

## L-Arabinose Transport Systems in *Escherichia coli* K-12†

DAVID KOLODRUBETZ‡ AND ROBERT SCHLEIF\*

Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254

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Mutations in the arabinose transport operons of *Escherichia coli* K-12 were isolated with the Mu *lac* phage by screening for cells in which  $\beta$ -galactosidase is induced in the presence of L-arabinose. Standard genetic techniques were then used to isolate numerous mutations in either of the two transport systems. Complementation tests revealed only one gene, *araE*, in the low-affinity arabinose uptake system. P1 transduction placed *araE* between *lysA* (60.9 min) and *thyA* (60.5 min) and closer to *lysA*. The operon of the high-affinity transport system was found to contain two genes: *araF*, which codes for the arabinose-binding protein, and a new gene, *araG*. The newly identified gene, *araG*, was shown by two-dimensional gel electrophoresis to encode a protein which is located in the membrane. Only defects in *araG* could abolish uptake by the high-affinity system under the conditions we used.

Novotnoy and Englesberg (15) first reported the existence of a low-affinity arabinose transport system in *Escherichia coli* B/r. Mutations in a gene they designated *araE* abolished the uptake of arabinose by this system. Subsequently, it was shown that strain K-12 contains two kinetically distinguishable systems for the transport of L-arabinose (20), both of which are inducible by *araC* protein. Brown and Hogg (4) then reexamined strain B/r and found that it also contains two inducible arabinose uptake systems. Apparently, the B/r strain first used by Novotnoy and Englesberg (15) lacked high-affinity transport. Brown and Hogg also determined that high-affinity transport is damaged by mutations in a gene they designated *araF*. This gene codes for the periplasmically located arabinose-binding protein (4). Both *araF* and *araE* map at loci distinct from each other and from the *araBAD* operon (15, 18).

It was the purpose of the work reported here to isolate and accurately map *araE* and *araF* mutations in *E. coli* K-12 and to isolate and map any additional genes required for arabinose transport. The existence of the two parallel arabinose uptake systems thwarts usual genetic attempts at isolation of mutations defective in either of the transport systems. We therefore turned to the Mu *lac* phage isolated by Casadaban and Cohen (6). The  $\beta$ -galactosidase gene of this phage is expressed only when the phage has inserted in the correct orientation in an

actively transcribing gene. This provided a means of isolating insertion mutations in genes regulated by the presence of arabinose. By using these insertion mutations, additional arabinose transport mutants were isolated and characterized.

### MATERIALS AND METHODS

**Strains, media, and chemicals.** Yeast-tryptone broth and yeast-tryptone plates, minimal salts medium for plates, and M9 minimal medium have been described previously (20). M10 is M9 plus  $5 \times 10^{-5}$  M  $\text{MnCl}_2$  (9). Sugars were added to 0.2% (except where noted otherwise), thiamine to 0.001%, thymine to 0.02%, amino acids to 0.005%, antibiotics to 20  $\mu\text{g}/\text{ml}$ , streptomycin to 200  $\mu\text{g}/\text{ml}$ , and 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactoside (X-gal) to 40  $\mu\text{g}/\text{ml}$ . All minimal media were supplemented with thiamine.

L-[ $^{14}\text{C}$ ]arabinose and [ $^{35}\text{S}$ ]H $_2$ SO $_4$  were from New England Nuclear Corp. or Amersham Corp.

Acrylamide, *N,N'*-methylene-bis-acrylamide, *N,N,N',N'*-tetramethylethylenediamine, and ammonium persulfate were obtained from Bio-Rad Laboratories. Nonidet P-40 was obtained from Shell Chemicals. Sodium lauryl sulfate was obtained from Gallard Schlesinger. Ultrapure urea was from Research-Plus or Schwarz/Mann. Ampholines were from LKB Instruments. All other chemicals were from Sigma Chemical Co. or Fisher Scientific Co.

All strains are derivatives of *E. coli* K-12 and are listed in Table 1.

**Enzyme assays.** Arabinose isomerase, arabinose-binding protein, and arabinose uptake by whole cells were assayed as described elsewhere (20; D. Kolodrubetz, Ph.D. thesis, Brandeis University, Waltham, Mass.).

**General genetic techniques. (i). Matings and transductions.** Some matings were done as described by Miller (14). Tray matings involving more than 10

† Publication no. 1381 from the Department of Biochemistry, Brandeis University, Waltham, Mass.

‡ Present address: Molecular Biology Institute, University of California, Los Angeles, CA 90024.

strains were done as described by Schleif (21). P1 transductions were done as described by Miller (14).

(ii) **Mutageneses.** Nitrosoguanidine mutagenesis was carried out as described by Vielmetter et al. (23). Mutagenesis by 2-aminopurine was done as described by Miller (14).

Mu *lac* lysates were made and used for infections as described by Casadaban and Cohen (6).

(iii) **Other methods.** Thy<sup>-</sup> mutants were isolated on plates by the trimethoprim method described by Miller (14).

Threonine, leucine, and histidine auxotrophs were selected by the cycloserine enrichment technique (14).

Strains were cured of Mu *lac* by the heat treatment technique described by Bukhari (5).

Strains were P1 transduced to RecA<sup>-</sup> by a lysate made on strain NK 5304 (11). Tet<sup>r</sup> colonies were selected and then scored for UV sensitivity (14).

(iv) **Isolating transport mutants by screening**

**for arabinose-dependent expression of the  $\beta$ -galactosidase of Mu *lac*.** DJK50 was infected with Mu *lac*, plated onto minimal glycerol-arabinose-X-gal-ampicillin plates, and incubated at 30°C. After 2 days of incubation, 1,000 blue colonies ( $\beta$ -gal<sup>+</sup>) were spot tested onto minimal glycerol-X-gal-ampicillin and minimal glycerol-arabinose-X-gal-ampicillin plates, incubated at 30°C, and scored for color after 48 h.

(v) **Isolation of transport mutants by selection for arabinose resistance of 10<sup>-4</sup> M arabinose.** Nitrosoguanidine, 2-aminopurine, Mu *lac*, and spontaneous mutagenesis were used to isolate arabinose-resistant mutants of *araD araE* or *araD araFG* strains. The mutagenized cells were plated onto minimal glycerol-10<sup>-4</sup> M arabinose (plus ampicillin for those cells mutagenized with Mu *lac*) and incubated at 35°C (32°C for Mu *lac* mutagenesis). After 2 days, colonies were picked and scored by complementation for having acquired *araA*, *araB*, or *araC* mutations.

TABLE 1. *E. coli* K-12 strains used<sup>a</sup>

| Strain                 | Genotype   | Comments or source (reference)   |
|------------------------|--|--|
| DJK1                   | F <sup>+</sup> <i>araC</i> <sup>+</sup> <i>B</i> <sup>+</sup> <i>A</i> <sup>+</sup> <i>D</i> <sup>+</sup> <i>leu</i> <sup>+</sup> <i>thr</i> <sup>+</sup> /Δ( <i>ara-leu</i> )498 <i>thi</i> -1 Δ <i>lac</i> -74   | RFS 740 (21)   |
| DJK4                   | F <sup>+</sup> <i>araC</i> <sup>+</sup> <i>4B</i> <sup>+</sup> <i>A</i> <sup>+</sup> <i>D</i> <sup>+</sup> <i>leu</i> <sup>+</sup> <i>thr</i> <sup>+</sup> /Δ( <i>ara-leu</i> )498 <i>thi</i> -1 Δ <i>lac</i> -74  | Spontaneous fucose-resistant isolate of DJK1   |
| DJK50                  | F <sup>+</sup> <i>araC</i> <sup>+</sup> Δ( <i>araBAD</i> )181/Δ( <i>ara-leu</i> )498 <i>thi</i> -1 Δ <i>lac</i> -74 <i>nal</i> -3  | RFS F <sup>+</sup> 181 (21) × nalidixic acid-resistant mutant of DJK1 (13)   |
| DJK51, DJK52           | F <sup>+</sup> <i>araC</i> <sup>+</sup> Δ( <i>araBAD</i> )181/Δ( <i>ara-leu</i> )498 <i>thi</i> -1 Δ <i>lac</i> -74 <i>nal</i> -3 <i>araG</i> ::Mu <i>lac</i> ( <i>lac Amp</i> <sup>r</sup> )  | This work  |
| DJK63, DJK70           | F <sup>+</sup> <i>araC</i> <sup>+</sup> 1336 <i>B</i> <sup>+</sup> <i>A</i> <sup>+</sup> <i>D</i> 591 <i>leu</i> <sup>+</sup> <i>thr</i> <sup>+</sup> /Δ( <i>ara-leu</i> )498 <i>thi</i> -1 Δ <i>lac</i> -74 <i>nal</i> -3 <i>araFG</i> 63,70                | Replaced episome in DJK51, DJK52 with episome from RFS 1336; then cured strains of Mu <i>lac</i> in <i>araFG</i> by heat treatment   |
| DJK75, DJK77           | F <sup>+</sup> <i>araC</i> <sup>+</sup> 1336 <i>B</i> <sup>+</sup> <i>A</i> <sup>+</sup> <i>D</i> 591 <i>leu</i> <sup>+</sup> <i>thr</i> /Δ( <i>ara-leu</i> )498 <i>thi</i> -1 Δ <i>lac</i> -74 <i>nal</i> -3 <i>araFG</i> 63,70 <i>araE</i> ::Mu <i>lac</i> | Resistant to 10 <sup>-4</sup> M arabinose by Mu <i>lac</i> insertion   |
| DJK322, DJK326         | F <sup>-</sup> : <i>araC</i> <sup>+</sup> 1336 <i>araD</i> 591 <i>thi</i> -1 Δ <i>lac</i> -74 <i>nal</i> -3 <i>araE</i> 78,81 <i>his</i> -182 <i>thr</i> -314  | From DJK75, DJK77; Ara regulatory region from DJK63 by P1 transduction, <i>araFG</i> region from DJK1 by P1 transduction, Mu <i>lac</i> in <i>araE</i> removed by heat curing, His <sup>-</sup> by Mu <i>lac</i> insertion and heat curing, Thr <sup>-</sup> by nitrosoguanidine |
| DJK323, DJK325         | F <sup>-</sup> : <i>araC</i> <sup>+</sup> 1336 <i>araD</i> 591 <i>thi</i> -1 Δ <i>lac</i> -74 <i>nal</i> -3 <i>ara</i> (FG)60 <i>thy</i> -176 <i>thr</i> -317  | From DJK75, DJK77; analogous to DJK322, DJK326 but <i>araE</i> region from DJK1 and Thy <sup>-</sup> from Mu <i>lac</i> insertion and heat curing  |
| DJK331, DJK337         | F <sup>-</sup> : <i>araC</i> <sup>+</sup> 1336 <i>araD</i> 591 <i>thi</i> -1 Δ <i>lac</i> -74 <i>nal</i> -3 <i>araE</i> 78,81 <i>his</i> -182 <i>thr</i> -314 <i>recA</i> 56 <i>srl</i> -1300::Tn10  | DJK322, DJK326 P1 transduced to Tet <sup>r</sup> Rec <sup>-</sup> with a lysate made on NK 5304  |
| DJK333, DJK335, DJK339 | F <sup>-</sup> : <i>araC</i> <sup>+</sup> 1336 <i>araD</i> 591 <i>thi</i> -1 Δ <i>lac</i> -74 <i>nal</i> -3 <i>srl</i> -1300::Tn10 <i>ara</i> (FG)60 <i>thy</i> -176 <i>thr</i> -317 <i>recA</i> 56  | DJK323, DJK325, DJK328 P1 transduced to Tet <sup>r</sup> Rec <sup>-</sup> with a lysate made on NK 5304  |
| DJK345                 | F <sup>+</sup> <i>araC</i> <sup>+</sup> 1336 <i>araD</i> 591 <i>leu</i> <sup>+</sup> /leu-6 <i>thr</i> /lysA22 <i>ser</i> A25 <i>rps</i> L133 mT12 <i>xyl</i> -7 <i>malA</i> 1 <i>gal</i> -6 <i>lacY</i> 1 <i>thi</i> -1                                     | Leu <sup>+</sup> Str <sup>r</sup> isolate of DJK63 × CGSC 5708   |
| NK 5304                | Hfr <i>srl</i> -1300::Tn10(Tet <sup>r</sup> ) <i>recA</i> 56 <i>ilv</i> -318 <i>thr</i> -300 <i>thi</i> -1 <i>spc</i> -300 <i>rel</i> -1   | From Paula Grasafe   |
| DJK371, DJK419         | F <sup>-</sup> : <i>araC</i> <sup>+</sup> 1336 <i>araD</i> 591 <i>thi</i> -1 Δ <i>lac</i> -74 <i>nal</i> -3 <i>ara</i> (FG)60 <i>araE</i> 371, <i>araE</i> 419 <i>thy</i> -176 <i>thr</i> -317   | Arabinose-resistant isolates of DJK323   |

<sup>a</sup> *nal*, Nalidixic acid resistant.

Strains whose arabinose resistance derived from mutation elsewhere were called arabinose transport mutants.

(vi) **Complementation testing.** To obtain episomes carrying the wild-type arabinose transport genes, several *E. coli* Genetic Stock Center (CGSC) strains were tested for their ability to mate with and complement arabinose transport mutants. Since several of the CGSC strains behaved like Hfr rather than F' strains, we enriched for episome-carrying strains by cycling the transferable markers through suitable *recA* strains. We obtained satisfactory episomes from CGSC4281, which carries *thyA*<sup>+</sup> and *araE*<sup>+</sup>, and CGSC5789, which carries *ara(FG)*<sup>+</sup> and *his*<sup>+</sup>.

Episomal transport mutations were isolated as chromosomal mutations and then recombined onto the appropriate episome. A functional transport system on the episome conferred arabinose sensitivity upon the strains used (*araD araFG* or *araD araE*). Recombinants arose spontaneously at a high frequency as arabinose-resistant colonies.

To test for complementation, an episome containing one transport mutation was introduced into *araD recA* cells containing another transport mutation. Merodiploids were selected on minimal glucose-threonine-tetracycline plates and then tested for complementation on minimal 10<sup>-4</sup> M arabinose-glycerol plates. Complementation yielded arabinose-sensitive exconjugants, and failure to complement was indicated by arabinose-resistant exconjugants.

(vii) **Isolation of additional arabinose-binding protein mutants.** Strains DJK331 and DJK337 were plated onto minimal glycerol-10<sup>-4</sup> M arabinose-threonine-histidine after either spontaneous or 2-aminopurine mutagenesis and incubated at 35°C. Colonies resistant to 10<sup>-4</sup> M arabinose were purified and retested for arabinose resistance. The 180 mutants resistant to 10<sup>-4</sup> M arabinose were then assayed for constitutive arabinose isomerase and binding protein. The 18 strains found which still had constitutive isomerase but which lacked arabinose-binding protein were used for complementation testing.

**Sample preparation and gel electrophoresis.** For experiments with <sup>35</sup>S-labeled proteins, cells were grown at 35°C in 15 ml of M10 medium with the appropriate supplements, except the medium contained 10<sup>-4</sup> M MgSO<sub>4</sub> and 10<sup>-3</sup> M MgCl<sub>2</sub>. Between 200 and 500 μCi of [<sup>35</sup>S]H<sub>2</sub>SO<sub>4</sub> was added to the cells at least six generations before harvesting. The entire volume of cells was harvested in late exponential growth (5 × 10<sup>8</sup> cells per ml), and protein samples were prepared as described by Ames and Nikaido (2), except the cells were disrupted by sonication. All samples were treated with RNase and DNase as described by O'Farrell (16), and solid urea was added to 9.5 M to all samples before dilution with sample dilution buffer. The cytoplasmic fraction consisted of the combined supernatants from the first three spins (41,000 × g). First- and second-dimension isoelectric focusing gels were poured and run as described by O'Farrell (16). Gels for non-equilibrium pH gradient electrophoresis were as described by O'Farrell et al. (17).

Gels were dried onto a piece of 3MM paper (Whatman, Inc.), using a Bio-Rad gel slab dryer. Gels with

<sup>35</sup>S-labeled proteins were dried, exposed to Kodak XR5 X-ray film, and then developed by standard procedures.

## RESULTS

**Initial isolation of arabinose transport mutants.** The *Mu lac* phage (6) provided a means of isolating arabinose transport mutants by screening lysogens of *Mu lac* for those which expressed β-galactosidase only in the presence of arabinose. Previous experiments have shown that the high-affinity arabinose transport system is not well induced in cells growing on arabinose (4, 20). However, in cells unable to metabolize arabinose, both the high- and low-affinity transport systems are induced over 100-fold by arabinose (D. Kolodrubetz and R. Schleif, submitted for publication). Thus, a strain deleted of the *araB*, *araA*, and *araD* genes was used, which also eliminates a background of *Mu lac* insertions in *araA*, *araB*, or *araD*.

About 1,230 *Mu lac* transductants of strain DJK50 were screened for arabinose-dependent β-galactosidase expression on X-gal plates. Eighteen strains displayed some degree of arabinose-dependent β-galactosidase expression, but only two strains showed reduced transport of arabinose, as compared with their parental strain. These two strains, DJK51 and DJK52, have *K<sub>m</sub>* values for arabinose transport of 6.5 × 10<sup>-5</sup> M (Fig. 1) and are therefore missing the high-affinity transport system. Both strains contained normal levels of arabinose-binding protein.

**Isolation of initial transport mutants in the second uptake system.** The high-affinity transport-negative strains were then used to isolate mutants in the low-affinity arabinose transport system, using the arabinose sensitivity of *araD* strains (8). To eliminate artifacts introduced by nonspecific arabinose uptake, these selections were performed at the lowest arabinose concentration at which the parental strains are still sensitive, 10<sup>-4</sup> M arabinose. An *araD* episome was introduced in place of the former episome of DJK51 and DJK52, and the strains were heat cured of *Mu lac* (5). The resulting strains, DJK63 and DJK70, lacking *araF* and *araG* activities, were then mutagenized by infection with *Mu lac*, and colonies resistant to 10<sup>-4</sup> M arabinose were selected. Of 21 candidates tested, the majority were either *araB* or *araC*. Of the 21 candidates, 2 were still constitutive for arabinose isomerase, contained active C, B, and A proteins, and yet showed less than 5% of the arabinose transport ability of the parent strains. These strains, DJK75 and DJK77, appear to be almost totally defective in arabinose transport, concentrating arabinose no more than 10-fold in

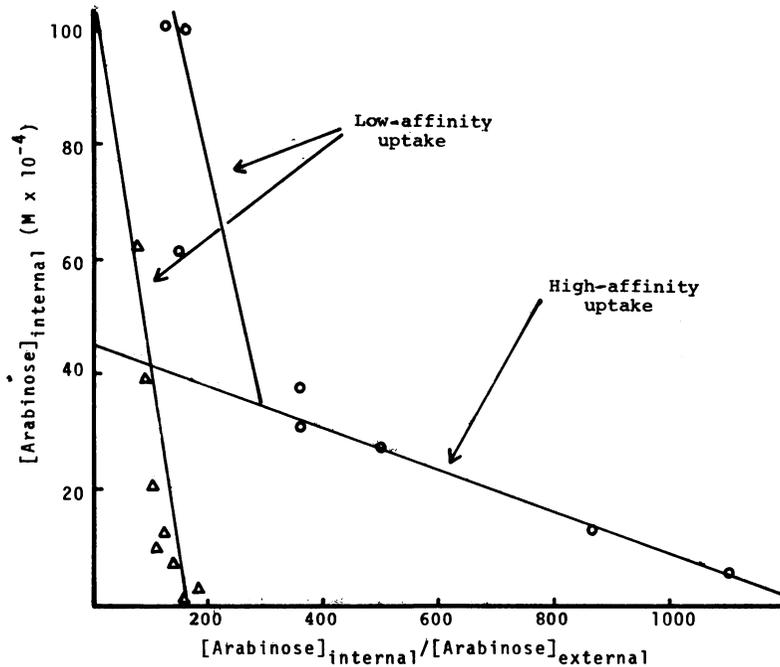


FIG. 1. Arabinose uptake by wild-type and mutant strains. Strains DJK50 (○) and DJK 51 (▽) were assayed for arabinose transport at various concentrations (from  $5 \times 10^{-7}$  to  $8 \times 10^{-5}$  M) of arabinose. The cells had been grown overnight in M10-glycerol-arabinose and were washed just before assaying in M10-glycerol. After being washed free of arabinose, the cells were grown for 1 h in M10-glycerol and then assayed.

the range of concentrations tested,  $5 \times 10^{-7}$  to  $8 \times 10^{-5}$  M. This small amount of residual transport may be due to low-level transport of arabinose via the methyl-galactoside permease (22) or may be an artifact of the assay system, such as nonspecific binding of arabinose to cells.

**Complementation testing of arabinose transport mutants.** From strains DJK75 and DJK77 we constructed strains DJK322, DJK323, DJK325, and DJK326, which are *araD araE* or *araD ara(FG)*. In addition, isogenic *recA* strains (DJK331, DJK333, DJK335, DJK337, and DJK339) were constructed. The sensitivity these strains display to the presence of  $10^{-4}$  M arabinose in growth medium permitted the convenient isolation of large numbers of mutants defective in either *araC*, *araB*, or *araA* or in the arabinose uptake systems. The uptake mutants were identified by their continued arabinose resistance after the introduction of an episome containing  $AraC^c AraB^+A^+D^-$ . The transport mutations were then crossed onto episomes for complementation testing.

In a cross of 25 independently isolated, episomal low-affinity transport mutants with 38 independent chromosomal low-affinity uptake mutants, none of the mating pairs complemented. All of the mutagens used to isolate

transport mutants were represented in this set of mutants. As a control, an episome containing the gene(s) for wild-type low-affinity uptake was shown to complement all 63 of these mutants. These results are best interpreted to mean that there is only one protein involved in low-affinity arabinose transport.

Complementation tests of high-affinity, transport-negative strains were done by crossing 23 high-affinity episomal mutants with a set of 62 independently isolated chromosomal high-affinity mutants. Rather unexpectedly, none of the mating pairs complemented each other, although an episome which carries the wild-type high-affinity uptake genes complements all of the mutations. This result is surprising, since both chromosomal and episomal mutants fall into two groups based on the presence or absence of arabinose-binding protein. Of the 56 mutants not mutagenized by *Mu lac*, 8 were binding protein negative.

To show that the small sampling of binding protein mutants is not merely an unusual subset of *araF* mutants, 16 more binding protein-minus mutants were found in 180 strains resistant to  $10^{-4}$  M arabinose. Fourteen of these originated from 90 arabinose-resistant mutants generated by spontaneous mutagenesis, and two were

found in 90 arabinose-resistant mutants isolated after 2-aminopurine mutagenesis. None of the 16 newly isolated, binding protein-negative strains complemented any of the chromosomal high-affinity transport mutants.

One possible explanation for these results is that the arabinose-binding protein has two domains, one involved in the binding of arabinose and the other involved in the transport of the sugar. A mutation in the former activity leads to the loss of both activities, but a mutation in the transport domain does not necessarily destroy the binding activity. This type of mutation is ruled out by our finding that a membrane protein, different from arabinose-binding protein, is absent from high-affinity, transport-minus, binding protein-plus mutants (see Materials and Methods).

In accordance with the nomenclature used with strain B/r (4), the gene for arabinose-binding protein is designated *araF*. We believe that the transport-negative, binding protein-plus strains lacking a membrane protein warrants christening a new gene, which we are naming *araG*. The gene order of *araF* and *araG* could be determined, since *Mu lac* causes polarity (7). Only 20% of the *Mu lac* insertions which inactivate high-affinity transport also destroy the binding activity of arabinose-binding protein. Thus, the arabinose-binding protein cannot be the second gene, so the gene order must be *araF-araG*. These complementation studies also establish that *araF* and *araG* are in the same operon, since *Mu lac* insertions in *araF* are *cis*-dominant in their inactivation of *araG*. From the gene order and the failure to find any complementation between *araF* and *araG* mutants, we concluded that, under our screening conditions, *araF* is not essential for uptake. Hence, for cells to be transport negative, they must either be *araG* or possess a polar *araF* which prevents expression of *araG*.

**Mapping the arabinose transport genes.** Based on the unpublished data cited by Hogg and Englesberg (10) for strain B/r, the *araE* gene maps near the *thyA* locus. To determine where *araE* maps in strain K-12, two *araE thyA* strains were transduced to *Thy*<sup>+</sup> with a P1 lysate made on an *Ara*<sup>+</sup> *Thy*<sup>+</sup> *Lys*<sup>-</sup> strain, DJK345. Table 2 shows the results which indicate the frequencies of occurrence of all of the possible types of recombinants. Examination of these data shows that the distance from *thy* to *araE* is about the same as the distance from *thy* to *lys*. Sketching the possible gene orders and the required crossovers and noting the frequency of occurrence of particular recombinants lead to the conclusion that the gene order is *thy-ara-*

TABLE 2. Number of *Thy*<sup>+</sup> transductants with P1 (*Ara*<sup>+</sup> *Thy*<sup>+</sup> *Lys*<sup>-</sup>)<sup>a</sup>

| Strain transduced  | No. of transductants                                |   |   |   |
|--|---|---|---|---|
|  | <i>AraE</i> <sup>-</sup><br><i>Lys</i> <sup>+</sup> | <i>AraE</i> <sup>+</sup><br><i>Lys</i> <sup>-</sup> | <i>AraE</i> <sup>+</sup><br><i>Lys</i> <sup>+</sup> | <i>AraE</i> <sup>-</sup><br><i>Lys</i> <sup>-</sup> |
| DJK371 ( <i>Thy</i> <sup>-</sup><br><i>AraE</i> <sup>-</sup> <i>Lys</i> <sup>+</sup> ) | 291   | 370   | 10  | 3   |
| DJK419 ( <i>Thy</i> <sup>-</sup><br><i>AraE</i> <sup>-</sup> <i>Lys</i> <sup>+</sup> ) | 258   | 437   | 25  | 7   |

<sup>a</sup> Cotransduction of *araE* and adjacent markers. Phage P1 was grown on *Ara*<sup>+</sup> *Thy*<sup>+</sup> *Lys*<sup>-</sup> strain DJK345 and used to transduce strain DJK371 or DJK419 to *Thy*<sup>+</sup>. These *Thy*<sup>+</sup> transductants were spot tested for lysine auxotrophy and the ability to grow on 10<sup>-3</sup> M arabinose as a sole carbon source.

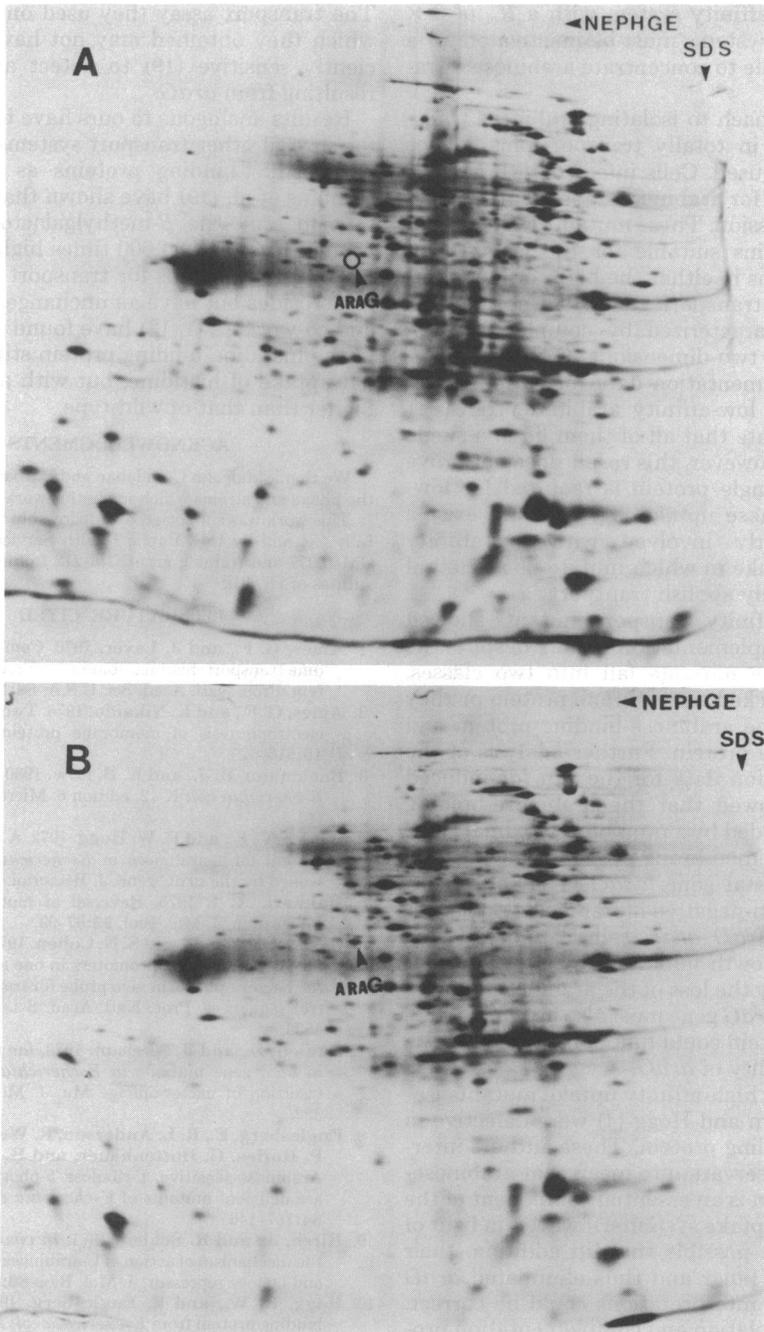
*lys*, with *ara* substantially closer to *lysA* (60.9 min) than to *thyA* (60.5 min) (3).

Since the episome from CGSC5789 contains the wild-type, high-affinity uptake genes, we can localize *araFG* to within several minutes of the *his* region (44 min). This is in agreement with the unpublished data cited in Parsons and Hogg (18) which show that *araF* maps near the *mgl* locus at 45 min in *E. coli* B/r.

**Identification of a membrane protein on two-dimensional gels as an arabinose transport protein, *araG*.** O'Farrell two-dimensional gel electrophoresis was performed on cytoplasmic extracts from *AraC*<sup>c</sup> or uninduced wild-type cells and on cells grown in the presence or absence of arabinose. By comparison with the electrophoretic mobility of the purified proteins, arabinose isomerase, ribulokinase, epimerase, and arabinose-binding protein were identified (data not shown). We observed no other C protein or arabinose-inducible cytoplasmic proteins. Therefore, the membrane proteins from an *AraC*<sup>c</sup> strain were compared with those from an uninduced wild-type strain (Fig. 2). One membrane protein was found to be reproducibly induced in an *araC*<sup>c</sup> strain. The protein has a molecular weight of about 37,000 and a pI of >7.0. Only this protein spot is missing from analogous gels prepared from mutants defective only in the high-affinity transport gene, *araG*. The spot is not altered in *araE* mutants. We concluded that *araG* codes for this membrane-bound protein.

## DISCUSSION

The isolation of arabinose transport-negative mutants in *E. coli* K-12 is difficult since the cells possess two independent arabinose transport systems (4, 20). There is the low-affinity system with a *K<sub>m</sub>* for arabinose transport of 5 × 10<sup>-5</sup> M



**FIG. 2. Membrane proteins inducible by C protein. (A), Membrane proteins from DJK1 (wild type); (B), membrane proteins from DJK4 (*araC*<sup>-</sup>). The location of the membrane protein reproducibly induced in the *araC*<sup>-</sup> cells, called *araG*, is indicated by the arrow. The cells were labeled with <sup>35</sup>S while growing in M10-glycerol and harvested as described in the text. The membrane proteins were prepared for gels as described (3) and then run on non-equilibrium pH gradient electrophoresis (NEPHGE) gels for 2,000 V·h. After equilibration, the proteins in the non-equilibrium pH gradient electrophoresis gels were electrophoresed on a 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel, and the gel was autoradiographed.**

and the high-affinity system with a  $K_m$  of  $3 \times 10^{-6}$  M. Both systems must be inactivated for a cell to be unable to concentrate arabinose intracellularly.

A new approach to isolating arabinose transport mutants in totally transport-plus strains was therefore used. Cells infected with Mu *lac* were screened for arabinose-dependent  $\beta$ -galactosidase expression. These mutants were used to construct strains suitable for the isolation of more mutations in either the high- or low-affinity arabinose transport genes. These mutants were then characterized by complementation testing and on two-dimensional O'Farrell gels.

The complementation data for 63 independently isolated low-affinity arabinose transport mutants indicate that all of them lie in a single gene, *araE*. However, this result does not prove that only a single protein is required for low-affinity arabinose uptake. There could exist a protein directly involved with low-affinity arabinose uptake in which mutations are lethal or do not totally abolish transport.

The high-affinity transport mutants showed only one complementation group despite the fact that these mutants fall into two classes. Either they lacked a membrane protein or they lacked both the arabinose-binding protein and the membrane protein. Further analysis of the complementation data for the Mu *lac*-induced mutations showed that the arabinose-binding protein is encoded by a promoter-proximal gene, *araF*, and the membrane protein is encoded by a promoter-distal gene, *araG*. That all of our binding protein-negative mutants were polar indicates that *araD araE* cells cannot become resistant to growth inhibition by  $10^{-4}$  M arabinose merely by the loss of the arabinose-binding protein. The *araG* gene must also be inactivated. The *araF* protein could function to increase the uptake capability of *araG*.

Three of six high-affinity uptake mutants isolated by Brown and Hogg (4) were defective in arabinose-binding protein. These authors interpreted this observation to mean that arabinose-binding protein is an essential component of the high-affinity uptake system. However, in light of our data, it is possible that, in addition, their mutants were polar and thus eliminated *araG* activity. Both interpretations could be correct. Our mutant isolation and complementation procedures utilized the arabinose sensitivity of *araD* mutants. Since such cells are not metabolizing arabinose, the uptake rate need not be high for cell growth to be blocked. However, for the isolation of their mutants, Brown and Hogg merely required sufficient attenuation of uptake to substantially inhibit metabolism of arabinose.

The transport assay they used on the mutants which they obtained may not have been sufficiently sensitive (19) to detect any transport resulting from *araG*.

Results analogous to ours have been reported for several other transport systems which have periplasmic binding proteins as components. Robbins et al. (19) have shown that strains mutant in only the  $\beta$ -methylgalactoside-binding protein have a  $K_m$  1,000 times higher than that for wild-type strains for transport of  $\beta$ -methylgalactosides but have an unchanged  $V_{max}$ . Ames and co-workers (1, 12) have found that mutants in the histidine-binding protein still have residual uptake of histidine, but with a  $K_m$  100-fold higher than that of wild-type.

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