

## The UP Element Is Necessary but Not Sufficient for Growth Rate-Dependent Control of the *Escherichia coli* *guaB* Promoter<sup>∇†</sup>

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**The *Escherichia coli* *guaB* promoter ( $P_{guaB}$ ) regulates the transcription of two genes, *guaB* and *guaA*, that are required for de novo synthesis of GMP, a precursor for the synthesis of guanine nucleoside triphosphates. The activity of  $P_{guaB}$  is subject to growth rate-dependent control (GRDC). Here we show that the A+T-rich sequence located between positions –59 and –38 relative to the *guaB* transcription start site stimulates transcription from  $P_{guaB}$  ~8- to 10-fold and, in common with other UP elements, requires the C-terminal domain of the RNA polymerase  $\alpha$  subunit for activity. Like the *rrnB* P1 UP element, the  $P_{guaB}$  UP element contains two independently acting subsites located at positions –59 to –47 and –46 to –38 and can stimulate transcription when placed upstream of the *lacP1* promoter. We reveal a novel role for the  $P_{guaB}$  UP element by demonstrating that it is required for GRDC. The involvement of the UP element in GRDC also requires the participation of sequences located at least 100 bp upstream of the *guaB* transcription start site. These sequences are required for down-regulation of  $P_{guaB}$  activity at lower growth rates.**

*Escherichia coli* RNA polymerase (RNAP) is a multisubunit complex consisting of an  $\alpha$  subunit homodimer, single  $\beta$ ,  $\beta'$ , and  $\omega$  subunits, and one of several  $\sigma$  subunits (14). The  $\alpha$  subunit contains an N-terminal domain ( $\alpha$ NTD; residues 8 to 232) and a C-terminal domain ( $\alpha$ CTD; residues 249 to 329) (5, 21, 44).  $\alpha$ NTD and  $\alpha$ CTD are separated by a flexible linker that is at least 13 amino acids long (5, 20, 25).  $\alpha$ CTD plays important roles in transcription activation by contacting a number of transcription factors (6, 18) or through interacting with an A+T-rich DNA sequence referred to as the UP element (16).

UP elements stimulate transcription by recruiting  $\alpha$ CTD to DNA (32, 43). The best-studied UP element spans the region from –59 to –38 at the *rrnB* P1 promoter (16). The *rrnB* P1 UP element contains two domains, a ~9-bp proximal UP element subsite located at positions –46 to –38 and a ~13-bp distal UP element subsite located at positions –59 to –47. Each subsite functions independently by recruiting one  $\alpha$ CTD (11). The amino acid residues in  $\alpha$ CTD that comprise the 265 determinant, including residues V264, R265, N268, N294, G296, K298, and S299, interact with UP element and A-tract sequences by contacting the DNA backbone, and R265 makes additional contacts with bases in the minor groove (4, 32, 43). These residues are the most important for *rrnB* P1 UP element utilization both in vivo and in vitro (13, 35). In the absence of a strong distal UP element subsite,  $\alpha$ CTD bound to a consensus or near-consensus proximal UP element subsite also makes productive contacts with region 4.2 of  $\sigma^{70}$  that serve to stimulate transcription (7, 35).

The *E. coli* *guaB* promoter ( $P_{guaB}$ ) regulates the transcrip-

tion of two genes, *guaB* and *guaA*, which together constitute the *guaBA* operon. The *guaB* and *guaA* genes encode IMP dehydrogenase and GMP synthetase, respectively, and are required for the biosynthesis of GMP from IMP (22, 42).  $P_{guaB}$  is regulated by the cyclic AMP (cAMP) receptor protein (CRP), and CRP binds to a ~300-bp DNA fragment containing  $P_{guaB}$  that includes a predicted CRP site centered at position –117.5 relative to the *guaB* transcription start site (19).  $P_{guaB}$  also contains putative binding sites for PurR and DnaA overlapping the core promoter region (23, 41). In addition, an A+T-rich sequence located between positions –59 and –38 at  $P_{guaB}$  matches the consensus 22-bp UP element sequence [5'-nnAA A(A/T)(A/T)T(A/T)TTTTnnAAAAnnn-3'; n represents a nonconserved position] at 13 out of 15 conserved positions (Fig. 1), but its role as an UP element has not been confirmed (10, 19).

The term growth rate-dependent control (GRDC) is used to refer to situations where the abundance of a particular transcript or protein product represents an increasing fraction of the cell mass at higher growth rates. For example, in *E. coli*, the synthesis rates of rRNA and tRNA increase in proportion to the square of the growth rate, with the result that stable RNA accounts for an increased fraction of the cellular mass with increasing growth rate (9, 15, 27). One consequence of GRDC of stable RNA synthesis is that the consumption of nucleoside triphosphates also increases in a growth rate-dependent manner (28). Because GMP is a precursor for the synthesis of GTP, it follows that the rate of synthesis of GMP should be regulated according to the growth rate. Consistent with this, it has been shown that  $P_{guaB}$  activity is subject to GRDC (8). The best-characterized promoter subject to GRDC that also contains an UP element is the *rrnB* P1 promoter, although the UP element plays no role in GRDC of this promoter (2, 28). In this work, we show that the A+T-rich sequence located upstream of the  $P_{guaB}$  –35 region does indeed function as an UP element and that, like the *rrnB* P1 UP element, it contains promoter-proximal and -distal subsites that are able to activate transcription

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(30), and also ligated to pRLG770 for *in vitro* transcription assays. All amplified sequences were checked by DNA sequencing.

**Measurement of transcription *in vivo*.** Dense starter cultures of bacteria containing single-copy promoter-*lacZ* fusions were diluted 100-fold into the same growth medium and grown at 37°C to an optical density at 600 nm (OD<sub>600</sub>) of 0.3 to 0.5. For GRDC experiments, starter cultures were grown overnight in medium supporting the lowest growth rate (i.e., using glycerol as the sole carbon source). Strains containing plasmids encoding WT  $\alpha$  and mutant  $\alpha$  derivatives were inoculated from fresh colonies into minimal medium at an OD<sub>600</sub> of ~0.007 and grown at 37°C to an OD<sub>600</sub> of ~0.335 to 0.45 in the presence of 100  $\mu$ g/ml ampicillin. Cells were permeabilized using chloroform-sodium dodecyl sulfate, and the  $\beta$ -galactosidase activity was determined using a published protocol (26). For GRDC experiments, cells were disrupted by sonication, and the  $\beta$ -galactosidase activity was normalized to the activity corresponding to a growth rate of 1 doubling per h, which was calculated from a linear regression curve fitted to a plot of  $\beta$ -galactosidase activity versus growth rate.

**Measurement of transcription *in vitro*.** Multiple-round transcription reactions were performed at 30°C for 20 min using a previously described method (36). Each 25- $\mu$ l reaction mixture contained 40 mM Tris-acetate (pH 7.9), 150 mM KCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol, 100  $\mu$ g/ml acetylated bovine serum albumin (Promega), 200  $\mu$ M ATP, 200  $\mu$ M GTP, 200  $\mu$ M CTP, 10  $\mu$ M UTP, and 5  $\mu$ Ci of [ $\alpha$ -<sup>32</sup>P]UTP (800 Ci/mmol; Perkin-Elmer). Each reaction mixture also contained 0.2 nM supercoiled template DNA (pRLG770 derivative) and either a commercially available WT *E. coli* RNAP holoenzyme (Epicenter) or *E. coli* core RNAP purified as described elsewhere (E. Campbell, S. Darst, S. I. Husnain, B. Nickels, M. S. Thomas, K.-A. Twist, and L. Westblade, unpublished data) and subsequently reconstituted with  $\sigma$ <sup>70</sup>. The latter form of RNAP contained either WT  $\alpha$  or a derivative lacking  $\alpha$ CTD (RNAP $\Delta\alpha$ CTD), and RNAP activity was standardized as described previously (Campbell et al., unpublished). Reactions were initiated with RNAP and terminated with 25  $\mu$ l of stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol). Samples, containing equal amounts of template DNA, were fractionated on a 5.5% acrylamide gel containing 7 M urea, and transcript abundance was quantified using a FujiFilm FLA3000 phosphorimager. The amount of transcript was determined by deducting the background activity in the lane from the value for the transcript abundance.

**DNase I footprinting.** DNase I footprinting was performed according to a published method (38). Derivatives of pBluescript II KS containing EcoRI-XhoI DNA fragments extending from position -133 to +36 of the *guaB* promoter were purified following electrophoresis in 6% acrylamide gels (25), labeled at the downstream (XhoI) end with [ $\gamma$ -<sup>32</sup>P]ATP (6,000 Ci/mmol; Perkin-Elmer) using T4 polynucleotide kinase, and purified using QIAQuick columns (Qiagen). Labeled DNA fragment (4 nM) was incubated at room temperature for 30 min in a buffer containing 20 mM HEPES (pH 8.0), 5 mM MgCl<sub>2</sub>, 50 mM potassium glutamate, 1 mM dithiothreitol, 500  $\mu$ g/ml acetylated bovine serum albumin, and 7% glycerol in the presence or absence of either 55 nM WT reconstituted RNAP or 119 nM reconstituted RNAP $\Delta\alpha$ CTD, in a final volume of 20  $\mu$ l, after which DNA fragments were digested with DNase I. Reactions were stopped using a buffer containing 0.3 M sodium acetate (pH 5.2) and 10 mM EDTA. Samples were purified by phenol-chloroform extraction and ethanol precipitation, and DNA fragments were separated in a 6% acrylamide-7 M urea sequencing gel. A Maxam-Gilbert G+A sequencing ladder was run alongside. Footprints were visualized using a FujiFilm FLA3000 phosphorimager.

## RESULTS

**Activation of P<sub>*guaB*</sub> *in vivo* by sequences located upstream of the core promoter elements.** To analyze the contribution to transcription of sequences present upstream of P<sub>*guaB*</sub>, promoter derivatives with different upstream endpoints were used to generate transcriptional fusions to the *lacZ* gene and introduced into the *E. coli* chromosome as a single copy. The full-length WT P<sub>*guaB*</sub> derivative used in this study contained promoter sequences extending upstream to position -253 relative to the *guaB* transcription start site. This promoter and a promoter derivative containing sequences up to -133 both harbored the putative CRP site. In the other promoter derivatives, the upstream deletion removed the putative CRP site. All promoter derivatives had a downstream endpoint located at

+36 (Fig. 2A). The promoter activity was determined by measuring the  $\beta$ -galactosidase activity in exponentially growing cells.

The results from the deletion analysis revealed that removal of the sequence from position -253 to -60 does not lead to any significant changes in the efficiency of transcription from P<sub>*guaB*</sub> (Fig. 2B). This suggests that sequences upstream of position -60 do not contribute significantly to transcription from P<sub>*guaB*</sub> in cells growing under the conditions employed. Transcription was also measured from a P<sub>*guaB*</sub> derivative with an upstream endpoint at position -37, which is referred to as the core *guaB* promoter (see the legend to Fig. 2). The core *guaB* promoter contains a "SUB" sequence in place of the putative P<sub>*guaB*</sub> UP element at positions -59 to -38 (Fig. 1). The SUB sequence has been shown to minimize the contribution to transcription from the recruitment of  $\alpha$ CTD to DNA (10, 30). Comparison of the activity of P<sub>*guaB*</sub>(-59 to +36) with core P<sub>*guaB*</sub> indicated that the putative P<sub>*guaB*</sub> UP element stimulates the core promoter approximately eightfold *in vivo* (Fig. 2B).

**Activation of P<sub>*guaB*</sub> *in vitro* by sequences located upstream of the core promoter elements.** To determine whether upstream *guaB* promoter sequences stimulate transcription in the absence of additional regulatory factors, multiple-round *in vitro* transcription reactions were performed using a subset of the promoter derivatives employed for the *in vivo* study (see above). Reactions were initiated with 10 nM RNAP. The results showed that, in the absence of other factors, sequences located upstream of position -59 do not contribute significantly to the transcriptional activity of P<sub>*guaB*</sub>. In contrast, deletion of the putative P<sub>*guaB*</sub> UP element gave rise to a ~6-fold decrease in transcription activity compared to that of the full-length *guaB* promoter (Fig. 2C). These results are in good agreement with the findings from the *in vivo* analysis (see above) and demonstrate that the putative P<sub>*guaB*</sub> UP element can function in the absence of additional regulatory factors.

**The putative P<sub>*guaB*</sub> UP element contains independently acting subsites.** We measured transcription both *in vivo* and *in vitro* from P<sub>*guaB*</sub>(-59 to +36), which contained the putative P<sub>*guaB*</sub> UP element sequence, and P<sub>*guaB*</sub> derivatives containing only the predicted P<sub>*guaB*</sub> proximal UP element subsite, only the predicted P<sub>*guaB*</sub> distal UP element subsite, or a consensus UP element (Fig. 1). The locations of the P<sub>*guaB*</sub> UP element subsites used here correspond to the proximal and distal UP element subsites defined in a previous study (11). The consensus UP element used in this analysis (the 4541 UP element [Fig. 1]) has been described previously (11). The results were compared to the transcription activity of core P<sub>*guaB*</sub>.

The results showed that the contributions of each tested sequence to transcription *in vivo* and *in vitro* are comparable (Fig. 3A and B). Whereas the putative P<sub>*guaB*</sub> UP element afforded an ~8- to 10-fold increase in transcription from P<sub>*guaB*</sub>, the putative P<sub>*guaB*</sub> proximal UP element subsite stimulated transcription ~5- to 6-fold and the putative P<sub>*guaB*</sub> distal UP element subsite afforded a ~3-fold increase in transcription. These results demonstrate that the putative P<sub>*guaB*</sub> UP element contains subsites that can function independently in stimulating transcription from P<sub>*guaB*</sub> and that together they function as coactivators of transcription. The consensus UP element stimulated transcription from P<sub>*guaB*</sub> ~11-fold, i.e., similar to the level afforded by the putative P<sub>*guaB*</sub> UP element.



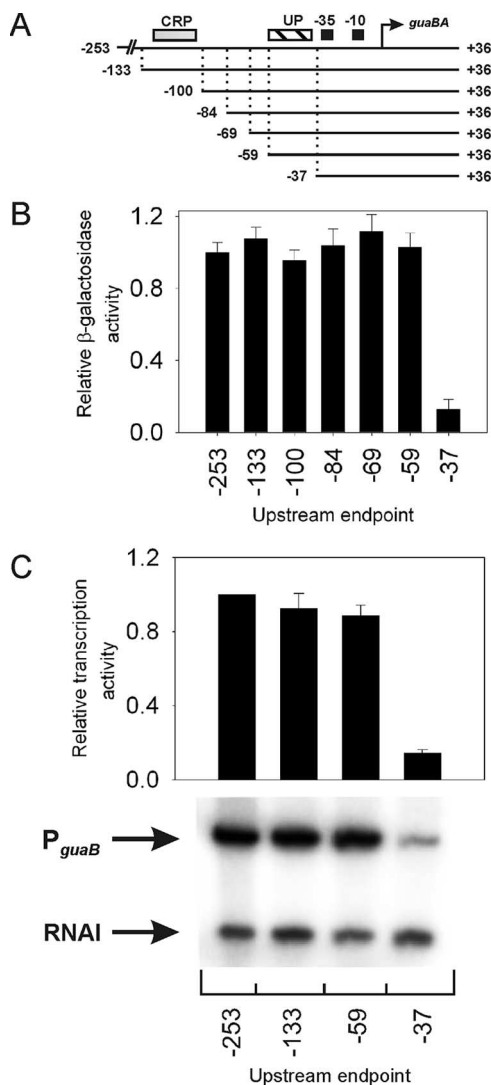


FIG. 2. Contributions of sequences upstream of the *guaB* promoter to transcription. (A) The WT *guaB* promoter (–253 to +36) and derivatives each having a different upstream endpoint were constructed. Deletion endpoints are indicated relative to the *guaB* transcription start site. The predicted CRP binding site centered at position –117.5, the putative  $P_{guaB}$  UP element (designated “UP”), the  $P_{guaB}$  –35 and –10 hexamers, and the *guaB* transcription start site are shown (diagram not to scale).  $P_{guaB}$ (–37 to +36) is referred to as core  $P_{guaB}$  and contains the SUB sequence from positions –59 to –38 (Fig. 1). (B) The  $\beta$ -galactosidase activity (in Miller units) specified by each promoter-*lacZ* fusion in strain VH1000 growing logarithmically in M9-glucose minimal medium is expressed relative to the activity of  $P_{guaB}$ (–253 to +36) (activity, 3,003 Miller units; relative activity, 1). (C) Multiple-round in vitro transcription assays were performed using a subset of *guaB* promoter derivatives with different upstream endpoints cloned into plasmid pRLG770, in the presence of 10 nM RNAP. The  $P_{guaB}$ -directed transcript produced from this plasmid contains ~180 nucleotides (RNA-I contains ~110 nucleotides). Each promoter activity was determined by measuring the transcript abundance and is expressed relative to the activity of  $P_{guaB}$ (–253 to +36), set at 1. In panels B and C, values are means and standard deviations from three independent experiments.

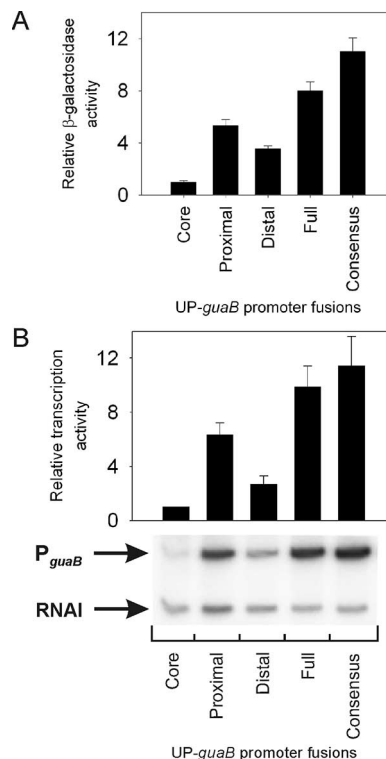


FIG. 3. Contribution of subsites within the  $P_{guaB}$  A+T-rich sequence to transcription. *guaB* promoter derivatives containing the consensus 4541 UP element located at positions –59 to –38, the complete 22-bp  $P_{guaB}$  A+T-rich element located at –59 to –38 (full), the predicted  $P_{guaB}$  proximal UP element subsite (positions –46 to –38), or the predicted  $P_{guaB}$  distal UP element subsite (positions –59 to –47), or lacking an UP element (core  $P_{guaB}$ ), were constructed. Core  $P_{guaB}$  contains a SUB sequence from position –59 to –38.  $P_{guaB}$  containing the proximal UP element subsite also contains a SUB sequence from position –59 to –47, and  $P_{guaB}$  containing the distal UP element subsite contains a SUB sequence from position –46 to –38. (A) The  $\beta$ -galactosidase activity (in Miller units) specified by each promoter-*lacZ* fusion in strain VH1000 growing logarithmically in M9-glucose minimal medium is expressed relative to the activity of core  $P_{guaB}$  (activity, 385 Miller units, relative activity, 1). (B) Multiple-round in vitro transcription assays were performed using each *guaB* promoter construct in plasmid pRLG770 in the presence of 5 nM RNAP. The activity of each promoter is expressed relative to that of core  $P_{guaB}$ , set at 1. In both panels, values are means and standard deviations from three independent experiments.

**Activation of the *lac* promoter in vivo by the putative  $P_{guaB}$  UP element.** The *rrnB* P1 UP element is capable of stimulating transcription from other promoter sequences (31). To determine whether this also holds true for the putative  $P_{guaB}$  UP element, transcription was measured in vivo from the core *lac* promoter (core *lacP1*) containing the putative  $P_{guaB}$  UP element located at positions –59 to –38. To compare the strength of activation due to the putative  $P_{guaB}$  UP element with that from other UP element sequences, transcription from core *lacP1* fused to either the *rrnB* P1 UP element or a consensus UP element was also quantified. The consensus UP element used here (the 4192 UP element [Fig. 1]) has been described previously (10). The results were compared to the transcription activity of the core *lac* promoter (i.e., *lacP1* without an UP element). The results show that the putative  $P_{guaB}$

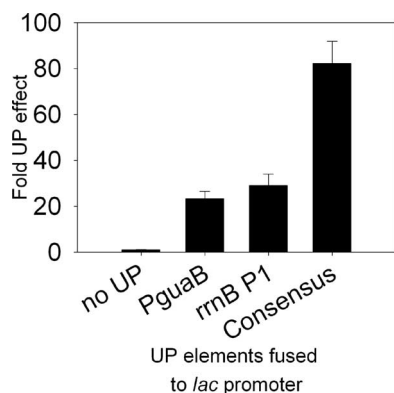


FIG. 4. Activation of the *lacP1* promoter by the  $P_{guaB}$  A+T-rich element. *lacP* promoter derivatives contain WT *lacP1* sequences from  $-37$  to  $+52$  and one of the following: the consensus 4192 UP element ("Consensus"), the *rrnB* P1 UP element, the  $P_{guaB}$  UP element, or no UP element, as indicated. UP element sequences are positioned from  $-59$  to  $-38$  relative to the *lacP1* transcription start site. The core *lac* promoter contains *lac* promoter sequences as far upstream as position  $-40$  and a SUB sequence from positions  $-58$  to  $-41$  (31). The  $\beta$ -galactosidase activity (in Miller units) specified by each promoter-*lacZ* fusion is the mean (and standard deviation) from three independent experiments and is expressed as a percentage of the activity of the core *lac* promoter (activity, 36 Miller units; relative activity, 1).

UP element stimulated transcription from core *lacP1*  $\sim 23$ -fold, i.e., at a much higher level than stimulation of the *guaB* promoter (Fig. 4). By comparison, the *rrnB* P1 UP element stimulated transcription from the core *lac* promoter  $\sim 30$ -fold, whereas the consensus UP element afforded an  $\sim 80$ -fold increase in promoter activity.

**Role of  $\alpha$ CTD in transcription from  $P_{guaB}$  in vivo.** To investigate whether  $\alpha$ CTD plays a role in transcription at  $P_{guaB}$ , transcription from  $P_{guaB}(-59$  to  $+36)$  and core  $P_{guaB}$  was measured in merodiploid cells containing the WT *rpoA* allele on the chromosome and a plasmid encoding either WT  $\alpha$  or a mutant derivative harboring an alanine substitution at position 265 ( $\alpha 265A$ ). The results show that the activity of  $P_{guaB}(-59$  to  $+36)$  did not differ significantly in the presence or absence of  $\alpha 265A$  (see Fig. S1A in the supplemental material). However, in the presence of  $\alpha 265A$ , transcription from core  $P_{guaB}$  increased  $\sim 2.9$ -fold over that for cells containing only WT  $\alpha$ . This can be explained by the rationale that a decrease in the activity of the putative UP element in the presence of  $\alpha 265A$  leads to a compensatory increase in transcription from core  $P_{guaB}$ . The effect of the mutant  $\alpha$  on UP element function was therefore quantified as described previously (13, 24, 33). Thus, in the presence of  $\alpha 265A$ , the stimulation of  $P_{guaB}$  due to the putative UP element was reduced by a factor of  $\sim 2.5$  (from 9.5-fold to 3.6-fold) (see Fig. S1B in the supplemental material).

**Role of  $\alpha$ CTD in transcription from  $P_{guaB}$  in vitro.** To demonstrate that  $\alpha$ CTD is required for stimulation of  $P_{guaB}$  by the A+T-rich element, transcription was also measured in vitro from  $P_{guaB}(-59$  to  $+36)$  and from core  $P_{guaB}$  in the presence of the RNAP holoenzyme containing WT  $\alpha$  or  $\alpha$  lacking  $\alpha$ CTD (RNAP $\Delta\alpha$ CTD). The results show that transcription from  $P_{guaB}(-59$  to  $+36)$  decreased  $\sim 10$ -fold in the presence of RNAP $\Delta\alpha$ CTD in comparison to the activity measured in the

presence of WT RNAP. On the other hand, the activity of core  $P_{guaB}$  did not differ significantly in the presence of either RNAP, and in each case the promoter activity was comparable to the activity of  $P_{guaB}(-59$  to  $+36)$  in the presence of RNAP $\Delta\alpha$ CTD (Fig. 5A). This indicates that the  $P_{guaB}$  A+T-rich element requires  $\alpha$ CTD to function in transcription activation.

DNase I footprinting analysis was employed to probe for  $\alpha$ CTD-DNA interactions at  $P_{guaB}$ . The putative  $P_{guaB}$  UP element contains a GC dinucleotide at positions  $-46$  and  $-45$  (Fig. 1) that is susceptible to cleavage by DNase I. In the presence of WT RNAP, cleavage at this position in  $P_{guaB}(-133$  to  $+36)$  is abolished, but this protection is lost in the presence of RNAP $\Delta\alpha$ CTD, indicating that  $\alpha$ CTD interacts with this region of the *guaB* promoter (Fig. 5B). Replacement of the putative  $P_{guaB}$  UP element with a SUB sequence [i.e.,  $P_{guaB}(-133$  to  $+36)$  (no UP)] did not abolish protection of the region corresponding to the proximal UP element subsite by WT RNAP (i.e., the SUB sequence was still bound by  $\alpha$ CTD).

**The  $P_{guaB}$  UP element is required for GRDC.** GRDC of  $P_{guaB}$  was examined by growing cells harboring single-copy  $P_{guaB}$ -*lacZ* fusions in media that supported different cellular growth rates and measuring the  $\beta$ -galactosidase activity. The activity of  $P_{guaB}(-133$  to  $+36)$  was found to increase in direct proportion to an increasing growth rate, i.e., a twofold increase in the growth rate was accompanied by a twofold increase in promoter activity (Fig. 6A). As expected, deletion of upstream sequences to position  $-37$  (core  $P_{guaB}$ ) resulted in a decrease in *guaB* promoter activity at all growth rates (Fig. 6B). However, the decrease was progressively more marked at higher growth rates, and this resulted in a complete loss of GRDC. To investigate whether the UP element contributes to GRDC, the UP element in  $P_{guaB}(-133$  to  $+36)$  was replaced by a SUB sequence [i.e.,  $P_{guaB}(-133$  to  $+36)$  (no UP)]. This manipulation also resulted in a loss of GRDC (as well as causing a sharp decrease in *guaB* promoter activity), suggesting that the UP element is required for GRDC (Fig. 6C). To determine whether sequences located upstream of the UP element are also required for GRDC, the activity of  $P_{guaB}(-59$  to  $+36)$  was measured at different cellular growth rates. The results demonstrated that sequences located upstream of the UP element are also required for GRDC (Fig. 6D). Moreover, at the lower growth rates (i.e.,  $\mu \leq 1.0$ ), this promoter derivative is at least 2.3-fold more active than  $P_{guaB}(-133$  to  $+36)$ , suggesting that sequences located upstream of the UP element are required for the repression of  $P_{guaB}$  in slowly growing cells. To localize these sequences more precisely, the activity of the  $P_{guaB}(-100$  to  $+36)$  promoter was also measured at different growth rates. The results show that this promoter derivative behaves similarly to  $P_{guaB}(-59$  to  $+36)$  (Fig. 6E), implying that sequences located between  $-133$  and  $-100$  participate in the GRDC of  $P_{guaB}$ .

## DISCUSSION

Previously, a 22-bp UP element-like sequence extending from position  $-59$  to  $-38$  relative to the *guaB* transcription start site was identified by comparison to the consensus sequence for UP elements (10, 19). We demonstrate here that this sequence does function as an UP element. First, this se-

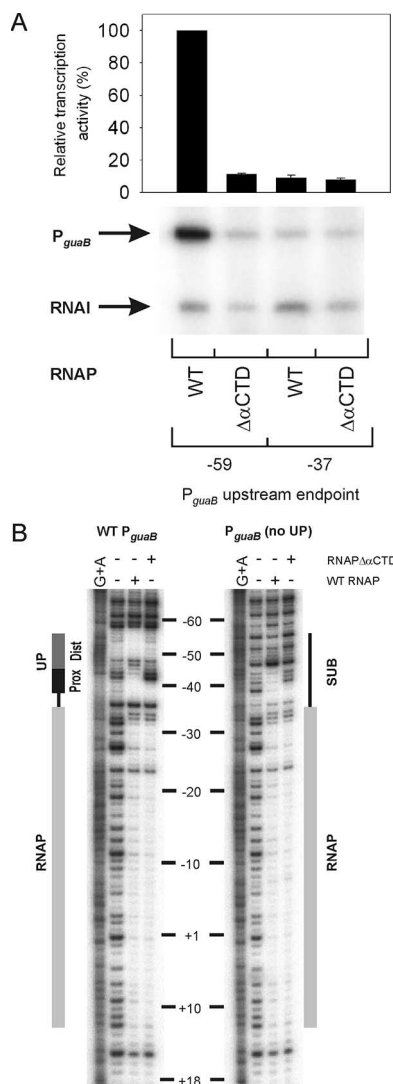


FIG. 5. Role of  $\alpha$ CTD in transcription from  $P_{guaB}$  in vitro. (A) Multiple-round transcription reactions were performed with the core *guaB* promoter and  $P_{guaB}$ (-59 to +36) in the presence of either 5 nM WT reconstituted RNAP or 11 nM reconstituted RNAP $\Delta\alpha$ CTD. The concentrations of the two RNAPs employed showed comparable activities, as measured by multiple-round transcription from the *lacUV5* promoter (data not shown). The activity of each promoter is expressed as a percentage of that of  $P_{guaB}$ (-59 to +36) in the presence of WT RNAP, set at 100%. Each promoter activity is the mean and standard deviation from three independent experiments. (B) DNase I footprinting was performed with the  $P_{guaB}$ (-133 to +36) DNA fragment (WT) and with  $P_{guaB}$ (-133 to +36) (no UP). Experiments were performed in the presence of either WT reconstituted RNAP or RNAP $\Delta\alpha$ CTD. The region protected by RNAP and the locations of the UP element and SUB sequence are indicated. Nucleotide positions are shown relative to the *guaB* transcription start site, and lanes containing the G+A ladder are indicated.

quence stimulates transcription from  $P_{guaB}$  ~8- to 10-fold both in vivo and in vitro. Moreover, it can activate other promoters such as *lacPI*, which it can stimulate at least 20-fold. The variation in transcription enhancement at different promoters is a feature of other UP elements (for example, a consensus

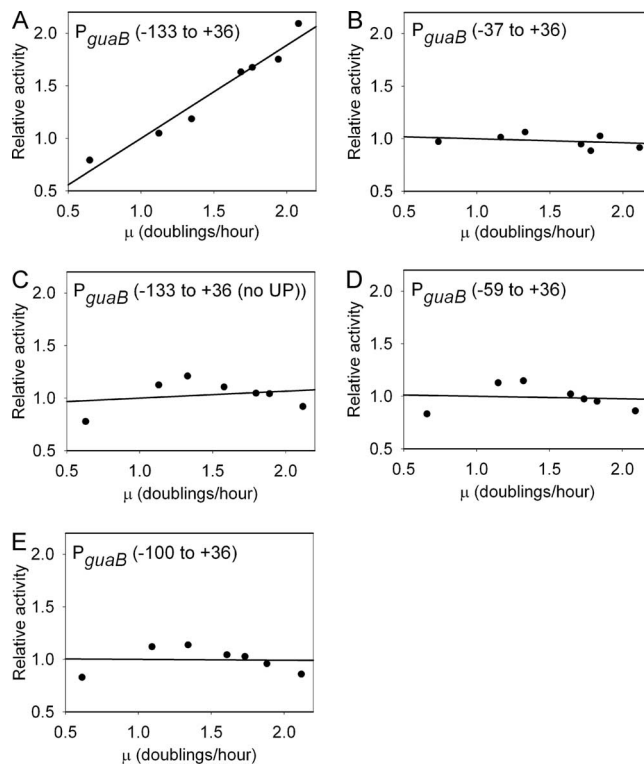


FIG. 6. Requirement of the  $P_{guaB}$  UP element for GRDC. The effects of growth rate on the activities of the indicated  $P_{guaB}$  derivatives were determined by growing strain VH1000 harboring fusions of these promoters to *lacZ* in different media and determining the  $\beta$ -galactosidase activities. The activity of each promoter was normalized to a value of 1.0 at a growth rate of 1 doubling per h. The  $\beta$ -galactosidase activity specified by each promoter (in Miller units) at a growth rate of 1 doubling per h was as follows:  $P_{guaB}$ (-133 to +36), 1,588;  $P_{guaB}$ (-37 to +36), 418;  $P_{guaB}$ (-133 to +36) (no UP), 419;  $P_{guaB}$ (-59 to +36), 3,573;  $P_{guaB}$ (-100 to +36), 3,009. Each data point represents the mean promoter activity and the mean growth rate. Means were calculated using data obtained from three independent experiments.

UP element stimulates transcription from  $P_{guaB}$  ~11-fold, from *rmB* P1 ~340-fold, and from *lacPI* ~80-fold in vivo) (11, 31). The lower contribution of the  $P_{guaB}$  UP element to transcription from  $P_{guaB}$  relative to that for *lacPI* is likely to be due to differences in promoter architecture that result in a higher activity of the core *guaB* promoter in comparison to *lacPI* (i.e., core  $P_{guaB}$  is ~10-fold more active than core *lacPI*). In particular,  $P_{guaB}$  has a consensus -10 sequence that is separated from the -35 region by 17 bp, whereas at *lacPI* the -10 sequence (which has a poorer match to the consensus sequence) is separated from the -35 region by an 18-bp spacer.

Second, the  $P_{guaB}$  UP element appears to contain independently functioning subsites that correspond in location to the previously identified proximal and distal subsites in the *rmB* P1 and consensus UP elements (11). Accordingly, we assume that each  $P_{guaB}$  UP element subsite functions by contacting a single  $\alpha$ CTD. As with the *rmB* P1 UP element, the  $P_{guaB}$ -proximal UP element subsite stimulates transcription to a greater degree than the distal subsite (11). It has been shown that  $\alpha$ CTD bound to consensus or near-consensus proximal UP element subsites functionally interacts with region 4 of  $\sigma^{70}$  and that



such interactions may account for the observation that proximal UP element subsites contribute more to transcription activation than distal UP element subsites (7, 35). Since the  $P_{guaB}$ -proximal UP element subsite matches the consensus sequence, it is possible that  $\alpha$ CTD- $\sigma^{70}$  interactions may be important for  $P_{guaB}$  UP element function.

Third, the activity of the  $P_{guaB}$  UP element requires  $\alpha$ CTD. Thus, deletion of  $\alpha$ CTD abolishes UP element-dependent transcription from  $P_{guaB}$  in vitro and is accompanied by a loss of protection of the UP element by RNAP. Although protection of non-UP element sequences located upstream of the core  $guaB$  promoter by WT RNAP was observed, these interactions do not contribute a strong stimulatory effect on transcription. This observation mirrors findings from a previous study that demonstrated protection of the region between  $-35$  and  $-70$  at the *lacUV5* promoter by  $\alpha$ CTD, although these sequences do not contain an UP element (34). Incorporation into RNAP of  $\alpha$  subunits containing an amino acid substitution in the DNA binding region ( $\alpha 265A$ ) was also shown to decrease UP element-dependent transcription from  $P_{guaB}$  in vivo. However, in this situation, UP element-independent transcription from the core  $guaB$  promoter fully compensated for the reduction in UP element function. This suggests that core  $P_{guaB}$  is subject to a feedback mechanism that can counter regulatory inputs acting outside of the core promoter region. Although a compensatory mechanism (involving the initiating nucleotide for transcription) operates under similar conditions at the *rmB* P1 promoter, it does not restore promoter activity completely in the presence of  $\alpha 265A$  (13, 39). This is probably due to the much larger negative effect that impairment of the  $\alpha$ CTD-UP element interaction exerts on *rmB* P1 activity.

The  $P_{guaB}$  UP element is absolutely required for GRDC of  $P_{guaB}$ . This contrasts with the situation at two other growth rate-regulated promoters (*rmB* P1 and the *leuV* promoter), where GRDC does not require the UP element (2, 3, 29). Our results also show that, in addition to the  $P_{guaB}$  UP element, sequences located between positions  $-133$  and  $-100$  are required for GRDC, and their role is to facilitate the inhibition of  $P_{guaB}$  at lower growth rates. This region contains a sequence exhibiting a good match to the CRP site consensus sequence, suggesting that CRP may participate in GRDC of  $P_{guaB}$ . These observations do not rule out the possibility that an additional factor(s) that binds to the region between  $-100$  and the UP element is involved. Therefore, GRDC of  $P_{guaB}$  may be explained by one of the following two general models. (i) Binding of one or more regulatory proteins to sequences located upstream of, or overlapping, position  $-100$  (for example, CRP) inhibits UP element function. This could occur directly or indirectly, by facilitating occupancy or remodeling of the region upstream of (and overlapping) the UP element by other DNA-binding proteins. (ii) The  $P_{guaB}$  UP element, with or without the participation of other DNA-binding proteins, may alter the orientation of the upstream DNA so as to allow a regulator bound near position  $-100$  to inhibit RNAP, perhaps by bringing it into close proximity to the core promoter region. In both models, DNA occupancy by at least one of the DNA-binding proteins, although not necessarily the factor binding upstream of position  $-100$ , would increase at lower growth rates. Work is currently under way to explore these possibilities.

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