DNA sequence of the araC regulatory gene from Escherichia coli B/r

C.Garrett Miyada, Arnold H.Horwitz, Laura G.Cass, Josef Timko and Gary Wilcox

Department of Microbiology and Molecular Biology Institute, University of California at Los Angeles, Los Angeles, CA 90024, USA

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ABSTRACT

The DNA sequence of the araC regulatory gene from Escherichia coli B/r has been determined by the base-specific chemical cleavage reactions of Maxam and Gilbert. An open reading frame is found which codes for a protein of 292 amino acids. A nonsense mutation, araC5, is shown to result from a G to A transition at nucleotide 429 converting the tryptophan codon TGG to the amber codon TAG. A deletion which does not recombine with any known point mutation in araC, Δ(araC0)719, removes all but the last 22 codons of the gene.

INTRODUCTION

The araC gene in Escherichia coli codes for a regulatory protein which controls the expression of at least six structural genes which are involved in the transport and catabolism of L-arabinose (1, 2). The araC protein regulates initiation of transcription of the araBAD operon (3) and in addition autogenously controls its own synthesis (4). The gene is transcribed in a clockwise direction on the standard E. coli linkage map (5). The DNA sequence of the araC promoter and the start site of araC mRNA have been determined (6). We have recently shown that araC mRNA contains a 150 nucleotide leader by sequencing the DNA corresponding to the promoter-proximal region of the araC gene (7). In order to precisely define the molecular basis by which araC protein controls gene expression it is necessary to determine the DNA sequence of the entire gene. In this paper we report the DNA sequence of the araC gene and the sequence changes resulting from a nonsense mutation and a deletion in araC.

MATERIALS AND METHODS

Materials

Restriction endonucleases Tag I and Ava I were purified by the methods of Sato et al. (8) and Murray et al. (9), respectively. Hae III was
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purchased from Calbiochem-Behring Corporation, La Jolla, CA. Ava II was from Bethesda Research Laboratories, Rockville, MD. Hinf I and Sau 3A were gifts from W. Salser and M. Komaromy, respectively, both of the Molecular Biology Institute, UCLA. Bacterial alkaline phosphatase (BAP) was purchased from Worthington Biochemical Corporation, Freehold, N.J.. Polynucleotide kinase was purchased from New England Biolabs, Beverly, MA. Reverse transcriptase was obtained from the National Cancer Institute, Bethesda, MD. (γ-32P) ATP was prepared as described by Johnson and Walseth (10). (α-32P) deoxynucleotides were purchased from Amersham, Arlington Heights, IL. Cronex 4 X-Ray film and Cronex Hi Plus intensifying screens were from E.I. DuPont de Nemours, Wilmington, DE.

Plasmids

Plasmids used for sequencing the araC gene and mutations in the araC gene were pLGC10, pLGCC20, pAH105, pAH25 and pCH11. Plasmids pLGC10, pLGCC20, and pCH11 have been described previously (7, 11). Plasmid pAH105 was constructed by subcloning Eco RI restriction fragments from plasmid pTB1 (12) into the Eco RI site of a modified pBR322 plasmid. Plasmid pAH105 was found among Ara+Ap^- transformants in an araC deletion background. Plasmid pAH25 was constructed by in vivo recombination of the araC5 nonsense mutation onto the ara deletion 738-containing plasmid, pAH17, as described by Horwitz et al. (11). Plasmids were grown in derivatives of E. coli K-12 strain RR1 and the DNA was purified as described by Norgard et al. (13).

Isolation and end-labeling of restriction fragments

Restriction fragments used for sequencing were purified on 5% polyacrylamide or 1.5% agarose gels buffered in TBE (10.8 gm Trizma-base, 5.5 gm boric acid, and 0.93 gm Na2EDTA per liter). The restriction fragments were visualized by U.V. shadowing (14), cut out of the gel, electroeluted out of the gel matrix, and concentrated by ethanol precipitation. DNA obtained from agarose gels was further purified after the electroelution step by passing it over a 0.6-1.2 ml DE-52 column (15). Restriction fragments were dephosphorylated using 1.4 units of bacterial alkaline phosphatase and incubating in 100-300 μl of 50 mM Tris-HCl, pH 8.0, for one hour at 65° C. The BAP was removed by successive phenol, phenol-chloroform, and ether extractions. Dephosphorylated restriction fragments were 5' end-labeled with (γ-32P) ATP and polynucleotide kinase essentially as described by Maxam and Gilbert (14). Restriction fragments were 3' end-labeled with (α-32P) dNTP and reverse transcriptase by filling in a 5' extended restriction site. The reverse transcription
reaction mixture included 5-20 pmoles of restriction fragment, 100 μCi of an appropriate (α-32P) dNTP (400 Ci/mmmole), 33 μM of any necessary cold dNTP, 6 mM Tris- HCl, pH 8.0, 10 mM MgCl2, 50 mM KCl, 1 mM dithiothreitol, 10% glycerol and 50 units of reverse transcriptase, in a volume of 30 μl. The reaction was incubated at 37°C for at least 90 min. and terminated as the above kinase reaction. Single end-labeled fragments were usually generated by a secondary restriction cut. Alternatively, strand separation was used to create single end-labeled restriction fragments.

**DNA sequence analysis**

Single end-labeled restriction fragments were subjected to the base-specific chemical cleavage reactions of Maxam and Gilbert (14). The G, G+A, A>C, C+T and C reactions were used. The products were analyzed on 0.4 mm thick gels (16) using acrylamide concentrations of 15-16%, 12% and 8%. The gels were developed at -70°C with or without intensifying screens. The final DNA sequence was analyzed by the computer program of Queen and Korn (17).

**RESULTS AND DISCUSSION**

**Sequence analysis of the wild-type araC gene**

The end-labeled restriction fragments used to determine the nucleotide sequence of the promoter-distal region of the araC gene are shown in Figure 1. As seen in the figure, the sequencing strategy employed resulted in the determination of the nucleotide sequence for both strands of DNA throughout the entire region. In addition, every restriction site used for

![Figure 1. DNA sequencing strategy for the promoter-distal region of the araC gene. Pertinent restriction sites are marked by vertical bars above the heavy horizontal line. The numbers below the heavy horizontal line represent the base pair number of the gene using the araC mRNA start as 1. The horizontal arrows above the line denote sequenced restriction fragments on the upper strand (5' to 3' reading left to right); the horizontal arrows below denote sequenced restriction fragments labeled on the lower strand. Open circles at the ends of the horizontal arrows represent 3'-end labeling; closed circles represent 5'-end labeling. The arrowheads extend as far as the sequence could be determined for that fragment.](image-url)
end-labeling or for a secondary restriction cut was sequenced across from a nearby restriction site. This eliminated the possibility of missing an internal sequence between two identical and adjacent restriction sites.

The entire nucleotide sequence for the \textit{araC} gene is shown in Figure 2. If the methionine codon centered at position 147 is taken as the first amino acid, the \textit{araC} gene codes for a protein of 292 amino acids with a molecular weight of 33,315. Two other potential methionine start codons in the same reading frame are centered at positions 127 and 138 and have been discussed in a previous paper (7). Isolated \textit{araC} protein run on SDS-polyacrylamide gels was found to have a subunit molecular weight of 28,000 (18). The apparent discrepancy between the molecular weight figures may be due either to aberrant mobility of \textit{araC} protein on SDS-polyacrylamide gels, proteolytic processing, or a combination of the two. The location of the Ava I site starting at position 961 within the open reading frame is consistent with the phenotype of the plasmid pLGClO. This plasmid contains the \textit{araC} promoter and \textit{araC} gene up to the Ava I site and is unable to complement \textit{araC} alleles (7). Since the coding region of \textit{araC} corresponding to the terminal 20 amino acids is not present in pLGClO, it is not surprising that no functional \textit{araC} protein is produced.

DNA sequence of an amber mutant

An \textit{araC} nonsense mutation, \textit{araC5} (19), was cloned onto a hybrid \textit{ara} plasmid by \textit{in vivo} recombination (11). The resulting recombinant plasmid, designated pAH25, was shown by the following criteria to contain the \textit{araC5} mutation. First, the plasmid failed to complement strain LA3, containing \textit{araC} deletion 719, but was able to complement strain LA304 which contains \textit{araC} deletion 719 and the amber suppressor mutation, supE. Second, the \textit{araC5} mutation was previously shown by genetic mapping to be located near the center of the \textit{araC} gene (19). An \textit{ara} point mutation on a plasmid can be readily mapped because recombination between a mutation on the plasmid and mutations on the chromosome can be detected as Ara+ papillae on indicator plates after two days of incubation (11). The \textit{araC5} mutation was shown to map in precisely the same location in \textit{araC} on the plasmid pAH25 as it did on the chromosome by transforming this plasmid into seven strains containing different \textit{araC} alleles and scoring for recombinants.

The base pair change resulting in \textit{araC5} was determined by DNA sequence analysis to be a G to A transition at position 429 in the \textit{araC} gene (see Figure 3). This base pair change results in the conversion of a tryptophan codon, TGG, to the amber codon, TAG, as was expected from the genetic
Figure 2. The nucleotide sequence of the araC gene and its corresponding protein product. The above figure combines sequencing data derived using the sequencing strategy shown in Figure 1 with previous sequence data (7). The nucleotide sequence is numbered in a 5' to 3' direction with the araC mRNA start site taken as 1. The inferred amino acid sequence is shown below the nucleotide sequence.
Figure 3. Autoradiograms of 8% polyacrylamide gels showing the position of the nonsense mutation araC5. (Left) The wild-type sequence from position 422 to 435. (Right) The araC5 sequence from position 422 to 435.

analysis of the mutation by Irr and Englesberg (19). The location of araC5, as determined by genetic mapping corresponds very closely to its location as determined by the DNA sequence analysis. Previous genetic mapping studies had placed araC5 at a point 40% of the distance through the araC gene (19). Combining the genetic mapping data with the known length of araC shown in Figure 2, we calculated that araC5 would be found near nucleotide position 400 which is very close to the actual location at position 429.

DNA sequence of a deletion mutant

Deletion 719 does not recombine with any known mutation in araC, removes the operator locus in the araBAD controlling site region and leaves the araBAD promoter intact (20). The Δ(araC)719 allele was cloned by in vivo recombination onto the plasmid pAH15 (11). The resultant plasmid, pCH11, was used to determine the sequence changes caused by the deletion. Plasmid pCH11 was restricted with BamH I, and the resulting 5'-ends labeled with (8-32P) ATP and polynucleotide kinase. After secondary restriction with Hinf I, the purified BamH I-Hinf I fragment was sequenced (see Figure 4). By comparison of the sequence shown in Figure 4 with the sequence shown in Figure 2 it can be concluded that Δ(araC)719 begins at nucleotide position -23 and ends at 949. Thus, this deletion removes all but the terminal 22 codons of araC.

In summary, we have completed the nucleotide sequence of the araC gene.
Figure 4. Autoradiogram of an 8% polyacrylamide gel showing the sequence of ara deletion 719.

An open reading frame containing 292 codons is terminated by a single ochre codon (TAA). Proof that the open reading frame codes for araC protein was obtained by sequencing the nonsense mutant, araC5. In addition, the ara deletion 719 was found to terminate within the coding region of the gene. The determination of the DNA sequence of the araC gene will facilitate further analysis of the structure-function relationships involved in the regulatory events mediated by araC protein.

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