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Reduction in Abortive Transcription from the λP_R Promoter by Mutations in Region 3 of the σ^{70} Subunit of *Escherichia coli* RNA Polymerase*

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Transcription initiation by *Escherichia coli* RNA polymerase at most promoters is associated with a reiterative synthesis and release of short abortive RNA products. We have investigated the mechanism of abortive RNA synthesis by using holoenzymes containing mutant σ^{70} subunits with changes in region 3 (S506F and P504L), which reduce the ratio of abortive to full-length products. Binary complexes formed by these mutant enzymes at a modified λP_R promoter contained a smaller fraction of open complexes than for normal polymerase, suggesting an involvement of region 3 in melting duplex DNA or in maintenance of the open complex. The half-lives of the majority of binary complexes formed by the mutant enzymes were less than 1 min, in contrast to 30 min for the wild-type complexes. The time courses of transcription and pulse-labeling assays showed that moribund complexes, which generate only abortive products (Kubori, T., and Shimamoto, N. (1996) *J. Mol. Biol.* 256, 449–457), were formed by the mutant enzymes. However, they accumulated to a lesser extent than for the wild-type enzyme, due both to faster dissociation and conversion into inactive complexes. This is the main cause of the low degree of abortive transcription displayed by the mutant enzymes on this promoter.

Abortive transcription by *Escherichia coli* RNA polymerase is characterized by reiterative synthesis and release of short transcripts of variable length in a template-dependent manner (1–5). This nonproductive process occurs at most promoters, even in the presence of high concentrations of all four substrates (6). During this process RNA polymerase does not usually dissociate from the template (7). The RNA cleavage factors GreA and GreB have been found to reduce the production of abortive transcripts while concomitantly increasing that of full-length transcripts at some weak promoters (8–10). Therefore, it is conceivable that there could be a regulatory switch operating at this initiation step that affects the efficiency of productive elongation.

The σ^{70} subunit is believed to be retained during abortive transcription, and the establishment of an elongation complex is correlated with release of σ subunit from the holoenzyme (11–13), which suggests a possible involvement of this subunit in abortive synthesis. Two spontaneously generated missense alleles have been isolated, *rpoD(P504L)* and *rpoD(S506F)*, whose mutations alter region 3 of the σ^{70} subunit and have been shown to compensate for the lack of ppGpp-dependent functions in a ppGpp^0 strain (14). It is known that region 3 can be cross-linked to the catalytic center of the enzyme (15) and becomes inaccessible from outside upon holoenzyme formation (16). Holoenzymes with these mutant σ subunits also exhibit reduced abortive initiation at several phage promoters (17). Thus their study should shed light on the mechanism of abortive initiation and the involvement of region 3 of σ^{70} in this process.

Recently Kubori and Shimamoto (18) demonstrated the existence of nonproductive complexes yielding only abortive products at modified λP_R and *lacUV5* promoters. These nonproductive complexes were termed "moribund complexes," since they are incapable of productive elongation and gradually become fully inactive. Consistent with the inactivation, these promoters yield significantly fewer than one full-length transcript per promoter in a single-round transcription (19). In the present study, we investigated the reduced output of abortive transcripts at a modified λP_R promoter (λP_RAL) caused by the σ^{70} mutations P504L and S506F. The faster dissociation and concomitant lesser accumulation of moribund complexes were found to be the major causes of the low levels of abortive synthesis by these two mutant enzymes.

EXPERIMENTAL PROCEDURES

Materials—Nucleoside triphosphates were obtained from Yamasa (Tokyo), and all radioisotopes were from NEN Life Science Products (Bethesda, MD). All other chemicals were of analytical grade. Restriction enzymes were purchased from Takara and Toyobo (Tokyo, Japan). *Taq* DNA polymerase was purchased from Boehringer Mannheim.

All the linear DNA templates were prepared from parent plasmids by the polymerase chain reaction amplification method. The construction of these plasmids has been described elsewhere (19). The templates carrying the λP_R promoter have 32 (λP_RAL32) or 73 bp[1] (λP_RAL73) A-less initial transcribed sequences, with total lengths of 190 and 230 bp, respectively. The transcription start site is situated 85 bp away from the upstream end of each DNA fragment. The original λP_R promoter partially overlaps the divergent λP_{RM} promoter in its upstream region. We inactivated P_{RM} by changing each of the bases −7A, −11T, and −12A into C (the positions are numbered with respect to the start site of P_{RM}) to produce the modified promoter λP_RAL. A 160-bp fragment harboring the T7A1 promoter (T7A1 DNA) was prepared as described previously (19). The run-off transcript produced on this template was 74 nucleotides in length. All the polymerase chain reaction products were purified on 8% polyacrylamide gels and eluted. Immobilized template DNA was prepared as described previously (16, 18, 19).

The wild-type holoenzyme and core enzyme were purified according to Gonzalez *et al.* (20) by using Biorex 70 (Bio-Rad) instead of phospho-

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[1] The abbreviation used is: bp, base pair(s).

cellulose. Mutant σ factors were purified from overexpressing strains (17) by the following modified procedure. After the ammonium sulfate fractionation, the samples were subjected successively to phenyl Toyopearl M, DEAE-Toyopearl M (TOSO; Tokyo, Japan) and phenyl-Superose HR (Amersham Pharmacia Biotech) column chromatography. The purity of the mutant σ factors was judged using a 7.5% polyacrylamide gels stained by the reverse staining method (21). Holoenzyme was reconstituted by mixing core enzyme with a 1.5 molar excess of mutant σ subunit. We confirmed that this amount of mutant σ was optimal (data not shown) and that a further increase in the amount did not significantly change the activities (17).

In Vitro Transcription Assay—All the transcription assays were carried out as described previously (18, 19) in T buffer (50 mM Tris-HCl, pH 7.9, 100 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1 mg/ml partially hydrolyzed casein) at 37 °C for 20 min. The concentration of the γ-^{32}P-initiating nucleotide (GTP or ATP) was 5 μM, whereas the other nucleoside triphosphates were 0.1 mM. Unless otherwise indicated, 40 μg/ml heparin was added simultaneously with the substrates. For pulse-labeling experiments in the presence of 100 mM NaCl, the RNA polymerase-promoter binary complexes were formed in T buffer. NaCl was then added in both the unlabeled and labeled substrate solutions.

The dissociation of the binary complexes was measured by trapping the dissociated enzyme with heparin (100 μg/ml) or with a 40-fold molar excess of T7A1 promoter-containing DNA (described in the text).

Gel Shift Assay—Binary complexes were formed in T buffer containing 12% glycerol at 37 °C for 10 min. Samples were then loaded onto a 4% polyacrylamide gel containing 6.75 mM Tris acetate, pH 8.0, and 1 mM EDTA. Electrophoresis was carried out for 1.5 h at a constant voltage of 150 V at room temperature in a circulating buffer system.

Permanganate Footprinting—Potassium permanganate footprinting was performed according to Suh *et al.* (22) with the following modifications. After the permanganate reaction, the samples were passed through Sephadex G-25 micro spin columns, treated with phenol/chloroform/isoamyl alcohol, and then precipitated with ethanol in the presence of sodium acetate and glycogen. DNA was cleaved by incubating the samples in 10% piperidine at 90 °C for 30 min, and the products were resolved on an 8% sequencing gel.

RESULTS

(a) Affinities of the Mutant Enzymes for the λP_R Promoter and the Lifetime of Their Binary Complexes—We examined the effects of the mutations in region 3 of σ^{70} on the stability of the binary complex of RNA polymerase with the λP_RAL promoter. First, a gel shift assay was employed in the absence of heparin to measure the apparent dissociation constants of complexes of the promoter with the wild-type and the two mutant holoenzymes. Essentially the same binding isotherm was observed for all three enzymes, so the mutations do not change the equilibrium constant for formation of the binary complex. The observed isotherm was sigmoidal, with a half-saturation around 20 nM (data not shown). This sigmoidal behavior is likely to be an artifact of the gel shift assay, because a single-round transcription assay using various concentrations of the wild-type enzyme yielded the usual hyperbola, with a half-saturation at 14 ± 3 nM (data not shown).

Heparin traps free RNA polymerase and therefore can be used to measure the rate of dissociation of binary complexes (7). We compared the heparin sensitivity of the binary complexes formed by the three enzymes. Complexes were formed by incubating the λP_RAL32 DNA with a nearly saturating concentration of each enzyme at 37 °C for 10 min, which was sufficient for the binding to reach equilibrium. Incubation was continued for another 10 min in the presence of 100 μg/ml heparin, and the gel shift assay was then performed. Only 7 and 13% of the binary complexes formed by the S506F and P504L enzymes, respectively, resisted heparin, whereas 60% survived in the wild-type case (data not shown). This suggests that a smaller fraction of the mutant enzymes form heparin-resistant complexes such as the open complex and/or that reversion from open to closed complex is more rapid.

Next we studied the dissociation kinetics of the binary complexes of wild-type and S506F enzymes with the λP_RAL32 promoter. In these experiments, binary complexes were first formed with λP_RAL32 at 37 °C, then further incubated for times ranging from 15 s to 20 min with either 100 μg/ml heparin or a 40-fold molar excess of T7A1 promoter as competitor. Finally, nucleotide substrates were added. The amounts of competitors used were enough to prevent the rebinding of the enzyme to λP_RAL32. We compared the dissociation kinetics using two different competitors because it has been reported that heparin is not a simple competitor for some promoters (23). The assay measures the fraction of open complex that has survived in the presence of a competitor for each period of time. We monitored the formation of both the full-length (32-mer) and abortive (9-mer) products.

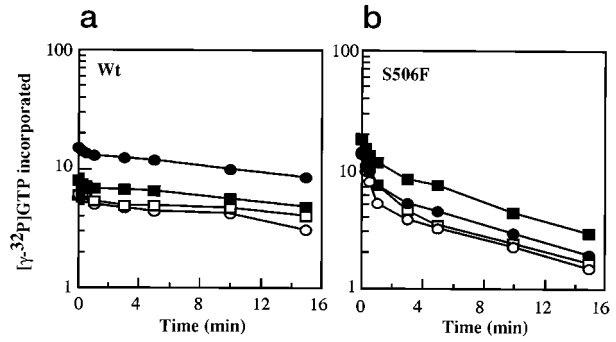

FIG. 1. **Dissociation of the binary complexes at the λP_RAL32 promoter.** The wild-type (*Wt*) holoenzyme (*a*) or S506F holoenzyme (*b*) were preincubated with λP_RAL32 for 10 min at 37 °C. They were then incubated with either 100 μg/ml heparin or a 40-fold excess of T7A1 DNA for the indicated times. Substrates were added next to initiate transcription reactions of 20 min. The amounts of transcripts were plotted against the time of incubation with the competitors. ATP was excluded from the substrate mixture so that a 32-mer transcript was the full-length product. The *filled circles* and *squares* show the amounts of 32-mer and 9-mer transcripts, respectively, when T7A1 DNA was used as competitor. *Open circles* and *squares* show the amounts of 32-mer and 9-mer, respectively, when heparin was used as competitor. The concentrations of enzyme and DNA were 35 and 12 nM, respectively, except that the concentration of template DNA was 5 nM when T7A1 DNA was used as competitor.

Figs. 1, *a* and *b* show the decay profiles for the two enzymes. The profile for the wild-type enzyme was almost monophasic, whereas that for the S506F mutant was biphasic and characterized by an early rapid decay. The kinetic parameters obtained are listed in Table I, proving that there is more than one type of binary complex of the S506F enzyme and that the major type is short-lived. Thus the back reaction into the free components is faster for the mutant enzyme, although the overall equilibrium constant of binary complex formation was not significantly different from wild type, as shown above by the gel shift assay in the absence of competitors.

Both heparin and T7A1 DNA exerted the same effect, within experimental error, as shown in Table I. Therefore in our system heparin acts as a simple competitor of the binary complexes by binding to free RNA polymerase. In the following sections all the interpretations of experiments using heparin assume this simple competition model.

(b) Promoter Melting by the Mutant Enzymes—Permanganate preferentially modifies thymine residues in single-stranded or distorted regions of DNA in open promoter complexes (24). In the presence of a saturating concentration of any of the enzymes, the thymine residues at −13, −10, −7, −4, −3, and +2 of the nontemplate strand and −8 and −9 of the template strand exhibited strongly increased permanganate sensitivity (Fig. 2). These residues were much more sensitive in the wild-type than in the mutant complexes, with S506F showing the least sensitivity. But the relative sensitivities of the residues in a given complex remained the same. These results

TABLE I

The values of the dissociation rate constants (k) for binary complexes of RNA polymerase carrying wild-type or S506F σ at the λP_RAL32 promoter

Dissociation rate constants (k) were obtained by fitting the data to single exponential or double exponential expressions of the forms $y = A \exp(-k_1 t)$ and $y = A \exp(-k_1 t) + B \exp(-k_2 t)$ for the wild-type and S506F enzymes, respectively. In the case of wild-type enzyme, the decay is monophasic, so it is designated by one rate constant.

Enzyme	Competitor		k_1	k_2
			min^{-1}	min^{-1}
Wild-type	T7A1	9-mer	0.027 ± 0.004	
		32-mer	0.032 ± 0.004	
Wild-type	Heparin	9-mer	0.023 ± 0.004	
		32-mer	0.030 ± 0.004	
S506F	T7A1	9-mer	2.1 ± 0.3	0.09 ± 0.01
		32-mer	1.6 ± 0.2	0.08 ± 0.01
S506F	Heparin	9-mer	1.8 ± 0.1	0.09 ± 0.01
		32-mer	2.3 ± 0.2	0.08 ± 0.01

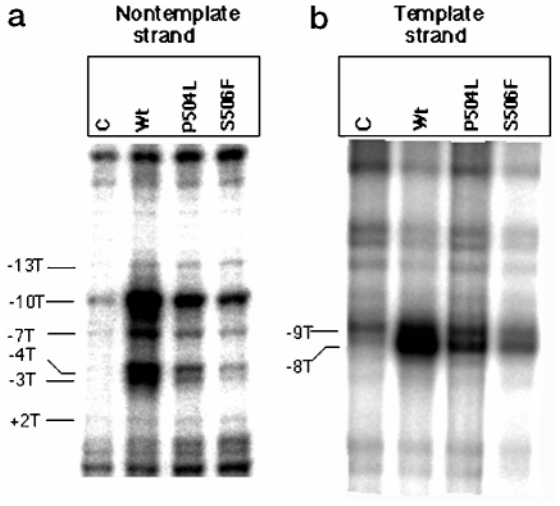

FIG. 2. **$KMnO_4$ footprinting of the binary complexes with λP_RAL promoter.** Footprinting experiments were done with linearized λP_RAL73 template labeled at the 5′ end of either the nontemplate (*a*) or the template (*b*) strand. A sequence ladder was used to identify the permanganate-sensitive bases (lanes not shown). Control lanes (*C*) show the pattern in the absence of enzyme. Enzyme and DNA concentrations were 35 and 12 nM, respectively. *Wt*, wild-type; *T*, thymine.

suggest that smaller fractions of the binary complexes are in the "open" form in the case of the mutant enzymes, especially S506F. These mutations in region 3 of σ^{70}, therefore, shift the equilibrium from the open toward the closed promoter state. This result is consistent with the presence of a large fraction of short-lived complexes among the binary complexes formed by the S506F mutant enzyme (Fig. 1); the major fraction of the mutant binary complexes is presumably in a closed state.

(c) Productive and Abortive Transcriptions by the Mutant Enzymes—The A-less template (λP_RAL32) was designed so that by excluding ATP during transcription, a 32-mer paused product would be formed. Both the full-length (32-mer) and the shorter abortive products of this template can easily be quantified on the same polyacrylamide gel (18). 5-mer and 9-mer transcripts were the major products observed along with the 32-mer using this template (Fig. 3*a*). These short 5- and 9-mers were indeed abortive products, as shown in section "d."

The amounts of products formed in the presence or absence of heparin are quantified in Fig. 3, *b* and *c*. At a nearly saturating concentration of the enzyme (~90% bound), both mutants produced less RNA than the wild-type enzyme (*panel b*). The amounts of abortive products (4–9-mer) were particularly reduced by the mutations, as is evident from the ratios of 5-mer

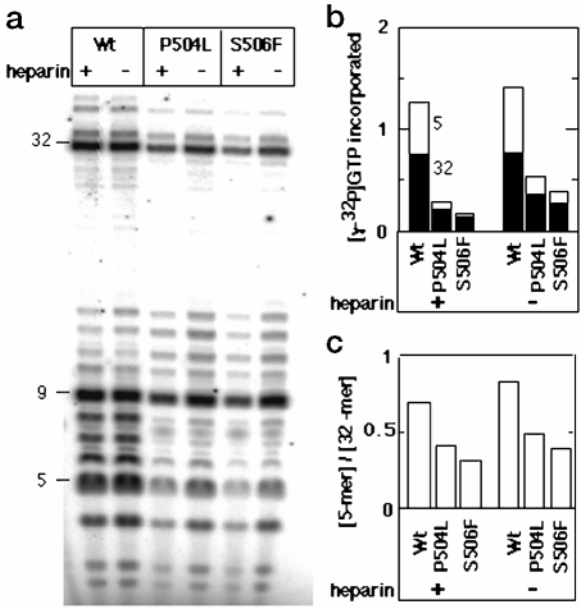

FIG. 3. **Transcription of λ_RAL32 template for 20 min.** *a,* transcripts synthesized by wild-type (*Wt*), P504L, and S506F holoenzymes in the presence (+) and absence (−) of heparin. The transcripts were labeled with [γ-^{32}P]GTP. The enzyme and DNA concentrations were 35 and 12 nM, respectively. *b,* amounts of 32-mer (*filled*) and 5-mer (*open*) products in the presence (+) and absence (−) of heparin are plotted in different scales. *c*, amounts of 5-mer products relative to those of the 32-mer full-length products.

to 32-mer transcripts (*panel c*). The 5-mer and 32-mer syntheses are, therefore, differentially inhibited by the mutations, suggesting the involvement of more than one pathway in the syntheses of these two products.

(d) Time Courses of Accumulation of Full-length and Abortive RNA Products of λP_RAL32—Next we examined the time course of accumulation of the transcripts generated by both free and immobilized λP_RAL32 templates in the presence of heparin. Templates that had been immobilized on avidin-acrylic beads were used to separate the released abortive products from those retained in the transcription complexes. After transcription with immobilized templates and brief centrifugation, the supernatant contained only released products. Fig. 4*a* shows that most of the products shorter than 12 nucleotides were released and, thus, indeed products of abortive transcription. This was true not only for S506F but also for the other two enzymes (data not shown). *Panels b* and *c* are plots of the time courses of accumulation of the two major products (5-mer and 32-mer) using the free template with the wild-type and S506F enzymes, respectively. The production of the full-length 32-mer transcript had a time lag of about 48 s for the wild-type and P504L enzymes and about 18 s for S506F enzyme, respectively (*panel c*). 32-mer synthesis by all the enzymes reached a plateau at around 10 min (data for P504L are not shown). In the case of the wild-type enzyme, the time course of 5-mer production had an initial burst phase and a slow phase continuing beyond 20 min (*panel b*). The latter slow accumulation has been interpreted as evidence for the formation of moribund complexes, which are defined as complexes capable of persistent production only of abortive transcripts. However, the slow accumulation phase was not observed or barely detectable with the mutants, and both 5-mer and 32-mer production reached plateaus within 10 min (*panel b*).

(e) Pulse-labeling Assay to Detect Moribund Complex—The analysis of time course data could be obscured if cleavage of transcripts were taking place. So we have used the pulse-labeling assay to confirm the difference between the temporal

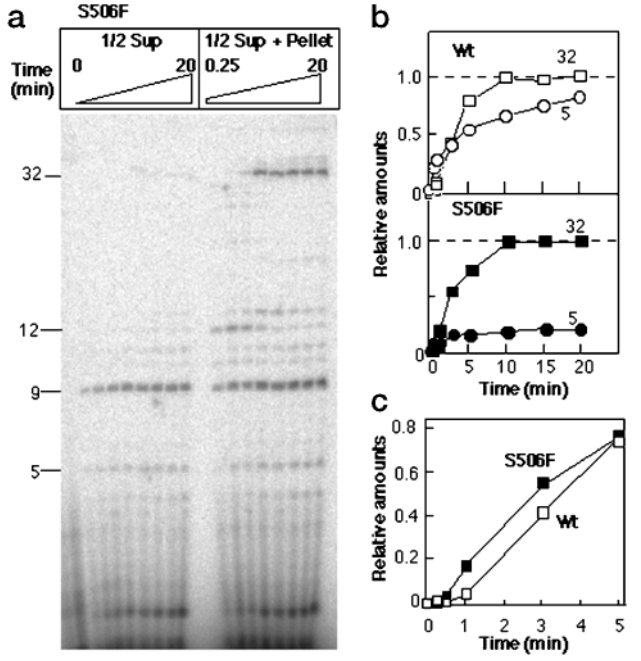

FIG. 4. **Time-courses of transcription of the λP_RAL32 template.** *a*, the immobilized λP_RAL32 template was transcribed by S506F for the indicated times, and reactions were then stopped with 25 mM EDTA. The reaction mixture was centrifuged, half the supernatant (*1/2 Sup*) was examined separately, and the rest was remixed with the pellet (*1/2 Sup + Pellet*). The *triangles* denote the time points as 15 and 30 s and 1, 3, 5, 10, 15, and 20 min. The enzyme and DNA concentrations were the same as in Fig. 3. *b*, the time courses of 32-mer (□) and 5-mer (○) synthesis by wild-type (*Wt*), and the time courses of 32-mer (■) and 5-mer (●) syntheses by S506F holoenzyme determined by experiments using nonimmobilized templates under the same conditions. *c*, the first 5 min of the time course of 32-mer synthesis by wild-type (□) and S506F (■) enzymes, respectively. In *panels b* and *c*, the amount of each transcript was normalized to the amount of 32-mer formed at 20 min. Experiments were done in the presence of heparin. In a previous study, the 9-mer was the transcript found to be most characteristic of the moribund complex, and it continued to be produced up to 20 min (18), but in the present study the persistent production of 5-mer was more pronounced. Although the reasons for this difference are unknown, it was associated with the use of different brands of radioisotope and/or with a change in the template (190-bp template instead of 1130 bp).

behaviors of abortive and full-length transcription. In this assay, transcription was first initiated with cold substrates, and then the γ-^{32}P-labeled initiating nucleotide was added at various time points, followed by transcription for a further 5 min. This assay can detect the initiation of abortive products even after the synthesis of full-length transcripts has ceased. Such a persistent initiation of short transcripts is characteristic of moribund complexes at the λP_RAL32 promoter (18).

The time course of synthesis of abortive products by the wild-type enzyme at the T7A1 promoter did not show any slow phase (data not shown). Therefore, moribund complexes (if any) do not accumulate at this promoter. To confirm the reliability of the pulse label assay, we used the T7A1 system as a control. As expected, incorporation of [γ-^{32}P]ATP was rapid, and neither full-length nor abortive transcripts were labeled after 3 min (Fig. 5*a*).

With the λP_RAL promoter, that the persistent initiation of 9-mer and 5-mer products after 32-mer initiation had ceased was observed in the case of the wild-type enzyme (Fig. 5*b*) but was almost undetectable (Fig. 5*c*) for S506F enzyme. Since most binary complexes of the mutant enzyme dissociate within 1 min and the released enzyme is trapped by heparin, the absence of persistent initiation might be an artifact caused by heparin, which was added with the substrates to prevent enzyme turnover. To avoid this potential artifact, we repeated the pulse-labeling experiments in the absence of heparin by using an alternative method to block turnover. λP_RAL32 template was kept in 3-fold excess over enzyme, and the elongation complex was stalled at +32 by excluding ATP. In a control experiment, to confirm the absence of turnover in this protocol, we added a template harboring a 73-bp A-less leader sequence (AL73 template) plus labeled substrates to the pre-incubated mixture of λP_RAL32 template, enzyme, and unlabeled substrates. We did not detect the 73-mer transcript, confirming that enzyme turnover had been blocked. The elimination of heparin did not significantly affect the persistent synthesis of abortive product by the wild-type enzyme (data not shown).

As shown in Fig. 6*a*, in the absence of heparin, S506F enzyme showed the persistent initiation of abortive transcripts. The ratios of abortive to full-length products increased with time for mutant enzyme (Fig. 6*b*) as for the wild-type (Fig. 5*b* and 6*d*). Although the S506F enzyme produced much smaller amounts of 5-mer RNA than the wild-type, the accumulation of 9-mer was more comparable with that for wild-type enzyme. Therefore S506F enzyme does form moribund complexes at the λP_R promoter, but the complexes are readily trapped by heparin after their rapid dissociation. The moribund complexes of the mutant are equilibrated with the free DNA and protein more rapidly than their wild-type counterparts, strongly suggesting the existence of binary complexes in moribund conformations.

If the interpretation of the pulse-labeling results mentioned above is correct, any conditions preferentially destabilizing wild-type moribund complexes should mimic the effect of the S506F mutation when combined with heparin. We performed the same assay with the wild-type enzyme in the presence (only during the pulse period) of 100 mM extra NaCl, so that the resulting total salt concentration was 100 mM KCl and 100 mM NaCl. We chose this condition because it maintained the level of full-length transcription and the length distribution of abortive products from this promoter, which were altered at 200 mM KCl or by higher concentrations of NaCl (data not shown). As shown in Fig. 6*c*, the persistent production of abortive products was significantly reduced in this condition, and the ratios of abortive to productive transcripts remained constant after 3 min (Fig. 6*d*). Therefore moribund complexes are salt-sensitive, indicating that ionic interactions play an important role in their stabilization.

DISCUSSION

Contribution of Region 3 of σ^{70} to Transcription Initiation—To understand the reasons for the reduced output of abortive transcripts by the σ^{70} mutants P504L and S506F (17), we examined the nature of the DNA-holoenzyme binary complexes, the abortive transcription process, and the accumulation of moribund complexes at the λP_RAL promoter. In the open complex at the natural λP_R promoter, about 14 bp of duplex DNA were melted in the −10 region (22). Recently, it has been shown that a σ^{70} fragment comprising amino acids 374–448, corresponding to a nearly complete region 2, can bind to the single-stranded form of the −10 region, specifically the nontemplate strand, with high affinity, and thereby stabilizes the melted duplex in this region (25). Here we present evidence that mutations in region 3 strongly affect the process of promoter melting or the stability of the open complex (Fig. 2) without changing the overall equilibrium constant of the binary complex formation. The predominant fraction of binary complexes of the mutants consists of a closed complex(es), which rapidly equilibrates with the free promoter and free holoenzyme.

Open and closed binary complexes are, each, populations containing several different species (26, 27). The rapid equilib-

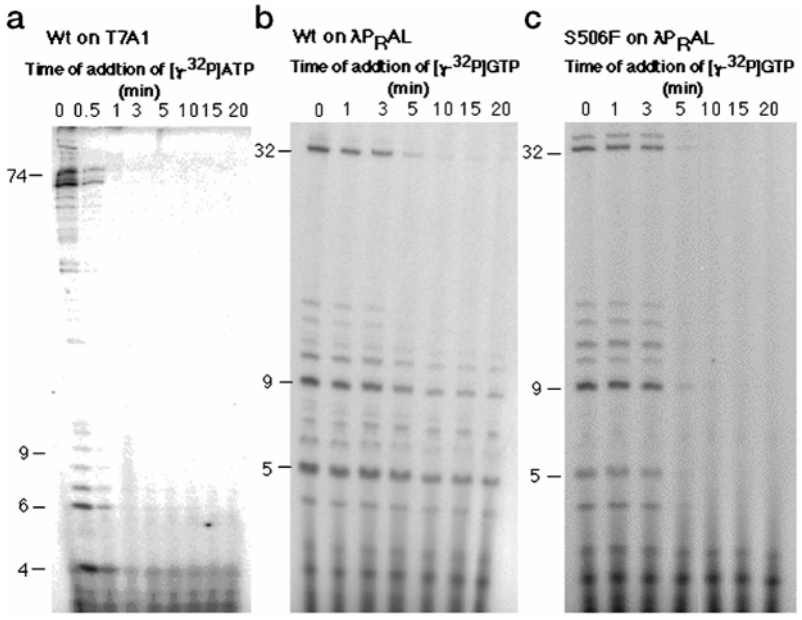

FIG. 5. **Pulse-labeling experiments in the presence of 40 μg/ml heparin.** Transcriptions were carried out in the presence of 35 nM wild-type (*Wt*) enzyme and 12 nM T7A1 (*a*) and λP_RAL32 (*b*) templates. *Panel c* is the same experiment with S506F enzyme on the λP_RAL32 template. See the text for details.

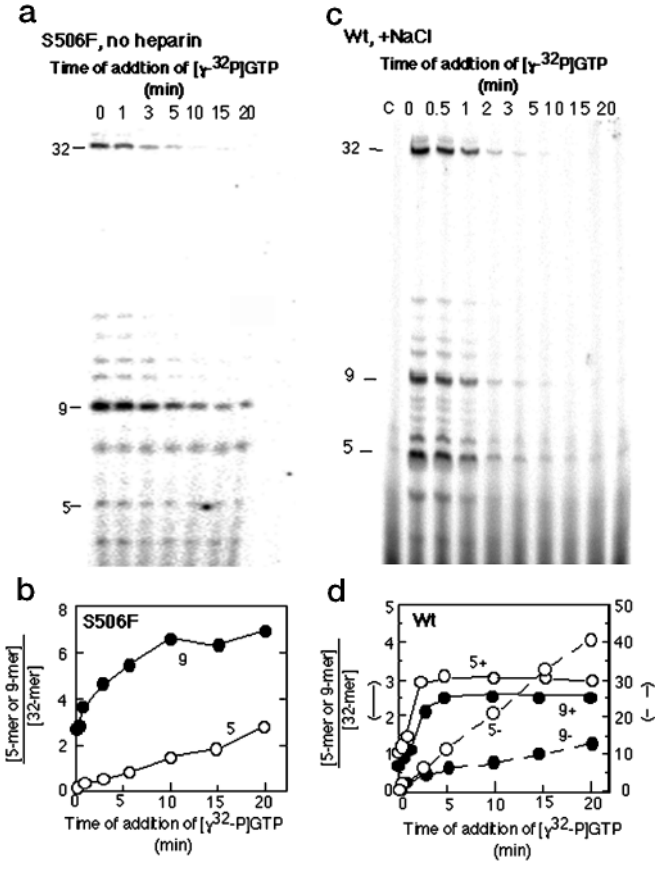

FIG. 6. **Pulse-labeling experiments in the absence of heparin or in the presence of additional salt.** *a*, λP_RAL32 template (105 nM) was transcribed S506F enzyme (35 nM) in the absence of heparin. See details in the text. *b*, the ratios of the amounts of 5-mer (○) and 9-mer(●) to that of 32-mer product shown in *panel a* are plotted against the time of addition of [γ-^{32}P]GTP. *c*, a similar assay using 12 nM DNA and 35 nM wild-type (*Wt*) enzyme in the presence of 40 μg/ml heparin. 100 mM NaCl was present during the labeling period. *d*, the ratios of the amounts of 5-mer (○, *5+*) and 9-mer (●, *9+*) to that of the full-length (32-mer) product shown in *panel c* are plotted. The *broken lines* are the same ratios of 5-mer (□, *5-*) and 9-mer (■, *9-*) without added NaCl derived from Fig. 5*b* as controls.

rium of moribund complex with free mutant enzyme further suggests the existence of binary complexes with moribund conformation. Such binary complexes should be a component of the abortive cycle of moribund complexes, because the cycle involves interconversion between ternary and binary complexes. An accurate assignment of species to the two phases observed in the decay curve of the mutant (Fig. 1*b*) is impossible. We conclude only that the mutation increases the rate of equilibration of open complex and moribund complex with their free components because both k_1 and k_2 for the mutant enzyme are larger than k_1 of the wild-type (Table I).

The lag time for productive transcription is reduced by the S506F mutation (Fig. 4*c*). A similar acceleration by this mutation has also been observed at the *gal*P2 promoter.[2] Therefore, a mutation in region 3 can accelerate the step(s) in the reaction pathway leading to productive transcription yet can simultaneously reduce the fraction of the open form in binary complexes and the overall yield of full-length product.

Mechanism of Reduction in Abortive Synthesis from the λP_RAL Promoter—At the λP_RAL promoter, abortive transcripts are continuously produced for more than 20 min, whereas synthesis of full-length 32-mer ceases in 5 min (Ref. 18; also in Fig. 5*b*). Due to this long duration of abortive cycling compared with productive RNA synthesis, it is reasonable to suggest that a significant fraction of the abortive products generated from this promoter are made by moribund or nonproductive binary complexes. Here we report that mutations in the σ subunit or the addition of high salt not only reduce abortive transcription but also increase the rate of equilibration of moribund complexes with free enzyme. A rapid exchange of moribund complexes with free enzyme facilitates their conversion into other complexes, including open complexes, the inactivated ternary complexes (19), and a complex with heparin (when it is added). These processes reduce the accumulation of moribund complexes and therefore reduce the output of abortive products. This appears to be the key reason for the observed decrease in abortive transcription by the σ mutants at this promoter. The existence of several alternative fates of mutant moribund complexes can also explain the absence of any concomitant increase in output of full-length products and the effects of heparin on full-length and abortive products. Recently, using DNA foot-

[2] V. J. Hernandez and M. Cashel, unpublished result.

printing, it has been shown that moribund complexes are promoter-bound species (19). Therefore, it is understandable that alteration of the RNA polymerase-promoter interaction by the mutations in the σ subunit (Figs. 1 and 2) should also influence the nature of the moribund complexes.

As already mentioned, the reduction of abortive synthesis by a decreased accumulation of moribund complexes did not cause a concomitant increase in productive transcription. Similarly the presence of high salt, which selectively destabilized the moribund complexes, did not affect productive transcription (Fig. 6*c*). This lack of correlation suggests that the abortive cycling associated with moribund complexes is not part of the sequential multistep initiation process leading to productive transcription. On the contrary, some moribund complexes convert into catalytically inactive complexes at this promoter (19). Although moribund complexes are very likely to be the major source of abortive products at this promoter, there is no evidence to prove or disprove the possibility that some abortive products arise from the productive pathway.

Since very few, if any, moribund complexes appear to accumulate at the T7A1 promoter, it seems that their formation depends on the promoter sequence. Evidently some promoters, such as λP_R, have a much greater tendency to form moribund complexes. It will be of great interest to evaluate how common is the formation of such complexes at promoters in general. Furthermore, structural characterization of moribund complexes will be essential for a full understanding of transcription initiation.

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Reduction in Abortive Transcription from the λP_R Promoter by Mutations in Region 3 of the ς^{70} Subunit of *Escherichia coli* RNA Polymerase

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