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# The *ara*C Promoter: Transcription, Mapping and Interaction with the *ara*BAD Promoter

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## Summary

**The start sites of the *ara*C and *ara*BAD gene messenger of E. coli were located by transcription in vitro from short DNA fragments, by high magnification electron microscopy and by genetic mapping. Transcription for these messengers proceeds in opposite directions from the start sites that are 150 base pairs apart. Transcription from the *ara*BAD promoter requires *ara*C protein plus arabinose and CAP protein plus cyclic AMP. In the experiments performed in vitro, inducing the *ara*BAD promoter represses activity of the *ara*C promoter.**

## Introduction

Synthesis of the enzymes required to metabolize L-arabinose in E. coli is regulated almost certainly at the point of initiation of transcription (Hirsh and Schleif, 1973, 1976a; Lee et al., 1974). The *ara*C protein participates in this regulation, functioning both positively to induce and negatively under other conditions to repress the transcription (Englesberg, Squires and Meronk, 1969; Kessler and Englesberg, 1969; Schleif and Lis, 1975). These properties have been demonstrated in a variety of genetic and physiological experiments (Englesberg et al. 1969; Kessler and Englesberg, 1969; Greenblatt and Schleif, 1971; Schleif and Lis, 1975). Additionally, the cyclic AMP receptor protein CAP is required for efficient transcription of the operon. Presumably, it also functions at the initiation step (Lis and Schleif, 1973; Lee et al., 1974).

The orientation of the *ara*C gene is opposite to the orientation of the *ara*BAD operon (Figure 1), and thus the *ara*C promoter could be adjacent to the *ara*BAD promoter (Wilcox, Boulter and Lee, 1974a). This raises the possibility of shared elements and therefore of direct interactions between the promoters. Direct evidence that the *ara*C promoter is complex was found by Casadaban (1976). In a specially constructed operon fusion strain, he found that the synthesis of *ara*C protein exhibited stimulation by the presence of cyclic AMP and CAP as well as repression of synthesis by the presence of *ara*C protein itself.

The components involved in regulation of the arabinose operon, *ara*C protein (D. Steffen and R. Schleif, manuscript submitted), CAP protein, RNA polymerase and a 1070 base pair DNA fragment containing the arabinose regulatory region (Lis and Schleif, 1975) are now available in purified form. An early study with these, high magnification electron microscopy, delineated the conditions necessary for formation of the initiation and repression complexes and determined their locations (Hirsh and Schleif, 1976a). Here we locate the promoter and initiation complex for transcription of the *ara*C gene by microscopy, verify in a purified system most of the regulatory properties of the operon that have been found in vivo, and show that transcription of the *ara*C and *ara*BAD messengers begins from the initiation complexes observed in the electron microscope. Extensive sliding or rolling of RNA polymerase from the binding sites determined microscopically to the transcription start sites is excluded.

Unexpectedly, the transcription experiments described in this paper also show an interaction between the promoter for synthesis of the *ara*C protein and the promoter for synthesis of the *ara*B, A and D proteins. When one promoter is active, the other is inactive! No conditions were found where both promoters were simultaneously active.

## Results

### Determination of Restriction Enzyme Cleavage Sites in the Arabinose Operon Regulatory Region

Transcription in vitro from a nested set of DNA sequences can, in principle, be used to locate RNA polymerase start sites. This approach is feasible in the arabinose operon, since a 1070 base pair DNA fragment containing the regulatory region may be purified (Lis and Schleif, 1975). The DNA may then be cleaved wth restriction enzymes and used as template for transcription experiments in which the sizes of the RNA transcripts are measured and assumed to be the distance from the start sites to the end of the DNA molecules. The cleavage sites of the restriction enzymes were determined by using three *ara* DNA fragments: the 1070 base pair DNA containing the entire regulatory region and two smaller fragments, one containing the left half of the 1070 base pair DNA and one containing the right half (Schleif and Lis, 1975). Figure 2 shows the regulatory region and the cleavage sites that were found within it for Bam I, Hae III, Hha I and Alu I. The location of the Hae III cleavage site within the *ara*B gene is known to lie very close to the beginning of the *ara*B gene, for the revertible point mutation *ara*B17 destroys the ability of Hae III to cleave there (Lis, 1975). This mutation is among a set of the earliest point mutations in the *ara*B gene (Schleif, 1972; Schleif and Lis, 1975).

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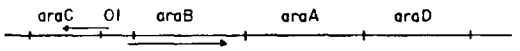


Figure 1. The Arabinose Operon

Transcription of messenger for *ara*C initiates in the regulatory region OI. In the presence of arabinose, *ara*C protein induces synthesis of the *ara*BAD messenger which initiates in the I region.

### Transcription from the *ara* Regulatory Region

Experiments performed in vitro using *lac* and lambda DNA as template have led to conditions allowing a high degree of specificity in the initiation of transcription (Majors, 1975; Meyer, Kleid and Ptashne, 1975). In these experiments, RNA polymerase is incubated with DNA and allowed to bind to promoters. Then heparin is added, followed 20 sec later by nucleoside triphosphates. The heparin inactivates free and weakly bound RNA polymerase molecules, but not those bound to most promoters. Thus at the time nucleoside triphosphates are added, only RNA polymerase molecules specifically bound at promoters remain active (Zillig et al., 1970).

To generate RNA molecules sufficiently short for detailed analysis, the *ara* DNA was cleaved with Hae III before transcription. A synthesis mixture containing DNA plus RNA polymerase yields a prominent transcript of 250 bases. A synthesis mixture containing DNA, RNA polymerase, CAP protein plus cyclic AMP, *ara*C protein and L-arabinose yields one prominent transcript of 45 base pairs (Figure 3). We shall show that the 250 base transcript is the initial portion of the messenger for *ara*C gene and the 45 base transcript is the initial portion of the messenger for the *ara*BAD operon.

In Figure 3, the *ara*C promoter appears to be more active than the *ara*BAD promoter. Much of this apparent difference results from the fact that an RNA molecule from the *ara*C promoter is 5 times as long and therefore 5 times as radioactive as an RNA molecule from the *ara*BAD promoter. The relative activities of the promoters in these experiments did not vary substantially when the concentration of RNA polymerase in the in vitro reactions was altered. Other alterations which might yield still greater activity of the *ara*BAD promoter or reduced activity of the *ara*C promoter have not yet been sought.

DNA uncut by Hae III directs transcription only of long RNA molecules, but once the DNA has been cut by Hae III, either the 250 or the 45 base transcript is synthesized. When the DNA is cut by Hha I in addition to Hae III, the 250 base transcript is shortened to about 185 bases, but is not shortened if Bam I is used in addition to Hae III. By reference to Figure 2, it can be seen that these facts locate the start site of the 250 base transcript which will be called the *ara*C transcript. The start site of the 45 base transcript RNA is determined by its disappearance following the Bam I cleavage, and is confirmed by its increase in size to about 75 bases when the DNA template has been cleaved with Alu I instead of Hae III.

### Regulated Properties of the *ara*C and *ara*BAD Transcripts

Synthesis of the 250 base *ara*C transcript requires only RNA polymerase and template. However, CAP protein and cyclic AMP together stimulate this transcription by about a factor of 2. Synthesis of the BAD transcript, the 45 base piece of RNA, requires template, CAP protein and cyclic AMP, *ara*C protein and L-arabinose, and RNA polymerase. Omission of any of these components eliminates detectable synthesis of this transcript. These properties are as expected from earlier experiments performed in vivo and in vitro (Sheppard and Englesberg, 1967; Greenblatt and Schleif, 1971; Wilcox et al., 1974b; Casadaban, 1976).

Most surprisingly, however, the synthesis of *ara*C transcript is reduced about a factor of 10 under the conditions yielding maximal *ara*BAD synthesis (Figure 3). Changes in reaction conditions which eliminate *ara*BAD synthesis, such as omission of *ara*C protein or substitution of D-fucose for L-arabinose, restore the *ara*C transcript to almost its prior level. Cleaving the DNA between the *ara*C and *ara*BAD promoters demonstrates that the repression effect on *ara*C requires integrity of the *ara*BAD promoter. Figure 2 shows that a Bam I cleavage site is on the edge of the initiation complex of the *ara*BAD messenger. As expected, cleavage at this site inactivates the *ara*BAD promoter. However, cleavage here restores substantial synthesis from the *ara*C promoter in the presence of *ara*C protein and L-arabinose (Figure 3). This indicates that the repression effect is mediated along the DNA rather than by a reduction in the concentration of free active RNA polymerase or CAP protein. To reduce the possibility of an artifact generated by inadequate RNA polymerase, despite the fact that RNA polymerase is present in about a 100 fold molar excess over *ara*BAD promoter, the experiment with and without Bam cleavage was repeated with RNA polymerase concentration increased 3 fold. The same results were obtained. It is not yet possible to determine whether the Bam cleavage restores full activity to the *ara*C promoter, since the activity of promoters could change if they are located near the ends of DNA fragments.

### Genetic Mapping of the Promoter for Synthesis of *ara*C Protein

The experiments described in the preceding sections locate a promoter active in the in vitro synthesis of an RNA species. This transcript appears to be

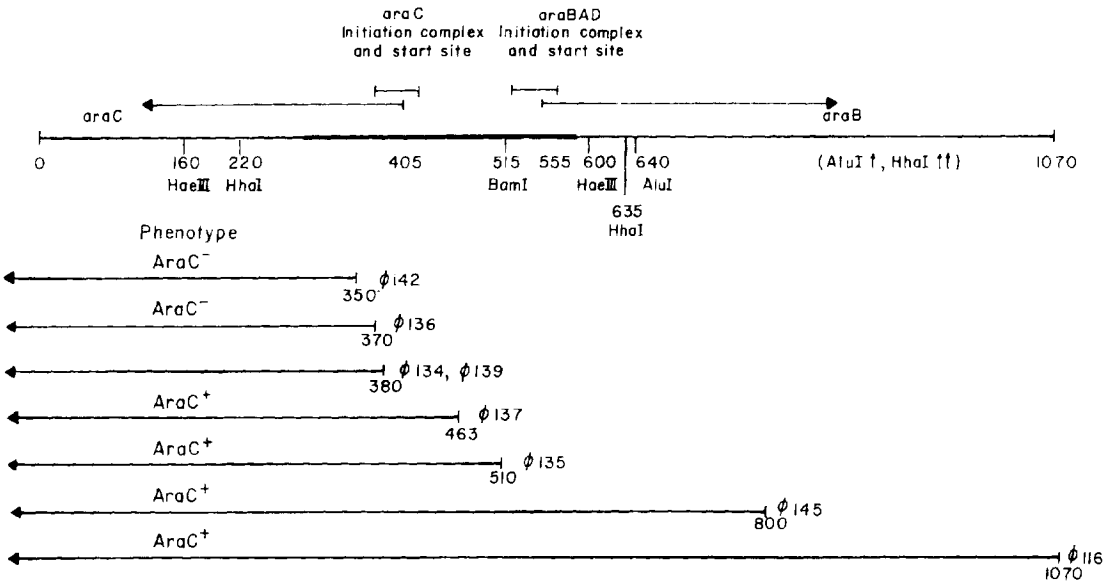


Figure 2. Topography of the *ara* Regulatory Region

The 1070 base pair *ara* DNA used in these experiments. It is made from heteroduplexes between φλ*para*114 and λ*para*116, and digestion with S1 nuclease (Lis and Schleif, 1975). The site of the initiation complex for *ara*BAD messenger was determined previously by electron microscopy (Hirsh and Schleif, 1976b), and the sites of the *ara*C messenger initiation complex and the start sites of transcription were determined in this work. The restriction enzyme cleavage sites of the Hae III, Alu I, Bam I and Hha 1 in the regulatory region are indicated. The locations of the Bam I and Hae III cleavages were determined as follows: Bam I cleavage yields fragments 515 and 655 base pairs long with the 515 fragment located on the left; Hae III cleavage yields fragments 160, 440 and 470 base pairs long with the 160 base pair fragment originating from the left end; combined digestion with Bam I and Hae III yields fragments 160, 355, 90 and 470 base pairs long. The Alu I and Hha I cleavage sites were determined by appropriate combined digestion or with the complete 1070 fragment and its left and right halves. The phage used in mapping the *ara*C promoter are shown. Each derives from φλ*para*C116 (Lis and Schleif, 1975) and is deleted fom the *ara*B side. The black line indicates the DNA remaining. The endpoints of the deletions were determined either by gel electrophoresis with a resolution ±10 base pairs (φ142, φ136, φ135, φ116) or by electron microscopy of appropriate heteroduplexes (Schleif and Lis, 1975).

the 5′ end of messenger for *ara*C protein. Here we describe experiments performed in vivo which locate the promoter for the *ara*C protein. Within the resolution of ±90 base pairs, the promoter active in vivo is located at the same site as the promoter active in vitro, and we conclude they are one and the same. The mapping was made possible by the existence of a collection of lambda *ara* transducing phage containing the *ara*C gene and different quantities of the *ara* regulatory region as displayed in Figure 2. All these phage were shown to contain an intact *ara*C structural gene, since each is capable of synthesizing active *ara*C protein under control of phage promoters. This phenomenon was shown to exist for these phage by D. Steffen and R. Schleif (manuscript submitted). We show below, however, that not all the phage contain the *ara*C promoter.

The assay of activity of the *ara*C promoter was performed by infecting lambda lysogenic AraC⁻ cells with these various *ara* phage. When the phage enter the cell, the presence of lambda repressor prevents DNA replication or initiation of transcription from phage early or late promoters. Thus any synthesis of *ara*C protein must reflect activity of the *ara*C promoter. The presence of *ara*C protein was detected by its ability to induce the *ara*BAD genes of the infected cell.

As shown in Table 1, the parental phage, λ*para*116, as well as those containing most of the regulatory region are able to synthesize *ara*C protein under these conditions. However, phage deleted of most of the regulatory region are unable to synthesize *ara*C protein. By reference to Figure 2, these facts locate a site outside the *ara*C gene necessary for normal synthesis of *ara*C protein.

In each of four independent repeats of the experiment, phage 135 and 137 yielded twice as much *ara*C activity as phage 116 and 145. It is tempting to ascribe this hypersynthesis to the absence of the functional *ara*BAD promoter. This could be an effect of the same nature as was discovered in the in vitro transcription from the *ara*C promoter. The additional complexities of this in vivo experiment dictate caution in this interpretation, however, for the effect could arise as a result of different lambda genes being removed at the other end of the deletions.

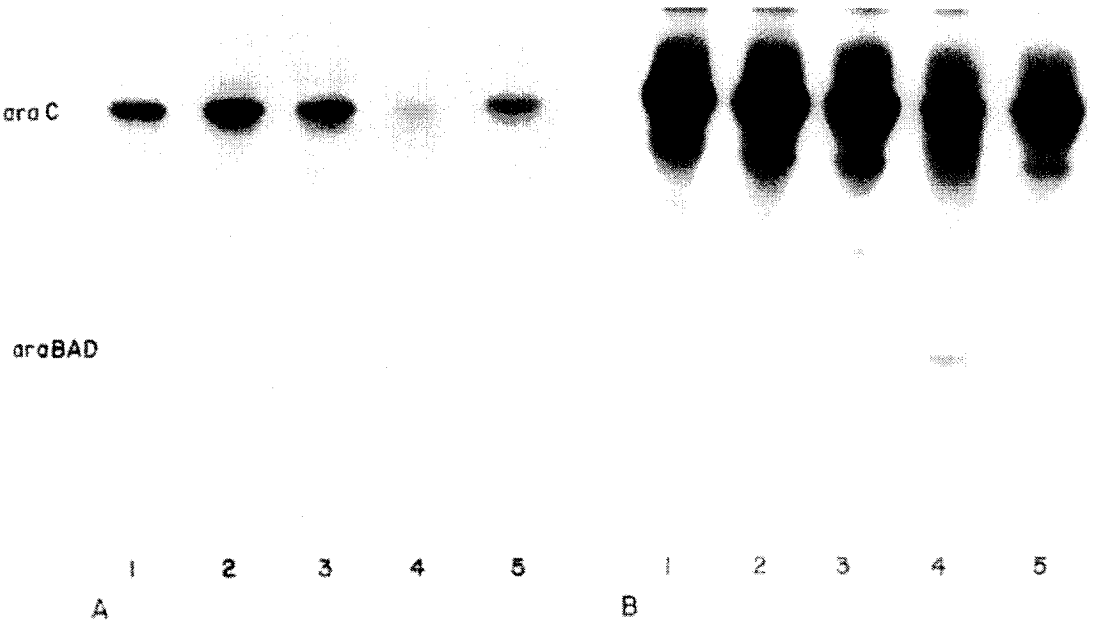


Figure 3. Autoradiogram of Radioactive *ara* RNA Transcripts

(A) 8 hr exposure of autoradiograms.

(B) 40 hr exposure of autoradiograms. Performed as described in Experimental Procedures with Hae III-cut *ara* DNA and RNA polymerase, CAP protein and *ara*C protein included as indicated. All reactions contained 100 mm L-arabinose and 1 mm cAMP.

(1) RNA polymerase.

(2) RNA polymerase and CAP.

(3) RNA polymerase and CAP. DNA cut by Bam I in addition to Hae III.

(4) RNA polymerase, CAP, *ara*C protein.

(5) RNA polymerase, CAP, *ara*C protein, Bam I-cut.

Quantitation of the transcripts from similar experiments shows that about 20% of the DNA molecules yield *ara*BAD transcripts.

### Location of the *ara*C Promoter by High Resolution Electron Microscopy

An earlier high resolution electron microscope study of the arabinose operon demonstrated the initiation complex for the *ara*BAD messenger and also a complex believed to be involved in repression of the BAD promoter. It failed to show the existence of the promoter for synthesis of C protein (Hirsh and Schleif, 1976b). This is not unexpected, since the promoter for the synthesis of C protein is likely to be "weak" and therefore may bind RNA polymerase slowly and/or weakly. The *ara* transcription experiments described here, however, yield considerable activity of the *ara*C promoter. The electron microscopy was therefore repeated under the conditions used in the *ara* transcription experiments. These conditions had previously been developed for transcription of the *lac* and lambda systems (Majors, 1975; Meyer et al., 1975). The changes, doubling the RNA polymerase concentrations, halving the DNA concentrations, tripling the incubation time and substituting heparin for poly(rI), would accelerate a slow binding reaction or increase the extent of a binding limited by an unfavorable equilibrium constant. Indeed, the changed conditions yield appreciable initiation complex detectable by microscopy (Figure 4).This complex is located at the start site of the *ara*C messenger, and as expected from the transcription experiments, its formation is stimulated about 2 fold by the presence of CAP protein and cyclic AMP. These complexes appear to be transcriptionally active since they vanish if nucleoside triphosphates are added.

### Discussion

Two types of experiments were used in this work to locate the start sites for transcription of the *ara*C and *ara*BAD messengers. Transcription in vitro from short DNA and accurate size measurements of the RNA placed the start sites with a precision of about 10 base pairs. The promoter for synthesis of the *ara*C protein was shown genetically to lie near the start site, and RNA polymerase was shown by electron microscopy to bind there. The start sites of transcription are located about 150 base pairs apart, and transcription proceeds from each in op-

Table 1. Genetically Mapping the *ara*C Promoter

| Phage Used to Infect *arc*C⁻ Cells | In Vivo *ara*C Activity | Conclusion |
|---|---|---|
| $\phi$142 | <62 | No *ara*C promoter |
| $\phi$136 | 250 | Possibly part of *ara*C promoter remains |
| $\phi$137 | 6250 | Functional *ara*C promoter, possibly hyperactive |
| $\phi$135 | 5600 | Functional *ara*C promoter, possibly hyperactive |
| $\phi$145 | 2600 | Functional *ara*C promoter |
| $\phi$116 | 2600 | Functional *ara*C promoter |

See Experimental Procedures. The column labeled "in vivo *ara*C activity" shows the monomers of arabinose isomerase per cell induced above the basal level of 150 at 120 min after infection.

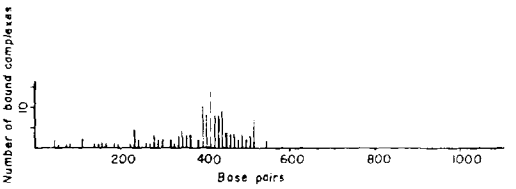


Figure 4. Locating the RNA Polymerase Binding Site for *ara*C Transcription by Electron Microscopy

Samples were prepared and visualized as described and referenced in Experimental Procedures. Shown is a histogram of the locations of RNA polymerase bound to the 1070 base pair DNA fragment depicted in Figure 2. These data are a composite of histograms from an experiment in which CAP protein was absent and from an experiment in which CAP protein was present. The binding frequencies in the two experiments were 0.08 and 0.15, respectively.

posite directions. It was therefore, unexpected that the high activity of the *ara*BAD promoter would lead to the low activity of the *ara*C promoter. This appears to be a direct interaction between the promoters, for cleaving the DNA with restriction endonuclease Bam I between the promoters removes the interaction.

Previous electron microscope studes (Hirsh and Schleif, 1976b) located a complex near the *ara*C promoter. This was postulated to be involved with repression of the *ara*BAD promoter, since it formed only in the presence of the anti-inducer D-fucose, was transcriptionally inactive and was located at a site predicted by genetic studies. Since this complex, which was identified as the *ara*BAD repression complex, is located close to the *ara*C promoter, it is possible that it also represses the *ara*C promoter or that instead it is a functional transcription complex on the *ara*C promoter. If the latter were true, the microscope experiments show that the complex must be slow to initiate transcription. This interpretation also raises the question of why the complex formed only in the presence of *ara*C protein. These questions of the relationship of the apparent repression complex to transcription from the *ara*C and *ara*BAD promoters have not been investigated in the work reported here.

As expected from the properties of the operon observed in cells, the activity of the *ara*BAD promoter in vitro requires *ara*C protein, CAP protein, arabinose and cyclic AMP, in addition to template DNA and RNA polymerase. The *ara*C promoter does not require the CAP protein, for it is active in vitro in its absence. This CAP independence is also displayed in vivo. Casadaban (1976) constructed a special strain with the $\beta$-galactosidase gene fused to the *ara*C promoter, thus facilitating assay of the activity of the *ara*C promoter. This fusion strain showed that the *ara*C promoter is active without CAP, but that it is stimulated about 3 fold by the presence of CAP. The transcription experiments and the electron microscopy described in this work show similar results. The fusion strain also showed a 10 fold reduction in the activity of the *ara*C promoter if active *ara*C protein was present. Our transcription experiments also show such a repression effect, but it is manifest only in the presence of arabinose.

The interaction between the *ara*C and *ara*BAD promoters shown in this work raises the possibility of action at a distance mediated via the DNA. Nonetheless, it is possible that protein—for example, *ara*C protein and/or CAP protein—bridges the gap between these two promoters and mediates the effect. This seems improbable, however, since the protein would need to span the distance of 100 bases which lies between edges of the polymerase molecules on the two promoters. Currently, the data favor the notion that the effect between the promoters is transmitted via the DNA directly. There are several ways this could come about. RNA polymerase could bind at one site and slide to another from which transcription begins. An interleaving of such rightward and leftward sites could produce a system in which only one of the two promoters is active at a time, or a structure of the DNA, such as base tilt, could be altered over an area by the binding of RNA polymerase to a promoter. Finally, the DNA could form a hairpin loop.

## Experimental Procedures

### DNA and Restriction Enzymes

The 1070 base pair DNA fragment containing the *ara* regulatory region was purified as described previously (Lis and Schleif, 1975). Digestions of this DNA by Bam I (from Baccilus amyloliquefaecens), Hae II (from Haemophilus aegyptius), Hha I (from Haemophilus haemolyticus) and Alu I (from Arthrobacter luteus) were performed as described by Haggerty and Schleif (1976), and sizes were measured on 2% agarose, or 4%, 8% or 12% acrylamide gels. The restriction enzymes were purified by precipitating DNA from the lysates with polyethylene glycol and NaCl and DEAE chromotography, or obtained from New England Biolabs. DNA length

standards were the sequenced *lac* 203 and *lac* 788 fragments (A. Maxam and W. Gilbert, personal communication).

### In Vitro Transcription

The procedures were modified as appropriate from Meyer et al. (1975) and Majors (1975). 20 μl reactions contained 50 mM KCl, 10 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM KEDTA, 1 mM DTT, 0.2 μg/ml DNA, from 5–15% glycerol, 1 mM cAMP, 100 mM L-arabinose or 50 mM D-fucose, 25 μg/ml RNA polymerase (gift from Richard Burgess), 1 μl of DEAE fraction of *ara*C protein (D. Steffen and R. Schleif, manuscript submitted), about 0.25 μg CAP protein (gift from John Majors), 250 μM ATP and GTP, 10 μM UTP, 2 μM $\alpha$-$^{32}$P CTP at 100 Ci/mmole (New England Nuclear). Concentrated buffer stock, water, DNA and sufficient restriction enzyme to digest the DNA completely were incubated at 37°C for 30 min and then at 65°C for 10 min to inactivate the enzyme. The remaining components at 0°C were then added in the order listed above. After 10 min of incubation at 37°C, heparin was added to a final concentration of 100 μg/ml, and 20 sec later, the triphosphates were added and incubated continued for 10 min at 37°C. 20 μl of deionized formamide were added, and bromophenol blue and xylene cyanol were added to 0.01%.The samples were electrophoresed on 12% or 5% acrylamide 7 M urea gels (Maniatis, Jeffrey and van de Sande, 1975) and visualized by autoradiography. Size standards were the *lac* 63 transcript from Hae III 203 (Majors, 1975), λOop 77 (Scherer, Hobon and Kossel, 1977), λpr110 from λpr Hae III 885 (Meyer et al., 1975 and personal communication) and λ "6S" 201 (Lebowitz, Weissman and Radding, 1971).

### High Magnification Electron Microscopy

Previously described procedures (Hirsh and Schleif, 1976a, 1976b) were followed, except that the buffers and protein concentrations which were used were the same as those in the transcription experiments.

### Mapping – Physical

The endpoints of the deletions entering the *ara* regulatory region were determined as described previously (Schleif and Lis, 1975) by heteroduplex analysis to a precision of about ±20 base pairs and by gel electrophoresis on 2% agarose gels to a precision of about ±10 base pairs. The DNA length standards were as above.

### Mapping – Genetic

Lambda-*ara* transducing phage containing the *ara*C gene and a deletion that could have inactivated the *ara*C promoter were scored by activity of this promoter. The cells, Δ*ara*C (λ) RFS935 (Schleif, 1972), were grown to 1 × $10^8$/ml at 35°C M10 medium (Nathanson and Schleif, 1975) containing 10 μg/ml thiamine, 0.2% glycerol and 1% casamino acids. At this time, $MgSO_4$ was added to 0.01 M, L-arabinose to 0.2% and phage to a multiplicity of 5. At 40, 80 and 120 min later, 2 ml samples were taken for assay of isomerase as described previously (Schleif, 1969).

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### References

Casadaban, M. (1976). Regulation of the regulatory gene for the arabinose pathway, *ara*C. J. Mol. Biol. *104*, 557–566.

Englesberg, E., Squires, C. and Meronk, F., Jr. (1969). The L-arabinose operon in *Escherichia coli* B/r: a genetic demonstration of two functional states of the product of a regulator gene. Proc. Nat. Acad. Sci. USA *62*, 1100–1107.

Greenblatt, J. and Schleif, R. (1971). Arabinose C protein: regulation of the arabinose operon in vitro. Nature New Biol. *233*, 166–170.

Haggerty, D. and Schleif, R. (1976). Location in bacteriophage lambda DNA of cleavage sites of the site-specific endonuclease from Bacillus amyloliquefaciens H. J. Virol. *18*, 659–663.

Hirsh, J. and Schleif, R. (1973). On the mechanism of action of L-arabinose C gene activator and lactose repressor. J. Mol. Biol. *80*, 433–444.

Hirsh, J. and Schleif, R. (1976a). High resolution electron microscopic studies of genetic regulation. J. Mol. Biol. *108*, 471–490.

Hirsh, J. and Schleif, R. (1976b). Electron microscopy of gene regulation: the L-arabinose operon. Proc. Nat. Acad. Sci. USA *73*, 1518–1522.

Kessler, D. P. and Englesberg, E. (1969). Arabinose-leucine deletion mutants of *Escherichia coli* B/r. J. Bacteriol. *98*, 1159–1169.

Lebowitz, P., Weissman, S. and Radding, C. (1971). Nucleotide sequence of a ribonucleic acid transcribed in vitro from λ phage deoxyribonucleic acid. J. Biol. Chem. *246*, 5120–5139.

Lee, N., Wilcox, G., Gielow, W., Arnold, J., Cleary, P. and Englesberg, E. (1974). In vitro activation of the transcription of *ara*BAD operon and *ara*C activator. Proc. Nat. Acad. Sci. USA *71*, 634–638.

Lis, J. (1975). Genetic, physiological and physical studies on the regulation of the L-arabinose operon of *Escherichia coli*. Ph. D. Thesis, Brandeis University, Waltham, Massachusetts.

Lis, J. and Schleif, R. (1973). Different cyclic AMP requirements for induction of the arabinose and lactose operons of *Escherichia coli*. J. Mol. Biol. *79*, 149–162.

Lis, J. and Schleif, R. (1975). The regulatory region of the L-arabinose operon: its isolation on a 1000 base-pair fragment from DNA heteroduplexes. J. Mol. Biol. *95*, 409–416.

Majors, J. (1975). Initiation of in vitro mRNA synthesis from wild-type lac promoter. Proc. Nat. Acad. Sci. USA *72*, 4396–4398.

Maniatis, T., Jeffrey, A. and van de Sande, H. (1975). Chain length determination of small double- and single-stranded DNA molecules by polyacrylamide gel electrophoresis. Biochemistry *14*, 3787–3793.

Meyer, B., Kleid, D. and Ptashne, M. (1975). λ Repressor turns off transcription of its own gene. Proc. Nat. Acad. Sci. USA *72*, 4785–4789.

Nathanson, N. M. and Schleif, R. (1975). Paucity of sites mutable to constitutivity in the *ara*C activator gene of the L-arabinose operon of *Escherichia coli*. J. Mol. Biol. *96*, 185–199.

Scherer, G., Hobon, G. and Kossel, H. (1977). DNA base sequence of the $p_o$ promoter region of phage λ. Nature *265*, 117–121.

Schleif, R. (1969). An L-arabinose binding protein and arabinose permeation in *Escherichia coli*. J. Mol. Biol. *46*, 185–196.

Schleif, R. (1972). Fine-structure deletion map of the *Escherichia coli* L-arabinose operon. Proc. Nat. Acad. Sci. USA *69*, 3479–3484.

Schleif, R. and Lis, J. (1975). The regulatory region of the L-arabinose operon: a physical, genetic and physiological study. J. Mol. Biol. *95*, 417–431.

Sheppard, D. and Englesberg, E. (1967). Further evidence for positive control of the L-arabinose system by gene *ara*C. J. Mol. Biol. *25*, 443–454.

Wilcox, G., Boulter, J. and Lee, N. (1974a). Direction of transcription of the regulatory gene *ara*C in *Escherichia coli* B/r. Proc. Nat. Acad. Sci. USA *71*, 3635–3639.

Wilcox, G., Meuris, P., Bass, R. and Englesberg, E. (1974b). Regulation of the L-arabinose operon BAD in vitro. J. Biol. Chem. *249*, 2946–2952.

Zillig, W., Zechel, K., Rabussay, D., Schachner, M., Sethi, V., Palm, P., Heil, A. and Seifert, W. (1970). On the role of different subunits of DNA-dependent RNA polymerase from *E. coli* in the transcription process. Cold Spring Harber Symp. Quant. Biol. *35*, 47–58.