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41. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.
42. We thank R. Rutkowski and M. Burgess for peptide synthesis, B. Santarsiero of Molecular Structures Corporation for collecting high resolution data from the p-LI crystals, and C. Carr, A. Cochran, D. Lockhart, Z. Y. Peng, B. Schulman, J. Staley, B. Tidor, and J. Weissman for discussions and critical reading of the manuscript. P.S.K. is a Pew Scholar in the Biomedical Sciences. This research was supported by grants from the National Institutes of Health (GM44162 to P.S.K. and GM48958 to T.A.) and by grants from the Lucille P. Markey Charitable Trust and the American Cancer Society (T.A.).

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A Third Recognition Element in Bacterial Promoters: DNA Binding by the α Subunit of RNA Polymerase

Wilma Ross, Khoosheh K. Gosink, Julia Salomon, Kazuhiko Igarashi, Chao Zou, Akira Ishihama, Konstantin Severinov, Richard L. Gourse*

A DNA sequence rich in (A + T), located upstream of the −10, −35 region of the *Escherichia coli* ribosomal RNA promoter *rrnB* P1 and called the UP element, stimulates transcription by a factor of 30 in vivo, as well as in vitro in the absence of protein factors other than RNA polymerase (RNAP). When fused to other promoters, such as *lacUV5*, the UP element also stimulates transcription, indicating that it is a separable promoter module. Mutations in the carboxyl-terminal region of the α subunit of RNAP prevent stimulation of these promoters by the UP element although the mutant enzymes are effective in transcribing the "core" promoters (those lacking the UP element). Protection of UP element DNA by the mutant RNAPs is severely reduced in footprinting experiments, suggesting that the selective decrease in transcription might result from defective interactions between α and the UP element. Purified α binds specifically to the UP element, confirming that α acts directly in promoter recognition. Transcription of three other promoters was also reduced by the COOH-terminal α mutations. These results suggest that UP elements comprise a third promoter recognition region (in addition to the −10, −35 recognition hexamers, which interact with the σ subunit) and may account for the presence of (A + T)–rich DNA upstream of many prokaryotic promoters. Since the same α mutations also block activation by some transcription factors, mechanisms of promoter stimulation by upstream DNA elements and positive control by certain transcription factors may be related.

The strength of promoters recognized by $E\sigma^{70}$, the most abundant of the *E. coli* RNAP holoenzymes, can be correlated to a considerable extent with their similarity to consensus recognition hexamers in the core promoter region, centered approximately 10 and 35 bp upstream of the start site of transcription, and the spacing between these hexamers (*1*). Nevertheless, it has been proposed that sequences outside of the core promoter region can modulate promoter activity (*2*). Upstream sequences have been shown to increase the activities of several *Escherichia coli* or *Bacillus subtilis* promoters in vitro in the absence of protein factors other than RNAP (*3*–*9*), and regions rich in (A + T) have been noted upstream of many promoters (*2*, *10*, *11*).

The *rrnB* P1 promoter is representative of a class of seven rRNA promoters in *E. coli* that together account for more than half of the transcription in the cell at high growth rates (*12*). Although the *rrnB* P1 core promoter is sufficient for specific initiation and for response to the two systems known to regulate transcription under different nutritional conditions—namely, growth rate–dependent control and stringent control (*13*, *14*)—the region upstream of the core promoter is largely responsible for its high activity (*4*, *9*, *13*, *15*, *16*). A 20–base pair (bp) region rich in (A + T), to which we refer as the upstream (UP) element, is located immediately upstream of the core promoter (Fig. 1). The UP element increases *rrnB* P1 activity by a factor of at least 30 in the absence of protein factors other than RNAP (*9*, *17*). The UP element is protected by RNAP in footprinting experiments, and replacement of the UP element with non-*rrnB* DNA results in severe reduction of protection in the upstream region (*8*, *18*, *19*). Therefore, the core and UP element together can be considered an extended promoter (Fig. 1) (*9*). The region adjacent to the UP element (between bp −60 and bp −150) contains binding sites for the activator protein Fis, which results in increasing the activity of the promoter by a factor of 10 (*8*, *16*, *20*, *21*). Fis is not required for stimulation of transcription by the UP element (*9*).

The σ^{70} subunit of RNAP holoenzyme ($\alpha_2\beta\beta'\sigma$) interacts with the −10 and −35 hexamers (*22*). However, the region or regions of RNAP required for UP element recognition have not been defined. On the basis of studies with mutant derivatives of the α subunit, it has been proposed that α interacts directly with certain transcription factors, leading to stimulation of promoter activity (*23*–*26*). We therefore used mutants of α to investigate the role of this subunit in UP element function although transcription activation in this case is achieved by a DNA element rather than by a trans-acting protein. Two mutant forms of the 329 amino acid α subunit, COOH-terminal truncations of 73 or 94 amino acids (α-256 or α-235, respectively), are stable in vivo and assemble into holoenzyme (*27*). Furthermore, reconstituted α-235 or α-256 core enzymes ($\alpha_2\beta\beta'$), prepared in vitro from purified subunits,

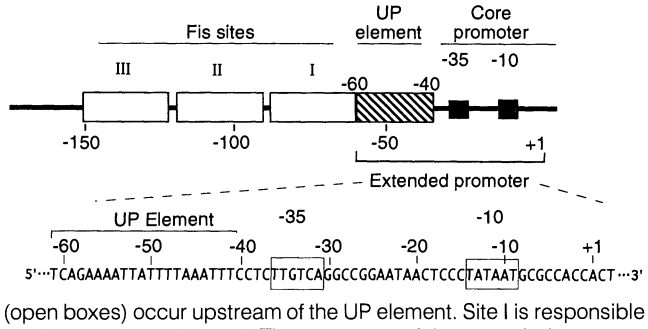

Fig. 1. The *rrnB* P1 promoter. The extended promoter region includes elements recognized by RNA polymerase: the core promoter, which consists of −10 and −35 consensus hexamers (filled boxes), and the UP element, which consists of bp −40 to −60 (shaded box) (*9*). Three binding sites for the transcriptional activator protein Fis (open boxes) occur upstream of the UP element. Site I is responsible for most of the activation by Fis at this promoter (*20*, *21*). The sequence of the extended promoter is indicated below the diagram.

W. Ross, K. K. Gosink, J. Salomon, and R. L. Gourse are in the Department of Bacteriology, University of Wisconsin–Madison, 1550 Linden Drive, Madison, WI 53706. K. Igarashi, C. Zou, and A. Ishihama are in the Department of Molecular Genetics, National Institute of Genetics, Mishima, Shizuoka 411, Japan. K. Severinov is at the Public Health Research Institute, 455 First Avenue, New York, NY 10016.

*To whom correspondence should be addressed.

retain wild-type catalytic activity (*23*) and, although they are defective in response to some transcription factors, the reconstituted mutant holoenzymes can initiate transcription at many promoters (*23, 24, 26, 28*). Strains containing these deletions are not viable in the absence of a wild-type α gene (*rpoA*), however, implying loss of an essential function (*27*).

Loss of UP element function in vitro and in vivo with α-mutant RNAPs. The effect of mutations in the COOH-terminal region of the α subunit on UP element–dependent stimulation of *rrnB* P1 transcription was tested in vitro with RNAPs reconstituted from purified subunits (*23, 29*). An *rrnB* P1 promoter, either containing or lacking the UP element, was inserted upstream of a transcription termination signal on supercoiled plasmids. The transcripts from these promoters were examined by denaturing gel electrophoresis (Fig. 2). With wild-type RNAP, the promoter containing the UP element (−88) was much more efficient than either of two promoters in which other sequences were substituted in place of the UP element (SUB or −41) (Fig. 2A, lanes 1 to 6) (*30*). However, with the mutant RNAPs containing α subunits truncated at the COOH-terminus (α-235 and α-256), expression from the promoter containing the UP element was the same as from promoters lacking the UP element: the UP element did not increase transcription (Fig. 2B, lanes 1 to 6) (*31*). The relatively inefficient transcription with the mutant RNAPs was similar to that of the *rrnB* P1 promoters lacking the UP element when transcribed with wild-type RNAP (Fig. 2, A and B).

An RNAP holoenzyme containing mutant α subunits with a single amino acid substitution, R265C (where Arg^{265} is replaced by Cys), was also tested. This α mutant is of particular interest because (i) it is defective in activation of transcription mediated by catabolite gene activator protein (CAP) (*32*) and oxidative stress regulator protein (OxyR) (*33*); and (ii) the Arg^{265} is ADP-ribosylated by bacteriophage T4, an event presumably involved in the shutoff of host RNA synthesis during phage infection (*34*). An RNAP reconstituted with α–R265C subunits was also unable to use the *rrnB* P1 UP element: The −88 and SUB promoters were transcribed with the same relatively low efficiency (Fig. 2C, lanes 7 to 10). Potential inhibitors in the mutant RNAP preparations cannot account for the reduced transcription of templates containing the UP element, because R265C RNAP did not prevent use of the UP element by the wild-type RNAP when both enzymes were present together (Fig. 2C, lanes 1 and 2).

Two other prominent transcripts encoded by the plasmid vector were generated in these experiments: RNA-I, a 108-nucleotide (nt) RNA from the plasmid origin of replication (*35*), and a set of larger transcripts (Fig. 2, A and B, lanes 9 and 10; Fig. 2C). RNA-I was transcribed efficiently by each of the mutant RNAPs. However, the larger transcripts were very inefficiently produced by the mutant enzymes and are discussed below.

The UP element is a separable promoter module that increases the activity of the *lac* core promoter in an *rrnB-lac* hybrid construct (*9*), and of the *lacUV5* core sequence in a similar hybrid promoter (Fig. 3, lanes 1 to 4) (*29*). Utilization of the UP element in the *rrnB-lacUV5* hybrid promoter depended on the COOH-terminus of α, since transcription of the promoters containing (*rrnB-lacUV5*) or lacking the UP element (*lacUV5*) was the same with each of the three α-mutant RNAPs (Fig. 3, lanes 5 to 8) (*31*). Thus, the selective defect of the mutant RNAPs in utilization of the UP element did not depend on the association of the UP element with a particular core promoter sequence.

The failure of RNAPs with mutant α subunits to utilize the UP element of the *rrnB* P1 promoter in vitro (Figs. 2 and 3) suggested that the mutant *rpoA* alleles should affect expression from promoters with UP elements in vivo. RNAP containing mutant α subunits, which were ex-

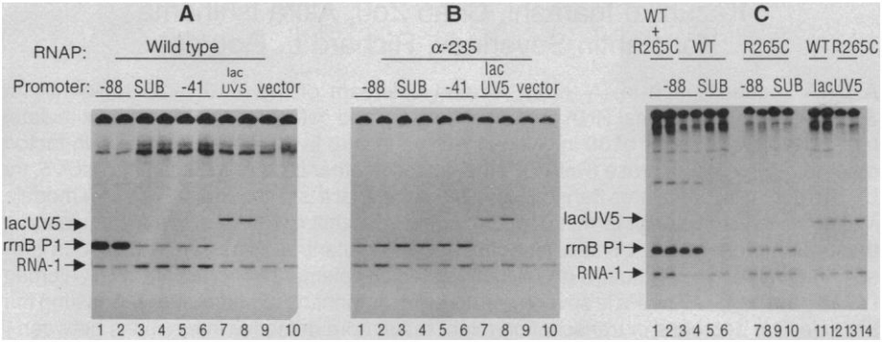

Fig. 2. In vitro transcription of *rrnB* P1 derivatives or of *lacUV5* with wild-type RNAP (**A**) or with RNAPs containing mutant α subunits, α-235 (**B**) or R265C (**C**). RNAPs, reconstituted from purified subunits (*23, 32*) and promoters carried on supercoiled plasmid DNA templates, were as indicated above each lane. Transcription reactions and denaturing gel electrophoresis were as described (*20, 29*). No Fis was present; promoters were −88: −88 to +1 *rrnB* P1, containing the UP element (pRLG862) (*20*); SUB: −88 to +1 *rrnB* P1 with the non-*rrnB* sequence 5′-GACTGCAGTGG-TACCTAGG-3′ substituted for the UP element sequence from −59 to −41 (pLR14) (*9*); −41: −41 to +1 *rrnB* P1, lacking the UP element, with vector sequence upstream of −41 (pWR55) (*29*); *lacUV5*, −60 to +40 (pRLG593) (*20*). Vector lanes contained pRLG770 with no promoter insert (*20*). Transcripts from *rrnB* P1 (~170 nt), or from *lacUV5* (~210 nt) (which end at the *rrnB* T1 terminator in the vector), and the vector-derived RNA-I transcript (*35*), are indicated with arrows. Concentrations of mutant and wild-type RNAPs (*29*), chosen to give similar amounts of *lacUV5* transcription (*49*), were (**A**) 2.6 nM wild-type RNAP; (**B**) 5.3 nM α-235 RNAP; (**C**) 0.8 nM wild-type or 0.8 nM R265C RNAP. The UP element did not stimulate transcription by the mutant RNAPs at any RNAP concentration tested (*29*).

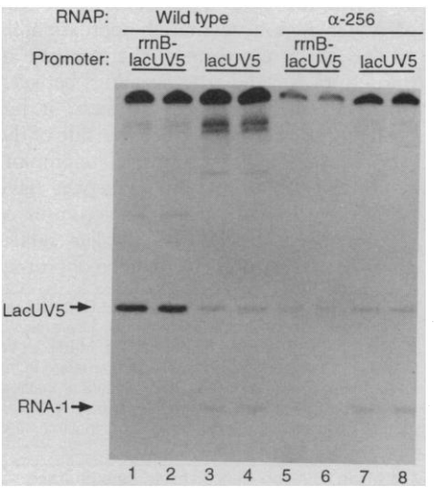

Fig. 3. In vitro transcription of an *rrnB* P1-*lacUV5* hybrid promoter or of the *lacUV5* promoter with reconstituted wild-type or α-256 RNAPs. The hybrid promoter consists of *rrnB* P1 upstream sequence (−88 to −37, containing the UP element) and *lacUV5* core region sequence (−36 to +40) (*29*). The *lacUV5* promoter, −60 to +40 (*20*), has its naturally occurring upstream sequences. The promoter and RNAP are indicated above each lane. Transcripts from the hybrid, *lacUV5* or RNA-I promoters are indicated with arrows (see Fig. 2). Transcription and gel electrophoresis conditions were as described (*29*). No Fis was present. Wild-type and α-256 RNAPs were used at 1.3 nM and 5.4 nM, respectively (*49*). The UP element did not stimulate hybrid promoter transcription at any mutant RNAP concentration tested (*29*).

pressed from plasmid encoded *rpoA* alleles and shown previously to assemble into holoenzyme in vivo (*27, 32*), were predicted to interfere in vivo with the function of RNAP containing wild-type α subunits (expressed from the chromosome) in the expression of an *rrnB* P1 promoter containing the UP element. The *rrnB* P1 promoter activity was monitored by measuring β-galactosidase activity from chromosomal *rrnB* P1 promoter-*lacZ* fusions (Table 1). The activity of the promoter containing the UP element (−61) was four to five times lower with each of the mutant alleles (Table 1, column 3). In contrast, the activity of the *rrnB* P1 promoter derivative lacking the UP element (−41) was not reduced, and in fact was approximately two times higher (Table 1, column 4) (*36*), indicating that production of the mutant subunits did not exert a nonspecific inhibitory effect on transcription.

The effect of the UP element on *rrnB* P1 promoter function in vivo is defined as the ratio of the activities of *rrnB* P1 promoters containing or lacking the element. In control strains containing wild-type *rpoA* alleles on both the plasmid and the chromosome, this ratio was 36 (Table 1, column 5), which is consistent with previous results (*9*). Expression of the mutant *rpoA* alleles reduced this ratio to about 4 (*36*). Thus, the COOH-terminal region of the α subunit was essential for the function of the UP element in transcription in vivo as well as in vitro.

Table 1. Effect of mutant *rpoA* alleles on *rrnB* P1 UP element function in vivo. β-galactosidase activities were determined (*20*) for *E. coli* strains lysogenic for lambda phage carrying *rrnB* P1 promoter–*lacZ* fusions [RLG957, −61 promoter, containing the UP element; or RLG2263, −41 promoter, lacking the UP element (*9*)], and carrying mutant or wild-type *rpoA* alleles expressed from the inducible *tac* promoter on multicopy plasmids (*27, 32*) in addition to the wild-type chromosomal *rpoA* gene. Strains were grown for approximately four generations in LB with ampicillin at 100 μg/ml and 0.25 mM isopropyl-β-D-galactoside (IPTG). The β-galactosidase activities of washed cells, expressed in Miller units (*56*), were corrected for a background activity observed with a *lacZ* construct that did not have a promoter [RLG1336; (*20*)] determined in the presence of each plasmid, and are the average of two determinations. The reduction in activity of the −61 promoter, and in the −61/−41 promoter activity ratios in the presence of the mutant α alleles varied by less than 10 percent in the two experiments. The *rpoA* plasmids were pLAW2, wild-type *rpoA* (*32*); pLAMC9, *rpoA*129 encoding the R265C α mutation, (*32*); pLAX185, wild-type *rpoA* (*27*); pLAD235 encoding the α-235 protein (*27*); and pLAD256, encoding the α-256 protein (*27*).

Plasmid	*rpoA* allele	Promoter activity		Ratio −61/−41
		rrnB −61	*rrnB* −41	
pLAW2	Wild type	799	22	36.3
pLAMC9	R265C	184	55	3.3
pLAX185	Wild type	946	26	36.4
pLAD256	Δ-256	269	64	4.2
pLAD235	Δ-235	295	61	4.8

Direct interactions between the UP element and the α-subunit of RNAP. We used footprinting experiments to confirm whether, as predicted from the transcription experiments (Figs. 2 and 3; Table 1), the mutant RNAPs were altered in their ability to interact with UP element DNA (Fig. 4) (*37*). Both the wild-type and the mutant RNAPs protected the core promoter region (−35 region to +20), but the two enzymes differed in their ability to protect the UP element. Cleavage of (A + T)–rich DNA by deoxyribonuclease I (DNase I) is relatively inefficient (*38*), but all DNase I accessible positions on each strand in the UP element region were protected by wild-type RNAP (*8, 18, 19*) (Fig. 4, A and B; Fig. 5B). In contrast, there was no evidence of protection in the UP element region by the mutant RNAPs (Fig. 4, A and B) (*39*). Hydroxyl radical footprinting experiments also indicated that the pattern of protection in the −40 to −60 region was altered by the mutant RNAPs; on both strands (Fig. 4, D and E), wild-type RNAP protected a 4- to 5-bp interval in the −50 to −55 region. However, the mutant RNAPs did not protect this region at all on the top strand, and on the bottom strand protection was significantly weaker than with the wild-type RNAP (*39*).

In contrast with the results obtained with the *rrnB* P1 promoter containing the UP element, DNase I footprints of an *rrnB* P1 core promoter derivative lacking the UP element (−41 to +1) showed little protection by either wild-type or mutant RNAP in the −40 to −60 region (Fig. 4C). Similarly, little protection was observed in the −40 to −60 region of the *lacUV5* promoter (*31, 40*). The subtle differences between the wild-type and mutant RNAP

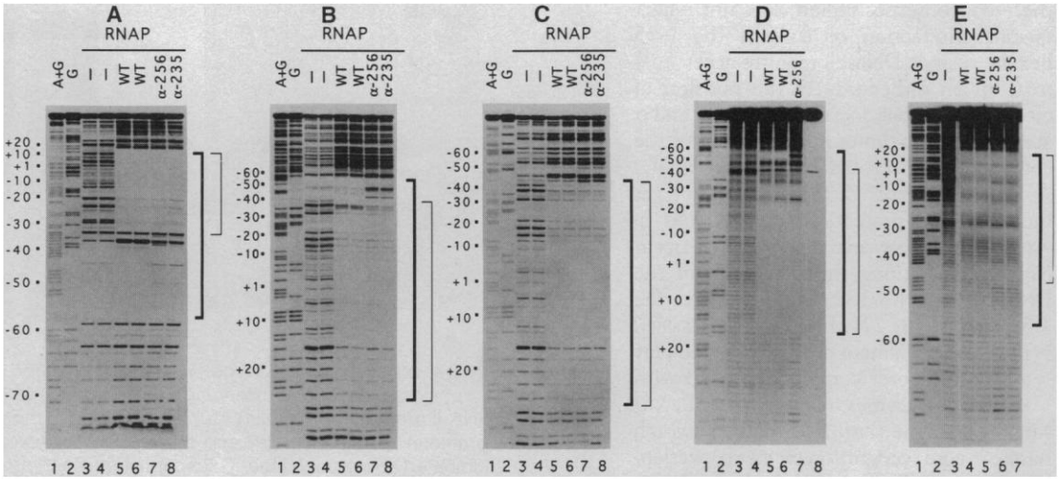

Fig. 4. DNase I (**A, B**, and **C**) or hydroxyl radical (**D** and **E**) footprints of wild-type or α-mutant RNAP complexes formed with *rrnB* P1 promoters containing (A, B, D, E) or lacking (C) the UP element. Areas protected by RNAP are marked with thick brackets (wild-type RNAP), or thin brackets (α-235 or α-256 RNAPs). The *rrnB* P1 promoters and strand being probed were (A) −88 to +50, bottom strand; (B) −88 to +1, top strand; (C) −41 to +1, top strand; (D) −88 to +50, top strand; (E) −88 to +50, bottom strand (*37*). Lanes 1 and 2 in (A to E) contain (A + G) and G sequence markers (*18*). The presence of wild-type (WT) RNAP, reconstituted α-mutant RNAP (α-235 or α-256), or no RNAP (−) is indicated. Wild-type RNAP was either native (A to D, lanes 5; E, lane 4) or reconstituted (A to D, lanes 6; E, lane 5). Promoter fragment preparation, RNAP-promoter complex formation, and cleavage with DNase I or hydroxyl radical are described (*37*). In (D), lane 8, the DNA fragment was untreated to reveal background nicking.

footprints in the −40 to −60 region of these two promoters (Fig. 4C) (*31, 40*) may reflect weak interactions of RNAP with DNA upstream of the −35 region in promoters lacking specific UP element sequences (*39*).

The transcription and footprinting results with RNA polymerases containing COOH-terminal deletions in the α subunit suggested that α might interact directly with the UP element. We found that purified α specifically protected a 25-bp segment containing the UP element of the *rrnB* P1 promoter (from −60 to approximately −35 on each strand) in DNase I footprinting experiments (Fig. 5, A and B) (*41*). The α subunit did not protect the core promoter region or an *rrnB* P1 promoter fragment lacking the UP element. The footprint with purified α extended slightly further upstream than that with RNAP holoenzyme (Fig. 5B), perhaps reflecting conformational differences in the α subunit when it is present in holoenzyme. We conclude that α is a site-specific DNA binding protein, and that the requirement for the COOH-terminal region of α in utilization of the UP element reflects direct interactions between α and upstream DNA.

The molecular details of the site-specific interaction between α and the UP element are not yet known. The Arg^{265} residue (Fig. 2C) and several others in the COOH-terminal region of α are required for UP element utilization, but these residues need not necessarily define sites of interaction with the DNA (*42*). Similarly, whether the base sequence in the UP element defines a backbone structure that is recognized by α, or whether there are direct contacts with the bases themselves, or both, remains to be determined.

The close proximity of the α footprint to the −35 consensus region does not reflect specific interaction of α with the −35 hexamer, since DNase I requires access to 4 to 6 bp on either side of the position of cleavage (*38*), but does suggest that α and σ are in close proximity in this region of the complex. Hydroxyl radical footprint analysis of RNAP–*rrnB* P1 promoter complexes indicates that protections in the −40 to −60 region, now attributable to α, occur close to and along the same face of the DNA helix as those attributable to σ in the −35 region (*18, 22*). Interactions between α and the UP element are also likely to play a role in promoter complexes formed with RNAP holoenzymes containing other σ subunits. At the *rrnB* P1 promoter (which has $E\sigma^{32}$ core recognition elements overlapping those for $E\sigma^{70}$), the UP element activates transcription by and is protected by $E\sigma^{32}$ (*43*). Furthermore, $E\sigma^{54}$ holoenzyme lacking the COOH-terminal region of α (α-256) has an altered interaction with the upstream region of the *glnA* promoter (*44*).

DNA elements requiring the COOH-terminus of α in other *E. coli* promoters. Upstream sequences have been shown to increase transcription from several other *E. coli* or *B. subtilis* promoters in the absence of factors other than purified RNAP (*3, 5–7*). In addition, footprint protection of some *E. coli* promoters by

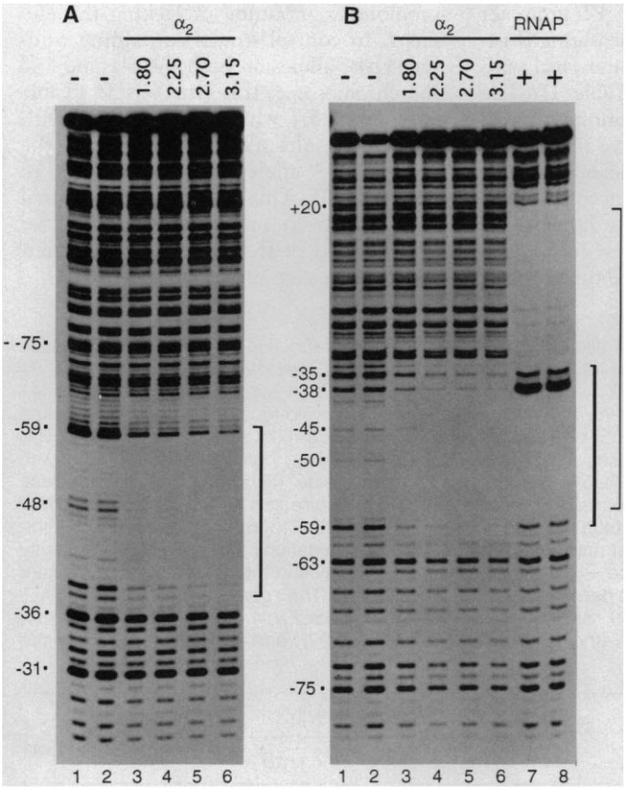

Fig. 5. DNase I footprints of α-*rrnB* P1 promoter complexes. Complexes were formed with promoter fragments ^{32}P labeled in either the top strand (**A**) or the bottom strand (**B**) and indicated amounts of purified α dimers (1.8 to 3.15 μg protein = 1.5 to 2.6 μM α dimer; lanes 3 to 6) or RNAP holoenzyme (10 nM; B, lanes 7 and 8), and were digested with DNase I (*37, 41*). Lanes 1 and 2 contain DNA fragment digested in the absence of added α dimer or RNAP. Protection of the UP element region by α is indicated by a thick bracket in each panel, and protection by RNAP holoenzyme is indicated by a thin bracket (**B**). DNA fragments resulting from DNase I cleavage at the indicated sequence positions were determined by comparison with sequence markers.

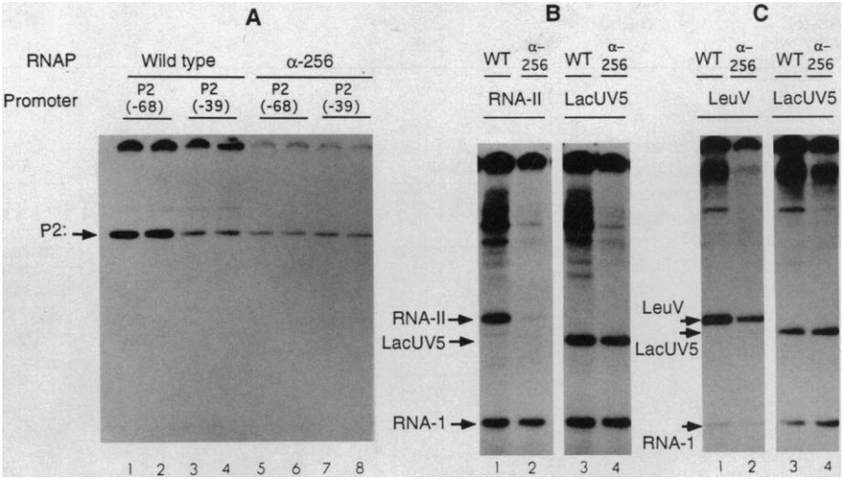

Fig. 6. In vitro transcription with wild-type (WT) RNAP or α-256 RNAP of (**A**) *rrnB* P2 promoters containing P2 upstream sequence extending to −68 (pWR5) (*29*) or to −39 (pRLG932) (*29*); (**B**) the cloned RNA-II promoter (pRLG934) (*29*) and the *lacUV5* promoter (pRLG593) (*20*); or (**C**) the *leuV* tRNA promoter (pRLG927) (*29*) and the *lacUV5* promoter. Promoters and WT or mutant RNAPs are indicated above each lane. Transcripts from each promoter are indicated with arrows. Conditions of transcription and gel electrophoresis were as described (*20, 29*). Reconstituted RNAP concentrations were: 0.9 nM wild type or 3.6 nM α-256 in (A); 2.6 nM wild type or 8.7 nM α-256 in (B); 2.6 nM wild type or 10.9 nM α-256 in (C). Experiments at other RNAP concentrations gave similar results (*29*).

wild-type RNAP extends further upstream than the core promoter region (*45*). These observations suggest that UP-like elements that interact with the α subunit might play a role in transcription from other promoters. We present evidence for such elements in three other *E. coli* promoters: *rrnB* P2, *leuV* and RNA II.

In the *rrnB* P2 promoter, the region upstream of the −35 hexamer significantly increases promoter activity in vivo (*46*). An *rrnB* P2 promoter extending to −68 was more efficiently expressed by wild-type RNAP in vitro than was an *rrnB* P2 promoter extending only to −39 (Fig. 6A). This increased efficiency was not observed with the RNAPs containing the mutant α subunits α-235, α-256, or R265C (Fig. 6A) (*31*); both the −68 and the −39 promoters were transcribed with the same low efficiency. Thus, the efficiency of *rrnB* P2 is due at least in part to a factor-independent effect of upstream DNA (an UP element) whose function requires the COOH-terminus of α.

The yield of a large plasmid vector-derived transcript was severely reduced relative to that of other promoters (*lacUV5* and RNA-I) in transcription reactions with the mutant RNAPs (Fig. 2, A and B). The heterogeneity and RNase H sensitivity of this transcript suggested that it might be the RNA-II transcript from the plasmid origin of replication (*31, 47*). Therefore, the RNA-II promoter was inserted into the same vector as used above (*29*), and transcribed with the wild-type and mutant RNAPs. Transcription from the RNA-II promoter was dependent on the COOH-terminus of α (Fig. 6B) (*31*). Similarly, the *leuV* tRNA (transfer RNA) promoter (*48*), was less efficiently transcribed with the mutant than with the wild-type RNAPs although the effect of the α mutations on *leuV* was not as great as on the *rrnB* P1 or RNA-II promoters (Fig. 6C).

The specific regions of the RNA-II and *leuV* promoters that are responsible for their dependence on the COOH-terminus of α have not been defined although by analogy with the *rrnB* P1 and P2 promoters (Figs. 2 and 6A) sequences upstream of the −35 region are likely to be involved. Consistent with this interpretation, sequences between −39 and −47 in the *leuV* promoter were previously shown to increase activity by a factor of 3 in vivo (*48*).

Some promoters appear to have a much smaller requirement, if any, for the COOH-terminal portion of α, for example, *lacUV5* and RNA-I (Fig. 2) (*23, 24*). However, analysis of deletion derivatives of such promoters would be necessary to determine whether upstream sequences in these promoters have any effect on transcription at all, and whether utilization of the upstream sequences requires the COOH-terminus of α (*49*). The subtle differences in footprint protection of the −50 region of the *lacUV5* promoter by wild-type and α-mutant RNAPs (*31, 40*) suggest that the upstream sequences may play some, although probably a much smaller, role in its activity.

In *rrnB* P1, the UP element is located between −40 and −60. However, *rrnB* P1 promoters lacking the upstream half of the UP element (extending only to −50 or −48) have transcription activities intermediate between promoters lacking (−41) or containing (−61) the full UP element (*9, 31*). In addition, the upstream section of the *rrnB* P1 UP element retains full activity in stimulating transcription and is specifically protected by RNAP in footprints when it is displaced upstream by one helical turn (11 bp) with an insertion at −46 (*8*). However, insertion of 5 bp at −46 (*8*) or deletion of 3 bp from −38 to −40 (*50*) results in loss of UP element function. The *rrnB* P1 UP element may, therefore, contain one or more sections whose effects may be separable. Thus, while UP elements must clearly be in the correct orientation relative to the other elements of the RNAP binding site (that is, the −10 and −35 hexamers), in other promoters UP elements may differ in their positions and in the magnitude of their effects.

Sequences rich in (A + T) in upstream promoter elements. Although the α subunit recognizes the *rrnB* P1 UP element, the precise sequence or structure (or both) that is recognized has yet to be defined. The DNA sequences of the four promoters for which we have identified a requirement for the COOH-terminus of α were aligned according to their −35 hexamers (Fig. 7A). Three of the upstream regions are very rich in (A + T), but no obvious consensus sequence is evident from this comparison. The *leuV* promoter, which had a lesser requirement for the COOH-terminus of α (Fig. 6C), is (A + T) rich only in its −40 to −50 region. In contrast, the upstream regions of the promoters in this study whose function is relatively independent of the COOH-terminus of α are not particularly rich in (A + T) [*lacUV5*, RNA-I, and four upstream deletion derivatives of the *rrnB* P1 or P2 promoters (Fig. 7B) (*49*)].

Sequences rich in (A + T), particularly A tracts, were noted previously in the −44 region of a substantial subset of *E. coli* promoters (*10*) and upstream of several strong *E. coli* promoters (*2, 11*), as well as of some promoters in Gram-positive bacteria (*3, 6, 51*). Increased promoter activity associated with naturally occurring or synthetic phased A tracts has been attributed to effects of DNA bending (*3, 7, 52*). Our

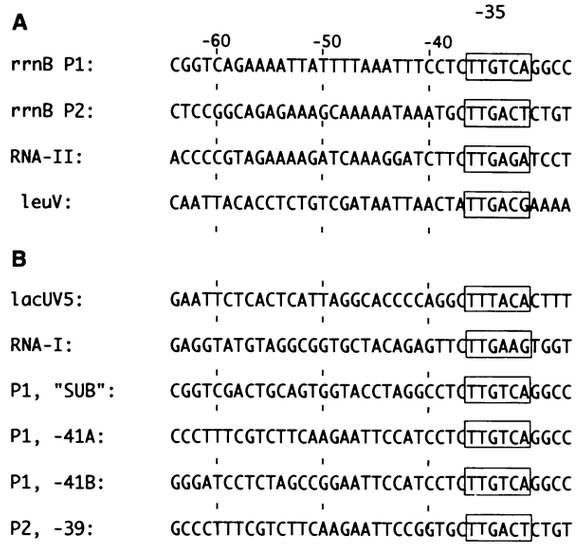

Fig. 7. Upstream sequences for sets of promoters whose activities are (**A**) affected by the α subunit mutations, or (**B**) relatively unaffected by these mutations (*49*). Sequences are aligned by their −35 region consensus positions. Sequences upstream of the promoter endpoint derive from plasmid DNA adjacent to the *EcoR*I promoter insertion site in either pRLG770 [(*20*), for *rrnB* P1 −41A and *rrnB* P2 −39] or pSL6 (*9*), for *rrnB* P1 −41B.

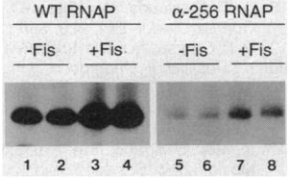

Fig. 8. The effect of Fis on transcription of the −88 to +50 *rrnB* P1 promoter by wild-type reconstituted RNAP (lanes 1 to 4) or α-256 RNAP (lanes 5 to 8). Transcription was as described (*20*), but with 100-μl reaction volumes, and samples were concentrated by ethanol precipitation prior to electrophoresis. Reconstituted RNAPs were used at 6.6 nM (wild type) or 43.6 nM (α-256). Where indicated, purified Fis protein (from R. Johnson) was present at a final concentration of 45 nM. Lanes 5 to 8 were exposed to film approximately three times longer than those in lanes 1 to 4, since the basal transcription by α-256 RNAP was much lower than that by wild-type RNAP, as shown in Fig. 2. Only the portion of the gel showing the *rrnB* P1 transcript is shown.

results suggest that, in at least some cases, the (A + T)–rich or curved DNA sequences present upstream of core promoters may contain specific binding sites for the α subunit of RNAP and that the α-DNA interactions might account for the effect of these sequences on transcription. At some promoters, DNA curvature might be required to facilitate the α-DNA interactions. Alternatively, the curvature detected in upstream regions may not precisely coincide with the specific sequence features that increase transcription. For example, the pronounced bend in the *rrnB* P1 promoter region previously detected by electrophoretic mobility analysis (*13*) lies well upstream of both the UP element and the principal Fis binding site (*53*), and thus does not appear to play a significant role in promoter activity. Nevertheless, although the UP element region of *rrnB* P1 itself does not display gross abnormalities in electrophoretic mobility (*53*), unusual structural characteristics in this (A + T)–rich sequence could be related to its recognition by RNAP.

Role of the UP element in positive control by activator proteins. The COOH-terminus of α is required for activation of transcription in vitro by a class of positive activator proteins (Class I activators), which have binding sites upstream of the core promoter region (*23, 24, 26, 33, 54, 55*). However, we found that Fis, a positive activator of *rrnB* P1 with a binding site centered at −71 (Fig. 1), did not require the COOH-terminus of α. Fis increased *rrnB* P1 transcription by the α-235 and α-256 RNAPs to approximately the same extent as it increased transcription by wild-type RNAP (three to four times in the experiment shown in Fig. 8). Thus, a binding site location well upstream of the core region is not sufficient to ensure that an activator protein will utilize the COOH-terminus of α for positive control (*44, 55*). Activator proteins not requiring the COOH-terminus of the α subunit may utilize contacts with another part of α, with the σ subunit (*26*), or with other subunits.

Interactions between α and the UP element are not essential for activation by Fis at *rrnB* P1. However, such interactions might play a role in the mechanisms of some other positive activators, since the R265C mutation in α inhibits activation by the UP element (Fig. 2C; Table 1), by CAP, cAMP (*32*), and by OxyR (*33*). For example, the COOH-terminal domain of α might simultaneously interact with closely adjoining surfaces of both a transcription factor and an upstream DNA element. In this model, interaction of α with a transcription factor might facilitate its interaction with a relatively poor upstream DNA sequence. In support of this model, different footprint patterns in the −50 region of the *lacUV5* promoter were observed in complexes formed with CAP and wild-type RNAP as compared to complexes formed with CAP and α-235 or α-256 RNAP (*40*). Alternatively, it is possible that the phenotypes of the α mutants might reflect interaction of α with either an activator protein or an upstream DNA element, depending on the promoter.

In summary, our results indicate that direct RNAP-DNA interactions with a promoter element upstream of the −35 region are mediated by the α subunit, and that these interactions can play a major role in the function of some promoters. The involvement of the α subunit in upstream DNA-mediated as well as transcription factor–mediated stimulation of transcription suggests that there might be shared features in these seemingly different mechanisms.

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tivity of the *rrnB* P1 core promoter in a manner analogous to the expression of the mutant α subunits [(*20*); R. A. Sharrock, R. L. Gourse, M. Nomura, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5275 (1985)].

37. ^{32}P–end-labeled promoter-containing restriction fragments (*20*) were incubated for 10 minutes at 37°C with 4.8 nM native wild-type RNAP [from D. Hager and R. Burgess, University of Wisconsin, Madison and characterized in (*4, 9*)], 13.2 nM reconstituted wild-type RNAP (*23*), 48 nM α-235 RNAP or 64 nM α-265 RNAP (*23*), in 25 μl of buffer containing 30 mM potassium glutamate, 10 mM tris-acetate, pH 7.9, 10 mM $MgCl_2$, 1 mM DTT BSA at 100 μg/ml, and the initiating nucleotides ATP (500 μM) and CTP (50 μM), which permit visualization of a heparin resistant complex ($RP_{A,C}$) at this promoter (*4, 9, 18, 19*). Heparin (final, 10 μg/ml) was added 30 seconds before cleavage by DNase I or hydroxyl radical. DNase I (Worthington) was added at 0.4 μg/ml for 30 seconds, and reactions were terminated, processed, and subjected to electrophoresis as described (*20*). Hydroxyl radical cleavage conditions were as described (*18*). Promoter fragments were 3′-end labeled with ^{32}P in the strands indicated in the legend to Fig. 4 as described (*20*): −88 to +50 promoter, Eco RI–Hind III fragment derived from pSL9 (*9*); −88 to +1 promoter, Eco RI–Xho I fragment derived from pSL13 (*9*); −41 to +1 promoter, SmaI-XhoI fragment derived from pLR5 (*9*).
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