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UP element-dependent transcription at the *Escherichia coli rrnB* P1 promoter: positional requirements and role of the RNA polymerase α subunit linker

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ABSTRACT

The UP element stimulates transcription from the *rrnB* P1 promoter through a direct interaction with the C-terminal domain of the RNA polymerase α subunit (αCTD). We investigated the effect on transcription from *rrnB* P1 of varying both the location of the UP element and the length of the α subunit interdomain linker, separately and in combination. Displacement of the UP element by a single turn of the DNA helix resulted in a large decrease in transcription from *rrnB* P1, while displacement by half a turn or two turns totally abolished UP element-dependent transcription. Deletions of six or more amino acids from within the α subunit linker resulted in a decrease in UP element-dependent stimulation, which correlated with decreased binding of αCTD to the UP element. Increasing the α linker length was less deleterious to RNA polymerase function at *rrnB* P1 but did not compensate for the decrease in activation that resulted from displacing the UP element. Our results suggest that the location of the UP element at *rrnB* P1 is crucial to its function and that the natural length of the α subunit linker is optimal for utilisation of the UP element at this promoter.

INTRODUCTION

Escherichia coli RNA polymerase holoenzyme (RNAP) is a multi-subunit complex consisting of an α subunit homodimer, single β, β′ and ω subunits, and one of several σ subunit species (1,2). During transcription initiation at many promoters, RNAP containing the major σ factor, σ^{70}, recognises two hexameric promoter elements located ~10 and 35 bp upstream of the transcription start point (+1) (3). Recognition of the –10 and –35 promoter elements requires regions 2.3–2.5 and 4.2 of σ^{70}, respectively (4,5). However, a third promoter element, the UP element, is frequently required for full promoter activity (6,7). UP elements interact directly with the C-terminal domain of the α subunit of RNAP (αCTD) (8,9) in holoenzymes containing σ^{70} or alternative σ factors (10–12).

UP elements consist of A+T-rich sequences located upstream of the –35 region at many promoters (6,8,13). At the most well-studied UP element-dependent promoter, *rrnB* P1, the UP element comprises DNA sequences from approximately positions –40 to –60 and is composed of two subsites centred at about –42 and –52 (termed the proximal and distal subsites, respectively), each of which can bind an αCTD (6,14–17). The interaction between αCTD and the UP element results in an increase in K_B, the initial equilibrium constant for RNAP binding to the promoter, but may also affect later steps in the pathway to open complex formation (16,18). The αCTD–UP element interaction is responsible for a 30–70-fold increase in *in vivo* promoter activity at *rrnB* P1 (8,16,17). The optimised UP element sequence ('consensus' UP element) contains alternating A- and T-tracts and is ~5-fold more effective than the wild type *rrnB* P1 UP element (17). UP elements can also be transposed as a separate module from one promoter to another where they retain their ability to stimulate transcription in a factor-independent manner (8,13,16).

Each α subunit consists of 329 amino acids organised in two independently folding domains (9,19). The N-terminal domain (αNTD; residues 8–231) contains determinants for dimerisation and assembly into RNAP (20,21) and plays a role in transcription activation at some promoters (22,23). αCTD (residues 249–329) (24) plays roles in transcription initiation, elongation and termination (25–27). During transcription initiation, αCTD provides a contact site for many transcription activators (25,28,29) and is directly involved in UP element recognition (8,30). αCTD folds into a $(HhH)_2$ domain (31) in which key residues interacting with DNA reside within the loop and second helix of HhH1 and HhH2, respectively (32). Based on genetic, biochemical and NMR studies of the αCTD–DNA interaction, and on the X-ray structure of the RuvA–

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DNA complex, the α $(HhH)_2$ domain is thought to interact in a sequence-specific way with bases in the minor groove and with the DNA backbone across the minor groove (30,32–35).

Several lines of evidence indicate that a flexible linker connects αCTD to αNTD. First, limited proteolysis studies pointed to an accessible region between amino acids 234 and 249 of *E.coli* α (9,19). Secondly, NMR analysis of an isolated C-terminal fragment of α (amino acids 233–329) showed that a region of at least 13 amino acids, extending from D233 to E248, exhibits a high degree of flexibility (36). This is consistent with the crystallographic data for αNTD, where the C-terminal limit of helix 3 is assigned to F231 (21). Thirdly, amino acid sequence alignments of α subunits from eubacteria and chloroplasts reveal a non-conserved sequence of variable length corresponding to amino acids V237–P251 of the *E.coli* α subunit (37). Consistent with its proposed role as a linker, the introduction of substantial deletions or insertions into this region does not affect the efficiency of assembly of α into RNAP, nor do they affect transcription from activator-independent and UP element-independent promoters (37,38). However, deletion of three amino acids from within the linker greatly reduces transcription from a promoter where the activator protein CRP binds to a site centred at –61.5 (Class I), whereas transcription is enhanced from a promoter where CRP binds at –41.5 (Class II). This result, together with the observation that αCTD tethered by a shorter linker is still recruited to DNA sequences upstream of the CRP binding site at the Class II promoter, implies that the α linker deletion compromises the motional freedom of αCTD rather than its inherent ability to bind DNA (37,38).

Although potential UP elements or UP element subsites have been identified in the –40 to –60 region at many promoters, in most cases such searches have not been extended to locations further upstream (6,8,13,17). The recent demonstration that αCTD is able to contact DNA near positions –43, –53, –63, –73, –83 and –93 at the *lac* promoter, unassisted by CRP (39), suggests that the length of the α linker is unlikely to restrict access of αCTD to UP elements located upstream of –60. However, the question of whether UP elements are able to stimulate transcription efficiently from distant locations has not been satisfactorily addressed.

Gourse and colleagues studied effects of UP element location before the UP element subsites were fully defined (15). They observed that upstream displacement of sequences between positions –47 and –87 by a single turn of the helix did not affect transcription from the *rrnB* P1 promoter, whereas displacement by two or more turns exerted an inhibitory effect on transcription. However, as the proximal UP element subsite was retained in its normal location in these early experiments, conclusions concerning displacement of the full UP element must be re-evaluated. In cases where UP elements have been identified upstream of the –40 to –60 region, DNA bending by accessory DNA binding proteins such as IHF is also required for full activity (e.g. 12,40,41), presumably to bring α binding sequences closer to the core promoter. Therefore, in this work, we have examined the effect of upstream displacement of the full UP element on transcription from the *rrnB* P1 promoter in the absence of accessory factors. We have also investigated the role of α linker length in UP element function at the *rrnB* P1 promoter and the possibility that lengthening the α linker might alleviate the negative effect of UP element displacement. Our results demonstrate that the position of the full UP element is crucial to its function at the *rrnB* P1 promoter and that the length of the α subunit linker is optimised for utilisation of UP elements in their normal position at *rrn* P1 promoters.

MATERIALS AND METHODS

rrnB P1 promoter fragments

The *E.coli* strains and plasmids used in this work are listed in Table 1. Standard molecular biology techniques for plasmid isolation and DNA manipulation were used throughout (42). All *rrnB* P1 promoter derivatives used for transcription assays or single copy *lacZ* fusion analysis were cloned as *Eco*RI–*Hin*dIII fragments into the transcription vector pRLG770 or λ phage system I (16), respectively, and possess a downstream end point at position +52 with respect to the *rrnB* P1 transcription initiation site. Upstream end points are at position –66 for promoter fragments present in pRLG4238, pRLG3278, pRLG4213, pRLG4214 and pRLG4210, and in the *lacZ* fusions present in RLG3074, RLG4192, RLG3097 and RLG2263, but contain different sequences from –38 to –59 (6,17). Promoter fragments present in plasmids pRLG4713–pRLG4717, pRLG4719 and pRLG4720 (and the corresponding *lacZ* fusions present in RLG4721–RLG4727) possess wild type *rrnB* P1 sequences up to position –37, upstream of which is a consensus UP element located at various positions. These promoter fragments were constructed by amplifying the *rrnB* P1 core promoter region from pRLG4210 using primer RLG1620 (5′-GCGCTACGGCGTTTCACTTC-3′) (13), which anneals downstream of the pRLG770 *Hin*dIII site, as the reverse primer in each case and one of a series of upstream primers of the type 5′-GCGC*GAATTC***GGAAAATTTTTTT-TAAAAAAGA**X-3′ (*Eco*RI site italicised and UP element emboldened) where 'X' corresponds to sequences inserted between the displaced UP element and position –37 of the *rrnB* P1 promoter (see Fig. 1) plus, in some cases, additional *rrnB* P1 core promoter sequences (–37 to –16) to allow primer annealing (Table 2). The consensus UP element present in these derivatives is a combination of the individual SELEX selected consensus proximal and distal UP element subsites, and differs from the version constructed by Estrem *et al.* (6) only by the substitution of a thymine residue for an adenine at position –46 (underlined).

Subunit purification and reconstitution of RNAP

His-tagged RNAP α subunits containing wild type and modified linkers were overproduced in strain BL21(DE3) harbouring pHTT7f1NHα and the pHTT7f1NHα derivatives pMGM2, pMGM5–pMGM11, pMGM31 and pMGM34, which encoded α subunits with shorter or longer interdomain linkers (37). Following 3 h of induction of the plasmid-borne phage T7 promoter, α subunits were purified by Ni^{2+}-affinity chromatography as described previously (30,43). Preparation of inclusion bodies of RNAP β, β′ and σ subunits from strains XL1-Blue (pMKSe2), BL21(DE3) (pT7β′) and BL21(DE3) (pLHN12σ), respectively, and reconstitution of RNAP were performed as described previously (43).

In vitro transcription

Multiple round transcription reactions were performed at 25°C for 20 min in a volume of 25 µl containing 150 mM NaCl, 40 mM

Table 1. Bacterial strains and plasmids

	Designation	Genotype/Relevant characteristics	Source/reference
E.coli strains			
	BL21(DE3)	F⁻ *ompT hsdS* ($r_B^-m_B^-$) *gal* λDE3 lysogen	ref. 51
	XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17* ($r_K^-m_K^+$) *supE44 relA1 lac* λ⁻ [F' *lacI*^{q}*ZΔM15 proAB*$^+$ Tn*10*]	ref. 52
	NK5031	F⁻ *lacZΔM5265 supF Nal*^{R}	ref. 53
	RLG1336	NK5031/λ M13 MCS-*lacZ*	ref. 44
	RLG2263	NK5031/λ core *rrnB* P1-*lacZ*[a]	ref. 16
	RLG3074	NK5031/λ wild-type *rrnB* P1-*lacZ*	ref. 17
	RLG3097	NK5031/λ core *rrnB* P1-*lacZ*[a]	ref. 17
	RLG4192	NK5031/λ consensus *rrnB* P1-*lacZ*[b]	ref. 17
	RLG4721	NK5031/λ consensus *rrnB* P1-*lacZ*[b]	This work
	RLG4722-RLG4727	RLG4721 with UP element displaced by 5, 11, 16, 22, 27 and 33 bp	This work
Plasmids[c]			
	pHTT7f1NHα	T7 ϕ10 promoter directing expression of *rpoA* on pET21a. $(His)_6$-tag coding sequence present between first two codons of *rpoA*	ref. 43
	pHTT7f1NHαΔ235	pHTT7f1NHα deleted for the C-terminal 94 codons of *rpoA*	ref. 43
	pMGM1	pHTT7f1NHα with *Bsu*36I site overlapping codons 233-235 of *rpoA*	ref. 37
	pMGM2, pMGM5, PMGM6, pMGM34	pMGM1 derivatives containing deletions of 3, 6, 9 and 12 amino acids, respectively, within the linker coding region of *rpoA*	ref. 37
	pMGM7-pMGM11, pMGM31	pMGM1 derivatives containing insertions of 3, 6, 16, 13, 32 and 10 amino acids, respectively, within the linker coding region of *rpoA*	ref. 37
	pMKSe2	pUC19 derivative carrying *rpoB* (encoding RNA polymerase β subunit) under control of the *lac* promoter	ref. 54
	pT7β'	pT76 derivative carrying *rpoC* (encoding RNA polymerase β' subunit) under control of the T7 ϕ10 promoter	ref. 55
	pLHN12σ	pBR322 derivative carrying *rpoD* (encoding RNA polymerase σ subunit) under control of the T7 ϕ10 promoter	ref. 56
	pLAW2	pBR322 derivative carrying *rpoA* under *lpp/lacUV5* promoter control	ref. 57
	pMGM12	pLAW2 derivative containing the *rpoA* gene from pMGM1	ref. 37
	pMGM13, pMGM16, pMGM17, pMGM35	pLAW2 derivatives in which *rpoA* contains the modified linker coding sequences from pMGM2, pMGM5, pMGM6 and pMGM34	ref. 37
	pMGM18-pMGM22, pMGM32	pLAW2 derivatives in which *rpoA* contains the modified linker coding sequences from pMGM7-pMGM11 and pMGM31	ref. 37
	pRLG770	pKM2 derivative in which the *rrnB* T1T2 terminator has been inserted downstream of the multiple cloning site	ref. 44
	pRLG3278	pRLG770 containing *rrnB* P1 promoter with consensus UP element[b]	ref. 6
	pRLG4210	pRLG770 containing core *rrnB* P1 promoter[a]	ref. 17
	pRLG4213	pRLG770 containing *rrnB* P1 promoter with consensus proximal UP element subsite	ref. 6
	pRLG4214	pRLG770 containing *rrnB* P1 promoter with consensus distal UP element subsite	ref. 6
	pRLG4238	pRLG770 containing the wild-type *rrnB* P1 promoter	ref. 17
	pRLG4713	pRLG770 containing *rrnB* P1 promoter with consensus UP element[b]	This work
	pRLG4714-pRLG4717, pRLG4719, pRLG4720	pRLG4713 with UP element displaced by 5, 11, 16, 22, 27 and 33 bp	This work

[a]The core *rrnB* P1 promoter fragment in RLG3097 and pRLG4210 differs from that present in RLG2263 due to the presence of the SUB sequence (GACTGCAGTGGTACCTAGGAAT) from –38 to –59 and *rrnB* P1 sequences from –60 to –66 in the former. RLG2263 retains *rrnB* P1 sequences up to position –41 and does not contain the SUB sequence.

[b]The consensus UP element present in RLG4192 and pRLG3278 has the sequence GGAAAATTTTTTTTCAAAAGTA (–59 to –38) and retains *rrnB* P1 promoter sequences from –60 to –66. The consensus UP element in RLG4721 and pRLG4713 has the sequence GGAAAATTTTTTT-TAAAAAAGA (–59 to –38) and does not retain additional upstream *rrn*B P1 sequences.

[c]All plasmids encode resistance to ampicillin.

Tris–acetate pH 7.9, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 100 μg/ml bovine serum albumin (BSA), 500 μM ATP, 200 μM CTP, 200 μM GTP, 10 μM UTP and 3–5 μCi [α-^{32}P]UTP (10 mCi/ml, 800 Ci/mmol). Each reaction also contained 0.6 nM supercoiled plasmid DNA, prepared using the Qiagen plasmid midi kit and purified further by phenol–chloroform extraction. All plasmid templates for transcription assays were derivatives of the transcription vector pRLG770 (44) and, therefore, also result in the production of the RNA-I transcript (108 nucleotides). Transcription originating from *rrnB* P1 promoter fragments terminate at the *rrnB* T1T2 terminator present on the vector and give rise to transcripts of ~220 nucleotides in length. Reactions were initiated by addition of RNAP and terminated with 25 μl of stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol). Samples were fractionated on a 5.5% acrylamide gel containing 7 M urea and transcript abundance was quantified using a PhosphorImager. Native RNAP was a generous gift of R. Landick and was 60 ± 10% active in binding to the λP_R promoter (45). RNAP reconstituted with the wild type α

Table 2. Upstream primers used to construct *rrnB* P1 promoters with displaced consensus UP elements

UP element displacement (bp)	Sequence of upstream primer[a]	pRLG770 derivative	λ system I lysogen
0	5'-XCTTGTCAGGCCGGAATAACTCC-3'	pRLG4713	RLG4721
5	5'-X**GGAAT**CTTGTCAGGCCGGAATAAC-3'	pRLG4714	RLG4722
11	5'-X**TACCTAGGAAT**CTTGTCAGGCC-3'	pRLG4715	RLG4723
16	5'-X**AGTGGTACCTAGGAAT**CTTGTC-3'	pRLG4716	RLG4724
22	5'-X**GACTGCAGTGGTACCTAGGAAT**C-3'	pRLG4717	RLG4725
27	5'-X***CAGGC*GACTGCAGTGGTACCTAGGAAT**C-3'	pRLG4719	RLG4726
33	5'-X***GCACCCCAGGC*GACTGCAGTGGTACCTAGGAAT**C-3'	pRLG4720	RLG4727

[a]The 5′ end of each primer has the sequence 5′-GCGCGAATTC**GGAAAATTTTTTTTAAAAAAGA**-3′, denoted by an 'X' in the table, containing an *Eco*RI site (underlined) and a consensus UP element (emboldened). The *rrnB* P1 –35 region and SUB sequences are underlined and emboldened, respectively, in the table. *rrnB* P1 derivatives in which the consensus UP element has been displaced by 5–22 bp contain part or all of the SUB sequence (17) whereas derivatives in which this element has been displaced by 27 and 33 bp contain, in addition, sequences from –37 to –47 of the *lac* P1 promoter (italicised and emboldened), which do not possess inherent UP element-like activity (13). Zero base pair displacement corresponds to the wild type location for the UP element, i.e. positions –38 to –59.

subunit was 55% as active as native RNAP. Reconstituted RNAPs were used at concentrations that resulted in equivalent transcription from the core *rrnB* P1 promoter present on pRLG4210 (4.4 nM native RNAP and 8–28 nM reconstituted RNAP) (see legend to Fig. 3).

Hydroxyl radical footprinting

Fragments containing the wild type *rrnB* P1 promoter were prepared for footprinting by first linearising CsCl-purified pRLG4238 DNA with *Hin*dIII, then removing the 5′ terminal phosphates with calf intestinal alkaline phosphatase and subsequently making a second cut with *Aat*II. The smaller, 205 bp, DNA fragment was purified from a preparative polyacrylamide gel and was labelled on the 5′ hydroxyl at the *Hin*dIII terminus with 10 μCi [γ-^{32}P]ATP (3000 Ci/mmol) using 0.5–1.0 U T4 polynucleotide kinase for 30 min at 37°C. Unincorporated nucleotides were removed by a pass through a Sephadex G-50 spin column and the purified labelled DNA used for footprinting. Binding reactions contained 10 mM Tris–HCl pH 8.0, 30 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 100 μg/ml BSA, 500 μM ATP, 50 μM CTP, 1.0–4.0 nM labelled DNA fragment and RNAP reconstituted with wild type α (21 nM), Δ6 α (45 nM), Δ9 α (65 nM), Δ12 α (73 nM) or Δ235 α (72 nM), and were incubated at 37°C for 30 min. Prior to hydroxyl radical treatment, heparin was added to a final concentration of 10 μg/ml for 30 s, whereupon hydroxyl radical treatment was performed as previously described (46). The products of the footprinting reaction were fractionated on 8% polyacrylamide sequencing gels that were calibrated with Maxam–Gilbert DNA sequence ladders and resultant footprinting patterns were analysed using a phosphorimager.

Determination of *in vivo* promoter activity

Analysis of *rrnB* P1 promoter activity *in vivo* was performed using strain NK5031 lysogenic for λ system I derivatives (16) in which *rrnB* P1 promoter variants were transcriptionally fused to *trpB′A-lacZ*. Construction of recombinant phages harbouring fusions of *lacZ* to *rrnB* P1 derivatives in which the consensus UP element had been displaced was carried out as described previously (47–49) and monolysogens of NK5031 (i.e. RLG4721–RLG4727) were identified by a combination of a PCR-based assay (17) and β-galactosidase measurement. Overnight cultures of the *lacZ* fusion strains were diluted 1:50 into pre-warmed Luria–Bertani broth [containing ampicillin (100 μg/ml) and 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) when harbouring pLAW2 derivatives expressing mutant *rpoA* alleles] and grown at 30°C with vigorous shaking for three to four generations (OD_{600} ~0.4–0.6). β-Galactosidase activity was determined by the method of Miller (50) using the chloroform–SDS procedure and values were corrected for background β-galactosidase activity by assaying a lysogen containing a promoter-less *lacZ* fusion (RLG1336). Results are presented as percentages, with the strain harbouring the consensus *rrnB* P1–*lacZ* fusion assigned a value of 100% in experiments utilising untransformed strains, and strains harbouring pLAW2 or pMGM12 assigned a value of 100% when measurements were performed on transformants harbouring pLAW2 derivatives.

RESULTS

Effect of UP element displacement on transcription from the *rrnB* P1 promoter *in vitro* and *in vivo*

To investigate the relationship between UP element location and UP element-dependent stimulation of transcription from the *rrnB* P1 promoter, a series of *rrnB* P1 promoter derivatives were constructed in which the full consensus UP element was displaced upstream from the *rrnB* P1 core promoter by distances corresponding to 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 turns of the DNA helix, i.e. 5, 11, 16, 22, 27 and 33 bp, respectively (Fig. 1). These promoters were cloned into the transcription vector pRLG770 and their activities *in vitro* were compared with that of an *rrnB* P1 promoter derivative containing the consensus UP element in the wild type position, and to the core *rrnB* P1 promoter, in which the UP element is replaced by a sequence devoid of UP element-like activity (17). The results showed that whereas the presence of the consensus UP element in the wild type location resulted in a 29-fold increase in promoter activity relative to the core promoter, displacement

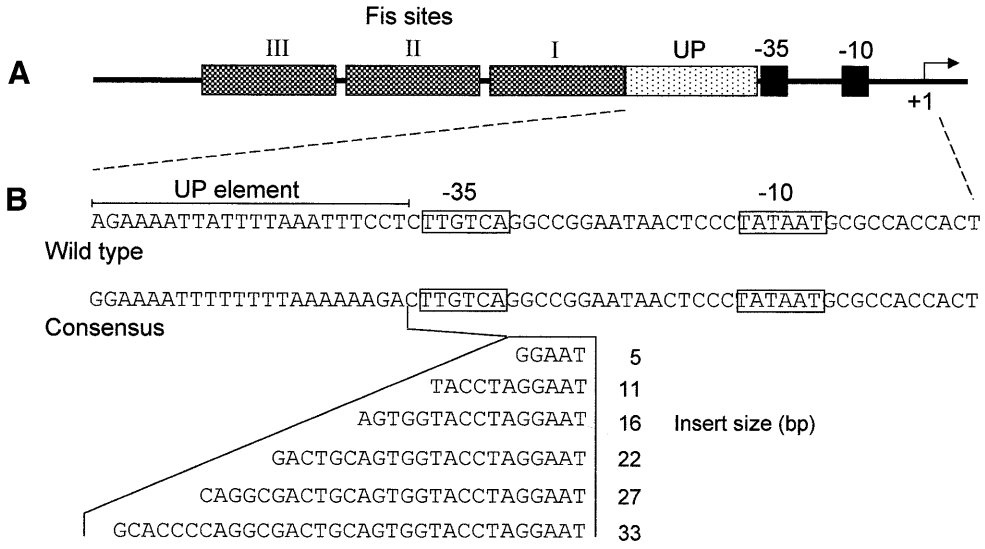


Figure 1. Displacement of the consensus UP element upstream of the *rrnB* P1 core region. (**A**) Organisation of the *rrnB* P1 promoter region. The *rrnB* P1 core region constitutes the DNA sequence from –37 to +1 with respect to the transcription initiation site (indicated with a bent arrow). Located immediately upstream of this region is the UP element (–38 to –59) and three tandem Fis sites. (**B**) DNA sequences from −59 to +3 of the wild type *rrnB* P1 promoter and of an *rrnB* P1 derivative containing the consensus UP element present in pRLG4713 and RLG4721. The −35 and –10 regions are boxed and the extent of the UP element sequence is indicated by a horizontal bar. DNA sequences inserted between positions –37 and –38 to displace the consensus UP element are indicated. In promoter derivatives where the UP element has been displaced by 5–22 bp these sequences comprise part or all of the SUB sequence (17), whereas derivatives in which this element has been displaced by 27 and 33 bp contain, in addition, sequences from –37 to –47 of the *lac* P1 promoter, which do not possess inherent UP element-like activity (13).

of the UP element by one helical turn (11 bp) resulted in only ~5-fold activation (Fig. 2A, compare lanes 2 and 4 with lane 1). Upstream displacement of the consensus UP element by 0.5, 1.5, 2, 2.5 or 3 turns of the DNA helix essentially abolished UP element-dependent stimulation (Fig. 2A). These results suggest that the location of the UP element at the wild type *rrnB* P1 promoter is optimal for UP element-dependent stimulation. The results also confirm the previous observation that activation of *rrnB* P1 by upstream sequences is face of the helix dependent (15).

To examine the effect of UP element displacement on transcription from *rrnB* P1 *in vivo*, the *rrnB* P1 promoter derivatives possessing displaced UP elements were cloned upstream of the *lacZ* gene on a recombinant λ phage and monolysogens were constructed (see Table 1). The results from β-galactosidase assays showed that UP element-dependent stimulation of transcription is maximal when the UP element is present in the wild type location (Fig. 2B). Displacement of the consensus UP element by 11 bp upstream from the wild type location gave rise to >20-fold decrease in transcriptional activity, whereas displacement of the UP element by any other distance, including displacement by only 5 bp, abolished UP element-dependent transcription (Fig. 2B). These results are fully consistent with the data obtained *in vitro* and emphasise the crucial importance of the position of the UP element to its function at *rrnB* P1.

Effect of α linker length on UP element-dependent transcription *in vitro*

To test the requirements for the RNAP α subunit interdomain linker at the *rrnB* P1 promoter *in vitro*, RNAPs were reconstituted using a series of 10 mutant α subunits possessing interdomain linkers of different lengths. These mutant derivatives have been described previously (37) and contain deletions of 3, 6, 9 and 12 amino acids (termed Δ3, Δ6, Δ9 and Δ12), and insertions of 3, 6, 10, 13, 16 and 32 amino acids (termed Ω3, Ω6, Ω10, Ω13, Ω16 and Ω32). Multiple round transcription assays were performed with template DNA containing the wild type *rrnB* P1 promoter, including its natural UP element, or *rrnB* P1derivatives possessing the consensus full UP element or either the consensus proximal or distal UP element subsites, instead of the *rrnB* P1 UP element (6,17). As transcription from the *rrnB* P1 core promoter in comparison to a control promoter, the RNA-I promoter, encoded on the same plasmid, was relatively unaffected by alterations to the length of the α interdomain linker, i.e. the ratio of transcripts originating from the core *rrnB* P1 and RNA-I promoters remained constant (Fig. 3A), transcription from the *rrnB* P1 core promoter was used to normalise the activities of the RNAP preparations containing interdomain linkers of different lengths.

At the wild type *rrnB* P1 promoter, a progressive decrease in RNAP activity was observed as the size of the deletion introduced into the α subunit linker was increased (Fig. 3B). The effect of linker deletion was similar for the *rrnB* P1 promoter containing the consensus UP element (Fig. 3C), although the extent of the decrease was less marked. Removal of 12 amino acids from the α linker resulted in an 80–90% decrease in transcriptional efficiency from the promoters with the wild type and consensus UP elements, almost as large a decrease as was observed following complete removal of the αCTD. Transcription from these promoters was much less sensitive to increases in the

length of the α linker. Thus, insertion of 16 amino acids resulted in only a 20–25% decrease in transcription from both promoters, and RNAP containing a 32 amino acid insertion in the α linker retained approximately half of wild type RNAP activity at these promoters.

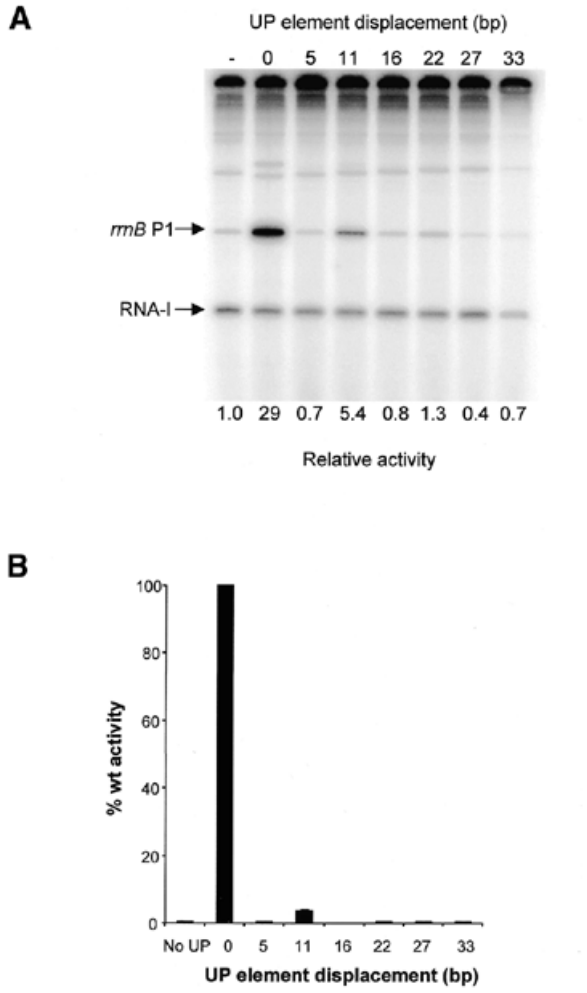


Figure 2. Effect of UP element displacement on transcription from *rrnB* P1 *in vitro* and *in vivo*. (**A**) Transcription gel showing the results of multiple round *in vitro* transcription reactions carried out with native RNAP on templates containing the *rrnB* P1 core promoter (pRLG4210) (denoted by a '–' sign), and the *rrnB* P1 promoter in which the consensus UP element is located at the normal position (pRLG4713) (indicated by a '0'), or displaced upstream by the indicated number of base pairs (pRLG4714–pRLG4720). The position of the transcripts derived from the vector-derived RNA-I promoter and the *rrnB* P1 promoter are indicated. Promoter activities, as determined from the transcript abundance in at least four transcription assays of the type shown, are presented below each gel lane as fold change in promoter activity relative to the core *rrnB* P1 promoter, where core promoter activity is assigned a value of 1.0. Values of less than unity arise through inhibition of core promoter activity. Standard deviations are within 18% of the mean and are omitted for clarity. (**B**) Effect of UP element displacement on transcription from *rrnB* P1 *in vivo*. β-Galactosidase activities were measured in lysogens of NK5031 harbouring a single copy *lacZ* fusion to the *rrnB* P1 core promoter (RLG3097) (denoted 'No UP') or the *rrnB* P1 promoter containing a consensus UP element located at the normal position (RLG4721) (indicated by a '0'), or displaced upstream of the normal location by the indicated number of base pairs (RLG4722–RLG4727). Values are expressed as a percentage of the activity in RLG4721 (100%) and are the means (with standard deviation) of three or more independent assays. 100% activity = 5780 Miller units.

The effect of altering α linker length on transcription from the *rrnB* P1 promoter containing only the consensus distal subsite or only the consensus proximal UP subsite was broadly similar to the overall pattern observed for the wild type *rrnB* P1 promoter, although the effect of deletion of three amino acids from the α linker was greater on transcription from *rrnB* P1 containing the proximal UP element subsite than on the other *rrnB* P1 derivatives (Fig. 3D and E).

Effect of α linker length on UP element-dependent transcription *in vivo*

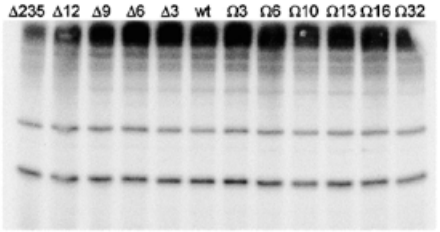
To investigate the effects of altering the length of the α interdomain linker on UP element-dependent transcription *in vivo*, plasmids expressing each of the mutant *rpoA* alleles were introduced into NK5031 harbouring single copy transcriptional fusions of the *lacZ* gene to various *rrnB* P1 promoter derivatives. β-Galactosidase activities were measured following induction of mutant α synthesis for three to four generations. In general agreement with our observations *in vitro*, decreasing the number of amino acids in the α linker caused a general decrease in the efficiency of transcription from the wild type *rrnB* P1 promoter, whereas only longer insertions within the linker (Ω16 and Ω32) decreased expression substantially (Fig. 4A). The overall pattern was similar at the consensus *rrnB* P1 promoter, although this promoter was less sensitive to changes in linker length than the wild type promoter (Fig. 4B).

Unlike the results *in vitro*, deletions of more than three amino acids from within the α linker strongly stimulated transcription from the core *rrnB* P1 promoter *in vivo* (Fig. 4C). Thus, cells containing RNAP in which the Δ12 α subunit was incorporated into RNAP gave rise to an ~15-fold increase in the β-galactosidase activity relative to cells expressing only wild type *rpoA*. Increasing the length of the α linker caused a gradual but much less profound increase in transcription from the core promoter. The increase in *rrnB* P1 core promoter activity *in vivo* can be interpreted in the context of our understanding of rRNA transcription regulation (58,59); i.e. decreased rRNA transcription (from decreased UP element function) leads to compensating derepression of *rrnB* P1 core promoter activity to keep total rRNA synthesis constant (see ref. 8 and Discussion). Figure 4D and E illustrates the effects of the altered linkers on UP element function *in vivo*, adjusted for these feedback effects. The fold increases in transcription resulting from the wild type *rrnB* P1 UP element (Fig. 4D) or from the consensus UP element (Fig. 4E) in the presence of each of the α linker mutants are presented as a percentage of the fold increases in transcription observed in the presence of wild type α. In this representation, it is obvious that the α linker deletions exert a strong negative effect on UP element function *in vivo*, consistent with their effects on UP element utilisation *in vitro*. Likewise, it is apparent that UP element-dependent function at *rrnB* P1 is also impaired by insertions within the α linker, although to a lesser degree.

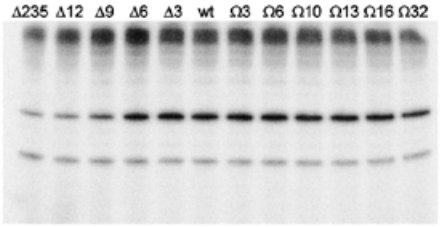
Effect of shortening the α linker on interactions between αCTD and the UP element at the *rrnB* P1 promoter

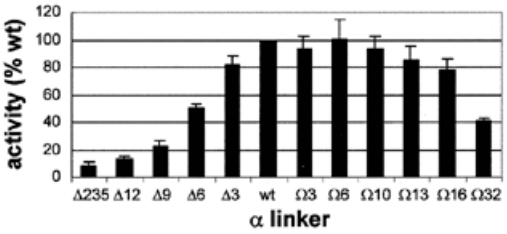
To determine whether there is a correlation between the effect of shortening the α interdomain linker on the activity of RNAP at the *rrnB* P1 promoter, and the ability of αCTD to bind the UP element, we carried out hydroxyl radical footprinting experiments on RNAP:*rrnB* P1 binary complexes. As previously

A core *rrnB* P1

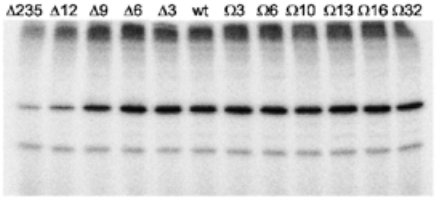


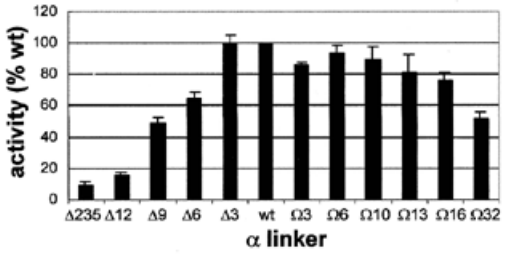
B wild type UP element



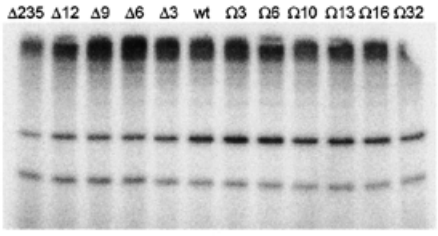


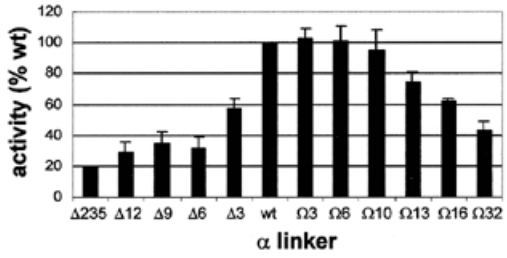
C consensus UP element



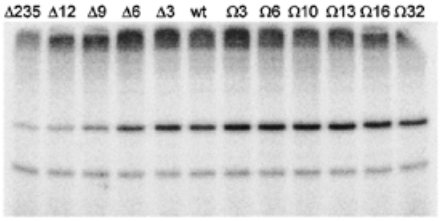


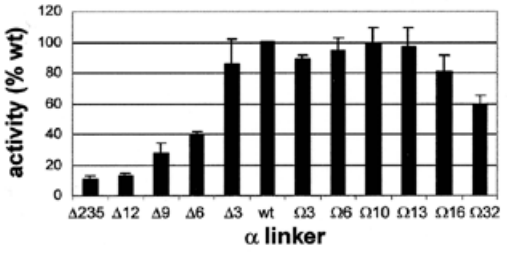
D proximal UP element subsite





E distal UP element subsite





observed, wild type RNAP protected a continuous region extending from approximately position –10 to +16 (not shown) and short regions around positions –20 and –30 (14) (Fig. 5A, lane 3, and B). In addition, short protected regions were observed around positions –43 and –53, corresponding to the sites of αCTD interaction within the proximal and distal UP element subsites (14,17). RNAP reconstituted with the Δ6 α derivative affords a similar degree of protection of the proximal and distal UP element subsites as wild type RNAP (Fig. 5A, lane 4, and B). However, protection of both UP element subsites was greatly diminished following deletion of a further three amino acids from the α linker (Δ9 RNAP) and was essentially abolished following deletion of 12 amino acids (Δ12 RNAP), without a concomitant reduction in the protection downstream of –40 (Fig. 5A, lanes 5 and 6, and B). RNAP reconstituted with the Δ235 α subunit failed to protect the UP element subsites from hydroxyl radical attack, as shown previously (8), confirming that the protections observed around –43 and –53 are due to αCTD (Fig. 5A, lane 7, and B). The pattern of protection produced in the core promoter region by each of the linker modified RNAPs and the Δ235 RNAP derivative was essentially identical to that afforded by wild type RNAP. These results indicate that as many as six amino acids can be deleted from the α interdomain linker without significantly compromising the ability of αCTD to engage the UP element subsites *in vitro*. The strong correlation between the transcriptional activity of the different RNAP deletion derivatives at *rrnB* P1 and their ability to protect the UP element subsites suggests that that the observed decrease in *in vitro* and *in vivo* transcriptional activity at the wild type *rrnB* P1 promoter upon shortening the α interdomain linker is due to a decreased ability to interact with the UP element.

Effect of increasing the length of the α interdomain linker on stimulation of transcription by displaced UP elements *in vitro* and *in vivo*

The footprinting experiments suggest that the 'reach' of αCTD is a function of linker length. Therefore, we used *in vitro* transcription assays to investigate whether tethering αCTD by a longer linker could improve the utilisation of displaced UP elements at *rrnB* P1. We observed no significant difference between the activity of RNAP reconstituted with the Ω13 α derivative and RNAP reconstituted with wild type α at the *rrnB* P1 promoter containing the UP element either in the normal position (as also shown in Fig. 3B) or when the UP element was displaced by one or two turns of the helix (Fig. 6A). A similar pattern was observed when RNAPs reconstituted with α mutants possessing 10 or 16 additional amino acids were compared with wild type RNAP (results not shown). We conclude that extending the α linker does not significantly enhance the response of RNAP to displaced UP elements at the *rrnB* P1 promoter *in vitro*.

To explore the effect of lengthening the α interdomain linker on the response of RNAP to displaced UP elements *in vivo*, the strains harbouring single copy fusions of *lacZ* to *rrnB* P1 promoters possessing displaced consensus UP elements were transformed with plasmids expressing wild type *rpoA*, or the Ω6 and Ω13 *rpoA* mutants. Consistent with our *in vitro* observations, β-galactosidase assays indicated that possession of an extended linker does not significantly increase the activity of RNAP at any of the promoters containing displaced UP elements (Fig. 6B).

Previous studies have demonstrated that displacement of the distal UP element subsite by one helical turn, while retaining the proximal subsite in its normal location, does not impair UP element-dependent transcription, whereas displacement of the distal subsite by two or three turns abolishes its contribution (15). To examine whether extending the α interdomain linker compensates for the negative effect of displacing the distal UP element subsite alone, we performed β-galactosidase measurements on a series of strains harbouring single copy fusions of the *lacZ* gene to *rrnB* P1 promoter derivatives in which the distal UP element subsite had been displaced by a half, one, two and three turns of the helix (15). Our results showed that the activity of all the promoter variants was unaffected in cells containing Ω6 or Ω13 α subunits (results not shown). These results suggest that extending the α subunit linker also does not compensate for the negative effect on transcription of displacement of the distal UP element subsite.

DISCUSSION

The experiments described here provide important information regarding the requirements for productive interactions between αCTD and UP elements. We have shown that upstream displacement of the full UP element by 5 bp or more greatly reduces UP element-dependent stimulation of transcription from *rrnB* P1. Our results are consistent with previous observations that αCTD can sometimes bind to the opposite face of the DNA helix to that occupied by the rest of RNAP, but that such interactions do not stimulate transcription and can in fact induce an inhibitory effect (60,61). As there appears to be a requirement for UP element:αCTD interactions to be located on the same face of the DNA helix at *rrnB* P1 as the rest of RNAP, adjacent to σ bound at the –35 region, it is possible that optimal UP element function requires contact between αCTD

Figure 3. (Previous page) Effect of α subunit linker length on transcription *in vitro* from *rrnB* P1 promoter derivatives. The left hand side of each panel shows the result of a typical transcription experiment assaying the activity of RNAPs reconstituted with wild type α or one of the mutant α subunits containing longer or shorter linkers, as indicated, at the core *rrnB* P1 promoter (**A**), and at *rrnB* P1 containing the wild type UP element (**B**), the consensus full UP element (**C**), the consensus proximal UP element subsite (**D**) and the consensus distal UP element subsite (**E**). RNAPs were used at a concentration which gave equivalent transcription from the *rrnB* P1 core promoter and were: Δ235α RNAP, 27.8 nM; Δ12α RNAP, 15.2 nM; Δ9α RNAP, 18.2 nM; Δ6α RNAP, 16.8 nM; Δ3α RNAP, 10.8 nM; wild type α RNAP, 8 nM; Ω3α RNAP, 12.6 nM; Ω6α RNAP, 12.2 nM; Ω10α RNAP, 19.6 nM; Ω13α RNAP, 14.6 nM; Ω16α RNAP, 19.8 nM; Ω32α RNAP, 27.0 nM. Transcripts arising from the *rrnB* P1 and RNA-I promoters are indicated by arrows. On the right of each transcription gel (B–E only) the relative abundance of the transcript originating from the corresponding *rrnB* P1 promoter derivative in the presence of each RNAP is shown (by definition, the relative abundance of transcripts originating from the *rrnB* P1 core promoter in the presence of each RNAP would be 100%). The values were calculated from at least three independent experiments and are presented as a percentage (with standard deviations) of transcript obtained with wild type RNAP.

and σ at some promoters. Recent evidence supports the importance of such an interaction at the *rrnB* P1 promoter containing the proximal UP element subsite (W.E.Ross and R.L.Gourse, unpublished results; 7,32) and at Class I CRP-dependent promoters (23; N.J.Savery, G.Lloyd, S.J.W.Busby, M.S.Thomas, R.H.Ebright and R.L.Gourse, unpublished results). Alternatively, it is possible that insertions between the –35 hexamer and the UP element result in perturbations in DNA structure that interfere with αCTD function.

Displacement of the full UP element by two (or more) turns upstream of *rrnB* P1 (i.e. moving the centres of the proximal and distal subsites to –64 and –75, respectively) essentially abolishes UP element-dependent stimulation, and displacement of the distal UP element subsite was shown previously to decrease the efficiency of αCTD binding (15). Conversely, experiments with promoters containing displaced CRP sites have revealed that αCTD can bind adjacent to CRP at positions as far as 85 bp upstream of the transcription start site (60,62). αCTD is also able to access naturally occurring UP elements located ~80 bp upstream of the *Pseudomonas putida Pu* promoter (12) or ~90 bp upstream of the λ P_L promoter (40,41). However, in these cases, the interaction between αCTD and more distant DNA binding sites is facilitated by the binding of the transcription factors CRP and IHF, respectively, to upstream sequences. Although αCTD can be crosslinked to DNA sites as far as 63, 73, 83 and 93 bp upstream of the *lac(ICAP)UV5* promoter in the absence of CRP (39), these presumably transient interactions do not facilitate transcription initiation.

Figures 3–5 indicate that there is a good correlation between α linker length, the ability of αCTD to access the UP element and the degree of UP element-dependent stimulation at the *rrnB* P1 promoter. Our observations on the effect of α linker deletion on transcription from the *rrnB* P1 promoter are also broadly similar to the results obtained by Fujita and colleagues (38), although they reported a more pronounced effect of the shorter deletion derivatives on transcription *in vitro*. In contrast, shortening the α subunit interdomain linker exerted a strong stimulatory effect on the core *rrnB* P1 promoter *in vivo* (Fig. 4C). This phenomenon was not observed for the UP element-independent *lacUV5* promoter (W.Meng, S.J.W.Busby and M.S.Thomas, unpublished results). The decreased efficiency of UP element utilisation by RNAPs containing deletions within the α subunit linker would be

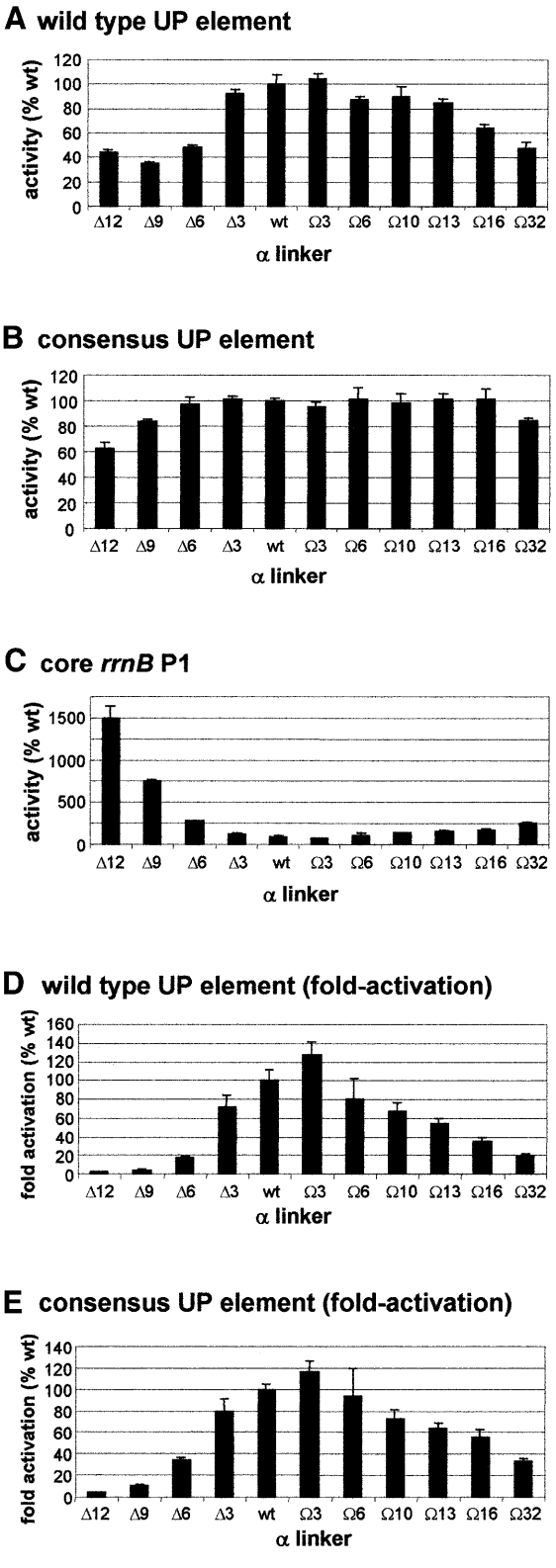


Figure 4. Effect of α subunit linker length on transcription *in vivo* from *rrnB* P1 promoter derivatives. β-Galactosidase activities were measured in lysogens of NK5031 harbouring a single copy *lacZ* fusion to (**A**) the *rrnB* P1 promoter with its natural UP element (RLG3074), (**B**) the *rrnB* P1 promoter with the consensus UP element (RLG4192) or (**C**) the *rrnB* P1 core promoter (RLG2263), in each case transformed with pMGM12, expressing wild type *rpoA*, or one of the pMGM12 derivatives expressing mutant *rpoA* alleles (pMGM13, pMGM16–pMGM22, pMGM32 or pMGM35), as indicated. For each *rrnB* P1 derivative, values are expressed as a percentage of the promoter activity in the presence of RNAP containing only wild type α, and are the means (with standard deviation) of three or more independent assays. 100% activity = 11.4, 1865 and 4983 Miller units, respectively, for the pMGM12 transformants of RLG2263 (core *rrnB* P1 promoter), RLG3074 (wild type *rrnB* P1 promoter) and RLG4192 (consensus *rrnB* P1 promoter). Fold activation due to the wild type (**D**) and consensus (**E**) UP elements in the presence of each mutant RNAP derivative is presented as a percentage of the fold activation in the presence of wild type RNAP (100%). Values were calculated by expressing the ratios of the wild type (or consensus) *rrnB* P1 promoter activity to the core *rrnB* P1 promoter activity for each RNAP derivative as a percentage of the ratio obtained in the presence of only wild type RNAP.

expected to result in a transient decrease in rRNA synthesis. As reported previously (8), when UP element function is compromised by mutations in *rpoA*, the activity of an *rrnB* P1 core promoter–*lacZ* fusion increases. For reasons that remain unclear, the magnitude of the 'derepression' observed in the presence of overexpressed linker modified α subunits was greater than the ~3-fold increase in core promoter activity measured in the presence of α subunits devoid of the C-terminal domain (8). The increase in UP element-independent promoter activity in the seven *rrn* operons responsible for rRNA biosynthesis in *E.coli* compensates for the decrease in UP element function and the resulting transient reduction in ribosome synthesis, a phenomenon referred to as 'rRNA feedback' (8,58,63). However, the effects of the α linker mutants on UP element function were apparent *in vivo* when the effects of rRNA feedback were taken into consideration (Fig. 4D and E).

Increasing the length of the α interdomain linker also impairs transcription from the *rrnB* P1 promoter, although RNAP appears less sensitive to linker insertions than deletions. This result indicates that the length of the wild type α interdomain linker is optimal for efficient utilisation of the UP element at *rrnB* P1. It is probable that the linker length adopted by bacterial RNAP results, at least in part, from selective pressure for UP element utilisation at rRNA promoters. In this context, it is interesting that chloroplasts contain α subunits possessing considerably longer linkers (37), suggesting that chloroplast RNAP is not subject to the same constraints as the bacterial enzyme.

Increasing the length of the linker by 16 amino acids (essentially doubling its length) would be expected to result in extending the 'reach' of αCTD by ~58 Å, enough to allow utilisation of UP elements positioned one or two turns upstream of the normal location (the pitch of B-form DNA is 34 Å per turn). As RNAP containing α subunits with linkers lengthened by up to 32 amino acids did not increase utilisation of displaced UP elements, it is possible that the α linkers containing insertions are not fully extended. Studies by Fujita and colleagues suggest, in fact, that segments of the α linker may adopt an ordered (helical) structure under certain conditions (38). In conjunction with the possible requirement for α–σ interactions and/or DNA structural requirements at the junction of the –35 region and UP element, the possibility that the linker has structure might explain why lengthening the linker does not extend the operational distance of αCTD with respect to UP element utilisation.

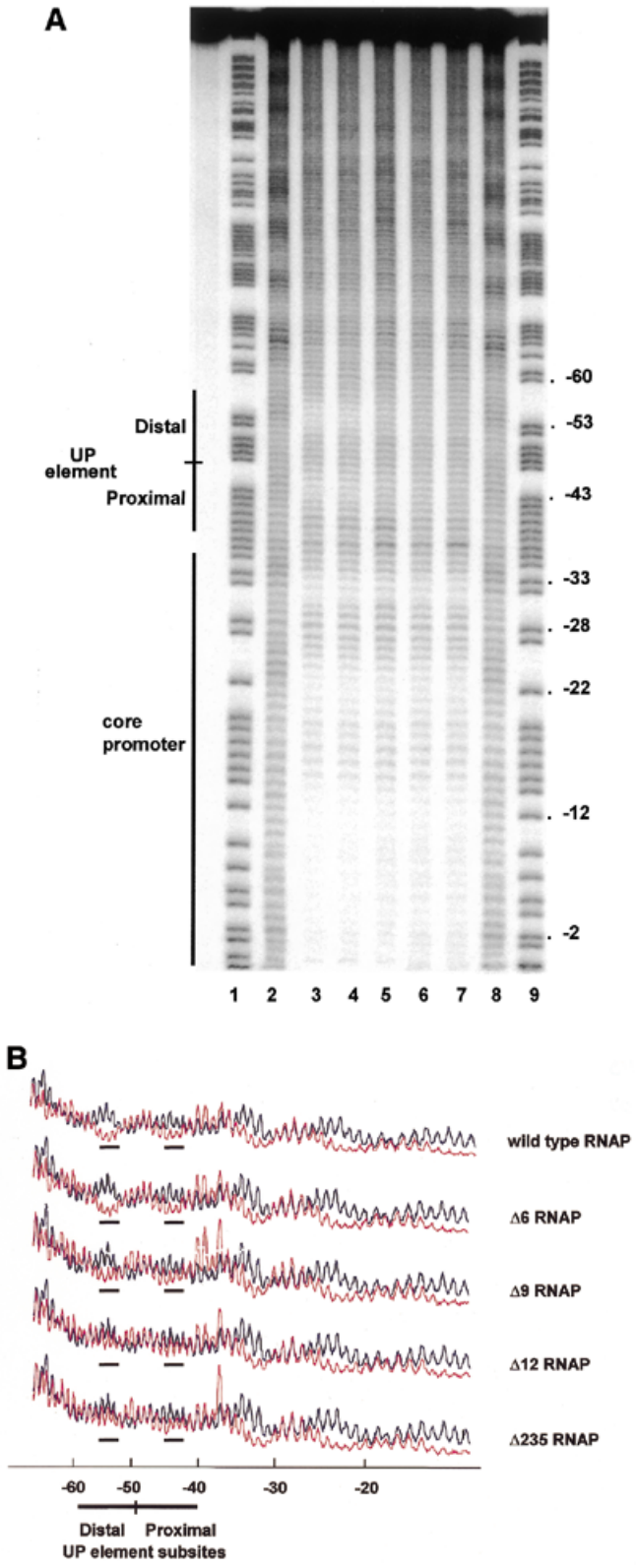


Figure 5. Hydroxyl radical cleavage of the wild type *rrnB* P1 promoter with wild type and mutant RNAPs. (**A**) Autoradiogram of a typical footprinting gel showing sites within the template (bottom) strand of the wild type *rrnB* P1 promoter which are protected from hydroxyl radicals by bound RNAP reconstituted with wild type or mutant α subunits. Lanes 1 and 9, Maxam–Gilbert G+A reaction; lanes 2 and 8, no RNAP; lane 3, wild type RNAP; lane 4, Δ6 RNAP; lane 5, Δ9 RNAP; lane 6, Δ12 RNAP; lane 7, Δ235 RNAP. Sequences comprising the core promoter element (as far downstream as +2) and the UP element subsites are indicated by black bars. (**B**) Scan of the footprinting pattern for each RNAP, reconstituted with the indicated α subunit (magenta trace), aligned in each case with the scan obtained in the absence of RNAP (black trace). The scans are averaged from three independent footprinting experiments. The regions in each scan corresponding to positions around –43 and –53 are underscored by a black bar.

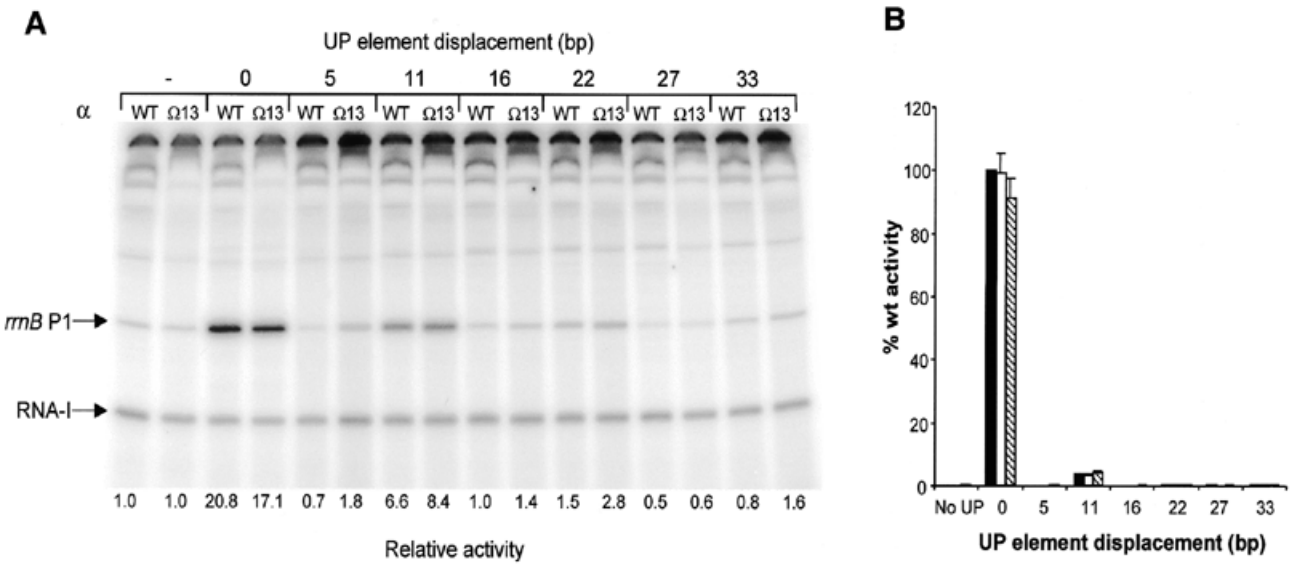


Figure 6. Effect of extended α subunit linker length on *in vitro* and *in vivo* RNAP activity at *rrnB* P1 promoters containing a displaced UP element. (**A**) Transcription gel showing the results of multiple round *in vitro* transcription reactions performed with RNAP reconstituted with wild type (WT) or Ω13 α subunits on templates containing the *rrnB* P1 core promoter (pRLG4210) (indicated by a '–' sign), and the *rrnB* P1 promoter in which the consensus UP element is located at the normal position (pRLG4713) (denoted by a '0'), or displaced by the indicated number of base pairs (pRLG4714–pRLG4720). The position of the transcripts derived from the RNA-I and *rrnB* P1 promoters are indicated. Promoter activities, as determined from the transcript abundance in at least four *in vitro* transcription reactions of the type shown, are presented below each gel lane as fold change in promoter activity relative to the *rrnB* P1 core promoter, where the core promoter activity is assigned a value of 1.0 for each RNAP tested. Values of less than unity arise through inhibition of core promoter activity. Standard deviations were within 22% of the mean and are omitted for clarity. (**B**) Effect of extended α subunit linker length on *in vivo* RNAP activity at *rrnB* P1 promoters containing a displaced UP element. β-Galactosidase activities were measured in lysogens of NK5031 harbouring a single copy *lacZ* fusion to the *rrnB* P1 core promoter (RLG3097) (indicated with a '–' sign) or the *rrnB* P1 promoter containing a consensus UP element located at the normal position (RLG4721) (denoted by a '0'), or displaced upstream of the normal location by the indicated number of base pairs (RLG4722–RLG4727). Each lysogen contained pLAW2, expressing wild type *rpoA* (black bars), or a pLAW2 derivative encoding the Ω6 α subunit (pMGM19; white bars) or the Ω13 α subunit (pMGM21; hatched bars). Values are expressed as a percentage of the activity in the RLG4721/pLAW2 transformant (100%) and are the means (with standard deviation) of three or more independent assays. 100% activity = 4897 Miller units.

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REFERENCES

1. Zhang,G., Campbell,E.A., Minakhin,L., Richter,C., Severinov,K. and Darst,S.A. (1999) Crystal structure of *Thermus aquaticus* core RNA polymerase at 3.3 Å resolution. *Cell*, **98**, 811–824.
2. Ebright,R.H. (2000) RNA polymerase: structural similarities between bacterial RNA polymerase and eucaryotic RNA polymerase II. *J. Mol. Biol.*, **304**, 687–698.
3. Mulligan,M.E., Hawley,D.K., Entriken,R. and McClure,W.R. (1984) *Escherichia coli* promoter sequences predict *in vitro* RNA polymerase selectivity. *Nucleic Acids Res.*, **12**, 789–800.
4. Darst,S.A., Roberts,J.W., Malhotra,A., Mark,M., Severinov,K. and Severinova,E. (1997) Pribnow box recognition and melting by *Escherichia coli* RNA polymerase. In Eckstein,F. and Lilley,D.M.J. (eds), *Nucleic Acids and Molecular Biology*, Vol. 11. Springer-Verlag, Berlin, pp. 27–40.
5. Barne,K.A., Bown,J.A., Busby,S.J.W. and Minchin,S.D. (1997) Region 2.5 of the *Escherichia coli* RNA polymerase σ^{70} subunit is responsible for the recognition of the 'extended –10' motif at promoters. *EMBO J.*, **16**, 4034–4040.
6. Estrem,S.T., Ross,W., Gaal,T., Chen,Z.W.S., Niu,W., Ebright,R.H. and Gourse,R.L. (1999) Bacterial promoter architecture: subsite structure of UP elements and interactions with the carboxy-terminal domain of the RNA polymerase α subunit. *Genes Dev.*, **13**, 2134–2147.
7. Gourse,R.L., Ross,W. and Gaal,T. (2000) UPs and downs in bacterial transcription initiation: the role of the alpha subunit of RNA polymerase in promoter recognition. *Mol. Microbiol.*, **37**, 687–695.
8. Ross,W., Gosink,K.K., Salomon,J., Igarishi,K., Zou,C., Ishihama,A., Severinov,K. and Gourse,R.L. (1993) A third recognition element in bacterial promoters: DNA binding by the α subunit of RNA polymerase. *Science*, **262**, 1407–1413.
9. Blatter,E.E., Ross,W., Tang,H., Gourse,R.L. and Ebright,R.H. (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.
10. Newlands,J.T., Gaal,T., Mecsas,J. and Gourse,R.L. (1993) Transcription of the *Escherichia coli rrnB* P1 promoter by the heat shock RNA polymerase (E sigma 32) *in vitro*. *J. Bacteriol*., **175**, 661–668.
11. Fredrick,K., Caramori,T., Chen,Y.F., Galizzi,A. and Helmann,J.D. (1995) Promoter architecture in the flagellar regulon of *Bacillus subtilis*: high-level expression of flagellin by the sigma D RNA polymerase requires an upstream promoter element. *Proc. Natl Acad. Sci. USA*, **92**, 2582–2586.
12. Bertoni,G., Fujita,N., Ishihama,A. and de Lorenzo,V. (1998) Active recruitment of σ^{54}-RNA polymerase to the *Pu* promoter of *Pseudomonas putida*: role of IHF and αCTD. *EMBO J.*, **17**, 5120–5128.
13. Ross,W., Aiyar,S.E., Salomon,J. and Gourse,R.L. (1998) *Escherichia coli* promoters with UP elements of different strength: modular structure of bacterial promoters. *J. Bacteriol*., **180**, 5375–5383.
14. Newlands,J.T., Ross,W., Gosink,K.K. and Gourse,R.L. (1991) Factor-independent activation of *Escherichia coli* rRNA transcription. II. Characterization of complexes of *rrnB* P1 promoters containing or lacking the upstream activator region with *Escherichia coli* RNA polymerase. *J. Mol. Biol.*, **220**, 560–583.
15. Newlands,J.T., Josaitis,C., Ross,W. and Gourse,R.L. (1992) Both FIS-dependent and factor-independent upstream activation of the *rrnB* P1 promoter are face of the helix dependent. *Nucleic Acids Res.*, **20**, 719–726.

16. Rao,L., Ross,W., Appleman,J.A., Gaal,T., Leirmo,S., Schlax,P.J., Record,M.T.,Jr and Gourse,R.L. (1994) Factor-independent activation of *rrnB* P1; an 'extended' promoter with an upstream element that dramatically increases promoter strength. *J. Mol. Biol.*, **235**, 1421–1435.
17. Estrem,S.T., Gaal,T., Ross,W. and Gourse,R.L. (1998) Identification of an UP element consensus sequence for bacterial promoters. *Proc. Natl Acad. Sci. USA*, **95**, 9761–9766.
18. Strainic,M.G., Sullivan,J.J., Velvis,A. and deHaseth,P.L. (1998) Promoter recognition by *Escherichia coli* RNA polymerase: effects of the UP element on open complex formation and promoter clearance. *Biochemistry*, **37**, 18074–18080.
19. Negishi,T., Fujita,N. and Ishihama,A. (1995) Structural map of the alpha subunit of *Escherichia coli* RNA polymerase: structural domains identified by proteolytic cleavage. *J. Mol. Biol.*, **248**, 723–728.
20. Igarashi,K., Fujita,N. and Ishihama,A. (1991) Identification of a subunit assembly domain in the alpha subunit of *Escherichia coli* RNA polymerase. *J. Mol. Biol.*, **218**, 1–6.
21. Zhang,G. and Darst,S. (1998) Structure of the *Escherichia coli* RNA polymerase α subunit amino-terminal domain. *Science*, **281**, 262–266.
22. Niu,W., Kim,Y., Tau,G., Heyduk,T. and Ebright,R.H. (1996) Transcription activation at class II CAP-dependent promoters: two interactions between CAP and RNA polymerase. *Cell*, **87**, 1123–1134.
23. Busby,S. and Ebright,R.H. (1999) Transcription activation by catabolite activator protein (CAP). *J. Mol. Biol.*, **293**, 199–213.
24. Jeon,Y.H., Negishi,T., Shirakawa,M., Yamazaki,T., Fujita,N., Ishihama,A. and Kyogoku,Y. (1995) Solution structure of the activator contact domain of the RNA polymerase α subunit. *Science*, **270**, 1495–1497.
25. Ishihama,A. (1993) Protein–protein communication within the transcription apparatus. *J. Bacteriol.*, **175**, 2483–2489.
26. Kainz,M. and Gourse,R.L. (1998) The C-terminal domain of the alpha subunit of *Escherichia coli* RNA polymerase is required for Rho-dependent transcription termination. *J. Mol. Biol.* **284**, 1379–1390.
27. Mah,T.-F., Kuznedelov,K., Mushegian,A., Severinov,K. and Greenblatt,J. (2000) The α subunit of *E. coli* RNA polymerase activates RNA binding by NusA. *Genes Dev.*, **14**, 2664–2675.
28. Igarashi,K. and Ishihama,A. (1991) Bipartite functional map of the *E. coli* RNA polymerase α subunit: involvement of the C-terminal region in transcription activation by cAMP-CRP. *Cell*, **65**, 1015–1022.
29. Busby,S. and Ebright,R.H. (1994) Promoter structure, promoter recognition, and transcription activation in prokaryotes. *Cell*, **79**, 743–746.
30. Gaal,T., Ross,W., Blatter,E.E., Tang,H., Jia,X., Krishnan,V.V., Assa-Munt,N., Ebright,R.H. and Gourse,R.L. (1996) DNA-binding determinants of the α subunit of RNA polymerase: novel DNA-binding domain architecture. *Genes Dev.*, **10**, 16–26.
31. Shao,X. and Grishin,N.V. (2000) Common fold in helix-hairpin-helix proteins. *Nucleic Acids Res.*, **28**, 2643–2650.
32. Ross,W., Ernst,A. and Gourse,R.L. (2001) Fine structure of *E. coli* RNA polymerase-promoter interactions: α subunit binding to the UP element minor groove. *Genes Dev.*, **15**, 491–506.
33. Murakami,K., Fujita,N. and Ishihama,A. (1996) Transcription factor recognition surface on the RNA polymerase α subunit is involved in contact with the DNA enhancer element. *EMBO J.*, **15**, 4358–4367.
34. Yasuno,K., Yamazaki,T., Tanaka,Y., Kodama,T.S., Matsugami,A., Katahira,M., Ishihama,A. and Kyogoku,Y. (2001) Interaction of the C-terminal domain of the *E. coli* RNA polymerase α subunit with the UP element: recognizing the backbone structure in the minor groove surface. *J. Mol. Biol.*, **306**, 213–225.
35. Ariyoshi,M., Nishino,T., Iwasaki,H., Shinagawa,H. and Morikawa,K. (2000) Crystal structure of the Holliday junction DNA in complex with a single RuvA tetramer. *Proc. Natl Acad. Sci. USA*, **97**, 8257–8262.
36. Jeon,Y.H., Yamazaki,T., Otomo,T., Ishihama,A. and Kyogoku,Y. (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.
37. Meng,W., Savery,N.J., Busby,S.J.W. and Thomas,M.S. (2000) The *Escherichia coli* RNA polymerase α subunit: length requirements for transcription activation at CRP-dependent promoters. *EMBO J.*, **19**, 1555–1566.
38. Fujita,N., Endo,S. and Ishihama,A. (2000) Structural requirements for the interdomain linker of α subunit of *Escherichia coli* RNA polymerase. *Biochemistry*, **39**, 6243–6249.
39. Naryshkin,N., Revyakin,A., Kim,Y., Mekler,V. and Ebright,R.H. (2000) Structural organization of the RNA polymerase-promoter open complex. *Cell*, **101**, 601–611.
40. Giladi,H., Murakami,K., Ishihama,A. and Oppenheim,A.B. (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.
41. Giladi,H., Koby,S., Prag,G., Engelhorn,M., Geiselmann,J. and Oppenheim,A. (1998) Participation of IHF and a distant UP element in the stimulation of the phage λ P_L promoter. *Mol. Microbiol.* **30**, 443–451.
42. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
43. Tang,H., Severinov,K., Goldfarb,A. and Ebright,R.H. (1995) Rapid RNA polymerase genetics: one-day, no-column preparation of reconstituted recombinant *Escherichia coli* RNA polymerase. *Proc. Natl Acad. Sci. USA*, **92**, 4902–4906.
44. Ross,W., Thompson,J.F., Newlands,J.T. and Gourse,R.L. (1990) *E.coli* Fis protein activates ribosomal RNA transcription *in vitro* and *in vivo*. *EMBO J.*, **9**, 3733–3742.
45. Barker,M.M., Gaal,T., Josaitis,C.A. and Gourse,R.L. (2001) Mechanism of regulation of transcription initiation by ppGpp. I. Effects of ppGpp on transcription initiation *in vivo* and *in vitro*. *J. Mol. Biol.*, **305**, 673–688.
46. Savery,N.J., Belyaeva,T. and Busby,S.J.W. (1996) Protein–DNA interactions. In Docherty,K. (ed.), *Gene Transcription: DNA Binding Proteins*, *Essential Techniques*. Wiley/BIOS Press, Chichester, UK, pp. 1–43.
47. Miura,A., Krueger,J.H., Itoh,S., de Boer,H.A. and Nomura,M. (1981) Growth-rate-dependent regulation of ribosome synthesis in *E. coli*: expression of the *lacZ* and *galK* genes fused to ribosomal promoters. *Cell*, **25**, 773–782.
48. Gourse,R.L., de Boer,H.A. and Nomura,M. (1986) DNA determinants of rRNA synthesis in *E. coli*: growth rate dependent regulation, feedback inhibition, upstream activation, antitermination. *Cell*, **44**, 197–205.
49. Gaal,T., Barkei,J., Dickson,R.R., deBoer,H.A., deHaseth,P.L., Alavi,H. and Gourse,R.L. (1989) Saturation mutagenesis of an *Escherichia coli* rRNA promoter and initial characterization of promoter variants. *J. Bacteriol.*, **171**, 4852–4861.
50. Miller,J. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
51. Studier,F.W. and Moffatt,B.A. (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.*, **189**, 113–130.
52. Bullock,W.O., Fernandez,J.M. and Short,J.M. (1987) XL1-Blue: a high efficiency plasmid transforming *recA Escherichia coli* strain with beta-galactosidase selection. *Biotechniques*, **5**, 376–378.
53. Guarente,L., Lauer,G., Robert,T.M. and Ptashne,M. (1980) Improved methods for maximising expression of a cloned gene: a bacterium that synthesizes rabbit β-globin. *Cell*, **20**, 543–553.
54. Severinov,K., Soushko,M., Goldfarb,A. and Nikiforov,V. (1993) Rifampicin region revisited—new rifampicin-resistant and streptolydigin-resistant mutants in the beta-subunit of *Escherichia coli* RNA-polymerase. *J. Biol. Chem.*, **268**, 14820–14825.
55. Zalenskaya,K., Lee,J.Y., Gujuluva,C.N., Shin,Y.K., Slutsky,M. and Goldfarb,A. (1990) Recombinant RNA polymerase-inducible overexpression, purification and assembly of *Escherichia coli rpo* gene-products. *Gene*, **89**, 7–12.
56. Savery,N.J., Lloyd,G.S., Kainz,M., Gaal,T., Ross,W., Ebright,R.H., Gourse,R.L. and Busby,S.J.W. (1998) Transcription activation at Class II CRP-dependent promoters: identification of determinants in the C-terminal domain of the RNA polymerase α subunit. *EMBO J.*, **12**, 3439–3447.
57. Zou,C., Fujita,N., Igarashi,K. and Ishihama,A. (1992) Mapping the cAMP receptor protein contact site on the α subunit of *Escherichia coli* RNA polymerase. *Mol. Microbiol.*, **6**, 2599–2605.
58. Gourse,R.L., Gaal,T., Bartlett,M.S., Appleman,J.A. and Ross,W. (1996) rRNA transcription and growth rate dependent regulation of ribosomal RNA synthesis in *Escherichia coli*. *Annu. Rev. Microbiol.*, **50**, 645–677.
59. Gaal,T., Bartlett,M.S., Ross,W., Turnbough,C.L.,Jr and Gourse,R.L. (1997) Transcription regulation by initiating NTP concentration: rRNA synthesis in bacteria. *Science*, **278**, 2092–2097.
60. Murakami,K., Owens,J.T., Belyaeva,T., Meares,C.F., Busby,S.J.W. and Ishihama,A. (1997) Positioning of two alpha subunit carboxy-terminal domains of RNA polymerase along promoter DNA by two transcription factors. *Proc. Natl Acad. Sci. USA*, **94**, 11274–11278.

61. Tagami,H. and Aiba,H. (1999) An inactive open complex mediated by an UP element at *Escherichia coli* promoters. *Proc. Natl Acad. Sci. USA*, **96**, 7202–7207.
62. Belyaeva,T., Rhodius,V., Webster,C. and Busby,S. (1998) Transcription activation at promoters carrying tandem DNA sites for the *Escherichia coli* cyclic AMP receptor protein: organization of the RNA polymerase α subunits. *J. Mol. Biol.*, **277**, 789–804.
63. Jinks-Robertson,S., Gourse,R.L. and Nomura,M. (1983) Expression of rRNA and tRNA genes in *Escherichia coli*: evidence for feedback regulation by products of rRNA operons. *Cell*, **33**, 865–876.