the antibody and σ^{70} . Similar effects were observed in the presence of the RNase inhibitor RNasin, arguing against RNase contamination of the antibody preparation (data not shown). The parallel inhibition of

FIG. 3. Effect of anti- σ^{70} antibody on transcription from P_{lys} . Each lane represents a separate transcription reaction. The sources of RNA polymerase (RNAP) were purified $E\sigma^{70}$ (lane 11) or step iv extracts lacking C (from MH9029; lane 1) or containing C (from MH9028; lanes 2 to 10). The supercoiled DNA templates were pWM57, containing P_{lys} (L), and pWM58, containing P_{tac} (T). The number of plus signs indicates the relative amounts of purified anti- σ^{70} monoclonal antibody (Ab) (2D4) or purified $E\sigma^{70}$ added to the reactions: for antibody, +, ++, and +++ represent 1.6, 3.2, and 5.4 μ g, respectively; for $E\sigma^{70}$, +, ++, and +++ represent 0.5, 1.0, and 1.5 μ g, respectively. To prevent overloading, lane 3 contains one-fourth the amount of sample in the other lanes. The positions of transcripts made from P_{lys} (~249 nt) and P_{tac} (~144 nt) are indicated at the left. The multiple bands presumably arose from termination site heterogeneity within the *spf* terminator.

both P_{lys} and P_{tac} activities implies that both are similar in their requirement for σ^{70} . Similar transcription inhibition was observed with another monoclonal antibody (3D3) directed against the putative core-binding region of σ^{70} (region 2) (27); however, P_{lys} activity was only partially restored by $E\sigma^{70}$ addition (data not shown). Direct inactivation of C by either antibody is unlikely, since no binding was detectable by Western blot (immunoblot) analysis (32) (data not shown).

A σ^{70} -depleted C protein fraction requires σ^{70} to activate

FIG. 4. Restoration of P_{lys} transcription by the addition of σ^{70} to a σ^{70} -depleted C-containing fraction. Each lane represents a separate transcription reaction performed in the presence of 25 U of RNasin (Promega Biotec) with supercoiled P_{lys} (L) or P_{tac} (T) templates (pWM57 and pWM58, respectively). C protein was added as 6 μ l of the σ^{70} -depleted heparin-agarose fraction (C fraction). The RNA polymerase components added are denoted $E\sigma$ (0.75 μ g of purified $E\sigma^{70}$), E (1.4 μ g of purified core polymerase), σ (0.6 μ g of purified σ^{70}), and E/σ (1.4 μ g of core polymerase and 0.6 μ g of σ^{70} added separately). Lane 1 contains one-third the amount of sample in the other lanes.

P_{lys} . The σ^{70} dependence of C activation was directly demonstrated by showing that transcription from P_{lys} by a C-containing but σ^{70} -depleted fraction could occur only after the addition of σ^{70} . During preparation of $E\sigma^{70}$ -enriched step iv extracts from the C-overproducing strain (MH9028), most of the C protein (as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was not precipitated along with $E\sigma^{70}$ by PEG. Since the PEG supernatant normally contains very little RNA polymerase, it represented a source of virtually σ^{70} -free C protein. To remove RNases and other potential transcription inhibitors, we partially purified the C protein from the PEG supernatant by fractionation through heparin-agarose.

This σ^{70} -depleted C fraction was then combined with purified RNA polymerase components in *in vitro* transcription reactions. Both P_{lys} and P_{tac} , either singly or in combination, were active in the presence of purified $E\sigma^{70}$ and the σ^{70} -depleted C fraction, demonstrating that C was active (Fig. 4, lanes 1 to 3). As observed previously (Fig. 3, lane 11), only P_{tac} was active when both promoter templates were incubated with $E\sigma^{70}$ alone (Fig. 4, lane 7). To show that core RNA polymerase was insufficient for C-promoted activation of P_{lys} , we added purified core and σ^{70} separately to reactions containing the C fraction. Although weak transcription from both promoters was observed with the core alone (Fig.

4, lane 5), further addition of σ^{70} resulted in a large increase in transcription from both promoters (Fig. 4, lane 4). These results demonstrate that the core preparation was functional and that the activity of both promoters was dependent on σ^{70} . It is unlikely that the small amount of transcription observed with the C fraction and core alone (Fig. 4, lane 5) was due to contaminating σ^{70} in the core preparation, since the core alone (without the C fraction) had no activity on P_{lac} (Fig. 4, lane 9) and since complete activity was restored upon the addition of σ^{70} (Fig. 4, lane 10). It seems more likely that the C fraction, although greatly depleted of $E\sigma^{70}$, still contained enough free σ^{70} to complex with added core and activate both promoters. The complete lack of transcription observed when purified σ^{70} was added to the C fraction (Fig. 4, lane 6) may reflect efficient core binding to DNA during the step iv extract preparation, with consequent effective precipitation by PEG.

DISCUSSION

Earlier analysis of the predicted C protein sequence did not indicate whether C is an activator protein or a sigma factor. The presence of weak homology to the helix-turn-helix motif (2, 17) implicated in DNA binding (3) suggested that C might be an activator, but limited homology to a region conserved in sigma factors (17) hinted that it might function as a sigma factor instead. The recent demonstration that C bound independently to conserved sequences in P_{lys} and P_{mom} (2) made it more likely that C is an activator.

In this work, we provide direct evidence that C is an activator and not a sigma factor (which should substitute for σ^{70}) by showing that transcription from a Mu late promoter requires C, core polymerase, and σ^{70} . The σ^{70} requirement was demonstrated by the inhibition of C-dependent transcription from P_{lys} with anti- σ^{70} antibodies and by the lack of C-dependent transcription from P_{lys} by core RNA polymerase; in both cases, transcription was restored by the addition of σ^{70} . The similarity of the 5' ends of P_{lys} RNAs made in vitro and in vivo makes it likely that the in vitro requirement for σ^{70} reflects a similar requirement in vivo; however, no studies have been done to assess the role of σ^{70} in Mu late transcription in vivo.

In one model for Mu late promoter activation, C binding to the consensus sequences would allow bound $E\sigma^{70}$ to form open complexes. Other activators, such as cI and cII of λ (12, 13), are believed to use this general mechanism. Alternatively, C may primarily enhance $E\sigma^{70}$ binding, perhaps by altering DNA structure, as suggested for the *E. coli* CAP protein (16). The proximity of the C- and $E\sigma^{70}$ -binding sites (2, 23) suggests that bound C and $E\sigma^{70}$ may interact directly. As yet there is no evidence for such interactions either on DNA or in solution. Although the partial coprecipitation of C with polymerase in the step iv extract might reflect direct protein interaction, it might also result from aggregation or binding of C to P_{lys} DNA (on pWM18) in the extract.

Although the three other Mu late promoters have not been tested, it seems likely that they will also require σ^{70} for C-dependent activation. Also yet unaddressed is the possibility that additional host factors may be required for late promoter activation. Such factors might have been present in the relatively crude fractions used to supply C in the transcription assays. Future studies of the structure and function of the C protein, its interactions with RNA polymerase, and the ionic, protein, cofactor, and DNA sequence requirements for Mu late promoter activity should enhance our understanding of the role that C plays in transcription activation.

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