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D-Arabinose Metabolism in *Escherichia coli* B: Induction and Cotransductional Mapping of the L-Fucose–D-Arabinose Pathway Enzymes

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D-Arabinose is degraded by *Escherichia coli* B via some of the L-fucose pathway enzymes and a D-ribulokinase which is distinct from the L-fuculokinase of the L-fucose pathway. We found that L-fucose and D-arabinose acted as the apparent inducers of the enzymes needed for their degradation. These enzymes, including D-ribulokinase, appeared to be coordinately regulated, and mutants which constitutively synthesized the L-fucose enzymes also constitutively synthesized D-ribulokinase. In contrast to D-arabinose-positive mutants of *E. coli* K-12, in which L-fuculose-1-phosphate and D-ribulose-1-phosphate act as inducers of the L-fucose pathway, we found that these intermediates did not act as inducers in *E. coli* B. To further characterize the *E. coli* B system, some of the L-fucose–D-arabinose genes were mapped by using bacteriophage P1 transduction. A transposon Tn*10* insertion near the *E. coli* B L-fucose regulon was used in two- and three-factor reciprocal crosses. The gene encoding D-ribulokinase, designated *darK*, was found to map within the L-fucose regulon, and the partial gene order was found to be Tn*10-fucA-darK-fucI-fucK-thyA*.

D-Arabinose, thought to be uncommon in the natural environment, is not usually metabolized by enteric bacteria, although in certain cases mutants that use D-arabinose as a sole carbon and energy source can be isolated. In *Escherichia coli* K-12, regulatory mutations of the L-fucose pathway allow growth on D-arabinose. *E. coli* degrades L-fucose to dihydroxyacetone phosphate and L-lactaldehyde by using the inducible enzymes L-fucose permease, L-fucose isomerase, L-fuculokinase (EC 2.7.1.51), and L-fuculose-1-phosphate aldolase (EC 4.1.2.17) (Fig. 1) (17–19). In *E. coli* K-12, metabolism of D-arabinose proceeds entirely by means of the L-fucose pathway enzymes, with D-arabinose eventually being degraded to dihydroxyacetone phosphate and L-glycolaldehyde. The apparent inducer of the L-fucose enzymes in *E coli* K-12 has been identified as the intermediate L-fuculose-1-phosphate (4), and the regulatory mutation that allows growth on D-arabinose results in induction of the L-fucose catabolic enzymes by D-ribulose-1-phosphate, an intermediate of D-arabinose metabolism (4, 30).

An alternative route of D-arabinose metabolism involves an enzyme of the ribitol catabolic pathway (26, 30). Ribitol is metabolized via a set of inducible enzymes through which ribitol is transported, oxidized by ribitol dehydrogenase (EC 1.1.1.56) to form D-ribulose, and then phosphorylated by D-ribulokinase (EC 2.7.1.47) to form D-ribulose-5-phosphate (Fig. 1).

Although *E. coli* K-12 does not normally contain the ribitol pathway, the genes that code for the ribitol enzymes can be transduced into K-12 (27). In strains containing both the L-fucose and ribitol pathways, D-arabinose is metabolized via L-fucose permease and L-fucose isomerase, yielding D-ribulose, which is a natural intermediate and the apparent inducer of the ribitol pathway. D-Ribulose is then phosphorylated by D-ribulokinase, and metabolism continues through the pentose phosphate pathway. While most of the D-ribulose formed from D-arabinose proceeds through the ribitol route, L-fuculokinase maintains a small pool of D-ribulose-1-phosphate and keeps the L-fucose enzymes induced.

As in *E. coli* K-12, *Klebsiella pneumoniae* and *K. aerogenes* strains grow on L-fucose naturally and can mutate to grow on D-arabinose. In contrast to K-12, all D-arabinose-positive mutants of *K. pneumoniae* PRLR3 and about half of the *K. aerogenes* W-70 D-arabinose-positive mutants examined were constitutive for the L-fucose enzymes. The remaining half of the W-70 isolates were inducible by D-ribulose-1-phosphate (26, 30). Both *Klebsiella* species contain the ribitol operon, and metabolism of the D-ribulose formed from the isomerization of D-arabinose proceeds primarily through the pentose phosphate pathway.

In contrast to *E. coli* K-12, wild type *E. coli* B and B/r strains can use D-arabinose without mutation, but cannot grow with L-fucose as a sole carbon and energy source. Boulter et al. reported that with *E. coli* B/r, the inability to metabolize L-fucose is caused by the lack of L-fuculose-1-phosphate aldolase activity (5), although L-fucose-utilizing mutants that have aldolase activity can be obtained by selecting for growth on that sugar. D-Arabinose is metabolized in *E. coli* B/r by enzymes that isomerize D-arabinose to D-ribulose and phosphorylate D-ribulose at the C-5 position (5), with metabolism continuing through the pentose phosphate pathway. The D-ribulokinase activity is separate and distinct from the L-fuculokinase of the L-fucose pathway. Additionally, *E. coli* B cannot metabolize ribitol and appears to lack the ribitol operon (27). The D-arabinose isomerase activity of *E. coli* B/r has been purified and shown to be very similar to the L-fucose isomerase of K-12 (6). D-Arabinose-negative mutants of wild-type *E. coli* B/r were isolated, and strains lacking D-arabinose isomerase activity were capable of inducing a D-ribulokinase activity when grown in the presence of D-arabinose (5).

The experiments described in this paper show that in *E. coli* B, L-fucose and D-arabinose are the apparent inducers of the L-fucose–D-arabinose enzymes, including the D-ribuloki-

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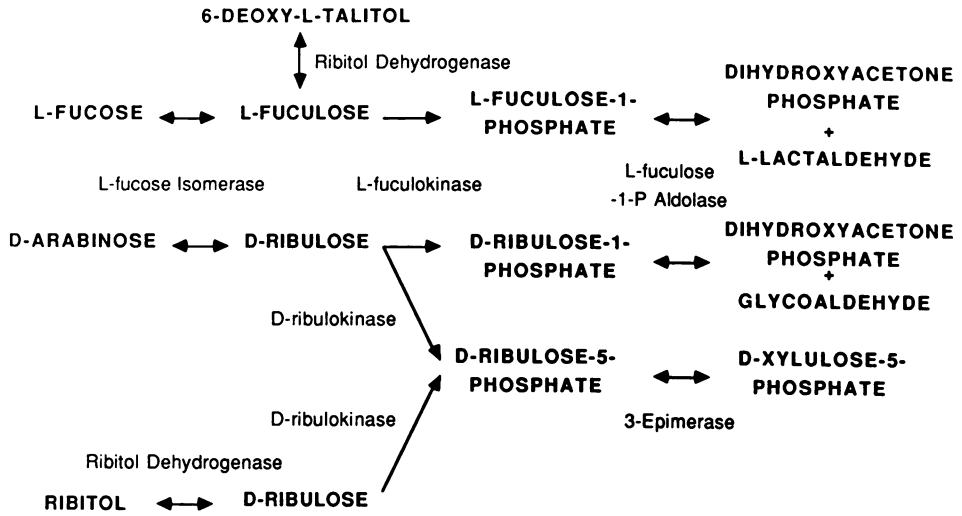

FIG. 1. Metabolic pathways for L-fucose, D-arabinose, ribitol, and 6-deoxy-L-talitol. L-Fuculose-1-P, L-Fuculose-1-phosphate.

nase activity, which phosphorylates D-ribulose at the C-5 position. In contrast to the induction pattern of *E. coli* K-12 and *Klebsiella* strains, it is shown that L-fuculose-1-phosphate and D-ribulose-1-phosphate do not act as inducers of these enzymes in *E. coli* B. It is also shown that the D-ribulokinase activity of *E. coli* B is coordinately induced with the L-fucose enzymes, and mutants which constitutively synthesize the L-fucose enzymes also constitutively synthesize D-ribulokinase. The isolation of mutations in some of the L-fucose–D-arabinose enzymes is described, as is the mapping of those mutated genes by bacteriophage P1 transduction.

MATERIALS AND METHODS

Bacterial strains, bacteriophage, and culture conditions. All *E. coli* B strains except WA837 were derived from the wild-type strain provided by C. Dowell, University of Massachusetts, Amherst. The strains derived for this study are listed in Table 1. Mutants unable to synthesize the kinases of the L-arabinose and L-rhamnose pathways were used in these experiments, as these enzymes are known to be capable of participating in D-arabinose or L-fucose metabolism (5, 11, 12, 22). Strain EM2022 and all strains constructed from it contained a mutation in the *araB* gene and thereby lacked L-ribulokinase (EC 2.7.1.16) activity. Additionally, EM2022 contained two mutations in the L-rhamnose regulon. The first mutation appeared to be in the *rhaD* gene, which encodes L-rhamnulose-1-phosphate aldolase (EC 4.1.2.19). The second mutation (*rha-4*) relieved the L-rhamnose toxicity associated with the *rhaD* mutation and resulted in the noninducibility of L-rhamnose isomerase (EC 5.3.1.14) and L-rhamnulokinase (EC 2.7.1.5) activities, suggesting that a regulatory mutation had occurred. The growth rates of EM2022 on L-fucose and D-arabinose were unchanged from those of the L-arabinose- and L-rhamnose-positive parent EM2001.

thyA mutants were isolated by trimethoprim selection as described by Miller (24). Bacteriophage P1 *cam* was prepared by heat induction as previously described (29). The use of P1 *cam* allowed temperature regulation of lysogeny as well as initial identification of the lysogenic state. Unless otherwise indicated, bacterial cultures were grown aerobically at 37°C in minimal salts medium (25) supplemented with 0.5% casein hydrolysate with or without 0.5% carbohydrate; 1.5% agar (Difco) was used for solid medium and 0.75% agar was used for soft medium.

Growth of cells for enzyme assays and growth rate measurements. Inocula for cultures were prepared by overnight growth in minimal salts with 0.5% casein hydrolysate broth medium. After growth, cells were diluted 1:100 into 20 ml of minimal salts medium containing 0.5% casein hydrolysate and 0.5% carbohydrate and grown to late log phase (OD_{660}, 0.8). Growth of cells was monitored by OD_{660} measurements. L-Fucose is toxic to L-fuculose-1-phosphate aldolase-negative mutants, presumably due to the accumulation of phosphorylated intermediates; therefore, in the preparation of aldolase mutant strains for enzyme assays, the inducing carbohydrate was not added until the culture had reached an OD_{660} of 0.4. To assay transport activity, cultures were grown to an OD_{660} of 0.6, harvested by centrifugation, and then suspended without washing in 10 mM KPO_4 (pH 7.0) buffer to a cell density of 10^9 CFU/ml. Cell suspensions were kept on ice until assayed.

Preparation of cell extracts. Cells were harvested and washed by centrifugation at 7,600 × *g* for 5 min at 4°C and then resuspended at 1/10 the original culture volume in 50 mM KPO_4 (pH 7.6)–10 mM magnesium acetate–1 mM EDTA–10 mM 2-mercaptoethanol buffer. Cell suspensions and extracts were kept on ice at all times. Cells were broken by sonication in a Braunsonic 1510 sonicator with two 15-s bursts at 40 W with a 30-s cooling period between bursts. Cells debris was removed by centrifugation at 36,000 × *g* for 20 min at 4°C. Protein concentration was determined by the Bradford method (7) with Bio-Rad reagents.

Coordinate induction of the L-fucose–D-arabinose enzymes. To study the induction patterns of L-fucose permease, L-fucose isomerase, L-fuculokinase, and D-ribulokinase, cultures of strain EM2022 were prepared as follows. The inoculum was prepared by overnight growth of EM2022 in 20 ml of 0.5% casein hydrolysate minimal medium. The overnight culture was harvested and suspended in 5 ml of minimal salts medium. These cells were added to a 1-liter Bellco Nephelo culture flask containing 100 ml of 0.5% casein hydrolysate minimal medium until the OD_{660} reached 0.2. The culture was incubated until the OD_{660} reached 0.6, at which time L-fucose was added to 0.5%. A 25-ml sample

TABLE 1. Bacterial strains[a] and bacteriophages

Strain or phage	Parent strain	Relevant genotype	Relevant phenotype
Strains			
EM2000		Wild-type *E. coli* B (*fucA1*)	Dar$^+$ Fuc$^-$
EM2001	EM2000	*fucA*$^+$	Fuc$^+$ Dar$^+$
EM2022	EM2001	*fucA*$^+$ *araB rhaD rha-4*	Fuc$^+$ Dar$^+$ Rha$^-$
EM2102	EM2022	*fucA2*	Fuc$^-$ Dar$^+$
EM2401	EM2022	*fucI1*	Fuc$^-$ Dar$^-$
EM2402	EM2022	*fucI6*	Fuc$^-$ Dar$^-$
EM2501	EM2022	*fucK4*	Fuc$^-$ Dar$^+$
EM2502	EM2022	*fucK28*	Fuc$^-$ Dar$^+$
EM2522	EM2502	*fucK28 darK2*	Fuc$^-$ Dar$^-$
EM2524	EM2502	*fucK28 darK110*	Fuc$^-$ Dar$^-$
EM2542	EM2502	*fucK28 fucI107*	Fuc$^-$ Dar$^-$
EM2543	EM2502	*fucK28 fucI203*	Fuc$^-$ Dar$^-$
EM2601	EM2022	*fucC2*	Fuc$^+$ constitutive
EM2602	EM2022	*fucC4*	Fuc$^+$ constitutive
EM2651	EM2601	*fucC2 fucK24*	Fuc constitutive, Fuc$^-$
EM2701	EM2022	*rtlC*(H)	Hypo-RDH
EM2740	EM2402	*fucI6 rtlC*(H)	Fuc$^-$ Dar$^-$ hypo-RDH
EM2002	EM2000	*thyA fucA1*	Thy Fuc$^-$
EM2004	EM2002	*fucA1 darK2*	Fuc$^-$ Dar$^-$ Thy$^+$ (from EM2202)
EM2005	EM2002	*fucA1 darK110*	Fuc$^-$ Dar$^-$ Thy$^+$ (from EM2204)
JM3000	K20	*rtlC*	Rtl enzymes constitutive
EM4700	JM3000	*rtlC*(H) *rtlK48*	Rtl$^-$
WA837	*E. coli* B	*met-100 hsdR1*	Fuc$^-$ Dar$^-$
Bacteriophages[b]			
P1 *cam*		P1::Tn*9 clr-100*	
λ NK561		λ *b*221 *c*I::Tn*10 O*am29 *P*am80	

[a] All bacterial strains were constructed during this study except as follows. Wild-type *E. coli* B was from the Phabagen collection (strain PC0037) of the Department of Molecular Cell Biology, Section of Microbiology, State University of Utrecht, Utrecht, the Netherlands, and was a gift from C. Dowell; JM3000 was from J. Bartkus (4); WA837 (34) was from the Coli Genetic Stock Center (strain 5613). Phenotypes: Fuc, L-fucose; Dar, D-arabinose; Rtl, ribitol; Thy, thymine requirement. Genotypes: *fucA*, L-fuculose-1-phosphate aldolase; *fucI*, L-fucose isomerase; *fucK*, L-fuculokinase; *darK*, D-ribulokinase; *fucC*, constitutive synthesis of *fucA*, *fucI*, *fucK*, and *darK* products and L-fucose permease; *rtlK48*, lacks D-ribulokinase from the ribitol pathway; *rtlC*, constitutive synthesis of ribitol dehydrogenase and D-ribulokinase of the ribitol pathway; *rtlC*(H), hypoconstitutive ribitol dehydrogenase (hypo-RDH); *rhaD*, lacks L-rhamnulose-1-phosphate aldolase; *rha-4*, loss of L-rhamnose isomerase and L-rhamnulokinase activities.

[b] From Silhavy et al. (29).

was removed (zero time point) and replaced with 25 ml of warm 0.5% casein hydrolysate–0.5% L-fucose minimal salts medium. At 30-min intervals, 25-ml samples were removed and replaced with fresh, warm medium. After each sampling, cells were prepared and assayed for permease activity, after which the remaining cells were centrifuged, resuspended, and broken by sonication for the determination of L-fucose isomerase, L-fuculokinase, and D-ribulokinase activities.

Isolation of carbohydrate-negative mutants. Mutants lacking the ability to use specific carbohydrates were generated by chemical mutagenesis with ethylmethanesulfonate (EMS) (24). After EMS treatment, negative mutants were enriched with penicillin (15) and then identified by plating on EMB (eosin-methylene blue [24]) agar which contained the carbohydrate of interest at 1%.

Enzyme assays. L-Fucose permease activity was measured by the transport of L-[1-^{14}C]fucose by a method similar to that described by Roberts et al. (28), with the following changes: 0.073 ml of a 10^9-CFU/ml cell suspension were added to 10 mM KPO_4 (pH 7.0) buffer for final volume of 0.73 ml. L-[1-^{14}C]Fucose was added to a final concentration of 100 μM, with a radioactive label concentration of 0.5 μCi/ml. Samples were filtered (0.45-μm pore size) and washed with cold KPO_4 buffer. Filters were placed into 10 ml of Filtron-X (National Diagnostics Co.), and radioactivity was measured in a scintillation counter. L-Fucose isomerase activity was measured by determining the rate of NADH oxidation in a coupled assay system with purified ribitol dehydrogenase (21). In extracts lacking ribitol dehydrogenase activity, D-ribulokinase and L-fuculokinase activities were measured by determining the rate of NADH oxidation in a coupled assay system with pyruvate kinase and lactate dehydrogenase (1), except that glutathione was replaced with dithiothreitol in the reaction mixtures. Purified ketosugars were used as substrates at 10 mM (1.5 μmol) final concentration. In extracts which contained induced levels of ribitol dehydrogenase, kinase activities were measured in a two-step assay which determined ADP formation (19). In both types of assays, L-fuculose was used as the substrate for measuring L-fuculokinase and D-ribulose was used as the substrate for measuring D-ribulokinase. Ribitol dehydrogenase activity was measured by the rate of NADH oxidation in the presence of D-ribulose (33). L-Fuculose-1-phosphate aldolase activity was measured by determining the rate of NADH oxidation in a coupled assay with α-glycerol phosphate dehydrogenase (EC 1.1.1.8) (21). Background NADH oxidase levels in crude extracts did not require ion-exchange chromatography, as had been described previously (21). In extracts which contained measurable ribitol dehydrogenase activity, aldolase activity was measured in a two-step assay which determined dihydroxyacetone phosphate formation (17). This assay was modified by the addition of iodoacetate, pH 7.0, to the reaction mixture to a final concentration of 1.5 mM.

Screening procedure for constitutive isomerase activity in transductional crosses. To determine the cotransduction frequency of the constitutive mutations *fucC2* and *fucC4*, transductants were screened for constitutive synthesis of L-fucose isomerase by the following method. Transductants

were plated on LB plates (24) containing tetracycline to select for the *zfj*::Tn*10* insert of the *fucC* donor, and colonies were allowed to grow to a diameter of 3 to 4 mm. A heavy amount of cell paste from an isolated colony was suspended in 0.25 ml of 100 mM KPO_4 (pH 7.6) buffer, and then 0.25 ml of lysis solution (200 mM KPO_4 [pH 7.6], 8% Triton X-100, 80 mM D-arabinose) was added to the cell suspension and lightly vortexed. This assay mixture was incubated in a 37°C waterbath for 60 min, after which the entire volume was measured for D-ribulose production by the cysteine-carbazole method (16).

Isolation of a Tn*10* near the L-fucose–D-arabinose genes. An *hsdR E. coli* B strain, WA837 (P1 *cam*), was mutagenized with λ NK561 as described by Silhavy et al. (29). WA837 was an atypical *E. coli* B strain in that it could not use D-arabinose, could not mutate to grow on D-arabinose or L-fucose, and did not possess detectable levels of any L-fucose–D-arabinose enzymes. The reason for the D-arabinose–L-fucose-negative phenotype of this strain is unknown.

Approximately 19,000 Tn*10* insertion mutants were pooled, and a P1 lysate was prepared from the pool by heat induction of the P1 *cam* phage. This lysate was used to transduce a *thyA* mutant of EM2022 (Fuc^+ Dar^+) to thymine prototrophy and tetracycline resistance by selection on 0.5% D-glucose–tetracycline minimal salts agar plates. A P1 *cam* lysate was made on tetracycline-resistant prototrophic transductants and used to transduce EM2502 (Fuc^- Dar^+) to tetracycline resistance. Transductants were scored for growth on L-fucose minimal medium.

Transduction procedures. Transduction procedures for bacteriophage P1 have been described elsewhere (14, 29). Tetracycline resistance (15 μg/ml, final concentration) was the selective marker in all two- and three-factor crosses. Donor strains for two- and three-factor crosses were constructed by transduction of *zfj*::Tn*10* from EM2022 *zfj*::Tn*10* to donor strains. Selection for transductants of the ribitol–D-arabitol region was done by growth on 0.5% D-arabitol minimal salts agar.

Chemicals and reagents. L-Fucose, D-arabinose, ribitol, D-arabitol, tetracycline hydrochloride, trimethoprim, and EMS were purchased from Sigma Chemical Co., and L-[1-^{14}C]fucose was from New England Nuclear Corp. 6-Deoxy-L-talitol was prepared by E. J. St. Martin (32). L-Fucose and L-fuculose contaminating the 6-deoxy-L-talitol preparation were separated by chromatography through a column of Dowex 50W-X4 (200/400 mesh) resin (in the Ca^{2+} form), eluting with distilled water (2). L-Fuculose was prepared from L-fucose by using whole cells as described previously (4). D-Ribulose was prepared from ribitol as described previously (25), and contaminating ribitol was removed by column chromatography as described above.

RESULTS

Induction of L-fucose–D-arabinose enzymes. Wild-type *E. coli* B strain EM2000 was capable of inducing L-fucose permease, L-fucose isomerase, L-fuculokinase, and D-ribulokinase activities when grown on casein hydrolysate in the presence of either L-fucose or D-arabinose. L-Fuculose-1-phosphate aldolase was not detectable under these conditions (Table 2). EM2000 could not grow on L-fucose owing to the lack of aldolase activity but could grow on D-arabinose, without the need for a mutation, at a rate of 2.6 h per doubling. Mutants which used L-fucose were obtained by plating 2×10^8 CFU/ml on 0.5% L-fucose minimal salts plates, and L-fucose-positive colonies arose at a frequency of 1.8×10^{-6} in 3 days. L-Fucose-positive mutants such as EM2022 retained their ability to grow on D-arabinose and induced an L-fuculose-1-phosphate aldolase activity when grown in the presence of L-fucose or D-arabinose (Table 2). L-Fucose-positive EM2022 grew on L-fucose and D-arabinose with growth rates of 2.4 and 2.2 h per doubling, respectively.

EM2022 was used in induction experiments, and L-fucose permease, L-fucose isomerase, L-fuculokinase, and D-ribulokinase were shown to be coordinately induced by either L-fucose (Fig. 2) or D-arabinose. Induction of the enzymes began by 30 min after addition of L-fucose and reached maximal levels by 3 h. *E. coli* K-12 has been shown to begin induction of the L-fucose enzymes by 1.5 h after the addition of L-fucose and not to reach maximal levels until at least 9 h after the addition of L-fucose (D. J. LeBlanc, Ph.D. thesis, University of Massachusetts, Amherst, 1970). The apparent inducer of the L-fucose enzymes in *E. coli* K-12 has been shown to be L-fuculose-1-phosphate (4). The rapid induction seen in *E. coli* B indicated that the inducer might be made, or accumulated earlier, during growth on L-fucose than it was in *E. coli* K-12.

Identification of L-fucose and D-arabinose as apparent inducers. L-Fucose-negative mutants of EM2022 lacking L-fucose isomerase, L-fuculokinase, or L-fuculose-1-phosphate aldolase were isolated after EMS mutagenesis and penicillin enrichment. These mutants fell into two classes regarding growth on D-arabinose. Class 1 L-fucose-negative mutants retained the ability to grow on D-arabinose and lacked either L-fuculokinase (EM2501, EM2502; Table 2) or L-fuculose-1-phosphate aldolase activity (EM2102; Table 2), but were capable of inducing the remaining enzymatic activities, including D-ribulokinase, when grown in the presence of either L-fucose or D-arabinose.

Class 2 mutants had lost the ability to use D-arabinose. These strains did not synthesize L-fucose isomerase but could induce the remaining enzymatic activities when grown in the presence of either L-fucose or D-arabinose (EM2401, EM2402; Table 2). The induction data obtained from the L-fucose isomerase-negative mutants showed that the actions of the isomerase and L-fuculokinase were not required for inducer synthesis and that L-fucose and D-arabinose were the apparent inducers of the L-fucose–D-arabinose pathway enzymes in *E. coli* B.

Since the L-fuculokinase of *E. coli* B phosphorylated D-ribulose with 73% of the efficiency seen with L-fuculose as the substrate (Elsinghorst and Mortlock, submitted for publication), some of the D-ribulokinase activity seen in extracts containing L-fuculokinase was due to this enzyme. The level of D-ribulose-phosphorylating activity in extracts containing both L-fuculokinase and D-ribulokinase was greater than the level of L-fuculose phosphorylation, which indicated that D-ribulokinase was also induced by L-fucose and D-arabinose. In order to verify the induction and regulatory pattern of D-ribulokinase, strains were constructed which lacked both L-fuculokinase and L-fucose isomerase. EM2502 [Fuc^- (*fucK*) Dar^+] was mutagenized with EMS, and D-arabinose-negative mutants were isolated. D-Arabinose-negative mutants fell into two classes. Class 1 mutants lacked D-ribulokinase activity (EM2522, EM2524; Table 2) but retained the ability to induce L-fucose permease, L-fucose isomerase, and L-fuculose-1-phosphate aldolase activities when grown in the presence of either L-fucose or D-arabinose. Class 2 D-arabinose-negative mutants lacked L-fucose isomerase activity (EM2542, EM2543; Table 2) but retained the ability to induce L-fucose permease, D-ribulokinase, and L-fuculose-

TABLE 2. Enzyme activities of L-fucose- and D-arabinose-induced cultures

Strain	Relevant genotype[a]	Growth substrate[b]	Sp act[c]				
			L-Fucose permease	L-Fucose isomerase	L-Fuculokinase	D-Ribulokinase	L-Fu-1-phosphate aldolase
EM2000	Wild type (*fucA1*)	D-Arabinose	2.85	400	100	310	<5
		L-Fucose	2.45	460	120	360	<5
EM2022	*fucA*$^+$	D-Arabinose	5.25	592	154	304	64
		L-Fucose	6.52	588	194	222	83
EM2102	*fucA2*	D-Arabinose	3.02	410	161	323	<5
		L-Fucose	3.00	534	206	381	<5
EM2401	*fucI1*	D-Arabinose	10.09	<5	330	380	101
		L-Fucose	11.91	<5	531	567	119
EM2402	*fucI6*	D-Arabinose	10.48	<5	284	366	94
		L-Fucose	17.18	<5	376	446	78
EM2501	*fucK4*	D-Arabinose	7.37	864	<5	164	59
		L-Fucose	9.43	1,060	<5	227	71
EM2502	*fucK28*	D-Arabinose	8.69	874	<5	264	68
		L-Fucose	12.37	892	<5	315	73
EM2522	*fucK28 darK2*	D-Arabinose	10.38	541	<5	<5	48
		L-Fucose	11.42	616	<5	<5	53
EM2524	*fucK28 darK110*	D-Arabinose	14.81	1,170	<5	<5	53
		L-Fucose	14.66	861	<5	<5	66
EM2542	*fucK28 fucI107*	D-Arabinose	ND[d]	<5	<5	249	50
		L-Fucose	ND	<5	<5	464	60
EM2543	*fucK28 fucI203*	D-Arabinose	12.27	<5	<5	502	72
		L-Fucose	12.24	<5	<5	326	90
EM2601	*fucC2*	Casein hydrolysate	26.04	1,185	431	744	121
EM2602	*fucC4*	Casein hydrolysate	20.94	1,947	538	835	138
EM2651	*fucC2 fucK24*	Casein hydrolysate	12.91	1,464	<5	350	ND

[a] Genotypes: *fucA*, L-fuculose-1-phosphate aldolase; *darK*, D-ribulokinase; *fucI*, L-fucose isomerase; *fucK*, L-fuculokinase; *fucC*, constitutive synthesis of *fucA*, *darK*, *fucI*, *fucK*, and L-fucose permease.

[b] All cultures were grown in minimal salts broth containing 0.5% casein hydrolysate with 0.5% D-arabinose or 0.5% L-fucose.

[c] Specific activity is expressed in the following units: L-fucose permease, nanomoles of L-[1-^{14}C]fucose transported per minute per milligram of cells (dry weight); L-fucose isomerase, nanomoles of L-fucose isomerized per minute per milligram of protein; L-fuculokinase and D-ribulokinase, nanomoles of substrate phosphorylated per minute per milligram of protein; L-fuculose-1-phosphate aldolase, nanomoles of dihydroxyacetone phosphate formed per minute per milligram of protein. Casein hydrolysate-grown cultures of all the strains listed (except those bearing *fucC* mutations) had undetectable levels of all the enzymes (<0.1 unit for permease and <5.0 units of all other enzymes).

[d] ND, Not determined.

1-phosphate aldolase activities when grown in the presence of either L-fucose or D-arabinose. The class 2 mutants showed that the action of the isomerase was not required for induction of D-ribulokinase and that L-fucose and D-arabinose were the apparent inducers of this enzyme.

L-Fuculose-1-phosphate and D-ribulose-1-phosphate are not inducers of the L-fucose pathway in *E. coli* B. To determine whether L-fuculose-1-phosphate and D-ribulose-1-phosphate could also act as inducers of the *E. coli* B enzymes, it was necessary to have them synthesized from substrates other than L-fucose or D-arabinose, since these sugars were known effectors. Therefore, the phosphorylated intermediates were formed intracellularly by using strains possessing a constitutive ribitol dehydrogenase and with 6-deoxy-L-talitol and ribitol as precursors, respectively. The novel methylpentitol 6-deoxy-L-talitol can be metabolized by using the ribitol and L-fucose pathway enzymes. Mutants that grow on this sugar constitutively synthesize ribitol dehydrogenase, which oxidizes 6-deoxy-L-talitol to L-fuculose. L-Fuculose is further metabolized by the L-fucose pathway enzymes (Fig. 1) (32).

The D-ribulokinase of the ribitol pathway would interfere with the interpretation of these induction experiments; therefore, a mutant blocked in this enzyme was isolated. JM3000, a ribitol-positive K-12 strain which synthesized ribitol dehydrogenase and D-ribulokinase constitutively (4), was mutagenized with EMS, and ribitol-negative mutants were isolated after penicillin enrichment. EM4700 was a ribitol-negative mutant of JM3000 which lacked D-ribulokinase activity and hypoconstitutively synthesized ribitol dehydrogenase but induced this enzyme to higher levels in the presence of ribitol. Growth on 6-deoxy-L-talitol requires a constitutive ribitol dehydrogenase (32), and the levels found in EM4700 were sufficient for growth on that substrate.

A P1 *cam* lysate of EM4700 was used to transduce its ribitol–D-arabitol region to EM2022 (Fuc$^+$ Dar$^+$) and EM 2402 [Fuc$^-$ Dar$^-$ (*fucI*)]. Ribitol dehydrogenase was used as an alternative route for the intracellular production of D-ribulose or L-fuculose by growth in the presence of ribitol or 6-deoxy-L-talitol, respectively. As L-fucose isomerase and L-fucose permease are not required for growth on either of these sugars, the regulation of the fucose pathway beyond the isomerase step could be studied. EM2740 [Fuc$^-$ Dar$^-$ (*fucI*)] (ribitol dehydrogenase constitutive) could not use either ribitol or 6-deoxy-L-talitol and could not induce any of the L-fucose or D-arabinose enzymes when grown in the presence of either ribitol or 6-deoxy-L-talitol (Table 3). Under these conditions, ribitol dehydrogenase would form D-ribulose and L-fuculose from ribitol and 6-deoxy-L-talitol, respectively. Basal levels of L-fuculokinase in this strain would lead to the formation of D-ribulose-1-phosphate or L-fuculose-1-phosphate, and if these intermediates could act as inducers, then increased levels of the remaining enzymes would have been measured.

Addition of L-fucose to the L-fucose isomerase-negative strain EM2740 [Fuc$^-$ Dar$^-$ (*fucI*), ribitol dehydrogenase constitutive] allowed growth on ribitol and on 6-deoxy-L-talitol (Fig. 3) by inducing the remaining enzymes, including L-fuculokinase and D-ribulokinase, which could then con-

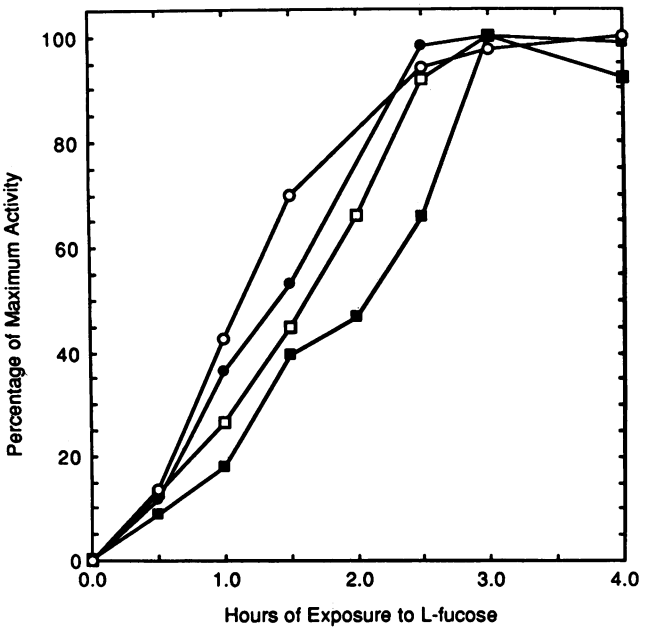

FIG. 2. Coordinate induction of L-fuculokinase, D-ribulokinase, L-fucose isomerase, and L-fucose permease in EM2022. The specific activities are expressed as a percentage of the maximum specific activity. Symbols and maximum specific activities: ○, L-fuculokinase, 200 nmol of L-fuculose phosphorylated per min per mg of protein; ●, D-ribulokinase, 341 nmol of D-ribulose phosphorylated per min per mg of protein; □, L-fucose isomerase, 800 nmol of L-fucose isomerized per min per mg of protein; ■, L-fucose permease, 71.2 nmol of L-[1-^{14}C]fucose transported per min per mg of cell dry weight.

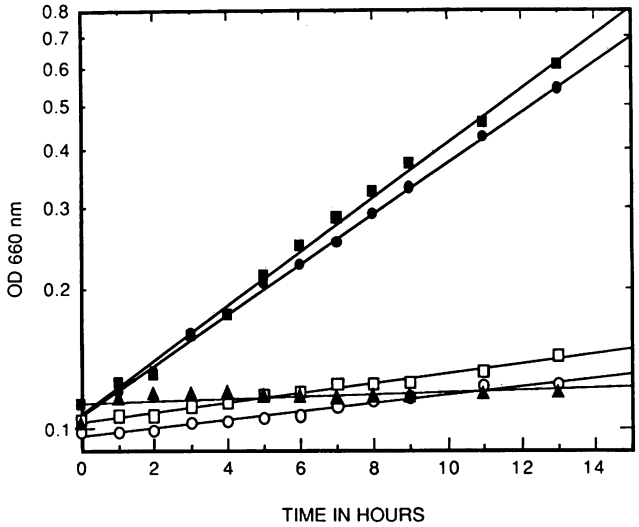

FIG. 3. Growth of EM2740 on ribitol and 6-deoxy-L-talitol when induced by L-fucose. Growth curves for EM2740 (Fuc$^-$ Dar$^-$ [lacks L-fucose isomerase], ribitol dehydrogenase constitutive) on the following carbohydrates: ○, 0.1% 6-deoxy-L-talitol; ●, 0.1% 6-deoxy-L-talitol plus 0.5% L-fucose; □, 0.1% ribitol; ■, 0.1% ribitol plus 0.5% L-fucose; ▲, 0.5% L-fucose.

tinue the catabolism of the D-ribulose or L-fuculose that was produced. Without L-fucose for induction, growth on ribitol and 6-deoxy-L-talitol did not occur (Fig. 3).

The ability of EM2740 to grow on ribitol and 6-deoxy-L-talitol after induction of D-ribulokinase and L-fuculokinase showed that intracellular pools of D-ribulose and L-fuculose were being formed by ribitol dehydrogenase. Additionally, strain EM2701 (Fuc$^+$ Dar$^+$; ribitol dehydrogenase constitutive) grew on ribitol or 6-deoxy-L-talitol as a sole carbon and energy source, which illustrated that ribitol dehydrogenase was capable of producing intracellular pools of D-ribulose and L-fuculose.

These experiments have shown that L-fuculose-1-phosphate and D-ribulose-1-phosphate do not act as inducers of the L-fucose–D-arabinose enzymes in *E. coli* B. Additionally, L-fuculose, D-ribulose, and D-ribulose-5-phosphate could not be inducers, since these compounds would also have been produced during these experiments.

Isolation of L-fucose constitutive mutants. After eight serial transfers of EM2022 (Fuc$^+$ Dar$^+$) in 0.1% L-fucose minimal medium (approximately 86 generations), mutants were isolated which showed improved growth on L-fucose. These mutants were initially identified by their colony morphology; fast-growing mutants produced colonies two to three times the size of the parent strain when grown on 0.1% L-fucose minimal plates. The growth rates of the fast-growing mutants on 0.5% L-fucose and D-arabinose were 1.4 and 1.2 h per doubling, respectively, whereas the growth rates of the inducible parent strain were 2.4 and 2.2 h per doubling, respectively.

Two independently isolated fast-growing mutants, EM 2601 and EM2602, were found to synthesize L-fucose permease, L-fucose isomerase, L-fuculokinase, D-ribulokinase, and L-fuculose-1-phosphate aldolase constitutively (Table 2). These activities were not inducible to higher levels by growth in the presence of L-fucose. L-Fuculokinase could phosphorylate D-ribulose; therefore, to verify the constitutive synthesis of D-ribulokinase, a mutant which lacked

TABLE 3. Enzyme activities of EM2740: induction by L-fucose, D-arabinose, ribitol, and 6-deoxy-L-talitol[a]

Growth substrate[b]	Sp act[c] (nmol/min per mg of protein)					
	L-Fucose permease	L-Fucose isomerase	L-Fuculokinase	D-Ribulokinase	Ribitol dehydrogenase	L-Fuculose-1-phosphate aldolase
CH	<0.10	<5	<5	<5	195	<5
CH + L-fucose	8.00	<5	124	198	213	88
CH + D-arabinose	3.80	<5	158	182	180	75
CH + ribitol	<0.10	<5	<5	<5	1,629	<5
CH + 6-d-talitol	<0.10	<5	<5	<5	380	<5

[a] EM2740 is L-fucose and D-arabinose negative owing to the lack of L-fucose isomerase (*fucI6*), has hypoconstitutive ribitol dehydrogenase, and lacks the ribitol pathway D-ribulokinase.

[b] Cultures were grown in 0.5% casein hydrolysate (CH) minimal salts broth containing, as indicated, 0.5% D-arabinose, 0.5% L-fucose, 0.5% ribitol, or 0.1% 6-deoxy-L-talitol (6-d-talitol).

[c] Specific activity is expressed as described in Table 2, footnote *c*, plus ribitol dehydrogenase, nanomoles of D-ribulose reduced.

TABLE 4. Cotransduction of L-fucose–D-arabinose alleles with *zfj*::Tn*10*[a]

Donor (*zfj*::Tn*10*)	Recipient	Phenotype scored	No. scored	No. recombinant	% Cotransduction	Location[b] (min)
EM2022	EM2000 (*fucA1*)	Fuc$^+$	2,048	1,074	52.4	59.66
	EM2102 (*fucA2*)	Fuc$^+$	587	299	50.9	59.68
EM2502 (*fucK28*)	EM2522 (*fucK28 darK2*)	Dar$^+$	846	336	39.7	59.85
	EM2524 (*fucK28 darK110*)	Dar$^+$	1,137	446	39.2	59.86
EM2022	EM2401 (*fucI1*)	Dar$^+$	682	259	38.0	59.88
	EM2402 (*fucI6*)	Dar$^+$	1,152	430	37.3	59.90
EM2502 (*fucK28*)	EM2542 (*fucK28 fucI107*)	Dar$^+$	944	348	36.9	59.90
	EM2543 (*fucK28 fucI203*)	Dar$^+$	2,032	764	37.6	59.89
EM2022	EM2501 (*fucK4*)	Fuc$^+$	742	265	35.7	59.93
	EM2502 (*fucK28*)	Fuc$^+$	2,178	762	35.0	59.95
EM2601 (*fucC2*)	EM2022	Constitutivity	200	62	31.0	60.05
EM2602 (*fucC4*)	EM2022	Constitutivity	200	59	29.5	60.09
EM2000	EM2002 (*thyA*)	Prototrophy	558	12	2.2	

[a] P1 *cam* lysate grown on each donor strain was used to transduce recipient strains to tetracycline resistance, and transductants were scored for growth on L-fucose (Fuc) or D-arabinose (Dar), depending on the cross. Constitutivity was determined by measuring L-fucose isomerase. Gene designations are described in Table 1, footnote *a*, plus *thyA*, thymidylate synthetase (located at 61 min).

[b] Distance from *zfj*::Tn*10* at 59.4 min calculated by the formula given in Ingraham et al. (20).

L-fuculokinase was isolated. L-Fucose-negative mutants of EM2601 were isolated after EMS mutagenesis and penicillin enrichment. EM2651, an L-fucose-negative mutant of EM 2601, lacked L-fuculokinase activity but synthesized constitutively all of the remaining enzymes, including D-ribulokinase (Table 2).

Mapping of the L-fucose–D-arabinose genes. A Tn*10* insertion was used as an external reference point to map the L-fucose–D-arabinose genes in two- and three-factor crosses, using bacteriophage P1. A Tn*10* insertion near the L-fucose–D-arabinose genes was isolated and found to be 31 to 52% cotransducible with the L-fucose genes and 2.2% cotransducible with the *thyA* locus at 61 min, placing the insert at 59.4 min on the *E. coli* chromosome, based on the formula given by Ingraham et al. (20). The designation *zfj*::Tn*10* was given to this insertion, as recommended by the rules of Chumley et al. (13). The results of two- and three-factor crosses are shown in Tables 4 and 5, respectively, and the gene order indicated by those crosses is shown in Fig. 4.

DISCUSSION

It is generally accepted that rapid growth is a highly selected characteristic in bacteria. An organism with a higher growth rate resulting in an equal final yield under identical growth conditions would be considered more efficient. In the development of a pathway for the catabolism of a carbon source, such as D-arabinose, selective pressures for rapid growth should direct the evolutionary steps taken during that development. *E. coli* B has a naturally evolved pathway for D-arabinose and grows on D-arabinose with a doubling time of 2.6 h. In contrast, D-arabinose-positive mutants of *E. coli* K-12 grow on D-arabinose with a doubling time of 4.2 h (3). The faster growth rate of *E. coli* B on D-arabinose suggests that it is further evolved towards a more efficient D-arabinose pathway. The similarities and differences between this pathway in *E. coli* B and experimentally evolved D-arabinose pathways in *E. coli* K-12, *K. aerogenes* W-70, and *K. pneumoniae* PRLR3 should be instructive in elucidating the evolutionary steps taken by nature during the development of a pathway.

The presence of the ribitol operon provides an advantage to *E. coli* K-12 strains growing on D-arabinose. While the doubling time on D-arabinose is approximately the same in *E. coli* K-12 strains with and without the ribitol operon (4.2 h), competition studies in D-arabinose chemostats have shown that ribitol-positive strains have a significant competitive advantage over ribitol-negative strains (3). Studies with *K. aerogenes* W-70 have shown that metabolism of D-arabinose via the ribitol pathway enzymes is more efficient than metabolism through the L-fucose pathway enzymes (31). It appears that a natural step towards the evolution of a true D-arabinose pathway would be phosphorylation of D-ribulose at the C-5 position.

Boulter et al. found that *E. coli* B/r had a D-ribulokinase which phosphorylated D-ribulose at the C-5 position (5). This observation was confirmed for the *E. coli* B D-ribulokinase by the identification of C-5 as the site of D-ribulose phosphorylation (data not shown). The D-ribulokinase activity induced by L-fucose and D-arabinose is not part of the ribitol operon since *E. coli* does not grow, and apparently cannot mutate to grow, on ribitol (27). The gene encoding D-ribulokinase was found to map within the L-fucose gene cluster at 60 min on the *E. coli* B chromosome, while the ribitol operon is located at 47 min in *E. coli* C (27). Furthermore, the ribitol operon is mutually exclusive with the galactitol operon (23), and *E. coli* B grows on galactitol.

In *E. coli* K-12 and *K. aerogenes* W-70, the apparent inducer of L-fucose permease, L-fucose isomerase, and L-fuculokinase is L-fuculose-1-phosphate (4, 30). D-Ribulose-1-phosphate acts as the alternate inducer in D-arabinose-positive mutants. D-Ribulose is the inducer of ribitol dehydrogenase and D-ribulokinase of the ribitol pathway (9). In contrast, L-fucose and D-arabinose were found to be the apparent inducers of L-fucose permease, L-fucose isomerase, L-fuculokinase, L-fuculose-1-phosphate aldolase, and D-ribulokinase in *E. coli* B. Additionally, L-fuculose-1-phosphate, D-ribulose-1-phosphate, L-fuculose, D-ribulose, and D-ribulose-5-phosphate do not act as inducers of any of the *E. coli* B enzymes, including D-ribulokinase. Boulter et al. isolated L-fucose isomerase-negative mutants of *E. coli* B/r which were capable of inducing a kinase activity when grown in the presence of D-arabinose (5). The kinase activity of these isomerase-negative mutants was not resolved into L-fuculokinase and D-ribulokinase activities, but the induction which was observed indicated that D-arabinose was an apparent inducer of the L-fucose–D-arabinose enzymes in *E. coli* B/r. While it has not been observed in the laboratory, a change in the L-fucose regulatory protein to accept either

TABLE 5. Reciprocal three-factor crosses of L-fucose–D-arabinose alleles[a]

Donor (*zfj*::Tn*10*)	Recipient	Phenotype scored	No. scored	No. recombinant	% Recombinant
EM2022	EM2524 (*fucK28 darK110*)	Fuc⁻ Dar⁺	1,098	115	10.5
EM2524 (*fucK28 darK110*)	EM2022		989	53	5.3
EM2524 (*darK110*)	EM2543 (*fucI203*)	Dar⁺	1,023	36	3.5
EM2543 (*fucI203*)	EM2524 (*darK110*)		1,087	101	9.3
EM2542 (*fucI107*)	EM2524 (*darK110*)	Dar⁺	951	8	8.4
EM2524 (*darK110*)	EM2524 (*fucI107*)		1,248	1	0.1
EM2022	EM2005 (*fucA1 darK110*)	Fuc⁻ Dar⁺	921	5	0.5
EM2005 (*fucA1 darK110*)	EM2022		1,083	50	4.6
EM2022	EM2522 (*fucK28 darK2*)	Fuc⁻ Dar⁺	957	77	8.0
EM2522 (*fucK28 darK2*)	EM2022		831	20	2.4
EM2542 (*fucI107*)	EM2522 (*darK2*)	Dar⁺	866	91	10.5
EM2522 (*darK2*)	EM2542 (*fucI107*)		894	12	1.3
EM2022	EM2004 (*fucA1 darK2*)	Fuc⁻ Dar⁺	749	15	2.0
EM2004 (*fucA1 darK2*)	EM2022		747	74	9.9
EM2022	EM2543 (*fucK28 fucI203*)	Fuc⁻ Dar⁺	1,005	35	3.5
EM2543 (*fucK28 fucI203*)	EM2022		1,027	1	0.1
EM2542 (*fucK28 fucI107*)	EM2022	Fuc⁻ Dar⁺	1,007	4	0.4
EM2022	EM2542 (*fucK28 fucI107*)		799	44	5.5
EM2402 (*fucI6*)	EM2502 (*fucK28*)	Fuc⁺	1,042	6	0.6
EM2502 (*fucK28*)	EM2402 (*fucI6*)		1,018	21	2.1
EM2000 (*fucA1*)	EM2402 (*fucI6*)	Fuc+	985	9	0.9
EM2402 (*fucI6*)	EM2000 (*fucA1*)		1,051	69	6.6
EM2402 (*fucI6*)	EM2501 (*fucK4*)	Fuc⁺	672	3	0.4
EM2501 (*fucK4*)	EM2402 (*fucI6*)		669	46	6.9

[a] P1 *cam* lysate grown on each donor strain was used to transduce recipient strains to tetracycline resistance, and transductants were scored for growth on L-fucose (Fuc) or D-arabinose (Dar), depending on the cross. Gene designations: see Table 1, footnote *a*.

D-arabinose or D-ribulose as an inducer has been predicted as an improvement for the D-arabinose pathway of *E. coli* K-12 (3).

A predicted step in the evolution of D-arabinose metabolism, which has been observed in the laboratory (3), is the loss of L-fuculose-1-phosphate aldolase activity (30). Aldolase activity is not required to synthesize the inducer of the L-fucose pathway enzymes in *E. coli* K-12 or *K. aerogenes* W-70. The selective advantage of an aldolase-negative mutation would be to increase the pools of D-ribulose-1-phosphate by preventing its metabolism, thereby allowing a higher level of L-fucose enzyme induction during growth on D-arabinose. Indeed, accumulation of sufficient inducer to allow maximal induction of the L-fucose enzymes appeared to be a problem for ribitol-positive *E. coli* K-12 strains and *K. aerogenes* W-70 grown on D-arabinose; L-fucose isomerase and L-fuculokinase were induced by D-arabinose to 14 to 50% of the levels found in L-fucose-induced cultures (3, 30, 31). In *E. coli* K-12 strains without the ribitol pathway, D-arabinose induced the L-fucose enzymes to 100% of the levels found in L-fucose-grown cells (21).

E. coli recognizes L-fucose and D-arabinose as the apparent inducers of the enzymes required for growth on D-arabinose and would not need an aldolase-negative mutation to increase the pools of inducer, as seen in *E. coli* K-12 and *K. aerogenes*. In fact, there appears to be a disadvantage to

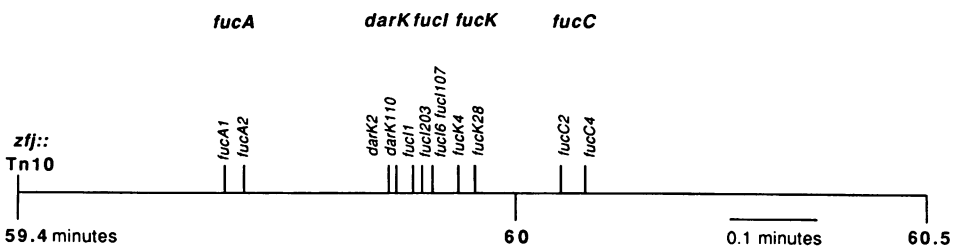

FIG. 4. Order of some of the L-fucose–D-arabinose genes in *E. coli* B. Order of genes based on results of two- and three-factor crosses. Gene designations: see Table 1, footnote *a*.

the aldolase-negative mutation, as seen by comparing the D-arabinose growth rates of L-fucose-positive and -negative *E. coli* B strains. Wild-type, L-fucose-negative strains grew on D-arabinose with a doubling time of 154 min, while L-fucose-positive strains grew on D-arabinose with a doubling time of 130 min. The improved D-arabinose growth rate seen in L-fucose-positive strains indicated that the accumulation of D-ribulose-1-phosphate by the wild type during growth on D-arabinose was detrimental to the cells. The detrimental effect of this accumulated intermediate was greater than the energy loss by metabolism of D-ribulose through the L-fucose pathway in the aldolase-positive revertant.

D-Arabinose-positive *E. coli* B could prevent metabolism of D-ribulose through the 1-phosphate route by a structural mutation in either the L-fuculokinase or aldolase activities, since they are not needed for inducer synthesis or growth on D-arabinose. Several wild-type *E. coli* B strains from different sources were surveyed and found to be unable to use L-fucose owing to the lack of aldolase activity. The absence of L-fuculokinase negative mutants and the lack of an obvious selective advantage to the aldolase-negative mutation suggest that the L-fucose–D-arabinose enzymes of *E. coli* B were originally induced by L-fuculose-1-phosphate and D-ribulose-1-phosphate and that a regulatory change allowing induction by L-fucose and D-arabinose came after the aldolase mutation.

A difference between the L-fucose pathways of *E. coli* B and K-12 is the relative ease with which constitutive mutants can be isolated from strain B. *E. coli* B mutants that constitutively synthesized L-fucose permease, L-fucose isomerase, L-fuculokinase, D-ribulokinase, and L-fuculose-1-phosphate aldolase were isolated during selection on L-fucose. Growth on L-fucose does not require the activity of D-ribulokinase, yet it was synthesized constitutively by the mutants, indicating that it is a member of the L-fucose regulon. The frequency of mutation to constitutive synthesis is unknown, but apparently a single mutation can result in this genotype, since it was transducible and two independently isolated mutations mapped to the same position in the L-fucose gene cluster, although the possibility of two closely linked mutations cannot be ruled out.

Two types of *E. coli* K-12 mutants which constitutively synthesized L-fucose permease, L-fucose isomerase, and L-fuculokinase have been isolated and described. One type of mutation was unlinked to the L-fucose gene cluster, while the other type was tightly linked to the L-fucose genes and appeared to be located in a regulatory protein (3, 4, 10). These closely linked regulatory mutations may be analogous to the constitutive mutations isolated from *E. coli* B. The unlinked mutation of *E. coli* K-12 results in an L-fucose-negative phenotype, with the L-fucose permease, isomerase, and kinase being synthesized constitutively, but leaving the aldolase noninducible (3) unless a previous regulatory alteration rendered the aldolase constitutive and phenotypically L-fucose positive (10). An unlinked regulatory mutation has not been isolated in *E. coli* B, but our constitutive mutation selection procedure would not have permitted the growth of such a mutant.

The genetic map of the *E. coli* B L-fucose genes was consistent with the order of those genes in *E. coli* K-12 (8; J. Bartkus and R. P. Mortlock, unpublished data). The gene encoding D-ribulokinase was found within the L-fucose gene cluster, specifically between the genes encoding L-fuculose-1-phosphate aldolase and L-fucose isomerase. The D-ribulokinase gene was designated *darK* in order to differentiate it from the D-ribulokinase of the ribitol pathway and the L-fuculokinase of the L-fucose pathway.

The gene encoding L-fucose permease (*fucP*) is also located in the L-fucose gene cluster (8; J. Bartkus and R. P. Mortlock, unpublished data). The position of *darK* relative to *fucP* is uncertain due to the lack of any L-fucose permease-negative mutants of *E. coli* B. It is unknown why permease mutants were not isolated by our mutant selection procedures, but perhaps transport of L-fucose and D-arabinose by nonspecific transport mechanisms was sufficient to allow slow growth, yet weak enough to avoid detection in L-fucose transport assays of uninduced cells. Such slow-growing mutants would not have been considered in our screening, which was for stable, nongrowing mutants. The product of the *fucO* gene, L-1,2-propanediol oxidoreductase (EC 1.1.1.77), was not studied in these experiments, and the location of the *fucO* gene in *E. coli* has not been determined. The genes of the L-fucose pathway in *E. coli* B appear to function as a single regulon, but the number of operons within that regulon is unknown.

These experiments suggest that *E. coli* B has evolved towards the metabolism of D-arabinose from an *E. coli* K-12-like ancestor, rather than that *E. coli* K-12 has evolved towards L-fucose metabolism from an *E. coli* B-like ancestor. The loss of aldolase activity and the natural recognition of D-arabinose by the L-fucose regulatory system in *E. coli* B are strong evidence that the L-fucose regulon of *E. coli* B is further evolved than that regulon in *E. coli* K-12.

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