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 vitro 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 adjacentlly 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 araO2 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.
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 $Delta cya$ 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. 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.

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 ($F^{-} AaraC-leu1022\Delta lac74 galK Str^{r} thi$). The deletions ended at: +44, −65, −94, −127, −159, −200 and −230.
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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REFERENCES


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