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An inactive open complex mediated by an UP element at *Escherichia coli* promoters

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ABSTRACT **A specific interaction between the α subunit of RNA polymerase and an A+T-rich upstream sequence (UP element) stimulates transcription at some promoters in *Escherichia coli*. We found that RNA polymerase formed a heparin-resistant nonproductive initiation complex at the *malT* promoter which has an A+T-rich upstream sequence that begins 9 bp upstream of the −35 region. Substitution of other sequences for the A+T-rich sequence eliminated both the formation of heparin-resistant complexes and α binding to the *malT* promoter. A 5-bp deletion between the A+T-rich sequence and the −35 region increased promoter activity. The UP element derived from the *rrnB* P1 promoter stimulated transcription of the *malT* core promoter when placed 4 bp upstream from the *malT* −35 region, but insertion of an additional 4 bp between the *rrnB* P1 UP element and the −35 element eliminated transcription activity without eliminating heparin-resistant complex formation. Similar UP element effects were observed in hybrids with the *lac* core promoter, even though the region around the transcription start site was melted in both productive and nonproductive complexes. We conclude that UP elements can mediate the formation of both productive and nonproductive open complexes, depending on their location with respect to the core promoter.**

Promoter recognition and open complex formation are central events in transcription initiation by RNA polymerase (RNAP) holoenzyme. In *Escherichia coli*, the RNAP σ subunit plays an essential role in these processes. The σ^{70} makes sequence-specific contacts to two hexameric elements located approximately 10 and 35 bp upstream of the transcription start site (1). The similarity of a promoter sequence to the −10 and −35 consensus hexamers (TATAAT and TTGACA, respectively) and to the consensus spacing between them (17 bp) is a major determinant of the strength of a promoter (2, 3). In addition, it was found recently (4, 5) that an A+T-rich sequence located upstream of the −35 hexamer (UP element) acts as a third RNAP recognition element in certain promoters.

The best characterized UP element is that of the *rrnB* P1 promoter, which increases promoter activity more than 30-fold *in vivo* (5). The C-terminal domain of the RNAP α subunit (αCTD), which functions as the target for a number of transcription factors (6, 7), binds directly to the UP element and is responsible for the specific interaction of the UP element with RNAP (5, 8–10). In addition, the effect of the UP element on a promoter activity correlates generally with its degree of similarity to the UP element consensus sequence (11). It has been estimated that approximately 3% of *E. coli* promoter sequences for mRNAs and 19% of promoters for stable RNAs contain a match to the consensus UP element sequence similar in extent to the *rrnB* P1 UP element (10). However, the functional significance of most of these sequences remains to be studied.

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We found previously (12) that RNAP formed a stable nonproductive complex at the *malT* promoter in the absence of added factors, whereas it formed a productive complex in the presence of the cAMP receptor protein (CRP). The nonproductive complex was resistant to the competitor heparin and made the abortive trinucleotide ApUpU from ApU and UTP, but it did not make a productive transcript in the presence of all four nucleoside triphosphates. The DNA region occupied by RNAP in the nonproductive complex was essentially the same as that in the CRP-dependent productive complex, although there was a significant difference in the DNase I protection pattern of the two complexes, particularly between −20 and −60. We noticed that the *malT* promoter contains an A+T-rich sequence upstream of the core promoter and that its location relative to the −35 hexamer is 5 bp further upstream of the −35 element than the *rrnB* P1 UP element. Here, we investigate how the location of an UP element affects the function of the *malT* promoter and the two hybrid promoters, *rrnB* P1 UP-*malT* and *rrnB* P1 UP-*lac*. We show that an UP element can mediate the formation of either a productive or a nonproductive complex, depending on its location with respect to the core promoter.

MATERIALS AND METHODS

Media and Growth Conditions. Cells were grown aerobically at 37°C in Luria–Bertani medium (13). When used, ampicillin was added at 50 μg/ml. Bacterial growth was monitored by determining the optical density at 600 nm.

DNAs and Proteins. The promoters used in this study are shown in Fig. 1. The *Hin*dIII end of the 247-bp *Eco*RI–*Hin*dIII fragment containing the wild-type *malT* promoter of pMT100 (12) was filled in and cloned between the *Eco*RI and *Sma*I sites of pUC19. The 261-bp *Eco*RI–*Xba*I fragment from the resulting plasmid was cloned into pLA100 to construct pMT201. pMT202 (Δ5 bp), pMT203 (−A/T), and pMT220 (*malT* H) were constructed from pMT201 by polymerase chain reaction mutagenesis. The 180-bp *Pvu*II–*Hin*dIII fragment containing the *lac* promoter derived from pUC19 was cloned between the *Sma*I and *Hin*dIII sites of pMS437C (14). The *Hin*dIII site of the resulting plasmid was filled in and an *Xba*I linker was inserted to construct pLA02. The *Bam*HI (−78), *Kpn*I (−45), and *Hin*dIII (−37) sites were introduced into the *lac* promoter region of pLA02 by polymerase chain reaction mutagenesis to form pLA100. To construct pLA200, the *Bam*HI and *Hin*dIII region of pLA100 was replaced by a 30-bp synthetic oligonucleotide containing the *rrnB* P1 UP element. To form pMT221 (UP4–*malT*), the *Eco*RI–*Hin*dIII region of pMT220 was replaced by the *Eco*RI–*Hin*dIII fragment derived from pLA200.

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Abbreviations: RNAP, RNA polymerase; αCTD, C-terminal domain of the RNAP α subunit; CRP, cAMP receptor protein; EMSA, electrophoretic mobility-shift assay.
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FIG. 1. Promoters used in this study. The −35 and −10 hexamers, and the CRP binding sites are underlined. The transcription start site is numbered as +1. The upstream A+T-rich sequence of the *malT* promoter appears in boldface. The UP element derived from the *rrnB* P1 appears in boldface italic.

To form pLA201 (UP4-*lac*), the *Bam*HI and *Hin*dIII region of pLA100 was replaced by a 29-bp synthetic oligonucleotide containing the *rrnB* P1 UP element. To construct pMT222 (UP8–*malT*) and pLA211 (UP8–*lac*), pMT221 and pLA201, respectively, were digested with *Hin*dIII and filled in. CRP and RNAP were purified as described previously (12). The α subunit of RNAP was purified, according to the method described (15), from cells harboring pGEMAX185.

Electrophoretic Mobility-Shift Assay (EMSA). Reaction mixtures (30 μl) containing a 5 nM DNA fragment and 30 nM RNAP were incubated in transcription buffer (20 mM Tris·HCl, pH 7.9/100 mM NaCl/3 mM $MgCl_2$/0.1 mM EDTA/0.1 mM DTT/5% glycerol/50 μM cAMP/50 μg/ml BSA) and assayed as described (12), except that the heparin challenge was performed by adding 3 μl of 50 μg/ml of heparin for 3 min. At the times indicated, 3 μl of NTP solution (1 mM ATP, UTP, or 1 mM each of the four NTPs) was added to the mixture before the heparin treatment. For α-subunit binding assays, α (0–0.4 μM) was incubated with a 5 nM DNA fragment for 40 min at 22°C in 20-μl reaction mixtures containing 50 mM Tris·HCl (pH 7.9), 50 mM NaCl, 3 mM DTT, and 5% glycerol; the complexes were analyzed by native gel electrophoresis at room temperature. The gel contained 6% polyacrylamide, 7.5% glycerol, and 45 mM Tris·borate (pH 8.3)/1 mM EDTA (TBE). The running buffer contained TBE and 2% glycerol.

***In Vitro* Transcription.** Complexes were formed as described above in 30 μl of transcription buffer, and transcription was started by adding 3 μl of a substrate solution containing 0.05 mM [α-^{32}P]UTP (5 μCi) and 0.5 mM each of ATP, GTP, and CTP. After 15 min of incubation, the reaction was terminated by adding 60 μl of phenol and 30 μl of stop buffer (0.6 M sodium acetate, pH 5.5/20 mM EDTA/200 μg/ml tRNA). The products were precipitated with ethanol and fractionated by electrophoresis on 8% polyacrylamide gels containing 8 M urea. Abortive initiation assays were performed at 37°C in 30-μl reaction mixtures containing transcription buffer, 5 nM UP4-*lac* or UP8-*lac* hybrid promoter DNA fragment, and 30 nM RNAP. The reaction was started by adding 3 μl of a solution containing 1 mM ATP and 0.1 mM [α-^{32}P]UTP (10 μCi). After 15 min of incubation, products were precipitated with ethanol and analyzed on 20% polyacrylamide/8 M urea gels.

Permanganate Footprinting. Fifty microliter reactions were incubated for 30 min at 37°C in a transcription buffer containing 1 nM ^{32}P-end-labeled DNA fragment and 110 nM RNAP. At the times indicated, 100 nM CRP was added before RNAP. The mixtures were treated with 5 μl of 50 μg/ml heparin for 3 min at 37°C. Two millimolar $KMnO_4$ was added for 1 min at 25°C, and the reaction was then quenched with 2-mercaptoethanol. After the addition of 20 μl of 1.5 M sodium acetate/20 mM EDTA/100 μg/ml tRNA, the mixture was precipitated with ethanol. DNA was cleaved by incubation in 1 M piperidine at 90°C for 30 min, and products were analyzed on a 6.5% polyacrylamide/8 M urea sequencing gel.

β-Galactosidase Assay. KI70 (Δ*lac*) and OK6201 (Δ*lac crp*$^-$) cells (16) harboring the promoter *lacZ* fusion plasmids were grown to an OD_{600} of 0.8. The β-galactosidase activity was determined as described (13).

S1 Nuclease Assay. Cells harboring the promoter-*lacZ* fusion plasmids were grown to an OD_{600} of 0.5 and RNA was prepared as described (17). *Eco*RI–*Xba*I fragments of pMT201 and pLA100, ^{32}P-labeled at the *Xba*I 5′ end were used as DNA probes for the *malT* and *lac* transcripts, respectively. RNA was hybridized with the DNA probes and treated with 100 units of S1 nuclease at 37°C for 15 min, and the reaction products were analyzed on 8% polyacrylamide/8 M urea gels.

RESULTS

The A+T-Rich Sequence Is Responsible for the Formation of Nonproductive Complex at the *malT* Promoter. In the absence of CRP, the *malT* promoter forms, with RNAP, a heparin-resistant binary complex that is unable to make run-off transcripts (12). The *malT* promoter has an A+T-rich sequence upstream of the −35 hexamer (Fig. 1). To examine the potential role of this A+T-rich sequence in the formation of the nonproductive complex, we constructed several variants of the *malT* promoter (Fig. 1) and analyzed RNAP-promoter complexes by EMSA. The nonproductive complex exhibits slightly more increased mobility than the productive complex formed after incubation of the *malT* promoter with both RNAP and CRP, and after being challenged with heparin (Fig. 2) (12). When the A+T-rich sequence was replaced by a G+C-rich sequence, formation of the nonproductive complex was markedly reduced (Fig. 2*A*, lanes 1 and 4). The reduction in the amount of nonproductive complex was even more significant in the *lac-malT* hybrid promoter when the *malT* A+T-rich sequence was replaced by the upstream region from the *lac* promoter (data not shown). We conclude that the A+T-rich sequence is required for the formation of the nonproductive heparin-resistant complex. The small amount of productive complex formed in the absence of CRP–cAMP was not affected by elimination of the A+T-rich sequence (lane 4).

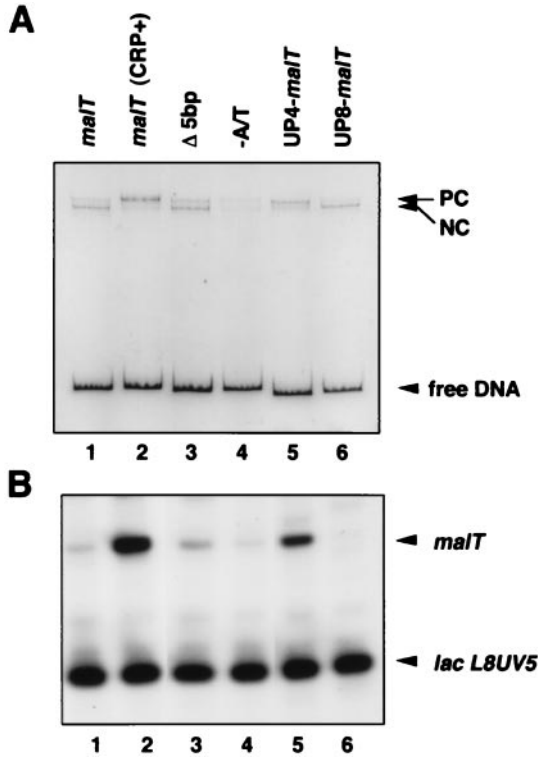

FIG. 2. Effect of the UP element on the *malT* core promoter *in vitro*. (*A*) EMSA for RNAP binding. The *Eco*RI–*Xba*I fragments containing the *malT* promoter derivatives were incubated with RNAP, treated with heparin, and analyzed by EMSA. In lane 2, CRP (50 nM) was added before RNAP. PC and NC represent the productive and nonproductive complexes, respectively. (*B*) *In vitro* transcription assay. The *Eco*RI–*Xba*I fragments containing the *malT* promoter derivatives were the templates, and the 205-bp *Eco*RI fragment containing the *lacL8UV5* promoter (39) was an internal control.

Effect of the Location of UP Elements on RNAP–*malT* Promoter Interaction. UP elements that have been characterized (to date) in detail facilitate formation of productive open complexes (refs. 4 and 5; see also ref. 30). The *malT* A+T-rich sequence exhibits a moderate similarity to the *rrnB* P1 (5) and consensus UP elements (10). We addressed the question of why the A+T-rich sequence leads to nonproductive complex formation at the *malT* promoter. We noticed that the rightmost end of the A+T-rich *malT*, with respect to the −35 hexamer, is 5 bp further upstream than that of the UP element in *rrnB* P1. Interestingly, analysis by EMSA revealed that the deletion of 5 bp between the A+T-rich sequence and the −35 region of the *malT* promoter moderately increased (about 2-fold) the amount of the upper band, corresponding to the productive complex (Fig. 2*A*, lanes 1 and 3). The Δ5-bp mutant promoter also exhibited a 2.5-fold increase in promoter activity (Fig. 2*B*, lane 3).

We next examined the effect of the location of the *rrnB* P1 UP on *malT* core promoter activity. We constructed two hybrid promoters (UP4–*malT* and UP8–*malT*) in which the *rrnB* P1 UP element was placed 4 and 8 bp, respectively, upstream of the *malT* −35 hexamer (Fig. 1). The two *rrnB* P1 UP–*malT* hybrid promoters were analyzed by EMSA. RNAP preferentially formed a lower mobility complex with the UP4–*malT* promoter whereas it formed predominantly a higher mobility complex with the UP8–*malT* promoter (Fig. 2*A*, lanes 5 and 6). The UP4–*malT* promoter was active in transcription *in vitro*, but little activity was detected with the UP8–*malT* promoter (Fig. 2*B*, lanes 5 and 6). These experiments clearly indicate that only a suitably located UP element

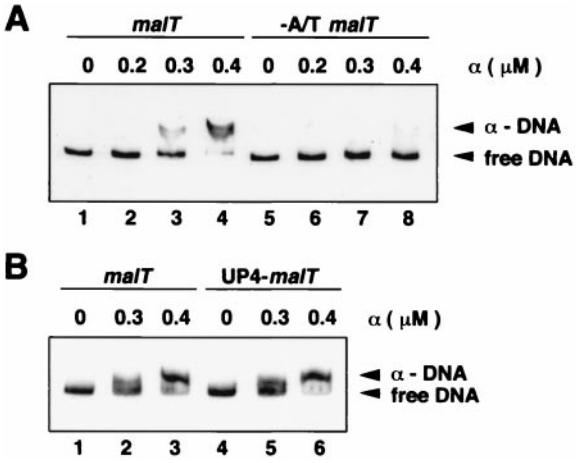

FIG. 3. EMSA for α subunit binding. (*A*) Comparison of the wild-type and −A/T *malT* promoters. The 261-bp *Eco*RI–*Xba*I fragment derived from pMT201 (lanes 1–4) or from pMT203 (lanes 5–8) was incubated with the indicated concentrations of α subunits. (*B*) Comparison of the wild-type *malT* and UP4–*malT* promoters. The *Eco*RI–*Xba*I fragment derived from pMT201 (lanes 1–3) or from pMT221 (lanes 4–6) was incubated with the indicated concentrations of α subunits.

stimulates the formation of a productive complex at the *malT* promoter, thereby activating transcription.

The Binding of the α Subunit to the A+T-Rich Sequence in the *malT* Promoter. To examine whether the *malT* A+T-rich sequence interacts with the α subunit, we performed EMSA with purified α subunits and DNA fragments containing the *malT* promoter variants. α formed a complex with the wild-type *malT* promoter (Fig. 3*A*, lanes 1–4), but much less complex was formed at the −A/T *malT* promoter (lanes 5–8). The affinity of the wild-type *malT* promoter for α subunits was approximately equivalent to that of the UP4-*malT* promoter, in which the *rrnB P1* UP element was fused to the *malT* core promoter (Figs. 1 and 3*B*).

The Transcription Initiation Region Is Melted in Nonproductive Complexes. To examine whether the region around the transcription start site was melted, we performed permanganate footprinting. The ^{32}P-labeled DNA fragment carrying the wild-type *malT* promoter was incubated with CRP–cAMP

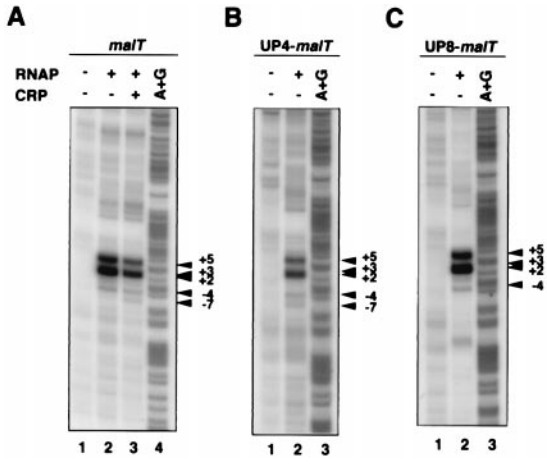

FIG. 4. Potassium permanganate footprinting of *malT* promoter derivatives. The 261-bp *Eco*RI–*Xba*I fragments derived from pMT201 (*A*), the 236-bp *Eco*RI–*Xba*I fragment derived from pMT221 (*B*), and the 240-bp *Eco*RI–*Xba*I fragment derived from pMT222 (*C*) were used for $KMnO_4$ footprinting. The arrowheads indicate the permanganate-sensitive sites. The numbers represent positions relative to the transcription start site.

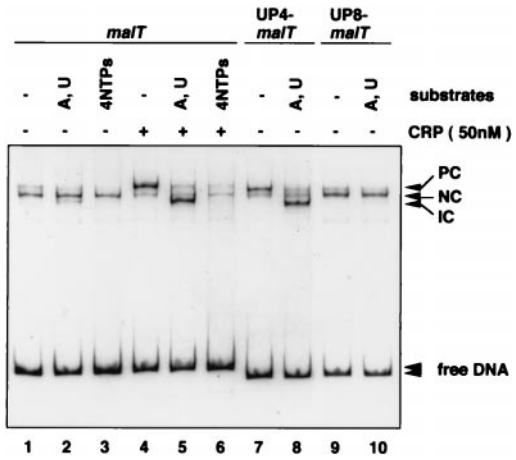

FIG. 5. Effect of ribonucleotides on RNAP-promoter complexes. The *Eco*RI–*Xba*I fragments containing the *malT* promoter derivatives were incubated with or without CRP (50 nM) in the presence of cAMP (50 μM) and then with RNAP. The mixtures were incubated with the indicated NTPs (1 mM each) for 15 min before treatment with heparin. PC, NC, and IC represent the productive, nonproductive, and initiated complexes, respectively.

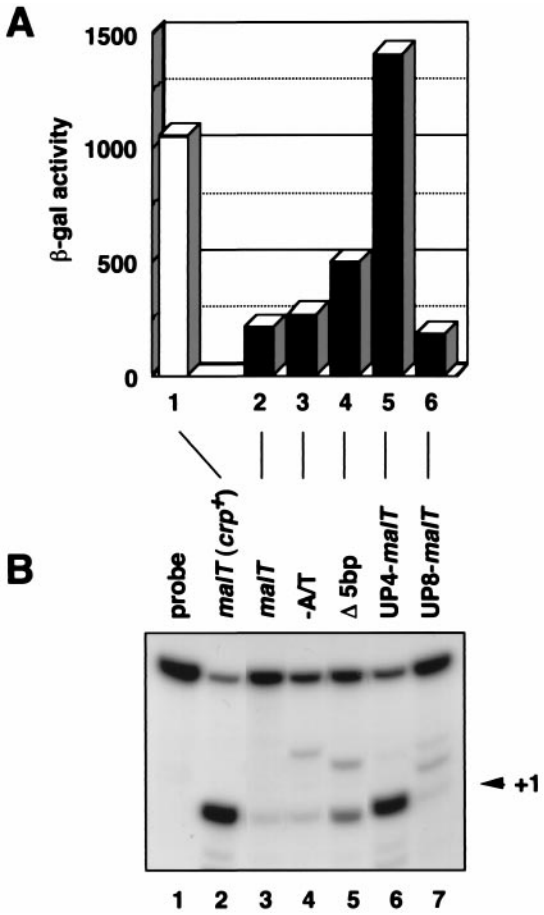

FIG. 6. Effect of the location of the UP element on promoter activity *in vivo*. (*A*) β-Galactosidase activity of the *malT-lacZ* fusions. The β-galactosidase activity of KI70 (Δ*lac*) (lane 1) and OK6201 (Δ*lac crp*$^-$) (lanes 2–6) cells harboring the *malT–lacZ* fusion plasmids were measured. Each bar is expressed in Miller units (13). The value is an average obtained from three independent experiments. (*B*) S1 assay. Total RNAs (100 μg) prepared from KI70 (Δ*lac*) (lane 2) and OK6201 (Δ*lac crp*$^-$) (lanes 3–7) cells harboring the indicated plasmids were subjected to S1 nuclease assay.

and/or with RNAP to form the productive or nonproductive complexes. After the addition of heparin, $KMnO_4$ modification was carried out. Permanganate preferentially modifies T residues in single-stranded regions of DNA (18). As shown in Fig. 4*A*, melting around the transcription start site was detected in both the productive and nonproductive complexes. The two functionally different complexes produced essentially the same melting pattern, although there was a slight difference in band intensity. The same melting pattern was also observed with the UP4-*malT* (Fig. 4*B*) and UP8-*malT* (Fig. 4*C*) promoters.

Effect of Ribonucleotides on Transcription Complexes. We next characterized the effects of ribonucleotides on wild-type *malT* open complexes with EMSA. The addition of the initiating nucleotides (ATP and UTP) should allow a productive complex to proceed to +8. In the presence of CRP, most of these initiated complexes had a mobility greater than that of the complexes formed in the absence of NTPs (Fig. 5, lanes 4 and 5). When all 4 NTPs were added in the presence of CRP, the amount of heparin-resistant complex was dramatically reduced, indicating that most of the RNAP ran off the template (lane 6). On the other hand, the addition of NTPs had essentially no effect on the complex formed in the absence of CRP (lanes 2 and 3). Similar experiments were done with complexes formed by the UP4-*malT* and UP8-*malT* promoters. Again a productive UP4-*malT* complex (lane 7) was converted to an initiated complex by the addition of ATP and UTP (lane 8), whereas a nonproductive UP8-*malT* complex (lane 9) remained unaffected (lanes 10).

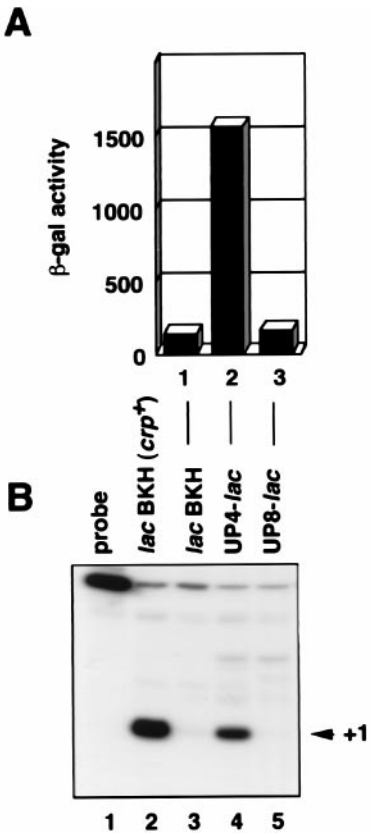

FIG. 7. Characterization of UP-*lac* hybrids promoters *in vivo*. (*A*) Effect of the location of UP elements on promoter activity. The β-galactosidase activity of OK6201 cells harboring the indicated promoter-*lacZ* fusion plasmids were determined. Bars are expressed in Miller units (13). The value is an average obtained from two independent experiments. (*B*) Analysis of the transcription start site. Total RNAs (100 μg) prepared from KI70 (Δ*lac*) (lane 2) and OK6201 (Δ*lac crp*$^-$) (lanes 3–5) cells harboring the indicated plasmids were subjected to S1 nuclease assay.

Effect of UP Element Location on *malT* Transcription *in Vivo*. To study the effect of an UP element on transcription from the *malT* promoter *in vivo*, β-galactosidase activities were measured in Δ*lac crp*$^-$ cells carrying the promoter-*lacZ* fusion plasmids (Fig. 6*A*). The β-galactosidase activity in Δ*lac crp*$^+$ cells carrying the *malT* promoter-*lacZ* fusion (pMT201) was about 5-fold higher than that in *crp*$^-$ cells (lanes 1 and 2). Elimination of the *malT* A+T-rich sequences caused little change in transcription in the absence of activation by CRP (compare lanes 2 and 3). On the other hand, the 5-bp deletion between the *malT* A+T-rich sequences and the −35 region increased basal transcription 2.5-fold (lanes 2 and 4). Furthermore, the *rrnB* P1 UP element at its normal position with respect to the −35 element markedly stimulated the *malT* core promoter (UP4-*malT*; lane 5), but not when fused 4 bp further upstream (UP8-*malT*; lane 6). Quantitative S1 nuclease assays confirmed that differences in β-galactosidase activities reflected differences in promoter strength (Fig. 6*B*).

Effect of an UP Element on the *lac* Core Promoter. To examine the effect of an UP element on the *lac* core promoter, *rrnB* P1 UP element-*lac* hybrid promoters were constructed, the promoters were fused to *lacZ* (Fig. 1), and promoter activities were assessed by measuring the β-galactosidase activity of the Δ*lac* cells harboring the fusion plasmids (Fig. 7*A*). The UP element markedly enhanced *lac* core promoter activity when placed 4 bp upstream of the −35 region as reported (4), whereas it had no effect when placed 8 bp upstream. S1 assays confirmed that the UP element affected transcription from *lac* P1 in the UP4-*lac* promoter construct (Fig. 7*B*).

We also used EMSA to test whether the *rrnB* P1 UP element could stimulate the formation of a heparin-resistant complex at the transcriptionally inactive UP8-*lac* promoter. As shown in Fig. 8*A*, the formation of heparin-resistant complexes was significantly increased at both the UP4-*lac* and UP8-*lac* promoters compared with the wild-type *lac* promoter. The heparin-resistant complex formed with the wild-type *lac* promoter in the absence of CRP-cAMP was at *lac* P2 (19, 20). The −35 region of P2 no longer existed in UP4-*lac* and UP8-*lac* promoters, suggesting that RNAP occupied P1 in these hybrid promoters. This was confirmed by DNase I footprinting (data not shown). In addition, permanganate footprinting revealed that a region around the P1 start point was melted in both hybrid promoter complexes, although DNA melting at the UP8-*lac* promoter was rather weak (Fig. 8*B*). We also performed abortive initiation assays on the UP-*lac* hybrid promoters complexes and the initiating nucleotides ATP and UTP. As shown in Fig. 8*C*, the pppApApUpU *lac* P1 product (19) was clearly produced from both promoters. Thus, the heparin-resistant complex formed at the UP8-*lac* promoter had the ability to produce abortive RNAs, but not run-off transcripts. We conclude from these results that the UP element increases RNAP complex formation at *lac* P1; whether these complexes are productive or nonproductive, however, depends on the location of the UP element, as in the case of the *malT* promoter.

DISCUSSION

The *rrnB* P1 UP element was identified as a third RNAP recognition element because it increased promoter strength both *in vivo* and *in vitro* (5). A major finding in the present study is that UP elements can lead to the formation of transcriptionally inactive open complexes when placed a half-turn upstream of the position where they stimulate transcription. In other words, the location of an UP element with respect to the core promoter is crucial in determining the nature of transcription complexes. The helical phase-dependent effect of the UP element on promoter function is analogous to that of upstream A-tracts (21–23) and CRP (24, 25).

It has been proposed that a UP element can contain two separable sections that can function independently and may be recognized by two flexible αCTD monomers (4, 10, 26). We observed that the *rrnB* P1 UP element could stimulate *malT* transcription when separated 14 bp from the −35 hexamer, although the level of activation was reduced compared with the UP4-*malT* promoter (data not shown). It is known that insertion of 5 bp at −46 or deletion of 3 bp from −38 to −40 results in loss of UP element function at the *rrnB* P1 promoter

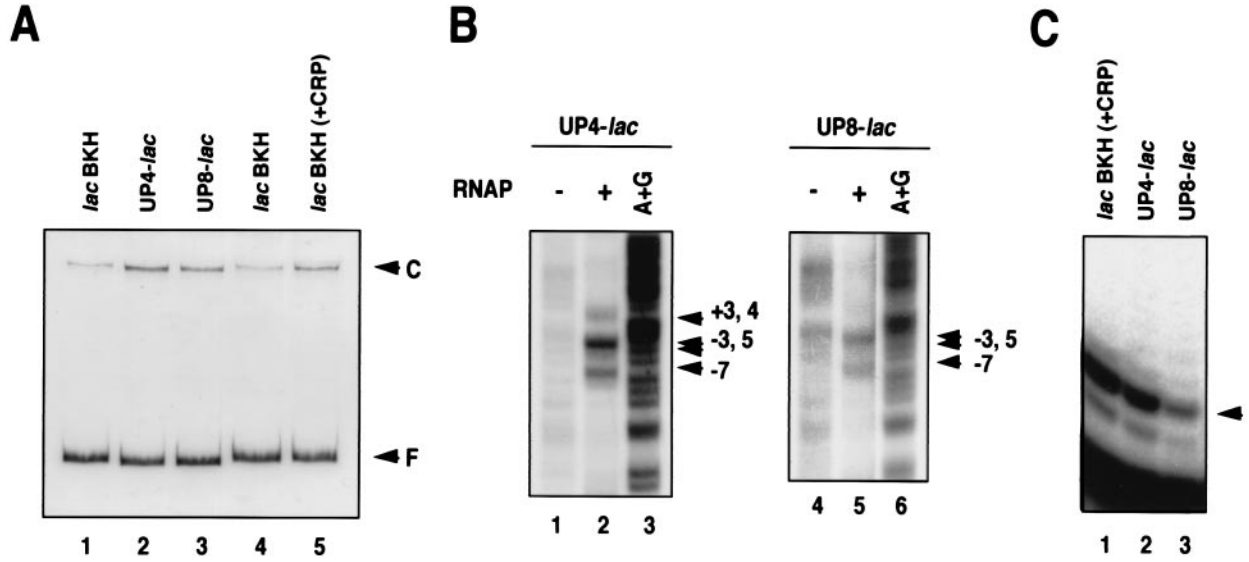

FIG. 8. Characterization of UP-*lac* hybrid promoters *in vitro*. (*A*) EMSA. The indicated *lac* promoter derivatives were incubated with RNAP. Lane 5 shows the CRP-dependent P1 complex of the wild-type *lac* promoter formed in the presence of CRP and cAMP. Lanes 1 and 4 correspond to the P2 complex of the wild-type *lac* promoter. (*B*) Potassium permanganate footprinting of UP4- and UP8-*lac* promoters. The arrowheads indicate the permanganate-sensitive sites. The numbers represent positions relative to the transcription start site of P1. (*C*) Abortive initiation assay. The products of the abortive initiation reaction were analyzed by electrophoresis on a 20% polyacrylamide gel containing 8 M urea. Lane 1 represents the products derived from the wild-type *lac* promoter in the presence of CRP and cAMP. The arrowhead indicates the major product, which presumably corresponds to the pppApApUpU product synthesized at the *lac* P1 promoter.

(27). It would be interesting to test whether RNAP forms nonproductive complexes at these mutant *rrnB* P1 promoters.

It was reported previously that RNAP could form two open complexes ($open_{upper}$ and $open_{lower}$), differing in electrophoretic mobility and in ability to escape abortive cycling at the *lacUV5* promoter, with the $open_{upper}$ being more proficient in escape (28, 29). The relative amounts of the two complexes varied with temperature, the $open_{lower}$ being favored at 16°C, and the major $open_{upper}$ at 37°C. In contrast, no temperature dependence of the ratios of the two complexes was observed in our system (data not shown).

How does an UP element lead to the formation of nonproductive complexes? It is known that upstream A-tracts, which were shown recently (30) to interact with α subunits, can inhibit transcription initiation at synthetic promoters (31). More recently, it was reported that UP elements can inhibit promoter clearance (32). The inhibition of promoter clearance by a transcription factor through overstabilization of RNAP binding has also been reported (33, 34). The RNAP–promoter interaction, however, is not enhanced in the nonproductive complexes at the *malT* promoter compared with the productive complexes (unpublished data). We prefer a model where the α subunit–UP interaction leads to a transcription complex with an inactive conformation. A similar model was proposed for the repression of the *gal* P1 promoter by GalR or LacI binding to an upstream operator (35). In addition, it is interesting to note that RNAP carrying a specific mutation in the β subunit forms a stable inactive open complex at a normal promoter (36).

What is the functional significance of the *malT* UP element and the nonproductive complex in the regulation of *malT* expression? We showed previously that the role of CRP in *malT* transcription is to lead to the formation of a productive open complex (12). We found that the *malT* nonproductive complex is converted to a productive complex by CRP and that CRP-dependent activation is markedly reduced if the promoter lacks its UP element (unpublished results). In other words, the major effect of CRP is not on the recruitment of RNAP to the promoter. One interpretation of our results is that the *malT* UP element recruits RNAP to the promoter, and that CRP predominantly acts at a post-recruitment step to make the complex productive. We cannot exclude the possibility, however, that CRP also helps the UP element recruit RNAP. The nonproductive complex could be a simple intermediate in the formation of the productive complex; or it could be the product of a branched pathway at an early stage in transcription initiation, a complex that must be disrupted before a productive complex can be formed (37).

One of the major roles of CRP is apparently to increase initial binding of RNAP through CRP–α subunit interaction at promoters where the CRP binding site is located, −61.5 (7, 38). We speculate that recruitment of RNAP by CRP may be insufficient to fully activate a promoter when the CRP site is located farther upstream (e.g., at −70.5, the site of *malT*). In this case, an UP element may be needed to facilitate recruitment. Further studies are needed to understand how CRP and UP elements cooperate to activate transcription.

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