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Studies on the structure and function of the beta-prime subunit of *Escherichia coli* RNA polymerase

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STUDIES ON THE STRUCTURE AND FUNCTION OF THE BETA-PRIME SUBUNIT OF *E. coli* RNA POLYMERASE

by

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A dissertation submitted to the Graduate Faculty in Biochemistry in partial fulfillment of the requirments for the degree of Doctor of Philosophy, The City University of New York.

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ABSTRACT

Studies on the structure and function

of the beta-prime subunit of *E. coli* RNA polymerase.

by

Jianying Luo

Adviser: Joseph S. Krakow

The *E. coli* RNA polymerase is a multisubunit enzyme. The holoenzyme active during initiation has the structure: α_2ßß'σ while the catalytic competent core enzyme has the structure: α_2ßß'. Monoclonal antibodies (mAbs) raised against the ß' subunit of the *E. coli* RNA polymerase were used to probe the structure and function of this subunit. Of the five anti-ß' monoclonal antibodies studied only mAb 311G2 is a strong inhibitor of RNA polymerase activity. This antibody binds to an epitope which is exposed in both the assembled holoenzyme and isolated ß' subunit. In contrast, the null antibodies bind to the free ß' subunit but very weakly to native RNA polymerase. It would appear that the ß' domain in which their epitopes reside is either conformationally altered or blocked due to interaction with other subunits in native RNA polymerase. In order to locate the positions of the epitopes for these 5 monoclonal antibodies, a series of overlapping deletion mutants has been constructed

by partial restriction and religation of the ß' gene present in pT7ß' (Zalenskaya *et al.*, Gene *89* (1990) 7-12). The presence of the epitopes for each of the anti-ß' monoclonal antibodies was assessed by Western blotting. The results indicate that the epitopes for mAb 340F11, mAb 370F3, mAb 371D6, and mAb 372B2 are located between amino acids 817 to 876. This region may be important in subunit-subunit interaction. The epitope for the inhibitory antibody, mAb 311G2, is located between amino acids 1047 to 1093. This region may be involved in the catalytic function of RNA polymerase.

Truncated ß' mutant proteins were also used to map the region on ß' involved in the assembly of RNA polymerase. Results suggest that the N-terminal region of ß' is involved in the assembly of core enzyme, and a region between aa 201 to 477 on ß' may be involved in the interaction between the ß' subunit and the σ subunit.

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ABBREVIATIONS

BSA:	bovine serum albumin;
cAMP:	3', 5' cyclic adenosine monophosphate;
CRP:	cyclic AMP receptor protein;
DTT:	dithiothreitol;
EDTA:	ethylenediamine tetraacetic acid;
ELISA:	enzyme-linked immunosorbent assay;
lac:	the lactose operon;
*lac*P$^+$:	wild-type *lac* promoter;
mAb:	monoclonal antibodies;
NFCM:	non-fat carnation milk
PAGE:	polyacrylamide gel electrophoresis;
PBS:	phosphate buffered saline;
SDS:	sodium dodecyl sulphate;
STS:	sodium tetradecyl sulphate;
TCA:	trichloroacetic acid;
TEMED:	N, N, N', N'-tetramethylethylenediamine;
Tris:	Tris-(hydroxymethyl) aminomethane.

INTRODUCTION

The DNA-dependent RNA polymerases are important enzymes involved in the earliest events of gene expression. The RNA polymerase from *E. coli* is a complicated multisubunit enzyme existing in two forms, core and holoenzyme. The catalytically competent core enzyme has the structure: α_2ßß' while the promoter-selective holoenzyme has the structure: α_2ßß'σ (Kumar, 1981). Both core and holoenzyme also contain two tightly bound zinc ions, one associated with the ß subunit, the other with the ß' subunit (Wu *et al.*, 1977). The ß and ß' subunits are encoded by the *rpoB* and *rpoC* genes within a polycistronic operon, while the α and σ subunits are encoded by two separated genes, *rpoA* and *rpoD* (von Hippel *et al.*, 1984). All four genes have been sequenced and cloned into expression vectors (Gribskov and Burgess, 1983; Zalenskaya *et al.*, 1990). The functional cycle of RNA polymerase is divided into three major steps, initiation, elongation, and termination (von Hippel *et al.*, 1984). During initiation, RNA polymerase holoenzyme binds to a promoter site with a binding constant (K_B) that is characteristic of each promoter to form a closed promoter complex. At optimal conditions, a closed promoter complex rapidly isomerizes to form an open promoter complex, in which about 12 base pairs of DNA around the transcription start site are unwound (McClure, 1985). The rate of isomerization is expressed with a rate constant, K_f, and is a unique property of individual promoters. A number of transcription regulators, such as CRP, λ repressor, and *lac* repressor, are known to modulate the K_B and K_f values at

specific promoters by directly interacting with DNA and RNA polymerase (McClure, 1985). The addition of the nucleotide substrates triggers several rounds of abortive initiation whereby each open promoter-polymerase complex makes and releases nested RNA oligomers up to 9 nucleotides in length. When the nascent RNA transcript is about 10-11 nucleotides long promoter clearance occurs. This involves the release of the σ factor, relinquishing of the promoter-anchoring contacts, and commencement of processive elongation by the core RNA polymerase with concomitant growth of the nascent RNA chain. Elongation continues until a pause site is reached. A pause site may be overridden by the ternary elongation complex, or it may lead to destabilization of the nascent RNA-DNA hybrid and subsequent dissociation of RNA polymerase and transcript from the DNA template (McClure, 1985; von Hippel *et al.*, 1984).

Due to the relative large size and the inability to obtain suitable three-dimensional crystals, the quaternary structure of *E. coli* RNA polymerase has not been determined by X-ray diffraction. However, various biochemical and physical techniques have been used to study the quaternary structure of *E. coli* RNA polymerase.

In cross-linking studies with bifunctional reagents of varying specificity and length, Hillel and Wu (1977) identified seven types of cross-linked products, α_2, αß, αß', $\alpha\sigma$, ßσ, ß'σ, and ßß' in holoenzyme. They presented a model for the holoenzyme consisting of spherical subunits in which ß and ß' form a tetrahedron-like structure together with the α subunit, which constitute a dimer. The σ subunit interacts with ß, ß' and one α subunit. Partial proteolysis studies support this model (Lowe and

Malcolm, 1976; Fisher and Blumenthal, 1980). Stender (1980) compared the accessibility of free subunits with their accessibility *in situ*, using antibodies raised against isolated subunits. He found the α subunit *in situ* to be much less accessible than in the unassembled state; for ß and ß' this difference is much smaller.

On the basis of small-angle neutron scattering data, Stockel *et al.* (1980a) presented a model in which the core enzyme is shown as an extended triangle of elongated α_2, ß and ß' subunits, of which the latter two are curved. In the holoenzyme (Stockel *et al.*, 1980b), the σ subunit with the shape of an elongated disc, nestles up to the core enzyme in a space-filling manner, making contacts with ß, ß' and one α subunit. The center of α_2, ß, ß', and σ in holoenzyme forms a tetrahedron.

Using small-angle X-ray scattering, Meisenberger and coworkers (1980b) have confirmed the assumption of Stockel that the two α subunits constitute a dimer. They extended the information of Stockel and coworkers by introducing a mass variation along the longitudinal axes of the subunits. In their model for the core RNA polymerase (Meisenberger *et al.*, 1980c), ß and ß' have a conical shape and are lying side by side with their thick ends in the same direction. α_2 is a curved, elongated disc with a deep crevice in the middle; the halves are attached to the thick ends of the ß and ß' subunits. The maximum dimension of α_2ß was found to be much larger than that of the core enzyme (30 nm vs 24 nm). It was proposed that the two α subunits in α_2ß are arranged angularly (not in a straight line), with the ß subunit contacting only one α subunit. After the binding of the ß' subunit to the other α subunit, the two α subunits in the core polymerase now are arranged straight. The free σ subunit is Y-shaped (Meisenberger *et al.*, 1980a). However, whether the σ subunit in

holoenzyme is Y-shaped remains unclear (Stockel *et al.*, 1980b; Lederer *et al.*, 1991). Through a neutron solution scattering study, Lederer *et al.* (1991) indicated that the σ subunit is arranged with respect to core enzyme so that the plane parallel to the disc of the σ is arranged vertically to the plane formed by the core substructure. The σ factor is buried in a groove of the core enzyme, probably between ß and ß'. In their model of holoenzyme dimers, two core enzymes of triangular shape were placed side by side in a C2-symmetry, the two σ subunits are positioned on the same side of the plane formed by the core-core substructure towards the top of the core triangles. Heumann *et al.* (1988) studied the complex of RNA polymerase and a 130 base pair promoter fragment of bacteriophage T7. They found that during the formation of the complex, the RNA polymerase becomes more compact while the DNA fragment does not undergo a gross conformational change. In their model for the complex of RNA polymerase and the promoter fragment, RNA polymerase is positioned on only one side of the DNA, with the elongated subunits ß and ß' aligned parallel to the DNA axis. The orientation of the triangularly shaped RNA polymerase on the DNA with respect to the direction of transcription was not determined by this method. However, an earlier experiment by Hirsh and Schleif (1976) had shown that wedge-shaped RNA polymerase molecules tightly bind to a DNA fragment containing the lambda *Pr* promoter, pointing toward the direction of transcription.

In the electron microscopic studies with polyclonal antibodies raised against α, ß, ß' and σ, Tichelaar *et al.* (1983) constructed a model for holoenzyme with dimensions of 9 nm x 16 nm. The framework of the enzyme is formed by two curved, elongated subunits crossing each other at about one-fourth of their length. They are

curved in the same direction forming a convex and a concave surface with α_2 located at the short ends. The σ subunit is hidden in the cavity at the concave side of core in contact with α_2. This model is in good agreement with the model of Stockel *et al.* (1980b) with some differences in the position of α subunits. The shapes proposed by Meisenberger *et al.* (1980a, b) for α_2 and σ also agree with this model.

Crystals of RNA polymerase suitable for X-ray analysis have not yet been obtained. However, a low-resolution (30 Å) three-dimensional structure of *E. coli* RNA polymerase has been determined using electron microscopy of two dimensional crystals formed on positively charged lipid layers (Darst *et al.*, 1989). The enzyme was shown as an irregularly shaped complex of 100 x 100 x 160 Å in size, consistent with the dimensions determined previously (Tichelaar *et al.*, 1983). The most striking feature of the model is a thumb-like projection that surrounds a cylindrical channel which bears a striking resemblance to the DNA binding-site cleft of the Klenow fragment of the *E. coli* DNA polymerase I. The channel, about 25 Å in diameter and 55 Å in length, is sufficient to accommodate around 16 base pairs of double-helical DNA in the B-form, which may relate to the 16-18 base pairs that become unwound in the transcriptional complex. It was suggested that the ß and ß' subunit may be involved in the formation of this DNA-binding channel.

The various techniques summarized above have generated important information regarding the dimensions, the gross shape as well as the spatial arrangement of subunits of RNA polymerase and implicated possible modes of interaction between subunits. However, the regions involved in subunit-subunit interactions and possible changes in subunit conformation attendant to enzyme

assembly remain largely undefined.

Rapid progress over the past few years on studies of *E. coli* RNA polymerase has greatly enriched our knowledge of the functional role of each subunit, the interaction between subunits and the mapping of functional domains on each subunit. A large amount of information has been derived for the σ and ß subunits, and more recently for the α subunit while much less attention has been given to the ß' subunit.

The activity of the σ subunit includes binding to the core RNA polymerase and activation of promoter recognition (Helmann and Chamberlin, 1988). It may also play a direct role in the isomerization of the closed promoter complex to the open promoter complex and in the inhibition of nonspecific DNA binding. In addition to the predominant σ^{70} factor, *E. coli* and other eubacteria have other σ factors which alter the promoter specificity of RNA polymerase. Sequence analysis of major and minor σ factors from a variety of organisms indicated that there are four conserved regions in the σ protein family, regions 1-4 (Lonetto *et al.*, 1992). The function of region 1 is not identified so far. Region 2 consists of four subregions, 2.1, 2.2, 2.3, and 2.4. Subregion 2.1, containing conserved hydrophobic and basic amino acids, was suggested to be involved in the binding of σ to the core RNA polymerase. Regions 2.1 and 2.3 have been proposed to participate in DNA strand opening on the basis of sequence similarity to a large family of eukaryotic RNA-binding proteins. Region 2.4 is implicated in recognition of the -10 region of the promoter by genetic studies. Region 3 is divided into two subregions. Subregion 3.1 bearing a weak resemblance to the helix-turn-helix DNA-binding motif while subregion 3.2 was implicated in core enzyme binding. Region 4 participates in recognition of the -35 promoter sequence

and consists of two subregions, 4.1 and 4.2. It was suggested that the highly conserved helix-turn-helix DNA binding motif of region 4.2 is stabilized by packing against the upstream amphipathic α-helix of region 4.1.

The ß subunit is the most intensively studied subunit in the core RNA polymerase. The ß subunit has been shown to form part of the catalytic site, being involved in binding to DNA template, nucleotide substrates as well as RNA products. It has been shown to be involved in elongation and termination of transcription. It was also indicated to interact with the σ subunit and ppGpp, participating in promoter selectivity and ppGpp sensitivity (Ishihama, 1988). The amino acid sequence of the ß subunit is well conserved during evolution. There are nine regions (A-I) conserved between the ß subunit of *E. coli* and the second-largest subunit of yeast RNA polymerase II, RPB2 (Sweetser *et al.*, 1987; Young, 1991). Region H contains the nucleotide binding site. The N-terminal third of the subunit was implicated to be especially important for proper folding and assembly of the ß subunit (Landick *et al.*, 1990). It was suggested that the N-terminal half of the ß subunit is involved in the assembly of core enzyme while the extreme C-terminus of ß is involved in interaction with the σ subunit (Glass *et al.*, 1986c; Glass *et al.*, 1988). Most rifampicin and streptolydigin resistant mutations have been mapped to the center of the ß subunit, between amino acids 500-570 (Jin and Gross, 1988). These mutations also cause a wide spectrum of alterations in the activity of RNA polymerase including binding of nucleotide substrates, elongation, both rho-dependent and rho-independent termination, and attenuation. One point mutation at aa 636 (Cys to Tyr) alters both promoter activity and transcription termination (Rockwell

and Gottesman, 1991). Two single amino acid changes which render RNA polymerase resistant to ppGpp inhibition were mapped to about aa 736 and aa 906 (Glass *et al.*, 1986a). The ß subunit includes a large dispensable region (aa 940-1040) that is absent in homologous RNA polymerase subunits from chloroplasts, eukaryotes and archaebacteria (Borukhov *et al.*, 1991a; Severinov *et al.*, 1992). This region overlaps with the *in vitro* promoter selectivity region identified by Glass *et al.* (1986b) between aa 956-1083. Lys1065 and His1237 were found located close to the binding site of the priming substrate in the RNA polymerase active center by a selective crosslinking technique (Mustaev *et al.*, 1991). Substitution at these two positions inhibits the transition from the initiation to the elongation stage of transcription.

Recently, a great deal of effort has been directed toward the elucidation of the function of the α subunit (Russo and Silhavy, 1992). Initially, the α subunit was assigned a role mainly in the assembly of the multisubunit complex, serving as a scaffold upon which the rest of the complex is built (Ishihama, 1981). The ADP-ribosylation of the α subunit at Arg-265 during bacteriophage T4 infection of *E. coli* suggested a possible role of α subunit in the regulation of transcription initiation. (Geiduschek and Kassavetis, 1988). Studies with anti-α monoclonal antibodies showed that some anti-α mAbs inhibit CRP-dependent transcription from the *lac* P^+ promoter almost completely while inhibiting CRP-independent transcription from *lac* UV5 promoter to a much lesser extent. In contrast, non-specific transcription from random templates was not markedly affected (Riftina *et al.*, 1989, 1990; Dalla Venezia and Krakow, 1990). These results suggested that the α subunit may play a role in the interaction between RNA polymerase and CRP as well as interaction

between σ factor and the core enzyme. This suggestion was supported by results from mutant RNA polymerases containing C-terminal truncated α subunits (Igarashi and Ishihama, 1991). It is clear now that the N-proximal region (aa 1-234) of the α subunit is responsible for core enzyme assembly (Igarashi *et al.*, 1991a) while the C-terminal region (aa 261-329) of the α subunit plays an important role in interaction with transcription regulators. Point mutations in *rpoA* have been described which affect regulation by at least eight different transcriptional regulators: CRP, CysB, AraC, MelR, OxrA/FNR, Ogr, δ, and OmpR. Two mutations at residue 261 prevent activation by CRP. Mutation E271K in *rpoA* caused pleiotropic metabolic defects by inhibiting activation by CysB, AraC, and MelR (Thomas and Glass, 1991). Mutations L289H, G311R, G311E, R317H, and W321ter affect transcription activation by OxrA under anaerobic conditions in *Salmonella typhimurium* (Lombardo *et al.*, 1991). The mutation of Leu-290 to His in *rpoA* prevents the growth of bacteriophage P2 by inhibiting expression of late phage genes, which are normally regulated by the *ogr* gene product (Christie *et al.*, 1986). The same mutation was also found to inhibit activation by a related δ gene product of the satellite phage P4 (Halling *et al.*, 1990). Several mutations (P322S, P323S, P323L) which cluster in the carboxy-terminus of the α subunit specifically interfere with the transcriptional control by OmpR and EnvZ, the two-component regulatory system that controls porin gene expression in *E. coli* in response to medium osmolarity (Slauch *et al.*, 1991). It is possible that the C-terminal region of the α subunit may fold into a domain with a number of contact points available for interaction with different regulatory proteins (Russo and Silhavy, 1992). Moreover, even with the same regulatory protein, there may be different

modes of interaction between the transcriptional regulator and RNA polymerase on different promoters. Evidence for this theory comes from studies with mutant *E. coli* RNA polymerase containing C-terminal truncated α subunits (Igarashi *et al.*, 1991b). This mutant enzyme was insensitive to the activation of transcription by CRP on the *lac* promoter and by OmpR on the *ompC* promoter *in vitro*. In contrast, truncation of the α subunits did not interfere with activation at CRP-dependent *galP1* promoter nor the PhoB-dependent *pstS* promoter, in both cases the activator binding site overlaps the -35 region of the promoter.

Progress on the elucidation of the function of the ß' subunit has been hindered by its large size, lack of specific inhibitors and lack of genetic markers. Our knowledge of ß' remains almost the same as 15 years ago. Based on the fact that the ß' subunit, the most positively charged subunit in RNA polymerase, is able to bind to DNA (Sethi and Zillig, 1970; Fukuda and Ishihama, 1974), it was suggested that ß' subunit may be involved in the interaction of RNA polymerase and the DNA template. This assumption is consistent with the observation that ß' binds to heparin, a polyanion which competes with DNA for a template-binding site on the RNA polymerase (Walter *et al.*, 1967). On the other hand, the contribution of ß' to the interaction between the σ subunit and core enzyme is demonstrated in reconstitution experiments, σ binding requires the previous integration of the ß' subunit (Palm *et al.*, 1975). It was also found that a very stable subassembly complex, ß'σ, can be isolated from *L. curvatus* RNA polymerase by phosphocellulose chromatography (Stetter and Zillig, 1974).

The sequence of the ß' subunit has been highly conserved during evolution.

Eight regions (A-H) of homology between the ß' subunit of *E. coli* and the largest subunit of various eukaryotic and archaebacterial RNA polymerases have been identified (Jokerst *et al.*, 1989; Young, 1991; Borukhov *et al.*, 1991b). Among the conserved regions are a basic region consistent with a DNA-binding domain (region C) and a consensus sequence (region A) characteristic of a potential zinc binding domain (Allison *et al.*, 1985). Mutation at residue 402 (Glu to Lys) suppresses a NusA mutant (nusA11), inferring that the ß' subunit is involved in transcription termination (Ito *et al.*, 1991, and unpublished data). Jin and Gross (1989) also reported that suppressors of some rho mutants have a mutation in the ß' subunit. Movement of the nascent transcript involves the interface between the ß and the ß' subunits, indicating that ß and ß' subunits participate in the formation of the active center of RNA polymerase (Hanna and Meares, 1983; Dissinger and Hanna, 1990). Borukhov *et al.* (1991b) have shown that the contact with the 3' terminus of the nascent transcript occurs within the region spanning Met-932 and Trp-1021 of the ß' subunit. Substitution of Asp to Lys at residue 1033 in ß' affects chromosomal replication in *E. coli* by altering the expression of the *DnaA* gene (Petersen and Hansen, 1991). A role for the ß' subunit in catalysis has been suggested by Lazcano *et al.* (1988). The strong inhibition effected by the anti-ß' mAb 311G2 (Rockwell and Krakow, 1985) and mAb PYN-1 (Nikiforov *et al.*, 1983) provides further evidence for the role of this subunit in RNA polymerase activity. The location of the domains on ß' involved in its function remains largely undefined.

The generation of monoclonal antibodies against unique antigenic determinants on the surface of a protein using the hybridoma technique (Kohler and

Milstein, 1975) has proved to be extremely powerful in unraveling the topological arrangement of subunits in multi-subunit proteins. Monoclonal antibodies can be used as specific probes to detect important regions involved in subunit-subunit interaction as well as to study possible conformational changes on a specific region of a subunit during enzyme assembly. Monoclonal antibodies raised against the α subunit of *Escherichia coli* RNA polymerase have generated valuable information on the topological arrangements of the two α subunits in the enzyme and the conformational changes occurring during core enzyme assembly (Riftina *et al.*, 1989). The first part of this thesis extends this technique to study the structure and function of the ß' subunit of the *E. coli* RNA polymerase using monoclonal antibodies specific to the ß' subunit. The effects of a set of anti-ß' monoclonal antibodies on RNA synthesis have been characterized, and their epitopes have been located.

The second part of this thesis describes reconstitution experiments using isolated wild-type α, ß, σ, and a set of truncated ß' proteins. The regions on ß' subunit involved in core enzyme assembly and interaction between the ß' subunit and the σ subunit (σ^{70}) have been identified.

MATERIALS AND METHODS

Source of materials: Reagents were obtained from: bovine serum albumin, 4-methylumbelliferyl β-D-galactopyranoside, 5-bromo-4-chloro-3-indolylphosphate (BCIP), heparin, phenol, thioglycolic acid, proteases and Nitro Blue Tetrazolium (NBT), Sigma Chemical Co.; 6-amino-n-hexanoic acid, BDH; Tween 80, J.T. Baker; non-fat powdered milk, Carnation Company; goat anti-mouse IgG-phosphatase, Kirkegaard and Perry Laboratories Inc.; goat anti-mouse IgG-β-galactosidase, Hyclone; TEMED and bis-acrylamide, Bio-Rad; acrylamide, Serva; nitrocellulose filters, Hoefer Scientific; restriction enzymes and T4 DNA ligase, Boehringer Mannheim Biochemicals; SeeKem and SeePlaque agarose, FMC; Geneclean Kit, U. S. Biochemical; NENSORB Prep columns, Du Pont; Cellulose (Cellex 410), Bio-Rad; STS, Pfaltz and Bauer Inc.; Silver Staining Kit, Bio-Rad.

Bacterial strains and plasmids: E. coli strains used were: HB101, K38/pGp1-2 (obtained from S. Tabor; Tabor and Richardson, 1985); BL21(DE3)pLysS, from Novagen; XL-1 Blue from Amersham; W3110lacI^{q}L8, (obtained from S. Knapp; Knapp *et al.*, 1990). Plasmids used were: pT7-ß' (obtained from A. Goldfarb; Zalenskaya *et al.*, 1990); pSEM-3 (obtained from S. Knapp; Knapp *et al.*, 1990); pGEMBC, pGEMAX, and pGEMD (obtained from A. Ishihama; Igarashi and Ishihama, 1991).

Preparation of DNA-cellulose column: The DNA-cellulose column was prepared by adsorption and UV-cross-linking as described by Litman (1968).

(1) Preparation of DNA solution: 2.7 g of calf-thymus DNA (Sigma) was teased apart with tweezers to facilitate dissolving. 200 ml of 0.02 M NaOH was used to dissolve DNA in order to reduce the viscosity of highly concentrated DNA solution. The DNA solution was stirred in the cold room overnight, its pH was neutralized by adding 200 ml 0.02 M HCl. The final DNA concentration was 6.5 mg/ml in 0.02 M NaCl.

(2) Acid-treatment of cellulose: 120 g of cellex 410 powder (Bio-Rad) was washed by stirring in 2000 ml of 1 N HCl for 30 min at room temperature. Washed cellulose was collected by filtering through Whatman paper (#1) by aspiration on a Buchner funnel. The cellulose was rinsed with H_2O, stirred again in 2000 ml of 1 N HCl for 20 min, washed exaustively with H_2O until no acid remained and then spread over the bottom of flat glass baking dishes (8 x 11) to dry in a stream of air from the hood blower overnight. The dried paste was then scraped from the dishes and ground into powder in a mortar before use.

(3) Preparation of DNA-cellulose: 150 ml of 6.5 mg/ml calf thymus DNA in 0.02 M NaCl was diluted to 500 ml with 0.01 M NaCl. 120 g of acid-treated cellulose powder was slowly added to this solution while stirring. After stirring for 30 min, the mixture was spread over the bottom of baking dishes, dried and powdered. The resulting material was lyophilized overnight. The cellulose was then mixed again with 360 ml of DNA solution containing 1 g DNA, stirred for 30 min, air-dried and lyophilized.

The cross-linking of DNA immobilized in cellulose was done by resuspending the cellulose in 2000 ml 95% grain alcohol, and slowly swirling the solution in 250 ml aliquots with a glass spatula under a UV-lamp (260 nm) for 20 min in a small baking dish. The distance from the UV-lamp to the surface of the suspension was 10 cm. The combined suspension was then filtered through a Buchner funnel and washed exaustively with washing buffer (1 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA) until the A_{260} of the filtrate was less than 0.05. The resulting DNA-cellulose was then lyophilized, resuspended in TGE buffer (20 mM Tris-Cl, pH 8.0, 5% glycerol, 0.1 mM EDTA) and packed into a column (300 ml). The column was washed with 2 M NaCl in TGE buffer and stored in cold room in TGE buffer containing 0.15 M NaCl and 0.02% NaN_3.

The amount of DNA bound to cellulose was assayed as the follows: 100 mg of DNA-cellulose was mixed with 5 ml of TE buffer and spun for 10 min at 1000 rpm. 2 ml of the supernate was used as the blank for UV-absorbance at 260 nm. The remaining 3 ml was vortexed, 2 ml was mixed with 2 ml concentrated HCl, boiled for 30 min, and spun for 10 min before determining the A_{260}. The DNA concentration was calculated as 3.5 mg/ml. Over all, more than 50% of starting amount of DNA was immobilized on the cellulose.

Preparation of DNA-agarose column: The DNA-agarose column was prepared as described by Schaller *et al.* (1972). 2.4 g of agarose (Sigma) was dissolved in 30 ml H_2O by heating in a microwave oven and cooled to 50°C. 25 ml of this 8% agarose solution was mixed with 25 ml of 12 mg/ml of calf thymus DNA in 0.02 M NaCl

which was heated to 50°C. The mixture in a closed bottle was shaken vigorously for 5 min to ensure the complete mixing of two solutions before allowing it to solidify in a shallow dish pre-cooled in ice. The gel was sliced into small cubes and then passed through a stainless steel mesh sieve (60-mesh) twice. The resulting material was resuspended in 0.01 M Tris-HCl (pH 7.5), 1 mM EDTA and 0.1 M NaCl, degassed for 2 hours, and packed into a column (100 ml) in the cold room. The column was washed with the same buffer until no DNA could be detected in the flow through. The column was either then equilibrated in the appropriate equilibration buffer before use, or stored at 4°C in the same buffer containing 0.02% NaN_3. 30-40 ml of buffer should be applied above the surface of the DNA-agarose in the column since it slowly swells during storage.

The amount of DNA trapped in agarose was determined by the following assay. 0.8 g of DNA-agarose (0.8 ml of packed column volume) was weighed, and added to 5 ml of 0.5 M $NaClO_4$. The mixture was boiled for 5 min to melt the agarose, and cooled to room temperature. The amount of DNA in upper solution was measured directly at 260 nm. As a blank, the same amount of agarose (without DNA added) was also treated with $NaClO_4$, boiled, and checked at 260 nm. The DNA concentration in the DNA-agarose was determined as 1 mg/ml.

Preparation of RNA polymerase: RNA polymerase was purified from *E. coli* K12 by a modification of the procedure of Burgess and Jendrisak (1975). Holoenzyme and core enzyme were resolved by chromatography on denatured calf thymus DNA-agarose (Lowe *et al.*, 1979). Protein concentration was determined using the

following extinction coefficients; core polymerase, $E^{1\%}_{280nm}$ = 5.8; holoenzyme, $E^{1\%}_{280nm}$ = 6.7 (Levine *et al.*, 1980).

Separation of RNA polymerase subunits: RNA polymerase subunits were prepared by chromatography of urea-dissociated core polymerase (5 mg) on Bio-Rex 70 (8 ml column volume) equilibrated in urea buffer (20 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 1 mM DTT and 7 M urea). The column was eluted with a 40 ml linear salt gradient from 0.1 M to 1 M NaCl in urea buffer. The ß' subunit was collected when the NaCl concentration reached 0.5 M. The α subunit was recovered in the flow-through and the ß subunit eluted at 0.2 M NaCl. Fractions containing each subunit were pooled separately and dialyzed against a buffer containing 20 mM potassium phosphate (pH 7.5), 1 mM EDTA, 150 mM NaCl, 5% glycerol and 0.1 mM DTT. Purified subunits were concentrated by dialysis against the same buffer containing 60% glycerol.

Partial digestion of RNA polymerase core enzyme: 2 μg of core enzyme was partially digested in 20 μl of 50 mM Tris-HCl (pH 8.0), 5% glycerol and 0.01 μg of trypsin or subtilisin for 30 min. at 37°C. The reaction was stopped by adding 4 μl of 20 mM PMSF. After addition of 3 μl of sample buffer containing 50% glycerol, 1% SDS, 0.05% bromphenol blue, and 3 μl of 14.3 M 2-mercaptoethanol, the mixture was heated in a boiling water bath for 2 min and resolved on a 10 % SDS polyacrylamide gel.

Gel electrophoresis (SDS-PAGE and STS-PAGE): SDS gel electrophoresis was performed as described by Laemmli (1970). Diluted protein samples or proteins dissolved in high salt were concentrated by TCA-precipitation before gel electrophoresis. Proteins were precipitated by adding TCA to 10% and sodium deoxycholate to 0.0025% followed by centrifugation at 4°C for 15 min after one hour incubation on ice. Pellets were solubilized in 1 x SDS sample buffer. (1 M Tris base may be used if the solution was too acidic).

Although the ß and ß' subunits can be fairly separated on a 7.5% SDS gel, the best separation was achieved by electrophoresis of core enzyme on STS (sodium tetradecyl sulfate) gels (10% acrylamide, 0.1% bisacrylamide) as described by Fisher and Blumenthal (1980). STS-PAGE is essentially the same as SDS-PAGE except that in the separation gel, stacking gel, and the electrode buffer, SDS was replaced by an appropriate amount of STS. A practical recipe is as follows: two stock solutions (solution A and B) were made. Solution A was made from 36.3 g of Tris, 4 ml of concentrated HCl and 0.23 ml of TEMED. The final volume was brought to 100 ml with H_2O after adjusting the pH to 8.0. Solution B contained 40% (w/v) acrylamide and 0.4% bisacrylamide. The separation gel was formed by combining 1.75 ml of solution B, 7 µl of 20% STS, 50 µl of 5% of APS, 4.3 ml of H_2O and 3.6 ml of solution A. The stacking gel was a mixture of 320 µl of 30% (w/v) acrylamide solution (acrylamide:bisacrylamide=29.2:0.8), 500 µl of 0.5 M Tris-Cl (pH 6.8), 1 ml of H_2O, 0.75 µl of 27% STS, 20 µl of 5% APS, and 2 µl of TEMED. The electrode buffer (1000 ml) was made from 14.4 g of glycine, 1 ml of 27% STS and 3.03 g of Tris base. The sample buffer for STS gels was the same as for SDS gels. The ß' subunit

migrates faster than the ß subunit on STS gels.

Preparation of monoclonal antibodies: Monoclonal antibodies were prepared as indicated in Rockwell *et al.* (1985). The anti-ß' polyclonal antibody was prepared from sera obtained from mice immunized against pure ß'. Immunoglobulin concentration was determined using the extinction coefficient: $E^{1\%}_{280nm} = 14.0$ (Ey *et al.*, 1978). The immunoglobulin isotype of each monoclonal antibody was determined using the Mouse Immunoglobulin Subtype Identification Kit from Boehringer Mannheim Corp.

Solid phase ELISA: Costar 96-well EIA polystyrene plates were coated with 1 μg of RNA polymerase or 0.5 μg of subunits in PBS (10 mM potassium phosphate, pH 7.2 and 150 mM KCl) at 4°C overnight. Remaining protein-binding sites in each well were blocked by incubation with 200 μl of PBS-BSA Buffer (2 mg/ml BSA in PBS) for 90 min at room temperature. The plates were washed three times with washing buffer containing 2 mg/ml BSA and 0.05% Tween 80 in PBS. Then, 100 μl supernatant of hybridoma culture medium or 5×10^{-10} M pure antibody was added to each well and incubated at 37°C for one hour. After washing the wells four times with the washing buffer, 100 μl of goat anti-mouse IgG-phosphatase (1/1000 dilution in PBS-BSA) was added and incubated at 37°C for one hour. The wells were again washed three times with washing buffer, 100 μl of substrate solution containing 1 mg/ml p-nitro-phenylphosphate in 0.1 M diethanolamine (pH 9.0), and 2.5 μM $MgCl_2$ was added and incubated for 45 to 60 min at room temperature. The reaction was

stopped by adding 100 μl of 1 M NaOH per well; the absorbance at 410 nm was determined using a Dynatech Microelisa Reader.

Native retardation gel assay for antigen-antibody complex: A modification of the method of Hedrick and Smith (1968) was used to identify the antigen-antibody complexes by native polyacrylamide gel electrophoresis. The RNA polymerase-monoclonal antibody complexes were formed by incubation of 1 μg of holoenzyme with the indicated amount of the antibody at 37°C for 30 min in 20 μl of 50 mM Tris-HCl (pH 8.0), and 5% glycerol. After addition of 1 μl of bromophenol blue (0.1 mg/ml), the mixture was loaded onto a native polyacrylamide gel system containing a 4% stacking gel (pH 6.8) and 6% running gel (pH 8.8) and a running buffer of 34 mM asparagine (pH 7.3) plus 0.01% (v/v) thioglycolic acid. Electrophoresis was performed at constant voltage of 200 volts for 1 hour and the gels were stained with Coomassie blue.

Monoclonal antibody binding constants: The dissociation constants of antigen-antibody complexes under native conditions were determined by an enzyme-linked immunosorbent assay (Friguet *et al.*, 1985). ELISA plates (MicroFluoro "β" Flat-bottom plates, Dynatech Labs) were incubated with 2 μg of core enzyme in PBS overnight at 4°C. Remaining protein binding sites were blocked by incubation with 200 μl/well of blocking buffer (PBS supplemented with 0.2% bovine serum albumin, 0.02% NaN_3) for 90 minutes at room temperature. The plates were then washed three times with the washing buffer (PBS supplemented with 0.2% bovine serum

albumin, 0.05% Tween 80, 0.02% NaN_3). The antigen (β' subunit, core or holoenzyme) at the concentrations indicated was incubated in solution with the indicated antibody at constant concentration at 37°C for 1 hour or at 4°C overnight to establish equilibrium. 100 μl aliquots were removed from these mixtures for binding to the plate-immobilized core enzyme. After incubation for 1 hour at 37°C, the plates were washed four times with washing buffer. 100 μl of goat anti-mouse IgG-β-galactosidase (1:1000 dilution in blocking buffer) were added to each well and incubated for 45 min at 37°C. After washing three times with the washing buffer, 100 μl of a solution containing 1 mg/ml of 4-methyl-umbelliferyl-β-D-galactopyranoside in 10 mM potassium phosphate (pH 7.5) and 1 mM $MgCl_2$ was added and the fluorescence of the product was determined with a Dynatech Microfluoro Reader.

Assay for d(A-T)$_n$-directed r(A-U)$_n$ synthesis: Wildtype core or holoenzyme or reconstituted RNA polymerase or RNA polymerase-monoclonal antibody complex in the amounts indicated in the legends were added to a mixture (final volume of 150 μl) containing 120 mM Tris-HCl (pH 7.8), 5 mM $Mg(OAc)_2$, 2 mM $MnSO_4$, 0.2 mM DTT, 0.5 μg DNA template, 160 μM ATP and ^{3}H-UTP. The reaction was performed at 37°C for 15 min, RNA transcripts were precipitated by adding 150 μl of cold 20% TCA and incubated at 0°C for 60 min. The precipitates were collected by filtering through glass fiber filters (Whatman GF/A) presoaked in 5% TCA and 50 mM sodium pyrophosphate. The filters were washed three times with 5% TCA and 50 mM sodium pyrophosphate, once with 95% ethanol; dried; and added to 5 ml

Scintisol before counting.

Abortive initiation assay: A modification of the assay of Malan *et al.* (1984) was used to determine the effect of monoclonal antibodies on transcription from *lac* UV5 and *lac* P^+ promoters. The reaction mixture (final volume 50 μl) contained: 40 mM Tris-HCl (pH 8.0), 100 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 2.5% glycerol, 2 pmol CRP, 5 nmol cAMP, 0.2 pmol *lac* P^+ or *lac* UV5 DNA fragment and the amount of RNA polymerase or RNA polymerase-mAb complex indicated in the legends. Monoclonal antibody-RNA polymerase complexes were formed by incubation of mAb and RNA polymerase in the ratios indicated in the legends at 37°C for 30 min. After additional incubation with the template for 10 min at 37°C, 1 mM ApA and 50 μM ^{3}H-UTP (320 cpm/pmol) were added. The reaction was terminated by addition of 10 μl of 0.5 M EDTA. The radioactive products were resolved by ascending paper chromatography in WASP solvent (water:saturated ammonium sulfate:2-propanol=18:80:2) with its pH adjusted to 8.0 by NH_4OH (Hansen and McClure, 1979). The amount of ApApUpU synthesized was estimated by determining the radioactivity of appropriate 1 cm segments in Scintisol.

Determination of effects of monoclonal antibodies on the reconstitution of core enzyme: RNA polymerase core enzyme was denatured either by incubation in the denaturation buffer (0.05 M Tris-HCl, pH 8.0, 10 mM DTT, 1 mM EDTA, 8 M urea) at 0°C for 2 hours or by dialysis against the same buffer for 3 hours at room temperature. Aliquots of denatured core enzyme were mixed without or with anti-ß'

monoclonal antibodies in the ratios indicated in the legends and dialysed against reconstitution buffer (0.05 M Tris-HCl, pH 8.0, 0.3 M KCl, 10 mM $MgCl_2$, 10 mM DTT, 20% glycerol and 0.2 mg/ml BSA) at 4°C overnight. The activity of reconstituted core enzyme in the absence or presence of antibodies was measured by the $d(A\text{-}T)_n$-directed $r(A\text{-}U)_n$ synthesis assay.

Western blot assay: Proteins were separated on a 10% SDS polyacrylamide gel following the procedure of Laemmli (1970). The proteins were transferred at 2.5 mA/cm^2 for 30 to 45 minutes to a nitrocellulose membrane using a MiniBlot-SDE (Millipore). Due to the high molecular weight of the β' subunit, pronase was used to facilitate its transfer to the nitrocellulose membrane (Gibson, 1981). After transfer, the nitrocellulose filter was dried, and a strip was cut for staining with 0.02% toluidine blue to check transfer efficiency. The nitrocellulose filter was incubated in 50 ng/ml of heparin in TBS (20 mM Tris-HCl, pH 7.4, and 0.9% NaCl) for 10 min at room temperature before blocking at 4°C overnight with gentle rotation in TBS buffer containing 5% NFCM, 2% fetal bovine serum, 0.02% NaN_3, and 0.1% (v/v) Tween 80. The blocked filter was washed three times with washing buffer (TBS with 0.5% NFCM) and then incubated with monoclonal antibody diluted in washing buffer at room temperature for 2 hours on a rocker. After washing four times, the filter was incubated in goat anti-mouse IgG-phosphatase (diluted 1:500 in washing buffer) for two hours at room temperature. After washing the filter three times, the signals were developed in a reaction buffer containing 0.1 M Tris-HCl (pH 9.5), 0.1 M NaCl, 50 mM $MgCl_2$, 0.33 mg/ml of NBT and 0.17 mg/ml of BCIP at room temperature for 5

to 20 min. The reaction was terminated by rinsing the filter with deionized water several times.

Construction of ß' mutants: 5 μg of pT7-ß' was partially digested in a 20 μl solution (the buffer recommended by Boehringer Mannheim Biochemicals) by a restriction enzyme having two or more cleavage sites in the *rpoC* gene. After one hour at 37°C, the volume of the reaction mixture was increased by addition of 0.3 M sodium acetate (pH 5.2) to 200 μl. After phenol-chloroform extraction and ethanol precipitation, the pellet was dissolved in 20 μl of TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA). From this, 4 μl was used for analysis on 0.8 % agarose gel, 12 μl for ligation at 14°C overnight in the presence of 1 unit of T4 DNA ligase and 4 μl was saved as a non-ligated DNA control for transformation. The ligation mixture as well as the non-ligated control were transformed into competent *E. coli* HB101 or XL-1 Blue prepared as described by Chung *et al.* (1989). Positive transformants were selected on LB-Amp plates and grown in liquid culture (LB-Amp) overnight. Plasmid minipreps were used for restriction enzyme analysis. Fourteen plasmids containing truncated *rpoC* (pJLB, pJLE1, pJLE2, pJLE3, pJLK1, pJLK2, pJLK3, pJLM1, pJLM3, pJLP3, pJLP4, pJLP6, pJLP7, and pJLP8) were made by this method.

The pT7-ß'-derived plasmids identified as containing a truncated *rpoC* were then transformed into either K38/pGp1-2, or BL21 plysS. Synthesis of the truncated ß' proteins was induced by either temperature shift from 30°C to 42°C in K38/pGp1-2 or by induction with 0.4 mM IPTG in BL21 plysS at an A_{600nm} of 0.6 to 1.5 for three hours. Total cell protein was prepared by lysing the cell pellet collected from a 1 ml

culture in 100 μl of electrophoresis sample buffer (0.1% SDS, 5% glycerol, 0.05% bromphenol blue plus 1% of 2-mercaptoethanol). After heatiing in a boiling water bath for 5 min 10 μl was used for analysis by SDS-polyacrylamide gel electrophoresis. For Western blotting, 1 μg of RNA polymerase core enzyme was added to 10 μl of cell extract prior to electrophoresis.

Mapping of antigenic determinants: For manipulation of the antigenic determinant regions on *rpoC*, the expression vector pSEM-3 (Knapp *et al.*, 1990) was modified as follows. Two oligodeoxynucleotides, GCCAAGGA and AGCTTCCTTGGCTGCA, were synthesized on an Applied Biosystems DNA Synthesizer and purified by using a NENSORB Prep column. The oligodeoxynucleotides (400 nmol of each) were phosphorylated using 10 units of T4 polynucleotide kinase and 1 mM ATP for 15 min at 37°C. After incubation at 70°C for 10 min, 20 nmol of each oligonucleotide were mixed and annealed at 37°C for 30 min, and then ligated to 1 μg of *Pst*I-*Hind*III digested pSEM-3. The resulting pSEM-4 has a *Sty*I site introduced into the polylinker region of pSEM-3. The *Sal*I-*Sty*I fragment of *rpoC* derived from pT7-ß' was inserted into the *Sal*I-*Sty*I site of pSEM-4 to form pJLSS. A fusion protein, pJLSS[ß-gal$^{1-375}$-ß'$^{877-1132}$], containing the N-terminal 375 amino acids of ß-galactosidase and a fragment of the ß' subunit (amino acids 877-1132) was expressed from pJLSS in W3110lacI^{q}L8 after induction with 1 mM IPTG as described by Knapp *et al.* (1990).

A frame-shift mutation at amino acid 1073 in *rpoC* was constructed by digesting pJLSS with *Bgl*II, filling in the ends with Klenow fragment, and ligated to form pJLSSB. When a *Sma*I linker, pGCCCGGGC (U. S. Biochemicals) was included

prior to ligation, pJLSSBS was formed and the reading frame of *rpoC* was restored. Fusion proteins containing the mutations in the ß' segment were expressed from pJLSSB and pJLSSBS in W3110lacI^{q}L8 as described above.

By replacing the *SalI-StyI* fragment in pT7ß' and pJLP7 with those from either pJLSSB or pJLSSBS, four more plasmids (pJLBB, pJLBS, pJLP7B, and pJLP7BS) with mutations in *rpoC* were formed.

Overexpression and purification of mutant and wildtype E. coli RNA polymerase subunits: For mutant ß' subunits, BL21 (λDE3) pLysS or K38/pGp1-2 were transformed with plasmids containing the mutant ß' constructions shown in Figure 5. Transformants were grown in 200 ml LB medium containing 200 μg/ml of ampicillin at 37°C (for BL21 cells) or at 30°C (for K38/pGp1-2 cells). Expression of mutant ß' proteins was induced at Klett 30 by adding IPTG to 1 mM (for BL21 cells) or by shifting the temperature from 30°C to 42°C (for K38/pGp1-2 cells). After induction for 3 hr, cells were harvested and stored at -80°C. For purification, frozen cells collected from 200 ml culture were resuspended in 1.5 ml "Lysis Buffer" containing 50 mM Tris·HCl (pH 8.0 at 4°C), 1 mM EDTA, and 0.1 mM Nacl. After adding PMSF to 0.27 mM, lysozyme to 0.3 mg/ml and sodium deoxycholate to 0.1%, the mixture was incubated on ice for 20 min with occasional mixing. The cell lysate was sonicated for 5 sec for 4 times and centrifuged at 15K rpm for 10 min. The pellet was resuspended in "Lysis Buffer" with a plastic spatula. After centrifugation at 15K rpm for 10 min, the pellet was washed with "Lysis Buffer" containing 0.2 mM NaCl, and extracted twice with "Extraction Buffer" containing 50 mM Tris-HCl (pH 8.0), 1 mM

EDTA, 10 mM DTT, 0.2 M KCl, 10 mM $MgCl_2$, 20 % glycerol and 6 M guanidine-HCl. The combined extract was centrifuged at 40K rpm for 90 min at 4°C in a Beckman TLS55 rotor. The supernatant was stored at -20°C and used directly for reconstitution of RNA polymerase.

Wildtype α and σ^{70} subunits were overexpressed in *E. coli* BL21 (λDE3) transformed with expression plasmids pGEMAX and pGEMD and purified as described by Igarashi and Ishihama (1991).

Wildtype ß and ß' subunits were expressed from BL21 (DE3) transformed with pGEMBC, purified in the same way as for mutant ß' proteins except for the following changes. The pellet formed after sonication and centrifugation was washed twice with a Triton buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.1 M KCl and 0.5% Triton. The final pellet was extracted four times with the same extraction buffer but with 6M urea substituted for guanidine-HCl. Since the urea-extraction buffer was less powerful than the guanidine-HCl extraction buffer, the extraction mixture was incubated on ice for one hour for each extraction.

Pooled protein extracts were diluted with a TGED-urea buffer (20 mM Tris-HCl, pH 8.0, 5% glycerol, 0.1 mM EDTA, 1 mM DTT and 6 M urea) to a salt concentration of 50 mM, spun at 10K rpm for 10 min and the supernatant was loaded onto a protein G-PAK HPLC column (Pharmacia) equilibrated in TGED-urea buffer with 0.05 M NaCl. The ß and ß' subunits were resolved with a linear salt gradient of 80 to 200 mM NaCl in TGED-urea buffer.

Reconstitution of RNA polymerase: For reconstitution of core enzyme, wildtype or

mutant ß' proteins were mixed with wildtype ß and α subunits in a molar ratio of 2:1:2 in a buffer containing 7 M deionized urea, 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 2 mM DTT, 0.1 mM EDTA, 0.2 M KCl and 10% glycerol. The final concentration of proteins was about 230 μg/ml. The mixture was dialyzed at 0°C for about 16 hours against a buffer containing 50 mM Tris-HCl (pH 8.0 at 4°C), 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.3 M KCl, 0.1 mM PMSF and 20% glycerol. After dialysis, the mixtures were incubated at 30°C for 30 min to activate premature core enzyme. The whole mixture was then microfuged at 4°C for 15 min to remove protein aggregates which may have formed during dialysis. For the reconstitution of holoenzyme, the protocol was basically the same as that for core enzyme except that the σ subunit was present in the dialysis mixture, and the molar ratio of α:ß:ß':σ was 2:1:2:1.

Immunoprecipitation assay: The supernatant of hybridoma cell culture medium containing anti-α mAb 125C6 (Riftina *et al.*, 1989) at concentration of about 5 mg/ml was used to detect any ß' mutant protein assembled into core or holoenzyme. At first, 2 mg of BSA was added to 0.5 ml of this supernatant, incubated at 37°C for half an hour to prevent possible nonspecific binding of antibody to proteins. Then, 30 μl of the centrifuged dialysate mixture was added to the BSA-blocked antibody solution and incubated at 37°C for 30 min. The antigen-antibody complex was recovered by incubating at room temperature for 20 min with 50 μl of 10% Protein A-Sepharose beads. (The Protein A-Sepharose beads were washed with PBS three times, resuspended in PBS containing 2 mg/ml BSA and incubated at room temperature for

20 min before adding to antigen-antibody mixture.) The antigen-antibody-Protein A Sepharose beads were washed with PBS four times, resuspended in 15 μl of 1 x SDS Sample buffer, boiled for 6 min. The supernatant was loaded onto a 15% SDS polyacrylamide gel. Gel electrophoresis was conducted at 200 volts at constant voltage for about one hour, and proteins on the gel were stained by Coomassie blue or silver-stain.

RESULTS

Properties of anti-ß' monoclonal antibodies. The properties of five monoclonal antibodies raised against the ß' subunit of *E. coli* RNA polymerase are summarized in Table II. Of the five antibodies studied, all have κ light chains and γ 1 heavy chain except mAb 340F11 which has γ 2a heavy chain. Only mAb 311G2 strongly inhibited initiation and elongation directed by *lac* promoters and d(A-T)$_n$. None of the other anti-ß' monoclonal antibodies inhibited *lac* UV5-directed activity while mAb 371D6 and mAb 372B2 partially inhibited the CRP-dependent reaction directed by a DNA fragment containing *lac* P1. No inhibition was seen when the reaction was run using a superhelical template containing *lac* P1 (data not shown). A lack of inhibition may reflect either the binding of a null antibody to a noncritical region of the assembled ß' or the inaccessibility of the epitope in the native RNA polymerase.

Effects of anti-ß' monoclonal antibodies on the reconstitution of core enzyme. Based on results of reconstitution of core enzyme from purified subunits or from subunits dissociated from intact enzymes, Ishihama (1981) has proposed the following sequence of subunit assembly: $\alpha + \alpha \rightarrow \alpha_2 + ß \rightarrow \alpha_2ß + ß' \rightarrow \alpha_2ßß'$ (premature core enzyme) $\rightarrow \alpha_2ßß'$ (mature core enzyme). In the inactive premature core enzyme the subunits are loosely associated. The temperature-dependent maturation is required for the formation of a structurally stable and functionally active RNA polymerase. Since anti-ß' mAbs 340F11, 370F3, 371D6, and 372B2 do not inhibit d(A-T)$_n$-directed

RNA synthesis, this assay was used to determine the possible effects of these four antibodies on recovery of active core enzyme reconstituted from a subunit mixture. The results presented in Table III indicate that mAb 372B2 strongly inhibits reconstitution of core enzyme and also partially inhibits the transition of premature core enzyme to the mature core enzyme. mAbs 370F3, 371D6, and 340F11 have no effect either on the reconstitution of core enzyme from subunit mixture or on the transition of premature core enzyme to mature core enzyme even when a high molar ratio of antibody to antigen was used. The results of the effects of the antibodies on the activity of mature core enzyme was similar to that of the native core enzyme (Tables II and III).

The binding of anti-ß' monoclonal antibodies to RNA polymerase. The binding of the anti-ß' monoclonal antibodies to RNA polymerase holoenzyme was assessed by a gel shift assay. The formation of an antigen-antibody complex is indicated by the altered mobility of RNA polymerase following native polyacrylamide gel electrophoresis. Figure 1 shows that only mAb 311G2 bound to RNA polymerase under the conditions used. The noninhibitory monoclonal antibodies did not bind to native RNA polymerase even though a high molar ratio of antibody to enzyme was used. The poorly defined protein bands seen above RNA polymerase in Lanes c-f are the free monoclonal antibody which do not migrate as a sharply defined band under the conditions of non-denaturing gel electrophoresis. The same poorly defined bands were also seen with antibodies alone on the native gel (data not shown). When these antibodies were assayed by SDS polyacrylamide gel electrophoresis the typical heavy

and light chain pattern was seen (data not shown).

The affinity of each of the anti-ß' monoclonal antibodies for their epitopes was determined by the indirect ELISA procedure devised by Goldberg and coworkers (Friguet *et al.*, 1985). The dissociation constants of the anti-ß' monoclonal antibodies for free ß', core or holoenzyme-associated ß' are presented in Figure 2A and 2B, and Table IV. There was a significant difference between the dissociation constants of the inhibitory mAb 311G2 (Figure 2A) and that of the non-inhibitory mAb 372B2 (Figure 2B). The results obtained with mAb 370F3, mAb 371D6 and mAb 340F11 are similar to those obtained with mAb 372B2 (Table IV). The mAb 311G2 bound with comparable affinity to both free ß' and the core or holoenzyme associated ß'. In contrast, the affinities of the null monoclonal antibodies for ß' in native core or holoenzyme were about 30 to 70-fold lower than that observed with the free ß' subunit (Table IV).

Western blot assay of protease-digested core enzyme. In order to assess the relation of the epitopes for the five anti-β' monoclonal antibodies studied, RNA polymerase core enzyme was partially digested with trypsin or subtilisin. The digested core enzyme was resolved by electrophoresis on a 10% SDS gel and the fragments were transferred to a nitrocellulose filter for blotting with the anti-β' monoclonal antibodies. Figures 3A and 3B show Western blots for digested core enzyme probed with the different anti-β' monoclonal antibodies. It is evident that mAb 311G2, mAb 370F3 and mAb 371D6 have distinctive band patterns. On the other hand, mAb 340F11 (IgG2a) and mAb 372B2 (IgG1) show similar band patterns; both of these

antibodies crossreact with the α subunit as does the anti-ß' polyclonal antibody (data not shown). This is apparently a consequence of denaturation of the α subunit for SDS polyacrylamide gel electrophoresis; neither monoclonal antibody reacts with native α in solid-phase ELISA assay (data not shown).

Epitope mapping of anti-ß' monoclonal antibodies. Localization of the epitopes for the anti-ß' monoclonal antibodies was carried out by preparing truncated ß' mutants. A series of overlapping deletion mutants was constructed by partial digestion and religation of the ß' gene present in pT7ß' (Fig.4; Zalenskaya *et al.*, 1990). Four deletion mutants (JLB and JLK1, JLK2, JLK3) were constructed by using restriction enzymes *Bgl*I or *Kpn*I (Figure 5). The reading frame for ß' translation was retained after religation for these four mutants. The region deleted was confirmed by restriction analysis. JLK2 and JLK3 both received 16 amino acids at the C-terminal end from the vector (Table V). Attempts were made to transform each into K38/pGp1-2 (Tabor and Richardson, 1985). pJLK2 and pJLK3 failed to transform K38/pGp1-2 (data not shown). These were transformed into BL21/pLysS (Studier *et al.*, 1990). In this strain a small amount of T7 lysozyme produced by the plasmid pLysS inhibits the T7 RNA polymerase so that the basal level of the mutant ß' will be very low before induction. Therefore, cells carrying a potentially toxic mutation will have a better chance to survive before induction. After induction of T7 RNA polymerase by temperature shift in K38/pGp1-2, or by adding IPTG to 0.4 mM to BL21/pLysS cells, the mutant ß' protein was expressed for about 3 hours. Extracts from the induced cells were resolved by SDS polyacrylamide gel electrophoresis and

stained with Coomassie Blue. Synthesis of the mutant proteins was confirmed by their migration at the expected size range (data not shown). The Western blots shown in Figures 6-9 indicate that all of the truncated ß' proteins were bound by the polyclonal anti-ß' antibody. JLB, JLK1 and JLK3 (data not shown) were not bound by any of the monoclonal antibodies. In contrast, JLK2 (data not shown) was bound by all of the monoclonal antibodies. The epitopes for the anti-ß' monoclonal antibodies were found within a relatively small region between amino acid residues 817-1072 (*Kpn*I-*Bgl*II). Another series of truncated ß' mutants was made in the same way as described above using *Eco*RI, *Mlu*I, and *Pvu*II. After identification of their deleted region in the ß' gene, four mutants JLE2, JLE3, JLM3, and JLP6 were selected for further study. The reading frame for ß' was retained in JLE2 and JLP6. The C-terminus of the ß' fragment JLE3 received 12 amino acids from the vector (Table V). The reading frame of ß' was changed in JLM3; as a result, 20 amino acids were added to the C-terminus of the truncated ß'. The results of the immunoblotting for JLE2, JLE3, JLM3, and JLP6 proteins (Fig. 7 and 8) are summarized in Table V. All were bound by the anti-ß' polyclonal antibody. mAb 311G2 bound to JLE2 and JLP6, but not to JLM3 and JLE3. mAb 340F11, mAb 370F3, mAb 371D6, and mAb 372B2 bound to JLE3 and JLM3, but not to JLE2 and JLP6. These results indicate that the epitope of mAb 311G2 may be located between amino acid 1047 (*Mlu*I site) and 1072 (*Bgl*II site), while the other anti-ß' monoclonal antibodies have their epitopes located between amino acids 817 (*Kpn*I site) and 929 (*Pvu*II site).

The fusion protein pJLSS[ß-gal$^{1-375}$-ß'$^{877-1132}$] was expressed from pJLSS and used for Western blotting. Only mAb 311G2 and the anti-ß' polyclonal antibody

bound to this fusion protein (Table V and Figure 9). This result indicates that all of the null inhibitory anti-ß' mAbs have epitopes located between amino acids 817 (*Kpn*I site) and 876 (*Sal*I site). pJLSSB with a frame-shift mutation at amino acid 1073 (*Bgl*II site) in *rpoC* was generated as described in Methods and Materials. The fusion protein expressed from pJLSSB contains a shorter fragment of the ß' subunit than that coded by pJLSS. mAb 311G2 did not bind to this protein while the anti-ß' polyclonal antibody still showed strong binding (data not shown).

pJLSSBS was formed through the insertion of a *Sma*I linker into pJLSSB. The reading frame in *rpoC* was restored in pJLSSBS, and a fusion protein with an insertion of a tetra-peptide (Arg-Pro-Gly-Asp) between amino acids 1073 and 1074 in the ß' segment was produced upon induction. The results of Western blotting (Figure 9) show that mAb 311G2 bound very weakly to this protein while the anti-ß' polyclonal antibody still showed strong binding. The epitope for mAb 311G2 appears to be located in the region of the *Bgl*II site which includes amino acid 1072.

Overexpression and purification of truncated ß' proteins. Plasmids containing truncated ß' gene (*rpoC*) were made by self-ligation of plasmid pT7ß' partially or completely digested by the indicated restriction enzymes. Figure 5 indicates the wildtype as well as truncated *rpoC* proteins. Expression of the truncated ß' proteins was conducted either by adding IPTG in BL21(DE3) cells or by temperature-shift in K38/pGp1-2 cells. Total cell extracts were used for SDS-polyacrylamide gel analysis to follow the induction time course. The expression of wildtype ß' was at the highest level after 90 min while most truncated ß' proteins reached the highest level of

expression after three hours of induction. Figure 10 shows a typical time-course for the expression of mutant ß' proteins. Mutant ß' proteins as well as wildtype ß' start to accumulate at 30 min after induction and reach the highest level after 3 hours of induction; then cells reach saturation phase and start to die (data not shown). All mutant ß' proteins shown in Figure 5 were expressed at high level (40% of the total cell protein) except JLK2, JLE1, and JLM1. JLk2 was expressed only at about 5% to 10% of the total protein (data not shown). JLE1 and JLM1 were never detected in cell extracts resolved on SDS-polyacrylamide gels. It is possible that the large truncation of the ß' subunit in these two mutants makes the mutant protein extremely unstable so that they are quickly degraded by proteases after induction. The possibility of toxicity of these two mutant ß' proteins is ruled out because plasmids which contain their coding sequence can be easily transformed into an expression host and maintained in the same host. After induction, total cell number did not decrease rapidly (data not shown).

The wildtype α, ß and ß' were purified to near homogeneity while the truncated ß' proteins were estimated to be about 80% pure. Figure 11 shows the electrophoretic pattern for the purified mutant ß' proteins. The size of each truncated ß' protein was in good agreement with those calculated from the deletion endpoints of the plasmid constructs.

***In vitro* assembly of mutant ß' proteins.** The assembly of truncated ß' proteins was carried out by an *in vitro* mixed reconstitution assay. Purified subunits were mixed in urea buffer in an appropriate molar ratio and then dialyzed against reconstitution

buffer. Reconstituted enzymes were immunoprecipitated using an anti-α monoclonal antibody and Protein A-Sepharose beads. Figure 12 shows that all of the truncated ß' mutants tested can assemble into core enzyme. JLE3 and JLM3 (data not shown) assemble into core enzyme weakly. This is indicated by the fact that the stoichiometry of the ß and the ß' mutant in the same lane is greater than 1:1. However, once they have incorporated into core enzyme, they can assemble further into holoenzyme. This is shown by the co-precipitation of the σ subunit in the same lane. Mutant ß' proteins JLBS, JLE3, JLK3, and JLP6 can assemble efficiently into holoenzymes. Three mutant ß' proteins, JLE2, JLP7, and JLP7B can assemble into core enzyme but failed to co-precipitate the σ subunit. The minor bands below σ in lanes g and h were not σ, but degraded fragments from JLP7 and JLP7B because they were also seen even when σ subunit was not included in the reconstituion mixture (data not shown). Therefore, they apparently have lost the region (Figure 5) which interacts with the σ subunit. In the immunoprecipitation experiments the dialyzed reconstitution mixture was centrifuged prior to addition of mAb 125C6 and Protein A-Sepharose to remove any possible mutant ß' aggregates. To assure that the coprecipitation of α, ß, and mutant ß' proteins by anti-α mAb 125C6 is a consequence of core enzyme assembly and not due to non-specific binding of the antibody to mutant ß' subunits immunoprecipitation was carried out with or without the ß subunit present during reassembly. Figure 13 shows that the assembly of mutant ß' proteins E3, P6, K3, and P7 was indeed ß-dependent. No mutant ß' proteins were precipitated when the ß subunit was absent from the dialysis mixture. Also, Lane j of Figure 13 shows the result of an immunoprecipitation of purified holoenzyme without antibody present

during immunoprecipitation process. BSA which was used to block non-specific sites on the Protein A-Sepharose was the only protein precipitated. Therfore, the possibility of non-specific precipitation of mutant ß' proteins by Protein A-Sepharose was ruled out. The assembly of other mutant ß' proteins (data not shown) is also ß-dependent. Since all mutant ß' proteins tested (JLBS, JLE2, JLE3, JLM3, JLK3, JLP6, JLP7, and JLP7B) were able to assembly into core enzyme, the region on the ß' subunit involved in core enzyme assembly may be located in the N-terminal third of this subunit. This assumption is supported by the fact that the N-terminal fragment of JLK3, JLE3, and JLP6 after hydroxylamine digestion can also be assembled into core enzyme (data not shown). The region on ß' subunit involved in interaction with the σ subunit may be located between aa 201 to 477 because this region is retained on mutant ß' proteins (JLBS, JLE3, JLM3, JLK3, and JLP6) which can bind to σ subunit, and deleted from those mutant proteins (JLE2, JLP7, and JLP7B) which cannot bind to the σ subunit.

RNA synthesis activity of reconstituted core enzyme containing mutant ß' proteins. The activity of assembled core enzyme forms containing mutant ß' proteins was determined by a mixed transcription assay. Purified subunits were mixed in urea buffer in an appropriate molar ratio and then dialysed against the reconstitution buffer. Aliquots of dialysate were incubated at 30°C for 30 min to activate premature core enzyme before analysis using the $d(A\text{-}T)_n$-directed synthesis of $r(A\text{-}U)_n$. Table VI shows that all core enzymes containing truncated ß' proteins lack activity for $d(A\text{-}T)_n$-directed RNA synthesis. For JLP7-containing core enzyme, this may be caused

by the deletion of the DNA-binding domain. For other truncated ß' mutants, this may be due to loss or damage of a region important in the enzyme activity. The mutant ß' JLBS which has a small insertion in ß' supported RNA synthesis as efficiently as the wildtype ß' subunit.

DISCUSSION

The anti-ß' monoclonal antibody 311G2 strongly inhibits both $r(A\text{-}U)_n$ synthesis directed by d(A-T)n and initiation directed by both *lac* P^+ promoter and *lac* UV5 promoter (Table II). It also strongly inhibits the elongation, but not DNA-template binding by RNA polymerase (Rockwell, 1986). mAb 311G2 has a comparable affinity for free ß' and core or holoenzyme-associated ß' subunit (Table IV). It also forms tight complex with the holoenzyme (Figure 1). Therefore, the epitope of mAb 311G2 is exposed both in core and holoenzyme. The strong inhibition of RNA polymerase activity but not DNA binding by mAb 311G2 suggests either that its epitope may be near the active site of RNA polymerase or that mAb 311G2 binds to a region removed from the active site and its binding to the ß' subunit results in a conformational change at or near the active site of the enzyme.

mAb 340F11 and mab 370F3 have no apparent effect on either the RNA synthesis directed by d(A-T)n (core enzyme activity) or the transcription initiation directed by *lac* P^+ and *lac* UV5 (holoenzyme activity) (Table II). They do not form a tight complex with the holoenzyme (Figure 1), and their affinity for core or holoenzyme associated ß' is much weaker than observed for the free ß' subunit (Table IV). However, they do not inhibit the reconstitution of core enzyme from a subunit mixture (Table III). The lack of inhibition of these antibodies on core enzyme reconstitution may reflect some conformational changes that may have happened in their epitope region during enzyme assembly. Therefore, these antibodies have a

much lower affinity for enzyme-associated ß' than for the free ß' subunit.

mAbs 371D6 and 372B2 partially inhibit initiation directed by *lac* P1, but not by *lac* UV5 (Table II). They do not inhibit synthesis directed by $d(A\text{-}T)_n$ (core enzyme activity) either. On the other hand, both have a much lower affinity for the core or holoenzyme associated ß' than for the free ß' subunit (Table IV). mAb 372B2 strongly inhibits the reconstitution of core enzyme from subunits and partially inhibits the transition of premature core to the active core enzyme, while mAb 371D6 does not affect either (Table III). It is possible that the epitope for mAb 372B2 is located in a region which is involved in subunit-subunit interaction and is not well exposed in core and holoenzyme. For mAb 371D6, its epitope may be changed conformationally after enzyme assembly. Both mAb 371D6 and mab 372B2 do not form tight complexes with holoenzyme (Figure 1). Their weak inhibition of initiation directed by *lac* P^+ but not by *lac* UV5 may indicate that the weak binding of these antibodies has created a steric hindrance which interferes with the interaction between RNA polymerase and the transcription activator protein, CRP. Therefore, the epitope region of these antibodies may be important in the interaction between RNA polymerase and transcription regulatory proteins.

The Western blot assays of the partially digested core enzyme show that all of the anti-ß' monoclonal antibodies bind to the denatured ß' peptide on nitrocellulose filters. mAb 340F11 and mAb 372B2 have similar patterns for binding to the ß' fragments, the remaining three anti-ß' monoclonal antibodies have binding patterns which differ from each other and from that of mAb 340F11 and mAb 372B2. The epitopes for mAb 340F11 and mAb 372B2 may be positioned closely on ß'. They

are not identical because these two antibodies behave differently in initiation and reconstitution assays and they belong to different IgG isotypes (Table II and III). The epitopes of the other three anti-ß' monoclonal antibodies are unrelated.

The location of the epitopes for the anti-ß' monoclonal antibodies studied was determined by Western blotting using a set of ß' deletion mutants. It is interesting that the epitopes of all five mAbs studied were located in a relatively narrow region between amino acids 817 and 1093. Epitopes of many monoclonal antibodies localized within a relatively short region were also observed by others (Nakamura *et al.*, 1989; Hodges *et al.*, 1988). These results imply that there may be an immunodominant site in the protein which can be well presented to the antibody producing system. However, the polyclonal antibody binds to all mutant ß' proteins.

The *E. coli* ß' subunit is related to the largest subunit of eukaryotic nuclear RNA polymerase II as well as to the large RNA polymerase components from archaebacteria (Allison *et al.*, 1985; Biggs *et al.*, 1985; Ahearn *et al.*, 1987; Puhler *et al.*, 1989; Nawrath *et al.*, 1990; Woychik and Young, 1990). Six major regions of homology have been noted for the largest subunit of RNA polymerase II and the *E. coli* ß' subunit (Allison *et al.*, 1985; Biggs *et al.*, 1985; Woychik and Young, 1990). Region IV covers amino acids 780-838 which overlaps with the epitope location of the non-inhibitory anti-ß' monoclonal antibodies studied in this report (amino acids 817-876). The epitope of mAb 311G2 is not located in any of the homologous regions although it is near the mutation at ß' residue 1033 involved in a domain implicated in replication control (Petersen and Hansen, 1991) and the region of ß' between Met-932 to Trp-1020 involved in the formation of the active pocket of RNA polymerase

(Borukhov *et al.*, 1991b). The data indicate that the binding site for mAb 311G2 involves residues in the vicinity of amino acid 1073 on the ß' subunit (between aa 1047 to 1093). Epitopes in native proteins are formed from 16 to 22 residues in a discontinuous array with a smaller subset of 5 to 6 residues contributing most of the binding energy (Laver *et al.*, 1990). Our mapping data has minimally identified the energetically significant epitopic domain. However, this epitopic domain needs not be functionally significant with regard to the inhibition effected by mAb 311G2 since binding of this antibody may disrupt other contacts important in the functional conformation of ß'. The epitope region of mAb 372B2 may possibly contribute a contact point between ß' subunit and other subunits; it is not well exposed in core or holoenzyme. On the other hand, epitopes of mAb 340F11, mAb 370F3 and mAb 371D6 may be well exposed in core or holoenzyme, but conformationally altered.

Since *E. coli* RNA polymerase is a multisubunit enzyme, and each subunit alone has no activity, the proper assembly of the enzyme is very important for the overall multiple functions of the whole complex. The region on the α subunit which is involved in enzyme assembly has been located to its N-terminal region from aa 1 to 234 (Igarashi *et al.*, 1991a). The N-terminal third of the ß subunit was suggested to be important for proper folding and assembly of ß subunit (Landick *et al.*, 1990) while its extreme C-terminus was shown to be involved in interaction with the σ subunit (Glass *et al.*, 1986c and 1988). Deletion of a well-conserved region (aa 371-340) of *E. coli* σ^{70} reduces its binding to core RNA polymerase (Lesley and Burgess, 1989), suggesting that this region of σ^{70} participates in binding to core enzyme. The region on the ß' subunit involved in enzyme assembly remains undefined.

In reconstitution and immunoprecipitation experiments, mutant ß' proteins JLBS, JLE2, JLE3, JLK3, JLP6, JLP7, and JLP7B can all assemble into core enzyme-like complexes. A comparison of the regions shared by these mutant proteins indicates that the N-terminal fifth of the ß' subunit is probably involved in core enzyme assembly.

According to previous results on the reconstitution of core enzyme from individual subunits and from subunit mixtures, Ishihama (1981) proposed that the assembly of core enzyme takes place in the following sequence: $\alpha + \alpha \rightarrow \alpha_2 + ß \rightarrow \alpha_2ß + ß' \rightarrow \alpha_2ßß'$. The incorporation of the ß' subunit into core enzyme requires preformed $\alpha_2ß$. An $\alpha_2ß'$ complex has not been detected in reconstitution assays (Zillig et al., 1976). Assembly of ß' subunit into core enzyme is evidently ß-dependent. Results of immunoprecipitation experiments with or without ß subunit present during reconstitution have clearly shown that the assembly of the mutant ß' proteins is indeed ß-dependent. Using small-angle X-ray scattering, Meisenberger *et al.* (1980c) found that the maximum dimension of $\alpha_2ß$ was much larger than that of the core enzyme. In their model for the $\alpha_2ß$ complex, the two α subunits are arranged angularly, with ß subunit contacting only one α subunit. In their model for core enzyme, the two α subunits arranged straight. The ß and ß' subunits have a conical shape and are lying side by side with their thick ends in the same direction. The α_2 in the core enzyme is a curved, elongated disc with a deep crevice in the middle with the two halves are attached to the thick ends of the ß and ß' subunits. My results on the assembly of mutant ß' proteins agree well with these models. It is possible that during the assembly of core enzyme from $\alpha_2ß$ and the ß', the N-terminal region of the

ß' subunit contacts the ß subunit first. This interaction between the ß and the ß' subunit may trigger conformational changes in the ß subunit; the changes in the ß subunit may then be transmitted to the α subunits. The conformational changes in the α subunits would force the two halves in the α_2 to become straightly arranged making the free half closer to the ß' subunit. Finally, the ß' subunit would interact with the α subunit and the core enzyme is assembled. The epitope for the anti-α monoclonal antibody used in this study, mAb 125C6, has been located in the C-terminal region of the α subunit between aa 310-329 (Sharif and Krakow, unpublished data). Its efficient precipitation of core or holoenzyme indicates that its epitope is well-exposed in at least one of the core or holoenzyme associated α subunits.

It is possible that multiple contact regions exsit between ß' subunit and α, ß subunits because the ß' subunit is a large protein. Cross-linking experiments have revealed extensive contact domains between ß and ß' subunits (Coggins *et al.*, 1977). With its epitope located in the C-proximal region of the ß' subunit, mAb 372B2 inhibits the reconstitution of core enzyme from subunit mixtures, and also partially inhibits the transition of premature core to mature core enzyme. However, the epitope region of mAb 372B2 may not be the region on the ß' subunit to initiate the assembly of ß' into core enzyme because the epitopes of other antibodies which do not inhibit the reconstitution of core enzyme are located in the same small region as that for mAb 372B2 (aa 817-876). The partial inhibition of mAb 372B2 on the transition from premature core to mature core enzyme also suggests that the epitope region of mAb 372B2 may be one of the contact regions between ß' and other

subunits, and the contact at this region happens at late stages during the assembly of core enzyme from α_2ß and ß'. The region which initiates the assembly of ß' into core enzyme may be located in the N-terminal part as indicated by immunoprecipitation data discussed above.

The isolated ß' subunit is the only subunit in RNA polymerase that is able to bind DNA (Sethi and Zillig, 1970; Fukuda and Ishihama, 1974). The σ subunit is involved in the specific binding of the holoenzyme to promoters while the core enzyme binds DNA templates only nonspecifically. Interaction of the σ subunit with ß' may play a role in DNA binding by RNA polymerase. Consistent with this assumption is an early finding indicating that a stable ß'σ subassembly can be isolated from *L. curvatus* RNA polymerase by phosphocellulose chromatography (Stetter and Zillig, 1974). In contrast, ß and α_2ß show little affinity for the σ subunit in reconstitution experiments (Fukuda and Ishihama, 1974; Palm *et al.*, 1975). The interaction between the ß' and σ subunits is very important for both assembly of holoenzyme and specific binding of holoenzyme to DNA. Immunoprecipitation data indicate that mutant ß' proteins JLBS, JLE3, JLK3, and JLP6 can assemble into holoenzyme-like complexes while JLE2, JLP7, and JLP7B can not. The region which is lost on JLE2, JLP7 and JLP7B is between aa 201-477 and is retained on JLBS, JLE3, JLK3, and JLP6. Therefore, this region may be important for the interaction between ß' and the σ subunit.

The σ subunit is released from the elongation complex when the nascent transcript reaches a critical length (Kumar and Krakow, 1975). It is possible that the ß' subunit may interact with other regulatory proteins subsequent to σ release. This

is supported by the finding that a mutation of ß' Glu-402 suppresses the temperature-sensitive phenotype of *NusA*11, a protein involved in transcription termination. Our assigned ß'-σ interaction region 201-477 on the ß' includes this mutation site as well as a conserved region (aa 348-380) which was found to be homologous to the DNA-binding domain of two prokaryotic DNA polymerases (Allison *et al.*, 1985).

Interactions between subunits in RNA polymerase are rather complicated. Results of crosslinking have indicated that ß and ß' have extensive contact regions, there are binding domains on ß and ß' for the σ subunit (Hillel and Wu, 1977). Protease digestion studies have shown that heparin, an RNA polymerase initiation inhibitor binding to ß', can disrupt the σ-ß association (Fisher and Blumenthal, 1980). On the other hand, the isolated ß subunit does not bind rifampicin, a transcription inhibitor, although all rifampicin-resistant mutations have been mapped to the ß subunit (Jin and Gross, 1988). The α_2ß subassembly exhibits only weak rifampicin binding and assembly of RNA polymerase is required for strong rifampicin binding. Other subunits could be involved either directly or indirectly in assembly of the rifampicin binding site. This is supported by a report (Kawai *et al.*, 1976) indicating that mutations in α and ß' subunits modulate the level of resistance to rifampicin by a Rif^r mutation located in *rpoB*. Crosslinking studies (Rice and Meares, 1978) indicate that the rifampicin binding site is in the vicinity of all of the other subunits of RNA polymerase. Rifampicin bound to RNA polymerase is freely accessible to solvent molecules but not to antibodies to rifampicin; the accessibility of rifampicin to solvent is markedly decreased by the removal of the σ subunit and somewhat decreased by DNA binding (Rice and Meares, 1982). It was suggested that the

rifampicin binding site is located in a crevice of the enzyme in the vicinity of the template binding domain held open by σ subunit.

Table IV shows that all core enzymes containing truncated ß' proteins lack activity in poly $d(A\text{-}T)_n$ directed RNA synthesis. For the P7-containing core enzyme, this may be caused by the loss of the DNA-binding domain. For other truncated ß' mutants, this may be a consequence of the loss or damage of a region important for enzyme activity. Loss of activity correlates with the truncation in the C-proximal region or the deletion of the C-terminal region. This is consistent with the following observations. A ß' mutation at Asp-1033 affects chromosomal replication control in *E. coli* by increasing transcription of the *DnaA* gene (Peterson and Hansen, 1991). The site on ß' which contacts the 3'-terminus of the RNA transcript was localized to a region spanning Met-832 to Trp-1020 (Borokhov *et al.*, 1991b). The epitope for an anti-ß' monoclonal antibody (mAb 311G2) which strongly inhibits transcription initiation and elongation but not DNA binding by RNA polymerase has been mapped between amino acids 1047-1093. Therefore, it is reasonable to assign the catalytically important region on the ß' subunit to be in its C-proximal part.

In summary, we have identified three important regions on the ß' subunit of *E. coli* RNA polymerase (Figure 14). The extreme N-terminal region is the primary region involved in the assembly of ß' subunit into core enzyme; the region covers aa 201-477 is involved in interacting with the σ subunit, *Nus* A protein and binding to the DNA template; the C-proximal region containing the epitope region of mAb 311G2 and the contact region to the 3'-terminus of RNA transcript is involved in the formation of the catalytic active site of the *E. coli* RNA polymerase.

Suggestions for future studies:

1. Truncated mutant ß' proteins can be used in Western blot assay to map epitopes of anti-ß' monoclonal antibodies prepared in other laboratories (Nikiforov *et al.*, 1983, Burgess *et al.*, 1986). This will help to further locate structurally or functionally important regions on the ß' subunit.

2. Many temperature-sensitive *E. coli* strains containing altered *rpoC* have been described previously (Miller *et al.*, 1976). Some of them (Ts4 and R120) have defects in enzyme assembly (Taketo and Ishihama, 1976; Gross *et al.*, 1977), while others (XH56 and rpoC110) have very low activity at the non-permissive temperature (Gross and Bautz, 1976; Kirschbaum, 1978). Plasmids containing truncated *rpoC* or *rpoC* deletions can be used to locate regions of mutation in *rpoC* in these mutant *E. coli* strains by plasmid rescue (Peterson and Hansen, 1991).

3. Random mutations in the epitope region of mAb 311G2 may generate important information on the mechanism of the contribution of ß' to the catalytic activity of RNA polymerase. Mutations in the epitope region of mAb 371D6 and mAb 372B2 may shed some light on possible interaction between ß' and transcriptional regulators.

4. Using the amino acid sequence of the ß' subunit, computer analysis could greatly help us to predict possible secondary structure and hydrophobicity or hydrophilicity of the whole ß' molecule or specific regions on the ß' subunit.

Table I. Properties of *E. coli* RNA polymerase subunits (modified from Burgess *et al.*, 1987).

Subunits	α	ß	ß'	σ	ω
Gene	*rpo*A	*rpo*B	*rpo*C	*rpo*D	*rpo*Z
Number of amino acids	329	1342	1407	613	90
Map position	73	90	90	67	?
MW (daltons)	36,511	150,615	155,159	70,262	10,105
Charge (at pH 7)	-14	-34	+6	-40	-4
Function	enzyme assembly, interaction with transcription regulators	active site	?	initiation specificity	?

Table II: Properties of anti-ß'monoclonal antibodies.

		effect of mAb on reaction directed by		
mAb	Ig class	$d(A\text{-}T)_n$	*lac* UV5	*lac* P^+
		% residual activity		
311G2	IgG1, k	18	4	2
370F3	IgG1, k	92	108	98
371D6	IgG1, k	82	109	50
372B2	IgG1, k	86	110	53
340F11	IgG2a, k	97	110	91

2 pmol of RNA polymerase core enzyme (for $d(A\text{-}T)_n$-directed transcription) or holoenzyme (for *lac* UV5 or *lac* P^+-directed transcription) was preincubated with 20 pmol of the indicated monoclonal antibody for 30 min at 37°C. 100% activity for $d(A\text{-}T)_n$-directed transcription stands for incorporation of 1 nmol of ^{3}H-UTP into TCA-precipitated RNA transcript. For *lac* UV5-directed initiation, 100% activity was the incorporation of 60 pmol of ^{3}H-UTP into abortive transcripts, while 100% activity for *lac* P^+-directed initiation was the incorporation of 30 pmol of ^{3}H-UTP into abortive transcripts.

Table III: Effects of anti-ß' monoclonal antibodies on reconstitution of RNA polymerase core enzyme.

	effect of mAb when incubated with		
antibody	subunits	premature core	mature core
		% recovery	
370F3	80 (64/1)	76 (64/1)	92 (64/1)
371D6	86 (20/1)	ND	83 (20/1)
372B2	13 (8/1)	51 (8/1)	75 (8/1)
340F11	89 (20/1)	90 (20/1)	99 (20/1)

Core enzyme was denatured by incubation in buffer containing 6 M guanidine-HCl for two hours at 4°C. The reconstitution mixtures contained 100 pmol of α subunit and 50 pmol each of ß and ß' subunits and antibody was added at the indicated stage of reconstitution. Reconstitution to form the premature core was carried out by dialysis for 16 hours at 4°C against reconstitution buffer. Incubation of the antibody with the premature or mature core enzyme was for 3 hours on ice followed by incubation for 40 min at 30°C. Aliquots (25 μl) containing 4 pmol of reconstituted core enzyme were assayed for d(A-T)$_n$-directed synthesis of r(A-U)$_n$. The activity of equal amount of core enzyme (4 pmol) reconstituted in the absence of antibodies was used as 100% activity, which represents the incorporation of 500 pmol of ^{3}H-UTP into TCA-precipitated RNA transcripts.

Table IV: Dissociation constants for binding of the anti-ß' monoclonal antibodies to free and RNA polymerase associated ß' subunit.

antibody	ß'	core	holoenzyme
mAb 311G2	1.5 nM	7.1 nM	16.7 nM
mAb 370F3	7.9 nM	521 nM	587 nM
mAb 371D6	13.1 nM	820 nM	550 nM
mAb 372B2	10.2 nM	273 nM	386 nM
mAb 340F11	3.6 nM	276 nM	168 nM

Conditions used are described in the legend to Figure 2A and 2B. The method of Friguet *et al.* (1985) was used to determine the K_d.

Legend to Table V

Construction of the various plasmids is presented in Figure 4. The Western blot results are shown in Figures 6-9. pJLSS expresses the fusion protein pJLSS[ß-gal$^{1-375}$-ß'$^{877-1132}$]. The * indicates the insertion of a tetra-peptide (Arg-Pro-Gly-Asp) between ß' amino acids 1073 and 1074. The "+" indicates the binding of a specific antibody to its antigen; The "–" indicates no binding; The "$\pm$" indicates weak binding. Unrelated amino acids added to the C-terminus of the following constructs are as follows (C-terminal amino acids in ß' are shown as capital letters, lower case letters represent unrelated residues):

pJLE3: LKLIDefddklsnmriksi

pJLK1: DDCGTelfddklsnmriksi

pJLK2: LIPGTelfddklsnmriksi

pJLM3: DGQTItrttrivcvavlrvklrlhrr

Table V. Binding of Monoclonal Antibodies to ß' Deletion Mutants and Fusion Protein.

	Δ bp	ß' aa	311G2	340F11	370F3	371D6	372B2	pAb
pJLB	742-3216	1-247:1033-1407	–	–	–	–	–	+
pJLE2	524-2959	1-174:987-1407	+	–	–	–	–	+
pJLE3	2960-4321	1-986	–	+	+	+	+	+
pJLK1	2448-4309	1-816	–	–	–	–	–	+
pJLK2	3279-4309	1-1093	+	+	+	+	+	+
pJLK3	2448-3278	1-816:1094-1407	–	–	–	–	–	+
pJLM3	3140-4088	1-1046	–	+	+	+	+	+
pJLP6	1345-2787	1-448:930-1407	+	–	–	–	–	+
	bp inserted into pSEM-4	ß' aa in fusion protein						
pJLSS	2629-3397	877-1132	+	–	–	–	–	+
pJLSSB	2629-3397	877-1072	–	–	–	–	–	+
pJLSSBS	2629-3397	877-1132*	±	–	–	–	–	+

Table VI. Activity of core enzymes containing mutant ß' proteins.

core enzyme	activity	assembly to core	binding to σ^{70}
WT ß'	100%	+	+
JLBS	73%	+	+
JLE2	2%	+	-
JLE3	3%	+	+
JLK3	2%	+	+
JLP6	2%	+	+
JLP7	2%	+	-
JLP7B	5%	+	-

Purified wildtype α, ß, ß' or mutant ß' were mixed in a molar ratio of 2:1:2 in 7 M urea buffer, dialyzed against the reconstitution buffer at 4°C overnight. Aliquots of each dialysis mixture (containing 10 pmol of total protein) were used for d(A-T)$_n$-directed r(A-U)$_n$ synthesis as described in "Materials and Methods". The activity of the core enzyme reconstituted from wildtype α, ß, and ß' were assumed as 100% activity which represents the incorporation of 28.6 pmol of UTP. For the assembly of mutant ß' proteins to core enzyme or holoenzyme, refer to the legend for Figure 12.

Figure 1. Binding of anti-ß' monoclonal antibodies to RNA polymerase holoenzyme. Incubation mixtures contained (final volume 20 μl): 50 mM Tris-HCl (pH 8.0), 0.1 mM dithiothreitol, 5% glycerol, 2.2 pmol RNA polymerase holoenzyme, 6.6 pmol mAb 311G2 or 22 pmol of the following antibodies: mAb 370F3 or mAb 371D6 or mAb 372B2 or mAb 340F11. The mixtures were incubated for 30 min at 37°C before resolution by native polyacrylamide gel electrophoresis. Lanes a and g, RNA polymerase; lane b, RNA polymerase + mAb 311G2; lane c, RNA polymerase + mAb 370F3; lane d, RNA polymerase + mAb 371D6; lane e, RNA polymerase + mAb 372B2; lane f, RNA polymerase + mAb 340F11.

a b c d e f g

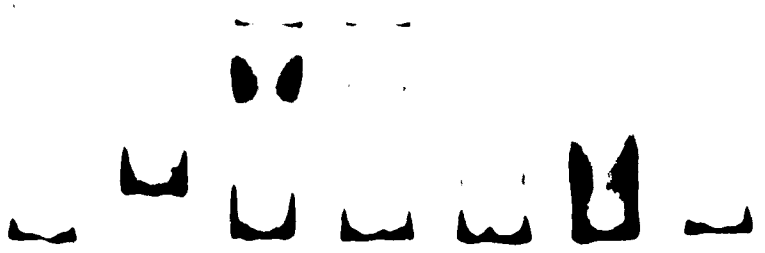


Figure 2A. Determination of the K_d for binding of anti-ß' mAb 311G2 to native RNA polymerase holoenzyme, core enzyme and ß' subunit. Decreasing concentrations of holoenzyme (from 1 μM), core enzyme (from 0.1 μM) or ß' subunit (from 0.01 μM) were incubated with a constant amount (0.5 nM) of mAb 311G2. a_o is the total antigen concentration and ν is the fraction of the bound antibody. Holoenzyme, ▲-▲; core enzyme, ●-●; ß' subunit, o-o.

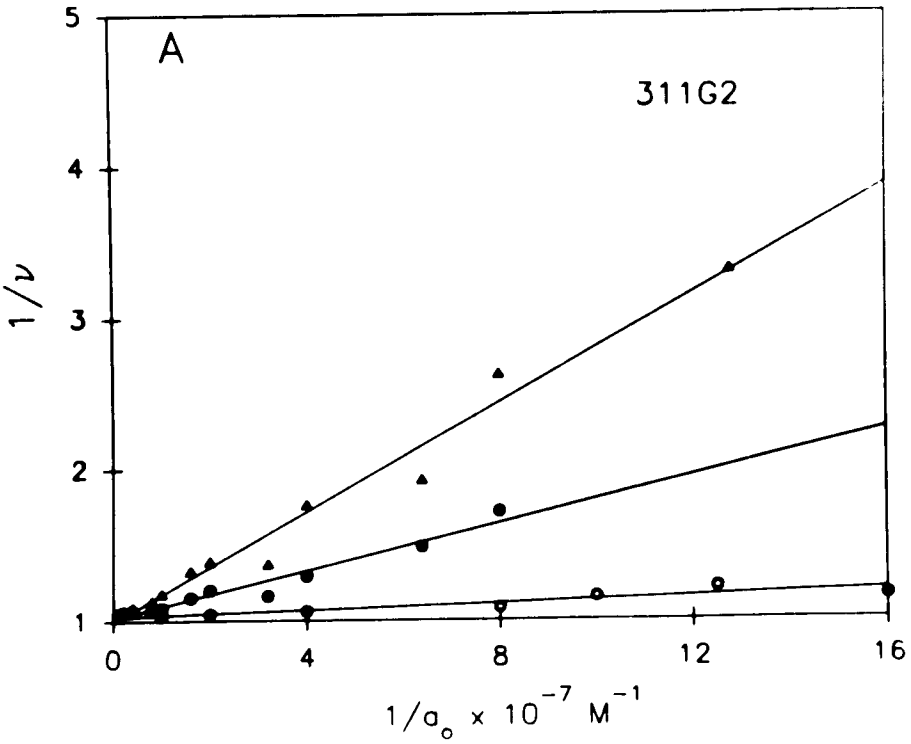

A
311G2
5
4
3
2
1
1/ν
0
4
8
12
16
1/a$_o$ x 10^{-7} M^{-1}

Figure 2B. Determination of the K_d for binding of anti-ß' mAb 372B2 to native RNA polymerase holoenzyme, core enzyme and ß' subunit. Decreasing concentrations of holoenzyme (from 1 μM), core enzyme (from 1 μM) or ß' subunit (from 0.1 μM) were incubated with a constant amount (0.5 nM) of mAb 372B2. a_o is the total antigen concentration and ν is the fraction of the bound antibody. Holoenzyme, ▲-▲; core enzyme, ●-●; ß' subunit, o-o.

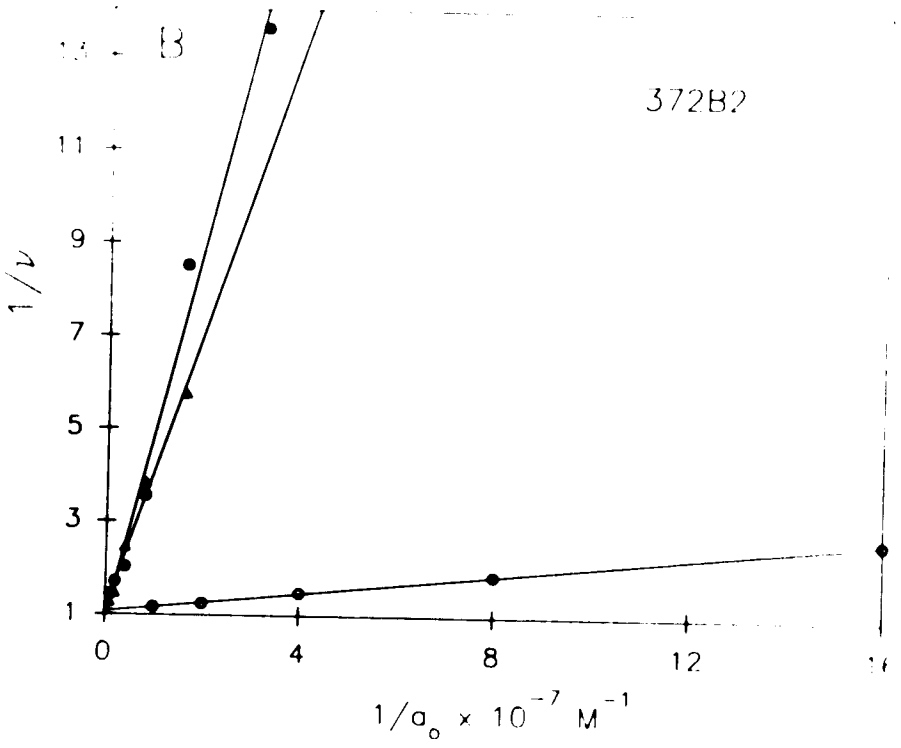

B
372B2
11
9
7
5
3
1
1/ν
0
4
8
12
1/a_o × 10^-7 M^-1

Figure 3A. Western blotting of trypsin-digested core enzyme with anti-ß' monoclonal antibodies. 2 μg of core RNA polymerase was partially digested for 30 min at 37°C in a 20 μl mixture containing 50 mM Tris-HCl (pH 8.0), 5 % glycerol, 0.01 μg of trypsin. The reactions were stopped by adding 4 μl of 20 mM PMSF and 6 μl of 5 x SDS Sample Buffer. Following electrophoresis the proteins were transferred to a nitrocellulose membrane. After incubation with the indicated monoclonal antibodies at room temperature for 2 hours, the immune complexes were visualized using goat anti-mouse IgG-alkaline phosphatase.

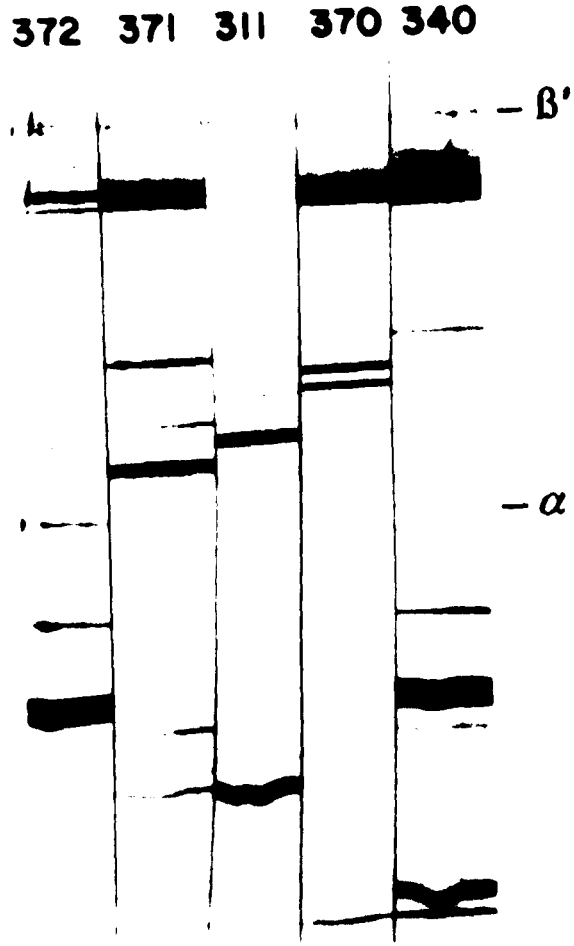

372 371 311 370 340
β'
α

Figure 3B. Western blotting of subtilisin-digested core enzyme with anti-ß' monoclonal antibodies. 2 μg of core RNA polymerase was partially digested for 30 min at 37°C in a 20 μl mixture containing 50 mM Tris-HCl (pH 8.0), 5% glycerol, 0.01 μg of subtilisin. The reactions were stopped by adding 4 μl of 20 mM PMSF and 6 μl of 5 x SDS Sample Buffer. Following electrophoresis the proteins were transferred to a nitro-cellulose membrane. After incubation with the indicated monoclonal antibodies at room temperature for 2 hours, the immune complexes were visualized using goat anti-mouse IgG-alkaline phosphatase.

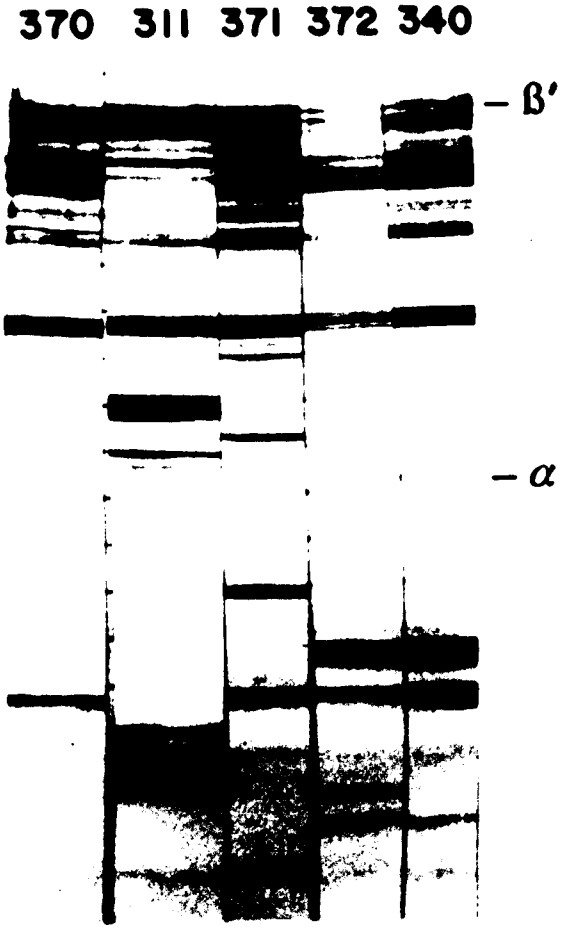

370 311 371 372 340
– β′
– α

Figure 4. Restriction map of pT7-ß' (Zalenskaya *et al.*, 1990) showing the sites used in constructing the deletion mutants used for mapping the positions of the anti-ß' epitopes.

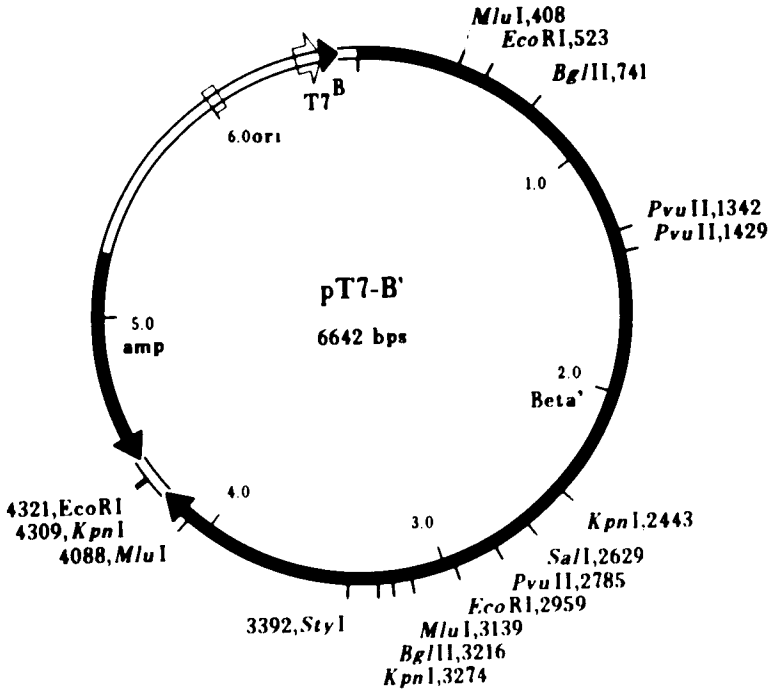

MluI,408
EcoRI,523
BglII,741
T7
B
6.0ori
1.0
PvuII,1342
PvuII,1429
pT7-B'
6642 bps
5.0
amp
2.0
Beta'
4321,EcoRI
4309,KpnI
4088,MluI
4.0
3.0
KpnI,2443
SalI,2629
PvuII,2785
EcoRI,2959
3392,StyI
MluI,3139
BglII,3216
KpnI,3274

Figure 5. Schematic representation of the ß' deletion mutants. The black boxes represent the segment(s) of the ß' retained while the dotted lines represent the region deleted in the construct. (Luo and Krakow, 1992; Luo *et al.*, 1992).

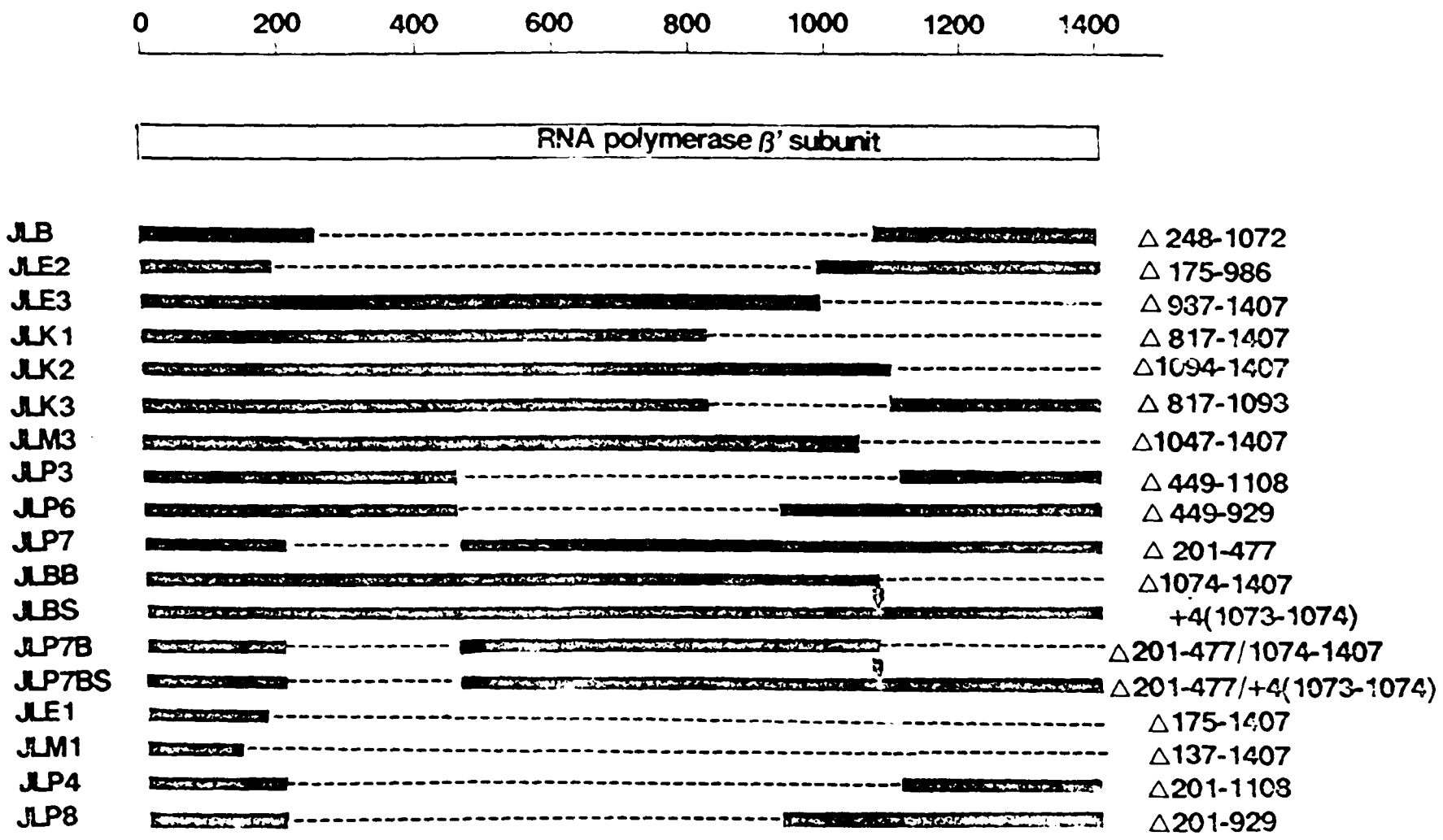

0
200
400
600
800
1000
1200
1400
RNA polymerase β' subunit
JLB
JLE2
JLE3
JLK1
JLK2
JLK3
JLM3
JLP3
JLP6
JLP7
JLBB
JLBS
JLP7B
JLP7BS
JLE1
JLM1
JLP4
JLP8
Δ 248-1072
Δ 175-986
Δ 937-1407
Δ 817-1407
Δ1094-1407
Δ 817-1093
Δ1047-1407
Δ 449-1108
Δ 449-929
Δ 201-477
Δ1074-1407
+4(1073-1074)
Δ201-477/1074-1407
Δ201-477/+4(1073-1074)
Δ175-1407
Δ137-1407
Δ201-1108
Δ201-929

Figure 6. Western blots of ß' fragments produced by pJLB and pJLK1. Synthesis of pJLB and pJLK1 proteins in *E. coli* was carried out as presented in Materials and Methods. Proteins were separated by electrophoresis on a 10% SDS polyacrylamide gel, transferred to nitrocellulose membranes and blotted with the indicated monoclonal antibodies and polyclonal anti-ß' antibody (pAb); 130 is the anti-α mAb 130C2.

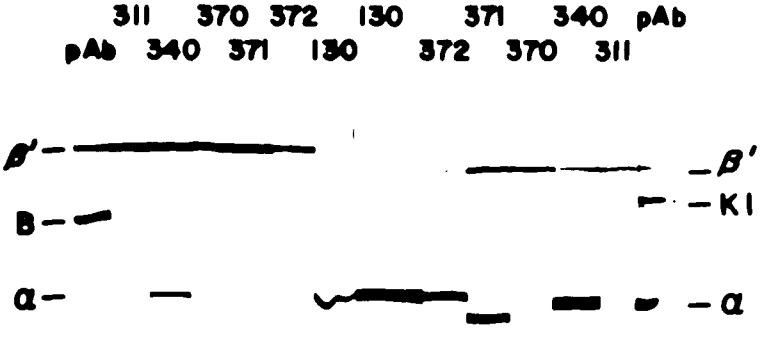

311 370 372 130 371 340 pAb
pAb 340 371 130 372 370 311
β'
β'
B
K1
α
α

Figure 7. Western blots of ß' fragments produced by pJLE2 and pJLE3. Synthesis of pJLE2 and pJLE3 proteins in *E. coli* was carried out as presented in Materials and Methods. Proteins were separated by electrophoresis on a 10% SDS polyacrylamide gel, transferred to nitrocellulose membranes and blotted with the indicated monoclonal antibodies and polyclonal anti-ß' antibody.

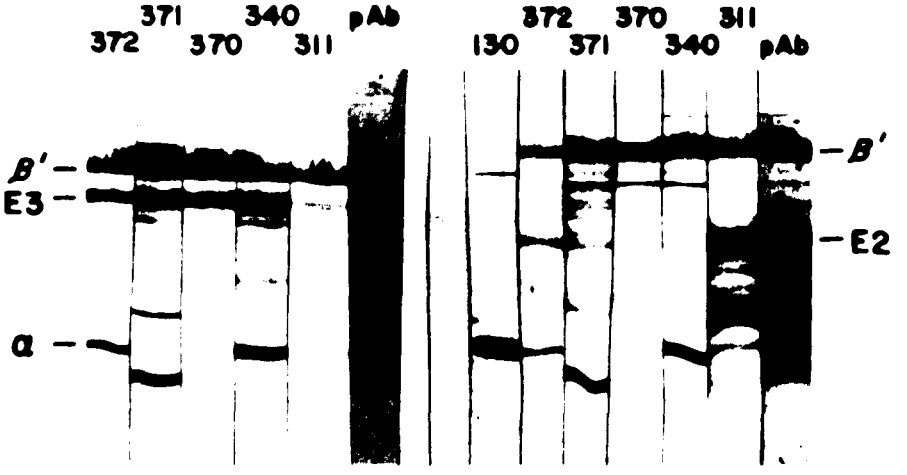

371
340
pAb
372
370
311
β'
E3
α
372
370
311
130
371
340
pAb
β'
E2

Figure 8. Western blots of ß' fragments produced by pJLP6 and pJLM3. Synthesis of pJLP6 and pJLM3 proteins in *E. coli* was carried out as presented in Materials and Methods. Proteins were separated by electrophoresis on a 10% SDS polyacrylamide gel, transferred to nitrocellulose membranes and blotted with the indicated monoclonal antibodies and polyclonal anti-ß' antibody.

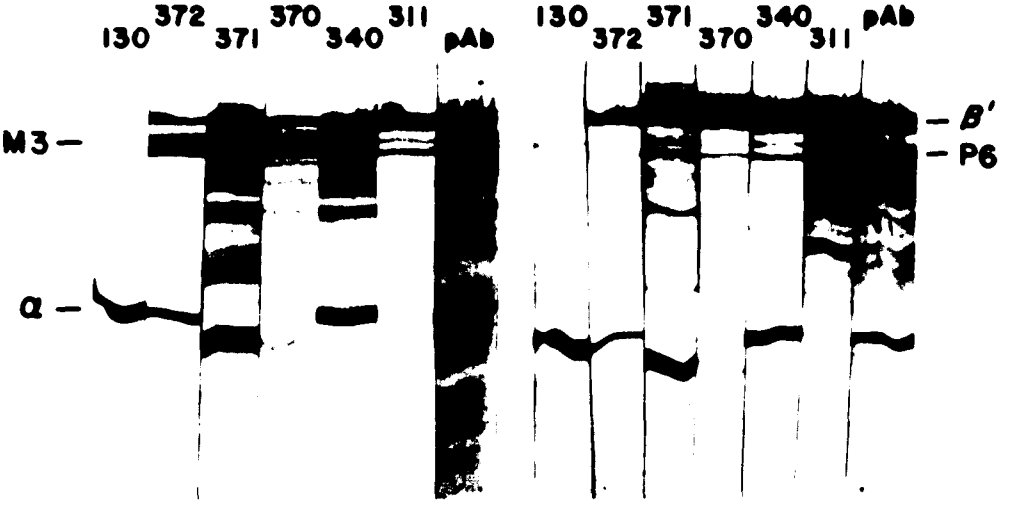

130
372
371
370
340
311
pAb
M3
α
130
372
371
370
340
311
pAb
B'
P6

Figure 9. Western blot of ß' fusion protein produced by pJLSS and pJLSSBS. Synthesis of pJLSS and pJLSSBS fusion proteins in *E. coli* was carried out as presented in Materials and Methods. Proteins were separated by electrophoresis on a 10% SDS polyacrylamide gel, transferred to nitrocellulose membranes and blotted with the indicated monoclonal antibodies and polyclonal anti-ß' antibody.

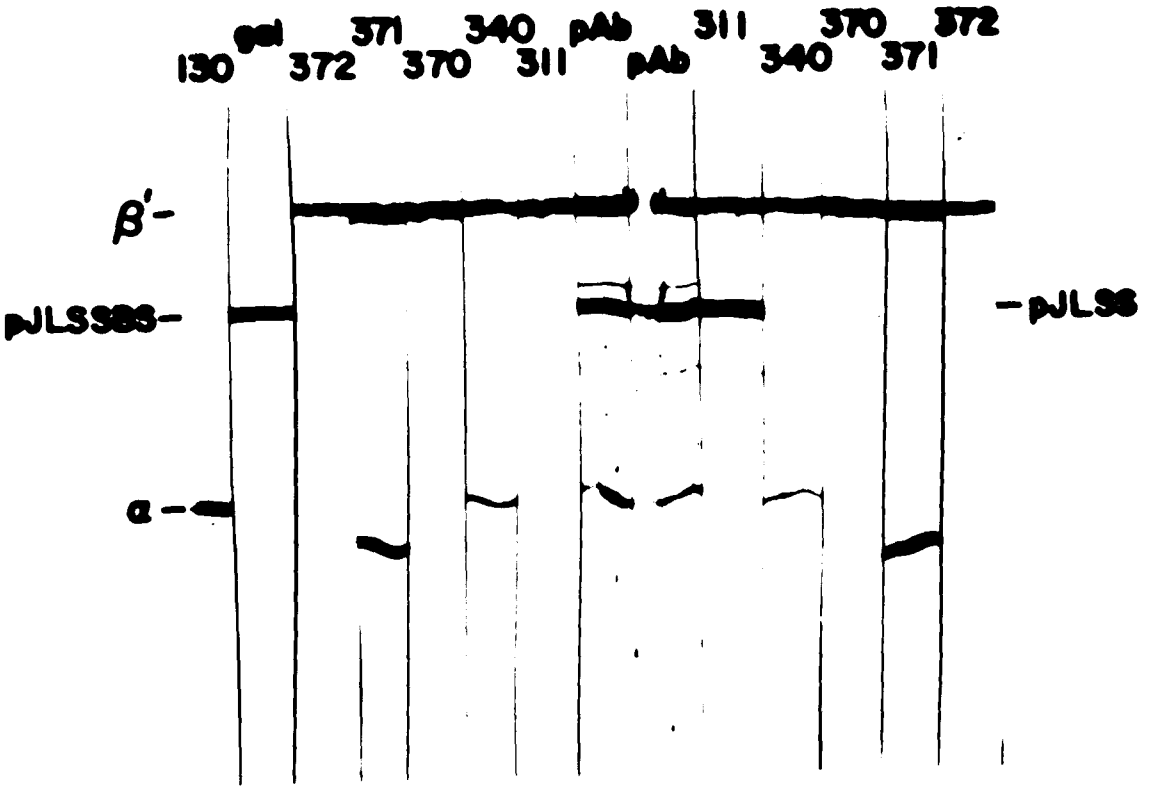

130
gal
372
371
370
340
311
pAb
pAb
311
340
370
371
372
β'-
pJLS985-
-pJL98
α-

Figure 10. Overexpression of mutant ß' proteins. K38/pGp1-2 cells were transformed with plasmids pJLB, pJLK1, pJLE2, pJLE3, and pJLM3. Mutant ß' proteins were induced by shifting the temperature of the 5 ml culture from 30°C to 42°C at 30 Klett units. 500 µl aliquots were removed from the culture at different times during induction, and analyzed by SDS-PAGE. Holo, purified holoenzyme standard; JLB, JLK1, JLE2, JLE3, and JLM3 are mutant ß' proteins; Lanes 1, 2, 3, 4, and 5 for each mutant protein indicate the samples removed at 0 min, 30 min, 1 hour, 2 hours, and 3 hours after induction.

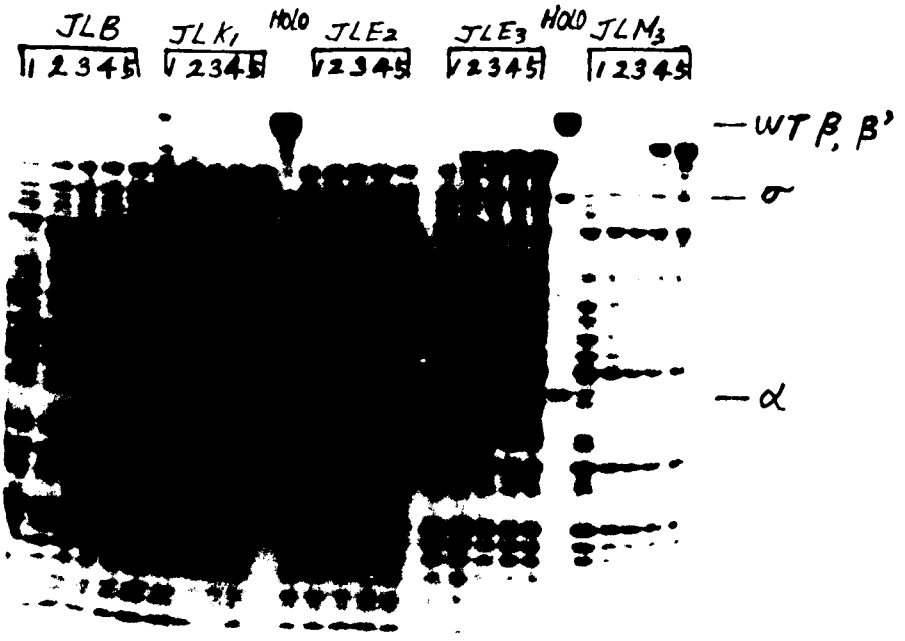

JLB
1 2 3 4 5
JLK1
1 2 3 4 5
HOLO
JLE2
1 2 3 4 5
JLE3
1 2 3 4 5
HOLO
JLM3
1 2 3 4 5
— WT β, β'
— σ
— α

Figure 11: Purified wildtype and mutant ß' proteins. Proteins were purified as described in "Materials and Methods". About 1.5 μg of protein was loaded in each lane of a 15% SDS gel. Lane a: Molecular weight markers (SDS-6H, Sigma); Lane b: JLP7B; Lane c, JLP7; Lane d: JLP6; Lane e: JLK3; Lane f: JLE3; Lane g: JLE2; Lane h: JLBS; Lane i: wildtype ß'; Lane j: RNA polymerase Holoenzyme.

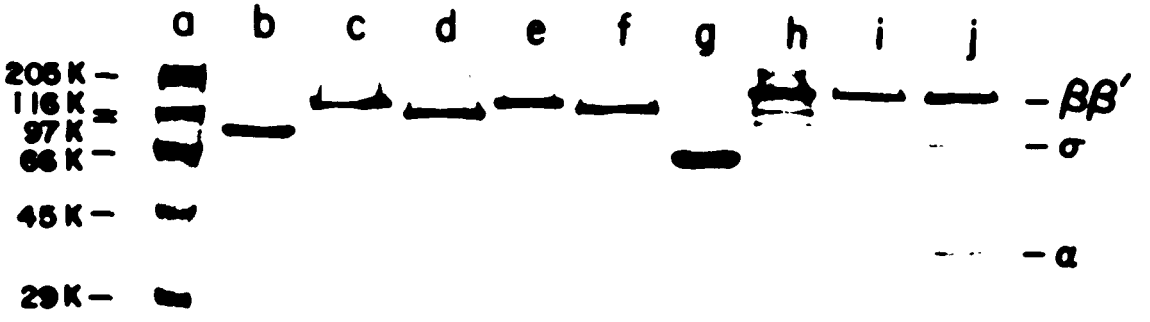

a
b
c
d
e
f
g
h
i
j
205K
116K
97K
66K
45K
29K
ββ′
σ
α

Figure 12: Immunoprecipitation of assembled "core" or "holoenzyme"-like complexes by anti-α mAb 125C6. Isolated subunits were mixed, dialyzed against reconstitution buffer, and incubated with anti-α mAb 125C6 as described in "Materials and Methods". Antigen-antibody complexes were precipitated by Protein A-Sepharose, resuspended in SDS-sample buffer and loaded on to a 15% SDS gel. Lane a, holoenzyme standard; lane b, α + ß + σ; lane c, α + ß + wildtype ß' + σ; lane d, α + ß + JLBS + σ; lane e, α + ß + JLE3 + σ; lane f, α + ß + JLK3 + σ; lane g, α + ß + JLP7 + σ; lane h, α + ß + JLP7B + σ; lane i, α + ß + JLP6 + σ; lane j, α + ß + JLE2 + σ. BSA, bovine serum albumin; H, IgG Heavy chain; L, IgG light chain; OA, chicken ovalbumin.


a b c d e f g h i j
ββ′–
σ–
α–
–BSA
–H
–OA
–L

Figure 13: The ß-dependence of the assembly of mutant ß' proteins into core or holoenzyme. A mixture of purified α, σ and mutant ß' with or without the ß subunit was dialyzed against the reconstitution buffer, immunoprecipitated with anti-α mAb 125C6, and analyzed on a 15% SDS gel. Lane a, holoenzyme standard; lane j, immunoprecipitation of holoenzyme without antibody (a negative control). Proteins in dialysate before immunoprecipitation with mAb 125C6 are as the following, Lane b, α + ß + JLE3 + σ; lane c, α + JLE3 + σ; lane d, α + ß + JLK3 + σ; lane e, α + JLK3 + σ; lane f, α + ß + JLP6 + σ; lane g, α + JLP6 + σ; lane h, α + ß + JLP7 + σ; lane i, α + JLP7 + σ.

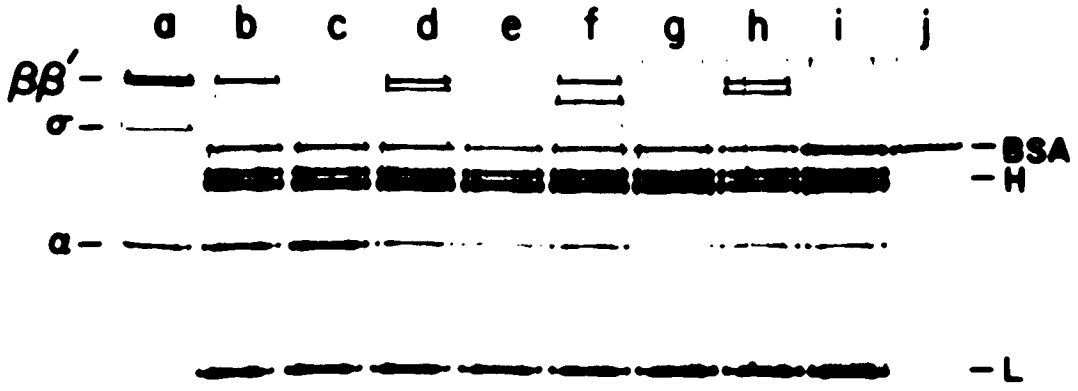
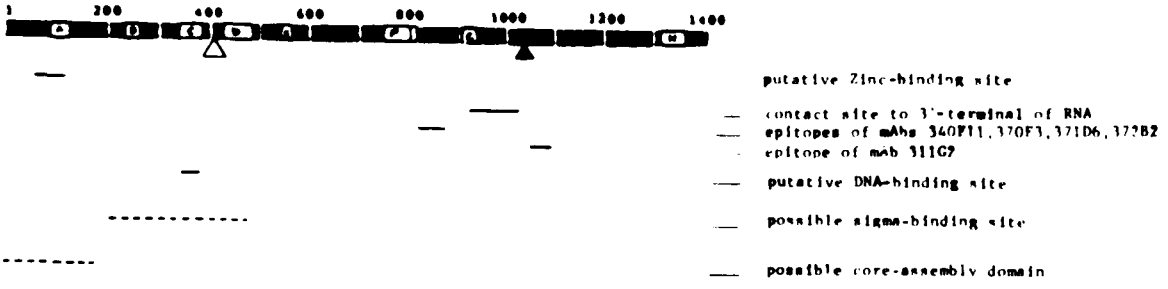

a b c d e f g h i j
ββ′
σ
α
BSA
H
L

Figure 14. Summary of interesting regions on the ß' subunit of the *E. coli* RNA polymerase. The *bar* at the *top* represents the 1407-amino acid-long ß' polypeptide, with *lettered boxes* symbolizing highly conserved sequence regions (Borukhov *et al.*, 1991b). "$_{\Delta}$" represents the point mutation at Glu-402 which suppresses the temperature-sensitive phenotype of NusA 11 (Nakamura, unpublished results); "▲" indicates the point mutation at Asp-1033 which affects chromosomal replication control (Peterson and Hansen, 1991).


1
200
400
600
800
1000
1200
1400
putative Zinc-binding site
contact site to 3'-terminal of RNA
epitopes of mAbs 340F11, 370F3, 371D6, 372B2
epitope of mAb 311G7
putative DNA-binding site
possible sigma-binding site
possible core-assembly domain

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