Regulatory Gene Control of Transcription of the L-Arabinose Operon in Escherichia coli*

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SUMMARY
The L-arabinose operon is under both positive and negative control by the product of its regulatory gene, araC. We have measured transcription of the arabinose genes by hybridization of 3H-labeled messenger RNA to single-stranded DNA from an arabinose transducing phage in order to study the effect of the regulatory gene on transcription. In strains in which the arabinose regulatory gene is completely nonfunctional due to deletion or nonsense mutation, only lack of activator, the positive controlling element, prevents expression of the arabinose operon. In these strains no mRNA from the arabinose operon can be detected, indicating that the positive control is exerted on the transcription process. Arabinose constitutive mutants which make functional activator in the absence of arabinose or which do not need the activator function for enzyme synthesis produce mRNA from the arabinose operon even in the absence of inducer. When an araC+ F' ara episome is introduced into these constitutive strains, making them diploid for the arabinose genes, the araC+ gene provides repressor function and transcription of the operon is shut off in the absence of inducer, demonstrating that negative control also functions at the transcription step. A number of araC mutants producing lower than wild type levels of enzymes from the arabinose operon were shown to have correspondingly lower levels of mRNA from the operon. These results are consistent with the hypothesis that the araC gene affects only the process of transcription of the arabinose operon.

The inducible L-arabinose operon of Escherichia coli is under dual control by its regulatory gene araC according to the following model. In the absence of arabinose, the araC gene product acts as a repressor of production of the arabinose-metabolizing enzymes L-arabinose isomerase, L-ribulokinase, and L-ribulose-5-P 4-epimerase which are coded for by genes araA, araB, and araD, respectively (1, 2). The site of action for repressor, the operator site, is defined by the genetic region araO (3). In the presence of arabinose, the repressor activity is lost, and the araC product becomes functional as an activator (1, 2). Activator is necessary for expression of the arabinose operon and exerts control at an initiator site defined by the araI genetic region (4, 5). The araC gene product is a protein (6). The genetic map order of the arabinose locus is araC araO araI araB araA araD; all are closely linked.

The model above does not detail the mechanisms by which the araC product carries out its two regulatory functions. Repression or activation could take place during the process of transcription of the arabinose genes to messenger RNA or during the process of translation of the mRNA to proteins. Recently, mRNA from the L-arabinose operon was detected by its binding to single strands of DNA from a defective λ80 bacteriophage, which carries the genes for the arabinose operon, and was found to be inducible. A culture of araC+ E. coli grown in the absence of arabinose contains little ara mRNA1 whereas a culture induced with arabinose produces relatively large amounts of ara mRNA (7). These results demonstrate that the process of transcription of the arabinose operon is regulated but do not determine whether the regulation is due to the repression mechanism, the activation mechanism, or both. The possibility also remains that the arabinose operon may be regulated also during the process of translation. This possibility seems especially pertinent for the araC protein because it clearly performs two different regulatory functions.

The processes of repression and activation of the arabinose operon can be separated by using various bacterial strains in which parts of the arabinose regulatory system have been altered by mutations. We have used the RNA-DNA hybridization technique to measure mRNA in such strains. In this way we are able to determine that both mechanisms affect transcription. We have looked for evidence that the araC gene protein may also control translation of ara mRNA. We find no evidence that altered araC proteins have any effect on translation of ara mRNA in a series of araC mutant strains.

Materials and Methods

Bacteria, Media, and Chemicals—The strain of bacteria used is described in Table I. The following solutions and media were used: annealing buffer, 0.5 M KCl, 0.01 M Tris-Cl, pH 7.3; 1% Casamino Acids medium (12): r-broth (9); lysing buffer, 5 mM EDTA, 0.15 M NaCl, 0.05 M Tris-Cl, pH 7.4; mineral arabi-

1 The abbreviations used are: ara mRNA, mRNA (messenger RNA) from the L-arabinose operon, operationally defined as that component of pulse-labeled RNA which hybridizes with DNA from bacteriophage λ80 but not with DNA from bacteriophage λ80; SSC, standard saline-citrate solution; TM buffer, Tris-magnesium sulfate buffer.
TABLE I

Strains of Escherichia coli used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>UP1002</td>
<td>araC leu-t</td>
</tr>
<tr>
<td>UP1012</td>
<td>araC5, nonsense mutation (9)</td>
</tr>
<tr>
<td>SB1114</td>
<td>araΔ786 (3)</td>
</tr>
<tr>
<td>SB3107</td>
<td>F' araB24/araB24 (2)</td>
</tr>
<tr>
<td>SB3123</td>
<td>F' araC61/araC61, temperature-sensitive mutation (10)</td>
</tr>
<tr>
<td>SB3140</td>
<td>araA2 araC67(C), resistant to bacteriophage T5, araC67, L-arabinose-negative (2)</td>
</tr>
<tr>
<td>SB7514</td>
<td>λ80dara C1857 S, λ80 C1857 S, heat-inducible double lysogen (7, 11)</td>
</tr>
<tr>
<td>J12097</td>
<td>araC(C) mutants selected from UP1002 by α-fucose through resistance (this paper)</td>
</tr>
<tr>
<td>J12112</td>
<td>araC(C) araCΔ786, selected from SB1114 (this paper)</td>
</tr>
<tr>
<td>J12129</td>
<td>araA284, deletion of araB araA araC genes, araD, genotype unknown (1)</td>
</tr>
<tr>
<td>J12122</td>
<td>λ80dara C1857 S, lysogen of J12112</td>
</tr>
<tr>
<td>J12123</td>
<td>araC67, temperature-sensitive mutation (10)</td>
</tr>
<tr>
<td>J12124</td>
<td>araC67, temperature-sensitive mutation (10)</td>
</tr>
</tbody>
</table>

Preparations and Immobilization of DNA on Nitrocellulose Filters—An overnight culture in t-broth of strain SB 7514, a heat-inducible double lysogen, was diluted to about 1 × 10^8 cells/ml and grown at 30° in a shaking water bath to 5 × 10^9 cells/ml. Phage production was induced by transferring the culture to 42° for 10 min. The culture was then incubated at 34° for 3 hours with vigorous shaking to allow phage production. The phage S mutation prevented lysis during this period. Cells were then harvested by centrifugation, resuspended in TMB buffer sufficient to concentrate them 20-fold, and lysed to release phages by adding 0.10 volume of chloroform and bubbling air through the suspension for 15 min. After clarification by two low speed centrifugations, portions of up to 5 ml of the phage preparation were brought to a density of 1.47 g/ml with CsCl and centrifuged for 24 hours at 23,000 rpm in a Beckman L3-50 ultracentrifuge in a type 50 rotor. Six-drop aliquots were collected from the bottom of the centrifuge tube into 0.5 ml of TMB buffer. Measurements of absorption at 260 nm showed that the density gradient formed separated the two phage types. Fractions containing the lighter λ80dara phage were pooled and dialyzed overnight against lysing buffer to remove CsCl. Phage DNA was extracted by incubating for 1 hour at 45° with 0.33 volume of phenol saturated with lysing buffer. Phenol was removed by dialysis overnight against SSC, and the DNA was banded on a CsCl density gradient (average density, 1.70 g/ml) by centrifuging for 48 hours at 35,000 rpm in a type 50 rotor. Ten-drop fractions were collected into 1 ml of SSC, and the DNA-containing fractions were dialyzed overnight against SSC.

To aid in detection and quantification during purification and use of the DNA, a small portion of the culture was labeled with 32P after phage induction, and the radioactive phages produced were mixed with the unlabeled phage.

Phage λ80 was prepared in a similar manner from the single lysogen J1212.

Purified DNA at a concentration of 1 µg per ml was melted to single strands by heating for 10 min at 95° in one tenth strength SSC and rapidly cooled by dilution into 5 volumes of ice-cold 4 × SSC. The DNA was passed through a Schleicher and Schuell type 16 27 mm filter by gravity filtration, and the filter was washed with 10 volumes of ice-cold 4 × SSC and then immersed in a solution of 0.02% bovine serum albumin, 0.02% Ficoll (approximate molecular weight, 400,000; Sigma Chemical Co.), 0.62% polyvinylpyrrolidone (average molecular weight, 360,000; Sigma Chemical Co.) in 4 × SSC for 45 min. After air-drying for 30 min, the filter was cut with a cork borer into pieces containing 1 µg of DNA and dried in air for 1 hour and at 60° overnight (13).

Preparation of Pulse-labeled RNA—Bacteria were cultured for at least two generations with forced aeration in 1% Casamino Acids medium. If the arabinose operon was to be induced during the labeling period, 0.4% arabinose was added 20 min prior to addition of the isotope. A portion of the culture was mixed with [3H]uridine (0.2 µg per ml final concentration; specific activities are designated for individual experiments) and after exactly 1.0 min it was poured over an equal volume of frozen, crushed culture medium containing 0.02% NaN₃. Capacity studies used to estimate mRNA levels for the arabinose operon in intact cells indicate a half-life of about 2 min for ara mRNA (12). Therefore, only a small fraction of the total ara mRNA synthesized should be lost before the end of the labeling period.

Crude lysates for RNA extraction were prepared as described previously and followed by DNase digestion (14). RNA was partially purified by repeated extractions with phenol saturated with lysing buffer. The aqueous phase was dialyzed overnight against annealing buffer. Acid precipitable radioactivity in the final RNA preparation was determined by scintillation counting (14).

DNA-RNA Hybridization—A filter containing single-stranded DNA was added to portions of [3H]RNA diluted to 1.0 ml in annealing buffer. Following incubation in tightly sealed tubes for 16 to 22 hours at 65°, the filter was washed with three 1-ml portions of annealing buffer. The filter was then incubated for 1 hour at 25° in 1 ml of 2 × SSC containing 20 µg of RNAse to digest RNA not tightly bound to DNA. The filter was then washed five times with annealing buffer, dried, and assayed for radioactivity by scintillation counting (13).

Hybridization of ara mRNA was calculated by subtracting the percentage of acid-precipitable counts of the RNA preparation which bound to λ80 DNA from the percentage which bound to λ80dara DNA.

Enzyme Levels—The specific activity of L-arabinose isomerase was determined as previously described (12). A reaction mixture contained, in 1.0 ml: glycylglycine (pH 7.0), 125 µmoles; L-arabinose, 150 µmoles; MnCl₂, 2.5 µmoles; cell-free extract; and water. The reaction was carried out at 30°. One unit of enzyme specific activity is 1 µ mole of L-ribulose formed per hour per mg of protein.

When L-arabinose isomerase and ara mRNA were measured
in the same induced cultures, the RNA was labeled 20 min after the addition of arabinose, but the cells for the enzyme assay were not taken until after 3 hours of induction.

Arabinose-constitutive Mutants—Spontaneous araC(C) mutants, constitutive for expression of the arabinose operon, were selected from the ara + strain UP1002 on mineral L-arabinose D-fucose L-leucine agar (1). Initiator constitutive mutants, ara(C) mutants, were isolated by the method of Englesberg et al. (4). A 4-mm diameter piece of filter paper saturated with the mutagen diethylsulphate was placed onto a mineral arabinose agar plate spread with about 10⁶ cells of the mutant araC766, which is able to grow on L-arabinose. After 1 week at 37°C, many small colonies had grown, especially in a zone near the mutagen. Mutants from colonies most distant from the diethylsulfate were purified on mineral arabinose agar and saved. All of the mutants isolated grew slowly on mineral arabinose agar and produced low constitutive levels of the arabinose isomerase. They appeared to be identical with ara(C) mutants isolated by Englesberg et al. (4).

All of the constitutive mutant strains analyzed for ara mRNA were also characterized by a series of genetic crosses with known deletion mutants. The presumptive evidence from these F'arab-mediated crosses suggests that the new mutants designated ara(C) bear lesions giving rise to D-fucose resistance in a region of the genome designated as the ara(C) gene (1). The parental strain used to generate the presumptive ara(C) mutants gives rise only to constitutive mutations in the initiator region of the operon. Crosses with these new mutants against two deletion mutants suggest, but do not prove, that the initiator region was mutated in each strain.

RESULTS

Transcription in araC-negative Strains—The levels of both ara mRNA and L-arabinose isomerase in a wild type strain of E. coli increase when arabinose is added to the culture medium (Table II). This induction of ara mRNA is consistent with the data of Wilcox et al. (7). Although no enzyme production is detected in the uninduced culture, some ara mRNA is measured by the hybridization assay in the same culture. However, strain araΔ804, in which most if not all of the arabinose operon is deleted (1) and which should therefore have little or no mRNA for the arabinose operon, also shows a similar low level of hybridization (Table II). Therefore, hybridization in the range of 0.01 to 0.03% probably comes from a source other than the arabinose operon. Possibly, bacterial genes from E. coli, in addition to the arabinose genes, have been incorporated into the arabinose transducing phage used. We therefore consider only levels of hybridization above 0.03% to indicate actual transcription of the arabinose operon.

The nonsense mutant, araC5, and two deletion mutants, araCΔ766 and araOCΔ710, produce very low levels of ara mRNA in the presence of inducer (Table II). Since these levels do not differ significantly from the wild type grown in the absence of arabinose, transcription of the arabinose operon does not seem to occur in these mutants.

Transcription in Repressed Partial Diploids—Mutations of the araC(C) type lead to constitutive production of the enzymes of the arabinose pathway (1). Therefore, ara mRNA must be produced in the absence of inducer in these mutants. The strain araA araC767(C) has a high constitutive level of ara mRNA due to the araC(C) mutation (Table III). Since this strain has a mutation in the L-arabinose isomerase structural gene, araA, corresponding constitutive production of this enzyme cannot be shown, but the strain does produce a high constitutive level of L-ribulokinase (2).

Upon insertion of an araC+ allele by construction of the partial diploid strain F'araB24/araA araC araC767(C), constitutive expression of the operon is repressed (2; Table III). Likewise, ara mRNA synthesis is repressed (Table III), and ara mRNA production takes place only in the presence of inducer. Therefore, the protein coded by the araC+ gene acts as a repressor of transcription of the arabinose operon.

The arabinose operon is also expressed constitutively as a result of another type of mutation. These mutations are designated ara(C) and affect the arabinose initiator region. Provided that repressor is absent, the arabinose-metabolizing enzymes are produced constitutively at low levels (3, 4).

We isolated a number of araC araC(C) mutants as described under "Materials and Methods." Two of the mutants tested had low constitutive levels of both ara mRNA and L-arabinose isomerase (Table IV). These low levels were significantly lowered by repressor produced from the araC+ gene in the partial
enzyme production is again indicative that our assay measures measured in these strains and compared, there was good correla-
tants having a range of constitutive enzyme levels were isolated finding lends further support to the idea that the uraC protein as described under “Materials and Methods.”

The nonsense mutation, araC5, and the deletion mutations, araCΔ766 and araOCΔ719, provide information about the role of activator in transcription of the arabinose operon. These mutants can produce none of the enzymes of the arabinose operon, even when arabinose is present, since they lack activator function. Because these strains at most would produce only partial araC proteins, they are unlikely to have any repressor activity. The absence of repressor function in araC5 and araOCΔ719 has been demonstrated by showing that they are recessive to araC(C) mutations (2). These araC-negative mutants are unable to produce ara mRNA. Therefore, the activator function which is not present in these strains must be necessary for transcription of the arabinose operon.

The effect of the repressor activity on transcription was studied in partial diploid strains in which an araC+ episome produces repressor activity in bacteria with chromosomal constitutive mutations. Since araC(C) mutants produce the enzymes from the arabinose operon in the absence of inducer, they have acti-
vator activity but not repressor activity in the absence of arabinose (1). When an araC" gene is introduced into an araC(C) strain by making it partially diploid with F'ara, the repressor is formed from the araC" gene, and enzyme synthesis is no longer constitutive but is inducible by arabinose. Presumably, activator is still present due to the araC(C) gene in these diploids, so regulation of the arabinose operon must be entirely due to the repression derepression mechanism. In the araC"/araC(C) diploid, the ara mRNA is also inducible (Table III), indicating that the araC repressor controls the transcription process.

Another possible interpretation of the dominance of araC" to araC(C), apart from blocking of activator function by the repressor, is that there may be actually very little activator function present. Hybrid oligomeric molecules of the araC protein which act like the wild type protein may be formed in the diploid. The subsequent lack of transcription may be due to absence of activator, not presence of repressor. This difficulty in interpreting the results of the preceding experiment is circumvented in the analysis of the araC(C) mutations. In strains in which the araC gene is entirely inactive due to deletion, araC(C) mutations can be selected which allow expression of the arabinose structural genes located cis to these mutations without the necessity for activator, provided that repressor is also absent (3, 4). The araC(C) mutations allow moderate levels of transcription to take place, but repressor produced from the episomal araC" gene in F'araB24/araC(C) araCΔ796 partial diploids decreases both transcription of the arabinose operon and production of L-arabinose isomerase (Table IV). Repressor must also act at the transcription step.

A consequence of the hypothesis that the araC gene regulates production of the arabinose metabolizing enzymes via regulation of transcription of the arabinose operon is that the rate of ara mRNA synthesis should limit enzyme production. Therefore, enzyme levels should be proportional to the amount of ara mRNA produced. Mutations of the araC regulatory gene which result in limited enzyme production should do so by limiting the rate of ara mRNA production. This prediction was tested in araC(TS) mutants induced at intermediate temperatures and in araC(C) mutants which produce submaximal constitutive levels of the arabinose enzymes. In these mutants, the araC protein must be modified because the araC gene is mutated, but enough activator function remains to allow at least some expression of the operon. In all strains tested, ara mRNA was correlated with L-arabinose isomerase levels. Difficulties in measuring low levels of binding accurately made it impossible to demonstrate an exact linear relationship in which the expression of the operon was at a low level, but no high level of ara mRNA was found in any strain with low enzyme activity. We, therefore, can find no evidence that the araC protein acts at the translational level for regulation of arabinose operon expression. In all cases studied, production of the enzymes of the arabinose operon seems limited only by the amount of ara mRNA which is produced. These findings lend strong support to the hypothesis that control of the arabinose operon occurs exclusively at the transcriptional step.

REFERENCES

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