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# Overproducing *araC* Protein with Lambda-Arabinose Transducing Phage

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**Summary.** *Escherichia coli* infected with bacteriophage lambda-arabinose transducing phage were tested as sources of *araC* protein. Infection of cells with such phage produces an intracellular concentration of *araC* protein up to 100 times that present in wild-type *E. coli*, apparently resulting from fusion of the *araC* gene to bacteriophage lambda promoters. Lysates from these phage-infected cells may be fractionated to yield another 100-fold enrichment in *araC* activity so that the total enrichment is 10,000-fold. A nonsense mutation in *araC* provided proof of the identification on gel electrophoresis of a band in the purified material. Biologically active *araC* protein is a dimer with 28,000 M.W. subunits.

The *araC* gene in these phage replaces the *int-xis* genes but is oriented in the opposite direction. Nonetheless, it appears to be transcribed in this position by the phage promoter $p_r$ via transcription the long way around. Furthermore, because *araC* gene is in this position, we were able to isolate phage on which the *araC* gene was under phage late gene control by deletion of the late gene transcription stop signals in the $b_2$ region.

## 1. Introduction

Some studies of the regulation of the L-arabinose operon of *Escherichia coli* require the operon specific positive regulator, *araC* protein in a pure state. The low intracellular concentration of this protein in wild-type *E. coli*, coupled with its instability have greatly hindered attainment of pure biologically active *araC* protein. Therefore, to facilitate this purification we have searched for sources more concentrated in *araC* protein.

One approach to this problem was suggested by the work of Miller et al. (1970) who found that cells infected with $\phi 80$ transducing phage containing the gene coding for the lactose repressor (*lacI*) contain 20 times the lactose repressor concentration as do cells containing the same gene on the bacterial chromosome. In this case overproduction was due to multiple copies of the *lacI* gene present in the cell through replication of the $\phi 80$ DNA.

When we attempted an analogous experiment with a lambda-arabinose transducing phage, we observed a 10-fold overproduction of *araC* protein. Further experiments suggested that this increase was due to fusion of the *araC* gene to a phage promoter rather than the production of multiple copies of the *araC* gene. The transducing phage structure permitted the subsequent isolation of a lambda arabinose transducing phage which directed a 100-fold increase in the intracellular concentration of *araC* protein.

In this work we show that the lambda-arabinose transducing phage are a suitable source from which to purify *araC* protein and that use of these phage infected cells increases both the yield and the specific activity of *araC* protein resulting from our purification.

We also present evidence indicating that on these transducing phage the early phage genes between *att* and *N* on the phage genome are transcribed in both directions, by phage promoters $p_1$ and $p_r$, such that a gene inserted in this region may be correctly transcribed regardless of its orientation.

## 2. Material and Methods

All inorganic salts were Fischer certified ACS or the purest grade supplied by Fisher. Other materials were obtained as follows: dex-

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**Table 1.** *Escherichia coli* and phage λ strains

| Strain | Relevant genotype | Source and comments |
|---|---|---|
| RFS1385 | *ara 498 ton 1385* | Derived from RFS726 (Lis and Schleif, 1973). A spontaneous mutant resistant to virulent Φ80 |
| RFS1491 | *tob araB1491 ton 1491* | Derived from KY4683 (Inoko and Imai, 1974). A spontaneous *araB* mutant was selected on minimal arabinose glycerol ribitol plates (Schleif, 1972). A mutant of the resulting strain resistant to Φ80 was then selected |
| JTL129 | *araB117 araC⁺ ton 129* | From John Lis. It is identical to JTL157 (Lis and Schleif, 1975a) with two exceptions: a) The strains contain different mutations in the arabinose operon, and b) JTL129 is sensitive to Φ80 |
| λ*paraC116* | *araC⁺ $C_1$857 S7* | Described by Lis and Schleif (1975a) |
| λ*paraC136* λ*paraC138* λ*paraC142* | *araC⁺ bst 136 $C_1$857* S7 | Described by Lis and Schleif (1975c) |
| λ*paraC163* | *araC240 $C_1$857 S7* | Described in Steffen (1977). An *araC⁻* nonsense mutant |

Genetic Abbreviations: Genes indicated as$^+$, for example *ara*$^+$, are wild-type. Genes indicated without this superscript, for example *tob*, are mutant. *ara*, *araB*, and *araC*, genes for arabinose utilization. *ton*, resistance to the phage Φ80 and T1. *tob*, see text and Inoko and Imai (1974). $C_1$, *S*, phage genes coding for phage repressor and a protein needed for cell lysis. *bst*, the stop signals in the $b_2$ region of phage lambda that stop the lambda late transcript. λp, nondefective (plaque forming) phage.

tran T500, Pharmacia Fine Chemicals; PEG 6000 and No. 20 dialysis tubing, Union Carbide; acrylamide, tetramethylenediamine and ammonium persulfate (all electrophoresis grade), Biorad Laboratories; ethylene diacrylate, Borden Chemical Company; mercaptoethanol, Eastman Chemical Company; antifoam Y-30 emulsion, Dow Corning; bovine chymotrypsinogen, Worthington Biochemicals. Fresh human hemoglobin was donated by Susan Moore. The remaining material were obtained from Sigma Chemical Company.

Culture media are described by Schleif (1969). Growth and harvesting of cells and phage is described by Lis and Schleif (1973). Growth of large quantities of cells in carboys is described by Lis and Schleif (1975). Bacterial and phage strains are described in Table 1.

### *Measurement of Cell Density*

Cell density was estimated by measuring the apparent absorbance of the cell suspension at 550 nm with a Zeiss PMQII spectrophotometer. Cell suspension were diluted to an apparent absorbance of less than 1.0 before measurement. For RFS1385 grown at 35° C in 1.33× concentrated YT medium, an apparent absorbance of 1.0 corresponds to approximately $2 \times 10^8$ cells/ml.

### *Production of Extracts for Assay of araC Protein*

*a) Growth of Cells.* Cells were grown at 37° C in 600 ml of rapidly shaking 1.33× concentrated YT medium to an apparent absorbance of 1.5. For growth of phage, the medium also contained 0.01 M $MgSO_4$ and 0.2% maltose (w/v). Cells were then either harvested or infected with $9 \times 10^{11}$ plaque forming units of phage (a multiplicity of infection of five). Cells were harvested by centrifugation at 4000 × g for ten minutes.

*b) Preparation of Crude Extracts.* All steps were carried out at 4° C. Two grams of cells were ground with two grams of alumina in a mortar and pestle for five to ten minutes, until a uniform sticky suspension resulted. This suspension was mixed with 2 ml of a solution of 50 mM Tris acetate pH 7.8, 300 mM potassium acetate, 10 mM magnesium acetate, 10 mM L-arabinose, 1 mM EDTA, 1 mM DTT, 100 μg/ml PMSF (predissolved at 10 mg/ml in ethanol before use), 10% glycerol (v/v). The buffer was added one milliliter at a time. To the resulting mixture was added 1.5 g of a suspension composed of 7.1% Dextran T-500 (w/w) and 28.4% PEG 6000 (w/w) in 4 M NaCl, 0.93 g additional NaCl, 0.16 ml $H_2O$ and 0.04 ml of 20% L-arabinose. The mixture was stirred for fifteen to thirty minutes and centrifuged at 16,000 × g for thirty minutes. The supernatant was dialyzed twice for one hour each time against fifty volumes of a solution of 10 mM Tris acetate pH 7.8, 60 mM potassium acetate, 14 mM magnesium acetate, 10 mM L-arabinose, 1 mM EDTA and 1 mM DTT.

### *Acrylamide Gel Electrophoresis*

Electrophoresis of protein solutions in 10% acrylamide gels containing sodium dodecyl sulfate (SDS) was performed by a minor modification (Steffen, 1977) of the method of Weber and Osborn (1969).

### *Determination of Protein Concentration*

The modification of the method of Lowry (1951) described by Albertsson (1971) was altered slightly to determine protein concentration (Steffen, 1977).

### *Assay for araC Protein*

*AraC* protein was assayed by its ability to stimulate in vitro protein synthesis of L-ribulokinase (an enzyme of the arabinose operon). In vitro protein synthesis was performed according to the method

**Table 2.** Purification of *araC* protein from RFS1385 infected with λ*paraC138*

| Purification step | Total protein (mg) | Total activity (units)[a] | Specific activity (units/mg) | Purification factor (fold) | Recovery of activity (%) |
|---|---|---|---|---|---|
| Crude extract | 10,000 | $1 \times 10^6$ | 100 | 1 | 100 |
| PEG-phosphate upper phase | 4,200 | $4.3 \times 10^5$ | 100 | 1 | 47 |
| Phosphocellulose eluate | 18 | $2.9 \times 10^5$ | 16,000 | 150 | 28 |
| DEAE Sephadex eluate | 4 | $4.2 \times 10^4$ | 11,000 | 100 | 4 |

[a] Units are defined such that a crude extract of cells possessing a wild-type *araC* gene and promoter (JTL129) contain 1 unit of *araC* activity per mg protein

of Zubay et al. (1970) as modified by Lis and Schleif (1973) with small changes described by Steffen (1977).

*Physical Purification of araC Protein*

All operations were carried out at 0° C to 5° C. Phosphocellulose was precycled for the minimum time recommended by the manufacturer. Each precycled batch of phosphocellulose behaves differently with regard to *araC* protein chromatography; consequently pilot runs were performed with each batch to determine the minimum concentration of phosphate required to elute *araC* protein. The value described below applies to one batch used for much of the purification we have done.

200 g of freshly grown cells were mixed with 200 grams of levigated alumina in a mortar and ground with a pestle until a uniform, extremely sticky texture was achieved. 200 mls of 50 mM Tris acetate, pH 7.8, 300 mM K acetate, 10 mM Mg acetate, 10 mM L-arabinose, 1 mM EDTA, 1 mM DTT, 100 μg/ml phenyl methyl sulfonylflouride, 10% (v/v) glycerol was added in fifty milliliter aliquots. The mixture of cells and buffer was mixed to homogeneity after each addition. To the resulting mixture, 148 g of a suspension composed of 71 g Dextran T-500, 284 g PEG 6000 and 645 g 4 M NaCl was then added along with 92.8 g NaCl, 16.4 ml distilled water and 3 ml 2 M L-arabinose. This mixture was stirred for thirty minutes and centrifuged at $16{,}000 \times g$ for thirty minutes. The supernatant of this centrifugation is referred to as the crude extract.

The crude extract was dialyzed on a rocking dialyzer for two one hour periods against 10 mM Tris acetate pH 7.8, 10 mM L-arabinose, 1 mM EDTA, 1 mM DTT, (three times the volume of extract). Next, 21 g of $K_2HPO_4$ (anhydrous) per 100 mls of dialyzed extract was added slowly with stirring. During addition, the pH was maintained at pH $8.0 \pm 0.1$ by the addition of concentrated $H_3PO_4$. After all the phosphate had dissolved, the extract was stirred an additional twenty minutes and centrifuged $10{,}000 \times g$ for twenty minutes. Centrifugation resulted in two liquid phases with solid material both at the interface and in a pellet. The total volume was estimated and the lower, phosphate rich phase was removed by aspiration. 100 mM $KPO_4$ pH 7.8, 10 mM L-arabinose, 1 mM EDTA, 1 mM DTT, 10% (v/v) glycerol (PC buffer) was added to the upper phase and solid material to produce a volume two-thirds of that present after centrifugation. The resulting suspension was mixed gently for five minutes and centrifuged at $800 \times g$ for ten minutes. The supernatant was diluted with PC buffer modified by reduction of the $KPO_4$ concentration to 10 mM to yield a conductivity equal to that of PC buffer. The diluted supernatant is referred to as the PEG-phosphate upper phase.

The PEG-phosphate upper phase was applied to a Whatmann phosphocellulose P-11 column ($5 \times 5$ cm) equilibrated with PC buffer. The column was washed with 700 mls of PC buffer and eluted with 250 mls buffer, PC buffer modified by increasing the $KPO_4$ concentration to 160 mM. The flow rate during loading, washing and eluting of the column was about 300 to 500 mls per hour. Fractions containing *araC* activity were pooled and are referred to as the phosphocellulose eluate.

The phosphocellulose eluate (10–40 mls) was passed through a Sephadex G-25 (medium) column ($2 \times 40$ cm) equilibrated with DEAE buffer (10 mM Tris acetate pH 9, 50 mM K acetate, 10 mM L-arabinose, 1 mM EDTA, 1 mM DTT, 10% (v/v) glycerol). Protein eluted from the column was applied to a DEAE Sephadex A-50 column ($1.2 \times 5$ cm) equilibrated with DEAE buffer. The column was washed with 25 mls of DEAE buffer modified to contain 100 mM K acetate and eluted with 50 mls of DEAE buffer modified to contain 160 mM K acetate. Loading, washing, and eluting of the column was done at a flow rate of approximately one milliliter per minute. The fractions containing *araC* activity were pooled and are referred to as the DEAE eluate.

The DEAE eluate was dialyzed for three hours on a rocking dialyzer against 250 mls of 10 mM Tris acetate pH 7.8, 60 mM K acetate, 15 mM Mg acetate, 1 mM EDTA, 1 mM DTT, 50% (v/v) glycerol, frozen by immersion in a solid $CO_2$-acetone bath and stored at −75° C. No activity was lost in freezing and thawing the sample. We have stored samples at −75° C for eighteen months with no loss in activity.

The results of a typical purification are summarized in Table 2.

*Analysis of araC Protein*

The purity of *araC* protein was determined by electrophoresing the purified protein in acrylamide gels containing SDS, staining the gels with commassie blue, measuring the absorbance at 580 nm of the gel with a gel scanner and comparing the area of the absorbance peak identified as being due to *araC* protein relative to the total absorbance of the gel.

Analytical chromatography of *araC* protein on Sephadex G-100 (fine) was done in a $1.2 \times 90$ cm column developed with 10 mM Tris acetate pH 7.8, 60 mM K acetate, 15 mM Mg acetate, 1 mM EDTA, 1 mM DTT. The mobility of BSA on the same column was determined in a separate run.

## Results

λ*paraC116* is a transducing phage in which some early DNA adjacent to the λ-att site has been replaced by *E. coli* DNA containing the *araC* gene (Fig. 1). When RFS1385 (a stain of *E. coli* lacking the arabinose operon) is infected with this phage, extracts are found to contain 10 times as much *araC* activity

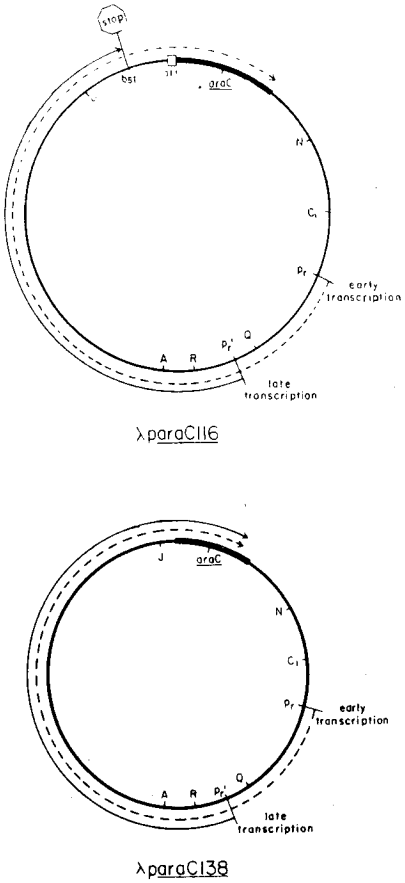


**Fig. 1.** A schematic map of the genome of λ*paraC116* and λ*para138*. ——— DNA derived from bacteriophage lambda; ——— DNA derived from *E. coli*; - - - - → Lambda early transcription; ——→ Lambda late transcription. The following genetic abbreviations are used: *N*, $C_I$, *Q*, *R*, *A* and *J* phage lambda genes; $p_r$, the phage rightward early gene promoter; $p'_r$, the phage late gene promoter; *bst*, the phage $b_2$ region stop signals; *att*, the prophage attachment site. λ*paraC136* and λ*paraC142* have similar maps to λ*paraC138*

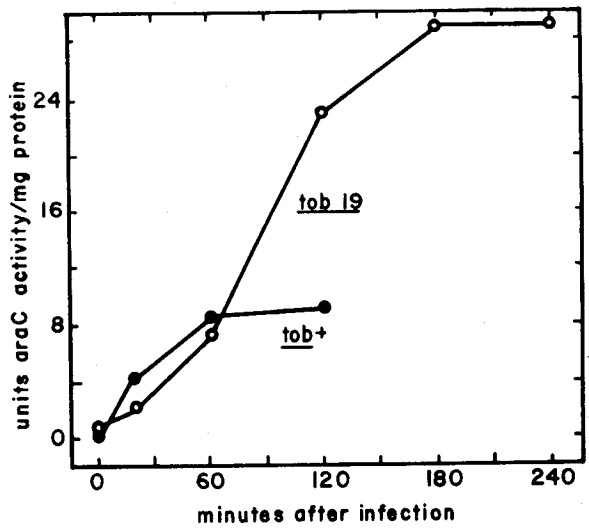


**Fig. 2.** Kinetics of appearance of *araC* activity. RFS1385 (—●—) or RFS1491 (—○—) were infected with λ*paraC116*, cells were harvested at the indicated times and crude extracts were prepared and assayed as described in Materials and Methods. The results of one experiment are presented. In several experiments we never observed a significant increase in *araC* activity in λ*paraC116* infected RFS1385 between 60 and 120 minutes after infection. These results are not influenced by in vivo degradation of *araC* protein. Addition of chloramphenicol to infected cells and incubation for 60 minutes results in loss of less than 1/3 of the *araC* activity present at the time of addition of the drug

as to extracts from cells (JTL129) containing a wild-type chromosomal *araC* gene. To decide if this increase is due to an increase in the number of *araC* genes produced by phage replication or rather due to fusion of the *araC* gene to a phage promoter, we examined the kinetics of appearance of *araC* activity in these ells. *AraC* activity ceases to increase within 60 minutes after infection (Fig. 2). The similarity between these kinetics and the kinetics of lambda early gene product synthesis (Pero, 1970) suggest that the observed increase in *araC* activity in λ*paraC116* infected cells might be due to fusion of the *araC* gene to a phage early promoter.

To test this possibility and to provide a more concentrated source of *araC* protein, we utilized a bacterial mutation, *tob*, which alters the kinetics of lambda early gene expression (Inoko and Imai, 1974). Lambda early protein synthesis fails to turn off in *tob*$^-$ bacteria, but rather continues throughout infection. We found that infection of a *tob*$^-$ stain of *E. coli* (RFS1491) with the arabinose transducing phage λ*paraC116* yielded kinetics of appearance of *araC* activity altered precisely as expected (Fig. 2). Like lambda early proteins, *araC* activity continues to increase for up to three hours after infection. This result implies that the *araC* gene on λ*paraC116* is transcribed by a lambda early promoter and thus provides a better source of *araC* protein than infection of wild-type cells with the *araC* phage. Hence extracts may be prepared containing 30 times as much *araC* protein as an uninfected wild-type cell.

Examination of the structure of λ*paraC116* and the direction of transcription of the *araC* gene (Fig. 1) leads to a startling conclusion. Transcription of *araC* on this phage likely occurs by RNA polymerase which initiates at promoter $p_r$ and which transcribes almost all the way around the genome and finally transcribes the lambda early gene region in the antisense direction: At least in this transducing phage, such genes appear to be transcribed in both directions.

Phage lambda also has a second promoter oriented such that RNA polymerase could transcribe the *araC* gene of λ*paraC116* in the sense (for *araC*) direction. This is the late gene promoter $p_r$. However, the kinetics of *araC* synthesis indicate that transcription from this promoter does not reach the *araC* gene.

Most likely this failure results from the late gene transcription stop signals in the $b_2$ region after the late genes and before the *araC* gene (Krell et al., 1972). We, therefore, tested three lambda arabinose transducing phage (*λparaC136*, *λparaC138*, and *λparaC142*) from which these stop signals had been deleted (Fig. 1) for their ability to direct *araC* protein synthesis. We found that cells (RFS1385) infected with these phage to be the most concentrated source of *araC* protein so far discussed. These infected cells contain 100 times more *araC* protein than an uninfected *E. coli*, 10 times more than *λparaC116* infected RFS1385 and 3 times more than *λparaC116* in tob$^-$ RFS1491.

To us the most straight forward explanation of these facts is that the $b_2$ stop signals allow the passage of RNA polymerases initiating at $p_r$ but do not allow the passage of polymerases initiating at $p_r'$. Transcription initiated at $p_r$ is blinded by the phage protein N to transcriptional stop signals (Franklin, 1974; Adhya et al., 1974). Deleting the stop signals allows transcription initiated at $p_r'$ to proceed through *araC*. Other effects could produce the results we observed. For example, the effect of the *tob* mutation in prolonging *araC* protein synthesis could derive from an alteration of the ionic conditions in the cell. Also, the effect of the deletions which removed the stop signals in the $b_2$ region could be indirect. Instead the stimulation could derive from removal of the phage *att* site and any interference this may have on RNA polymerase initiation at the promoter for *araC* protein. Other possibilities also exist and the point is not definitely resolved. For our purposes the mere overproduction was adequate.

### *Use of Phage-Infected E. coli as a Source of araC Protein*

Using the procedure described in Materials and Methods, *araC* protein was purified from three different phage infected cell systems; *λparaC116* infected RFS1385, *λparaC116* infected RFS1491 and *λparaC138* infected RFS1385. In each case extracts were prepared three hours after infection. Uninfected *E. coli* contains so little *araC* protein that we felt attempting purification from this source would be unproductive. We found that all three phage infected cell systems were satisfactory sources for purification. *AraC* protein could be purified 100-fold from all of them and the purified protein was stable for more than a year at −70° C when stored in 50% (v/v) glycerol. The same physical purification produced a 100-fold increase in the specific activity of all systems.

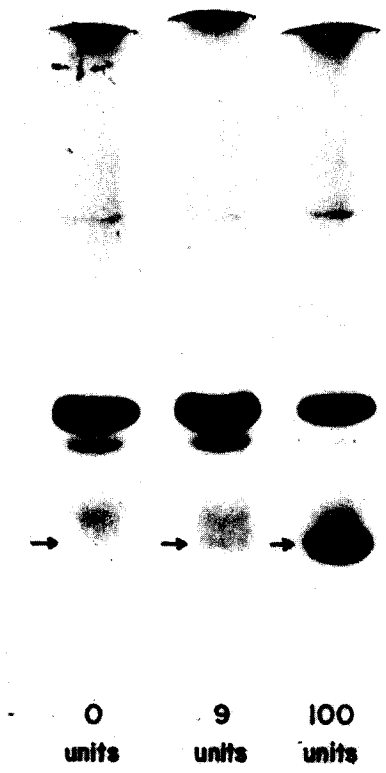


**Fig. 3.** Identification of *araC* protein on SDS polyacrylamide gels. Extracts of RFS1385 infected with (a) *λaraC163*, producing zero units of *araC* activity per milligram protein, (b) *λparaC116*, producing nine units of *araC* activity per milligram protein, or (c) *λparaC138*, producing one hundred units of *araC* activity per milligram protein, were purified as described in Materials and Methods and electrophoresed in SDS acrylamide gels. The gels were stained with Coomassi Blue and photographed on Kodak high contrast copy film. The direction of electrophoresis is from top to bottom

### *Use of Lambda-Arabinose Transducing Phage to Physically Identify araC Protein*

Three *λparaC* phage were used to identify a protein band separated by SDS acrylamide gel electrophoresis as *araC* protein. *λparaC163*, a phage containing a nonsense mutation in its *araC* gene, leads to cell extracts containing no detectable *araC* activity. *λparaC116*, a phage on which *araC* gene transcription has to cross the $b_2$ stop signals, leads to cell extracts containing 10 units of *araC* activity per mg protein. *λparaC138*, a phage on which the $b_2$ stops signals are deleted, leads to cell extracts containing 100 units of *araC* activity per mg protein. When extracts of cells (RFS1385) infected with each of these phage is purified as described in Materials and Methods, and when these purified extracts are electrophoresed in SDS acrylamide gels, the patterns of Figure 3 result. One protein band clearly is absent from the *λparaC163* extract, present in the *λparaC116* extract and present in increased quantities in the *λparaC138*

extract. This band we identify as *araC* protein. Identification of the *araC* protein allows us to determine that the *araC* protein purified from λ*paraC138* infected RFS1385 (the 100-fold overproducer) is about 20% pure.

Using the subunits of *E. coli* RNA polymerase, BSA, LDH and bovine chymnotrypsinogen as standards, *araC* protein migrates in these gels like a protein with molecular mass of 30,000. Since *araC* activity co-chromatographs with a mobility similar to BSA on a Sephadex G-100 column, we can additionally conclude that the biologically active form of *araC* protein is a dimer.

## Discussion

### *a) Absence of a Gene Dosage in the Synthesis Rate of araC Protein*

Infection of cells with the phage λ*paraC116* leads to synthesis of *araC* protein. At about 30 min after the infection, further synthesis of *araC* activity ceases. At this time there are about 50 copies of unpackaged lambda DNA in the cell (Canter and Smith, 1970) and if each were functional, we would expect a substantial synthesis of *araC* protein to continue. One explanation for these kinetics is the following: The *araC* gene has been functionally fused to the early lambda promoter $p_r$ and *araC* protein is able to repress its own synthesis, most likely by repressing transcription from its own promoter. Evidence for the repression effect has been provided by a special gene fusion constructed by Casadaban (1976) in which the *araC* promoter was fused to the β-galactosidase gene. When active *araC* protein was introduced into such cells, the synthesis of β-galactosidase was repressed by a factor of ten. Evidence that the *araC* gene in phage λ*paraC116* has been fused to the lambda early gene promoter $p_r$ comes first from the observed kinetics of *araC* synthesis-they parallel the kinetics of early gene activity, and second from the effect of a mutation affecting early gene regulation. In bacteria containing this mutation (*tob*) the lambda early genes do not shut off 30 minutes after infection as is normal. The synthesis of *araC* protein from λ*paraC116* also does not shut off. The structure of the phage λ*paraC116* (Lis and Schleif, 1975a) and the orientation of the *araC* gene (Wilcox et al., 1974) are compatible with this explanation. Other explanations for the hypersynthesis are consistent with the findings, and one is presented in the Results section.

### *b) The Effect of bst on araC Transcription*

The promoter $p_r$ is not the only promoter on lambda properly oriented to transcribe the *araC* gene on phage λ*paraC116*; the late gene promoter $p'_r$ is also suitably oriented. However, transcription from this promoter should not reach the *araC* gene, for in the $b_2$ region is a set of stop signals (which we call *bst*) that block this transcription (Krell, et al. 1972). We confirmed the existence of these stop signals by deleting the $b_2$ region and showing that synthesis of *araC* protein increased as would be expected if it were now also being transcribed under control of the $p'_r$ promoter. This effect was found for several different deletions, λ*paraC136*, λ*paraC138* and λ*paraC142*. As in the case of the overproduction of *araC* in the *tob* mutant, other explanations than stop signals may be the actual reason for overproduction of *araC* in these deletions.

### *c) Identification of araC Protein in Acrylamide Gels*

*AraC* protein was identified as a band on sodium dodecyl sulfate gel electrophoresis by its absence when the infecting phage possessed a nonsense mutation in the *araC* gene, its presence when the infecting phage was the "normal" λ*paraC116*, and its greatly increased presence when the infecting phage was deleted of the $b_2$ stop, bst, and therefore should hypersynthesize *araC* protein. This identification might be thought to suffer from the possibility that the band on the gels derives not from the *araC* gene, but instead from a gene transcriptionally downstream from the *araC* gene. In this position, its synthesis might be thought to parallel the synthesis we actually observed. However, the absence of synthesis of the band when the *araC* gene contained a nonsense mutation excludes this possibility. Ordinarily a nonsense mutation would introduce polarity and depress the synthesis of gene products downstream in the operon. However, the N-directed RNA polymerase ignores such polarity introducing signals and a downstream gene will be expressed whether or not a nonsense mutation exists upstream.

A second cause for a misidentification cannot be excluded, but is highly artificial. Suppose the protein identified as *araC* protein is in fact some protein under *araC* control. Being a positive regulator, elevated levels of *araC* protein could lead to hypersynthesis of the hypothetical protein. This line of reasoning is reduced in strength by the following observations. The protein cannot be any of the enzymes known in arabinose metabolism (*araB*, *araA*, *araD*) as the

genes coding for these enzymes are absent from both the phage and cells. Secondly, this protein would need to be synthesized at a rate proportional to *araC* concentration up to a rate 100 times normal. Thirdly, *araC* protein is known to act as a positive regulatory regulator only in the presence of the inducer L-arabinose. Growth of phage infected cells was performed in the absence of arabinose.

### *d) Suitability of λparaC Phage as a Source of araC Protein*

We have shown that cells infected with lambda-arabinose transducing phage are a suitable source for purification of biologically active *araC* protein. The phage infected cell extracts contain neither any activities which interfere with the purification nor does alteration of the control properties of the phage alter the spectrum of contaminating proteins so as to reduce the power of the physical purification. In fact, the physical purification and genetic enrichment described here are complementary. Their individual effects on specific activity of *araC* protein are multiplicative, leading to an overall 10,000-fold increase in *araC* protein specific activity.

Furthermore, when *araC* protein is purified from these phage infected cells, the resulting purified protein is sufficiently free of contaminating activities for at least some rather critical applications. For example, such *araC* protein has been used in studies by electron microscopy of protein-DNA complexes on the arabinose operon (Hirsh and Schleif, 1976) and studies on the quality and size of RNA transcripts made in vitro of the arabinose operon (Hirsh and Schleif, submitted).

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