

Escherichia coli Promoters with UP Elements of Different Strengths: Modular Structure of Bacterial Promoters

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The α subunit of *Escherichia coli* RNA polymerase (RNAP) participates in promoter recognition through specific interactions with UP element DNA, a region upstream of the recognition hexamers for the σ subunit (the -10 and -35 hexamers). UP elements have been described in only a small number of promoters, including the rRNA promoter *rrnB* P1, where the sequence has a very large (30- to 70-fold) effect on promoter activity. Here, we analyzed the effects of upstream sequences from several additional *E. coli* promoters (*rrnD* P1, *rrnB* P2, λp_R , *lac*, *merT*, and RNA II). The relative effects of different upstream sequences were compared in the context of their own core promoters or as hybrids to the *lac* core promoter. Different upstream sequences had different effects, increasing transcription from 1.5- to ~ 90 -fold, and several had the properties of UP elements: they increased transcription *in vitro* in the absence of accessory protein factors, and transcription stimulation required the C-terminal domain of the RNAP α subunit. The effects of the upstream sequences correlated generally with their degree of similarity to an UP element consensus sequence derived previously. Protection of upstream sequences by RNAP in footprinting experiments occurred in all cases and was thus not a reliable indicator of UP element strength. These data support a modular view of bacterial promoters in which activity reflects the composite effects of RNAP interactions with appropriately spaced recognition elements (-10 , -35 , and UP elements), each of which contributes to activity depending on its similarity to the consensus.

Promoter sequences involved in recognition by *Escherichia coli* RNA polymerase (RNAP) were identified from comparisons of a large number of known promoters and from mutational analyses (28, 29, 38, 62). These sequences, the -10 and -35 hexamers (5' TATAAT 3' and 5' TTGACA 3', respectively), are recognized by the σ^{70} subunit of RNAP (11). The strength of a promoter correlates generally with its degree of identity to these sequences and with the length of the spacer between them (the homology score [42]), although exceptions to this rule have been described (7, 26).

It was proposed more than 10 years ago that optimal transcription activity could be achieved by different combinations of promoter elements, including not only the -10 and -35 hexamers, but also upstream and downstream regions (7). In accord with this suggestion, RNAP protects regions both upstream and downstream of the -10 and -35 hexamers in footprints (8, 45, 47, 56), and A+T-rich sequences upstream of the -35 hexamer in several *E. coli* or *Bacillus subtilis* promoters were found to increase transcription *in vitro* in the absence of accessory proteins (3, 19, 31, 37, 39, 50, 54). Phased A-tracts inserted upstream of the -35 region in various promoter constructs were also shown to increase transcription (6, 12, 24).

The A+T-rich region upstream of -40 in the rRNA promoter *rrnB* P1, the UP element, increases transcription 30- to 70-fold by binding the RNAP α subunit (13, 50, 53). A consensus UP element sequence was determined by using *in vitro* selection for upstream sequences that promote rapid RNAP binding to the *rrnB* P1 promoter, followed by *in vivo* screening for high promoter activity. The consensus UP element consists of alternating A- and T-tracts (13). UP elements matching the consensus increased promoter activity as much as 326-fold, about 5-fold more than the wild-type *rrnB* P1 UP element. UP

elements were also identified in other promoters, for example, the flagellin (*hag*) promoter of *B. subtilis* (18), the P_{L2} promoter of phage lambda (25), and the P_e promoter of phage Mu (61), although the effects of these elements were not as large as that of *rrnB* P1. UP elements also function in promoters recognized by RNAP holoenzymes with alternate σ factors (18).

UP elements are not as highly conserved as the -10 and -35 elements and were not described in studies comparing the large sets of *E. coli* promoters used to define the consensus hexamers (28, 29, 38). However, A+T-rich sequences were identified as a prominent feature of a subset of *E. coli* promoters (the -44 motif [23]), and a recent *E. coli* promoter analysis (48) identified two A+T-rich regions at upstream positions corresponding to those crucial for UP element function (14). A+T-rich upstream sequences were also identified in compilations of *B. subtilis* and *Clostridium* promoters (27, 30).

We have proposed that UP elements may be a recognition feature in many bacterial promoters (13, 53), but in most promoters, the role of upstream sequences has not been evaluated experimentally. Therefore, in this paper, we have examined the role of upstream sequences from six promoters (*rrnB* P2, *rrnD* P1, RNA II, *merT*, *lac*, and λp_R). We find that several of the sequences function as UP elements and that their effects on promoter activity differ, correlating generally with the degree of similarity to the UP element consensus sequence. These results support the model that bacterial promoters consist of at least three modules, not just -10 and -35 elements. We also show that upstream protection in footprints is not a reliable indicator of UP element function.

MATERIALS AND METHODS

Strains and plasmids. The strains and plasmids used in this study are listed in Table 1. Single-copy promoter-*lacZ* fusions were constructed by inserting promoters as *EcoRI-HindIII* fragments into either of two phage lambda *lacZ* fusion vectors (system I for promoters with higher activities or system II for promoters with lower activities [50]). Monoclonogenic strains were identified by comparison of the β -galactosidase activities of several independent candidates and by a PCR test (13). System I phages carrying λp_R promoter-*lacZ* fusions contain the

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Genotype	Source or reference
Strains		
NK5031	$\Delta lacM5262 supF$ Nal ^r	52
λ system I lysogens		
RLG 957	NK5031/ λ <i>rmB</i> P1(-61 to +50)- <i>lacZ</i>	52
RLG 2263	NK5031/ λ <i>rmB</i> P1(-41 to +50)- <i>lacZ</i>	50
RLG 3074	NK5031/ λ <i>rmB</i> P1(-66 to +50)- <i>lacZ</i>	13
RLG 3269	NK5031/ λ <i>rmD</i> P1(-41 to +1)- <i>lacZ</i>	This work
RLG 3271	NK5031/ λ <i>rmD</i> P1(-60 to +1)- <i>lacZ</i>	This work
RLG 4250	NK5031/ λ i^{21} λ p_R (-61 to +20)- <i>lacZ</i>	This work
RLG 4251	NK5031/ λ i^{21} λ p_R (-42 to +20)- <i>lacZ</i>	This work
RLG 4252	NK5031/ λ <i>rmB</i> P2(-68 to +252)- <i>lacZ</i>	This work
RLG 4253	NK5031/ λ <i>rmB</i> P2(-39 to +252)- <i>lacZ</i>	This work
λ system II lysogens		
RLG 3280	NK5031/ λ i^{21} λ p_R (-66 to -38)- <i>lac</i> (-37 to +52)- <i>lacZ</i>	This work
RLG 3281	NK5031/ λ <i>merT</i> (-66 to -38)- <i>lac</i> (-37 to +52)- <i>lacZ</i>	This work
RLG 3282	NK5031/ λ RNA II(-65 to -38)- <i>lac</i> (-37 to +52)- <i>lacZ</i>	This work
RLG 3283	NK5031/ λ <i>rmB</i> P2(-65 to -38)- <i>lac</i> (-37 to +52)- <i>lacZ</i>	This work
RLG 4280	NK5031/ λ <i>lac</i> (-60 to +52)- <i>lacZ</i>	This work
RLG 4281	NK5031/ λ <i>lac</i> (-47 to +52)- <i>lacZ</i>	This work
RLG 4282	NK5031/ λ <i>rmB</i> P1(-88 to -38, $\Delta 72$)- <i>lac</i> (-37 to +52)- <i>lacZ</i>	This work
RLG 4288	NK5031/ λ <i>lac</i> (-40 to +52)- <i>lacZ</i>	This work
Plasmids		
pRLG 770	Vector (no insert)	52
pRLG 1820	<i>rmB</i> P1(-88 to -38, $\Delta 72$)- <i>lac</i> (-37 to +52)	50
pRLG 942	<i>rmB</i> P2(-65 to -38)- <i>lac</i> (-37 to +52)	This work
pRLG 941	<i>merT</i> (-66 to -38)- <i>lac</i> (-37 to +52)	This work
pRLG 940	RNA II(-65 to -38)- <i>lac</i> (-37 to +52)	This work
pRLG 939	λ p_R (-66 to -37)- <i>lac</i> (-36 to +52)	This work
pRLG 1821	<i>lac</i> (-47 to +52)	50
pRLG 2227	<i>rmB</i> P1(-88 to -38, $\Delta 72$)- <i>lacUV5</i> (-37 to +39)	This work
pRLG 947	<i>rmB</i> P2(-65 to -38)- <i>lacUV5</i> (-37 to +39)	This work
pRLG 946	<i>merT</i> (-65 to -38)- <i>lacUV5</i> (-37 to +39)	This work
pRLG 945	RNA II(-65 to -38)- <i>lacUV5</i> (-37 to +39)	This work
pRLG 943	λ p_R (-65 to -37)- <i>lacUV5</i> (-36 to +39)	This work
pRLG 593	<i>lacUV5</i> (-60 to +39)	52
pRLG 936	λ p_R (-42 to +20)	This work
pRLG 2229	λ p_R (-61 to +20)	This work
pRLG 934	RNA II(-150 to +50)	53
pRLG 938	RNA II(-42 to +50)	This work
pRLG 3266	<i>rmD</i> P1(-60 to +1)	This work
pRLG 3267	<i>rmD</i> P1(-41 to +1)	This work

immunity region of phage 21, introduced from λ i^{21} phages in vegetative crosses. System II phages all contain the immunity 21 region. Plasmids used for in vitro transcription were derivatives of pRLG770 (52) and contained *EcoRI-HindIII* promoter fragments inserted ~170 bp upstream of an *rmB* T1 terminator.

EcoRI-HindIII promoter-containing fragments were obtained by PCR from plasmid templates containing other derivatives of the same promoter, except as noted. Upstream primers for PCR contained an *EcoRI* site and the upstream 25 to 30 nucleotides of the promoter. The downstream primer (RLG1620) contained vector pRLG770 sequence (5'-GCGCTACGGCGTTTCACTTC-3') about 40 bp downstream of the *HindIII* site for insertion of the promoter fragment. *rmD* P1 promoter fragments were obtained by PCR from plasmid pRLG3246 [*rmD* P1 (-61 to +10)] (22). The λ p_R (-60 to +20) promoter fragment was obtained from *EcoRI* and *HindIII* digestion of pBR80 (58) and

contained about 120 bp of pBluescript vector sequence both upstream and downstream of the promoter.

Hybrid promoters containing upstream elements from different sources fused to either the *lac* or the *lacUV5* core promoter at position -37 were constructed by PCR with plasmids pRLG1821 (50) and pRLG593 (53) as templates. Upstream primers contained an *EcoRI* site, the desired UP element sequence, and *lac* core promoter sequence from -37 to -17. The downstream primer was RLG1620 (see above). The upstream sequences of the hybrid-*lac* promoters are shown in Fig. 5.

Promoter activity determinations. Promoter activities were determined in vivo by measurement of β -galactosidase activities in strains lysogenic for λ carrying the promoter-*lacZ* fusions. Cultures were grown for 4 or more generations in Luria-Bertani medium at 30°C (for system I lysogens) or at 37°C (for system II lysogens), and mid-logarithmic-phase cells were used to measure activities as described previously (41).

In vitro transcription was carried out essentially as described previously (52, 53) in reaction mixtures containing 50 ng of supercoiled plasmid DNA template, 10 mM Tris-Cl (pH 7.9), 10 mM MgCl₂, 1 mM dithiothreitol, and 100 μ g of bovine serum albumin per ml. Reaction mixtures also contained either 30 mM KCl (see Fig. 2B and C and 3) or 50 mM KCl (Fig. 2A) and the following nucleoside triphosphate (NTP) concentrations: Fig. 2A, 500 μ M ATP, 50 μ M GTP and UTP, 10 μ M CTP, and [α -³²P]CTP; Fig. 2B, 500 μ M ATP, 50 μ M UTP, 10 μ M GTP and CTP, and [α -³²P]GTP; Fig. 2C, 100 μ M ATP, CTP, and GTP, 10 μ M UTP, and [α -³²P]UTP; Fig. 3, 400 μ M ATP, 100 μ M GTP and UTP, 10 μ M CTP, and [α -³²P]CTP. [α -³²P]NTPs were from DuPont and were used at about 5 μ Ci per reaction.

DNase I footprinting. DNA fragments were prepared by digestion of plasmid DNAs (pRLG2227, pRLG947, pRLG946, pRLG945, pRLG943, and pRLG593 [Table 1]) with *HindIII* (at promoter position +40), labelling of the top (non-template) strand with Sequenase (Amersham) and [α -³²P]dATP (DuPont), and further digestion with *AatII* (77 bp upstream of the *EcoRI* site at the upstream end of the promoter). Fragments were gel isolated and purified and concentrated with Elutip D columns (Schleicher & Schuell). RNAP or α subunit complexes with promoter fragments (0.5 nM) were formed at 26°C in a mixture of 30 mM KCl, 40 mM Tris-acetate (pH 7.9), 10 mM MgCl₂, 10% glycerol, and 100 μ g of bovine serum albumin per ml and were digested with DNase I at 2 μ g/ml for 30 s. Heparin (10 μ g/ml) was added to RNAP-promoter complexes prior to DNase I digestion. Processing and electrophoresis of samples were performed as described previously (52, 53).

RNAPs and α subunit. Wild-type, $\alpha 265A$, and $\alpha 235$ RNAPs were obtained from A. Ishihama (53) or were reconstituted from purified subunits as described previously (21).

RESULTS

We tested whether a series of promoters contained UP elements by (i) measuring the effects of their upstream sequences in vivo with promoter-*lacZ* fusions, (ii) determining the effects of their upstream sequences in vitro with wild-type and α -mutant RNAPs, and (iii) characterizing the interactions between the upstream sequences and RNAP or purified α subunit in vitro by DNase I footprinting. We were particularly interested in whether the effects of different UP elements on transcription would correlate with the number of sequence matches to the recently defined UP element consensus (13) and whether footprints would be a reliable indicator of the presence or absence of an UP element.

Effects of upstream sequences on transcription in vivo. The effects of upstream sequences on promoter activity in vivo were determined for four promoters, chosen because previous in vitro data indicated that their upstream sequences might increase their activities in vivo. For two of the promoters, upstream sequences were protected by RNAP in footprints (λ p_R and *lac* [8, 34]), and for the other two promoters, upstream regions stimulated promoter activity in vitro (*rmB* P2 and *rmD* P1 [53, 54]). Derivatives of each promoter containing either native or substituted upstream sequences (Fig. 1) were fused to *lacZ*, and their activities were determined by measuring β -galactosidase levels in strains containing chromosomal copies of the fusion constructs (Table 2). For each of the promoters, the derivative with native upstream sequences had more activity than the derivative with substituted sequences, although the magnitudes of the effects were very different. The *rmD* P1 upstream sequence increased transcription dramatically (ap-

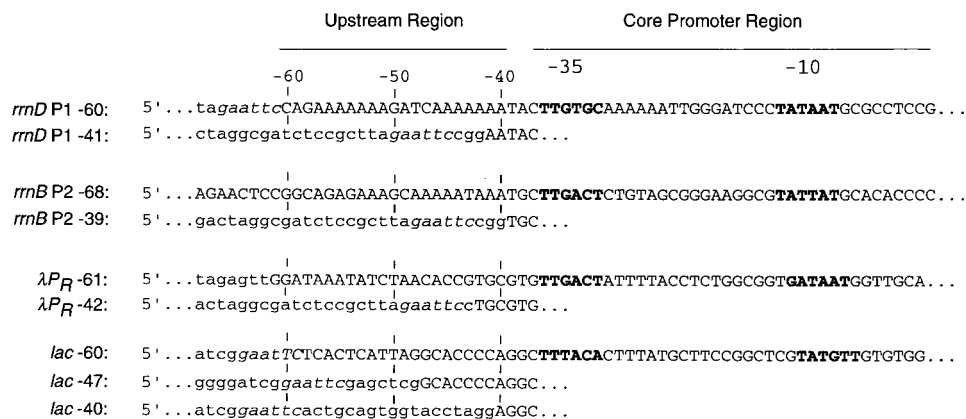


FIG. 1. Sequences of derivatives of four *E. coli* promoters, *rmD* P1, *rmB* P2, λp_R , and *lac*, containing either native or substituted upstream sequence. Promoters are designated by the position of the upstream-most native position (e.g., *rmD* P1 -60 has native sequence to -60). Native sequences are represented by uppercase letters, and substituted sequences are represented by lowercase letters. *Eco*RI sites at the junction of promoter and vector sequences are italicized. The -10 and -35 hexamers are shown in boldface. Substituted sequences for *rmD* P1 -41, *rmB* P2 -39, λp_R -42, and *lac* -47 are from the lambda phage vector into which they were cloned for in vivo activity measurement (Table 1). For the *lac* -40 promoter, the substituted sequence was the SUB sequence (50). The phage vector sequences and the SUB sequence were previously characterized in the context of the *rmB* P1 promoter and did not affect transcription (50, 53).

proximately 90-fold), even more than the previously characterized *rmB* P1 UP element (Table 2) (13, 50). The *rmB* P2 upstream sequence also increased transcription, but to a much lesser extent (3.3-fold), while the λp_R and *lac* upstream sequences had only very small effects (1.7- and 1.5-fold, respectively). The *lac* promoter sequences responsible for the 1.5-fold effect appeared to include the region upstream of -47, since *lac* promoters with upstream endpoints of -40 or -47 had slightly lower activities than the -60 derivative.

Upstream sequences affect transcription directly. Upstream sequence effects on transcription in vivo could result from direct interactions with RNAP or from effects of transcription factors. To distinguish between these possibilities, upstream sequence function was characterized in vitro (Fig. 2). Transcription of the *rmD* P1 promoter containing its native upstream sequence (to -60) was much stronger than that of the promoter with native sequence only to -41 (Fig. 2A, lanes 1 to 4), indicating a direct effect of the upstream region on RNAP. This effect was not seen with a mutant RNAP defective in UP element recognition ($\alpha R265A$ RNAP [21]) (Fig. 2A, lanes 5 to 8), indicating that the *rmD* P1 -41 to -60 sequence functions as an UP element. This *rmD* P1 upstream region corresponds to the position of the *rmB* P1 UP element (50) and partially overlaps a region in *rmD* P1 previously found to stimulate its function in vitro (-50 to -89 [54]). The *rmD* P1 UP element had a greater effect than the *rmB* P1 UP element in vitro (51), consistent with their relative effects in vivo (Table 2). Under these in vitro conditions, the effects were not as large as those observed in vivo (Table 2 and Fig. 2A) (13, 53). However, for *rmB* P1, larger effects of the UP element were observed at higher-salt and lower-RNAP concentrations, and kinetic studies have revealed in vitro effects similar to those observed in vivo (13, 50). We expect that a similar situation would be true for *rmD* P1.

The *rmB* P2 upstream sequence examined above (Table 2) was previously shown to increase transcription in vitro in the absence of additional factors and to require the α subunit C-terminal domain (α CTD) for its effect (53). Thus, we conclude that *rmB* P2 has an UP element with a modest degree of function in vivo.

The λp_R and *lac* upstream sequences had very little effect on transcription in vivo. Nevertheless, we examined λp_R in vitro by using derivatives with native (to -61) or substituted (to

-42) upstream sequences to distinguish whether possible inhibitory factors might have obscured detection of stimulatory effects in vivo (Fig. 2B). No effect of the native upstream sequence was observed. We did not examine the *lac* upstream sequences in vitro, since this promoter's activity in the absence of the activator protein, CRP, was too low to be quantified by this assay (see below).

An additional promoter, RNA II, which makes the primer for ColE1 plasmid replication, was also included in our study. This promoter (with native upstream sequences to -150) was not efficiently transcribed by α -mutant RNAP in previous transcription experiments, suggesting that it might contain an UP element (53). To further characterize its upstream sequences,

TABLE 2. Effect of native upstream sequences on promoter activity in vivo

Strain	Promoter	Upstream endpoint	β -Galactosidase activity (Miller units) ^a	Relative activity ^b
RLG 3271	<i>rmD</i> P1	-60	2,050	93.0
RLG 3269	<i>rmD</i> P1	-41	22	1.0
RLG 4252	<i>rmB</i> P2	-68	10,915	3.3
RLG 4253	<i>rmB</i> P2	-39	3,432	1.0
RLG 4250	λp_R	-61	5,002	1.7
RLG 4251	λp_R	-42	2,833	1.0
RLG 4280	<i>lac</i>	-60	66	1.5
RLG 4281	<i>lac</i>	-47	35	0.8
RLG 4288	<i>lac</i>	-40	44	1.0
RLG 3074	<i>rmB</i> P1	-66	1,642	59.0
RLG 957	<i>rmB</i> P1	-61	1,016	36.0
RLG 2263	<i>rmB</i> P1	-41	28	1.0

^a Measured as described in reference 41. Values are the average of at least two determinations that differ by <10%. Values should be compared within each set of derivatives of the same promoter, but not between different promoter groups, since promoter downstream endpoints, and thus the initial transcribed regions, differ. *lac* promoter constructs are in λ vector system II, and *rmD* P1, *rmB* P2 and λp_R promoter constructs are in λ vector system I (see Table 1 and Materials and Methods).

^b Activities are expressed relative to the shortest derivative of each promoter.

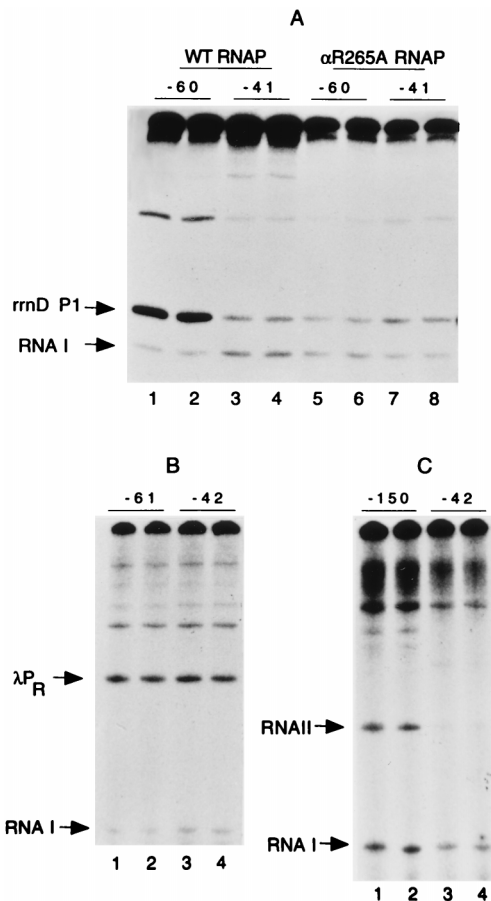


FIG. 2. In vitro transcription of promoters containing or lacking native upstream sequences. (A) *rrmD* P1 promoters with upstream endpoints of -60 or -41 (plasmid templates pRLG3266 and pRLG3267) transcribed with 0.5 nM wild-type (WT) RNAP (lanes 1 to 4) or 0.5 nM $\alpha R265A$ mutant RNAP (lanes 5 to 8). (B) λp_R promoters with upstream endpoints of -61 or -42 (plasmid templates pRLG2229 and pRLG936) transcribed with 1 nM wild-type RNAP. (C) RNA II promoters with upstream endpoints of -150 or -42 (plasmid templates pRLG934 and pRLG938) transcribed with 2 nM wild-type RNAP. In each experiment, transcripts were separated on a 5% acrylamide-7 M urea gel, and the promoter-specific and the vector-encoded RNA I transcripts are indicated.

we constructed an additional promoter derivative with sequences extending only to -42 and compared the activities of the two promoters in vitro (Fig. 2C). The native upstream sequences increased transcription by wild-type RNAP in the absence of other protein factors, indicating that the RNA II upstream sequence functions directly. Although the RNA II upstream sequence was not examined in the context of its own promoter in vivo, it stimulated the activity of the *lac* core promoter (see below). We also observed that expression of plasmid-encoded α subunits defective in DNA binding and UP element function reduced the maintenance of ColE1 plasmid derivatives, suggesting that the RNA II UP element does function to stimulate its promoter in vivo (21).

Relative strengths of different UP elements in the context of the same core promoter. The widely varying effects of different upstream sequences on transcription (Table 2) could reflect differences in their interactions with the α subunit and/or differences in the capacity of the core promoters to respond to an UP element (i.e., core promoter mechanisms could be limited to different extents by a step or steps affected by UP elements).

To compare directly the relative strengths of several upstream sequences, their effects on the same core promoter were determined with hybrid promoters. The upstream sequences from *rrmB* P2, RNA II, λp_R , and *merT* were fused to the *lac* core promoter. The *merT* sequence was included in the study, since it was protected by RNAP in footprinting experiments (46). The *lac* core promoter was used for the hybrid promoter constructs, since we showed previously that it responds to the *rrmB* P1 UP element in an *rrmB* P1-*lac* hybrid (50) (Table 3).

The activities of the hybrid promoters were compared in vivo with that of the *lac* promoter without an UP element (-40 *lac* [Table 3]). The *rrmB* P1 UP element had the largest effect, increasing *lac* transcription ~ 33 -fold, consistent with previous observations (50). The *rrmB* P2, RNA II, and *merT* sequences increased transcription 12.8-, 4.9-, and 2.0-fold, respectively, while the λp_R upstream region did not increase *lac* promoter activity significantly.

The *rrmB* P1, *rrmB* P2, RNA II, and *merT* upstream sequences affected *lac* promoter activity directly, since the hybrid promoters had greater activity in vitro than the *lac* promoter without an UP element (Fig. 3A) (50). Transcription from the *lac* core promoter (-40 *lac*) and from λp_R -*lac* was not detectable (Fig. 3A, lanes 1 and 2) (51). Thus, the activities of the hybrid promoters in vitro were consistent with their relative activities in vivo: *rrmB* P1-*lac* > *rrmB* P2-*lac* > RNA II-*lac* > *merT*-*lac* > λp_R -*lac* (Table 2 and Fig. 3A). The stimulation of *lac* promoter activity by the upstream sequences in vitro was dependent upon the DNA binding function of the RNAP α subunit, since no transcription from the hybrid promoters was observed with RNAP lacking the α CTD ($\Delta 235$ RNAP [Fig. 3B, lanes 1 to 5]), although the mutant enzyme was proficient in transcription of *lacUV5* (lane 6).

Interaction of upstream elements with the RNAP α subunit.

The experiments presented above identified a requirement for the DNA binding function of the α subunit for upstream sequence function, suggesting that as with the *rrmB* P1 UP element, α CTD interacts directly with these sequences. We confirmed this conclusion by footprinting with hybrid promoters in which the upstream sequences were fused to the *lacUV5* core promoter (Fig. 4). (*lacUV5*, a promoter with a 2-bp substitution mutation in *lac* that creates a consensus -10 hexamer, was used to improve promoter occupancy by RNAP; we assumed that the *lacUV5* mutation did not affect the α subunit-UP element interaction directly.)

Protection of the *rrmB* P1 UP element when fused to the *lacUV5* promoter (Fig. 4A) was comparable to its protection in the context of its own core promoter (53). The A+T-rich UP element was cleaved inefficiently by DNase I, as expected (lane

TABLE 3. Effect of upstream sequences from other promoters on *lac* core promoter activity in vivo

Strain	Promoter ^a	β -Galactosidase activity (Miller units) ^b	Relative activity ^c
RLG4282	<i>rrmB</i> P1- <i>lac</i>	1,235	33.3
RLG3283	<i>rrmB</i> P2- <i>lac</i>	492	12.8
RLG3282	RNA II- <i>lac</i>	186	4.9
RLG3281	<i>merT</i> - <i>lac</i>	78	2.0
RLG3280	λp_R - <i>lac</i>	41	1.1
RLG4288	<i>lac</i> (-40)	38	1.0

^a Promoters contained *lac* core promoter sequence (-37 to $+52$) and sequence from the indicated promoters upstream of -37 (Fig. 5).

^b Measured as described in reference 41. Values are averages of at least two determinations that differ by $<10\%$.

^c Activities are relative to that of the *lac*(-40) promoter.

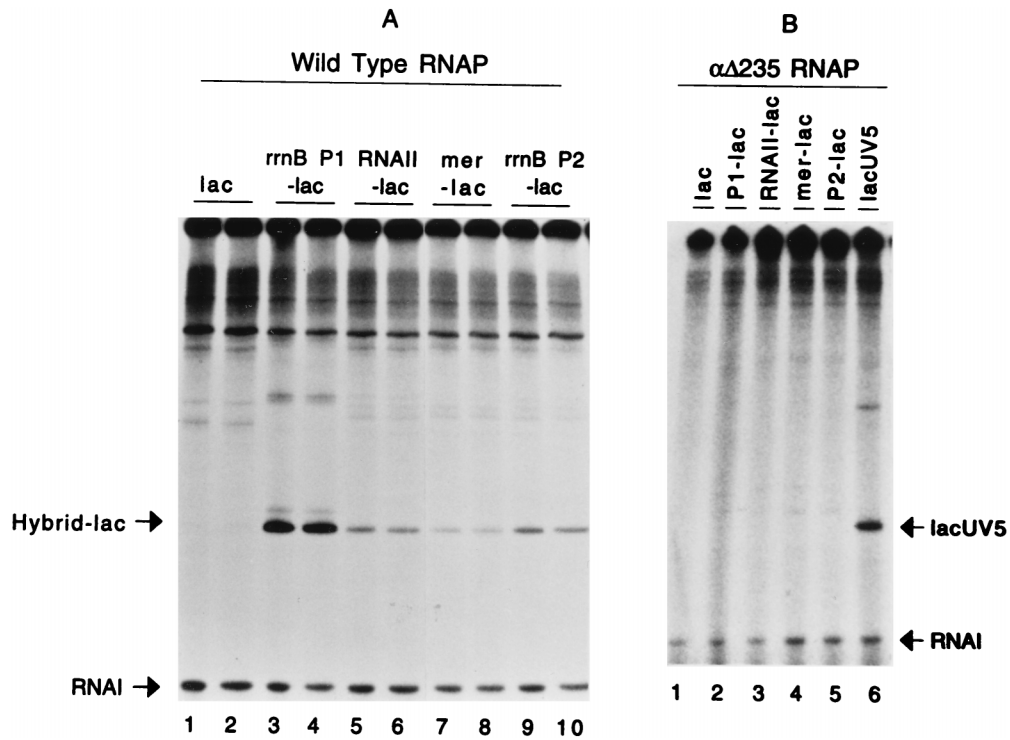


FIG. 3. In vitro transcription of *lac* and hybrid-*lac* promoters with wild-type RNAP (A) or $\alpha\Delta235$ mutant RNAP (B). The upstream sequences of the hybrid promoters and their junction with the *lac* core promoter (at position -37) are shown in Fig. 5. Transcripts were separated on 5% acrylamide-7 M urea gels, and hybrid promoter, *lacUV5*, and vector-encoded promoter RNA I transcripts are indicated. Duplicate samples are shown in panel A. The RNAP concentrations were 2 nM (A) and 8 nM (B). Plasmid templates for transcription were as follows: *lac*, pRLG1821; *rrnB* P1-*lac*, pRLG1820; RNA II-*lac*, pRLG940; *merT*-*lac*, pRLG941; *rrnB* P2-*lac*, pRLG942; and *lacUV5*, pRLG593.

2) (53, 57), but several protected positions were detected in the presence of wild-type RNAP (lane 3). This protection was not observed with RNAP lacking the α CTD (lane 4).

Protection was also observed upstream of -40 in wild-type RNAP footprints of each of the other hybrid promoters and of *lacUV5* with its native upstream sequence (Fig. 4B, lane 2, and

C to F, lanes 3). In each case, upstream sequence protection required the α CTD (Fig. 4B, lane 1, and C to F, lanes 4). Protection in three of the promoters occurred in two short regions (approximately -41 to -43 and -50 to -53) that correspond to the proximal and distal positions protected against hydroxyl radical cleavage in the *rrnB* P1 UP element

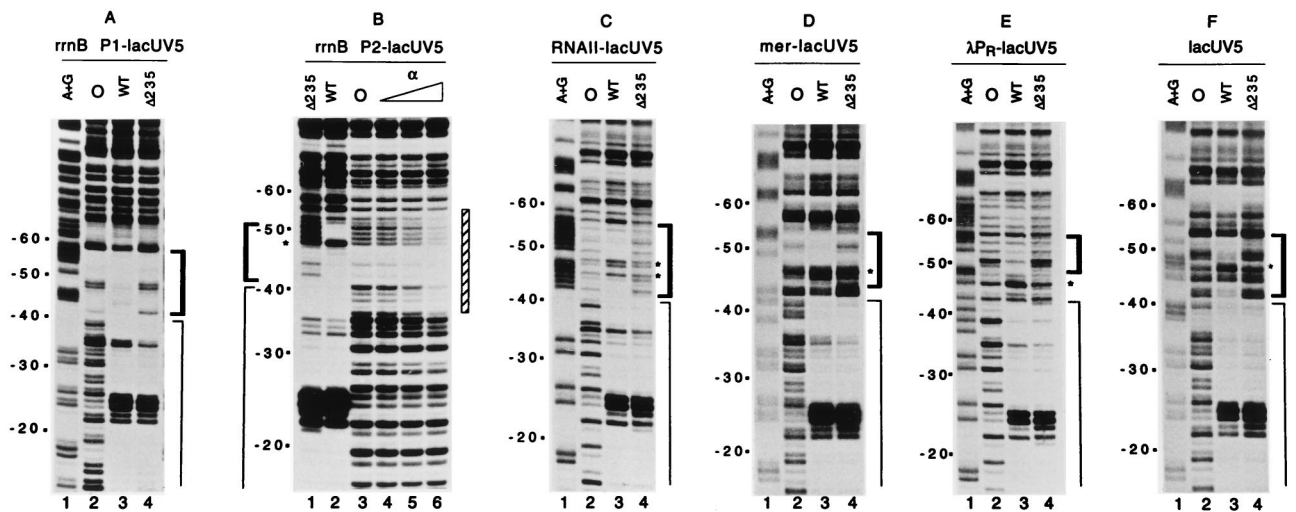


FIG. 4. DNase I footprints of hybrid-*lacUV5* promoters containing various different upstream sequences (A to E) and of *lacUV5* with its native upstream sequence (F). In each case, the top (nontemplate) strand was radiolabelled at position +40. A+G, sequence markers; 0, no RNAP; WT, wild-type RNAP (40 nM); $\Delta235$, $\alpha\Delta235$ mutant RNAP (40 nM). The core promoter-protected regions are indicated by a thin line, and upstream regions protected by wild-type RNAP, but not by $\alpha\Delta235$ mutant RNAP, are indicated with a thick line. Positions in the upstream regions hypersensitive to DNase I are indicated by asterisks. In panel B, *rrnB* P2-*lacUV5* was also tested in the presence of purified α subunit (2 to 8 μ M, lanes 4 to 6). The region protected by the α subunit is indicated with a hatched bar.

	-60	-50	-40	-35	Relative Activity
-40- <i>lac</i>	tctccggtc	gactgc AgTg	Tacct Ag	gagggc	1.0
-60- <i>lac</i>	atcgggaa	tctc AcTca	TTagg	caccccagggc	1.5
λp_R - <i>lac</i>	tcgcaagggat	AAATATc	Taac	accggtgcgctc	1.1
<i>mer</i> - <i>lac</i>	tctcaggtt	tctcc AAATTg	TTTT	tcc At Atcgc	2.0
<i>RNAII</i> - <i>lac</i>	ttcgaccccg	t AgAAA agaT	caaagg	Atcttc	4.9
<i>rrnBP2</i> - <i>lac</i>	ttcactccggc	AgAgAa Agcaaaaa	ATAA atgc		12.8
<i>rrnBP1</i> - <i>lac</i>	tgcgcggtc	cag AAAAT TATTTT	aa A tttcctc		33.3
<i>rmD</i> P1	tagaatttc	cag AAAAA a	gagTcaa AAAA atac		93.0*
Consensus (from in vitro selection)		AA A	AAA TTT T TTTT	--AAAA	

FIG. 5. Sequences of promoter upstream regions compared to the UP element consensus (from reference 13). Upstream sequences shown are those in the hybrid-*lac* promoters (Tables 1 and 3 [see Materials and Methods]), except for *rmD* P1, which is from the *rmD* -60 promoter (Fig. 1). Matches to the consensus are indicated in uppercase and boldface type. Vector-derived sequences are underlined, and native upstream sequences not matching the consensus are in lowercase type. The -35 hexamer regions are represented by open boxes. Positions accessible or hypersensitive to DNase I cleavage (Fig. 4) are indicated by arrows. The relative activities of the upstream sequences represent their function in the context of the hybrid-*lac* promoters (Table 3), except for *rmD* P1 (indicated with an asterisk), which was determined in the context of its own core promoter (Table 2).

(*lacUV5*, RNA II-*lacUV5*, and *rrnB* P2-*lacUV5*) (Fig. 4B, C, and F) (13, 45). In the *merT* and λp_R upstream sequences, protection occurred in the distal region (-51 to -53), but was not as evident in the proximal region (-41 to -43). An additional partially protected region (around -60) occurred in some of the footprints (e.g., *lacUV5* and λp_R -*lacUV5*). In each promoter, positions around -44 to -48 were accessible and, in some cases, were hypersensitive to DNase I (see Fig. 5 for sequences). The core region of each hybrid promoter (Fig. 4) was protected by both the wild-type and $\alpha\Delta 235$ RNAPs, and as observed previously for *lacUV5* (34), contained sites in the spacer region (at -24 and -25) that were hypersensitive to DNase I cleavage.

We also tested the binding of purified α subunit to the three UP elements with moderate effects on transcription, *rrnB* P2, RNA II, and *merT*. Specific protection of the *rrnB* P2 UP element was observed from \sim -36 to -53 (Fig. 4B, lane 6). Approximately fourfold-higher α subunit concentrations (4 to 8 μ M) were required for protection of the *rrnB* P2 UP element than for protection of the *rrnB* P1 UP element in the same experiment (51, 53). At higher concentrations of α subunit, the *rrnB* P2-protected region extended further upstream, to approximately -62, similar to the boundary observed with the *rrnB* P1 UP element (51, 53). Protection of the *merT* UP element region was observed at \sim 8 μ M α subunit, while specific protection of the RNA II upstream region was not observed (51).

DISCUSSION

UP elements of different strengths. We identified upstream sequences in several *E. coli* promoters that had the characteristics of UP elements: they increased transcription in vivo as well as in vitro in the absence of factors besides RNAP, and their function depended on their interaction with the α subunit of RNAP. Effects of the different upstream sequences examined here varied widely, by a factor of almost 100. We arbitrarily define as UP elements only those sequences that increased transcription twofold or more in vivo. We do not define the *lac* and λp_R upstream sequences as UP elements, since they affected transcription in vivo only slightly, these

effects were defined relative to the function of an arbitrary "neutral" sequence, and their effects were not observed in vitro. Although they did not significantly affect promoter function, the *lac* and λp_R sequences were protected in footprints with wild-type RNAP, and this protection was α CTD dependent (Fig. 4). Thus, footprint protection of an upstream sequence is not sufficient to define a functioning UP element in the absence of other evidence.

The negligible effect of the *lac* upstream sequence on promoter activity is consistent with previous observations on the effects of promoter substitution mutations in this region (16) and of α subunit mutations on *lacUV5* activity in vitro (53). Although α CTD interactions with *lac* upstream DNA are insufficient to increase transcription in the absence of CRP, they have been observed in footprinting experiments performed in the presence of RNAP and CRP (34) and appear to play a role in activator-dependent transcription by contributing to the overall stability of the activator-RNAP-promoter complex (9, 21, 59).

Similarity to consensus as a predictor of UP element function. Effects of upstream sequences on transcription correlate generally with the extent of their similarity to the consensus UP element (Fig. 5). The consensus sequence contains two conserved regions, an 11-bp distal region [5' -57 to -47, AAA(a/t)(a/t)T(a/t)TTTT] and a 4-bp proximal region (-44 to -41, AAAA) (13). Mutational analyses indicate that specific positions within the consensus sequence (-51 to -53 and -41 to -43) are most critical to function (14) and that each region can function alone, with the proximal region conferring larger effects on the *rrnB* P1 core promoter (>100-fold) than the distal region (\sim 15-fold [14]).

The two strongest UP elements, *rmD* P1 and *rrnB* P1, match the consensus exactly in one of the two regions and contain some matches in the other. The *rmD* P1 UP element (\sim 90-fold effect) matches the proximal region consensus exactly and the distal region at 7 of 11 positions (Fig. 5). It lacks the distal region T-tract, which likely accounts for its three- to fourfold-lower activity than that of the consensus UP element. The *rrnB* P1 element contains an exact match to the consensus in the distal region, but fewer matches in the proximal region; its somewhat smaller stimulatory effect (33-fold) may reflect the

smaller effects on transcription of the distal region compared to the proximal region.

The UP elements with moderate to low activity (*rmB* P2, RNA II, and *merT*) contain less extensive similarity to the consensus. The *rmB* P2 upstream sequence contains three of four A residues found in the proximal consensus (Fig. 5) and also contains A residues at -45 and -46, an additional feature of some strong proximal sequences (14). However, it contains only 4 of 11 distal consensus positions and is not protected against DNase I cleavage upstream of -51. We therefore suggest that its function may be attributable largely to proximal region interactions. The effect of the *rmB* P2 UP element on *lac* promoter activity (~12-fold) was similar to that observed in another study in which mutant *lac* promoters containing a series of A residues in the proximal upstream region conferred an α CTD-dependent increase in promoter activity (9).

The RNA II and *merT* UP elements match the consensus better in the distal than in the proximal region (Fig. 5), which may account in part for their relatively small effects on transcription. We also note that the RNA II UP element contains two recognition sites for the Dam methylase (GATC). It has been proposed previously that DNA methylation plays a role in controlling the RNA II promoter, although it is not known whether the GATC sites in the UP element, in addition to a third GATC site in the -35 region, contribute to regulation (60). The *rmD* P1 UP element also contains a GATC sequence.

The two upstream sequences with negligible effects on transcription (λp_R and *lac*) have no proximal region matches to consensus and contain distal regions with either little similarity to the consensus (*lac*) or mismatches at critical positions (λp_R). A substitution mutation in λp_R , C to T at -51 (p_{RM116}), that was previously reported to increase transcription threefold (17) results in a match to the consensus at eight contiguous positions.

Determinants of UP element strength. Differences in the degree of UP element function are likely to reflect several factors, including (i) the relative affinities of sequences for α subunit, (ii) the exact positioning of the sites with respect to the other promoter elements (40, 44), and (iii) the extent to which a particular core promoter mechanism is rate limited at a step affected by α subunit-DNA interaction. The affinities of two UP elements (*rmB* P1 and *rmB* P2) for purified α subunit differed by about fourfold (Fig. 4) (51), and this may account for the difference in their effects on the same core promoter (hybrid-*lac* promoters [Table 3]). However, the relative affinities of different sequences for purified α subunit must be interpreted with caution, since binding of α subunit alone might not be a reliable indicator of α subunit binding as part of the RNAP holoenzyme.

The *lac* and *rmB* P1 core promoters responded similarly to the *rmB* P1 UP element, as well as to phased A-tracts (1, 50). However, differences in the promoter mechanisms may explain why the *rmB* P2 UP element had a greater effect on the *lac* core promoter than on its own core promoter (12.8-fold versus 3.3-fold, respectively [Tables 2 and 3]). Other core promoter sequences may respond less well to and, in some cases, may even be inhibited by α CTD-DNA interactions. For example, an upstream A-tract (presumably an α subunit binding site [see below]) was reported to increase the activity of one synthetic core promoter, but to inhibit the activity of a second, clearance-limited core promoter (12). However, none of the upstream sequences analyzed in our study had negative effects on promoter activity.

A-tract sequences and α subunit recognition. Existing data suggest that the proximal and distal consensus sequences may

each represent an α CTD monomer binding site (14). Each of these sequences contains an A-tract, and although details of the α CTD-DNA interaction remain to be defined, the unusual structural features of A-tract DNA (reviewed in reference 63) may play a role in its recognition by α subunit. The upstream sequences in our study that functioned as stronger UP elements (e.g., RNA II, *rmB* P2, *rmB* P1, and *rmD* P1) contain an A- or T-tract at least 4 nt in length, consistent with the role of an A-tract in recognition by α subunit.

We have found that the previously observed stimulatory effects of multiple phased A-tracts on transcription (24, 49) depend upon interaction with the RNAP α subunit (1). Although multiple phased A-tracts result in the macroscopic curvature that confers aberrant gel electrophoretic mobility (35), this macroscopic curvature does not appear to be essential for UP element function. A single A-tract in the -40 region can have a large effect on transcription (14), and some UP elements (e.g., *rmB* P1) do not display such curvature (20).

Sites of enhanced DNase I cleavage have been observed in the footprints of proteins known to bend DNA, such as FIS and CRP (15, 34). The enhanced DNase I cleavage sites in the upstream regions of several of the promoter-RNAP complexes analyzed here (Fig. 4 and 5) and in a consensus UP element (13) suggest that DNA distortion occurs upon α subunit binding. These upstream hypersensitive sites also indicate that one face of the DNA helix is accessible to other proteins (DNase I in this case) when α subunit is bound and that α subunit and an activator protein could interact simultaneously on different surfaces of an upstream sequence, as suggested for the Ada protein at the *ada* and *aidB* promoters (36).

UP element position and size. The upstream sequences characterized in this and previous work were located primarily between -40 and -60 (Fig. 1 and 5 and Tables 2 and 3). However, DNA sequences smaller than 20 bp in length can function as UP elements to increase transcription. For example, the proximal or distal portions of the *rmB* P1 UP element confer partial UP element function (14, 44, 50). Similarly, some of the UP elements described in this paper (e.g., *rmB* P2 [described above]) are likely to utilize only a limited portion of the upstream region for α subunit interactions.

Sequences upstream of -60 may also interact directly with RNAP and contribute to transcription in some promoters. In the *rmB* P1 promoter, sequence upstream of -60 increases activity about 1.5-fold in vivo in the absence of the activator protein FIS (13, 50), and the distal portion of the *rmB* P1 UP element (the -50 region) retains full function and protection in footprints when moved one turn of the DNA helix upstream of its normal position (44). RNAP also protects DNA upstream of -60 in some promoters, for example, in the *B. subtilis hag* promoter (18). The α CTD can also affect transcription when positioned several turns upstream of its normal location in complexes formed with the activator protein CRP (4, 43, 55). This variability in positioning of α subunit binding sites most likely results from the flexible linker connecting the α CTD to the α subunit N-terminal domain (5, 32).

The downstream boundary of the UP element region is at around position -40, since sequence between -38 and -40 was not strongly conserved in the in vitro-selected UP elements (13), and substitutions at these positions had only minor effects on transcription (14). We note that the identity of the residue directly adjacent to the -35 hexamer (-37 in our numbering system [see Fig. 5]) is very important to transcription of the *rmB* P1, λp_R , and *lac* promoters (2, 10, 33). The effects of this residue on *rmB* P1 function are independent of the α CTD, suggesting that it plays a role in σ , not α subunit, interactions (2).

Modular structure of promoters. In summary, we conclude that UP elements occur in a variety of promoters, where they make different contributions to promoter strength. Thus, promoters can be considered as modular composites of a series of at least three RNAP recognition elements: the -10 and -35 hexamers and the UP element. (Our studies do not exclude the possibility of additional RNAP recognition determinants as well, e.g., in downstream regions [7].) Together these RNAP recognition elements confer appropriate basal activity for a particular promoter in the absence of transcription factors. Individual promoters need not contain significant information in all promoter modules, and many promoters have evolved to utilize transcription factors that respond to specific environmental signals in lieu of a particular interaction.

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REFERENCES

- Aiyar, S. E., R. L. Gourse, and W. Ross. Upstream A-tracts increase bacterial promoter activity through interactions with the RNA polymerase α subunit. Submitted for publication.
- Aiyar, S. E., R. L. Gourse, and W. Ross. Unpublished observations.
- Banner, C. D., C. P. Moran, Jr., and R. Losick. 1983. Deletion analysis of a complex promoter for a developmentally regulated gene from *Bacillus subtilis*. *J. Mol. Biol.* **168**:351–365.
- Belyaeva, T. A., V. A. Rhodius, C. L. Webster, and S. J. W. Busby. 1998. Transcription activation at promoters carrying tandem DNA sites for the *Escherichia coli* cyclic AMP receptor protein: organisation of the RNA polymerase α subunits. *J. Mol. Biol.* **277**:789–804.
- Blatter, E. E., W. Ross, H. Tang, R. L. Gourse, and R. H. Ebright. 1994. Domain organization of RNA polymerase α subunit: C-terminal 85 amino acids constitute a domain capable of dimerization and DNA binding. *Cell* **78**:889–896.
- Bracco, L., D. Kotlarz, A. Kolb, S. Diekmann, and H. Buc. 1989. Synthetic curved DNA sequences can act as transcriptional activators in *Escherichia coli*. *EMBO J.* **8**:4289–4296.
- Bujard, H., M. Brenner, U. Deuschle, W. Kammerer, and R. Knaus. 1987. Structure-function relationship of *Escherichia coli* promoters, p. 95–103. In W. S. Reznikoff et al. (ed.), RNA polymerase and the regulation of transcription. Elsevier, New York, N.Y.
- Craig, M. L., W.-C. Suh, and M. T. Record, Jr. 1995. HO $^{\cdot}$ and DNase I probing of E σ^{70} RNA polymerase- σ^{70} promoter open complexes: Mg $^{2+}$ binding and its structural consequences at the transcription start site. *Biochemistry* **34**:15624–15632.
- Czarniecki, D., R. J. Noel, Jr., and W. S. Reznikoff. 1997. The -45 region of the *Escherichia coli lac* promoter: CAP-dependent and CAP-independent transcription. *J. Bacteriol.* **179**:423–429.
- Dickson, R. C., J. Abelson, P. Johnson, W. S. Reznikoff, and W. M. Barnes. 1977. Nucleotide sequence changes produced by mutations in the *lac* promoter of *Escherichia coli*. *J. Mol. Biol.* **111**:65–75.
- Dombroski, A. J., W. A. Walter, M. T. Record, Jr., D. A. Siegle, and C. A. Gross. 1992. Polypeptides containing highly conserved regions of transcription initiation factor σ^{70} exhibit specificity of binding to promoter DNA. *Cell* **70**:501–512.
- Ellinger, T., D. Behnke, R. Knaus, H. Bujard, and J. D. Gralla. 1994. Context-dependent effects of upstream A-tracts. Stimulation or inhibition of *Escherichia coli* promoter function. *J. Mol. Biol.* **239**:466–475.
- Estrem, S. T., T. Gaal, W. Ross, and R. L. Gourse. 1998. Identification of an UP element consensus sequence for bacterial promoters. *Proc. Natl. Acad. Sci. USA* **95**:9761–9766.
- Estrem, S. T., W. Ross, S. Chen, T. Gaal, W. Niu, R. H. Ebright, and R. L. Gourse. Unpublished observations.
- Finkel, S. E., and R. C. Johnson. 1992. The Fis protein: it's not just for DNA inversion anymore. *Mol. Microbiol.* **6**:3257–3265.
- Flatow, U., G. V. Rajendrakumar, and S. Garges. 1996. Analysis of the spacer DNA between the cyclic AMP receptor protein binding site and the *lac* promoter. *J. Bacteriol.* **178**:2436–2439.
- Fong, R. S., S. Woody, and G. N. Gussin. 1994. Direct and indirect effects of mutations in lambda P_{RM} on open complex formation at the divergent P_R promoter. *J. Mol. Biol.* **240**:119–126.
- Fredrick, K., T. Caramori, Y.-F. Chen, A. Galizzi, and J. D. Helmann. 1995. Promoter architecture in the flagellar regulon of *Bacillus subtilis*: high-level expression of flagellin by the σ^D RNA polymerase requires an upstream promoter element. *Proc. Natl. Acad. Sci. USA* **92**:2582–2586.
- Frisby, D., and P. Zuber. 1991. Analysis of the upstream activating sequence and site of carbon and nitrogen source repression in the promoter of an early-induced sporulation gene of *Bacillus subtilis*. *J. Bacteriol.* **173**:7557–7564.
- Gaal, T., L. Rao, S. T. Estrem, J. Yang, R. M. Wartell, and R. L. Gourse. 1994. Localization of the intrinsically bent DNA region upstream of the *E. coli rrmB* P1 promoter. *Nucleic Acids Res.* **22**:2344–2350.
- Gaal, T., W. Ross, E. E. Blatter, H. Tang, X. Jia, V. V. Krishnan, N. Assa-Munt, R. H. Ebright, and R. L. Gourse. 1996. DNA-binding determinants of the α subunit of RNA polymerase: novel DNA-binding domain architecture. *Genes Dev.* **10**:16–26.
- Gaal, T., M. S. Bartlett, W. Ross, C. L. Turnbough, Jr., and R. L. Gourse. 1997. Transcription regulation by initiating NTP concentration: rRNA synthesis in bacteria. *Science* **278**:2092–2097.
- Galas, D. J., M. Eggert, and M. S. Waterman. 1985. Rigorous pattern-recognition methods for DNA sequences. Analysis of promoter sequences from *Escherichia coli*. *J. Mol. Biol.* **186**:117–128.
- Gartenberg, M. R., and D. M. Crothers. 1991. Synthetic DNA bending sequences increase the rate of in vitro transcription initiation at the *Escherichia coli lac* promoter. *J. Mol. Biol.* **219**:217–230.
- Giladi, H., K. Murakami, A. Ishihama, and A. B. Oppenheim. 1996. Identification of an UP element within the IHF binding site at the $P_{L1-P_{L2}}$ tandem promoter of bacteriophage λ . *J. Mol. Biol.* **260**:484–491.
- Grana, D., T. Gardella, and M. M. Susskind. 1988. The effects of mutations in the *ant* promoter of phage P22 depend on context. *Genetics* **120**:319–327.
- Graves, M. C., and J. C. Rabinowitz. 1986. In vivo and in vitro transcription of the *Clostridium pasteurianum* ferredoxin gene. Evidence for "extended" promoter elements in gram positive organisms. *J. Biol. Chem.* **261**:11409–11415.
- Harley, C. B., and R. P. Reynolds. 1987. Analysis of *E. coli* promoter sequences. *Nucleic Acids Res.* **15**:2343–2361.
- Hawley, D. K., and W. R. McClure. 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. *Nucleic Acids Res.* **11**:2237–2255.
- Helmann, J. D. 1995. Compilation and analysis of *Bacillus subtilis* σ^A -dependent promoter sequences: evidence for extended contact between RNA polymerase and upstream promoter DNA. *Nucleic Acids Res.* **23**:2351–2360.
- Hsu, L. M., J. K. Giannini, T.-W. C. Leung, and J. C. Crosthwaite. 1991. Upstream sequence activation of *Escherichia coli argT* promoter in vivo and in vitro. *Biochemistry* **30**:813–822.
- Jeon, Y. H., T. Yamazaki, T. Otomo, A. Ishihama, and Y. Kyogoku. 1997. Flexible linker in the RNA polymerase alpha subunit facilitates the independent motion of the C-terminal activator contact domain. *J. Mol. Biol.* **267**:953–962.
- Josaitis, C. A., T. Gaal, W. Ross, and R. L. Gourse. 1990. Sequences upstream of the -35 hexamer of *rrmB* P1 affect promoter strength and upstream activation. *Biochim. Biophys. Acta* **1050**:307–311.
- Kolb, A., K. Igarashi, A. Ishihama, M. Lavigne, M. Buckle, and H. Buc. 1993. *E. coli* RNA polymerase, deleted in the C-terminal part of its α -subunit, interacts differently with the cAMP-CRP complex at the *lacP1* and at the *galP1* promoter. *Nucleic Acids Res.* **21**:319–326.
- Koo, H.-S., H.-M. Wu, and D. M. Crothers. 1986. DNA bending at adenine-thymine tracts. *Nature* **320**:501–506.
- Landini, P., and M. R. Volkert. 1995. RNA polymerase α subunit binding site in positively controlled promoters: a new model for RNA polymerase-promoter interaction and transcriptional activation in the *Escherichia coli ada* and *aidB* genes. *EMBO J.* **14**:4329–4335.
- Lavigne, M., M. Herbert, A. Kolb, and H. Buc. 1992. Upstream curved sequences influence the initiation of transcription at the *Escherichia coli* galactose operon. *J. Mol. Biol.* **224**:293–306.
- Lisser, S., and H. Margalit. 1993. Compilation of *E. coli* mRNA promoter sequences. *Nucleic Acids Res.* **21**:1507–1516.
- McAllister, C. F., and E. C. Achberger. 1988. Effect of polyadenine-containing curved DNA on promoter utilization in *Bacillus subtilis*. *J. Biol. Chem.* **263**:11743–11749.
- McAllister, C. F., and E. C. Achberger. 1989. Rotational orientation of upstream curved DNA affects promoter function in *Bacillus subtilis*. *J. Biol. Chem.* **264**:10451–10456.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Mulligan, M. E., D. K. Hawley, R. Enriken, and W. R. McClure. 1984. *Escherichia coli* promoter sequences predict in vitro RNA polymerase selectivity. *Nucleic Acids Res.* **12**:789–800.
- Murakami, K., J. T. Owens, T. A. Belyaeva, C. F. Meares, S. J. Busby, and A. Ishihama. 1997. Positioning of two alpha subunit carboxy-terminal domains of RNA polymerase at promoters by two transcription factors. *Proc. Natl. Acad. Sci. USA* **94**:11274–11278.
- Newlands, J., C. Josaitis, W. Ross, and R. L. Gourse. 1992. Both FIS-dependent and factor-independent upstream activation of the *rrmB* P1 promoter are face of the helix dependent. *Nucleic Acids Res.* **20**:719–726.
- Newlands, J. T., W. Ross, K. K. Gosink, and R. L. Gourse. 1991. Factor-

- independent activation of *Escherichia coli* rRNA transcription. II. Characterization of complexes of *mmB* P1 promoters containing or lacking the upstream activator region with *Escherichia coli* RNA polymerase. *J. Mol. Biol.* **220**:560–583.
46. O'Halloran, T. V., B. Frantz, M. K. Shin, D. M. Ralston, and J. G. Wright. 1989. The MerR heavy metal receptor mediates positive activation in a topologically novel transcription complex. *Cell* **56**:119–129.
 47. Ozoline, O. N., and M. A. Tsyganov. 1995. Structure of open promoter complexes with *Escherichia coli* RNA polymerase as revealed by the DNase I footprinting technique: compilation analysis. *Nucleic Acids Res.* **23**:4533–4541.
 48. Ozoline, O. N., A. A. Deev, and M. V. Arkhipova. 1997. Non-canonical sequence elements in the promoter structure. Cluster analysis of promoters recognized by *Escherichia coli* RNA polymerase. *Nucleic Acids Res.* **25**:4703–4709.
 49. Perez-Martin, J., F. Rojo, and V. de Lorenzo. 1994. Promoters responsive to DNA bending: a common theme in prokaryotic gene expression. *Microbiol. Rev.* **58**:268–290.
 50. Rao, L., W. Ross, J. A. Appleman, T. Gaal, S. Leirimo, P. Schlax, M. T. Record, and R. L. Gourse. 1994. Factor independent activation of *mmB* P1. An "extended" promoter with an upstream element that dramatically increases promoter strength. *J. Mol. Biol.* **235**:1421–1435.
 51. Ross, W. Unpublished observations.
 52. Ross, W., J. F. Thompson, J. T. Newlands, and R. L. Gourse. 1990. *E. coli* Fis protein activates ribosomal RNA transcription *in vitro* and *in vivo*. *EMBO J.* **9**:3733–3742.
 53. Ross, W., K. K. Gosink, J. Salomon, K. Igarashi, C. Zou, A. Ishihama, K. Severinov, and R. L. Gourse. 1993. A third recognition element in bacterial promoters: DNA binding by the alpha subunit of RNA polymerase. *Science* **262**:1407–1413.
 54. Sander, P., W. Langert, and K. Mueller. 1993. Mechanisms of upstream activation of the *mmD* promoter P₁ of *Escherichia coli*. *J. Biol. Chem.* **268**:16907–16916.
 55. Savery, N. J., V. A. Rhodius, H. J. Wing, and S. J. W. Busby. 1995. Transcription activation at *Escherichia coli* promoters dependent on the cyclic AMP receptor protein: effects of binding sequences for the RNA polymerase α subunit. *Biochem. J.* **309**:77–83.
 56. Schickor, P., W. Metzger, W. Werel, H. Lederer, and H. Heumann. 1990. Topography of intermediates in transcription initiation of *E. coli*. *EMBO J.* **9**:2215–2220.
 57. Suck, D., and C. Oefner. 1986. Structure of DNase I at 2.0 Å resolution suggests a mechanism for binding to and cutting DNA. *Nature* **321**:620–625.
 58. Suh, W.-C., W. Ross, and M. T. Record, Jr. 1993. Two open complexes and a requirement for Mg²⁺ to open the λP_R transcription start site. *Science* **259**:358–361.
 59. Tang, H., K. Severinov, A. Goldfarb, D. Fenyo, B. Chait, and R. H. Ebricht. 1994. Location, structure, and function of the target of a transcriptional activator protein. *Genes Dev.* **8**:3058–3067.
 60. van Putten, A. J., R. de Lang, E. Veltkamp, H. J. J. Nijkamp, P. van Solingen, and J. A. van den Berg. 1986. Methylation-dependent transcription controls plasmid replication of the CloDF13 *cop-I*(Ts) mutant. *J. Bacteriol.* **168**:728–733.
 61. van Ulzen, P., M. Hillebrand, M. Kainz, R. Collard, L. Zulianello, P. van de Putte, R. L. Gourse, and N. Goosen. 1997. Function of the C-terminal domain of the alpha subunit of *Escherichia coli* RNA polymerase in basal expression and integration host factor-mediated activation of the early promoter of bacteriophage Mu. *J. Bacteriol.* **179**:530–537.
 62. Youderian, P., S. Bouvier, and M. M. Susskind. 1982. Sequence determinants of promoter activity. *Cell* **30**:843–853.
 63. Young, M. A., J. Srinivasan, I. Goljer, S. Kumar, D. L. Beveridge, and P. H. Bolton. 1995. Structure determination and analysis of local bending in an A-tract DNA duplex: comparison of results from crystallography, nuclear magnetic resonance, and molecular dynamics simulation on d(CGCAAAA ATGCG). *Methods Enzymol.* **261**:121–144.