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Dual Control of a Common L-1,2-Propanediol Oxidoreductase by L-Fucose and L-Rhamnose in *Escherichia coli*

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Anaerobic growth of *Escherichia coli* on L-fucose or L-rhamnose as the sole source of carbon and energy depends on the regeneration of NAD from NADH by disposing the intermediate L-lactaldehyde as L-1,2-propanediol. The two parallel pathways, with their own permeases and enzymes encoded by two widely separated gene clusters, appear to share a single enzyme that catalyzes the formation of L-1,2-propanediol. Although this oxidoreductase is encoded by a gene at the *fuc* locus, the enzyme is inducible by both L-fucose and L-rhamnose. The inducibility by L-rhamnose is controlled by a gene at the *rha* locus with no other known functions, since the aerobic growth rate on L-rhamnose remains normal. L-1,2-Propanediol oxidoreductase activity is inducible only anaerobically, and the effect of the two methylpentoses operates at different levels: L-fucose exerts its influence post-transcriptionally; L-rhamnose exerts its influence transcriptionally.

Escherichia coli can grow aerobically and anaerobically on L-fucose or L-rhamnose as a sole source of carbon and energy. The two compounds are structurally similar, differing only in the stereoconfiguration at carbons 2 and 4. The inducible pathways for the metabolism of the two compounds show striking parallelism (Fig. 1). Both pathways are mediated sequentially by a permease (20; J. Power, personal communication), an isomerase (18, 36, 39), a kinase (9, 22, 37, 40), and an aldolase (10, 11, 17, 30, 31). The stereochemical difference of the intermediates disappears with cleavage of the phosphorylated ketose intermediate, yielding dihydroxyacetone phosphate and L-lactaldehyde. Aerobically, L-lactaldehyde is converted to L-lactate by an NAD-linked dehydrogenase (34); anaerobically, L-lactaldehyde is reduced to L-1,2-propanediol, which is excreted into the medium (12). By sacrificing the aldehyde as an alcohol, the two extra reducing equivalents of the methylpentose are disposed of. The two sets of proteins in the trunk pathways and their respective positive activators are encoded by widely separated gene clusters: the *fuc* locus at minute 60.2 (16) and the *rha* locus at minute 87.7 (28). In addition, the *fuc* locus appears to contain structural genes for L-lactaldehyde dehydrogenase and L-1,2-propanediol oxidoreductase (1, 12, 19–21, 24, 28, 32, 33; Y.-M. Chen, unpublished data).

After the aldolase step, the maintenance of separate proteins by the two pathways should no longer be necessary if there is a mechanism for either L-fucose or L-rhamnose to induce a common set of gene products. Indeed, several lines of evidence suggest that a single L-1,2-propanediol oxidoreductase serves both pathways and the activity of this enzyme is inducible anaerobically by either L-fucose or L-rhamnose: (i) the enzyme proteins associated with the two pathways are indistinguishable with respect to electrophoretic mobility, molecular weight (two identical subunits of 39,000), and kinetic properties (3); (ii) the enzymes from L-fucose and L-rhamnose-induced cells show immunochemical identity in the Ouchterlony double diffusion test in agar (4); and (iii) a mutant (strain ECL32) lacking the enzyme whose synthesis is associated with the L-fucose system (12, 34, 35) fails to grow anaerobically on L-rhamnose (3). A surprising discovery was then made with an immunochemical assay: whereas L-rhamnose induces the oxidoreductase protein only anaerobically, L-fucose induces it both aerobically and anaerobically, but the aerobically induced protein is enzymatically inactive (4). Posttranscriptional control of the oxidoreductase activity was thus proposed. This view was confirmed by the use of three independent operon fusions constructed by joining the promoter of the presumptive oxidoreductase gene (*fucO*) to the structural genes for lactose utilization (*lacZYA*). β-Galactosidase of the ϕ(*fucO-lac*) hybrid operons is inducible by L-fucose both aerobically and anaerobically (8). Moreover, in merodiploids bearing both *fucO*$^+$ and ϕ(*fucO-lac*), the oxidoreductase activity is inducible only anaerobically, but β-galactosidase remains inducible both aerobically and anaerobically. Thus, the absence of respiratory control in the expression of ϕ(*fucO-lac*) cannot be attributed to a polarity effect of the fusion on a distal gene encoding a product with autogenous regulatory function in transcription (7). We report here the existence of the gene *rhaF* (*F* for liason with the L-fucose system) with no other apparent function than to activate in *trans* the *fucO* gene.

MATERIALS AND METHODS

Chemicals. L-Lactaldehyde was prepared by the reaction of ninhydrin with D-threonine (41). L-Fucose, L-rhamnose, and *O*-nitrophenol-β-D-galactoside were obtained from Sigma Chemical Co., St. Louis, Mo. Vitamin-free casein acid hydrolysate was from ICN Nutritional Biochemicals, Cleveland, Ohio. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside was from Bachem, Inc., Torrance, Calif. MacConkey agar base (without lactoses) was from Difco Laboratories, Detroit, Mich. All other chemicals were commercial products of reagent grade.

Bacteria and phage. *E. coli* K-12 strain ECL116 was used as the parental stock. Transduction was carried out with P1(*vir*) (26). Pertinent genetic characteristics and sources of the *E. coli* K-12 strains and phages used are given in Table 1.

For the isolation of *rhaF*::Mu d1 operon fusion mutants (6), a 2.5-ml suspension of 2×10^9 overnight grown cells of an ECL116 derivative selected for faster anaerobic growth on L-rhamnose was infected with Mu d1 phage at a multiplicity of 0.1. After infection, 0.5 ml of the suspension was

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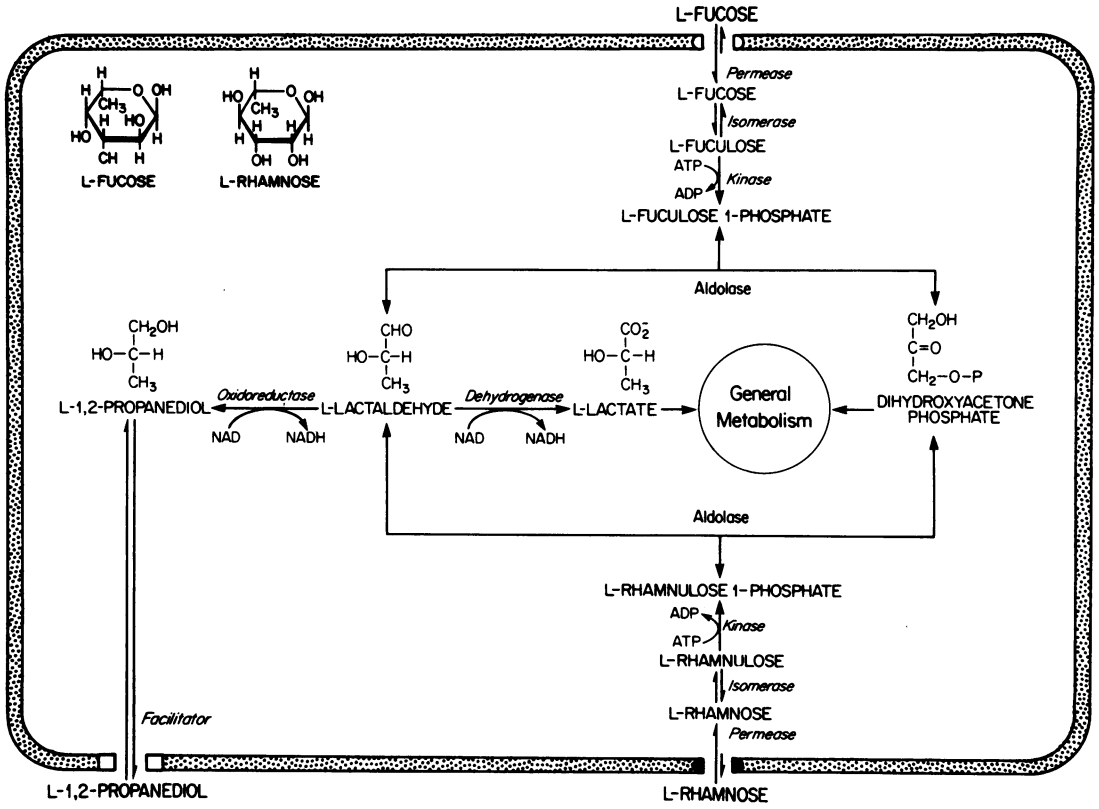

FIG. 1. Pathways of L-fucose and L-rhamnose dissimilation by *E. coli*.

incubated aerobically in 20 ml of mineral medium containing ampicillin (20 μg/ml) and glucose (0.4%) for 6 h. The cells were collected by centrifugation, washed, suspended in fresh medium of the same composition, and grown anaerobically overnight. Samples of cells were plated on MacConkey agar containing L-rhamnose (1%), ampicillin, and 5-bromo-4-chloro-3-indolyl-β-D-galctoside (40 μg/ml) and incubated anaerobically for 2 days. The plates were inspected immediately after removal from the sealed jars, and pale-colored colonies were marked. The plates were then kept in refrigeration for at least 2 h to allow spontaneous oxidation of 5-bromo-4-chloro-3-indole to the blue indigoid product. Colonies originally noted to be pale and which subsequently turned purplish blue were picked as presumptive mutants with defective L-rhamnose fermentation. Among these colonies, two independent fusion mutants that grew on L-rhamnose aerobically but not anaerobically were found. Their β-galactosidase activity was inducible by L-rhamnose. To stabilize genetically the hybrid operons, the two Mu dl fusion strains were lysogenized with λ pl(209) (23). The respective λ lysogens without the Mu dl prophage, strains ECL333 and ECL335, were isolated by growth of the cells at

TABLE 1. *E. coli* K-12 and bacteriophage strains

Strain	Genotype	Source or reference
E. coli		
ECL116	F⁻ Δ*lacU169 thi endA hsdR*	B. Magasanik and (2)
ECL327	F⁻ Φ[*fucO-lac*::λp(209)] Δ*lacU169 thi endA hsdR*	(8)
ECL333	F⁻ Φ[*rhaF*-1-*lac*::λp(209)] Δ*lacU169 thi endA hsdR*	This study
ECL335	F⁻ Φ[*rhaF*-2-*lac*::p(209)] Δ*lacU169 thi endA hsdR*	This study
ECL339	F⁻ Δ*lacU169 thi endA hsdR* Δ(*rha-pfkA*)*15 zig-1*::Tn*10*	This study
ECL345	F′ 143 (*tyrA*⁺ *fucO*⁺ *lysA*⁺)/F⁻Φ[*fucO-lac*::λp1(209)] *recA* Δ*lacU169 thi endA hsdR*	(7)
ECL358	F⁻ Φ[*fucO-lac*::λp(209)] Δ(*rha-pfkA*) 15 *zig-1*::Tn*10* Δ*lacU169 thi endA hsdR*	This study
MAL103	F⁻ Mu dl Mu cts Δ(*proAB lacIPOZYA*)X111 *rpsL*	(6)
DF929	HfrC Δ(*rha-pfkA*) 15 *zig-1*::Tn*10 glpK phoA8 relA1 tonA22* T_2^r (λ)	D. F. Fraenkel and (13)
Phage		
P1 *vir*		(26)
Mu dl	cts *trp'CBA' lac'OZYA'* Tn*3* Ap^r	From *E. coli* MAL103
λ pl(209)	::(+ Mu') trp'BA-Δ*W209-lac'OZY*	M. Casadaban
λ *vir*		J. R. Beckwith
λ *c*Ih80		J. R. Beckwith

TABLE 2. L-1,2-Propanediol oxidoreductase activities in extracts of cells grown anaerobically in inducing media

Strain	Relevant genotype	L-1,2-Propanediol oxidoreductase activity	
		L-Fucose medium	L-Rhamnose medium
ECL116	*fucO*$^+$ *rha*$^+$	900	400
ECL327	Φ(*fucO-lac*) *rha*$^+$	56[a]	60[a]
ECL339	*fucO*$^+$ Δ*rha*	850	44
ECL345	F′ *fucO*$^+$/Φ(*fucO-lac*) rha$^+$	2,000	360

[a] The residual activities in extracts of the fusion strains were attributed to another enzyme (8).

42°C. They were shown to be sensitive to ampicillin, to retain ϕ(*rhaF-lac*), and to be sensitive to λ *vir* but immune to λ *c*Ih80.

A deletion covering the *rha* locus, Δ(*rha-pfkA*)*15*, was transduced into strain ECL116 to give strain ECL339 by P1 grown on strain DF929 (6). The transductant was selected for drug reistance on tetracycline (20 μg/ml)–LB agar (*zig-1*::Tn*10* is 80% linked to the deletion) and scored for *rha* on MacConkey–L-rhamnose agar. The same deletion was transduced into strain ECL327 to give strain ECL358 by P1 grown on strain ECL339. Transfer of the deletion into the final recipient was confirmed by its normal growth on glycerol or L-fucose but impaired growth on glucose (*pfkA*), failure of growth on L-rhamnose both aerobically and anaerobically, and the absence of L-rhamnose isomerase activity in extracts of cells grown in the inducing medium.

Growth of cells. Aerobic growth was carried out in a 2,000-ml flask containing 150 ml of mineral medium (38), with appropriate supplements, and agitated on a rotary shaker. Anaerobic growth was carried out in 150-ml flasks filled to the top with medium, tightly capped, and gently stirred by a magnet. Anaerobic incubation of cells on solid medium was carried out in sealed jars under an H_2-CO_2 atmosphere (GasPak; BBL Microbiology Systems, Cockeysville, Md.). All strains were grown at 37°C, except those carrying Mu dl fusions, which were grown at 30°C. Inducing medium for physiological studies contained L-fucose or L-rhamnose (0.2% for aerobic growth and 0.4% for anaerobic growth) plus vitamin-free casein acid hydrolysate (0.1%), pyruvate (30 mM), and thiamine (2 μg/ml). The methylpentose was omitted from noninducing medium.

Preparation of cells extracts and enzyme assays. Cells were harvested from exponentially growing cultures at 100 to 150 Klett units (no. 42 filter) and washed once with 0.1 M potassium phosphate (pH 7.0). The pellet was weighed and dispersed in four volumes of 2.5 mM glutathione–0.1 M potassium phosphate (pH 7.0). The dispersed cells in the tube were disrupted (for 1 min per ml of suspension) in a model 60 W ultrasonic disintegrator (MSE) while being chilled in a −10°C bath. The resultant mixture was centrifuged at 100,000 × *g* for 2 h at 4°C, and the supernatant fraction was used for enzyme assays. L-1,2-Propanediol oxidoreductase activity was measured by the rate of decrease in extinction coefficient at 340 nm (E_{340}) in an assay mixture consisting of 0.5 mM lactaldehyde, 0.1 mM NADH, and 100 mM sodium phosphate (pH 7.0) in a final volume of 1 ml. L-rhamnose isomerase activity was determined by the rate of L-rhamnulose formation by the cysteine-carbazole method (15). Specific activities of the oxidoreductase and the isomerase are expressed in nanomoles per minute per milligram of protein. Protein concentrations in cell extracts were estimated by a phenol reagent (25). β-Galactosidase activity was measured and expressed in units by the method of Miller (27).

RESULTS

Inducibility of L-1,2-propanediol oxidoreductase activity. If there is only a single L-1,2-propanediol oxidoreductase, disruption of the *fucO* gene by fusion with *lacZYA* in strain ECL327 should abolish anaerobic induction of the enzyme activity not only by L-fucose but also by L-rhamnose. Table 2 shows that this is true. By comparison, deletion of the *rha* region in strain ECL339 resulted only in the failure of L-rhamnose to induce the activity. As expected, strain ECL327 grew anaerobically on neither L-fucose nor L-rhamnose, whereas strain ECL339 grew anaerobically on L-fucose but not L-rhamnose (data not shown). In strain ECL345, carrying the wild-type *fuc* region on the F plasmid and the ϕ(*fucO-lac*) on the chromosome, induction of the oxidoreductase by both sugars was restored. However, it is not clear why there is hyperinduction of the enzyme by L-fucose but not at all by L-rhamnose.

Inducibility of β-galactosidase in ϕ(*fucO-lac*) strains. Consistent with the report that the L-1,2-propanediol oxidoreductase protein was inducible both aerobically and anaerobically by L-fucose, but only anaerobically by L-rhamnose (4), induction of the β-galactosidase activity of the ϕ(*fucO-lac*) in strain ECL327 by L-fucose was independent of the respiratory condition of growth (7), whereas induction of this activity by L-rhamnose required anaerobiosis (Table 3). Deletion of the *rha* locus in strain ECL327 to give strain ECL358 abolished the ability of L-rhamnose to induce the β-galactosidase under both respiratory conditions with no appreciable effect on the induction pattern by L-fucose.

Enzyme induction in ϕ(*rhaF-lac*) strains. Deletion of the *rha* region might prevent *fucO* induction by L-rhamnose because of failure to produce the effector molecule, the activator protein for the *rha* system, and possibly also a specific gene product interacting with *fucO*. If there exists at the *rha* locus a gene whose product serves only to regulate the expression of *fucO*, then mutations in such a gene should prevent anaerobic growth on L-rhamnose but not on L-fucose. The ϕ(*rhaF-lac*) strains ECL333 and ECL335 satisfy this phenotype. In addition, when their L-rhamnose isomer-

TABLE 3. β-Galactosidase activities in extracts of cells grown aerobically or anaerobically in inducing media

Strain	Relevant genotype	β-Galactosidase activity[a]			
		L-Fucose medium		L-Rhamnose medium	
		$+O_2$	$-O_2$	$+O_2$	$-O_2$
ECL327	Φ(*fucO-lac*) *rha*$^+$	1,600	1,900	250	1,400
ECL358	Φ(*fucO-lac*) Δ*rha*	1,200	1,700	15	26

[a] 1 U (Miller) = 0.67 nmol min^{-1} mg^{-1} of protein (cell extract).

TABLE 4. L-1,2-Propanediol oxidoreductase and β-galactosidase activities in extracts of Φ(*rhaF-lac*) strains grown in inducing media

Strain	L-1,2-Propanediol oxidoreductase activity		β-Galactosidase activity[a]		
	L-Fucose medium	L-Rhamnose medium	L-Fucose medium	L-Rhamnose medium	
	$-O_2$	$-O_2$	$-O_2$	$+O_2$	$-O_2$
ECL333	800	30	7[b]	85	130
ECL335	770	40	5	30	30

[a] 1 U(Miller) = 0.67 nmol min^{-1} mg^{-1} of protein (cell extract).
[b] Not significantly different from noninduced value.

ase activity was tested, it was found to be normally inducible in both cases (from a basal level of 93 to an induced level of 1,300 U). Table 4 shows that L-1,2-propanediol oxidoreductase activity in the fusion strains was no longer inducible anaerobically by L-rhamnose but remained fully inducible by L-fucose. On the other hand, the β-galactosidase activity of the fusions was not inducible by L-fucose but was slightly inducible by L-rhamnose both aerobically and anaerobically to about the same extent. To map the fusions, strains ECL333 and ECL335 were infected with P1 grown on strain ECL339, and tetracycline-resistant transductants were selected. Among the transductants of strain ECL333, 81% (63 of 83) were rhamnose negative. The corresponding linkage for strain ECL335 was 95% (84 of 88). Thus, the fusions are situated at the *rha* locus, and the *rhaF* product is necessary for the induction of L-1,2-propanediol oxidoreductase by L-rhamnose. Furthermore, *rhaF* is coinduced when the other structural genes of the L-rhamnose system are highly expressed.

DISCUSSION

The view that *fucO* is the gene encoding L-1,2-propanediol oxidoreductase is also compatible with the strength of its promoter. The induced β-galactosidase activity in the ϕ(*fucO-lac*)-bearing strain ECL327 (as well as for two other independent fusion strains [see reference 8]) of about 1,000 to 2,000 U compares favorably with an activity of 5,000 U (50,000 monomers per cell) induced by isopropyl-β-D-thiogalactoside in cells bearing the wild-type *lac* operon and grown on glycerol (5). In contrast, the induced β-galactosidase activity in the two ϕ(*rhaF-lac*)-bearing strains ECL333 and ECL335 of about 30 to 130 is compatible with fusions to genes encoding regulator proteins. For example, a fusion of *lacZ* to the promoter of *araC*, encoding the activator of the L-arabinose operon, produced 55 β-galactosidase units (5), and among 11 protein fusion strains of *lacZ* to *malT*, encoding the activator of the maltose regulon, the one most productive of β-galactosidase activity gave 80 U (14). Nonetheless, decisive evidence showing that mutations in *fucO* can alter the structure of L-1,2-propanediol oxidoreductase (thermolability, for example) is not yet available.

Even if the simple working model of a shared L-1,2-propanediol oxidoreductase with the control by L-fucose acting posttranscriptionally and the control by L-rhamnose acting transcriptionally is correct, several additional questions remain to be asnwered. (i) Is *fucO* acted on directly by the general positive regulator, the *fucR* product (24, 33), which controls the expression of the operons of the trunk pathway? (ii) What is the mechanism that determines the catalytic ability of the L-fucose-induced oxidoreductase protein? Is this mechanism encoded by a gene of the *fuc* system, and is the gene product a protein which is sensitive to redox conditions? (iii) Is the *rhaF* product, which controls the expression of only *fucO*, an enzyme that forms an effector, or is the protein itself a regulator? Is this protein redox sensitive? Finally it would also be interesting to know whether or not a single L-lactaldehyde dehydrogenase is shared by the *fuc* and *rha* systems.

The parallel mechanisms employed for the dissimilation of L-fucose and L-rhamnose and the similarity of the chemical structures of the corresponding intermediates in the two pathways suggest common descent of the genes. The *fuc* and *rha* gene clusters are about 90° apart, which would suggest that the two systems appeared along with genome duplication (29). But what could be the basis for the evolution of such dissimilar mechanisms for the control of L-1,2-propanediol oxidoreductase? Eventually, a challenging problem would be to propose a sequence of events that can explain the persistence of this molecular Siamese twinship.

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