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Location of a Structural Gene for Xylose-H$^+$ Symport at 91 Min on the Linkage Map of *Escherichia coli* K12*

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Mutations in the xylose-H$^+$ transport activity of *Escherichia coli* K12 were isolated using Mud(Ap^R*lac*). The initial selection was for simultaneous acquisition of ampicillin and xylose resistance in an *fda* background. Colonies were then screened for xylose-inducible β-galactosidase and for growth on xylose of their *fda*$^+$ derivatives. Two of the xylose-positive derivatives were shown to be impaired in xylose-H$^+$ symport in whole cells and in xylose transport into subcellular vesicles. Their xylose transport in whole cells showed increased sensitivity to arsenate. The site of prophage insertion was mapped to 91.4 min on the *E. coli* genome between *pgi* and *malB*. It is proposed that the gene for the xylose-H$^+$ symport system be called *xylE*.

The transport of xylose into *Escherichia coli* was first described by David and Wiesmeyer (1). A subsequent study of xylose transport, although mainly in *Salmonella typhimurium*, deduced from nonlinear Lineweaver-Burk plots that more than one xylose transport system occurred in *E. coli* (2). One of these was found to be proton-linked, energized by respiration, insensitive to arsenate, and retained in subcellular vesicles (3). These characteristics distinguished it from a second general type of transport system that contained a periplasmic binding protein, was energized by a phosphorylated compound, was sensitive to arsenate, and was absent from subcellular vesicles (see *e.g.* Refs. 4–6). Recently, Ahlem *et al.* (7) characterized a xylose-binding protein in *E. coli*, although the transport process it presumably facilitated was not studied. Overall, the observations of Shamanna and Sanderson (2), Lam *et al.* (3), Ahlem *et al.* (7), and the present paper are consistent with the operation of (at least) two transport systems for xylose in *E. coli*, one proton-linked and the other containing a binding protein.

The structural (*xylA xylB*) and regulatory (*xylR*) genes for catabolism of xylose were located at min 78–79 on the linkage map of *E. coli* or *S. typhimurium* (8, 9). In *S. typhimurium*, a gene, *xylT*, coding for xylose transport activity was very close to these genes, but perhaps functioned as a separate operon (10). However, we now show that an *E. coli* gene coding for the xylose-H$^+$ transport system maps at 91.4 min, between *pgi* and *malB*. This locus is designated *xylE*, for reasons discussed below.

The work to be described is in three parts: first, the isolation of Mud(Ap^R*lac*) insertions into a *xyl* gene other than *xylA*, *xylB*, or *xylR*; second, the demonstration that two of the *xyl*::Mud(Ap^R*lac*) mutants had lost xylose-H$^+$ symport activity and therefore, by definition, the gene *xylE*; and third, mapping of the ampicillin-resistance determinant inserted in the *xylE* gene of the mutants.

EXPERIMENTAL PROCEDURES

Organisms—The *E. coli* strains used are listed in Table I. Inocula were prepared by aerobic growth in 5–15 ml of nutrient broth (13 g/liter) for 6–9 h. Cells (2–10 ml) were transferred to salts medium (11) supplemented with 80 μg/ml of histidine and 20 mM glycerol or 10 mM xylose or both as required. Culture volumes were 200 ml in 250-ml flasks (transport and β-galactosidase assays), 400 ml in 500-ml flasks (sugar-H$^+$ measurements), or 1200 ml in 2000-ml flasks (vesicle preparations). Incubation was at 30 °C in a Gallenkamp orbital incubator operating at 200–220 rpm. Cultures of absorbance at A_{680} = 0.7–1.6 were harvested by centrifugation and depleted of energy reserves as described elsewhere (3, 11) except that 150 mM KCl, 5 mM MES[1] was used for washing and resuspension of 200-ml cultures.

Mutagenesis—Infection of strain JM2087 by Mud(Ap^R*lac*)I or Mud(Ap^R*lac*)II was carried out as described by Casadaban and Cohen (12). Infected cultures were diluted 10-fold with nutrient broth, divided into 10 samples, and incubated at 30 °C overnight to allow phenotypic expression. Samples of 0.2 ml from each culture were plated onto selection medium (salts plus histidine, glycerol, xylose, and ampicillin). Colonies growing on this medium were then patched out on the same medium and screened on various media (see text). Single colonies of appropriate strains were isolated.

Assay for β-Galactosidase—Plates were inverted over chloroform (about 2 ml) for 10 min, and then a 2.5-ml soft agar overlay containing 4 mM *O*-nitrophenyl β-D-galactoside was poured evenly over the colonies; those containing β-galactosidase activity turned yellow after a few minutes at room temperature.

Cells grown in liquid culture, washed, and resuspended in ice-cold 150 mM KCl, 5 mM MES, pH 6.5 (A_{680} = 20), were disrupted by sonication (about six bursts for 30 s with 30 s intervals, 1-cm diameter probe, MSE sonicator) until the A_{680} was 2–8% of the initial value. Replicate samples (50 and 100 μl) were assayed for β-galactosidase as described below. In some experiments, the harvested cells were resuspended in either KCl/MES or Z buffer (13), sonicated, and sedimented at 30,000 × *g* for 10 min. The supernatant "cytoplasmic fraction" was kept, while the pellet, "membrane fraction", was resuspended in 40–50 ml of Z buffer and resedimented four times. The final pellet was resuspended to an A_{680} of approximately 1. Replicate samples (25–100 μl) were assayed (below).

β-Galactosidase assays were measured by the method of Miller (13), after dilution of samples to 1 ml with Z buffer. Activity units were calculated by Miller's formula, except for assays of subcellular vesicles when substitution of 2.12 for 1.75 provided better correction for absorption by the biological material.

Preparation of Subcellular Vesicles—Spheroplasts of the appropriate strain were made by the method of Witholt *et al.* (14). Subcellular vesicles were then prepared as described by Kaback (15) and stored at −80 °C.

Measurement of Energized Sugar Transport—Transport of 50 μM ^{14}C-labeled xylose, lactose, or maltose (Amersham International) into intact cells of *E. coli* was measured as described by Lam *et al.* (3).

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[1] The abbreviation used is: MES, 2-(*N*-morpholino)ethanesulfonic acid.

TABLE I

E. coli K12 strains used

Strain	Genotype	Comments
AB264	(Muc⁺)	From B. Bachmann[a]
EJ14	As JM2087 but *xylE*::Mud(Ap^R*lac*)I	JM2087 became Xyl^R on Gly
EJ15	As JM2087 but *fda*⁺	P1.K10 × JM2087
EJ18	As EJ14 but *fda*⁺	P1.K10 × EJ14
EJ20	As JM2235 but *xylE*::Mud(Ap^R*lac*)I	P1.EJ18 × JM2235
EJ21	As EJ20 but *arg*⁺	Hfr JM559 × EJ20
EJ22	As JM2349 but *xylE*::Mud(Ap^R*lac*)I	P1.EJ21 × JM2349
EJ23	As PO85 but (Muc⁺)	From PO85
EJ25	As EJ23 but (Mud(Ap^R*lac*)I)	P1.EJ18 × EJ23
EJ26	As EJ23 but *zjb*::Tn10	P1.NB1 × EJ25
JM559	Hfr Cavalli Δ(*his gnd*) *fda*^{ts}	Lam *et al.* (3)
JM1742	Hfr Cavalli Δ(*his gnd*) *fda*^{ts} *sor*⁺	Unpublished[b]
JM2087	Δ(*his gnd*) Δ*lac araD fda ptsF ptsM rpsL*	From MC4100
JM2235	*thr leu argH pheA cysA his trp mtl malA gal lac rpsL* (Muc⁺)	From PA309
JM2333	As JM2087 but *xylE*::Mud(Ap^R*lac*)II	JM2087 became Xyl^R on Gly
JM2336	As JM2333 but *fda*⁺	P1.K10 × JM2333
JM2349	*argH metA aceA* (Muc⁺)	Derived from PA505-5
JM2362	As JM2349 but *xylE*::Mud(Ap^R*lac*)II	P1.JM2333 × JM2349
MAL103	Δ(*pro lac*)X111 *araD rpsL araB*::Muc^{ts} (Mud(Ap^R*lac*)I)	Casadaban and Cohen (12)
MAL315	Δ(*pro lac*)X111 *araD rpsL* Δ(*ara leu*) 7697 *mal*::Muc^{ts} (Mud(Ap^R*lac*)II)	Casadaban cited in Beny *et al.* (35)
NB1	*pgi zwf zjb*::Tn10	From J. Scaife[c]
PO85	Hfr *malB107 his*	From P. Oliver[d]

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[b] M. C. Jones-Mortimer, unpublished observations.
[c] J. Scaife, Department of Molecular Biology, University of Edinburgh.
[d] P. Oliver, Department of Genetics, University of Cambridge.

Initial rates were calculated from a sample taken 15 s after addition of sugar.

Transport of 40 μM [^{14}C]xylose or [^{14}C]lactose into subcellular vesicles was energized by 20 mM ascorbate + 0.1 mM phenazine methosulfate (15) + oxygen and measured as described by Horne and Henderson (16). The total volume of the suspension was 0.25 or 0.5 ml, and 0.05-ml samples were taken and washed with 0.1 M LiCl at timed intervals.

Measurement of Sugar-promoted pH Changes—Bacterial suspension equivalent to 16–18 mg (dry mass) was diluted into a total volume of 2.7 ml of de-aerated 150 mM KCl, 2 mM glycylglycine at 25 °C, contained in a glass cell very similar to that described by Henderson *et al.* (11). Additions of 20 μl of 0.5 M de-aerated sugar solutions were made when the pH electrode immersed in the suspension indicated a low drift rate at pH values varying between 5.6 and 6.3. Deflections were converted to ΔH^+ by calibration with 3 μl of air-free standard 0.01 M NaOH. Further experimental details were as described by Henderson *et al.* (11). Measurements were made within 6 h of harvesting for strain JM2336 and within 30 h for other strains.

Genetical Techniques—Conjugation was performed essentially as described by Miller (13). Streptomycin (100 μg/ml) was used to counter-select the Hfr strain. All the selection media contained Thr, Phe, Cys, His, and Trp. Leu, Arg, glucose, mannitol, or maltose were added as appropriate. Amino acid supplements were at concentrations of 20–80 μg/ml, monosaccharides at 10 mM, disaccharides at 5 mM, and ampicillin at 25 μg/ml.

Transductions were done by the method of Miller (13), with appropriate supplements at the concentrations above, and 30 mM acetate, 20 mM succinate, or 10 μg/ml of tetracycline as necessary. For strain EJ14, *malB* alleles were scored on His, succinate, and maltose when *malB*, but not *malB*⁺, colonies would grow. The reason was the *fda* lesion in strain EJ14, whereby uptake of maltose into *malB*⁺ strains would inhibit growth through accumulation of toxic fructose bisphosphate. The absence of maltose uptake into *malB* strains would permit growth on the succinate. The method for scoring *pgi* alleles in strain EJ14 took advantage of its *fda gnd* background, where *pgi*, but not *pgi*⁺, colonies would grow on glucose 6-phosphate (17). The reason was that toxic fructose bisphosphate could not be formed directly from glucose 6-phosphate in *pgi gnd* mutants, although the cells could grow by its metabolism to trioses via 6-phosphogluconate; the indirect synthesis of fructose bisphosphate by an aldolase in the condensing direction (18) did not appear to be fast enough for it to reach toxic concentrations. However, when the cell was *pgi*⁺, fructose bisphosphate would rapidly be formed and inhibit cell growth.

Deletion mapping was undertaken as follows. Five independent cultures of strains EJ14 and JM2333 were grown in nutrient broth overnight at 30 °C. A sample (0.2 ml) of each culture was spread on minimal medium supplemented with His, Met, Ade, glycerol, xylose, lactose, and casamino acids and incubated at 42 °C for 24 h. Xylose and lactose together select against the xylose-inducible *lac* operon of Mud(Ap^R*lac*), because the organism was *fda*. The temperature of 42 °C also selected against Mu because the repressor protein of Mud(Ap^R*lac*) was temperature-sensitive.

Protein Assays—Protein concentrations were measured by the method of Schaffner and Weissmann (19).

Reproducibility of Measurements—All measurements on one preparation were made in duplicate. However, transport and β-galactosidase activities varied by a factor of up to 2 between different preparations of the same strain, so all values represent the means of determinations on at least two batches of intact cells or subcellular vesicles. Standard errors have been omitted for clarity.

RESULTS

Isolation of Mutants—*E. coli* strain JM2087 (*fda lac*) was infected with Mud(Ap^R*lac*)I or Mud(Ap^R*lac*)II and grown to segregate mutations. Individual mutants resistant to ampicillin and xylose were then selected on minimal medium using glycerol as carbon source. Colonies from 20 independent selections were replica-plated onto medium containing (i) histidine + xylose (growth would indicate reversion to *fda*⁺), (ii) histidine + xylose + glycerol, (iii) histidine + glycerol, and (iv) nutrient broth + ampicillin. Cells on plates ii and iii were tested for β-galactosidase activity (see "Experimental Procedures"). Colonies from eight independent selections had xylose-inducible β-galactosidase, indicating that the Mud(Ap^R*lac*) phage had inserted into a *xyl* gene with the correct orientation and, in the case of Mud(Ap^R*lac*)II, reading frame.

Assuming the existence of two separate transport systems for xylose (see Introduction), a mutation in one of these can be distinguished from a mutation in the *xylA* or *xylB* genes by making the organism *fda*⁺; a mutant with one transport system intact will then grow on xylose, but a mutant with a lesion in *xylA* or *xylB* will still not grow on xylose. Accordingly, each of the mutants was made *fda*⁺ by transduction. Of the five independent Mud(Ap^R*lac*)I-induced mutations that had xylose-inducible β-galactosidase, two (EJ17 and EJ18) grew on xylose after becoming *fda*⁺. Strain JM2336, one of three Mud(Ap^R*lac*)II mutants, behaved similarly.

β-Galactosidase Activities in the E. coli Strains—Strain JM2087 was made *fda*⁺ as described under "Experimental Procedures." One resulting transductant, strain EJ15, was then used as a wild type control for comparison with the mutant strains EJ18 and JM2336.

Each strain was grown in liquid culture on glycerol or on glycerol + xylose. Measurement of β-galactosidase activities in sonicated cells confirmed that the Mud(Ap^R*lac*) insertions had introduced xylose-inducible β-galactosidase (Table II). Furthermore, over 50% of the β-galactosidase activity of strain JM2336 was retained in membrane preparations made by differential centrifugation and washing of the sonicated cell extract (Table III). This compared with an average of 3% retained in membranes from strain EJ18 (Table III). Subcellular vesicles made from strain JM2336 showed about 7.5-fold higher β-galactosidase activity than similar vesicles prepared from strain EJ18 (Table III). These results implied that the fused gene product was bound to the membrane of strain JM2336, unlike the predominantly cytoplasmic location of the normal β-galactosidase activity (20).

Xylose and Lactose Transport in the E. coli Strains—Each of the strains EJ15, EJ18, and JM2336 was grown on glycerol or on glycerol + xylose and tested for xylose induction of xylose and lactose transport. Consistent with its *lac* deletion genotype, the control strain EJ15 showed no significant lactose transport but did have high levels of xylose-inducible xylose transport (Table II). After insertion of Mud(Ap^R*lac*), the mutant strains had both acquired xylose-inducible lactose transport (Table II), and their ability to transport xylose was moderately impaired (Table II).

A preliminary kinetic analysis showed a nonlinear Lineweaver-Burk plot for xylose uptake into strain EJ15 and approximately linear plots for strain EJ18. The data were consistent with the loss of a low affinity xylose transport system of apparent K_m = 170 μM from strain EJ15 and retention of a high affinity system of apparent K_m = 0.3–3 μM in strain EJ18; these latter values agreed with those of 0.2–5 μM reported for the xylose-binding protein system (7). A detailed kinetic study awaits construction of a XylE⁺ XylF⁻ organism. If strains EJ18 and JM2336 had retained a binding protein transport system, xylose transport should be more sensitive to inhibition by arsenate, to which proton-linked transport systems are insensitive (4–6). Thus, the proton-linked lactose transport (21) in strains EJ18 and JM2336 was even stimulated by arsenate (Fig. 1), and xylose transport into wild type strain EJ15 was insensitive to arsenate (Fig. 1). However, xylose transport into the mutant strains EJ18 and JM2336 was relatively inhibited (Fig. 1). The extent of inhibition by arsenate was rather variable in all strains, depending on the concentration of substrate used, the pH, and the nature of ancillary respiratory substrates (data not shown). Nevertheless, all the results were consistent with the loss of an arsenate-insensitive, and therefore potentially H⁺-linked, xylose transport system as a result of the Mud(Ap^R*lac*)

TABLE II

Transport and β-galactosidase activities of E. coli strains

The *E. coli* strains were grown and harvested, and activities were measured as described under "Experimental Procedures." The mean value of each activity is given with the number of different cultures tested in parentheses.

Strain	Growth substrates	Xylose transport	Lactose transport	β-Galactosidase
		nmol/mg/min	*nmol/mg/min*	*units/mg*
EJ15	Glycerol + xylose	13.5 (8)	0.5 (2)	3 (3)
	Glycerol	0.2 (2)	0.4 (2)	3 (2)
EJ18	Glycerol + xylose	7.0 (10)	6.5 (3)	742 (4)
	Glycerol	0.1 (2)	0.4 (2)	4 (2)
JM2336	Glycerol + xylose	11.2 (3)	4.36 (3)	592 (3)
	Glycerol	0.3 (3)	0.9 (3)	2 (2)

TABLE III

Location of β-galactosidase in Mud(Ap^{R}lac) strains

Strain EJ18 or JM2336 was grown on glycerol + xylose. Cytoplasmic fraction, membrane fraction, and subcellular vesicles were prepared and their β-galactosidase activities were measured as described under "Experimental Procedures." The average total β-galactosidase activity for cytoplasm plus membrane fraction was 202,318 units (3) for strain EJ18 and 68,756 units (5) for strain JM2336. The figures in parentheses are the number of cultures tested.

Strain	β-Galactosidase activity		
	Cytoplasm	Membrane	Membrane vesicles
	%	%	*units/mg protein*
EJ18	97 (5)	3 (5)	473 (3)
JM2336	45 (3)	55 (3)	3543 (6)

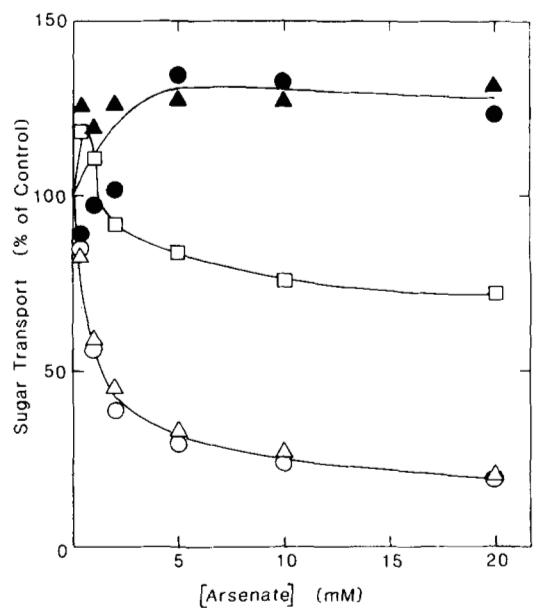

FIG. 1. **Effect of arsenate concentration on xylose transport into *E.coli* strains.** Strain EJ15 (□), EJ18 (○ and ●), or JM2336 (△ and ▲) was grown on glycerol + xylose, washed, depleted, and resuspended in 150 mM KCl, 5 mM MES, pH 6.5, to $A_{680} \approx 5$. Samples (0.2 ml) were mixed with appropriate proportions of 100 mM K arsenate, pH 6.5, and 150 mM KCl, 5 mM MES, pH 6.5, to give the indicated arsenate concentrations in a final volume of 0.5 ml including 10 mM glycerol. After a 3 min exposure and bubbling with air, 180 μM [^{14}C]xylose (□, ○, and △) or [^{14}C]lactose (● and ▲) was added and samples (0.2 ml) taken at 15 s and 2 min for measurement of sugar accumulation (see "Experimental Procedures"). The points are averages of duplicate measurements on two separate cultures of strain EJ15 and one culture of strain EJ18 or JM2336. Control values (nanomoles/mg/2 min) were 26.8 (□), 13.7 (○), 11.2 (△), 14.5 (●), and 10.2 (▲).

insertions in strains EJ18 and JM2336.

Xylose-promoted pH Changes—The appearance of an alkaline pH change when a sugar substrate was added to a suspension of energy-depleted cells was diagnostic of sugar-H^+ symport (21, 22). Xylose-induced strain EJ15 showed such an alkaline pH change when xylose was added (Fig. 2 and Table IV). The effect was reproducible (Table IV), and the rates and extents achieved were comparable to those reported previously for xylose-H^+ symport (3). As observed before (3), the alkaline pH change reversed to a net acid efflux due to anaerobic metabolism of the sugar (Fig. 2).

Xylose did not promote an alkaline pH change with xylose-induced *E. coli* strains EJ18 or JM2336 (Fig. 2 and Table IV). Instead, there was an almost immediate acidification (Fig. 2), showing that the sugar had nevertheless rapidly entered the cells and could be metabolized. Thus, strains EJ18 and JM2336 had lost xylose-H^+ symport activity as a result of the Mud(Ap^R*lac*) insertion.

An alternative explanation for the disappearance of xylose-H^+ symport might be that the Mu insertion invoked an unspecific change, for example leakiness of the membrane to protons. However, this was not so because LacY proton symport activity was unimpaired, as evidenced by the extensive alkaline pH change promoted by its nonmetabolizable substrate, methyl-β-D-thiogalactoside (Fig. 2 and Table IV). This control experiment was a valuable by-product of the use of Mud(Ap^R*lac*) insertion mutants (see also next section).

Xylose Transport into Subcellular Vesicles—The activity of the LacY, AraE, XylE, and GalP sugar-H^+ transport systems was retained in right-way round subcellular vesicles (3, 6, 15, 16, 23). Their activities were energized by respiration, and the energization was prevented by uncoupling agents. Similarly, subcellular vesicles prepared from strain EJ15 retained xylose transport (Fig. 3) which required respiratory substrate and was sensitive to uncoupling agents (data not shown).

By contrast, there was no respiration-energized xylose transport into vesicles made from strain EJ18 or JM2336 (Fig. 3). This provided independent support for the conclusion that the Mud(Ap^R*lac*) insertions were in a gene or genes responsible for xylose-H^+ symport activity. The absence of energized xylose transport was not due to an unspecific effect, because the vesicles from either strain (EJ18 or JM2336) showed respiration-dependent lactose uptake (Fig. 3), which was absent from strain EJ15 (Fig. 3). Therefore, the Mud(Ap^R*lac*) insertions had inactivated a membrane-bound xylose transport system and introduced the LacY transport system.

Conjugation Mapping—The *xylE*::Mud(Ap^R*lac*)I mutation was first transferred into the multiple auxotroph F^- Muc^+ lysogen JM2235 (by P1 transduction), selecting for ampicillin resistance. The colonies obtained were screened for xylose-inducible β-galactosidase to ensure that Mud(Ap^R*lac*) had not moved, and one (designated EJ20) was used as recipient for conjugation with the Hfr Cavalli strain JM559, known to be $XylE^+$ (3). Since 51 out of 79 Arg^+ recombinants were Ap^S,

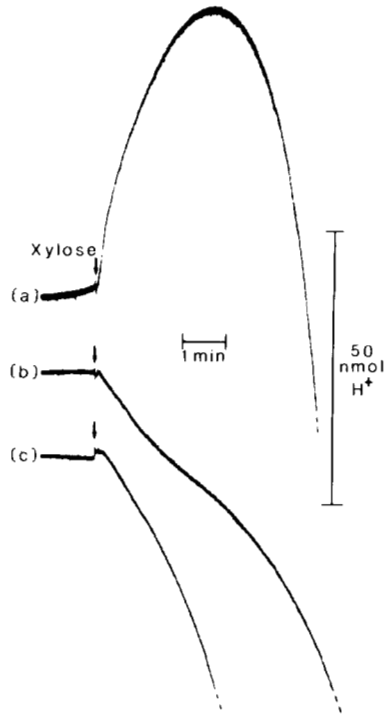

FIG. 2. **Xylose-promoted pH changes in *E. coli* strains.** Cultures of *E. coli* EJ15 (*a*), EJ18 (*b*), or JM2336 (*c*) were grown on glycerol + xylose, harvested, energy-depleted, and resuspended in 150 mM KCl, 2 mM glycylglycine. The pH of separate suspensions of each strain was recorded, and 10 μmol of xylose were added at the point shown by the *arrow*. An upward deflection indicated an alkaline pH change. For further details, see "Experimental Procedures" and Refs. 3 and 11.

TABLE IV

Alkaline pH changes promoted by xylose or methyl-β-D-thiogalactoside

The *E. coli* strains were grown on glycerol + xylose, and sugar-promoted alkaline pH changes were measured on the energy-depleted cells as described under "Experimental Procedures" and illustrated in Fig. 2. Mean values are given with the number of measurements in parentheses. At least two separate cultures of each strain were used. TMG, methyl-β-D-thiogalactoside.

Strain	H^+ uptake	
	Xylose	TMG
	nmol/mg	
EJ15	2.66 (16)	0.10 (8)
EJ18	0.03 (8)	3.78 (7)
JM2336	0.12 (6)	2.53 (5)

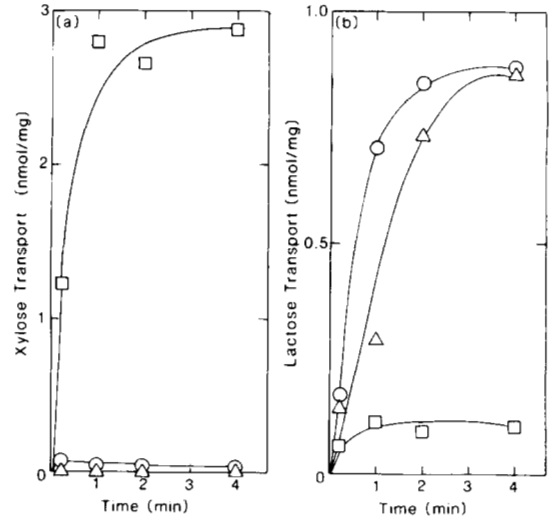

FIG. 3. **Xylose and lactose transport into subcellular vesicles of *E. coli* strains.** Vesicles were prepared from strain EJ15 (□), EJ18 (○), or JM2336 (△) grown on glycerol + xylose. The transport of 40 μM [^{14}C]xylose (*a*) or 40 μM [^{14}C]lactose (*b*) was energized by ascorbate + phenazine methosulfate and measured as described under "Experimental Procedures." Each point is the mean of at least four measurements on at least two separate vesicle preparations.

Mud(Ap^R*lac*) was inserted fairly close to *argH* at min 89.5. There was no discernible linkage to *leu* (min 1.8) or to *mtl* (min 80.7). The latter absence of linkage appeared significant since all other known *xyl* genes map close to *mtl* (9).

Transduction Mapping—A series of transductions was conducted to locate the position of *xylE*::Mud(Ap^R*lac*) more exactly. The gene *argH* is at 89.5 min; the initial transduction investigated the region towards 90.5–91.5 min for which strains with appropriate markers were readily available.

Phage P1 grown on strain EJ21 (Table I) was used to infect the Muc$^+$ lysogen JM2349 (*argH metA aceA*). The results and conclusions were as follows. None of the Arg$^+$ or Met$^+$ transductants examined was Ap^R. When the selection was for Ace$^+$, 2 out of 67 colonies were Ap^R, 63 were Met$^+$, and 12 were Arg$^+$. This confirmed the known order of *argH metA* and *aceA* and suggested a position for *xylE* beyond *aceA*. This position was confirmed by selecting Ap^R transductants and screening their Met and Ace phenotypes (Table V). The four-crossover class was Met$^+$ Ace$^-$ Ap^R, so *xylE* mapped clockwise from *aceA*. Similar results were obtained with strain JM2333 (*xylE*::Mud(Ap^R*lac*)II) as donor (Table V). A five-point cross with this *xylE* allele (data not shown) confirmed this gene order and indicated that *xylE* mapped clockwise from *sor*; the *sor* gene is located between *aceA* and *pgi* (24).

The location of *xylE* relative to *pgi* and *malB* was investigated. First, P1 grown on strain NB1 (*pgi zjb*::Tn10) was used to transduce strain EJ14 (*xylE*::Mud(Ap^R*lac*)I) to tetracycline resistance. Of 79 transductants scored, 13 became Ap^S, whereas only one became *pgi* (for the method of scoring, see "Experimental Procedures"). The Tn10 insertion was therefore closer to the Mu insertion than to *pgi*. Second, P1 grown on strain EJ26 (*malB zjb*::Tn10) was used to transduce strain EJ14 to tetracycline resistance, and the progeny were scored for ampicillin resistance and for maltose resistance (*malB*, see "Experimental Procedures"). Of 160 transductants scored, 62 were Ap^S *malB* (donor type), 85 were Ap^R *malB*$^+$ (recipient type), and 13 were Ap^R *malB*. No Ap^S *malB*$^+$ recombinants were obtained. This class would have constituted a four-crossover class, but in view of the proximity of *malB* to *xylE* and of the relatively small size of the sample, it is not surprising that no such recombinants were obtained. From this experiment, *malB* mapped between *zjb*::Tn10 and *xylE*. Therefore, the order of the four genes must be

pgi xylE malB zjb::Tn10.

Deletion Mapping—Ellwood and Nomura (25) showed that *E. coli* was tolerant of deletions encompassing many genes in the *purD* region, provided of course that the induced auxotrophic requirements were met. The location of a Mu prophage, and hence of the gene into which it was inserted, may be determined by isolating mutants from which Mu has been deleted and screening for the loss of neighboring genetic markers. Such deletions were detected in separate experiments with strain EJ14 and JM2333 (see "Experimental Procedures").

The results with strain EJ14 (Table VI) showed that Mu, and hence *xylE*, was located between *aceA* and *pgi* or between *pgi* and *malB*. The ambiguity was not resolved because of the absence of the deletion class *pgi*$^+$ *malB*, which would have indicated Mu lay between these two markers. With strain JM2333, the deletion analysis indicated that Mu was located between *pgi* and *malB* (assuming that the diagnostic strain (last line of Table VI) was a deletion mutant rather than an inversion).

The consensus from all the mapping data was the gene order

argH metA aceA sor pgi xylE::Mud(Ap*lac*) *malB zjb*::Tn10.

The Xylose Mutation Was Not in a Maltose Gene—In view of the proximity of the *xylE* gene to the *malB* genes, which specify membrane proteins for maltose transport, the possible involvement of the latter in xylose transport was eliminated by the experiment shown in Table VII. This showed that neither xylose transport (in strain EJ15) nor β-galactosidase activity (in strain EJ18) was induced by maltose; furthermore, maltose transport was not induced by xylose. In the control experiments, xylose transport was induced by xylose, and maltose transport was induced by maltose. Hence, the *xyl* genes, including *xylE*, were expressed independently of the *mal* genes and could not be part of the *malB* operon.

DISCUSSION

It can be difficult to select mutations in genes for transport processes, especially when there is more than one transport system for a single substrate. The use of *fda* strains has several advantages. First, it provides a positive selection for mutants impaired in either transport or metabolism, because the ac-

TABLE V

Location of xylE by P1-mediated transduction

P1 grown on strain EJ21 (*xylE*::Mud(Ap^R*lac*)I) or JM2333 (*xylE*::Mud(Ap^R*lac*)II) was used to transduce strain JM2349 (*xylE*$^+$ *argH metA aceA*) to Ap^R and scored for inheritance of the *met* and *ace* alleles (see "Experimental Procedures"). The number in each class is given.

Markers scored		Donor	
metA	*aceA*	EJ21	JM2333
+	+	6	13
+	−	0	0
−	+	5	6
−	−	69	141

TABLE VI

Location of xylE by deletion analysis

Thermoresistant Lac$^-$ derivatives of strains EJ21 (*xylE*::Mud(Ap^R*lac*)I) and JM2333 (*xylE*::Mud(Ap^R*lac*)II) were tested for the loss of markers near *xylE* (see "Experimental Procedures").

Markers scored					No. of independent mutants from	
purD	*metA*	*aceA*	*pgi*	*malB*	EJ21	JM2333
−	−	−	−	+	5	3
+	−	−	−	+	5	2
+	+	−	−	+	5	1
+	+	−	−	−	3	0
+	+	+	−	+	5	0
+	+	+	−	−	1	0
+	+	+	+	−	0	1

TABLE VII

Independent induction of xylose and maltose transport

A single batch of each strain was grown on the indicated substrates and harvested, and activities were measured in duplicate as described under "Experimental Procedures."

Strain	Growth substrates	Xylose transport	Maltose transport	β-Galactosidase
		nmol/mg/min	*nmol/mg/min*	*units/mg*
EJ15	Glycerol + xylose	14.9	0.7	4
EJ15	Glycerol	0.2	0.4	3
EJ15	Glycerol + maltose	0.3	12.2	2
EJ18	Glycerol + xylose	5.8	0.6	1251
EJ18	Glycerol	0.2	0.7	6
EJ18	Glycerol + maltose	0.2	9.6	12

cumulation of phosphorylated sugar is toxic to the cell. Second, conversion of sugar-resistant strains to *fda*$^+$ should reveal two classes of mutants if the starting strain had two transport systems; one *fda*$^+$ class would grow on the sugar because it was impaired in one only of the transport systems, whereas the other *fda*$^+$ class would not grow because it was impaired in at least one of the metabolic enzymes. Thus, mutants in one only of the transport systems could be obtained. Third, this technique has general application for selecting mutants in the transport or metabolism of any sugar utilizing the Embden-Meyerhof pathway. The selective pressures are probably complex, because a mutation in one of two operative transport systems would be expected only to decrease, not abolish, the uptake of substrate. In fact, selection against one transport system may be enhanced if the other is at low activity due to catabolite repression. In the work here, glycerol was used as the carbon source because it was reported to repress a presumably binding protein-dependent xylose transport system (1). Ahlem *et al.* (7), however, found that the xylose-binding protein was still produced when *E. coli* was grown on xylose and glycerol.

The efficiency of the selection was enhanced in several ways by the use of Mud(Ap^R*lac*) for mutagenesis. First, the mutation frequency within a lysogenic population is 50–100 times higher and Mu insertions are more stable than spontaneous mutations (26). Second, the xylose inducibility of β-galactosidase, which can easily be tested on plates, provides an additional means of detecting mutants. Third, it introduces an ampicillin-resistance determinant into the gene, and Ap^R is often a much easier phenotype to score than the phenotype of the mutated gene. Fourth, Mud(Ap^R*lac*)II mutants can be used to identify and characterize the products of the genes into which fusion has occurred (see *e.g.* Refs. 27–30).

In both our independently derived mutants, the Mud(Ap^R*lac*) insertion introduced ampicillin resistance, xylose-inducible β-galactosidase, and lactose transport while simultaneously abolishing xylose-promoted alkaline pH changes and vesicular xylose transport. This provided direct evidence that a gene responsible for xylose-H$^+$ symport was the site of the insertion. Furthermore, its relatively high K_m value and its insensitivity to arsenate were properties typical of proton-linked sugar transport systems (see *e.g.* Refs. 4–6). The mapping of the inserted Ap^R marker revealed the location of the *xylE* gene near 91 min; both mutations mapped at the same locus by a variety of techniques. However, the *xyl* operon is in a different position (near 80 min) in both *E. coli* and *S. typhimurium* (8, 9). In *E. coli*, a xylose transport gene (*xylP*) was described but not mapped (1). In *S. typhimurium*, which did not exhibit a xylose-H$^+$ transport activity (3), a gene (*xylT*) involved in xylose transport was also located near 80 min, but probably in a separate operon from the other *xyl* genes (10). It is probable, therefore, that *xylT* codes for a component of the binding protein transport system. We now propose that the genes for xylose-H$^+$ symport be called *xylE* and the genes for the binding protein transport system be collectively called *xylF*, to conform with the nomenclature of the genes *araE* and *araF* for two arabinose transport systems (29–34), the former being proton-linked and the latter involving a binding protein (6). We are undertaking experiments to confirm the location of the *xylF* gene in *E. coli*.

Since the *xylE* mutants described here still exhibited xylose-inducible xylose transport and metabolism, they were different from a previously isolated xylose transport-negative strain, VL18 (3). This strain had lost transport of labeled xylose, the xylose-promoted pH change, and the acid efflux indicative of xylose metabolism (compare Fig. 2 of this paper with Fig. 1 of Ref. 3). The lesion in VL18, which was located near *mtl* (80.7 min), would therefore appear to be in the positive regulatory gene *xylR*.

The separate genetic location of the two xylose transport systems may have some general evolutionary or regulatory significance, since such separation was also found for the two galactose transport systems, *galP* near 64 min and *mglP* near 45 min, and the two arabinose transport systems, *araE* and *araF* near 45 and 61 min, respectively (9). It confirmed that the xylose-H$^+$ transport system was different from the other sugar-H$^+$ transport systems of *E. coli*, including *lacY*, which mapped at 8 min (9).

The location of the *xylE* gene and the availability of a fused *xylE lacZ* gene product should now facilitate the isolation and structural analysis of a xylose-H$^+$ transport protein.

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Location of a structural gene for xylose-H+ symport at 91 min on the linkage map of Escherichia coli K12.

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