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LETTERS TO THE EDITOR

Deletion Analysis of the *Escherichia coli ara* P_C and P_{BAD} Promoters

Deletions extending various distances into the *ara* P_C-P_{BAD} regulatory region were studied to define the sites required *in vivo* for the activity of these promoters. Deletions from the P_C side entering the CRP site, which is located from −80 to −120 with respect to the P_{BAD} transcription start site, reduced activity of this promoter. Similarly, deletions entering this site from the P_{BAD} side reduced activity of the P_C promoter. Cyclic AMP receptor protein bound at this site apparently functions to stimulate transcription of both flanking promoters.

Transcription of the L-arabinose operon *araBAD* in *Escherichia coli* is both positively and negatively regulated by *araC* protein in addition to being positively regulated by the cyclic AMP receptor protein CRP (Sheppard & Englesberg, 1967; Englesberg *et al.*, 1969; Greenblatt & Schleif, 1971; Lis & Schleif, 1973; Wilcox *et al.*, 1974). The adjacent, but divergently oriented promoter P_C (Fig. 1) is also activated by CRP, but only about fourfold rather than the 40-fold seen with P_{BAD} (Casadaban, 1976).

Although CRP has long been known to be an activator of transcription from a number of promoters, its mechanism of action has been unclear. A simple, unique interaction between CRP bound to DNA and an adjacently bound RNA polymerase seems to be excluded by the fact that in the *lac* operon CRP binds behind RNA polymerase at positions −50 to −70, in the *gal* operon it binds beside polymerase at positions −25 to −50, and in the *araBAD* operon *araC* protein is interposed between CRP and RNA polymerase (Majors, 1977; Taniguchi *et al.*, 1979; Ogden *et al.*, 1980; Lee *et al.*, 1981). Furthermore, from the order of binding sites along the DNA, CRP from this same position in the *araC* system might be expected to stimulate transcription from the P_C promoter.

A 440 base-pair piece of the arabinose operon has been cloned in either orientation in front of the *galK* gene (Dunn *et al.*, 1984) in a plasmid where the *galK* gene otherwise lacks a functional promoter (McKenney *et al.*, 1981). Consequently, assay of galactokinase in strains containing the plasmid permits simple quantitation of either *ara* P_{BAD} or P_C promoter activities. Deletions were then isolated from the P_C side in the plasmid in which P_{BAD} was fused to *galK* or from the P_{BAD} side in the plasmid with P_C fused to *galK* (Dunn *et al.*, 1984). Thus the effect of the deletions on either promoter could be quantitated. Plasmids containing these deletions were used in the accompanying paper (Hahn *et al.*, 1984) to infer that one of the roles of CRP in the *araBAD* operon is to help overcome the repression that is established by *araC* protein in conjunction with the $araO_2$ site. We show here that CRP also stimulates transcription when repression is not possible, and that it appears to function in both directions when bound to a single site.

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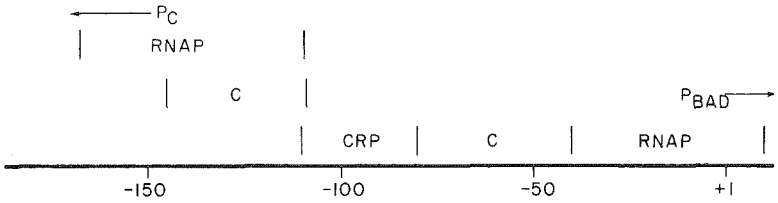

FIG. 1. The *araCBAD* regulatory region showing the binding sites for RNA polymerase, *araC* protein, and cyclic AMP receptor protein. Nucleotide positions are given with respect to the first nucleotide of the P_{BAD} transcript.

The results from assaying P_C activity in strains with deletions extending various distances toward P_C are presented in Figure 2. The strain with the deletion ending at -65 (i.e. -82 relative to P_C) in *araI* expresses P_C normally and shows the same twofold self-repression that is seen from the undeleted, pTD4 plasmid (data not shown). This shows that the *araI* site is not required for the repression of P_C. A deletion extending to position -94 (i.e. -53 relative to P_C) reduces the P_C promoter activity by threefold. Since this deletion ends within the CRP site located from -78 to -107, and since the normal CRP stimulation of P_C is three- to fivefold, it is likely that the deletion inactivates the CRP stimulation of P_C. This was tested by examining the CRP-cAMP dependence of the remaining P_C activity. To do this, the strain was made incapable of synthesizing its own cAMP by the introduction of a deletion of the adenyl cyclase gene. Whereas in the Δ*cya* strains, the undeleted P_C promoter showed the normal threefold stimulation by the addition of cAMP, the P_C promoter in the strain with the deletion up to position -94 showed no cAMP stimulation. This shows that the CRP binding site located from -78 to -107 functions on the CRP-cAMP stimulation of P_C *in vivo*.

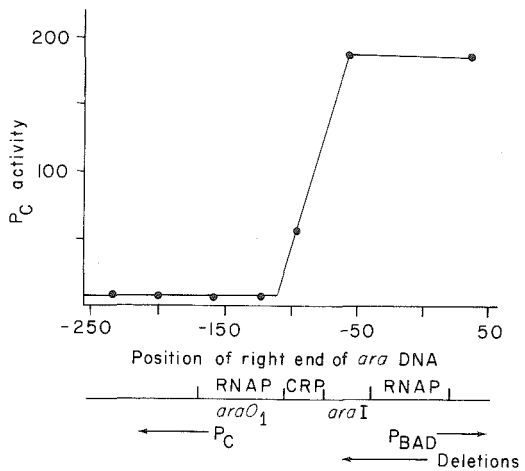

FIG. 2. The *araC* promoter activities in galactokinase units assayed as described (Hahn *et al.*, 1984) from a set of deletion plasmids containing the *ara* P_C-*galK* fusion in strain SH321 (F^- Δ*araC-leu1022* Δ*lac74 galK* Str^r *thi*). The deletions ended at: $+44$, -65, -94, -127, -159, -200 and -230.

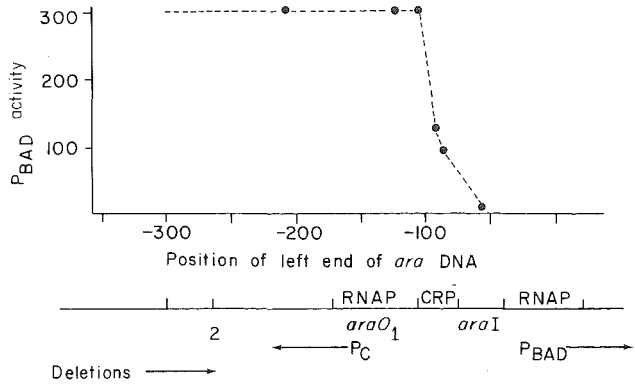

FIG. 3. Induced *araBAD* promoter activities in galactokinase from a set of deletion plasmids containing the *ara* P_{BAD}-*galK* fusion in strain SH322 (F^- *leu* Δ*lac74 galK* Str^r *thi*) grown in the presence of 0·2% (w/v) L-arabinose. The deletions ended at positions −222, −127, −107, −94, −84 and −57.

Finally, a deletion extending to −127 eliminates P_C activity. This deletion endpoint is 21 base-pairs upstream from the P_C start site (Lee *et al.*, 1981; Stoner & Schleif, 1984) and therefore removes the −35 region of the P_C RNA polymerase binding site.

The results obtained from assaying P_{BAD} inducibility in a set of deletions extending various distances toward P_{BAD} are presented in Figure 3. Deletions ending upstream of −107 leave P_{BAD} normally inducible, whereas those ending closer to the transcription start site display impaired inducibility. In particular, the deletions ending in the region to which CRP binds leave the operon only partially inducible. The effect is not greater because repression is also eliminated by the deletions (Hahn *et al.*, 1984). This result suggests that CRP-cAMP acting from this site stimulates transcription of the P_{BAD} promoter. Deletions that entered the *araI* region left P_{BAD} uninducible.

The above results show that the cyclic AMP receptor protein binding site that is located from position −78 to −107 functions both for CRP-cAMP stimulation of P_C and for CRP-cAMP stimulation of P_{BAD}. CRP bound at this site therefore stimulates activity of RNA polymerase molecules transcribing from flanking divergently oriented promoters.

The initial footprinting experiments (Ogden *et al.*, 1980) showed the presence of a second CRP binding site in the *ara* regulatory region between −120 and −146. Since this site showed lower affinity for CRP than that located between −78 and −107, and by comparing preliminary transcription results with DNase footprinting results, it was postulated not to be involved in activation of either P_C or P_{BAD} (Lee *et al.*, 1981). The data presented here are consistent with this conclusion, since a deletion from the P_C side ending in the −120 to −146 region left P_{BAD} normally inducible, and since a deletion coming from the other direction and ending in the −78 to −107 region removed all cAMP dependence of P_C.

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