JOURNAL OF BACTERIOLOGY, Feb. 1969, p. 769-775
Vol. 97, No. 2

Printed in U.S.A.

Relationships Between the Regulation of the Lactose and Galactose Operons of *Escherichia coli*

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Received for publication 13 November 1968

A group of structurally related compounds, including galactose, fucose, and a number of galactosides, are regulatory effectors for both the *lac* and *gal* operons of *Escherichia coli*. Although a common set of effectors exists, each operon appears to be regulated independently of the other. Experiments with various regulatory mutants have shown, first, that the presence of the proteins of one operon is without effect on the regulation of the other and, second, that the influence an effector has on one operon is independent of the presence or the functional state of the regulatory genes of the other operon. It is unlikely, therefore, that the two operons share a common regulatory macromolecule. Both *gal* R^- and *gal* o^c regulatory mutants are equally resistant to repression by glucose and galactosides. It has been possible to show, in the *gal* operon, that induction and repression are competitive processes. For this operon, the differential rate of enzyme synthesis is set by the relative intracellular concentrations of inducer (fucose) and repressor (isopropylthiogalactoside).

The *lac* and *gal* operons code for enzymes which act sequentially in metabolism, and their regulation appears to be closely related. Both of the metabolic products of the *lac* operon are active in the regulation of *gal*; galactose acts as an inducer and glucose acts as a catabolite repressor. Conversely, among the metabolic products of the *gal* operon, uridine diphosphogalactose or an immediate derivative acts as a repressor of the *lac* operon (13). In addition, several compounds are active in the regulation of both operons. The *lac* inducers such as methylthiogalactoside (TMG) and isopropylthiogalactoside (IPTG) are repressors of the *gal* operon (2), and the inducers of *gal*, galactose and fucose, are weak inducers of the *lac* operon (16, 17).

We have investigated whether the sharing of a common set of effectors by these two operons reflects the existence of a common regulatory macromolecule. The data indicate that no such site exists. This being so, it has been possible to determine whether in *gal* the level of enzyme synthesis reflects an internal competition between induction and repression, and whether there is any difference between *gal* R^- and *gal* o^c mutants in their response to regulatory effectors.

MATERIALS AND METHODS

Chemicals. The following chemicals were purchased: TMG (methyl-β-D-thiogalactoside) and IPTG (isopropyl-β-D-thiogalactoside) from Mann Research Laboratories, New York, N.Y.; melibiose from Nutritional Biochemicals Corp., Cleveland, Ohio; *p*-aminophenyl-β-D-galactoside from Pierce Chemical Co., Rockford, Ill.; ^{14}C-galactose from the National Bureau of Standards, Washington, D.C.; glucose-free galactose from Sigma Chemical Co., St. Louis, Mo.; lysozyme from Worthington Biochemical Corp., Freehold, N.J.; and D(+)fucose from General Biochemicals Corp., Chagrin Falls, Ohio. Methyl-β-D-galactoside, propyl-β-D-galactoside, phenyl-β-D-galactoside, phenyl-β-D-thiogalactoside, and *p*-aminophenyl-β-D-thiogalactoside were gifts from S. Spiegelman; *o*-nitrophenyl-β-D-fucoside was obtained from H. V. Rickenberg. Additional *o*-nitrophenyl-β-D-fucoside was purchased from Cyclo Chemical Corp., Los Angeles, Calif. The samples of galactose and fucose had less than 0.2% impurities as tested by thin-layer chromatography with either *n*-butyl alcohol-acetone-water (40:50:10, v/v) or methylethyl ketone-glacial acetic acid-methanol (60:20:20, v/v) as solvent systems.

Bacterial strains. The genetic constitutions, sources, and strain numbers of the *Escherichia coli* stocks are listed in Table 1.

Culture medium. Cells were aerated at 37 C in M9 minimal medium (10 mg of gelatin per liter, 10^{-3} M $MgSO_4$, 3×10^{-6} M $FeCl_3$, 8×10^{-2} M NaCl, 1.9×10^{-2} M NH_4Cl, 2.2×10^{-2} M KH_2PO_4, and 4.8×10^{-2} M Na_2HPO_4) with 1 g of glycerol per liter as carbon source. To produce catabolite repression, 1 g of glucose per liter was substituted for glycerol. The medium was supplemented as required with 0.5 μg of thiamine per ml and individual amino acids at 20 μg/ml each.

TABLE 1. *Strains of E. coli*

Strain	Genetic constitution	Source
W3110	*gal*$^+$ *lac*$^+$	J. Weigle
3000	*gal*$^+$ *lac*$^+$	H. Revel
3300	*lac i*$^-$	H. Revel
C600	*gal*$^+$ *lac y*$^-$	J. Weigle
B78A	*gal R*$^-$ *lac y*$^-$	G. Buttin
C81-2	*gal o*^{c}	G. Buttin
HfrH	*gal*$^+$	
W2241	*lac y*$^-$	E. Lederberg
W2244	*lac z*$^-$	E. Lederberg
W4132	*lac* deletion	E. Lederberg
S165	*gal* deletion	S. Adyha

Experimental conditions. Cells were grown from an inoculum of less than 10^6 cells/ml to approximately 2×10^8 cells/ml, chilled, centriuged in the cold, and resuspended in fresh medium at 4×10^7 to 8×10^7 cells/ml. Portions of this cell suspension with appropriate additions of inducers and repressors were then incubated for an additional 2 to 3 generations (approximately 4 hr). After incubation, the cultures were chilled, and chloramphenicol (50 μg/ml) was added to prevent further protein synthesis. Samples from these cultures were assayed for β-galactosidase. For the assay of galactokinase, the cells were centrifuged and resuspended in M9 medium with the following changes: absence of carbon source, the addition of 10^{-2} M dithiothreitol as an enzyme preservative, and replacement of the phosphate buffer by 0.1 M tris-(hydroxymethyl)aminomethane (Tris)-chloride (*p*H 7.8).

Assays. The assay of β-galactosidase was adapted from the procedure of Lederberg (7) as previously described (12). Enzyme activity is expressed as nanomoles of *o*-nitrophenol liberated per minute at 30 C.

Galactokinase activity was assayed by a modification of Sherman's method (14). The formation of the reaction product, ^{14}C-galactose-1-phosphate, was measured by determining the amount of radioactivity that was bound to anion-exchange paper after elution of the substrate, ^{14}C-galactose. Cells to be assayed (2×10^6 to 2×10^7) were lysed by exposing them for 5 min at 37 C to a mixture of 2.5×10^{-4} M ethylenediaminetetraacetate, 0.1 mg of lysozyme per ml, and 0.03 M Tris-acetate buffer (*p*H 8.0) in a final volume of 75 μliters. Substrate mixture (25 μliters) was then added, to give a final concentration of 3×10^{-3} M ^{14}C-galactose (specific activity, 0.1 μc/μmole), 0.1 M Tris-chloride buffer (*p*H 7.8), 4×10^{-3} M NaF, 4×10^{-3} M $MgCl_2$, and 1.7×10^{-3} M adenosine triphosphate. After incubation for 60 min at 37 C, the reaction was stopped by immersing the tubes in a boiling-water bath for 1 min. The total reaction mixture was then adsorbed to 2-cm^2 pieces of diethylaminoethyl cellulose paper (Whatman DE 81). The unreacted galactose was eluted by washing the papers with water while they were in the reaction tubes. The papers were oven-dried, and the residual ^{14}C-galactose-1-phosphate was counted in a Nuclear-Chicago liquid scintillation counter. The number of counts fixed was proportional to reaction time and enzyme concentration over the experimental range, and duplicate assays were within 5% of each other. Enzyme activity is expressed as micromoles of galactose-1-phosphate formed per hour at 37 C.

The differential rate of synthesis of each enzyme during a period of growth is expressed as the increment in enzyme activity divided by the corresponding increment in cell mass. Cell mass was measured in optical density units at 550 nm in a Zeiss PMQII spectrophotometer. An optical unit of 1.0 is equal to 6.5×10^8 cells/ml and 0.24 mg of protein/ml.

Intracellular IPTG concentrations were measured by exposing growing cultures to 5×10^{-4} M ^{14}C-IPTG having a specific activity of 0.05 μc/μmole. At intervals, 2-ml samples were removed, filtered onto prewetted membrane filters (Millipore Corp., Bedford, Mass.), and washed at room temperature with 2 ml of M9 minimal medium without sugar. The filters were air-dried and counted. Uptake is expressed as nanomoles of IPTG accumulated per milliliter, divided by the absorbance of the culture.

For the measurement of intracellular fucose concentrations, cultures in the presence of 50 μg of chloramphenicol per ml were exposed to 3×10^{-3} M ^{3}H-fucose having a specific activity of 9.3×10^{-2} μc/μmole. After 10 min of incubation at 37 C, 3-ml samples were treated as for IPTG uptake. Uptake is expressed as nanomoles of fucose accumulated per milliliter, divided by the initial absorbance of the culture.

RESULTS

Relationship between the operons. In studying the relationship between the *lac* and *gal* operons, it is first necessary to establish whether synthesis of the proteins of one operon interferes with synthesis of the proteins of the other. This cannot be tested for by attempting to induce both operons simultaneously, since the inducers of *lac* are the repressors of *gal*. However, constitutive mutants which produce high levels of the proteins of one operon can be tested for their ability to be induced to synthesize the proteins of the other. For the *gal* operon, we compared strain 3300 *i*$^-$, which produces the enzymes of the *lac* operon constitutively, and the parental strain 3000 *i*$^+$, which does not. Fucose induced galactokinase synthesis equally in these two strains (Table 2), showing that active synthesis of the proteins of the *lac* operon does not interfere with induction of the *gal* operon. Likewise, for the *lac* operon, the induced rate of β-galactosidase synthesis was nearly the same in *gal* constitutive mutants and in their respective parental inducible strains (Table 2). It was necessary to use a high concentration of IPTG to induce the *lac* operon in the C600 strain and its *R*$^-$ derivative because these strains lack the β-galactoside permease. From the results obtained with constitutive mutants, we concluded

TABLE 2. *Induction of one operon compared in an uninduced wild-type culture and constitutive mutants of the other operon*

Strain	Inducer	Rate of enzyme synthesis	
		gal[a]	*lac*[b]
3000 *lac*$^+$	3×10^{-3} M fucose	2.4	
3300 *lac* i^-		2.6	
C600 *gal*$^+$ *lac* y^-	10^{-2} M IPTG		2,000
B78A *gal* R^- *lac* y^-			1,600
HfrH *gal*$^+$	5×10^{-5} M IPTG		2,500
C81-2 *gal* o^c			3,100

[a] Units of galactokinase.
[b] Units of β-galactosidase.

that the enzymes of both operons can be formed simultaneously, and that the presence of the proteins of one operon does not interfere with the synthesis of the proteins of the other operon.

Since the proteins of one operon do not interfere with the induction of the other, we can ask whether the *lac* and *gal* operons are related by sharing a common regulatory macromolecule, or whether the two operons possess independent systems of regulation which recognize each other's effectors. For example, the binding of IPTG to a single molecular species could result in induction of the *lac* operon and repression of the *gal* operon, or, alternatively, IPTG could interact with two different molecular species, one responsible for *lac* induction and the other for *gal* repression. To choose between these alternatives, we tested the regulation of each operon in strains in which the regulatory system of the other operon is either mutated or deleted.

Galactokinase proved to be normally inducible in a strain carrying a deletion which included the *i*, *o*, and *z* and part of the *y* cistrons of *lac* (Table 3), indicating that the regulatory protein required for *gal* induction is not coded for within the deleted portion of the *lac* operon.

The induced synthesis of galactokinase was repressed in a wild-type strain by all of the strong *lac* inducers tested (Table 3, column 2). In the deletion mutant, all strong *lac* inducers continued to repress galactokinase synthesis, but only by 30 to 50% (Table 3, column 4). McBrien and Moses (9) have also reported that IPTG represses the *gal* operon in a *lac* deletion mutant. The reduced ability of galctosides to repress galactokinase probably is due to the absence of galactoside permease. Incomplete repression also was observed in strain W2241, which lacks permease (Table 3, column 8), whereas strong repression was observed in strain W2244, which contains an active permease but lacks β-galactosidase (Table 3, column 6). From these results, we concluded that the protein(s) which recognizes fucose as a *gal* inducer and galactosides as *gal* repressors must be coded for outside of the *lac* operon.

The ability of phenyl-galactoside, aminophenyl-galactoside, and methyl-galactoside to induce the *gal* operon should be noted. In each case, induction required the presence of a functional β-galactosidase (Table 3), suggesting that some reaction product was the true inducer of the *gal* operon. This product may not have been galactose itself, since propyl-galactoside, which supports the growth of cells, and presumably is also split, did not induce *gal*. It is possible that the action of β-galactosidase on these compounds generates a transgalactosylation product which induces the *gal* operon.

Effectors of the *lac* operon were tested in mutants in which the *gal* regulatory genes are mutated or deleted. As shown in Table 2, IPTG induced β-galactosidase synthesis in strains carrying either a *gal* o^c mutation or a *gal* R^- mutation. A strain which carries a deletion of the *gal* operon covering the operator, epimerase, transferase, and part of the kinase genes has been isolated recently (Shapiro, *personal communication*). In this strain, IPTG continues to act as an inducer of β-galactosidase, and fucose, in the presence of IPTG, continues to act as a repressor of β-galactosidase. IPTG alone at 5×10^{-5} M induced β-galactosidase synthesis to 6,700 units in this strain, and the further addition of 10^{-1} M fucose reduced the rate of synthesis to 3,300 units. [Fucose, although a weak inducer of the *lac* operon, acts to reduce the rate of β-galactosidase synthesis when present in high concentrations. A previous publication (17) gives an explanation for this paradoxical behavior.]

Combining the results of these experiments we concluded that the regulatory macromolecule which recognizes compounds as *gal* effectors is not coded for by the genes determining the *lac* regulatory system, and the regulatory macromolecule which recognizes compounds as *lac* effectors is not coded for by the genes determining the *gal* regulatory system.

Response of gal regulatory mutants to effectors. Two types of *gal* regulatory mutations have been isolated. One is an operator constitutive mutant, which maps adjacent to the three structural genes of the operon and was selected by resistance to TMG repression, and the other is a regulator constitutive mutant, which maps at a considerable distance from the structural genes, and was selected by resistance to glucose repression (2, 3). We have tested to see whether these mutants differ

TABLE 3. *Effect of compounds on galactokinase synthesis*

Compound[a]	Rate of galactokinase synthesis							
	Wild type		*lac* deletion		z^-		y^-	
	−Fu[b] (1)	+Fu (2)	−Fu (3)	+Fu (4)	−Fu (5)	+Fu (6)	−Fu (7)	+Fu (8)
No addition	0.16	1.52	0.40	4.55	0.26	3.28	0.23	3.08
Galactose	0.71	—	—	—	—	—	—	—
strong lac inducers								
Isopropyl-β-D-thiogalactoside	0.08	0.08	0.28	1.59	0.17	0.12	0.17	2.09
Methyl-β-D-thiogalactoside	0.06	0.07	—	1.58	0.11	0.10	0.17	1.18
Propyl-β-D-galactoside	0.08	0.08	—	2.29	0.18[c]	0.12	—	2.38
Methyl-β-D-galactoside	0.69	0.62	—	1.24	0.18	0.31	—	1.08
lac anti-inducers								
o-Nitrophenyl-β-D-fucoside	0.12	0.50	—	2.94	—	2.24	—	2.35
Phenyl-β-D-thiogalactoside	0.08	0.25	—	3.56	—	1.61	—	2.64
weak lac inducers								
Phenyl-β-D-galactoside	0.75	1.13	—	3.67	0.20	2.78	0.38	2.76
p-Aminophenyl-β-D-galactoside	1.12	—	—	4.13	—	—	0.22	—
p-Aminophenyl-β-D-thiogalactoside	0.12	1.42	—	4.00	—	3.32	—	2.99

[a] Galactose was present at 10^{-2} M; all other compounds, at 10^{-3} M. The strains used were W3110, *lac*$^+$ *gal*$^+$; W2241, *lac* y^-; W2244 *lac* z^-; and W4132, *lac* deletion.
[b] Fucose was used at 3×10^{-3} M.
[c] Based on a separate experiment.

in any manner in their response to effectors. Both were resistant to repression by galactosides (Table 4). The mutants were partially resistant to repression by glucose, and this repression could not be reversed at high inducer concentrations (Table 5). The catabolite repression of regulatory mutants differs from that seen in wild-type cells where repression is more complete and is readily reversed by high concentrations of inducer (Table 5).

Competition between induction and repression. In the *lac* operon, anti-inducers inhibit enzyme synthesis in induced *lac*$^+$ cells (11), and weak inducers inhibit synthesis in both *lac* i^- and *lac*$^+$ cells (16, 17). In each case, the inhibition is overcome by high concentrations of inducer (16, 17). That is, induction and repression of β-galactosidase synthesis appear to be competitive processes. Since it now appears that effectors such as IPTG influence the regulation of the *gal* operon directly, and not by acting through the *lac* system, we can inquire whether induction and repression are competitive in the *gal* operon also. As shown in Fig. 1, the fucose-induced synthesis of galactokinase was repressed by IPTG, and this repression was overcome by further increasing the concentration of fucose. In this experiment, galactokinase formation was a linear function of cell growth under all conditions, indicating that both repression and its reversal represent changes in the steady-state rate of enzyme synthesis. When the rate of galactokinase synthesis was measured at various fucose and IPTG concentrations (Table 6), the two compounds acted competitively, and the concentration of fucose required to induce a given rate of synthesis was proportional to the amount of IPTG present.

TABLE 4. *Effect of galactosides on constitutive galactokinase synthesis*

Strain	Rate of galactokinase synthesis[a]			
	No addition	+IPTG	+TMG	+APTG
B78A *gal* R^-	3.68	4.21	3.62	3.41
C81-2 *gal* o^c	7.63	6.00	4.91	7.34

[a] Compounds were present at 10^{-3} M. APTG, aminophenylthiogalactoside.

The competition between inducer and repressor did not result from interference between these compounds at the level of entry into the cell. Direct measurements of intracellular fucose concentrations showed that IPTG did not decrease the uptake of fucose (Table 7). Thus, IPTG did

not repress the *gal* operon by preventing the entry of inducer. In contrast, the catabolite repressor glucose is an effective inhibitor of fucose entry (1).

TABLE 5. *Effect of glucose on galactokinase synthesis*

Strain	Carbon source	Rate of galactokinase synthesis	
		No addition	+ 10^{-1} M fucose
W3110 *gal*$^+$ + 3×10^{-3} M fucose	Glycerol	1.12	1.65
	Glucose	0.22	1.15
B78A *gal* R^-	Glycerol	3.22	—
	Glucose	1.24	1.57
C81-2 *gal* o^c	Glycerol	5.16	—
	Glucose	2.04	2.62

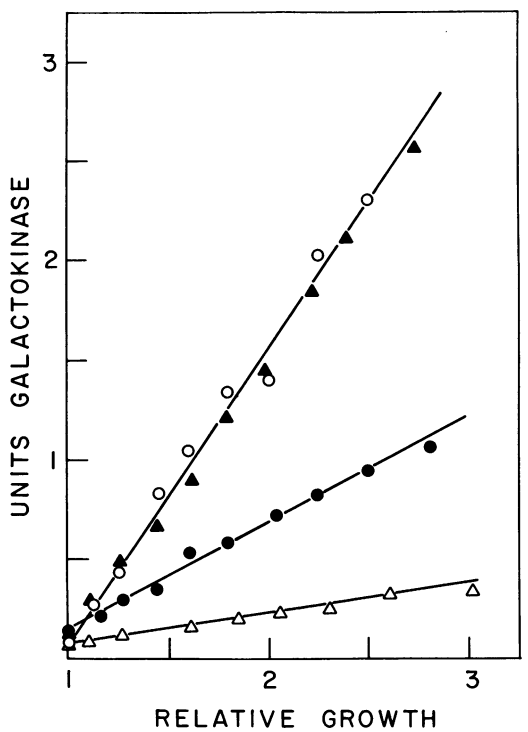

FIG. 1. *Relationship between galactokinase formation and cell growth. Wild-type strain W3110 was grown with the following additions: none (△), 5 × 10^{-3} M fucose (○), 5 × 10^{-3} M fucose and 5 × 10^{-5} M IPTG (●), and 10^{-1} M fucose and 5 × 10^{-5} M IPTG (▲). Units of galactokinase are expressed as micro moles of galactose-1-phosphate liberated per hour at 37 C, divided by the optical density of the cells at the beginning of the experiment.*

TABLE 6. *Repression and reversal of repression of galactokinase synthesis in wild-type strain W3110*

Fucose concn (M)	IPTG concn (M)			
	0	10^{-4}	3×10^{-4}	10^{-3}
0	0.14[a]	0.17	0.19	0.16
10^{-4}	0.35	0.19	0.21	0.22
10^{-3}	0.96	0.21	0.19	0.29
3×10^{-3}	1.12	0.30	0.23	0.27
10^{-2}	1.16	0.57	0.30	0.28
3×10^{-2}	1.30	1.26	0.61	0.39
10^{-1}	1.65	1.46	1.15	0.56

[a] Results show the rate of galactokinase synthesis.

TABLE 7. *Accumulation of fucose in the presence and absence of IPTG*[a]

Cells	Fucose accumulated (nmoles)	
	− IPTG	+10^{-3} M IPTG
3000 *gal*$^+$	4.1	4.0
3000 *gal*$^+$ grown in 3×10^{-3} M fucose	4.6	3.6

[a] Cells in the presence of chloramphenicol were exposed to 3×10^{-3} M fucose at 9.3×10^{-2} μc/μmole for 10 min.

Fucose can partially inhibit IPTG accumulation, but this does not appear to be the mechanism by which it antagonizes IPTG repression. Measurement of IPTG uptake by cells fully induced for the *lac* operon showed some inhibition of galactoside permease by 10^{-1} M fucose. Moreover, fucose partially repressed the formation of *lac* operon proteins, including galactoside permease. In combination, these two effects lowered the intracellular IPTG concentration in cells growing in the presence of fucose. However, as the following experiment shows, this inhibition of IPTG entry is not sufficient to explain the ability of fucose to reverse the IPTG repression of *gal*. Two cultures grown in radioactive IPTG, one with 3×10^{-3} M fucose and the other with 10^{-1} M fucose, were assayed at intervals for galactokinase and intracellular IPTG. For both cultures, the IPTG concentration per cell increased during the course of the experiment (Fig. 2). However, in each culture, the rate of galactokinase synthesis remained constant, being 0.22 units at 3×10^{-3} M fucose and 1.34 units at 10^{-1} M fucose. It is apparent that in the culture with the low fucose concentration even the lowest IPTG concentration measured must have been enough to saturate the repression mechanism for the *gal* operon. In

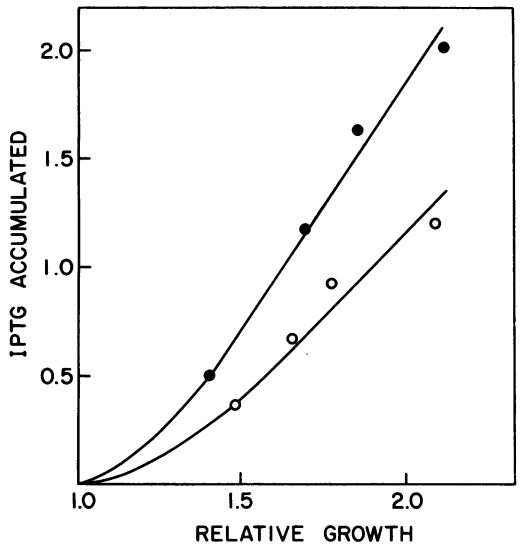

FIG. 2. *Effect of fucose on IPTG accumulation Symbols: ●, 5 × 10⁻⁴ M IPTG and 3 × 10⁻³ M fucose; ○, 5 × 10⁻⁴ M IPTG and 10⁻¹ M fucose. The rates of galactokinase synthesis were 0.22 and 1.34, respectively. IPTG accumulation is expressed as nanomoles of intracellular IPTG, divided by the optical density of the culture at the time of sampling. Strain 3000 was used.*

the culture with 10^{-1} M fucose, the intracellular concentration of IPTG reached values which exceeded those required to produce maximal repression in the culture with 3×10^{-3} M fucose; nevertheless, the rate of galactokinase synthesis was high and remained constant. Thus, fucose must act by some internal mechanism to reverse the repression caused by IPTG.

From these experiments, we concluded that an intracellular competition exists between induction and repression in the *gal* operon.

DISCUSSION

The *lac* and *gal* operons not only code for enzymes which act sequentially in metabolism but the same family of compounds act as regulatory effectors for both. Nevertheless, the data presented argue against the existence of a single regulatory molecule common to both operons. Various galactosides function as *gal* repressors in a *lac* deletion strain and as *lac* inducers in strains mutated or deleted for the *gal* regulatory genes. Similarly, fucose functions as a *gal* inducer in a *lac* deletion strain and as a *lac* repressor in strains mutated or deleted for the *gal* regulatory sites. In each case, a mutation which destroys the ability of one operon to respond to an effector has no effect on the sensitivity of the other. If a common regulatory element is absent, the similarity of regulatory sites in the two operons may reflect their evolutionary origin from a common ancestor. The regulatory and structural genes of one operon might have arisen by duplication of the other operon, followed by evolutionary divergence.

For both operons, the rate of enzyme synthesis is set by the relative intracellular concentrations of both inducer and repressor molecules. Other operons may also be controlled by a competition between inducers and repressors. Ornithine transcarbamylase is not only repressed by arginine but is also "derepressed" by glutamate, a precursor of the pathway, and the effects reported appear to be competitive (6). Sulfite reductase synthesis is repressed by cysteine or methionine. In the absence of these compounds, the enzyme is derepressed; yet the further addition of serine (10) or L-djenkolic acid (4) causes an additional increase in the rate of enzyme formation. In the mandelate pathway, the first three enzymes are induced by mandelate, and are repressed by the end products benzoate, catechol, succinate, and acetate (15). Alanine dehydrogenase is induced by alanine and repressed by nicotinic acid, and in this case also induction and repression appear to be competitive (5).

Adhya and Echols (1) and Lengeler (8) have suggested that the glucose repression of the *gal* operon results from the inhibition of inducer entry. However, competition at the level of entry does not account for the induction and repression of the *lac* and *gal* operons by various galactosides. We have shown that in these cases the competition occurs inside the cell.

The sensitivity of operons to both inducers and repressors may well be a general phenomenon. Just as a cell does not require the enzymes of a metabolic pathway when the end product is supplied exogenously, a cell also has little use for the enzymes of a pathway unless the substrate is available. If operons are metabolically regulated, so to speak, from both ends, the classification of enzymes as repressible or inducible becomes arbitrary. The distinction between inducible and repressible systems probably depends on whether the balance set by endogenous effectors is expressed as a relatively low or high rate of enzyme synthesis.

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