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Three Kinds of Controls Affecting the Expression of the *glp* Regulon in *Escherichia coli*

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Three kinds of control mechanisms govern the expression of the members of the *glp* regulon for glycerol and *sn*-glycerol 3-phosphate (G3P) catabolism in *Escherichia coli* K-12: specific repression by the product of the *glpR* gene; catabolite repression; and respiratory repression (the effect exerted by exogenous hydrogen acceptors). The operons of the *glp* system show different patterns of response to each control. By growing in parallel a mutant strain with temperature-sensitive repressor ($glpR^{ts}$) and an isogenic control with a deletion in the regulator gene at progressively higher temperatures, it was possible to show that the synthesis of aerobic G3P dehydrogenase (*glpD* product) is far more sensitive to specific repression than that of either glycerol kinase (*glpK* product) or G3P transport (*glpT* product). Conversely, in the strain with a deletion in the regulator gene, the syntheses of glycerol kinase and G3P transport are more sensitive to catabolite repression than that of the aerobic G3P dehydrogenase. The levels of the two flavoprotein G3P dehydrogenases vary in opposite directions in response to changes of exogenous hydrogen acceptors. For example, the ratio of the aerobic enzyme to the anaerobic enzyme (specified by *glpA*) is high when molecular oxygen or nitrate serves as the hydrogen acceptor and low when fumarate plays this role. This trend is not influenced by the addition of cyclic adenosine 3',5'-monophosphate to the growth medium. Thus, respiratory repression most likely involves a third mechanism of control, independent of specific or catabolite repression.

Glycerol and *sn*-glycerol 3-phosphate (G3P) (16) are dissimilated by *Escherichia coli* K-12 through a converging pathway schematized in Fig. 1. The glycerol branch begins with an entry process involving facilitated diffusion (31, 32) which is followed by an adenosine 5'-triphosphate (ATP)-dependent phosphorylation (11, 13, 38, 42, 43). The G3P branch begins with an active transport process (10). Both branches provide the common substrate for either the aerobic G3P dehydrogenase (22, 39) or the anaerobic G3P dehydrogenase (18, 20), depending on the nature of the electron acceptor available (19). Both dehydrogenases convert G3P to dihydroxyacetone phosphate (4, 20, 39), a metabolite of the glycolytic network. The gene for glycerol facilitator (*glpF*) and the gene for glycerol kinase (*glpK*) appear to be of a single operon (1) located at minute 76 on the chromosome according to Taylor (7, 37). The gene for the G3P transport system (*glpT*) and the gene for the anaerobic G3P dehydrogenase (*glpA*) are located next to each other at minute 43 (5, 19) as indicated on the Taylor map, perhaps also belonging to a single operon. The gene for aerobic G3P dehydrogenase (*glpD*) is found in a third region at minute 66, adjacent to the *glpR* locus specifying the repressor which regulates the expression of all the known operons of the *glp* system (5) and which responds to G3P as the effector (12) (Fig. 2). Two other controls exist for the expression of members of the *glp* regulon; these are catabolite repression and the effect exerted by the electron acceptor serving the G3P flavo-dehydrogenases. This latter effect will be referred to as respiratory repression. Results from earlier studies indicated that the *glp* operons can be distinguished from each other by their response patterns to the three control mechanisms (5, 19, 21, 42).

Attempts were made in this study to characterize the relative sensitivity of each *glp* operon to the three regulatory mechanisms by systematic comparisons of appropriate mutants grown under well-controlled conditions.

MATERIALS AND METHODS

Bacterial strains. The genotypes of the *E. coli* K-12 strains used in these experiments are described in Table 1. Strain 75, an aerobic G3P dehydrogenase-

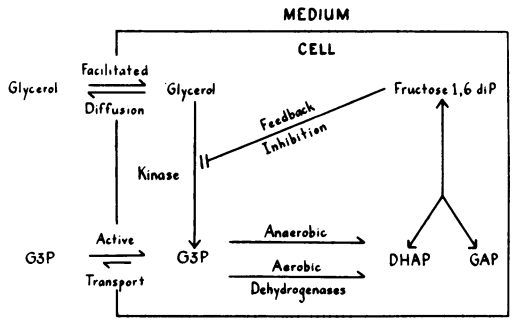

FIG. 1. *The converging catabolic pathway for glycerol and G3P in E. coli K-12. DHAP stands for dihydroxyacetone phosphate and GAP, D-glyceraldehyde 3-phosphate.*

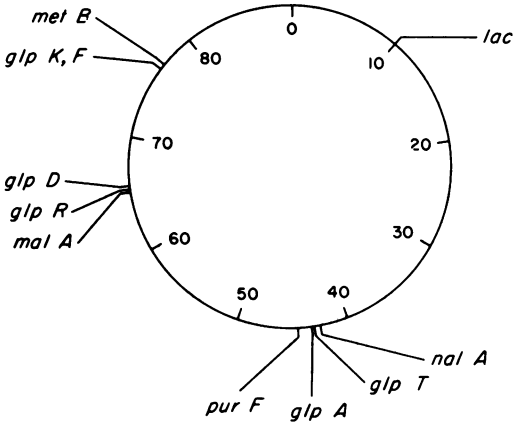

FIG. 2. *Genetic map of E. coli (1) showing the scattered locations of the glp genes. K denotes glycerol kinase; F, glycerol facilitator; D, aerobic G3P dehydrogenase; T, G3P transport; A, anaerobic G3P dehydrogenase; and R, repressor.*

negative derivative of strain 72, was isolated by W. S. Kistler as an aerobic glycerol-negative clone following ethyl methanesulfonate treatment (10) and aerobic penicillin counterselection in the presence of glycerol and G3P (5). Strain 402 was constructed by infecting strain 203 (*glpR*$^+$, *glpD*$^-$, *glpA*$^-$) (19) with transducing bacteriophage P1 grown on strain 72 (*glpR*^{del}, *glpD*$^+$, *glpA*$^+$) and selecting for aerobic growth on glycerol minimal medium. Enzymatic analysis confirmed that the transductant had acquired the aerobic G3P dehydrogenase activity and produced both this enzyme and glycerol kinase constitutively. Like the donor strain, the transductant is maltose-negative, and thus can be assumed to carry the same small deletion covering portions of both the *glpR* and *malA* genes. G3P dehydrogenase-negative strains were kept on agar slants containing 1% glucose, 1% casein amino acids (1% CAA) in minimal medium.

Media and growth conditions. The same mineral medium (36) was used in all experiments. As carbon sources with low catabolite repression, either casein acid hydrolysate at a final concentration of 0.3% (salt-free, vitamin-free grade from Nutritional Biochemicals Co., Cleveland, Ohio), or a synthetic mixture was used. The latter medium contains L-alanine, L-aspartate, and L-glutamate (2 g/liter each) as principal ingredients and the following amino acids as supplements (concentrations in mg/liter): L-arginine (hydrochloride), 60; cysteine (hydrochloride) 13; glycine, 30; L-histidine, 10; L-isoleucine, 30; L-phenylalanine, 30; D,L-serine, 60; L-threonine, 30; L-tryptophan, 10; L-tyrosine, 20; L-valine, 30. Stock amino acid solutions were sterilized by filtration (0.45 μm pore size; Millipore Corp., Bedford, Mass.). All amino acids used were from Calbiochem., La Jolla, Calif. (A grade). For anaerobic studies, the mineral medium was supplemented with 30 mM pyruvate and 0.1% CAA (19).

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

Strain no.	Parent	Reference	Genotype *glp*				
			K	T	A	D	R
1	K-12	22	+	+	+	+	+
89	1	5	+	+	+	+	n[a]
72	89	5	+	+	+	+	del
252	89	5	+	+	+	+	ts
75	72	[b]	+	+	+	−	del
402	203, 72	[b]	+	+	−	+	del

[a] The abbreviations n, del, and ts stand for noninducible, deletion and temperature-sensitive, respectively.

[b] For further details see Materials and Methods.

Unless otherwise stated, growth of cells was at 37 C. Aerobic cultures (250-ml volume) were grown in 2-liter Erlenmeyer flasks agitated in a New Brunswick Scientific Model G 25 incubator shaker operated at approximately 240 rpm. Anaerobic cultures were grown in 250-ml screw-capped Erlenmeyer flasks completely filled with growth medium, stirred gently by a small magnetic bar at 70 to 100 rpm.

Aerobic cultures were started with 1% inocula from an overnight aerobic culture grown on 0.3% CAA. Anaerobic cultures were started with 1% inocula from an overnight anaerobic culture grown on 30 mM pyruvate and 0.1% CAA in the absence of any special electron acceptor.

Cell harvest and preparation of extracts. Cells were harvested from exponentially growing cultures at a turbidity of approximately 100 Klett units (corresponding to 0.1 mg of bacterial protein per ml) measured in a Klett-Summerson colorimeter with a number 42 filter.

For assay of G3P transport, 0.1 ml of chloramphenicol (2 mg/ml) was added to a 5-ml culture sample before the cells were collected by centrifugation at about 1,000 × *g* for 14 min at room temperature. The cell pellet was suspended in 2.5 ml of 10 mM potassium phosphate (pH 7.0), 0.85% NaCl and 40 μg of chloramphenicol per ml, and held in a 30 C water bath until assayed. G3P transport activity remained unchanged for at least 90 min.

For enzymatic assays, 200 to 220 ml of cultures were poured into ice-chilled tubes containing 5 ml of chloramphenicol solution (2 mg/ml) and centrifuged at 2,000 × *g* for 20 min at 3 to 5 C. The cell pellet was resuspended in chilled saline and recentrifuged and

stored overnight in a −20 C freezer. Thawed cells were suspended in 20 mM potassium phosphate buffer (pH 7.0) at 5 to 10 mg of bacterial protein per ml and disrupted by sonic oscillations as previously described (9), allowing 1 min of treatment per ml of suspension. The cell extracts were clarified by centrifugation at 12,000 × *g* for 20 min at 3 to 5 C. Triplicate or quadruplicate determinations of enzyme activity were made within 4 h after preparation of the extract.

Glycerol kinase assay. Glycerol kinase activity was assayed in 1 ml of a reaction mixture containing 10 mM glycerol, 20 mM ATP, 20 mM $MgCl_2$, 1 mM nicotinamide adenine dinucleotide (NAD), 180 mM glycine buffer (titrated to pH 9.5 with NaOH), and 300 mM hydrazine-hydrochloride (pH 9.5), 25 μg of crystalline rabbit muscle G3P dehydrogenase (C. F. Boehringer & Soehne, Mannheim, Germany) and up to 0.1 ml of cell extract. Absorbance was monitored in a Gilford recording spectrophotometer at 340 nm against a control containing all of the above components except muscle G3P dehydrogenase. The sensitivity of this assay ranged from 2 to 200 nmol of NADH produced per min. The employment of glycine instead of carbonate (22) as a buffer improved the reproducibility of the assay.

Aerobic and anaerobic G3P flavoprotein dehydrogenases. To determine aerobic G3P dehydrogenase activity, the reaction mixture previously described was supplemented with Triton X-100 at a final concentration of 0.2% to improve solubility of the reduced form of the tetrazolium acceptor dye (19). To determine the activity of the anaerobic G3P dehydrogenase, triplicate assays were performed first without and then with added flavines. The means and variances for the two sets of measurements were computed, and the significance of differences were evaluated with the *t* test. The increment of activity caused by the presence of 10^{-5} M flavin-adenine dinucleotide and 10^{-3} M flavin mononucleotide was attributed to the anaerobic enzyme (19).

G3P transport. Since the assay used in previous studies gave values that are 1 to 2% of those predicted from the observed growth rates on G3P (22), a revised method which revealed much higher specific transport activity was adopted for the present study. A 50-μliter cell suspension was added to 200 μliters of a 10 mM potassium phosphate buffer (pH 7.0) containing 0.145 M NaCl and 0.02 μCi of ^{14}C-G3P diluted with carrier compound (Sigma grade X, D,L-α-glycerophosphate, i.e., glycerol 1-P and glycerol 3-P) to give a final concentration of 0.2 mM of the L-isomer of G3P. The reaction mixture was incubated in a test tube at 30 C, and at 45-s intervals, a 50-μliter sample was transferred by an Eppendorf automatic pipet onto the center of a filter (0.65 μm pore size; Millipore HAWP, Bedford, Mass.) mounted on a sintered glass disk over a vacuum. The wet spot on the filter was washed with 4 drops (approximately 0.2 ml) of 0.85% NaCl delivered by a Pasteur pipet. Washing was complete within 7 s after sampling. The filter was dissolved in a scintillation vial containing Bray's solution (2) for counting of radioactivity. Transport rates were generally linear over the first 2 min of assay.

Chemical methods. ^{14}C-*sn*-glycerol-3-phosphate stocks were freed from possible contaminating glycerol by adsorption on columns of Dowex-1 AG 1 X-2 formate at 3 to 5 C. After washing with water, the G3P was eluted with formic acid by using a linear gradient of 0 to 5 M. The fractions containing ^{14}C-G3P were pooled, concentrated by lyophilization, dissolved again in 1 mM $NaHCO_3$, and stored at −20 C.

Unlabeled G3P and G1P (D,L-α-glycerophosphate, Sigma Chemical Co., grade X) was recrystallized twice from a 76% ethanolic solution (F. Ruch, personal communication), a procedure which reduced the glycerol content from nearly 4% to less than 0.2%.

G3P content was determined enzymatically in a 1-ml reaction mixture containing 50 mM $NaHCO_3$ (pH 8.3), 2 mM NAD, 0.1 mg phenazine methosulfate (PMS), 0.05 mg of [3(4,5 dimethylthiazolyl 1-2) 2,5 diphenyl tetrazolium bromide] (or MTT), and 2 mg of Triton X-100, and up to 0.1 ml of solution containing G3P. The reaction was initiated by adding 25 μg of crystalline rabbit muscle G3P dehydrogenase and monitored at 570 nm until the rate of change in absorbance was less than 0.017 optical density units per min. A molar extinction coefficient of 1.7×10^4 (19) was used to calculate the G3P concentration.

Glycerol content (5–50 nmol in 10–50 μliters) in the presence of greater than 100-fold molar excess of G3P was determined enzymatically in a 1-ml reaction mixture containing 40 mM potassium phosphate buffer (pH 7.0), 4 mM $MgCl_2$, 1 mM ATP, 5 mM phosphoenolpyruvate, 0.10 to 0.15 mM NADH, 10 μg of canine muscle L-lactate dehydrogenase, 10 μg of rabbit muscle pyruvate kinase, and 5 μg of *Candida mycoderma* glycerol kinase. (All enzyme preparations were from C. F. Boehringer & Soehne, Mannheim, Germany.) The absorbance was monitored at 340 nm. In this assay, it is essential that the glycerol sample be added last, after the enzymatic blank reactions (probably due to traces of ADP in the ATP solution, and traces of pyruvate in phosphoenolpyruvate solutions) are complete.

Protein determination. The procedure of Herbert, Phipps, and Strange (14) designed to determine the protein content of bacterial suspensions was used for intact cells and for cell extracts. Bovine serum albumin (crystallized, Pentex Corporation, Kankakee, Ill.) served as a standard.

RESULTS

Specific repression. Cultures of strain 252 (*glpR*^{ts}) were grown over a temperature range from 26.5 to 42 C on a low catabolite-repressive medium consisting of synthetic amino acids. Since the *glpR*^{ts} allele was derived from a *glpR*^{n} (noninducible) allele by selection of a clone that grew on glycerol at 42 C but not at 25 C, the *glp* operons are expected to be progressively derepressed as the growth temperature increases. Possible effects of temperature on transcription and translation per se are nulled by comparison with the isogenic but repressor-deleted strain 72 (*glpR*^{del}) grown under identical conditions. The

data are presented as specific activity repression ratios defined as (specific activity of strain 72/specific activity of strain 252) − 1.0. Assuming the specific activities are proportional to the concentration of operons not repressed and available for transcription, and that non-cooperative binding of a single molecule of repressor to the operator is sufficient to cause repression, then

$$\text{S.A. [Strain 72]/S.A. [Strain 252]} - 1.0 = (\text{O-R})/(\text{O}) = (K_a)\,(\text{R})$$

where S.A. stands for specific activity, (O), (R), and (O-R), respectively, represent the concentrations of operator, repressor, and the operator-repressor complex, and K_a is the association constant for the operator-repressor complex. According to this model, a repression ratio of zero would represent a fully derepressed state, with higher ratios indicating increasing degrees of repression of gene expression.

As can be seen from the results presented in Fig. 3, at the uppermost temperature, strain 252 behaved like a fully constitutive mutant. As the growth temperature decreased, which should result in the increase of the concentration of functional repressor, progressive repression of the three operons did occur as expected. Expecially noteworthy is the more severe repression of the *glpD* gene relative to those of the *glpK* and *glpT* genes, a fact that suggests different binding constants of the three operator regions for the repressor.

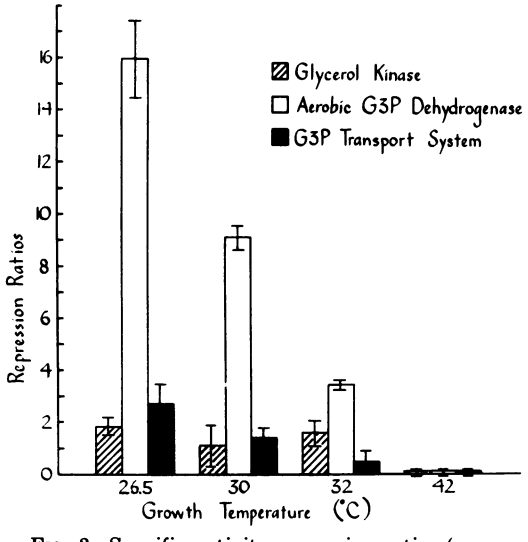

FIG. 3. *Specific activity repression ratios (see explanation and equation in text) for glycerol kinase, aerobic G3P dehydrogenase, and G3P transport activity as a function of growth temperature. Strain 72 (glpR^{del}) and strain 252 (glpR^{ts}) were grown side by side in SAA medium at each temperature. The ratio of zero indicates full derepression in the temperature-sensitive strain.*

Catabolite repression. The patterns of catabolite repression of the various operons of the *glp* system were examined in strain 72 (*glpR*^{del}) grown on a synthetic mixture of amino acids plus or minus glucose. The synthetic amino acid mixture was found to give higher constitutive enzyme levels than the casein amino acid medium employed in an experiment described earlier (21). Glycerol kinase, the G3P transport system and G3P dehydrogenase showed decreasing sensitivity to repression by glucose (Table 2). The catabolite repressibility of anaerobic G3P dehydrogenase was similar to that expected of G3P transport, as measured in strain 75 which lacks the aerobic dehydrogenase (Tables 2, 3).

Respiratory repression. Because glycerol facilitator, glycerol kinase, and the G3P transport play essential roles irrespective of whether the cells are growing aerobically or anaerobically, the study of the effect of electron acceptors on enzyme synthesis was focussed on the two G3P dehydrogenases. To insure unambiguous deter-

TABLE 2. *Catabolite repression*[a]

Supplement	Glycerol kinase	Specific activities[b]	
		G3P transport	Aerobic G3P dehydrogenase
A, None	2.59 ± 0.05	0.407 ± 0.008	0.266 ± 0.010
B, Glucose (10 mM)	0.072 ± 0.008	0.093 ± 0.020	0.123 ± 0.008
Ratio (A/B)	36.0 ± 4.0	4.4 ± 0.9	1.7 ± 0.1

[a] Strain 72 (*glpR*$^-$) was grown at 37 C on synthetic amino acid medium with or without 10 mM glucose.

[b] Specific activities are in micromoles substrate converted to product per minute per milligram of protein at 30 C in the case of the enzymes, and micromoles G3P taken up per milligram of cell suspension protein per minute in the case of G3P transport.

TABLE 3. *Catabolite repression of anaerobic G3P dehydrogenase in a constitutive strain lacking aerobic G3P dehydrogenase*

Supplement[a]	Anaerobic GeP dehydrogenase activity[b]
None	0.008 ± 0.001
Glucose (10 mM)	<0.001

[a] Strain 75 (*glpD*$^-$) was grown on SAA medium aerobically at 37 C, with and without 10 mM D-glucose.

[b] Micromoles dye reduced per minute per milligram of protein ± standard deviation.

mination of the individual activity of these two dehydrogenases, strain 402 (*glpA*$^-$, *glpR*^{del}) and strain 75 (*glpD*$^-$, *glpR*^{del}), each possessing only one of the enzymes, were grown under the same conditions. The level of aerobic G3P dehydrogenase was maximal when cells were grown with oxygen as the external hydrogen acceptor (Fig. 4). The level of anaerobic G3P dehydrogenase was maximal when the cells were grown with fumarate as the exogenous hydrogen acceptor (Fig. 5). Anaerobically, nitrate, either alone or in the presence of fumarate, repressed the levels of the anaerobic dehydrogenase back to the range found in aerobically grown cells. Even though the data also show that anaerobically, in the absence of any added hydrogen acceptor, the levels of anaerobic G3P dehydrogenase approach those found in the presence of fumarate, it should be remembered that the pyruvate-casein amino acid medium contains fumarate-yielding compounds such as aspartate (0.7 mM) which may be converted to fumarate plus NH_3 by aspartase. The trends of change in the levels of aerobic G3P and anaerobic G3P dehydrogenase can be more clearly discerned when the average values of pooled experiments for each growth condition are presented in juxtaposition (Fig. 6). The *glpD* gene product is low under just those circumstances where the *glpA* gene product is high.

The fact that constitutive levels of the two

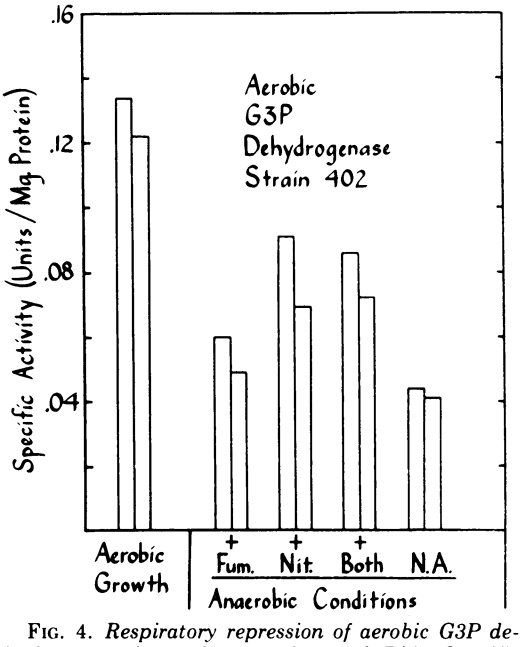

FIG. 4. *Respiratory repression of aerobic G3P dehydrogenase in strain 402 (glpA*$^-$, *glpR*^{del}*). Specific enzyme activity is expressed in micromoles tetrazolium dye reduced per minute per milligram of protein at 30 C. Hydrogen acceptors added to anaerobic growth media: 10 mM sodium fumarate (+ Fum); 10 mM* KNO_3 *(+ Nit); 10 mM fumarate and 10 mM nitrate (both); none added (N.A.).*

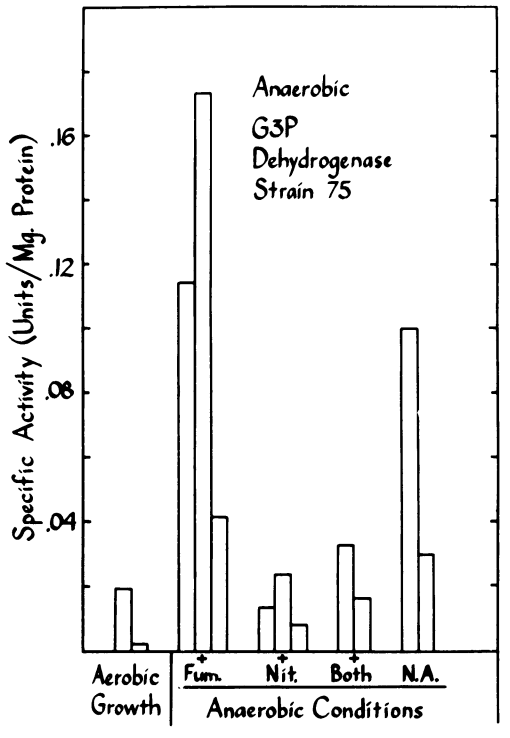

FIG. 5. *Respiratory repression of anaerobic G3P dehydrogenase of strain 75 (glpD, glpR*^{del}*). Units and abbreviations as in Fig. 4.*

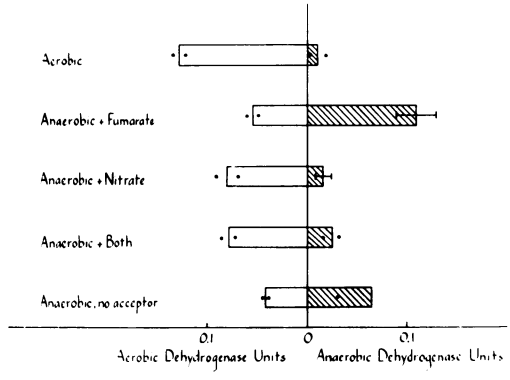

FIG. 6. *Respiratory repression of the two G3P dehydrogenases. Composite data on aerobic dehydrogenase (obtained from strain 402) and anaerobic dehydrogenase (obtained from strain 75) are plotted. Units are in micromoles per minute per milligram of protein. Bars represent the average of at least two separate experiments. The individual determinations are shown by ●. Where three experiments were performed, standard deviations are presented.*

dehydrogenases can change in opposite directions excludes catabolite repression as an explanation. This conclusion was tested by growing cells on glucose, aerobically or anaerobically, in the presence or absence of cyclic AMP. The level of glycerol kinase was used as an indicator for the severity of catabolite repression operating under the various conditions and the level of ethanol dehydrogenase was used as an indicator for the degree of respiratory repression (23). The levels of anaerobic G3P dehydrogenase and ethanol dehydrogenase, in contrast to that of glycerol kinase, were not significantly influenced by externally added cyclic AMP (Table 4).

Number of glp operons. During the course of this study, it was noticed that the products of the two closely located *glpA* and *glpT* genes seem to vary coordinately (Fig. 7). Thus, these two genes probably belong to a single operon, as in the case of *glpF* and *glpK*.

DISCUSSION

Differential sensitivity of the *glp* operons to specific repression (induction) and to catabolite repression point to differences in the operator and promoter regions of the three gene clusters of the *glp* regulon. These differences may have been the result of natural selection to safeguard the cells against metabolic embarrassments. The greater severity of specific repression of aerobic G3P dehydrogenase relative to the repressions of glycerol kinase and G3P transport may be desirable for two reasons: (i) to prevent breakdown of G3P derived from biosynthesis in the absence of exogenous glycerol or G3P, aided further by the fact that the catabolic G3P dehydrogenases have poorer affinities for the substrate than the biosynthetic enzyme, G3P acyl transferase; (ii) to minimize resistance to induction of the *glp* regulon. High kinase-dehydrogenase and G3P transport-dehydrogenase basal activity ratios would allow rapid buildup of the inducer concentration when there is external glycerol or G3P. This second condition, however, is not without hazards; over-accumulation of G3P can be growth inhibitory, as illustrated by a mutant lacking aerobic G3P dehydrogenase (6), or a regulatory mutant (*glpR*^{n}) in which the repression cannot be overcome by the inducer (N. Cozzarelli, Ph.D. Thesis, Harvard University, Cambridge, Mass. 1966).

Preferential shutdown of the synthesis of glycerol kinase and G3P transport over that of G3P dehydrogenase by catabolite repression may be another safeguard against flooding the metabolic pool of G3P while cells are switched to favored carbon sources.

Reciprocal fluctuations in the levels of the two G3P flavoprotein dehydrogenases in re-

TABLE 4. *Specific activities*[a] of three enzymes in strain 75 grown on glucose ± cyclic AMP

Growth condition	Cyclic AMP	Glycerol kinase	Anaerobic G3P dehydrogenase	Ethanol dehydrogenase
Aerobic	−	146 ± 41	1.0 ± 0.3	2.7 ± 0.4
Aerobic	+	729 ± 118	6.3 ± 0.1	3.5 ± 1.6
Anaerobic	−	25 ± 4	40 ± 4	75 ± 4
Anaerobic	+	153 ± 6	42 ± 7	70 ± 8

[a] Millimicromoles per minute per milligram of protein.

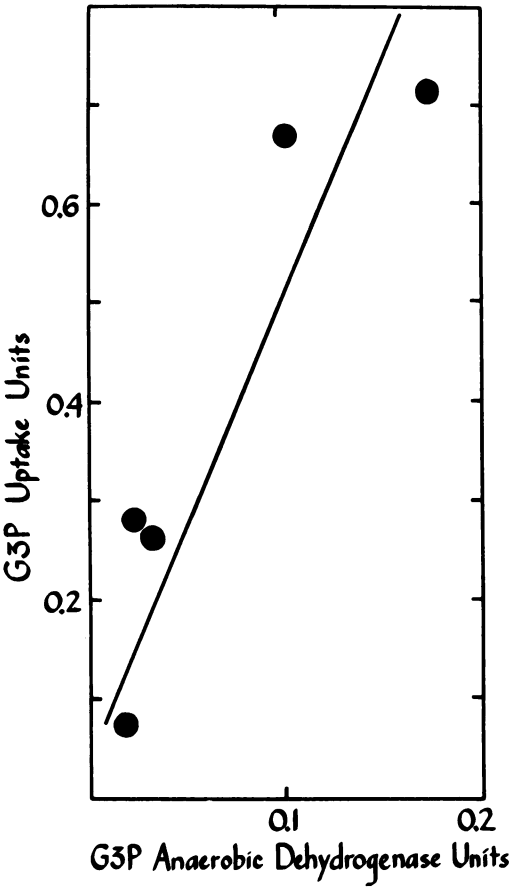

FIG. 7. *Correlation of G3P anaerobic dehydrogenase activity with G3P transport activity. Strain 75 was grown under five conditions with different hydrogen acceptors as in Fig. 4, 5, 6. Abscissa: G3P uptake units (micromoles per minute per milligram of protein). Ordinate: G3P anaerobic dehydrogenase activity units (micromoles per minute per milligram of protein).*

sponse to changes in the nature of available hydrogen acceptors, independently of specific and catabolite repression, point to a third class of control mechanism. The term respiratory repression is proposed to describe this phenomenon. This kind of control has been observed in studies of a number of bacterial enzymes (40). Apparently, through this mechanism the hierarchy of the synthesis of proteins participating in the electron transfer reactions is established. Interestingly, oxygen, which presumably offers maximal returns for energy generation, prevents the induction of the respiratory nitrate reductase (3, 25–29, 33, 34) as well as fumarate reductase (3, 15, 17, 24). In the absence of oxygen, nitrate prevents the induction of fumarate reductase which has the lowest energy-yielding potential (3, 41).

A teleonomic explanation (8, 30) for the higher levels of the G3P transport system and anaerobic G3P dehydrogenase attained during growth in the presence of fumarate may be advanced. It has been estimated that up to 3 mol of ATP may be produced per mol of nitrate reduced to nitrite during "nitrate respiration" (35). The reduction of fumarate to succinate in "fumarate respiration," on the other hand, is unlikely to yield, for thermodynamic reasons, more than one mole of high energy phosphate per mole of fumarate reduced. In this laboratory, we have observed that fumarate supports less total growth on G3P than does nitrate when provided on an equimolar basis (W. Freedberg, unpublished data), even though the exponential growth rates in the two cases are nearly the same (19). The increased levels of G3P transport and anaerobic dehydrogenase, when fumarate serves as the exogenous electron acceptor, may be essential to compensate for the lower efficiency for anaerobic energy-generation. It must also be pointed out, however, that the glycerol kinase activity of cells grown in the presence of fumarate is generally about one-half that of cells grown in the presence of nitrate, as shown initially by Kistler and Lin (19) and as confirmed here. In this case, no functional advantage is obvious for this behavior, apart from noting that glycerol kinase is generally produced in excess and that over-phosphorylation is prevented by feedback inhibition.

On the whole, it would seem that the various structural genes belonging to the *glp* regulon of *E. coli* are separably controlled in their expression so as to insure metabolic balance under a variety of conditions. For this purpose, three different mechanisms of controls are employed. On top of these, a kinetic feedback control at the enzymatic level can also be mobilized.

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