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# Cross-Induction of the L-Fucose System by L-Rhamnose in *Escherichia coli*

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**Dissimilation of L-fucose as a carbon and energy source by *Escherichia coli* involves a permease, an isomerase, a kinase, and an aldolase encoded by the *fuc* regulon at minute 60.2. Utilization of L-rhamnose involves a similar set of proteins encoded by the *rha* operon at minute 87.7. Both pathways lead to the formation of L-lactaldehyde and dihydroxyacetone phosphate. A common NAD-linked oxidoreductase encoded by *fucO* serves to reduce L-lactaldehyde to L-1,2-propanediol under anaerobic growth conditions, irrespective of whether the aldehyde is derived from fucose or rhamnose. In this study it was shown that anaerobic growth on rhamnose induces expression of not only the *fucO* gene but also the entire *fuc* regulon. Rhamnose is unable to induce the *fuc* genes in mutants defective in *rhaA* (encoding L-rhamnose isomerase), *rhaB* (encoding L-rhamnulose kinase), *rhaD* (encoding L-rhamnulose 1-phosphate aldolase), *rhaR* (encoding the positive regulator for the *rha* structural genes), or *fucR* (encoding the positive regulator for the *fuc* regulon). Thus, cross-induction of the L-fucose enzymes by rhamnose requires formation of L-lactaldehyde; either the aldehyde itself or the L-fuculose 1-phosphate (known to be an effector) formed from it then interacts with the *fucR*-encoded protein to induce the *fuc* regulon.**

L-Fucose and L-rhamnose are dissimilated by *Escherichia coli* in parallel ways (Fig. 1). The trunk pathway for each compound is mediated by a permease (21; J. Power, personal communication), an isomerase (18, 41, 44), a kinase (12, 23, 42, 45), and an aldolase (13, 14, 17, 35, 36). The two sugars differ in the stereoconfiguration at carbons 2 and 4, but structural differences in the intermediates disappear with cleavage of the phosphorylated ketose by the aldolase, yielding, in both cases, dihydroxyacetone phosphate and L-lactaldehyde.

Aerobically, L-lactaldehyde is converted by an NAD-linked dehydrogenase to L-lactate, which is oxidized to pyruvate for further metabolism (15, 39); anaerobically, L-lactaldehyde is reduced to L-1,2-propanediol, which is excreted into the medium (15, 40). The sacrifice of the aldehyde as a hydrogen sink increases the portion of dihydroxyacetone phosphate that can be utilized as a carbon and energy source.

The catalytic proteins in each trunk pathway and the corresponding positive regulatory protein are encoded by a single gene cluster: the *fuc* locus at minute 60.2 (1, 7, 16, 37, 38) and the *rha* locus at minute 87.7 (1, 34). The structural genes of the *fuc* system appear to be organized as a regulon comprising at least three operons (7, 20–22), with L-fuculose 1-phosphate as the effector (3). The *rha* system responds to L-rhamnose as the effector (34).

A common enzyme of broad function, encoded by the *ald* gene, which is linked to neither the *fuc* nor the *rha* locus, is responsible for the dehydrogenation of L-lactaldehyde to L-lactate (Y.-M. Chen, Y. Zhu, and E. C. C. Lin, J. Bacteriol., in press). Likewise, the reduction of L-lactaldehyde to L-1,2-propanediol is catalyzed by a common enzyme. However, the gene encoding this enzyme, *fucO*, is a member of the *fuc* regulon (5, 6, 9, 11, 15). How rhamnose causes the induction of *fucO* is the subject of this report.

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## MATERIALS AND METHODS

**Chemicals.** L-Lactaldehyde was prepared by the reaction of ninhydrin with D-threonine (46). L-Rhamnulose was prepared from a solution of L-rhamnose heated with pyridine (26). L-[U-$^{14}$C]rhamnose (45 to 60 mCi per milliatom of carbon) was purchased from Research Products International Corp., Mount Prospect, Ill. L-Fucose, L-rhamnose, and D-xylose were obtained from Sigma Chemical Co., St. Louis, Mo. Vitamin-free casein acid hydrolysate (CAA) was from ICN Nutritional Biochemicals, Cleveland, Ohio. The pyruvate kinase–L-lactate dehydrogenase mixture was from Boehringer Mannheim Biochemicals, Indianapolis, Ind. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) was from Bachem Inc., Torrance, Calif. All other chemicals were commercial products of reagent grade.

**Bacteria and phage.** The *E. coli* strains used are described in Table 1. Eight independent clones of strain ECL116 were mutagenized with ethyl methanesulfonate (28). Following overnight growth of the treated populations in glucose-mineral medium, a portion of each culture was enriched in rhamnose-negative mutants by the streptozotocin selection method (25). Survivors were plated on MacConkey agar containing 1% rhamnose as the sugar. Pale-colored colonies were screened on minimal agar containing rhamnose, fucose, or glucose. Those that failed to grow only on rhamnose were collected and examined for the specific nature of the lesion. Deficiencies of L-rhamnose permease, L-rhamnose isomerase, and L-rhamnulose kinase activities were detected by examining cells grown on CAA in the presence of rhamnose. Lack of L-rhamnulose 1-phosphate aldolase activity was diagnosed by inhibition of growth on glycerol in the presence of rhamnose (excessive accumulation of a phosphorylated sugar causes stasis). Impairment of the activator protein encoded by *rhaC* (recently resolved into two genes, *rhaR* and *rhaS* [see Results]) was revealed by pleiotropic defects in the enzymes of the rhamnose pathway (34) and by complementation with a plasmid, pJTC9, bearing

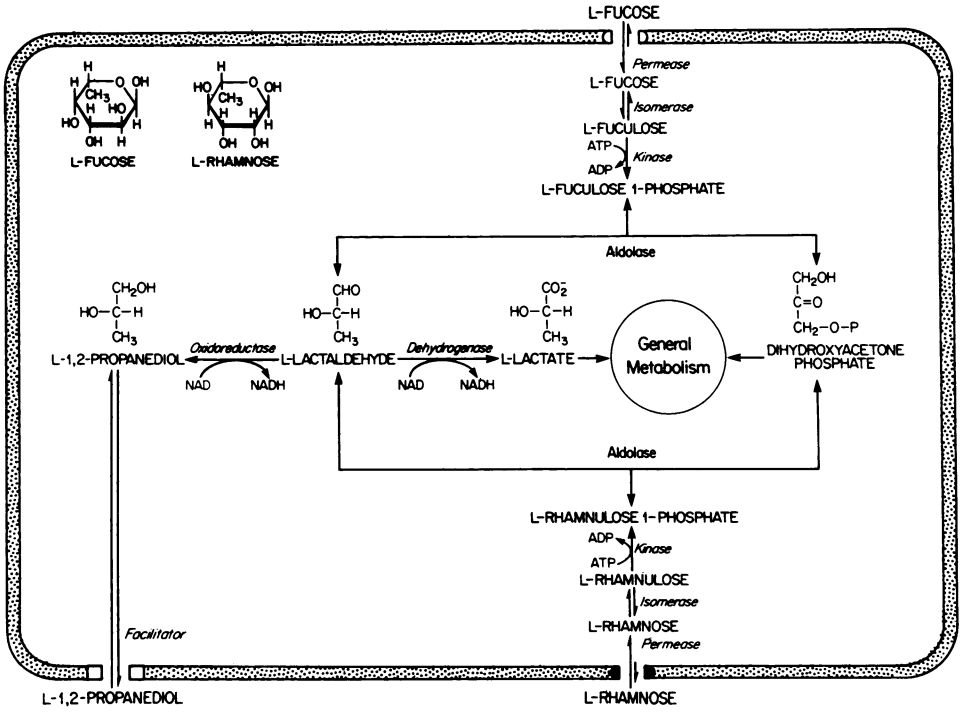


FIG. 1. Catabolic pathways for L-rhamnose and L-fucose in *E. coli*.

*rhaR*$^+$ *rhaS*$^+$ (see Construction of plasmids, below). Table 2 summarizes the characterization of the rhamnose-negative mutants. Strain ECL378 (Tn*10* 80% linked to *rha*$^+$) was used as a donor by P1 *vir* transduction (30) to test whether or not the mutations in rhamnose-negative strains ECL714 (*rhaB*), ECL715 (*rhaA*), ECL716 (*rhaD*), and ECL717 (*rhaR*) were at the *rha* locus. Strain ECL378 was constructed by transducing the Tn*10* from strain ECL339 (*zig-1*::Tn*10* Δ*rha*) to strain ECL116 (*rha*$^+$) by selection on LB agar containing tetracycline and scoring for red colonies on MacConkey-rhamnose-tetracycline agar.

The following procedure was used to generate an *arg* deletion extending into the *fuc* region. The *arg*::Tn*10* in strain ECL357 was transduced into strain ECL56, in which all of the fucose enzymes were expressed constitutively. A Tc$^r$ Arg$^-$ Fuc$^+$ (constitutive) transductant was used for generating *fuc* deletion mutants by selection against the Tn*10* conferring tetracycline resistance (4). Strain ECL478, identified as a Fuc$^-$ clone on MacConkey-fucose agar, was found to lack significant activities of all of the fucose enzymes and was thus presumed to have sustained a deletion extending from *arg* through the entire *fuc* region. To place a Tn*10* close to the *arg-fuc* deletion, the following procedure was used. Strain ECL289 (*eno fuc*$^+$ *argA*::Tn*10*) was first selected for the excision of Tn*10* (4). A derivative obtained (*eno fuc*$^+$ Δ*argA7*) was used as the recipient for transduction with a P1 lysate prepared from a population with random Tn*10* insertions in the chromosome. A Tc$^r$ Eno$^+$ transductant was in turn used as the donor of the two markers with the *eno fuc*$^+$ Δ*argA7* strain as the recipient. A transductant, ECL479, was found to have a Tn*10* 94% linked to *eno* and 46% linked to *argA*. Strain ECL479 (*zfj3*::Tn*10*) was used as a transduction donor to place the Tn*10* close to the Δ(*fuc-argA*) of strain ECL478. The Δ(*fuc-argA*) was then transduced into strain ECL116 by selecting for Tc$^r$ and scoring for Fuc$^-$ and Arg$^-$. Strain ECL366 (Δ[*fuc-argA*] *zfj3*::Tn*10*) was thus obtained.

Strain ECL711 was obtained by transducing the *rhaD62* mutation in strain ECL484 (by selecting for a Tn*10* placed nearby) into strain ECL326.

**Growth conditions.** Quantitative comparisons of enzyme activities were carried out with extracts of cells grown anaerobically at 37°C in 150-ml flasks filled to the top with medium, tightly capped, and gently stirred by a magnet. A mineral solution (43) supplemented with CAA (0.5%), pyruvate (30 mM), and thiamine (2 μg/ml) was used as the noninducing medium. The inducing medium also contained fucose or rhamnose (0.4%). For growth of strains carrying a plasmid vector, ampicillin (200 μg/ml) was added to the medium. For selecting or scoring antibiotic resistance, ampicillin was added to 200 μg/ml and tetracycline was added to 20 μg/ml. For the preparation of plasmid and chromosomal DNA, the cells were grown to stationary phase in LB medium.

**Preparation of cell extracts and enzyme assays.** Cells harvested from an exponentially growing culture (150 to 200 Klett units; no. 42 filter) were centrifuged and washed once with 0.1 M potassium phosphate (pH 7.0). The final pellet was weighed and dispersed in 4 volumes of the same buffer, and the cells were disrupted (1 min/ml of suspension) in a tube by a model 60 W ultrasonic disintegrator (MSE) while being chilled in a −10°C bath. The extract was centrifuged at 100,000 × *g* for 2 h at 4°C, and the supernatant fraction was used for enzyme assays.

L-Fucose isomerase and L-rhamnose isomerase activities were determined from the initial rate of ketose formation by the cysteine-carbazole method (46). L-Rhamnulose kinase activity was determined from the rate of NADH oxidation in the presence of L-rhamnulose, ATP, the pyruvate kinase–L-lactate dehydrogenase mixture (8 μg/ml), and phosphoenolpyruvate (12). L-1,2-Propanediol oxidoreductase activity was assayed by the rate of L-lactaldehyde-dependent oxidation of NADH (11). Protein concentrations in cell extracts

TABLE 1. *E. coli* strains

| *E. coli* strain | Genotype | Source or reference |
|---|---|---|
| Crookes | Wild type | ATCC 8739 |
| K-12 ECL1 | HfrC *phoA8 relA1 fhuA22* $T2^r$ (λ) | 27 |
| K-12 ECL56 | HfrC *phoA8 relA1 tonA22* $T2^r$ (λ) ($Fuc^+$ and constitutive) | 21 |
| K-12 ECL289 | HfrC *eno argA*::Tn*10 relA1 tonA22* $T2^r$ (λ) | 7 |
| K-12 ECL476 | HfrC *fucA514 phoA8 relA1 tonA22* $T2^r$ (λ) | T. Chakrabarti |
| K-12 ECL477 | HfrC *fucR501 phoA8 relA1 tonA22* $T2^r$ (λ) | Y.-M. Chen |
| K-12 ECL478 | HfrC Δ(*fuc-argA*)*1 phoA8 relA1 tonA22* $T2^r$ (λ) | This study |
| K-12 ECL479 | HfrC *zfj3*::Tn*10* Δ*argA7 relA1 ton22* $T2^r$ (λ) | This study |
| K-12 ECL484 | $F^+$ *rhaD62 metB1* | 34 |
| K-12 ECL116 | $F^-$ Δ*lacU169 endA hsdR thi* | 2 |
| K-12 ECL326 | $F^-$ Φ[*fucO1-lac*::λ p1(209)] Δ*lacU169 endA hsdR thi* | 11 |
| K-12 ECL333 | $F^-$ Φ[*rhaF1-lac*::λ p1(209)] Δ*lacU169 endA hsdR thi* | 10 |
| K-12 ECL335 | $F^-$ Φ[*rhaF2-lac*::λ p1(209)] Δ*lacU169 endA hsdR thi* | 10 |
| K-12 ECL339 | $F^-$ Δ(*rha-pfkA*)*15 zig-1*::Tn*10* Δ*lacU169 endA hsdR thi* | 10 |
| K-12 ECL357 | $F^-$ *arg*::Tn*10 recB21 thyA36* | M. Syvanen |
| K-12 ECL366 | $F^-$ Δ(*fuc-argA*)*1 zfj3*::Tn*10* Δ*lacU169 endA hsdR thi* | This study |
| K-12 ECL378 | $F^-$ *zig-1*::Tn*10* Δ*lacU169 endA hsdR thi* | This study |
| K-12 ECL711 | $F^-$ Φ[*fucO-lac*::λ p1(209)] *rhaD62 zig-1*::Tn*10* Δ*lacU169 endA hsdR thi* | This study |
| K-12 ECL714 | $F^-$ *rhaB101* Δ*lacU169 endA hsdR thi* | This study |
| K-12 ECL715 | $F^-$ *rhaA502* Δ*lacU169 endA hsdR thi* | This study |
| K-12 ECL716 | $F^-$ *rhaD701* Δ*lacU169 endA hsdR thi* | This study |
| K-12 ECL717 | $F^-$ *rhaR702* Δ*lacU169 endA hsdR thi* | This study |

were estimated with bovine serum albumin as a standard (29). Specific activities of the isomerase, the kinase, and the oxidoreductase are expressed in nanomoles per minute per milligram of protein at 25°C.

For determination of L-rhamnose permease activity, cells grown aerobically in mineral medium supplemented with 0.2% rhamnose and 0.5% CAA were incubated for 1 min in a medium containing 20 μM labeled substrate. The radioactivity retained by the cells, washed on a filter disk, was determined (8). The rate of accumulation of radioactivity was linear for 2 min. Permease activity is expressed in nanomoles of rhamnose uptake per minute per milligram (dry weight) of cells at 25°C.

**Construction of plasmids.** Standard molecular cloning procedures were followed (31). The plasmid pfuc20 was constructed by inserting into pBR322 a 3.4-kilobase (kb) *Bam*HI-*Eco*RI fragment that contained *fucR* but no intact structural genes of the *fuc* regulon (Y.-M. Chen, Y. Zhu, and E. C. C. Lin, submitted for publication). Plasmid pJTC9 was constructed by inserting the 2.2-kb *Bam*HI-to-*Eco*RI fragment of plasmid pJTC5 (J. F. Tobin and R. F. Schleif, J. Mol. Biol., in press), a plasmid containing the rhamnose operons, into the polylinker of plasmid pGC2 (32). Plasmid pJTC40 was constructed by cutting plasmid pJTC9 at the unique *Bgl*II restriction site that lies within the *rhaS* gene, filling in the site with the Klenow fragment of DNA polymerase I, and religating the blunt-ended molecule. This procedure inserts four base pairs at the restriction site and generates a termination codon with the *rhaS* coding sequence.

Transformation of competent bacteria with plasmids was carried out by the calcium chloride procedure (31).

**Preparation of DNA.** Plasmid DNA was prepared from 1.5 ml of LB culture by the alkaline lysis method (31). Chromosomal DNA was prepared from 20 ml of LB culture. The harvested cells were washed, pelleted, and suspended in 2.0 ml of 1.5 M NaCl–100 mM EDTA–20 mM Tris hydrochloride at pH 8.0. The suspension was incubated in the presence of lysozyme (1 mg/ml) for 30 min at 37°C. After the preparation was frozen (chilled by dry ice in ethanol) and thawed, 80 μl of 3 M Tris hydrochloride (pH 8.8) and 230 μl of 10% *N*-lauroyl sarcosine were added. The contents were gently mixed by stirring with a Pasteur pipette. The mixture was extracted with phenol equilibrated with 10 mM Tris hydrochloride (pH 8.0) by gentle shaking for 30 min. The supernatant fraction was retrieved, and the extraction procedure was repeated twice. The aqueous fraction was then extracted three times with ether. Any residual ether was removed under a stream of nitrogen. The sample was heated to 60°C for 10 min and dialyzed overnight against two changes of 0.1 mM EDTA–10 mM NaCl–10 mM Tris hydrochloride at pH 8.0.

**Southern transfer and hybridization.** DNA fragments were labeled by nick translation (31). Chromosomal DNA (10 μg) was digested with 100 U of *Eco*RI, precipitated with ethanol, and electrophoresed on a 0.8% agarose gel. Southern transfers were then performed (31) with dextran T-500 added to the hybridization buffer at 0.1 g/ml.

## RESULTS

**Anaerobic inducing effects of fucose and rhamnose.** Because the *fuc* structural genes are partitioned in three operons (*fucPIK*, encoding L-fucose permease, L-fucose isomerase, and L-fuculose kinase, respectively; *fucA*, encoding L-fuculose 1-phosphate aldolase; and *fucO*, encoding L-1,2-propanediol oxidoreductase [11, 21, 22; Chen et al., submitted], cells of a wild-type K-12 Hfr strain (ECL1) were grown anaerobically on CAA and pyruvate, with or without rhamnose, to discover whether cross-induction of the fucose system was limited to L-1,2-propanediol oxidoreductase. Enzyme assays of the cell extracts revealed that rhamnose cross-induced not only the oxidoreductase but also the

TABLE 2. Characterization of rhamnose-negative mutants

| *E. coli* strain and genotype | Enzyme sp act[a] | | | Growth response to rhamnose[b] |
|---|---|---|---|---|
| | L-Rhamnose permease | L-Rhamnose isomerase | L-Rhamnulose kinase | |
| ECL714 *rhaB* | 11 | 3,600 | 0 | R |
| ECL715 *rhaA* | 11 | 0 | 570 | R |
| ECL716 *rhaD* | 5 | 2,600 | 320 | S |
| ECL717 *rhaR* | 0.2 | 5 | 0 | R |

[a] The cells were grown aerobically in mineral medium containing 0.2% rhamnose and 0.5% CAA.

[b] Growth was tested on agar containing 0.2% glycerol with or without 0.2% rhamnose. Abbreviations: R, resistant to rhamnose as indicated by normal colony sizes; S, sensitive to rhamnose as indicated by subnormal colony sizes.

TABLE 3. Anaerobic induction of the isomerases and L-1,2-propanediol oxidoreductase by fucose and rhamnose in various strains and mutants

| *E. coli* strain and relevant genotype | Inducer added to growth medium | Enzyme sp act | | |
|---|---|---|---|---|
| | | L-Rhamnose isomerase | L-Fucose isomerase | L-1,2-Propanediol oxidoreductase |
| Crookes *fuc*$^+$ *rha*$^+$ | None | 50 | 35 | 70 |
| | Fucose | 200 | 3,000 | 1,300 |
| | Rhamnose | 12,000 | 3,500 | 1,200 |
| ECL1 *fuc*$^+$ *rha*$^+$ | None | 30 | 20 | 40 |
| | Fucose | 20 | 2,000 | 950 |
| | Rhamnose | 7,500 | 1,900 | 900 |
| ECL116 *fuc*$^+$ *rha*$^+$ | None | 20 | 20 | 40 |
| | Fucose | 30 | 1,500 | 900 |
| | Rhamnose | 6,000 | 1,200 | 500 |
| ECL339 Δ(*rha-pfkA*) | Fucose | 30 | 1,500 | 850 |
| | Rhamnose | 230 | 25 | 40 |
| ECL366 Δ(*fuc-argA*) | Fucose | 20 | 20 | 40 |
| | Rhamnose | 5,000 | 40 | 34 |

permease, the isomerase, the kinase, and the aldolase (data not shown).

An examination of two other *E. coli* stocks, a K-12 F$^-$ strain (ECL116) and strain Crookes (19), indicated that the gratuitous induction of the fucose trunk pathway by rhamnose was not an idiosyncratic property of the K-12 Hfr strain used. In all three strains, rhamnose induced not only L-rhamnose isomerase but also L-fucose isomerase and L-1,2-propanediol oxidoreductase (Table 3). In contrast, fucose induced L-fucose isomerase and L-1,2-propanediol oxidoreductase but not L-rhamnose isomerase. The levels of the fucose enzymes induced by fucose or rhamnose were similar.

Chemical contamination of the rhamnose stock by fucose was ruled out by the inability of the same rhamnose preparation to induce L-fucose isomerase and L-1,2-propanediol oxidoreductase in strain ECL339 with a *rha* deletion. Fortuitous activity of L-rhamnose isomerase on fucose was excluded by the failure of rhamnose to induce any fucose-isomerizing activity in strain ECL366 with a *fuc* deletion. Thus, either rhamnose itself or one of its metabolites cross-induces the *fuc* regulon.

**A derivative of rhamnose as the inducer of the *fuc* regulon.** Four *rha* mutants, defective in different genes, were analyzed for induction of the fucose enzymes by rhamnose. A defect in the *rhaA* gene, encoding L-rhamnose isomerase, prevented induction of L-fucose isomerase and L-1,2-propanediol oxidoreductase by rhamnose. Thus, rhamnose itself is incapable of inducing the *fuc* regulon. The ability of rhamnose to induce L-fucose isomerase and L-1,2-propanediol oxidoreductase was also abolished by a mutation in *rhaB*, encoding the kinase; *rhaD*, encoding the aldolase; or *rhaR*, encoding the activator protein (Table 4). Rhamnose, therefore, has to be metabolized to or beyond L-lactaldehyde to induce the *fuc* genes.

Because the supply of L-lactaldehyde was limited, a small-scale qualitative test for its inductive effect was carried out with strain ECL326, bearing Φ(*fucO-lacZ*). About $10^3$ cells were spread uniformly on four agar plates containing CAA (200 μg/ml) and X-Gal (40 μg/ml), a chromogenic substrate of β-galactosidase. A sterile filter disk was then placed on the center of each agar plate and impregnated with 10 μmol of fucose, rhamnose, L-lactaldehyde, or D-xylose. After aerobic incubation for about 36 h at 37°C, the colonies that formed around the disk charged with fucose, rhamnose, or L-lactaldehyde were blue. In contrast, the colonies on the plate with D-xylose were uniformly colorless (data not shown). In a control experiment with strain ECL711, a transductant of strain ECL326 which inherited a *rhaD* mutation abolishing L-rhamnulose 1-phosphate aldolase activity, β-galactosidase was induced by fucose and L-lactaldehyde but not by rhamnose.

L-Lactaldehyde may act directly as an effector for induction of the *fuc* regulon, or it may induce indirectly by giving rise to L-fuculose 1-phosphate through the reversible reaction catalyzed by the aldolase encoded by *fucA*. The equilibrium constant for [L-lactaldehyde][dihydroxyacetone phosphate]/[L-fuculose 1-phosphate] is 0.46 mM (17), and the intracellular concentration of dihydroxyacetone phosphate in *E. coli* is generally about 0.2 mM (27). Consequently, if the activity of L-fuculose 1-phosphate aldolase is not limiting, the ratio of L-fuculose-1-phosphate/L-lactaldehyde can approach 0.4. To determine whether L-lactaldehyde or L-fuculose 1-phosphate is responsible for induction of the *fuc* regulon by rhamnose, we tested a *fucA* point mutant, ECL476 (<1% aldolase activity when grown under inducing conditions). In this mutant, rhamnose was still able to induce L-fucose isomerase and L-1,2-propanediol oxidoreductase (Table 4). Although the result suggests that L-lactaldehyde serves as an alternative effector for the *fuc* regulon, a definitive conclusion cannot be made until it can be established that the *fucA* mutation in the test strain is nonleaky and that no significant activity is contributed by other enzymes in the cell. A small residual enzyme activity in vivo would suffice to build up an inducing concentration of L-fuculose 1-phosphate in the metabolic cul de sac.

As expected, two fucose-negative mutants revealed to be defective in *fucI* by complementation with the cloned gene were induced in its fucose permease (fuculose kinase activity could not be readily assayed because rhamnulose kinase also acted on fuculose [12]). Likewise, two fucose-negative mutants revealed to be defective in *fucK* by complementation with the cloned gene were induced in its fucose permease and fucose isomerase by rhamnose (data not shown).

TABLE 4. Activities of the isomerases and L-1,2-propanediol oxidoreductase in *rha* and *fuc* mutants grown anaerobically

| Strain (plasmid) and relevant genotype | Rhamnose in growth medium | Enzyme sp act (U) | | |
|---|---|---|---|---|
| | | L-Rhamnose isomerase | L-Fucose isomerase | L-1,2-Propanediol oxidoreductase |
| ECL116 *rha*$^+$ *fuc*$^+$ | − | 30 | 20 | 40 |
| | + | 6,000 | 1,200 | 500 |
| ECL715 *rhaA502* | − | 10 | 10 | 20 |
| | + | 10 | 10 | 15 |
| ECL714 *rhaB101* | − | 20 | 20 | 30 |
| | + | 3,600 | 20 | 40 |
| ECL716 *rhaD701* | − | 20 | 110 | 40 |
| | + | 2,000 | 180 | 50 |
| ECL717 *rhaR702* | − | 0 | 0 | 20 |
| | + | 5 | 0 | 20 |
| ECL476 *fucA514* | − | 10 | 10 | 50 |
| | + | 6,900 | 1,700 | 290[a] |
| ECL477 *fucR501* | − | 40 | 10 | 60 |
| | + | 9,800 | 10 | 90 |
| ECL477 *fucR501* (pfuc20 *fucR*$^+$) | − | 2 | 40 | 70 |
| | + | 9,600 | 2,900 | 1,400 |

[a] An activity of 180 U was observed in cells induced with fucose. The subnormal level might be attributable to divergent but overlapping promoter regions of *fucA* and *fucO* (Chen and Zhu, unpublished data).

**The role of the *fucR* gene in the induction of the *fuc* regulon by rhamnose.** The possibility of L-lactaldehyde acting as an alternative inducer of the *fuc* regulon in turn raises the question of whether an independent regulator gene exists for control of the *fuc* regulon by rhamnose. If so, inactivation of *fucR* should not abolish the cross-inducing activity of rhamnose. When strain ECL477, a *fucR* mutant, was grown anaerobically in the presence of rhamnose, neither L-fucose isomerase nor L-1,2-propanediol oxidoreductase was induced (Table 4). The lost ability was restored by transformation with a plasmid (pfuc20) bearing the *fucR*$^+$ gene but no intact *fuc* structural genes. It therefore seems that rhamnose induces the fucose system by virtue of being a precursor of an effector that interacts with the *fucR* product.

**The *rhaF* locus is *rhaR*.** It was reported that disruption of a gene in the *rha* region by a *lac* fusion prevented induction of *fucO* by rhamnose. The suggestion was made that the fusion occurred in a gene, *rhaF*, that encoded an activator of *fucO* (10). A subsequent finding that the fusion reduced the inducible level of L-rhamnose isomerase, together with the results on cross-induction described in the present study, raised the possibility that the fusion affected the activator gene for the *rha* structural genes.

The *rhaC* locus, previously thought to encode the activator protein for the *rha* system (34), has recently been found to contain two genes: *rhaR* and *rhaS*. The protein encoded by *rhaR* is sufficient for activating the *rhaBAD* operon (Tobin and Schleif, in press). The location of Φ(*rhaF-lacZ*) was first determined by a Southern transfer experiment. Chromosomal DNAs prepared from the wild-type strain (ECL116) and the two mutants (ECL333 and ECL335) bearing independent *lac* fusions were digested with *Eco*RI. A 0.9-kb fragment of the *rhaS* gene produced by *Eco*RI and *Bst*EII cuts (Fig. 2) was used as a probe to hybridize with the *Eco*RI-digested chromosomal DNA of each strain. Whereas the hybridizing fragment from the wild-type strain was 6.8 kb, the corresponding fragments from the Φ(*rhaF-lac*) mutants were only 5.4 kb (Fig. 3). Hence, the *lac* fusion introduced a second *Eco*RI site in the *rha* region. Since there is only a single *Eco*RI site in the entire *rha* region close to the promoter of the *rhaS* and only a single *Eco*RI site in the coding region of the *lacZ* gene, the shortened chromosomal fragment should be the product of cuts at the promoter of *rhaS* and the site within *lacZ*. The *rhaS* and *rhaR* genes span about 1.8 kb. The distance from the fusion joint to the *Eco*RI site in *lacZ* is deduced to be about 4 kb. The *lacZ* in Φ(*rhaF-lacZ*), therefore, should be fused to the end of the gene *rhaR*. Furthermore, when plasmid pJTC9, bearing only *rhaR* and *rhaS*, was introduced into strains ECL717 (*rhaR702*) and ECL333 (Φ[*rhaF-lac*]), the structural genes of both the *rha* and *fuc* systems became inducible by rhamnose (Table 5). The plasmid pJTC40, bearing *rhaR* without a functional *rhaS*, was also effective in complementing the defect in the two strains. Hence, the *lac* fusion was indeed within the *rhaR* gene, and the disruption was near its 3′ end. Subnormal activation of the *rha* operon would result in reduced metabolic flow through the rhamnose pathway, lowering the steady-state concentration of L-lactaldehyde. It might be noted that the presence of multicopies of both *rhaR* and *rhaS* resulted in hyperinducibility of L-rhamnose isomerase but not that of L-fucose isomerase and L-1,2-propanediol oxidoreductase, the induction of which is evidently limited by the single copy of *fucR*. The presence of multicopies of *rhaR* alone did not result in hyperinducibility

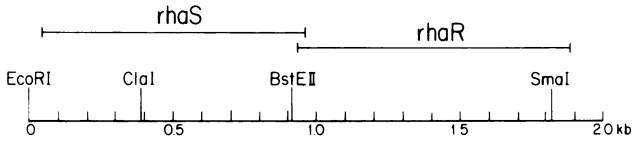


FIG. 2. Restriction map of *rhaR* and *rhaS* genes.

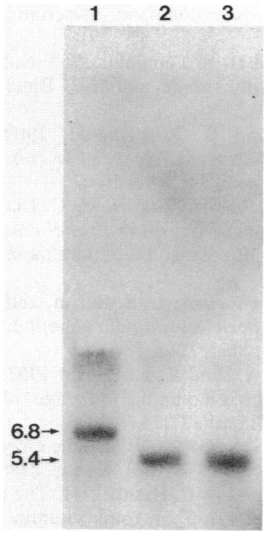


FIG. 3. Autoradiogram of Southern blot hybridization between the $^{32}$P-labeled *Eco*RI-*Bst*EII fragment from the *rhaS* gene and *Eco*RI restriction digests of chromosomal DNA. Numbers on the left indicate the sizes of the hybridized chromosomal fragments in kilobases. Lanes: 1, DNA from strain ECL116; 2, DNA from strain ECL333; 3, DNA from strain ECL335.

of L-rhamnose isomerase. It is not certain whether this is because the two genes products have a synergistic effect or because in the cells harboring pJTC40 the function of the *rhaR* gene product was interfered with by an incomplete product of the *rhaS* gene. It might also be noted that β-galactosidase was induced by rhamnose in strain ECL333 (Φ[*rhaF-lac*]) (10; Table 5). These induction patterns indicated that the regulators of the *rha* system are under positive autogenous regulation. This interpretation is consistent with the results of S1 mapping experiments examining transcription from the *rhaS* and *rhaR* genes (Tobin and Schleif, in press).

## DISCUSSION

Aside from being shared and jointly regulated in its synthesis by fucose and rhamnose, L-1,2-propanediol oxidoreductase has a more unusual regulatory feature. Since the enzyme activity is useful for hydrogen disposal during anaerobic growth but is wasteful of carbon and energy source during aerobic growth, a mechanism is necessary for regulation of catalytic activity according to the cellular respiratory state. This is accomplished by posttranslational modification, first shown by the presence of a low specific activity of the enzyme but undiminished levels of the immunochemically cross-reacting material in cells grown aerobically on fucose. Cells grown aerobically on rhamnose, however, were observed not to be induced in this protein (5, 6, 9, 11). Aerobically, rhamnose probably failed to cross-induce *fucO* because the metabolic flow rate through the trunk pathway was inadequate for maintaining the necesssary L-lactaldehyde level. The aerobic doubling time on rhamnose was 200 min, in contrast to the 80-min doubling time supported by fucose (Y. Zhu, unpublished data).

A previous study using the technique of *lac* fusion implicated a regulatory gene in the *rha* locus for anaerobic cross-induction of *fucO*. In two strains bearing an independently formed Φ(*rhaF-lac*), anaerobic induction of *fucO* by rhamnose no longer occurred (10). The results from Southern blot hybridizations and the complementation experiments in the present study revealed that the *lac* segment, instead of being fused to a distinct regulatory gene, *rhaF*, was joined to the 3′ end of *rhaR* with only partial impairment of its function. As a consequence, rhamnose could no longer adequately induce its trunk pathway. The more severe reduction in the ability of rhamnose to induce *fucO* (>10-fold) than in its ability to induce *fucI* (<4-fold) and *rhaA* (<4-fold) can only be tentatively explained. It is possible that the subnormal induction of L-1,2-propanediol oxidoreductase still gave sufficient enzyme activity to meet the need of a lowered metabolic flow rate through the rhamnose trunk pathway and that, when the effector-activator complex was limiting, *fucI* was expressed at a higher level than *fucO* because of a difference in promoter affinity.

The collective phenotypes of all of the mutations studied—*rhaA*, *rhaB*, *rhaD*, *rhaR*, and *fucR*—are consistent with the view that rhamnose induces the *fuc* system by giving rise to L-lactaldehyde (or L-fuculose 1-phosphate). Induction of the fucose system by rhamnose was previously noted in *Klebsiella pneumoniae*, but the cause was unexplored (E. J. St. Martin, Ph.D. thesis, University of Massachusetts, Amherst, 1975). The simplest model is that the effector produced from rhamnose combines with the *fucR* gene

TABLE 5. Complementation of Φ(*rhaF-lacZ*) in strain ECL333 by *rhaR*$^+$

| Strain (plasmid) | *rha* genotype | | Rhamnose in growth medium | Enzyme sp act[a] | | | |
|---|---|---|---|---|---|---|---|
| | Host | Plasmid | | L-Rhamnose isomerase | L-Fucose isomerase | L-1,2-Propanediol oxidoreductase | β-Galactosidase |
| ECL717 | *rhaR702* | | − | 0 | 0 | 22 | |
| | | | + | 5 | 0 | 20 | |
| ECL717(pJTC9) | *rhaR702* | *rhaR*$^+$ *rhaS*$^+$ | − | 20 | 10 | 50 | |
| | | | + | 18,000 | 1,600 | 1,000 | |
| ECL333 | Φ(*rhaF-lacZ*) | | − | 0 | 10 | 15 | 15 |
| | | | + | 2,000 | 450 | 85 | 250 |
| ECL333(pJTC9) | Φ(*rhaF-lacZ*) | *rhaR*$^+$ *rhaS*$^+$ | − | 0 | 20 | 27 | 35 |
| | | | + | 17,000 | 1,600 | 990 | 1,000 |
| ECL333(pJTC40) | Φ(*rhaF-lacZ*) | *rhaR*$^+$ *rhaS*$^-$ | − | 0 | 30 | 17 | 30 |
| | | | + | 7,000 | 1,500 | 800 | 1,000 |

[a] All cultures were grown anaerobically with rhamnose as the inducer.

product to turn on the *fuc* regulon. A more complex model would be that the cross-induction involves L-lactaldehyde as the effector instead of L-fuculose 1-phosphate and that induction by L-lactaldehyde requires physical interaction between the regulatory proteins of the *fuc* and *rha* systems. Indirect evidence for interaction between the regulatory proteins was provided by the observations that mutations in *fucR* which render the *fuc* regulon inducible by D-arabinose (24) were sometimes accompanied by defects in rhamnose utilization (St. Martin, Ph.D. thesis) and that selection for growth on the rare sugar L-galactose, an analog of L-fucose, resulted in loss of the ability to grow on rhamnose (Zhu and Lin, manuscript in preparation).

The advantage of cross-induction of the fucose pathway by rhamnose through a common metabolite or effector is mechanistic simplicity. Such a mechanism, however, exacts a price of gratuitous synthesis of the enzymes in the fucose trunk pathway when only rhamnose is present. The absence of a control by which rhamnose can selectively activate *fucO* might indicate that during the evolutionary history of *E. coli* rhamnose was seldom present without fucose. Since both sugars are frequent constituents of polysaccharides (33), synthesis of the fucose enzymes in the trunk pathway might rarely be gratuitous. Alternatively, the lack of a more elegant mechanism for the cross-induction indicates that the *rha* and *fuc* systems are in the process of progressive, or retrogressive, evolution.

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