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Upstream A-tracts increase bacterial promoter activity through interactions with the RNA polymerase α subunit

(A-tract DNA/DNA curvature/UP element)

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ABSTRACT **Upstream A-tracts stimulate transcription from a variety of bacterial promoters, and this has been widely attributed to direct effects of the intrinsic curvature of A-tract-containing DNA. In this work we report experiments that suggest a different mechanism for the effects of upstream A-tracts on transcription. The similarity of A-tract-containing sequences to the adenine- and thymine-rich upstream recognition elements (UP elements) found in some bacterial promoters suggested that A-tracts might increase promoter activity by interacting with the α subunit of RNA polymerase (RNAP). We found that an A-tract-containing sequence placed upstream of the *Escherichia coli lac* or *rrnB* P1 promoters stimulated transcription both *in vivo* and *in vitro*, and that this stimulation required the C-terminal (DNA-binding) domain of the RNAP α subunit. The A-tract sequence was protected by wild-type RNAP but not by α-mutant RNAPs in footprints. The effect of the A-tracts on transcription was not as great as that of the most active UP elements, consistent with the degree of similarity of the A-tract sequence to the UP element consensus. A-tracts functioned best when positioned close to the −35 hexamer rather than one helical turn farther upstream, similar to the positioning optimal for UP element function. We conclude that A-tracts function as UP elements, stimulating transcription by providing binding site(s) for the RNAP αCTD, and we suggest that these interactions could contribute to the previously described wrapping of promoter DNA around RNAP.**

Promoters used by the major form of *Escherichia coli* RNA polymerase (RNAP), Eσ^{70}, generally contain two hexamers located about 10 and 35 bp upstream of the start site of transcription, which are recognized by the σ^{70} subunit (1). In addition, sequences upstream of the −35 hexamer in some promoters in *E. coli* as well as in other bacterial species increase transcription in the absence of accessory proteins (2–9). These upstream sequences are generally A+T-rich, and some contain multiple A-tracts in phase with the DNA helical repeat (phased A-tracts). Phased A-tracts inserted upstream of the −35 hexamer in synthetic hybrid promoters have also been reported to increase transcription (10–14). Because phased A-tracts result in macroscopic DNA curvature [i.e., intrinsic bends whose angle increases with the number of A-tracts (15, 16)], the effects of A-tracts on transcription often have been attributed to direct effects of DNA bending, even though a mechanism for such an effect was not clear (4, 5, 7, 8, 10–14, 17–19).

Upstream sequences are not as extensively conserved as the −10, −35 hexamers in *E. coli* promoters (1) and were not considered as an RNAP recognition element until recently (9, 20). However, an A-tract positioned at about −40 (21, 22) or A+T-rich sequences at about −40 and −50 (23) were noted in subsets of promoters. Some conservation of alternating A- and T-tracts was also noted in the upstream region of *Bacillus subtilis* promoters (24). Whereas many promoters contain A+T-rich upstream sequences, in most cases a contribution of these sequences to promoter function has not been demonstrated directly.

In the *E. coli rrnB* P1 promoter, an upstream A+T-rich sequence functions as a promoter recognition element, the UP element, which increases transcription 30- to 70-fold by increasing the initial equilibrium constant and perhaps by increasing later steps in the RNAP-promoter interaction (9, 25). The UP element is recognized by the RNAP α subunit (20), and mutations in the C-terminal domain of α (αCTD) that prevent DNA binding eliminate UP element function (20, 26). UP elements have been characterized in a number of other promoters as well (20, 27–30). Recently, a consensus UP element sequence was determined by using an *in vitro* selection procedure with *E. coli* RNAP followed by an *in vivo* screen for high transcription activity. The most active (consensus) UP element increased promoter activity more than 300-fold and contained two A-tracts separated by a T-tract (25).

The sequence similarity between phased A-tracts and UP elements, particularly the consensus UP element (Fig. 1), suggested that phased A-tracts might function as UP elements by interacting directly with RNAPα. To test this possibility, we analyzed the transcription and RNAP binding properties of synthetic hybrid promoters containing upstream phased A-tracts. We found that phased A-tracts increased transcription when fused to the *lac* and *rrnB* P1 core promoters both *in vitro* and *in vivo*, and that stimulation of transcription and protection of A-tracts in footprints required the αCTD. Our results provide a clear mechanism for the effects of phased A-tracts on transcription: A-tracts function as UP elements to stimulate transcription through DNA–protein interactions. Although some facet of A-tract structure is likely to be a contributing factor in DNA recognition by α, in most cases the macroscopic curvature conferred by multiple phased A-tracts is unlikely to be a primary determinant of promoter stimulation.

MATERIALS AND METHODS

Hybrid Promoters. A-tract-*lac* or A-tract-*rrnB* P1 promoters (Fig. 1) were constructed by PCR by using plasmid templates carrying the *lac* (pRLG1821) or the *rrnB* P1 (pRLG2230) promoter, a downstream primer complementary to the vector (27), and an upstream primer encoding an *Eco*RI site, the A-tract sequence, and ≈20 nt of *lac* or *rrnB* P1 promoter sequence. Primer sequences are available on request. A *Hin*-

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Abbreviations: RNAP, RNA polymerase; αCTD, C-terminal domain of α.
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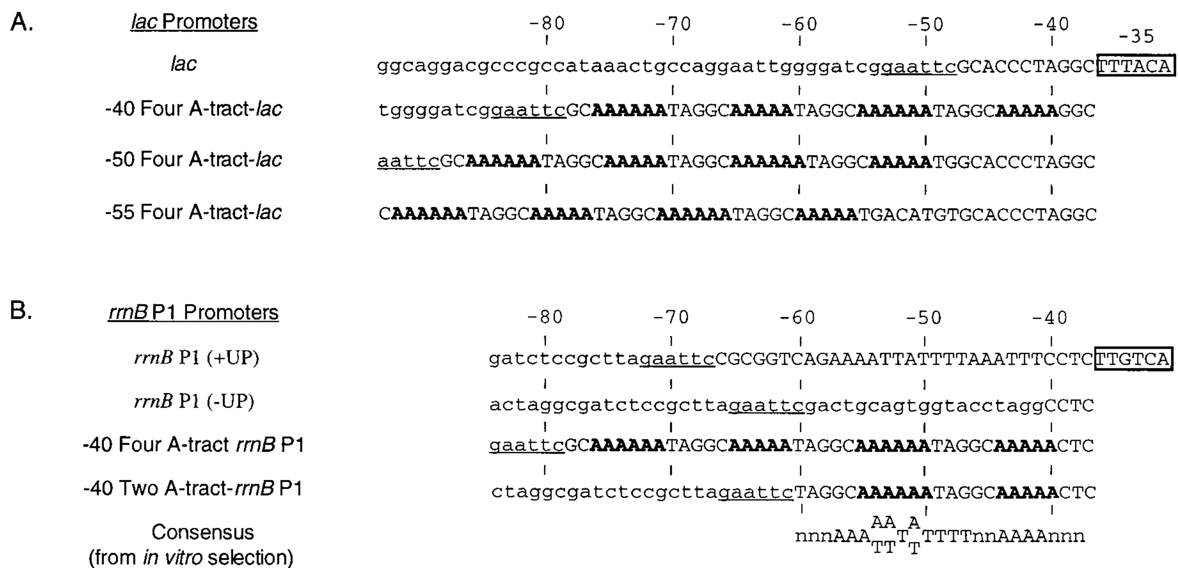

FIG. 1. Sequences of the upstream regions of *lac* and *rrnB* P1 promoter derivatives. Promoter and upstream A-tract-containing sequences are in uppercase. Phased A-tracts are in boldface. Lowercase sequence upstream of the *Eco*RI cloning site (underlined) is from the phage λ vectors in the promoter-*lacZ* fusion constructs (see *Methods*). Promoter sequences downstream of the −35 hexamer (to +52 for *lac* derivatives or +50 for *rrnB* P1 derivatives) are not shown. (*A*) The *lac* core promoter contains *lac* sequence downstream from −47. *Lac* sequence from −41 to −47 does not affect promoter activity (27). Hybrid A-tract *lac* promoters contain an upstream phased A-tract sequence (11) and *lac* sequence downstream from either −39 (−40 A-tract *lac*) or −47 (−50- and −55-A-tract *lac*). (*B*) The *rrnB* P1 promoters contain either the native *rrnB* P1 UP element [+UP, with *rrnB* P1 sequence downstream from −66; (9, 20)], the nonfunctional "SUB" sequence (also lowercase) from −59 to −41 [−UP; (9)], or a phased A-tract sequence (four A-tract as in *A* or a shorter two A-tract sequence) and *rrnB* P1 sequence downstream from −39. The consensus UP element sequence (25) is shown for comparison.

dIII cloning site was constructed at the downstream boundary of the promoter sequence (+52 for *lac* derivatives or +50 for *rrnB* P1 derivatives). PCR products were digested with *Eco*RI and *Hin*dIII, gel purified, and cloned into the vectors pRLG770 (31) and phage λ (see below). Promoter sequences were confirmed after PCR amplification from these vectors.

Determination of Promoter Activities *in Vivo*. Strains monolysogenic for phage λ that carry promoter-*lacZ* fusions were constructed in *E. coli* strain NK5031 by using fusion system I for *rrnB* P1 derivatives or system II for *lac* derivatives (9). Promoter activities were determined by measuring β-galactosidase levels (32) in cultures grown exponentially for four to five generations in Luria–Bertani medium.

Purification of α and Reconstitution of RNAP. N-terminal histidine-tagged α subunits (wild type, Δ235, or R265A) were overexpressed, purified, and reconstituted into RNAP as described (26, 33).

***In Vitro* Transcription.** *rrnB* P1 derivatives were transcribed at 22°C for 15 min in 25-μl reactions containing 0.4 nM supercoiled plasmid/1 nM wild-type or 3 nM α-mutant RNAP/150 mM NaCl/40 mM Tris·acetate (pH 7.9)/10 mM $MgCl_2$/1 mM DTT/100 μg/ml of BSA/500 μM ATP/50 μM CTP/10 μM GTP/10 μM UTP/0.2 μM [α-^{32}P] UTP 800 Ci/mmol (DuPont/NEN). Transcripts were visualized on 5% acrylamide-7 M urea gels as described (31). Transcription of *lac* promoters was as for *rrnB* P1 except that the buffer contained 30 mM KCl and 10 mM Tris·acetate (pH 7.9), and samples were ethanol precipitated overnight at −20°C with 20 μg of glycogen (Boehringer Mannheim) before electrophoresis. Gels were quantified by using a Molecular Dynamics Phosphorimager or were exposed to XAR-5 film (Kodak). Abortive synthesis from hybrid *lac* promoters was carried out as described (11).

Footprinting. Promoter fragments were from derivatives of pSL6 (34), pRLG4270 for four A-tract-*rrnB* P1 and pRLG4271 for two A-tract-*rrnB* P1. Fragments were prepared by digestion of plasmids with *Bam*HI, 3′ end-labeling with [α-^{32}P]-dGTP (800 Ci/mmol; DuPont), and T7 DNA polymerase (Sequenase, Amersham), digestion with *Xho*I, gel purification, and concentration using Elutip-D columns (Schleicher & Schuell). RNAP-promoter complexes were formed with 1 nM DNA and 10 nM wild-type RNAP or 30 nM mutant RNAP (αΔ235 or R265A) for 20 min at 22°C in 10 mM Hepes/100 mM NaOAc/0.1 mM EDTA/1 mM DTT/10 mM $MgCl_2$/500 μM ATP/50 μM CTP and were treated with DNase I (1.25 μg/ml for 30 sec) as described previously (31, 35). Complexes were then isolated on 5% native acrylamide gels, DNA was eluted by diffusion and purified by using Elutip-D columns, resuspended in gel-loading solution (31) and run on 10% acrylamide-7 M urea gels. Gels were exposed to XAR 5 film (Kodak) or analyzed by using a Phosphorimager (Molecular Dynamics).

RESULTS

A-Tract Hybrid Promoters. To test the hypothesis that phased A-tracts increase transcription through interactions with the α subunit of RNAP, we constructed promoter derivatives containing phased A-tracts fused upstream of the *lac* core promoter (A-tract-*lac*) or the *rrnB* P1 core promoter (A-tract-*rrnB*; Fig. 1). The A-tract sequence used was shown previously to stimulate transcription from the *lac* promoter in a position-dependent manner *in vitro* (11) and contains four A-tracts (A_5 or A_6) with 3′ ends positioned 10 or 11 bp apart, in phase with the helical repeat. This sequence was estimated to confer an overall bend of approximately 72° (18° per A-tract) (11, 15, 16).

Two of our hybrid *lac* promoters (Fig. 1*A*) correspond to promoters that exhibited maximal (−50-A-tract-*lac*) or minimal (−55-A-tract *lac*) transcription activity *in vitro* in the previous studies (11). A third hybrid-*lac* promoter contained its first A-tract positioned at −40 (−40-A-tract-*lac*), a position suggested by the importance of an A-tract in the −40 region of the consensus UP element sequence (25). Hybrid *rrnB* P1 promoters contained either two or four phased A-tracts, with the proximal A-tract positioned at −40 (Fig. 1*B*).

Upstream A-Tracts Increase Promoter Activity *in Vivo*. Promoter activities were determined *in vivo* by using chromosomal promoter–*lacZ* fusions (Table 1). The A-tract sequence had a great effect on *lac* promoter activity when positioned at −40, increasing transcription 20-fold over that from the *lac* core promoter (Table 1). The same A-tract sequence had only a 5-fold effect when positioned 10 bp farther upstream at −50 and had no effect when positioned "out-of-phase" at −55, consistent with the previous observations using similar constructs (11). The *rrnB* P1 UP element was about 2-fold more active in stimulating the *lac* promoter than the phased A-tracts (−40-A-tract *lac*), increasing its activity about 40-fold (Table 1) (9).

The phased A-tract sequences not only increased *lac* promoter activity but also increased *rrnB* P1 core promoter activity *in vivo* (Table 1). The four A-tract sequence increased *rrnB* P1 activity 19-fold, whereas the native *rrnB* P1 UP element increased activity 43-fold (Table 1). Thus, the *rrnB* P1 UP element had about a 2-fold greater effect than the phased A-tracts positioned at −40 with each of the two promoter systems tested, *lac* and *rrnB* P1. The two A-tract upstream sequence increased *rrnB* P1 transcription nearly as much as the four A-tract sequence (15- vs. 19-fold; Table 1), indicating that the promoter distal A-tracts in the four A-tract sequence play only a small role in the stimulation of transcription.

Role of the α Subunit of RNAP in Stimulation of Transcription by A-tracts *in Vivo*. To determine whether the A-tract stimulation of transcription required αCTD-upstream DNA interactions, A-tract-*rrnB* P1 promoter activity was compared with *rrnB* P1 (−UP) core promoter activity in strains carrying multicopy plasmids expressing wild-type α or α mutants that abolish UP element function [R265A or αΔ256; (20, 26, 36)]. Stimulation of transcription by upstream A-tracts decreased from 16-fold in the presence of wild-type α to about 3-fold in the presence of the α mutants (Table 2). This effect was very similar to the reduction in *rrnB* P1 UP element function in the presence of mutant α plasmids (Table 2) as observed previously (20, 26). Overexpression of mutant α subunits resulted in a 2-fold increase in *rrnB* P1 core (−UP) promoter activity, in addition to the decrease in A-tract *rrnB* P1 and +UP *rrnB* P1 activities. The increased *rrnB* P1 core promoter activity was previously attributed to derepression of a feedback regulatory system compensating for the loss of *rrn* UP element function [(20); see also ref. 38]. The observed reduction in UP element or A-tract effects is an underestimate of the full effect of the α mutations, because these strains contain both wild-type (chromosomal) and mutant (plasmid-encoded) α alleles.

Table 1. Effects of A-tracts on transcription *in vivo*

Strain	Promoter*	Activity†	Relative activity§
lac promoters			
RLG4281	*lac* core	33 ± 2.1	1
RLG4277	−40 A-tract *lac*	656 ± 14.6	20
RLG4278	−50 A-tract *lac*	165 ± 5.9	5
RLG4279	−55 A-tract *lac*	30 ± 2.8	1
RLG4282	*rrnB* P1 UP *lac*	1309 ± 2.6	40
rrnB promoters			
RLG2263	*rrnB* P1 (−)UP	26 ± 9.5	1
RLG4272	two A-tract-*rrnB* P1	378 ± 6	15
RLG4273	four A-tract-*rrnB* P1	501 ± 9	19
RLG3074	*rrnB* P1 (+)UP	1120 ± 13	43

*Promoters are described in Fig. 1 and in ref. 9.
†β-galactosidase activity in Miller units (32); averages ± standard error from at least three experiments. Values were corrected for a background of 26 ± 1 Miller units for system I fusions in B. Background was <1 Miller unit for system II fusions in A. Absolute values of system I and system II fusions should not be compared directly.
§Values are expressed relative to the promoter lacking an UP element in each set.

Table 2. Effect of α subunit mutations on A-tract stimulation of *rrnB* P1 *in vivo*

rpoA allele*	*rrnB* P1 +UP†	*rrnB* P1 −UP†	4 A-tract-*rrnB* P1†	UP element effect§	A-tract effect§
WT	1209	31	499	39.0	16.1
Δ256	261	60	167	4.4	2.8
WT	1243	31	503	40.1	16.2
R265A	272	59	159	4.6	2.7

*Plasmid-encoded α alleles provided from pLAX185 (WT) and pLAD256 (Δ256) (37) or pHTf1α (WT) and pHTf1αR265A (26).
†β-galactosidase activities in Miller units (32). Strains were: *rrnB* P1 +UP, RLG3074; *rrnB* P1 −UP, RLG2263 or four A-tract-*rrnB* P1, RLG4272 and contained the indicated *rpoA* plasmids. Activities, corrected for background, are the average of two determinations that differed by less than 10%.
§UP element and A-tract effects on transcription are the ratio of the activities of *rrnB* P1 +UP or four A-tract-*rrnB* P1 to the activity of the *rrnB* P1 core promoter (−UP).

The αCTD is Required for Stimulation of Transcription *in Vitro*. To determine whether the A-tract effects on promoter activity *in vivo* (Tables 1 and 2) reflect a direct effect of α subunit interactions with upstream A-tract sequences, independent of other accessory protein factors, we carried out *in vitro* transcription and footprinting with wild-type and α-mutant RNAPs.

The phased A-tract sequences increased transcription from the *lac* promoter with wild-type RNAP in a position-dependent manner *in vitro*, and the relative activities of the hybrid promoters correlated with their *in vivo* activities (Fig. 2*A*). The A-tract sequence had the largest effect on transcription *in vitro* when positioned at −40, had a smaller effect when positioned at −50, and had no detectable effect when positioned at −55 (Fig. 2*A*, lanes 7–12). The effect of the A-tracts (positioned at −40) was about 2-fold less than that of the *rrnB* P1 UP element (Fig. 2*A*, lanes 3, 4, 7, 8). These results confirm that stimulation of transcription by A-tracts does not require protein factors other than RNAP. A second slightly larger transcript observed with the −40 A-tract-*lac* promoter (Fig. 2*A*, lanes 7, 8) probably derives from A-tract stimulation of an overlapping promoter, *lac* P2, as noted previously (11).

When the *lac* promoter derivatives were transcribed with αΔ235-mutant RNAP lacking the αCTD, no stimulation of promoter activity by the A-tract sequences or by the *rrnB* P1 UP element was observed [Fig. 2*B*; see also ref. 39]. Each of the hybrid promoters was transcribed very poorly by mutant RNAP (Fig. 2*B*, lanes 3–12), although this RNAP transcribed *lacUV5* (lanes 1, 2) and RNA I (all lanes) efficiently in the same experiment.

Similar experiments were carried out with the set of *rrnB* P1 derivatives (Fig. 3). Consistent with previous results (20, 25), the *rrnB* P1 and consensus UP elements stimulated transcription by wild-type RNAP (about 25- and 45-fold, respectively; compare lanes 1, 4, 7) but did not stimulate transcription by two α-mutant RNAPs (R265A and αΔ235; compare lanes 2, 3 with 5, 6 and 8, 9). The three enzymes transcribed the *rrnB* P1 core promoter (−UP) with equal efficiency [Fig. 3, lanes 1–3; (20)]. The four A-tract and two A-tract sequences increased *rrnB* P1 transcription with wild-type RNAP (about 10- to 15-fold; compare lanes 1, 10, 13), consistent with their effects *in vivo* (Table 1). However, the A-tract sequences did not increase transcription by either of the α-mutant RNAPs (compare lanes 2, 3, 11, 12, 14, 15). We conclude that the A-tract sequence functions like an UP element; i.e., its effects are dependent on the DNA-binding function of the α subunit.

We also found that the A-tract sequence and the *rrnB* P1 UP element increased the synthesis of abortive products from the

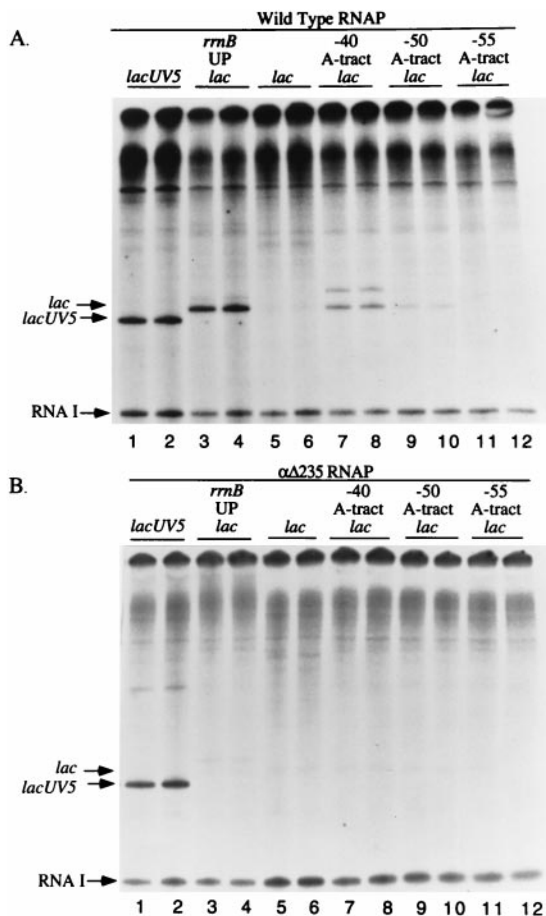

FIG. 2. *In vitro* transcription of wild-type *lac* or *lac*-hybrid promoters with (*A*) wild-type RNAP or (*B*) α-mutant RNAP (αΔ235). Duplicate samples are shown. Transcripts from the *lacUV5*, *lac*, and vector-encoded RNAI promoters are indicated with arrows. The *lacUV5* transcript is ≈10 nt shorter than *lac* transcripts because of a different promoter downstream endpoint (+39). Plasmid templates were pRLG593 [*lacUV5*; (31)]; pRLG1820 [*rrnB* P1 (−88 to −37, Δ72)-*lac*(−36 to +52); (9)], pRLG1821 [*lac* −47 to +52; (9)], pRLG4258 (−40 A-tract-*lac*; Fig. 1), pRLG4260 (−50 A-tract-*lac*; Fig. 1), and pRLG4262 (−55-A-tract-*lac*; Fig. 1).

hybrid *lac* promoters with wild-type RNAP (data not shown), consistent with the effects on productive transcription (Fig. 2) and with previous reports (11). With αΔ235-mutant RNAP, abortive product formation from the A-tract-*lac* and *rrnB* UP-*lac* hybrid promoters was dramatically reduced (by at least 90%; data not shown), although a low level of synthesis above that from the *lac* core promoter was observed. The significance of this small residual effect remains to be determined.

Protection of the A-Tract Regions by RNAP in Footprints Requires the αCTD. The αCTD requirement for the A-tract effects on transcription *in vivo* (Table 2) and *in vitro* (Figs. 2, 3) is consistent with a direct interaction of the α subunit with these sequences, as observed with UP elements (20). To determine whether RNAP protects the A-tract region in an αCTD-dependent manner, we carried out DNase I footprints of complexes formed by wild-type or α-mutant RNAP and the two A-tract or four A-tract *rrnB* P1 hybrid promoters (Fig. 4*A* and *B*). Wild-type RNAP protected the core region of each promoter (−35 to +20), as well as several positions in the region containing the first and second A-tracts [−43 to −45 and −54 to −56, with DNase I accessible positions at about −48. DNase I accessible sites within UP element-protected regions have been observed previously (27)]. In addition, partial protection of positions in the third and fourth A-tract

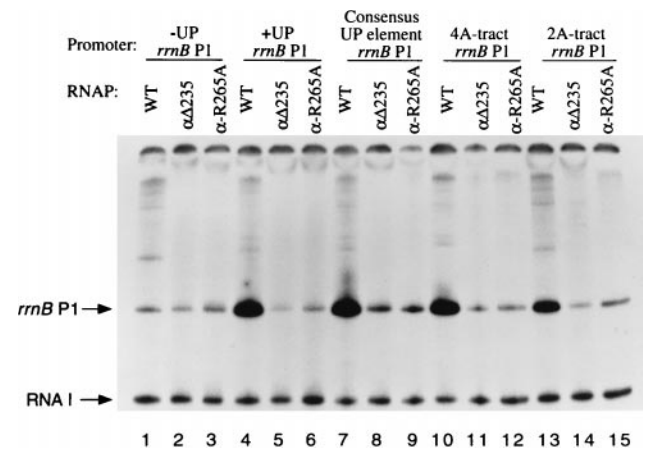

FIG. 3. *In vitro* transcription of *rrnB* P1 promoter derivatives with wild-type or α-mutant (αΔ235 or R265A) RNAPs. The consensus UP element-*rrnB* P1 promoter contains the sequence of the 4192-UP element, 5′AAAATTTTTTTTCAAAAGTA from −57 to −38 (25). Transcripts from the RNAI and *rrnB* P1 promoters are indicated by arrows. Plasmid templates were pRLG2230 [−UP; *rrnB* P1 (−41 to +50), Fig. 1], pRLG4238 [+UP; *rrnB* P1 (−66 to +50), (25)], pRLG3278 [UP element 4192-*rrnB* P1; (25)], pRLG4268 (four A-tract-*rrnB* P1; Fig. 1), and pRLG4269 (two A-tract-*rrnB* P1; Fig. 1).

regions of the four A-tract promoter was also observed (Fig. 4*B*, lane 5) and is similar to protection patterns observed with some UP elements (see *Discussion*).

The upstream A-tract regions were not protected in footprints with the α-mutant RNAPs (αΔ235 and αR265A; Fig. 4*A*, lane 5; 4*B,* lanes 6, 7), although these RNAPs protected the core promoter regions. These results indicate that the αCTD is required for interaction of RNAP with the A-tract regions, as observed for UP elements (20, 25, 27). The partial protection of the core promoter upstream of the −10 element by the mutant RNAPs (Fig. 4) was also observed with the wild-type *rrnB* P1 promoter (W.R., unpublished results). Because the heparin stable RNAP-promoter complexes were gel isolated in these experiments, the partial protection may reflect indirect effects of the α mutants on RNAP-core promoter interactions.

Similar results were obtained by using hydroxyl radical footprinting (data not shown), and the A-tract region positions protected by wild-type RNAP (at −40 to −44 and −51 to −55) were the same as those protected in the *rrnB* P1 UP element (25, 35). In addition, as with the *rrnB* P1 and consensus UP elements (20, 25), protection of the A-tract region was observed in footprints with high concentrations of purified α (data not shown).

DISCUSSION

A-Tracts Increase Transcription by Binding to the RNAP α Subunit. We found that phased A-tracts placed upstream of either of two core promoters (*lac* or *rrnB* P1) increased promoter activity both *in vivo* and *in vitro* in a position-dependent manner. These results are consistent with previous studies on a variety of promoters indicating that A-tracts stimulate transcription (e.g., refs. 2, 4, 11, 12, 19). By several criteria we showed that the A-tract-containing sequences function as recognition elements for RNAP, interacting with the αCTD in a manner analogous to UP elements in *rrnB* P1 and other bacterial promoters (20, 27–29).

The phased A-tract sequence was approximately 2-fold less effective in stimulating transcription than the *rrnB* P1 UP element and 5- to 10-fold less effective than the best UP elements *in vivo* (25), consistent with its degree of similarity to the UP element consensus. When positioned at −40, where it increased transcription most effectively, the A-tract sequence

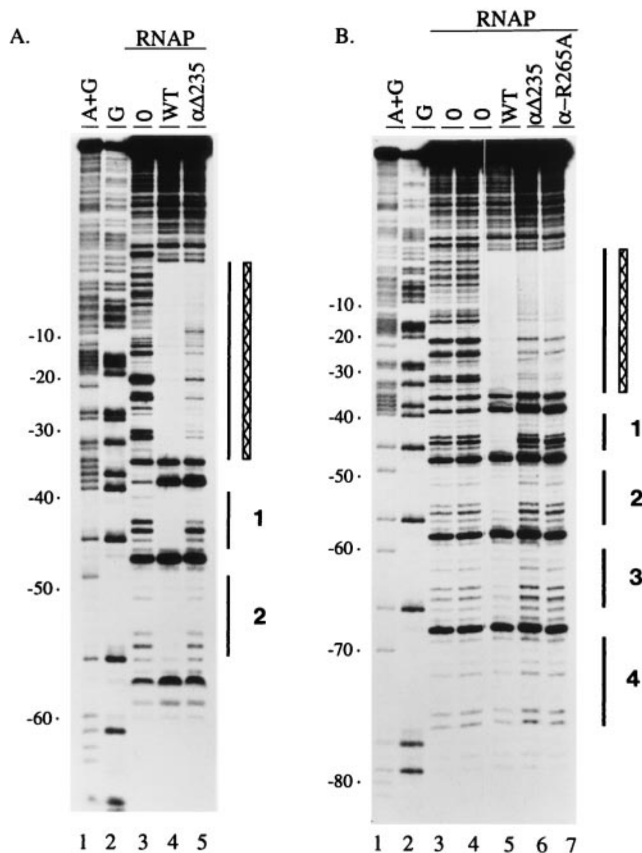

FIG. 4. DNase I footprints of complexes formed by (*A*) the two A-tract-*rrnB* P1 promoter or (*B*) the four A-tract-*rrnB* P1 promoter with wild-type RNAP (10 nM) or mutant RNAP (αΔ235 or R265A; each 32 nM). Control samples lacking RNAP (0) are in lanes 3 (in *A* and *B*) and 4 (in *B*). Regions fully or partially protected by wild-type RNAP (WT; vertical bars) and by α-mutant RNAPs (αΔ235 or αR265A; hatched boxes) are indicated. The phased A-tract-containing regions in the two A-tract promoter (*A*) are labeled 1 and 2, and in the four A-tract promoter (*B*) are labeled 1–4, where 1 represents the promoter proximal A-tract region (see Fig. 1 for sequences). A+G and G sequence markers were prepared as described in (40). Promoter fragments were 3′-end labeled in the bottom (template) strand at a site just upstream of the A-tract regions.

matches the consensus at nine of fifteen positions. These include four important A residues at positions −41 to −44 and five of seven consensus residues between −49 and −55 but do not include the T-tract between those regions (Fig. 1). In comparison, the *rrnB* P1 UP element matches the consensus at 12 of 15 positions (25). The extent of transcription stimulation by other UP elements also correlates qualitatively with their degree of similarity to the consensus (25, 27).

Our data suggest that the interaction of αCTD with the A-tract sequence is similar to its interaction with other UP elements. Substitution of alanine for arginine at the surface-exposed α residue 265 (R265A) abolished the function of the phased A-tract sequence as well as of the *rrnB* P1 and consensus UP elements [Fig. 4; (25, 26, 36)]. In addition, the same upstream DNA positions were protected in both the A-tract sequence and the *rrnB* P1 UP element in footprints with wild-type RNAP (20, 25, 35).

Effects of A-Tract Number and Location on αCTD Interactions. The position-dependent effects of A-tracts on transcription are consistent with properties of UP elements. The major determinants of the *rrnB* P1 UP element occur between −40 and −60, although sequences upstream of −60 can increase transcription another 2-fold (9, 25). The proximal region of the consensus UP element (the −40 region) makes the largest contribution to function and can stimulate transcription greatly independent of the rest of the UP element (S. T. Estrem, T. Gaal, W. Ross, W. Niu, R. H. Ebright, and R. L. Gourse, unpublished results). Consistent with these properties, the A-tract sequence stimulated transcription best when positioned at −40 (Table 1; Fig. 2*A*), and the promoter proximal two A-tracts were responsible for most of the A-tract effects (Table 1; Fig. 3).

We suggest that αCTD-dependent interaction of RNAP with the third and fourth A-tracts may also account for the small effect of these sequences on promoter activity. Small effects of sequences upstream of −60 and interaction of these sequences with αCTD have been observed previously for other promoters (29, 41–43). The affinity of αCTD for the third and fourth A-tract region may be greater than any affinity it might have for the nonspecific vector sequence in the analogous position in the two A-tract promoter, thus accounting for the small difference in the activities of the two and four A-tract promoters.

The flexibility in positioning of the αCTD is thought to result from the domain structure of the α subunit, in which a flexible linker tethers the αCTD to the N-terminal domain (44, 45). Properties of RNAPs containing only one αCTD suggest that a single αCTD can bind to and protect a region corresponding to half of the *rrnB* P1 UP element (S. T. Estrem, T. Gaal, W. Ross, W. Niu, R. H. Ebright, and R. L. Gourse, unpublished results). Partial protection of multiple regions by wild-type RNAP (containing two αCTDs) may result either from occupancy of different regions in different molecules in the population of DNA fragments or from short-lived αCTD interactions with different regions on the same DNA fragment during the time course of the footprinting reaction.

The face-of-the-helix dependence of A-tract effects [Table 1; Fig. 2; (11, 12, 46)] is consistent with the similar position dependence of the distal region of the *rrnB* P1 UP element (41). This position dependence of the α subunit binding site in the UP element most likely derives from constraints on the positioning of the αCTD with respect to the rest of RNAP, as observed for positioning of activator protein binding sites [e.g., cyclic AMP receptor protein (CRP); (47, 48)] because the contacts between RNAP and DNA in other regions of the promoter are restricted to one side of the helix (35, 49).

Other Effects of A-tracts on Transcription. Upstream A-tracts functioned as UP elements to stimulate transcription from the *lac* and *rrnB* P1 core promoters in this work. However, at other core promoters with mechanisms rate-limited at different steps in the pathway to productive transcription, complexes may be affected differently by UP element sequences. For example, at a promoter limited in promoter escape, the presence of upstream A-tracts reduced promoter activity (12). Similarly, αCTD interactions with transcription factors can sometimes limit transcription (50, 51).

Relationship of DNA Bending to UP Element Function. We have shown here that A-tracts function as UP elements through DNA–protein interactions with the αCTD. We suggest that these interactions contribute to the previously described wrapping of DNA around RNAP in promoter complexes (14, 52). However, several lines of evidence suggest that the macroscopic DNA bending associated with multiple in-phase A-tracts is not a requirement for the stimulatory effect on transcription: (*i*) sequences containing only one A-tract are sufficient to stimulate transcription greatly [S.T. Estrem, T. Gaal, W. Ross, W. Niu, R.H. Ebright, and R.L. Gourse, unpublished results; (12)]; (*ii*) a 2-fold increase in the number of A-tracts increases the level of transcription activation only slightly (Table 1); and (*iii*) some UP elements (e.g., *rrnB* P1) display little or no DNA curvature (53).

Although macroscopic curvature is not essential for the A-tract effects on transcription, the unusual structural features of A-tract DNA, including a narrow minor groove and a high

degree of propeller twist (reviewed in ref. 54) may play a role in α recognition. It is not yet known whether recognition of UP elements (and their A-tracts) by α involves base-specific interactions or features of the DNA backbone structure or both.

Our results do not exclude the possibility that the macroscopic curvature associated with multiple-phased A-tracts may play a different or additional role in transcription at some promoters. For example, curved DNA functions as a "coactivator" to facilitate interaction between distantly bound enhancer proteins and $E\sigma^{54}$ (55, 56). In addition, we cannot eliminate the possibility that there may be cases where A-tracts influence transcription by a "structural transmission" effect such as that proposed for Integration Host Factor (IHF) binding at the *ilv*P_G promoter (57). However, we suggest that in most cases upstream A-tracts increase transcription through DNA–protein interactions with the αCTD and should therefore be considered UP elements.

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