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Upstream Sequence Activation of *Escherichia coli argT* Promoter in Vivo and in Vitro†

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ABSTRACT: *Escherichia coli argT* promoter in a *galK* fusion construct is shown by BAL 31 deletion to require its upstream region for high in vivo activity. The extent of activation conferred by the upstream sequence from −130 to −38 is 25-fold. A spontaneous mutant containing a T to G transversion at −37 (i.e., the T-37G promoter) shows a similar requirement; however, the upstream sequence producing a 10-fold effect spans only −130 to −60. The difference in upstream sequence boundaries between the wild-type and T-37G promoters suggests the possible existence of two activating elements. Gel mobility investigation points to the presence of bent DNA in the *argT* promoter, and the bent center was localized to the −90 to −95 region by circular permutation analysis. The role of the upstream activating sequence (UAS) in promoter function was probed by competitive transcription experiments in vitro. Results of this type of analysis indicate that the full UAS activates transcription through a combined effect on K_B and k_2. Of these, K_B is significantly strengthened by the proximal element, and k_2 is stimulated to a smaller extent by the distal element. The evidence from deletion analysis, gel mobility investigation, and competitive transcription together support a "two-element" model of UAS function for the *argT* promoter.

To probe the regulation of a gene, it is necessary to first understand the activity of its promoter. Research over the past decade has made clear that many of the signals of regulation reside within the promoter region. Prokaryotic promoters recognized by $E\sigma^{70}$ have been shown to contain two hexameric consensus elements at the −35 and −10 regions separated optimally by 17 base pairs (bp)[1] of spacer DNA (Rosenberg & Court, 1979; Siebenlist et al., 1980; Hawley & McClure, 1983; Harley & Reynolds, 1987). Abundant biochemical and genetic analyses point to these elements as the recognition signals for RNA polymerase binding to the promoter [reviewed by McClure (1985)]. RNA polymerase σ-subunit mutations that compensate for changes in the consensus nucleotides further substantiate the role of these hexameric sequences in transcription initiation (Helmann & Chamberlin, 1988; Gardella et al., 1989; Siegele et al., 1989; Zuber et al., 1989).

For many phage and cellular messenger RNA promoters, a direct correlation has been demonstrated in vitro between the facility of open complex formation, as measured by the product K_Bk_2, and the agreement of the hexameric sequences to consensus, as meausred by the homology score (Mulligan et al., 1984). This relationship suggests that the consensus nucleotides provide the maximum fit in promoter–polymerase binding. On the basis of the K_Bk_2 values, this diverse set of promoters can be ordered on a scale of intrinsic selectivity by RNA polymerase that varies over 4 orders of magnitude (Mulligan et al., 1984).

A subset of the above promoters has also been analyzed systematically in vivo (Bujard et al., 1987). The comparison reveals a lack of correspondence between the in vivo promoter strength and the promoter hierarchy established in vitro (Deuschle et al., 1986). For example, *tac*, the synthetic consensus promoter, was found not to be the most active promoter in vivo (Bauer et al., 1988; Bujard et al., 1987); instead, it was shown that sequences downstream from +1 can play a significant role in determining activity. This finding coupled with the realization that no naturally occurring *E. coli* promoters utilize the consensus sequence led to the suggestion that promoter activities are optimized, rather than maximized, in vivo (McClure, 1985; Bujard et al., 1987).

Many factors have been found to contribute to promoter optimization in vivo, some exerting generalized effects while others influencing unique sites. These factors include the role of activator and repressor proteins, the degree of template

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[1] Abbreviations: OAc, acetate; amp, ampicillin; ATP, adenosine 5′-triphosphate; bp, base pair(s); BSA, bovine serum albumin; CMP, cytidine 5′-monophosphate; CTP, cytidine 5′-triphosphate; DTT, dithiothreitol; EtdBr, ethidium bromide; Na_2EDTA, ethylenediaminetetraacetic acid disodium salt; gal, galactose; GalK, galactokinase; gal-1-P, galactose 1-phosphate; GTP, guanosine 5′-triphosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; KGlu, potassium glutamate; nt, nucleotide(s); NTP, ribonucleoside triphosphate; PAGE, polyacrylamide gel electrophoresis; PolIk, DNA polymerase I Klenow fragment; RNAP, RNA polymerase; rms, root mean square; SD, standard deviation; Tris, tris(hyroxymethyl)aminomethane; UAS, upstream activating sequence; UTP, uridine 5′-triphosphate; XC, xylene cyanol.

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superhelicity, the active RNA polymerase concentration, and the participation of flanking sequence elements in promoter function (von Hippel et al., 1984; McClure, 1985; Reznikoff et al., 1985; Bujard et al., 1987). For a given promoter, its activity under any growth condition would be the balanced outcome of a specific combination of the above factors.

As a class, the stable RNA promoters of *E. coli* have been found to exhibit high strength in vivo; the *rrnABP*$_1$ and *leu*V promoters are among the strongest cellular promoters known (Bujard et al., 1987; Bauer et al., 1988). While a variety of factors ensure the in vivo activity of P$_1$ (Gourse et al., 1986), our knowledge concerning the regulation of other stable RNA promoters is less thorough. One factor which has been shown to influence the in vivo strength of many stable RNA promoters involves upstream sequence signals (Lamond & Travers, 1983; Bossi & Smith, 1984; van Delft et al., 1987; Bauer et al., 1988; Lukacsovich et al., 1989). For several of these promoters, the associated upstream elements were shown to activate gene expression in part by intrinsic bending; for others, the involvement of activator proteins has been demonstrated (Vijgenboom et al., 1988; Nachaliel et al., 1989). Mechanistically, how the UAS and DNA bending contribute to promoter activation is not clearly known.

In this study, we report on the upstream sequence requirement for the *argT* tRNA gene promoter in *Escherichia coli*. This analysis reveals the possible existence of two activating elements. The UAS is shown to bend intrinsically, and the bending locus is centered at the −90 to −95 region. By performing competitive transcription as a function of RNA polymerase concentration, the proximal and distal portions of the UAS were found to stimulate promoter function by separately enhancing K_B and k_2, respectively.

Experimental Procedures

Bacterial Strains and Plasmids. *E. coli* strain N100 (*pro his galK recA*) and plasmid pDS26T were obtained from M. Rosenberg. Plasmid pDS26T is a derivative of pDS20 containing a λt_0 terminator downstream of the *galK* gene (Schumperli et al., 1982). Plasmid pGC4, containing a truncated *rrnA* operon preceding the *galK* structural gene, is a kind gift of M. Cashel (Sarmientos & Cashel, 1983). Designations of all derivatives of plasmid pMR1 are described in the legend to Figure 1B,C.

Construction of the argT Promoter–galK Fusion Plasmid. The *argT* promoter spanning nucleotides −129 to +28 (see Figure 1A) was recovered on a *Hae*III–*Pvu*II fragment from plasmid pLC25-25 (Hsu et al., 1984). This fragment was blunt-end-ligated into *Eco*RI- and *Hin*dIII-digested, PolIk-treated pDS26T DNA to replace the *gal* promoter (Maniatis et al., 1982). Transformation of competent N100 cells gave rise to the fusion clone pMR1.

Plasmid and Fragment DNA Preparation. Supercoiled plasmid DNA was prepared from alkaline lysate (Birnboim & Doly, 1979) by two rounds of CsCl–EtdBr density gradient centrifugation, dialyzed extensively against TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA), and stored at 4 °C for transcription purposes. Fragment DNA used as transcription templates was recovered by electroelution from agarose gels in TAE buffer (40 mM Trizma base, 20 mM acetic acid, and 2 mM Na_2EDTA, pH 8.1), adsorbed to and eluted from NENSORB 20 columns (DuPont/New England Nuclear) as described by Johnson et al. (1986). The concentration of DNA was determined by the absorbance reading at 260 nm; 1 A_{260} unit was equated with 50 μg/mL DNA.

Deletion Mutagenesis. Plasmid pMR1y1 or pMR1y6 DNA (4 μg) linearized at the upstream *Eco*RI site was treated with 0.3 unit of BAL 31 (New England Biolabs) at 30 °C for 5–10 min in a 50-μL volume in the recommended buffer. After the reaction was quenched with EDTA, truncated DNA was adapted with *Eco*RI linkers, restricted with *Eco*RI, recircularized with T4 DNA ligase, and used to transform N100 cells. Transformants harboring upstream deletions were identified by sizing the *Eco*RI–*Sma*I-liberated promoter fragment by PAGE (5%; 19:1). The deletion end point in each mutant was determined by DNA sequencing. Designation of the various deletion derivatives appears in the legend to Figure 1B.

Tandem Dimer Construction. Tandem dimer of the wild-type *argT* promoter was constructed by using pCLy1L DNA as the starting plasmid. The 554 *Ssp*I–*Hin*dIII fragment (see map of Figure 1C) was treated with PolIk and ligated into pCLy1L/*Ssp*I vector DNA. A tandem dimer clone, pTDy1, was identified when a 558 bp fragment was released upon digestion of the plasmid DNA by six different restriction enzymes (i.e., *Aat*II, *Eco*RI, *Dra*I, *Sma*I, *Sna*BI, *Taq*I; see Figure 1B).

DNA Sequencing. DNA sequencing by the Sanger dideoxy terminator method (Sanger et al., 1977) was performed with double-stranded plasmid templates using a pBR322 *Eco*RI primer (New England Biolabs). The sequencing protocol was adapted from the Promega Biotec Manual (1986).

Galactokinase Assay. Fresh bacterial lysates were made from log-phase cultures (A_{650} = 0.4–0.6) grown in M9-supplemented medium (Miller, 1972). GalK assay was conducted to ensure the linearity of the response to enzyme concentration and time as described (Wilson & Hogness, 1966; McKenney et al., 1981). GalK units were expressed in nanomoles of gal-1-P formed at 32 °C per minute per milliliter of cells at an A_{650} of 1. Plasmid copy number variation among different lysates was corrected by normalizing GalK to the β-lactamase level determined by the spectrophotometric assay of Ross and O'Callaghan (1975).

Gel Mobility Assay. The mobility of *argT* promoter fragment and its various derivatives was assessed relative to the following molecular weight standards: pBR327/*Hin*fI digest, φX174/*Hae*III digest, and pBR327/*Hae*III digest. Electrophoresis was carried out at 4 or 25 °C (i.e., room temperature) in 7% (83:1) polyacrylamide gels in TAE buffer at 5–6 V/cm (Fried & Crothers, 1981). DNA bands were visualized by EtdBr staining.

In Vitro Transcription Reaction. RNA polymerase holoenzyme, prepared by the method of Gonzalez et al. (1977), was 100% saturated with σ^{70} and assayed to contain 40–65% active molecules at the time of preparation (Chamberlin et al., 1979). The enzyme was the generous gift of K. Arndt and B. Krummel in M. J. Chamberlin's laboratory. In vitro transcription reactions were routinely performed in 20–40-μL volume containing 18 nM template DNA in 30 mM K^+-HEPES, pH 8.0, 10 mM $MgCl_2$, 0.2 M KGlu, 1 mM DTT, 100 μg/mL nuclease-free BSA, 2.7 mM ATP, 1.4 mM UTP, 1.1 mM GTP, and 0.7 mM [α-^{32}P]CTP at a specific activity of 300–700 cpm/pm (Leirmo et al., 1987; Chamberlin et al., 1983). RNA polymerase concentrations and times used in each assay are given in the individual figure legends. In mixed-template reactions, the two competing templates were equimolar at 9 nM each. For most assays, the reaction mixture was first preincubated at the indicated temperature for 5 min, and RNA polymerase was added with quick mixing to commence the reaction. To terminate transcription, sample aliquots (20–40 μL) were first diluted to 100-μL volume to contain 30 mM EDTA and 0.42 mg/mL yeast carrier RNA; total RNA was then recovered by phenol/chloroform ex-

tractions and ethanol precipitation. For transcript quantitation, RNA was fractionated in denaturing polyacrylamide gels and autoradiographed, and the ^{32}P-labeled bands were excised for scintillation counting.

Results

The goal of the present study is to assess the role of upstream sequence in *argT* promoter function. This requirement was probed first in vivo by BAL 31 deletion using an *argT* promoter–*galK* fusion plasmid.

galK Fusion Plasmids Containing argT Promoters. The *argT* promoter–*galK* fusion was constructed as described under Experimental Procedures. The clone, pMR1, was originally isolated as a pink colony from a MacConkey/gal/amp indicator plate (McKenney et al., 1981) and was shown by DNA sequencing of the promoter region to contain the wild-type *argT* sequence. However, during subsequent propagation of this plasmid strain, the occurrence of red and white colonies on MGA agar was noted. To investigate the nature of these plasmids, a red and white colony each was picked from MGA plates, grown, and streaked repeatedly on L/amp agar to obtain the two isolates designated pMR1y1 and pMR1y6. While both isolates grew at comparable rates in L/amp broth or agar, they differed drastically when grown on MGA plates. Strain pMR1y1 grows slowly to form a small white colony after 2 days, while strain pMR1y6 forms a large magenta red colony after just one overnight of incubation.

The two strains showed little difference in plasmid copy number per cell (data not shown). DNA sequencing revealed that pMR1y1 contained the wild-type *argT* promoter sequence while pMR1y6 has undergone a T to G transversion at the −37 position (data not shown). Normalized GalK levels in pMR1y1 and pMR1y6 lysates were 1060 ± 123 (1 SD) units/mL (n = 12) and 62 ± 21 (1 SD) units/mL (n = 6), respectively. The large reduction in promoter activity due to the T-37G mutation confirms the importance of the highly conserved 5′-T residue in the −35 hexameric consensus of prokaryotic promoters (Hawley & McClure, 1983).

Throughout subsequent handling, these two isolates behaved as stable clones. Plasmid DNA prepared from these strains at different times did not show further sequence changes in the promoter region. Also, various plasmid DNA preparations can be used to transform fresh N100 competent cells to reproducibly yield a homogeneous population of transformants on MGA plates; this latter evidence eliminates chromosomal DNA changes as a possible factor in the stabilization of these plasmid strains. Although current available evidence does not allow us to rule out nucleotide changes in the vector portion of these plasmids, the above criteria suggest that the principal difference between pMR1y1 and pMR1y6 is likely the point mutation in the *argT* promoter.

Presently, the factors that destabilized pMR1 or stabilized pMR1y1 are not known exactly. We suspect that destabilization was brought about by the inadvertent prolonged exposure of pMR1 to a galactose-containing medium (i.e., MGA agar). Were this the case, the judicious avoidance of such an exposure should stabilize pMR1y1; this was in fact observed. In our workings with pMR1y1 and pMR1y6 since their isolation, both clones have been stable to continuous propagation in L-broth media by all criteria mentioned above. Given the stability of pMR1y1 and the wild-type nature of its promoter insert, subsequent analyses of this plasmid are deemed relevant to the understanding of *argT* promoter activity in vivo.

Upstream Region of the argT Promoter Is Required for Activity in Vivo. BAL 31 deletion was performed on both pMR1y1 and pMR1y6 DNA to probe the upstream sequence

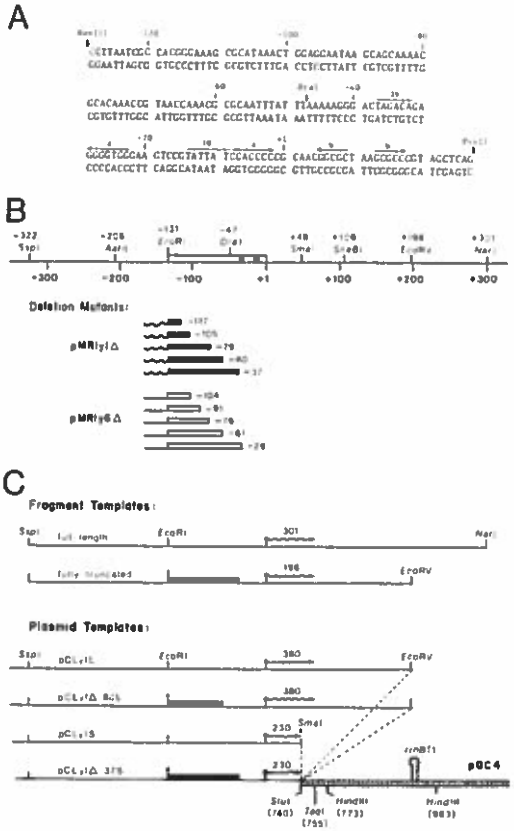


FIGURE 1: (A) Sequence of the *argT* promoter on the *HaeIII–PvuII* fragment. Numbering was relative to the transcriptional start site (+1). The −35 and −10 hexameric consensus elements are indicated. Two pairs of inverted repeats, a and b, are marked by overline arrows. (B) Map of the *argT* promoter in the fusion plasmid pMR1y1 and its various truncated derivatives. Map coordinates are assigned relative to the transcriptional start site (+1). The open box represents the *argT* promoter, and the vertical solid bars correspond to the −35 and −10 hexamers. Upstream deletion from the *Eco*RI site generated pMR1y1Δ and pMR1y6Δ plasmids; the numbering appearing after the Δ sign corresponds to the nucleotide retained after BAL 31 deletion. The extent of deletion in each is shown as the solid (pMR1y1Δ) or open (pMR1y6Δ) bars. The series of pMR1y1Δ promoters were not recloned to normalize the upstream vector background (as indicated by the squiggle lines) since consistent GalK measurement could be obtained for these plasmids. Recloning, however, was crucial for the pMR1y6Δ series (straight lines) in order to obtain reliable GalK measurement. (C) Templates for in vitro transcription. Wild-type "full-length" fragment template was excised from pMR1y1 by *SspI–NarI* double digestion; mutant "truncated" templates were recovered from the pMR1y1Δ plasmids by *SspI–Eco*RV cuts. The expected runoff transcripts from the wild-type and truncated promoters were 301 and 198 nt, respectively. The *SspI–Eco*RV fragment from pMR1y1Δ-37 was designated the "fully-truncated" promoter. Supercoiled plasmid templates were constructed by fusing the *Eco*RI–*Sma*I or *Eco*RI–*Eco*RV fragment from the pMR1y1 plasmids to the pGC4/*Eco*RI–*Stu*I vector to replace the $rrnAP_1P_2$ promoters (stippled bar; Sarmientos & Cashel, 1983). The resultant plasmids are referred to as pCLy1 plasmids with similar Δ designations to denote upstream deletion. Downstream fusion (dotted lines) leads to transcription termination at the *rrnB*T1 terminator. "L" or "S" attached to each plasmid template refers to downstream joining via either the *Eco*RV or the *Sma*I site, respectively, to yield the "Long" (~380 nt) or "Short" (~230 nt) terminated transcripts.

requirement for the *argT* promoters. Two sets of mutants were obtained, and the extent of deletion in each is diagrammed in Figure 1B. Next, GalK level was determined for each mutant to examine the relationship between upstream deletion and *argT* promoter activity.

In measuring GalK levels, it was necessary to reclone the truncated promoters from the pMR1y6Δ series in order to obtain consistent values. For these extremely weak promoters, recloning normalized the upstream vector sequence and con-

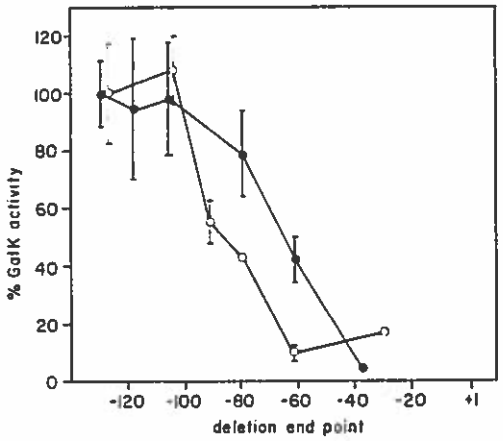


FIGURE 2: Upstream sequence requirement for *argT* promoter activity in vivo. GalK level, normalized to β-lactamase activity, was measured in N100 cells harboring a pMR1y1Δ plasmid (filled circles) or a pMR1y6Δ plasmid (open circles). Each was in turn normalized to the GalK activity of the full-length promoter in each series to obtain percent GalK activity; 100% GalK levels for pMR1y1 and pMR1y6 were 1060 ± 123 (1 SD; $n = 12$) and 62 ± 21 (1 SD; $n = 6$) units/mL, respectively. For the pMR1y1Δ series, GalK determination was performed 3–6 times; the error bar (solid line) represents 1 SD calculated by the rms method. For the pMR1y6Δ plasmids, GalK measurement was done twice; the error bar (dotted line) represents the range of the two values.

comitantly the background transcriptional noise. The background noise due to the upstream vector sequence was 10% of pMR1y6, as shown by the Δ-61 clone. A complementary deletion from the downstream *Sma*I site to −30 resulted in 11% of the pMR1y6 GalK expression (data not shown) and confirmed the absence of an interfering promoter in the upstream flanking region of the *argT* sequence itself. A second complication often encountered in working with a weak promoter involves the formation of new promoter signals due to deletion/fusion/recloning manipulations. Such mutants are detected by DNA sequencing, and their resultant GalK activity is interpreted with caution; an example of such a mutation was found in pMR1y6Δ-29 (see Figure 2). Given these appropriate controls, the deletion results obtained with the pMR1y6Δ series of promoters are considered valid. For the strong pMR1y1Δ series of promoters, interpretation of the GalK data was not complicated by transcription from weak endogenous promoters, and recloning was not crucial in obtaining reliable GalK measurement.

In Figure 2, the average GalK level (2–12 determinations) measured for each mutant was plotted as a function of its deletion end point. Despite substantial variability associated with each, the overall results clearly showed a strong dependence of the activity of *argT* promoters on the span of upstream DNA. Both wild-type and T-37G promoters exhibited this requirement with activation of 25- and 10-fold, respectively.

While both wild-type and T-37G promoters were stimulated by upstream sequences, the boundaries of the respective UAS differed. This conclusion stands even when the large variability of the data points is taken into account. This difference points to the possible existence of two activating elements within the UAS region: a proximal element spanning nucleotides −60 to −38 and a distal element encompassing the remainder of the UAS from −60 to −130. Interestingly, the two promoters appear to be differentially dependent on the upstream region; while the T-37G promoter relies heavily on the distal element for activity, the wild-type promoter derives most of its activation from the proximal element.

Anomalous Gel Mobility of the argT Promoter Fragment.

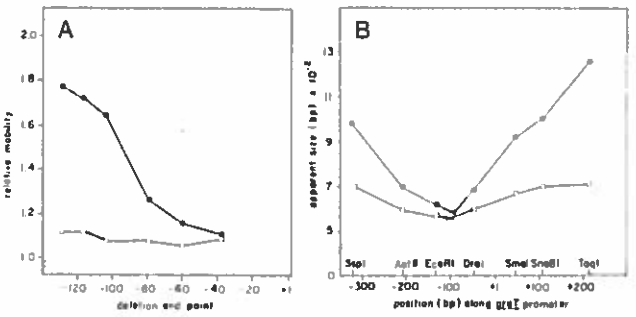


FIGURE 3: (A) Mobility anomaly of wild-type and truncated *argT* promoter fragments. Various pCLy1Δ-L plasmids (see description in legend to Figure 1C) were digested with *Ssp*I and *Hin*dIII to liberate the *argT* promoter fragments. The digests were electrophoresed in 7% (83:1) polyacrylamide gels in TAE buffer at a voltage gradient of 5–6 V/cm either at 25 °C (i.e., room temperature; open circles) or at 4 °C (solid circles) against three sets of molecular weight standards: pBR327/*Hin*fI digest, φX174/*Hae*III digest, and pBR327/*Hae*III digest. The apparent lengths are extrapolated from the gel profiles (not shown). The expected lengths of the various *argT* promoter fragments are 554, 544, 532, 506, 487, and 464 bp for pCLy1L, Δ-117L, Δ-115L, Δ-79L, Δ-60L, and Δ-37L, respectively. Mobility anomaly is plotted as relative mobility (the ratio of apparent versus expected fragment lengths) versus the deletion end point. (B) Location of the bent center. Construction of the tandem dimer clone pTDy1 was described under Experimental Procedures. Single digests of pTDy1 DNA with *Aat*II, *Eco*RI, *Dra*I, *Sma*I, *Sna*BI, and *Taq*I were electrophoresed either at 25 °C (open circles) or at 4 °C (solid circles) as described in (A) above. The apparent size of the promoter fragment liberated by a restriction enzyme is plotted as a function of the position of that enzyme cut site; the reference monomer length of the *argT* promoter is 554 bp. The resultant curves allowed the extrapolation of the bent center to nucleotide −92 ± 3 bp.

The existence of an UAS for the *argT* promoter prompted us to examine the mobility of various promoter fragments by PAGE. A preliminary experiment using the 179 bp *Eco*RI–*Sma*I fragment of pMR1y1 showed that it migrated with the apparent sizes of 224, 220, and 187 bp at 4, 21, and 65 °C, respectively, in 7% (83:1) polyacrylamide gels (data not shown). At 4 °C, the mobility of the *argT* promoter fragment was about 25% slower than expected. Although mobility anomaly diminished with increasing temperature as expected, it was not completely abolished even at 65 °C.

To determine a possible correlation between altered mobility and the extent of UAS, promoter fragments were liberated from various pCLy1Δ-L plasmids by *Ssp*I–*Hin*dIII double digestion. The pCLy1Δ-L plasmids contain recloned pMR1y1Δ promoters and differ from one another solely by the upstream deletion in the *argT* promoter region (see Figure 1C). The selected enzyme cuts, *Ssp*I and *Hin*dIII, further "internalized" the upstream region, thus maximizing the bending effect. Fragment mobility was assessed at both 4 and 25 °C in 7% (83:1) gels, and the result is shown in Figure 3A, where the relative mobility (the ratio of apparent vs expected molecular size) was plotted as a function of the deletion end point. This graph illustrates the following points. One, the full-length *argT* promoter displayed a large deviation from expected mobility, by as much as 70%. Two, the mobility anomaly is very sensitive to temperature as evidenced by the parallel gel runs at 4 vs 25 °C. Three, mobility anomaly correlated positively with the extent of the upstream region, and progressive removal of the upstream sequence gradually restored normal gel behavior. Together, these observations strongly implicate the existence of a bent conformation in the upstream DNA.

Localization of the Bent Center. When the tandem dimer plasmid pTDy1 was used, the bending locus associated with the upstream region of the *argT* promoter was localized by

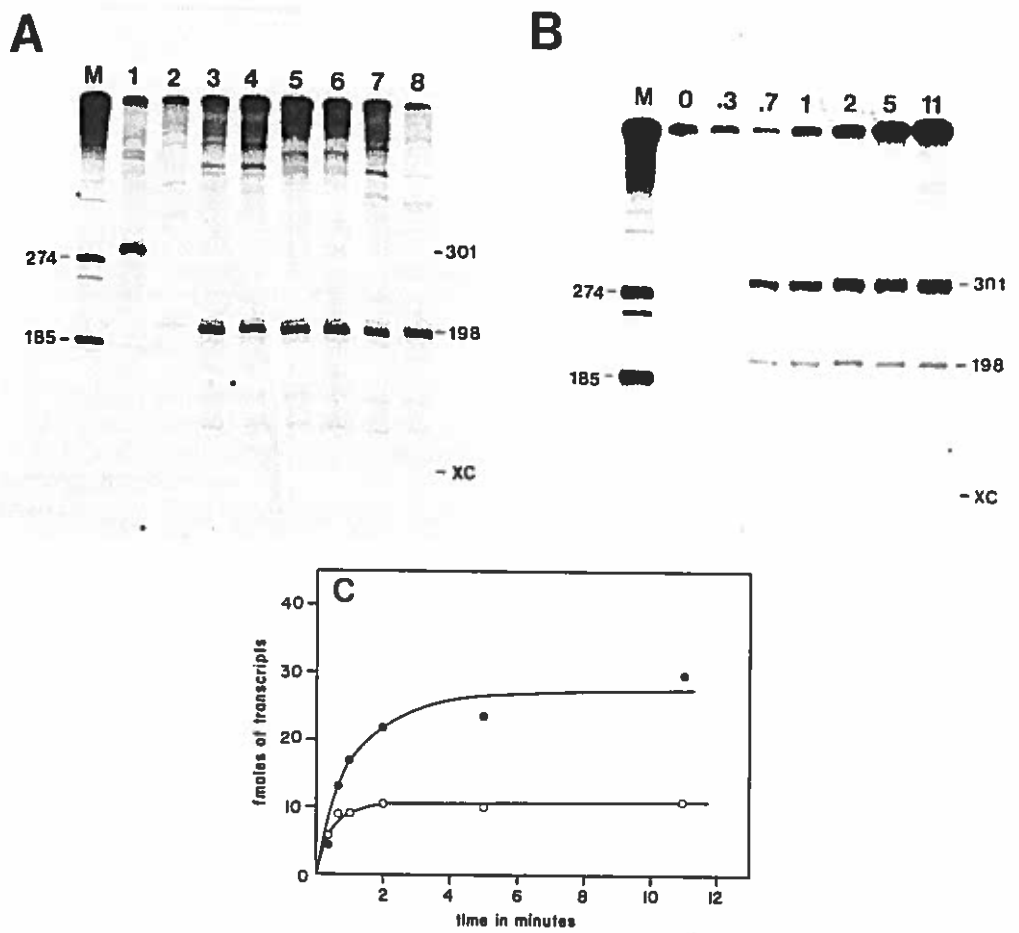


FIGURE 4: In vitro transcription of the *argT* promoters on fragment templates. Transcription reactions were performed at high [NTP] as described under Experimental Procedures using [α-^{32}P]CTP as the labeling nucleotide. Labeled RNA was fractionated in 6% (19:1)/7 M urea/polyacrylamide gels along with RNA markers and autoradiographed. RNA standards (lane M) consisted of a runoff transcript (274 nt) and a terminated transcript (185 nt) from a *Sal*I-linearized pLPG126 plasmid (from R. Reynolds). Expected runoff products from the *argT* promoter at the *Nar*I or *Eco*RV sites were 301 and 198 nt, respectively. (A) Single template transcription. Reactions of 40-μL volume containing 18 nM each of DNA and RNAP was incubated for 5 min at 30 °C. The promoter fragments were derived from plasmids (1) pMR1y1, (2) pMR1y6, (3) pMR1y1Δ-117, (4) pMR1y1Δ-105, (5) pMR1y1Δ-79, (6) pMR1y1Δ-79, (7) pMR1y1Δ-60, and (8) pMR1y1Δ-37. (B) Time course of competitive transcription. The reaction was performed in a 300-μL volume containing 9 nM each of the full-length and fully truncated promoters (see Figure 1C) and 18 nM RNAP at 30 °C; 40-μL aliquots were sampled at 0 min (before RNAP addition) and 0.3, 0.7, 1, 2, 5, and 11 min. (C) Quantitation of the gel shown in (B). Labeled RNA bands were excised as 0.7-cm slices and counted. Background counts from adjacent slices around each RNA band were subtracted graphically. Corrected cpm was converted to femtomoles of transcripts according to the equation: Δ cpm of RNA/(specific activity of [α-^{32}P]CTP in cpm per fmol/number of CMP residues in the RNA transcript). Femtomoles of transcripts plotted (vertical axis) refers to the amount of RNA in each 40-μL sample aliquot.

the circular permutation assay of Wu and Crothers (1984). As expected of bent DNA, the mobility of the 554 bp *argT* promoter fragment was found to vary according to the relative position of the bending locus defined by the restriction enzyme cut sites utilized. As a particularly striking example of mobility anomaly, the *Taq*I fragment migrated with an apparent length of 1260 bp at 4 °C, more than twice its actual size. The greater mobility deviation at the lower temperature resulted in the pronounced dip in Figure 3B, thus allowing the extrapolation of the bent center to nucleotide -92 ± 3 bp.

Transcription of Individual Fragment Templates. In pMR1y1Δ-37, the UAS was completely removed without damaging the −35 box. The GalK level primed by this "core" promoter in vivo was only 4% of the wild type. This finding was puzzling since the −35 and −10 elements of the *argT* promoter show reasonably good fit to the prokaryotic consensus. To understand how *argT* promoter achieves its optimal activity in vivo, transcriptional analysis was performed to first define the effect of the UAS in vitro.

As diagrammed in Figure 1C, all DNA templates used in this study are identical from nucleotides −37 to +46; this region includes the consensus elements and the nucleotides that are unwound during open complex formation and initiation. With these templates, any differential effect in overall transcription must stem from sequence signal(s) removed during upstream deletion.

With the use of individual fragment templates, transcription in vitro was carried out at a RNAP to DNA template ratio of 1:1, and the result is shown in Figure 4A. The transcripts obtained were of the expected sizes: 301 nt from the *Nar*I runoff, and 198 nt from the *Eco*RV runoff. The level of transcription derived from pMR1y6 (lane 2) was reduced to ~14% of the wild-type level, consistent with the much reduced in vivo activity of the T-37G promoter (~6% of wild type). In contrast, the truncated promoter fragments from the pMR1y1Δ plasmids all supported transcription to the same extent, at ~100–120% of the full-length promoter (quantitation data not shown). Thus, when individual templates were assayed, the UAS appeared to have little effect on *argT* promoter transcription in vitro.

Time Course of Competitive Transcription from Fragments. When equimolar amounts of the full-length and the fully truncated promoters from pMR1y1 and pMR1y1Δ-37, respectively, were used in the same reaction mixture, the two

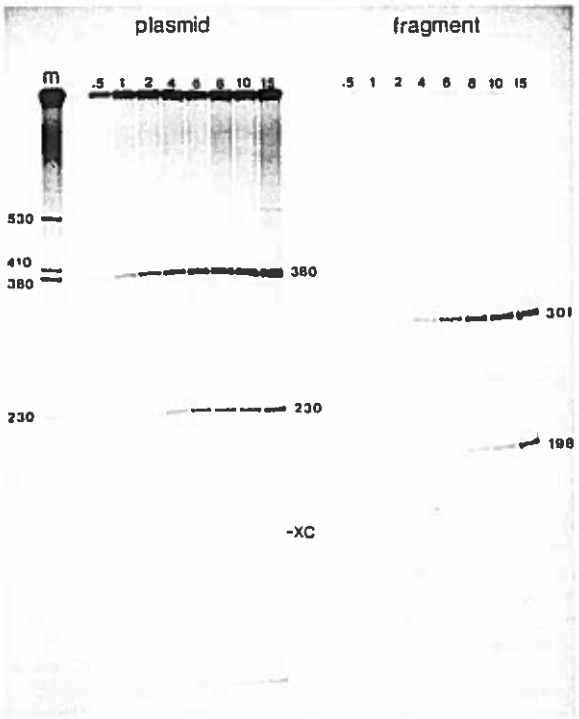


FIGURE 5: Competitive transcription from plasmid or fragment templates. The full-length and fully truncated promoters were allowed to compete in the fragment or supercoiled plasmid configuration. Reactions were performed at high [NTP] condition for 5 min at 37 °C in 20-μL volume containing 9 nM each of the template DNAs. RNA polymerase was added last to commence the reaction. The number above each lane indicates the RNAP:DNA ratio in the reaction; the ratios used, 0.5, 1, 2, 4, 6, 8, 10, and 15, corresponded to final [RNAP] of 9, 18, 36, 72, 108, 144, 180, and 270 nM, respectively. Lane M consists of standard terminated RNAs transcribed from a mixture of plasmids: pGC4 (530 and 410 nt), pCLy1L (380 nt), and pCLy1Δ-37S (230 nt). The autoradiograph shows the RNA separation obtained from a 5% (19:1)/7 M urea/polyacrylamide gel. XC indicates the position of the xylene cyanol dye.

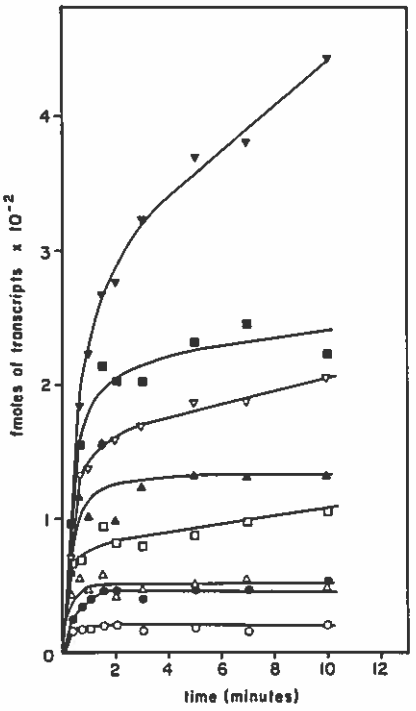


FIGURE 6: Effect of [RNAP] on the time course of competitive transcription. Transcription reactions were performed at 37 °C in high [NTP] reaction conditions as described under Experimental Procedures. For each time course, the reaction was performed in a volume of 100 μL containing 9 nM each of pCLy1L and pCLy1Δ-37S DNA as templates. RNAP was added last to commence the reaction. Aliquots of 10 μL were sampled at 0.3, 0.7, 1, 1.5, 2, 3, 5, 7, and 10 min, processed as described under Experimental Procedures, and fractionated in 5% (19:1)/7 M urea/polyacrylamide gels. RNA bands of 380 and 230 nt were excised and quantitated as described in the legend to Figure 4C. RNAP:DNA ratios examined were 2 (circles), 5 (upright triangles), 8 (squares), and 12 (downward triangles). Filled symbols, 380-nt transcript from UAS(+) promoter; open symbols, 230-nt transcript from UAS(−) promoter.

templates were allowed to compete for RNA polymerase as a function of time. Figure 4B shows the time course obtained at a RNAP:DNA ratio of 1.

Qualitatively, the truncated promoter was poorly transcribed during competitive transcription and the accumulation of product RNA leveled off soon into the reaction. In comparison, transcription of the full-length promoter continued on to yield more product. To substantiate this interpretation of the gel profile, radioactive counts associated with each RNA band were determined to account for the different number of CMP residues in each transcript. The quantitative data, plotted in Figure 4C, indicate that transcription from both promoters initially proceeded with comparable linear rates, but both leveled off a short time into the reaction. At ~1 min, transcription from the truncated promoter had reached a plateau. For the full-length promoter, the plateau was reached after 2–3 min. At the plateau, there were 2.5 times more transcripts from the full-length promoter than the fully truncated promoter. This result suggests that the UAS(+) promoter enjoys a substantial advantage when it must compete for RNA polymerase.

Time Course of Competitive Transcription as a Function of [RNAP]. Although the presence of the UAS confers high activity to the wild-type promoter, the kinetics of competitive transcription obtained were unexpected for reactions performed under high [NTP] conditions that can support multiple rounds of transcription. To investigate these unusual kinetics, additional time courses were performed to examine the extent of RNA polymerase recycling as well as how the enzyme molecules were partitioned between the competing templates.

For all subsequent experiments, supercoiled plasmids replaced promoter fragments as templates due to the abundance and relative ease of their preparation. As shown in Figure 5, similar transcriptional results were obtained from plasmid or fragment promoters. Except for the emergence of plasmid *rep* RNA, major reaction products at the 5-min time point were mainly transcripts derived from the competing *argT* promoters.

To explore the kinetics of competitive transcription as a function of [RNAP], equimolar mixtures of UAS(+) [pCLy1L] and UAS(−) [pCLy1Δ-37S] DNA were transcribed at RNAP:DNA ratios of 2, 5, 8, and 12, and the resultant time courses are shown in Figure 6. Two conclusions are immediately apparent from this set of data. First, the initial linear rate of transcription varied positively with the [RNAP] and was slightly greater for the UAS(+) versus the UAS(−) promoters at all RNA polymerase concentrations used. Second, despite the high RNA polymerase concentrations used, the kinetics still tended to level off. At RNAP:DNA ratios of 2 and 5, the plateau was reached sharply at ~1 min for the UAS(−) promoter and at ~2 min for the UAS(+) promoter. At RNAP:DNA ratios of 8 and 12, however, the time courses resulted in biphasic curves. The biphasic incorporation proceeded with a sharp initial phase lasting 1 min or so and continued as a linear second phase with a much reduced slope. At a RNAP:DNA ratio of 12, the slope of the second phase was about $^1/_{17}$ that of the first. The occurrence of the second phase is most likely due to recycling of the RNA polymerase molecules; its greatly reduced slope suggests that only a small

Table I: Time Course of Competitive Transcription as a Function of [RNAP][a]

RNAP:DNA ratio	fmol of promoter DNA[b]	fmol of RNAP[c]	fmol of transcripts at t = 5 min			no. of times promoter used[d]	no. of RNA chains/ RNAP[e]
			UAS(+)	UAS(−)	total		
2	180	360	46	20	66	0.37	0.18
5	180	900	130	50	180	1	0.20
8	180	1440	224	92	316	1.75	0.22
12	180	2160	320[a]	160[a]	480	2.67	0.22

[a] These numbers are extrapolated plateau values of transcripts, in femtomoles, at the 5-min point from UAS(+) and UAS(−) promoters (see Figure 6). [b] Femtomoles of promoter DNA assumes the existence of one promoter, i.e., the *argT* promoter, per plasmid circle. [c] Femtomoles of RNAP does not take into account the percent active molecules. [d] Number of times promoter used = total femtomoles of transcripts (column 6)/femtomoles of promoter DNA (column 2). [e] Number of RNA chains/RNAP = total femtomoles of transcripts (column 6)/femtomoles of RNAP (column 3).

fraction of the RNA polymerase molecules were able to do so. Thus, even under conditions of high [NTP], RNA polymerase did not recycle effectively at the *argT* promoters. This finding is quite unexpected.

In Table I, the quantitative data from these time courses are presented to further confirm the lack of recycling by RNA polymerase. Two parameters are of interest in this analysis. One is the average number of times a promoter was utilized during the reaction; this number increased from 0.37 to 2.67 over RNAP:DNA ratios of 2–12. Thus, at low RNA polymerase concentrations, promoter usage was fractional, while at high RNA polymerase concentrations, multiple transcription from each promoter ensued. Despite the increased promoter usage at higher RNAP:DNA ratios, the number of RNA chains formed per RNA polymerase molecule present in each reaction was found to be approximately constant; an average value of 0.21 was obtained under all RNA polymerase concentrations studied. The constancy of this second parameter can be interpreted most simply to mean that the RNA polymerase preparation contained ~21% of active molecules when assayed with the *argT* promoter (Chamberlin et al., 1979).

The results of Figure 6 and Table I, therefore, point out two unusual features of the kinetics of *argT* promoter transcription: one, that each active RNA polymerase molecule was able to transcribe the *argT* promoter once; two, the travel across the transcription unit by all active RNAP molecules was completed in a single burst of synthesis lasting 1–2 min.

Competitive Transcription as a Function of [RNAP] Revealed the Role of Upstream Elements. Supercoiled plasmids pCLy1L, pCLy1Δ-60L, and pCLy1Δ-37S, containing the full UAS, the proximal UAS element, or no UAS, respectively, were transcribed individually or in pairs at increasing RNA polymerase concentrations to examine the dependence of *argT* promoter activity on upstream DNA. The RNAP:DNA ratios varied from 0.5 to 15.

Individually, all three plasmid templates were transcribed efficiently with nearly identical slopes (Figure 7A). The UAS(−) template displayed a small initial lag which was readily overcome above a RNAP:DNA ratio of 1 (squares). The curves for pCLy1L (circles) and pCLy1Δ-60L (triangles) showed no lags and were essentially indistinguishable throughout until saturation set in at high [RNAP]. At the plateau, pCLy1L gave rise to 20% more transcripts than pCLy1Δ-60L.

When competing in pairs, the differential pattern of transcription for the two templates in each pair serves to elucidate the role of the missing element in the more truncated promoter. The results of competitive transcription experiments are shown in Figure 7B–D. In panel B, the competition between the full-length and the fully truncated promoters showed mixed effects, with the truncated promoter displaying both an initial lag at low RNAP:DNA ratios and a slower rate of transcript

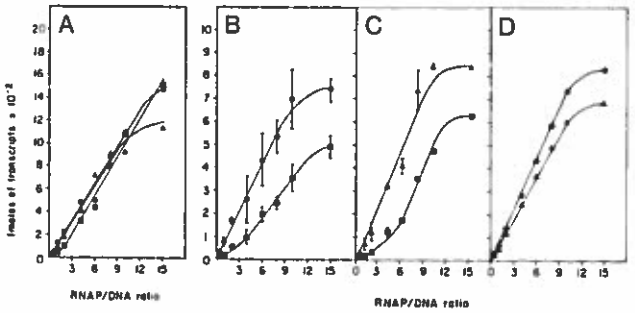


FIGURE 7: Transcription of plasmid templates singly or in competition as a function of [RNAP]. Reaction conditions and quantitation procedures were as described in the legend to Figure 4. Plasmid templates were pCLy1L or pCLy1S (circle), pCLy1Δ-60L (triangles), and pCLy1Δ-37S (squares). (Panel A) Transcription of individual templates; (panel B) pCLy1L vs pCLy1Δ-37S; (panel C) pCLy1Δ-60L vs pCLy1Δ-37S; (panel D) pCLy1S vs pCLy1Δ-60L. Results of panel B plot the mean of four separate experiments; the error bars correspond to 1 SD calculated by the rms method. Panel C represents the average of two separate experiments; the vertical bars are indicative of the variability observed. Panel D presents the data from one of three separate experiments performed using this pair of templates.

accumulation at intermediate RNAP:DNA ratios. The existence of a transient transcriptional lag indicates that the truncated template is rate limited at the K_B step when [RNAP] is low. When [RNAP] is sufficiently high to counter the K_B effect, any decrease in the slope of the truncated promoter relative to that of the wild type suggests rate limitation at the isomerization step due to deletion of the UAS (see Discussion). The composite effect displayed by the fully truncated promoter is thus consistent with an activating role of the *argT* UAS both in polymerase binding and in promoter–polymerase complex isomerization during transcription initiation.

In panel C, the difference between the two promoters is the proximal UAS element. The truncated promoter suffers a severe lag at low [RNAP] but recovers at sufficiently high [RNAP] to yield a parallel rate of incorporation. This pattern suggests the proximal region to be involved solely in facilitating the binding of RNA polymerase to the promoter by enhancing K_B. The enhancing effect, judging by the initial slopes of the two curves, is estimated to be about 5-fold.

In panel D, the differentiating factor between the competing templates is the distal UAS element. Both templates were transcribed without a lag, indicating a similar dependence of promoter–polymerase binding on RNA polymerase concentration. The overall rate of transcription by the truncated promoter, however, suffers reproducibly by ~1.3-fold, pointing to a small role of the distal UAS element in stimulating k_2 in vitro.

Discussion

Like many stable RNA promoters in *E. coli*, the *argT* tRNA gene promoter was found in the present study to also require its upstream sequence for full activity in vivo. In vitro,

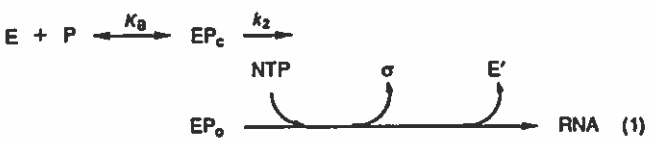
the removal of UAS affected both the rate and extent of transcriptional expression. The UAS of *argT* promoter spans a 90 bp region upstream of the −35 hexameric consensus sequence. Several lines of evidence suggests that it may comprise two structurally and functionally distinct elements.

The first indication that two elements might exist came from parallel analyses of the upstream sequence requirement by wild-type *argT* and mutant T-37G promoters; whereas the wild-type promoter was dependent on the entire upstream region for full activity, the dependence shown by the T-37G promoter was completely abolished when deletion reached −60 (Figure 2). On the basis of this comparison, we assigned the region from −130 to −60 as the distal element, and the region from −60 to −38 as the proximal element.

Structurally, the two elements differ substantially in sequence characteristics and DNA conformation. The 23 bp proximal region includes a 14 bp AT tract, and it appears to contribute minimally to DNA bending (Figure 3A). The distal element, on the other hand, is implicated to form a highly bent conformation judging from its degree of mobility anomaly. Consistent with this, the base sequence of this seven-turn region shows the occurrence of approximately in-phase A_{2-4} nucleotides conducive to DNA bending (Koo et al., 1986). The best center, localized to the −90 to −95 region, further coincides with the midpoint of the distal UAS (Figure 3B). Functionally, it was deduced from competitive transcription experiments that the two upstream elements activate transcription by different mechanisms. The proximal element stimulates transcription by a K_B effect, that is, by stimulating the binding of RNA polymerase to the promoter (Figure 7C), and the distal element enhances transcription at the k_2 step, by facilitating the isomerization to open complex (Figure 7D). In addition to the evidence presented here, Bauer et al. (1988) have previously alluded to the possibility of two activating components in the upstream region of the *leuV* promoter.

In this study, the roles of the UAS elements during transcription initiation were determined by competitive transcriptional analysis since individual promoter templates, regardless of the extent of upstream deletion, gave barely detectable differences. Competition, therefore, magnified the effect of the missing UAS. Results of competitive transcription revealed a sharp dependence on RNA polymerase concentration by a promoter lacking the proximal UAS region; this dependence, on the other hand, was not detected at all in a promoter lacking the distal UAS region. Instead, the latter promoter gave a reproducibly slower rate of transcription at all RNA polymerase concentrations. On the basis of the derivation shown below, the [RNAP]-dependent effect was deduced to be due to an alteration in K_B, and the [RNAP]-independent effect, to k_2.

When the extent of transcription was plotted as a function of RNA polymerase concentration (see Figure 7), the slope of the curve for each template is $\Delta N/\Delta[E]$, where N is the plateau level of transcripts accumulated in 5 min of reaction time and [E] is the concentration of RNA polymerase. According to the two-step model of transcription initiation (Chamberlin, 1974) shown in eq 1 where E and E′ stand for



(1)

RNA polymerase holoenzyme and core enzyme, respectively, and P for promoter DNA, the efficacy of closed (EP_c) and open (EP_o) promoter–polymerase complex formation can be described, respectively, by the equilibrium binding constant, K_B, and the forward isomerization rate constant, k_2, when [NTP] is high. At a given [RNAP], the amount of transcripts obtained is proportional to $[EP_o]$ through the proportionality constant k_i, the rate constant of transcript initiation. In turn, $[EP_o]$ is dependent on [E] and [P] through the proportionality constants, K_B and k_2. As a result, eq 2 is attained:

$$N = k_i K_B k_2 [E][P] \quad (2)$$

For each template, the slope of the curve relating transcript accumulation to [E] becomes

$$\Delta N/\Delta[E] = k_i K_B k_2 [P] \quad (3)$$

In a competitive reaction, although each template proceeds with an incorporation rate as described by eq 3, the relative rate of transcription from the two templates, X and Y, can be expressed as a ratio and simplified to eq 4 due to two

$$(\Delta N/\Delta[E])_X/(\Delta N/\Delta[E])_Y = (K_B)_X(k_2)_X/(K_B)_Y(k_2)_Y \quad (4)$$

features built into the reaction design. One, the competing templates were used at equimolar amounts so that $[P]_X$ and $[P]_Y$ canceled. Two, since all versions of the *argT* promoter used in the transcription studies are identical in the region from −37 to +46 (see Figure 1C), the k_i value, intrinsic to a promoter and its initiating sequence (Gralla et al., 1980), should further by identical for all *argT* promoter templates in the same configuration.

According to eq 4, the slopes of the two curves from the competing templates in the same reaction mixture are related to each other through variations in K_B and/or k_2. When the slopes are equal (and parallel), the two promoters form open complexes with similar ease and stability as indicated by the product $K_B k_2$. A drop in either K_B, k_2, or both yields a less active promoter. Of these two constants, because K_B varies according to RNA polymerase concentration and k_2 is independent, factors affecting K_B or k_2 can be distinguished on the basis of titration against [RNAP]. An element that solely affects K_B, when deleted, would be predicted to yield a weaker promoter that exhibits a transcriptional lag at low [RNAP], but a restored parallel rate of transcription at RNA polymerase concentrations high enough to compensate for the difference in K_B. Such an effect was obtained in Figure 7C. On the other hand, a UAS(−) promoter whose activity is decreased due to a drop in k_2 alone would show no lag at all, but rather, the amount of transcript derived from this promoter would be lower at all concentrations of RNA polymerase; this outcome was observed in Figure 7D. By this rationale, a truncated promoter missing a UAS element that affects both K_B and k_2 should yield a composite curve during competition such that it both displays a transcriptional lag at low [RNAP] and a decreased slope at higher [RNAP]; this pattern was in fact obtained in Figure 7B.

Although the competitive transcription experiment described in this report does not yield absolute values for each of the constants involved, it nevertheless proves to be a useful assay in uncovering the relative effect of a mutation on K_B and/or k_2. To determine the values of K_B and k_2 for the UAS(+) and UAS(−) *argT* promoters, kinetics studies using the abortive initiation assay are in progress (Johnston et al., 1976; McClure, 1978).

During the in vitro characterization of the *argT* promoters, an unusual feature of its transcription was noted; namely, under the high [NTP] reaction condition adopted to achieve steady-state levels of transcription (Chamberlin et al., 1983; Arndt & Chamberlin, 1988), RNA polymerase failed to re-

cycle readily at this promoter. Instead, as summarized in Table I, transcription occurred by way of a one-time passage of the active RNA polymerase molecules through the *argT* promoters, regardless of the concentration of RNA polymerase used.

The exact cause of this observation was not determined in this study. However, several factors capable of altering the kinetics of RNA polymerase recycling have been implicated by previous studies; these include inactivation of RNA polymerase by heat denaturation (Hinkle & Chamberlin, 1972; Williams & Chamberlin, 1977), the inhibition of RNA polymerase by product RNA (Krakow, 1966), and the titration of core polymerase by binding to random sites on DNA (Williams & Chamberlin, 1977). A most relevant consideration in the reported experiments is the use of the weaker *argT* promoter (i.e., weaker than T7 A1) which can affect the kinetics of promoter site selection and allow more effective competition by other promoters present on the template DNA (Chamberlin et al., 1983; Arndt & Chamberlin, 1988). Furthermore, since most of our transcriptional analysis was performed with plasmid templates, the effect of supercoiling and the potential titration of RNA polymerase holoenzyme by a large number of specific or nonspecific sites on the vector DNA cannot be ignored (von Hippel et al., 1984). In addition, it is pertinent to point out that little is known regarding RNA polymerase release and recycling in the high-salt potassium glutamate reaction condition used (Roe & Records, 1985; Leirmo et al., 1987). Future studies directed at understanding the transcriptional kinetics of the *argT* promoter will resolve the question of whether a combination of the above factors was operable or perhaps a novel type of transcriptional regulation applies for this stable RNA promoter.

Regarding the activating role of the proximal UAS element, similar AT-rich sequences found at comparable positions upstream of many bacterial promoters have been found to stimulate the activity of these promoters (Banner et al., 1983; Travers, 1984; Graves & Rabinowitz, 1986; Bujard et al., 1987; Plaskon & Wartell, 1987). Interestingly, for $rrnBP_1$ and P_{tyrT}, two stable RNA promoters that exhibited the UAS requirement, DNase I footprinting of the promoter regions showed extended upstream protection by RNA polymerase to nucleotides −61 to −63 at the proximal UAS region, about 5–10 bases longer than that observed for most mRNA promoters (Gourse, 1988; Travers et al., 1983). This footprinting evidence supports our present conclusion that the proximal UAS of the *argT* promoter functions by enhancing K_B, most likely through additional stabilizing contacts with the RNA polymerase molecule. These contacts, however, must be secondary to the polymerase contacts at the consensus hexamers since the proximal UAS in pMR1y6Δ-61 was inconsequential in activating the T-37G promoter (see Figure 2).

The distal UAS, although located upstream of the proximal element, was found to activate k_2 which governs the second step in transcription initiation. This arrangement deviates from the predominant view that functional units of prokaryotic promoters are largely arranged asymmetrically on the linear sequence of DNA according to the order of their utilization during transcription initiation. This discrepancy, however, is presently reconciled by the finding that the distal UAS in the *argT* promoter assumes a highly bent structure. Thus, after RNA polymerase has become bound to the consensus hexamers and the proximal UAS, the distal bent DNA may further exert its role either by looping around the polymerase molecule to stabilize the open complex, thereby stimulating k_2, or, alternatively, by sequestering or securing the polymerase molecule already in the open conformation such that it disfavors k_{-2}. Although the extent of activation by the distal UAS is small, investigation of its detailed mechanism may shed further light on how DNA bending confers activation (Travers, 1989).

Together, the two UAS elements were found to confer a 7–8-fold activation to the *argT* promoter in vitro (i.e., 5–6 fold by the proximal UAS and 1.3-fold by the distal UAS). This level of activation is much smaller than the 25-fold effect observed in vivo. One explanation for this discrepancy may be an underestimation in vitro due to the unusual kinetics of RNA polymerase recycling and the unknown salt effect imposed by the reaction medium. This disparity, however, hints at a second possibility that, in vivo, upstream activation of the *argT* promoter may require the participation of ancillary factors. Among stable RNA promoters, the UAS of *thrU-tufB* promoter was shown to be the binding site for the activator protein EFTu·GDP complex even though intrinsic bending was not detected (Vijgenboom et al., 1988). Recently, Nachaliel et al. (1989) were able to demonstrate specific binding by nonribosomal proteins associated with the 30S subunit to the UAS region of the $rrnAP_1$ promoter. Given these precedents, the potential requirement for UAS binding protein(s) at the *argT* promoter cannot be ruled out.

By combining deletion and transcriptional analyses, we have been afforded a preliminary view of how the *argT* promoter might interact with the RNA polymerase molecule (Darst et al., 1989). On the basis of the evidence presented here, a model can be proposed where the open complex engages ~150 bp of the *argT* promoter sequence from −130 to +20. The 3′ half (from −60 to +20) including the consensus hexamers and the proximal UAS element most likely make specific and strong contacts with the polymerase molecule. The 5′ half from −130 to −60, possibly in conjunction with ancillary proteins, in turn can add to the stability of the open complex via bending. Critical proof of this model will require footprinting analysis of the *argT* promoter as well as investigation of the activating effect of the UAS as a function of its rotational and linear relationship to the core promoter (McAllister & Achberger, 1989).

In concluding, it is pertinent to point out that the competitive edge enjoyed by the UAS(+) promoter in vitro is most significant at low concentrations of RNA polymerase. This observation has direct relevance in understanding the activity of *argT* promoter in vivo since in the cell, not only is the free RNA polymerase concentration limiting but also the presence of myriads of other competing templates cannot be ignored (Ingraham et al., 1983; McClure, 1985).

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REFERENCES

Arndt, K. M., & Chamberlin, M. J. (1988) *J. Mol. Biol. 202*, 271–285

Banner, C. D. B., Moran, C. P., & Losick, R. (1983) *J. Mol. Biol. 168*, 351–365.
Bauer, B. F., Kar, E. G., Elford, R. M., & Holmes, W. M. (1988) *Gene 63*, 123–134.
Birnboim, H. C., & Doly, J. (1979) *Nucleic Acids Res. 7*, 1513–1523.
Bossi, L., & Smith, D. M. (1984) *Cell 39*, 642–652.
Bujard, H., Brenner, M., Deuschle, U., Kammerer, W., & Knaus, R. (1987) in *RNA Polymerase and the Regulation of Transcription* (Reznikoff, W. S., et al., Eds.) pp 95–103, Elsevier, New York.
Chamberlin, M. J. (1974) *Annu. Rev. Biochem. 43*, 721–775.
Chamberlin, M., Nierman, W., Wiggs, J., & Neff, N. (1979) *J. Biol. Chem. 254*, 10061–10072.
Chamberlin, M., Kingston, R., Gilman, M., Wiggs, J., & deVera, A. (1983) *Methods Enzymol. 101*, 540–568.
Darst, S. A., Kubalek, E. W., & Kornberg, R. D. (1989) *Nature 340*, 730–732.
Deuschle, U., Kammerer, W., Gentz, R., & Bujard, H. (1986) *EMBO J. 5*, 2987–2994.
Fried, M., & Crothers, D. M. (1981) *Nucleic Acids Res. 9*, 6505–6525.
Gardella, T., Moyle, H., & Susskind, M. M. (1989) *J. Mol. Biol. 206*, 579–590.
Gonzalez, N., Wiggs, J., & Chamberlin, M. (1977) *Arch. Biochem. Biophys. 187*, 404–408.
Gourse, R. L. (1988) *Nucleic Acids Res. 16*, 9789–9809.
Gourse, R. L., de Boer, H. A., & Nomura, M. (1986) *Cell 44*, 197–205.
Gralla, J. D., Carpousis, A. J., & Stefano, J. E. (1980) *Biochemistry 19*, 5864–5869.
Graves, M. C., & Rabinowitz, J. C. (1986) *J. Biol. Chem. 261*, 11409–11415.
Harley, C. B., & Reynolds, R. P. (1987) *Nucleic Acids Res. 15*, 2343–2361.
Hawley, D. K., & McClure, W. R. (1983) *Nucleic Acids Res. 11*, 2237–2255.
Helmann, J. D., & Chamberlin, M. J. (1988) *Annu. Rev. Biochem. 57*, 839–872.
Hinkle, D. C., & Chamberlin, M. J. (1972) *J. Mol. Biol. 70*, 187–195.
Hsu, L. M., Klee, H. J., Zagorski, J., & Fournier, M. J. (1984) *J. Bacteriol. 158*, 934–942.
Ingraham, J. L., Maaloe, O., & Neidhardt, F. C. (1983) *Growth of the Bacterial Cell*, Sinauer Associates, Inc., Sunderland, MA.
Johnson, M. T., Read, B. A., Monko, A. M., Pappas, G., & Johnson, B. A. (1986) *BioTechniques 4*, 64–70.
Johnston, D. E., & McClure, W. R. (1976) in *RNA Polymerase* (Losick, R., & Chamberlin, M., Eds.) pp 413–428, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Koo, H.-S., Wu, H.-M., & Crothers, D. M. (1986) *Nature 320*, 501–506.
Krakow, J. S. (1966) *J. Biol. Chem. 241*, 1830–1834.
Lamond, A. I., & Travers, A. A. (1983) *Nature 305*, 248–250.
Leirmo, S., Harrison, C., Cayley, D. S., Burgess, R. R., & Records, M. T., Jr. (1987) *Biochemistry 26*, 2095–2101.
Lukacsovich, T., Gaal, T., & Venetianer, P. (1989) *Gene 78*, 189–194.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning, a Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
McAllister, C. F., & Achberger, E. C. (1989) *J. Biol. Chem. 264*, 10451–10456.
McClure, W. R. (1978) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5634–5638.
McClure, W. R. (1985) *Annu. Rev. Biochem. 54*, 171–204.
McKenney, K., Shimatake, H., Court, D., Schmeissner, U., & Rosenberg, M. (1981) in *Gene Amplification and Analysis* (Chirikjian, J. G., & Papas, T. S., Eds.) Vol. 2, pp 383–415, Elsevier, New York.
Miller, J. H. (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Mulligan, M. E., Hawley, D. K., Entriken, R., & McClure, W. R. (1984) *Nucleic Acids Res. 12*, 789–800.
Nachaliel, N., Melnick, J., Gafny, R., & Glaser, G. (1989) *Nucleic Acids Res. 17*, 9811–9822.
Plaskon, R. R., & Wartell, R. M. (1987) *Nucleic Acids Res. 15*, 785–796.
Reznikoff, W. S., Siegele, D. A., Cowing, D. W., & Gross, C. A. (1985) *Annu. Rev. Genet. 19*, 355–387.
Roe, J.-H., & Records, M. T., Jr. (1985) *Biochemistry 24*, 4721–4726.
Rosenberg, M., & Court, D. (1979) *Annu. Rev. Genet. 13*, 319–353.
Ross, G. W., & O'Callaghan, C. H. (1975) *Methods Enzymol. 43*, 69–85.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Sarmientos, P., & Cashel, M. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 7010–7013.
Schumperli, D., McKenney, K., Sobieski, D. A., & Rosenberg, M. (1982) *Cell 30*, 865–871.
Siebenlist, U., Simpson, R. B., & Gilbert, W. (1980) *Cell 20*, 269–281.
Siegele, D. A., Hu, J. C., Walter, W. A., & Gross, C. A. (1989) *J. Mol. Biol. 206*, 591–603.
Travers, A. A. (1984) *Nucleic Acids Res. 12*, 2605–2616.
Travers, A. A. (1989) *Annu. Rev. Biochem. 58*, 427–452.
Travers, A. A., Lamond, A. I., Mace, H. A. F., & Berman, M. L. (1983) *Cell 35*, 265–273.
van Delft, J. H. M., Marinon, B., Schmidt, D. S., & Bosch, L. (1987) *Nucleic Acids Res. 15*, 9515–9530.
Vijgenboom, E., Nilsson, L., & Bosch, L. (1988) *Nucleic Acids Res. 16*, 10183–10197.
von Hippel, P. H., Bear, D. G., Morgan, W. D., & McSwiggen, J. A. (1984) *Annu. Rev. Biochem. 53*, 389–446.
Williams, R. C., & Chamberlin, M. J. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 3740–3744.
Wilson, D., & Hogness, D. (1966) *Methods Enzymol. 8*, 229–240.
Wu, H.-M., & Crothers, D. M. (1984) *Nature 308*, 509–513.
Zuber, P., Healy, J., Carter, H. L., III, Cutting, S., Moran, C. P., Jr., & Losick, R. (1989) *J. Mol. Biol. 206*, 605–614.