JOURNAL OF BACTERIOLOGY, Nov. 1969, p. 809-816
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Vol. 100, No. 2
Printed in U.S.A.

Catabolite Repression Gene of *Escherichia coli*

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Received for publication 15 August 1969

A catabolite repression gene (*cat*) which alters the sensitivity of *Escherichia coli* to catabolite repression has been mapped by transduction and shown to be located between the *pyrC* and *purB* genes. When the *cat-1* mutation was studied in a number of genetic backgrounds, the results showed that this mutation affects the synthesis of more than one catabolic enzyme but does not completely eliminate catabolic repression under all conditions. It is suggested that this mutation may cause a block in the accumulation of the catabolite effector. Our experiments show that this effector is not glucose-6-phosphate.

The differential rate of β-galactosidase (EC 3.2.1.23) synthesis in *Escherichia coli* is reduced by the presence of certain sugars such as glucose. If the rate of anabolism is limited, any source of carbon can severely repress the synthesis of this enzyme (15). This phenomenon, which affects many other catabolic enzymes, has been termed catabolite repression (14); it occurs, apparently, whenever the intracellular concentration of catabolites is excessive (14).

Loomis and Magasanik (11) isolated a mutant of *E. coli* K-12, strain LA12, in which the *lac* operon is insensitive to catabolite repression. Because strain LA12 grows more slowly on glucose than its parent, the possibility had to be considered that the decreased metabolism of glucose is responsible for this effect. They therefore isolated a mutant capable of the usual rapid growth on glucose from strain LA12, which they called strain LA12G. In this organism, too, glucose failed to repress β-galactosidase; it still caused repression of tryptophanase and amylomaltase. In this strain, β-galactosidase is insensitive to catabolite repression even when anabolism is reduced by nitrogen starvation. Low-frequency conjugation experiments suggested that the mutation responsible for the resistance of β-galactosidase to catabolite repression, *cat-1*, is near the *trp* operon on the genetic map (13). Examination of stable merodiploids of the region showed that the wild-type gene (cat^+) is *trans*-dominant to the mutant allele (cat^-).

We report here a closer mapping of this gene. [The gene *cat-1* was originally designated CR (10–12), but the nomenclature has been changed to conform to the rules suggested by Demerec et al. (2).]

In the course of these experiments, we observed that recombinants that had received the *cat-1* gene by transduction from strain LA12G grew more slowly on glucose than their parents; in these recombinants, glucose fails to repress tryptophanase.

While these experiments were in progress, Rickenberg et al. (17) reported that in an F^- mutant of strain LA12G, strain L9, which had been obtained from LA12G by treatment with acridine orange (13), not only β-galactosidase but also tryptophanase and amylomaltase are completely insensitive to catabolite repression by glucose. These authors also examined strain LA12G and found β-galactosidase to be insensitive to repression by glucose. Tryptophanase and amylomaltase were sensitive to repression by glucose, though they appeared to be somewhat less sensitive than in the wild strain. Thus, their results with strain LA12G do not differ greatly from those of Loomis and Magasanik (11).

Rickenberg et al. (17) also found that in all strains all three enzymes are severely repressed by glucose-6-phosphate. They concluded that the *cat-1* mutation is not specific for β-galactosidase, but presumably reduces the ability of the cell to form a general effector of catabolite repression from glucose. Our results, reported in this paper, are in general agreement with this conclusion. We can, in addition, offer a tentative explanation for the difference in the expression of the *cat-1* allele in strain LA12G and other strains that carry it.

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MATERIALS AND METHODS

Chemicals. Isopropyl-thio-β-galactoside (IPTG) and o-nitrophenyl-β-D-galactoside (ONPG) were obtained from the Mann Research Laboratories, New York, N.Y. ^{14}C-leucine and ^{14}C-arginine were purchased from New England Nuclear Corp., Boston, Mass. Glucose-6-phosphate was obtained from Sigma Chemical Co., St. Louis, Mo., and glucose-6-phosphate dehydrogenase, from Boehringer Mannheim Corp. The dye 5-bromo-4-chloroindolyl-β-D-galactoside was obtained from Calbiochem, Los Angeles, Calif.

Medium. Minimal medium was made by adding the following to 1 liter of water: K_2HPO_4, 10 g; $MgCl_2$, 0.2 g; Na_2SO_4, 0.2 g; NaCl, 5.0 g; sodium citrate, 0.2 g; $FeSO_4$, 0.46 mg; $CaCl_2$, 4.4 mg; and thiamine, 1.0 mg. The pH was adjusted to 7.0 with HCl. Carbon sources were added at 0.4%; $(NH_4)_2SO_4$ was present at 0.2% as nitrogen source except as indicated. Streptomycin sulfate was added at a concentration of 100 μg/ml when required.

LB medium was made by adding to 1 liter of water, 10 g of tryptone, 5 g of NaCl, 5 g of yeast extract, and 1 ml of 5 N solution of NaOH.

Solid indicator medium for catabolite repression of β-galactosidase was prepared from minimal medium by adding agar (1.5%), glucose (1.0%), and lactose (0.2%). In some cases, a dye, 5-bromo-4-chloroindolyl-β-D-galactoside (20 μg/ml), which yields a blue product when hydrolyzed by β-galactosidase, was included. This compound does not induce the *lac* operon. When the dye was omitted, colonies were examined for induced levels of β-galactosidase by exposing them to toluene vapor for several minutes and then layering them with ONPG as described by Loomis and Magasanik (10). This medium will distinguish between colonies of *cat-1* and cat^+ lac^+ ($I^+Z^+Y^+$) bacteria because β-galactosidase is induced in the *cat-1* bacteria but not in cat^+ bacteria under these conditions (9).

Bacterial strains. The bacterial strains used in this study are listed in Table 1.

Transduction by phage P1. Bacteriophage P1kc was obtained from S. E. Luria. Transducing phage were recovered from cells growing in LB medium according to the method of J. Rothman (Ph.D. Thesis, Massachusetts Institute of Technology, Cambridge, 1965) with the modification that $CaCl_2$ was added to a final concentration of 5×10^{-3} M. Recipient bacteria in the exponential phase of growth, at a titer of 5×10^8 bacteria/ml, were exposed to transducing phage at a multiplicity of infection of 10. This mixture was incubated at 37 C for 15 min. The cells were then washed, resuspended in saline, and plated on the appropriate selection plates.

Induction of enzymes. β-Galactosidase was induced by the addition of IPTG to a final concentration of 10^{-3} M; tryptophanase was induced by the addition of L-tryptophan to 0.4 mg/ml.

β-Galactosidase assay. β-Galactosidase activity was measured by the method reported by Loomis and Magasanik (10).

TABLE 1. *Bacterial strains*

Strains of *Escherichia coli* K-12	Pertinent characteristics[a]	Source or reference[b]
3.000	Hfr H lac+ ($I^+Z^+Y^+$)	a
3.300	Hfr H lac+ ($I^-Z^+Y^+$)	a
X7014-L	lac+ ($I^+Z^+Y^+$) pyrC− purB− Sm^r	b
X140-L	lac+ ($I^+Z^+Y^+$) pyrD− Sm^r	c
U160	F− lac+ ($I^+Z^+Y^+$) trp− Sm^r	a
U146	F− lac+ ($I^+Z^+Y^+$) trp− Sm^r	a
AB311	Hfr lac− ($I^+Z^+Y^-$) thr− leu− Sm^r	a
DF2000	Hfr lac+ ($I^+Z^+Y^+$) pgi− zwf−	d
LA-12	F+ lac+ ($I^+Z^+Y^+$) cat-1	e
LA-12G	F+ lac+ ($I^+Z^+Y^+$) cat-1	e
T-9	F− lac+ ($I^+Z^+Y^+$) cat-1	Obtained from LA-12G by acridine orange treatment (6)
T-3	F− lac+ ($I^+Z^+Y^+$) cat-1 thr− leu− Sm^r	Recombinant of AB311 and T-9

[a] The sex, *lac*, and streptomycin symbols are identical to those used by Loomis and Magasanik (10). *Cat* refers to a gene for insensitivity to catabolite repression. Thr−, leu−, and trp− refer to the inability to synthesize threonine, leucine, and tryptophan, respectively. Strains where these are indicated were supplied with the amino acids in the growth media at a concentration of 20 μg/ml. *PurB*, *pyrC*, and *pyrD* represent mutations in specific genes involved in the synthesis of purines and pyrimidines, respectively. The medium of strains carrying the *purB* mutation was supplemented with adenine (50 μg/ml) and guanine (50 μg/ml). *PyrC* and *pyrD* mutants received 50 μg of uracil per ml. The *pgi*− and *zwf*− symbols refer to the lack of activity of phosphoglucose isomerase and phosphoglucose dehydrogenase, respectively.

[b] (a) S. E. Luria; (b) a Lac+ derivative of strain X7014 supplied by J. Beckwith; (c) a Lac+ derivative of strain X140 supplied by E. R. Signer; (d) D. G. Fraenkel (4, 5); (e) Loomis and Magasanik (11).

Tryptophanase assay. The activity of tryptophanase (EC 4.1.1.27) was determined according to the method of Bilezekian et al. (1) by measuring indole formation from L-tryptophan.

Protein synthesis. The rate of protein synthesis was estimated by determining the rate of uptake of a radioactive amino acid according to the method of Nakada and Magasanik (16).

β-Galactosidase synthesis during nitrogen starvation. The cells were grown on various carbon sources, in minimal medium with any growth requirements added, to exponential phase. They were then collected on membranes (Millipore Corp., Bedford, Mass.), washed with twice the volume of warm 0.85% saline solution, and resuspended in minimal salts medium lacking a nitrogen source. Supplements and carbon sources were added as indicated in the text. IPTG, 10^{-3} M, and a radioactive amino acid were added. Samples were taken at 0, 10, 20, 40, and 80 min for assay of β-galactosidase and determination of the radioactivity incorporated into protein. The differential rate of enzyme synthesis, then, represents the rate of enzyme synthesis relative to the rate of protein synthesis.

Measurement of cellular glucose-6-phosphate. Cells in the exponential phase of growth were examined. The cells (2×10^8 to 5×10^8) were collected on a membrane filter (0.45-μm pore), washed with 2 ml of warm carbon-free medium, and immediately extracted with boiling water. This procedure was completed within 45 sec. The cell extract was then evaporated to dryness under an air stream, the residue was resuspended in the appropriate buffer, and the glucose-6-phosphate content was assayed enzymatically by use of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) according to the method of Horecker and Wood (7). Standard curves were prepared with known amounts of glucose-6-phosphate replacing the cells. This technique gave results which were linear in relation to the number of cells assayed. Reproducibility on duplicate samples was generally within 10%.

Measurement of growth rates. Cells were grown overnight in minimal medium containing the appropriate supplements and glucose, glycerol, or glucose-6-phosphate as the sole source of carbon. They were then diluted 1:100 into fresh medium and grown through several generations into exponential phase. Growth was followed by measuring the optical density at 530 nm with a Klett-Summerson spectrophotometer.

RESULTS

Mapping of the cat gene. Low-frequency conjugation experiments have placed the *cat* gene near the *trp* genes (13). However, these genes are not cotransducible by phage P1 (13). Signer et al. (18) have recently shown that the *purB*, *pyrC*, and *pyrD* genes map near the *trp* loci in *E. coli* K-12. Therefore, we investigated whether the *cat* gene is cotransducible with these markers.

A phage P1 lysate of strain LA-12G, a prototrophic strain which carries the *cat-1* mutation, was used to transduce a cat^+ strain, X7014-L, which has mutations in the *purB* and *pyrC* genes. Transductants to purine or pyrimidine independence were selected independently and then scored for the two unselected markers. The data given in Table 2 represent the total of two separate experiments and clearly show that the *cat* gene is cotransducible with both the *purB* and *pyrC* genes. Of 225 transductants selected for pyrimidine independence, 22% inherited the catabolite-insensitive (Cat^-) character, whereas only 4% received the $purB^+$ gene. When $purB^+$ was the selected marker, 27% of the transductants inherited the Cat^- phenotype and 4% were $pyrC^+$. Over 90% of the transductants which inherited both the $purB^+$ and $pyrC^+$ loci from strain LA-12G also inherited the *cat-1* mutation.

In addition, the *cat* marker was not cotransduced with the *pyrD* marker. When strain X140-L, which has a mutation in *pyrD1*, was transduced by a phage P1 lysate of strain LA-12G, not one of more than 1,000 transductants selected for pyrimidine independence also inherited the Cat^- character from strain LA-12G. Thus, it appears that the *cat* gene is centrally located between the *pyrC* and *purB* loci (Fig. 1).

Growth characteristics of cat-1 mutants. Many of the transductants of strain X7014-L which received the *pyrC* or *purB* genes from strain LA-12G do not grow as rapidly as the parental strains (Table 3); this reduction in growth rate

TABLE 2. *Transduction of the cat gene*[a]

Selected marker	Resulting characteristics			No. of transductants
	pyrC	*cat*	*purB*	
pyrC	+	−	+	9
	+	−	−	41
	+	+	−	174
	+	+	+	1
purB	+	−	+	7
	−	−	+	52
	−	+	+	160
	+	+	+	1

[a] Strain X7014-L ($pyrC^-$ cat^+ $purB^-$) was transduced by phage P1 grown on strain LA-12G ($pyrC^+$ *cat-1* $purB^+$) as described in Materials and Methods.

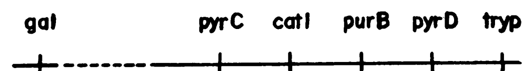

FIG. 1. *Partial linkage map of E. coli. Distances are not drawn to scale; based on the data of Signer et al. (18) and that of Table 2.*

TABLE 3. *Growth rates of various catabolite-sensitive and -insensitive strains*

Strain	Relevant genotype			Mass doubling time (min)		
	cat	*purB*	*pyrC*	Glycerol	Glucose	Glucose-6-phosphate
Parental strains						
3,000	+	+	+	90	65	55
X7014-L	+	–	–	85	60	60
U160	+	+	+	120	80	—
U146	+	+	+	90	60	—
LA-12	–	+	+	100	85	60
LA-12G	–	+	+	90	65	60
T-9	–	+	+	90	65	60
Transductants of strain X7014-L[a]						
T-70	+	–	+	90	70	—
T-71	–	–	+	85	160	—
T-56	+	+	–	130	100	100
T-55	–	+	–	160	360	140
T-58	+	+	–	130	120	100
T-57	–	+	–	170	360	160
T-59[a]	+	+	–	120	100	100
T-41	+	+	+	130	95	—
T-40	–	+	+	170	340	—
Recombinants of X7014-L[b]						
T-62	+	+	+	80	110	—
T-67	+	+	+	180	120	—
T-63	+	+	–	220	180	80
T-64	–	+	–	180	360	120
T-61	–	+	+	120	220	—
T-65	–	+	+	120	220	—
Other recombinants[c]						
T-1 (AB311 × T-9)	–	+	+	80	60	65
T-2 (AB311 × T-9)	+	+	+	80	60	65
T-3 (AB311 × T-9)	–	+	+	80	60	65
T-6 (3.300 × T-3)	–	+	+	80	60	65
T-16 (LA-12G × U160)	–	+	+	130	110	—
T-17 (LA-12G × U146)	–	+	+	90	60	—

[a] A phage P1 lysate of strain LA-12G was used in the construction of all transductants except that of strain T-59 which involved a lysate of strain 3.000.

[b] Strain LA-12G was the donor strain in the construction of all recombinants except that of strain T-67 in which strain 3.000 replaced strain LA-12G.

[c] These strains were derived from the crosses shown in parentheses. Strains T-1, T-2, and T-3 were selected for lac$^+$ Sm^r and then screened for *thr*, *leu*, and *cat* characters. Strain T-6 was selected for thr$^+$ leu$^+$ Sm^r and screened for the *lac*$^+$ (I$^-$Z$^+$Y$^+$) character. Strains T-16 and T-17 were selected for trp$^+$ Sm^r phenotype.

is generally greater in strains in which the *cat-1* mutation of strain LA-12G is integrated than in those which retain the wild-type *cat* allele. Moreover, while the parental strains and those transductants which remained catabolite-sensitive grow more rapidly when glucose or glucose-6-phosphate replaces glycerol as the sole source of carbon, the *cat-1* transductants grow considerably more slowly on glucose or glucose-6-phosphate than on glycerol. Similar results were observed in recombinants for *pyrC* or *purB* isolated from a conjugation experiment involving strain X7014-L (Table 3).

However, other strains which carry the *cat-1* mutation and do grow as rapidly as the wild-type strain when either glucose or glycerol serves as carbon source have been obtained from conjugation experiments involving other strains (Table 3) and selection for more distant markers. It appears that, when selection is for the closely linked *purB* or *pyrC* markers, the *cat-1* recombinants from LA-12G-derived strains grow slowly on glucose; when selection is for more distant markers, the *cat-1* recombinants grow well on glucose. Therefore, LA-12G appears to carry a second mutation that compensates for

the poor growth on glucose resulting from the *cat-1* mutation. Thus, it is apparent that the loss of sensitivity to catabolite repression in *cat-1* mutants is not caused merely by a gross reduction in the rate of catabolism of the source of carbon.

Specificity of the cat-1 mutation. Studies with strains LA-12 and LA-12G, in which the *cat-1* mutation was originally isolated, suggested that the mutation did not affect the catabolite sensitivity of tryptophanase or amylomaltase (11). However, when the *cat-1* mutation is transferred by transduction or conjugation to other strains of different genetic background, the catabolite sensitivity of tryptophanase is affected as well as that of β-galactosidase (Table 4).

Hsie and Rickenberg (8) have reported mutants of *E. coli* in which glucose does not repress the formation of several catabolic enzymes. They found that glucose-6-phosphate would give rise to catabolite repression in these mutants. As can be seen in Table 4, glucose-6-phosphate represses both β-galactosidase and tryptophanase in strains carrying the *cat-1* mutation as well as in cat^+ strains.

Nitrogen starvation in the presence of any source of carbon represses β-galactosidase in cat^+ but not in *cat-1* strains (11, 15). However, we have found (Table 5) that either uracil or leucine starvation of auxotrophic strains carrying the *cat-1* mutation results in severe repression of β-galactosidase when glucose or glycerol is present. In addition, when a leucine-requiring *cat-1* strain (T-72) is starved simultaneously for both nitrogen and leucine, glucose represses β-galactosidase 95%. Thus, it is apparent that the block in catabolite repression can be overcome by appropriate restrictions of anabolism. It is possible that *cat-1* mutation affects the accumulation of an effector of catabolite repression from glucose.

Relation of glucose-6-phosphate to catabolite repression. Studies with a mutant of *E. coli* lacking phosphoglucose isomerase (EC 5.3.1.9) have indicated that a high concentration of glucose-6-phosphate is not necessary for catabolite repression of the *lac* operon (12). However, Rickenberg and co-workers (8) have recently suggested that glucose-6-phosphate can directly effect catabolite repression. We have tested this hypothesis in strain DF2000 which lacks glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and phosphoglucose isomerase activities (4, 5). Growth of this strain in the presence of glucose or glucose-6-phosphate results in a much higher internal concentration of glucose-6-phosphate than is found in wild-type cells, but it does not result in repression of β-galactosidase (Table 6). Moreover, when this strain is starved for nitrogen, the presence of glycerol and gluconate represses β-galactosidase whereas glucose is without effect (Table 7). Clearly, a high concentration of glucose-6-phosphate is neither necessary nor sufficient for catabolite repression of β-galactosidase.

DISCUSSION

The *cat-1* mutation which alters the sensitivity of the *lac* operon in *E. coli* to catabolite repression has been genetically mapped by transduction with phage P1. The mutation occurs at a locus between *pyrC* and *purB* near the *trp* locus of *E. coli* (Fig. 1).

The ability to co-transduce the *cat-1* mutation

TABLE 4. *Catabolite sensitivity of tryptophanase*

Strain	Relative differential rate[a]					
	β-Galactosidase			Tryptophanase		
	Glycerol	Glucose	G-6-P	Glycerol	Glucose	G-6-P
cat^+ strains[b]						
3000	100	66	25	100	10	<1
3.300	100	60	15	100	5	—
X7014-L	100	45	—	100	5	—
cat-1 strains[b]						
LA-12G	100	100	20	100	25	—
T-1	100	100	20	100	110	<1
T-6	100	100	20	100	55	—
T-64	100	100	—	100	100	—
T-71	100	100	—	100	100	—

[a] Values are relative to the rate in the control culture with glycerol as carbon source. G-6-P = glucose-6-phosphate.
[b] The relevant genotypes can be seen in Table 3.

TABLE 5. *Repression of β-galactosidase in cat-1 mutant strains*

Strain[a]	Growth requirements	Starved for	Carbon source present	Relative differential rate[b]
T-1	None	Nitrogen	None	100
		Nitrogen	Glycerol	100
T-73	Uracil	Uracil	None	100
		Uracil	Glycerol	<5
		Uracil	Glucose	<5
T-72	Leucine	—	None	100
		Nitrogen	None	100
		Nitrogen	Glucose	90
		Leucine	None	90
		Leucine	Glucose	<5
		Leucine, nitrogen	None	100
		Leucine, nitrogen	Glucose	5

[a] Strain T-1 was isolated as a streptomycin-resistant prototroph carrying a *cat-1* mutation from a mating of strain AB 311 and T-9. Strain T-72 is a *cat-1* leucine auxotroph obtained from this same mating. Strain T-73 was isolated as a uracil auxotroph after ethylmethyl sulfonate treatment (11) of strain T-1.

[b] The rates were determined as described in Materials and Methods. The values are relative to the rate in the control culture in a carbon-free medium lacking any nutritional supplements. In the experiments with T-1 and T-73, ^{14}C-labeled leucine, 0.05 μc per 10 μg per ml, was added for determination of the differential rate. In the experiment with strain T-72, ^{14}C-labeled arginine (0.25 μc per 10 μg per ml) was present for this determination.

with either a purine or pyrimidine gene has allowed us to construct a series of strains carrying the *cat-1* mutation in different genetic backgrounds. Study of these transductants has indicated that the introduction of a short segment of genetic material carrying the *cat-1* mutation into the bacterial chromosome impairs the ability of the organism to grow rapidly on glucose. Such recombinants, in contrast to those that have not received that *cat-1* gene, but only the neighboring *purB* or *purC* regions, grow considerably more slowly on glucose than on glycerol.

In the slow-growing *cat-1* recombinants, neither β-galactosidase nor tryptophanase is subject to repression by glucose. This is hardly surprising since it is generally accepted that catabolite repression is not due to glucose itself but to catabolites derived from the rapid metabolism of glucose (14). It is considerably more interesting that some strains have the *cat-1* character, but grow as rapidly on glucose as the *cat*$^+$ wild strain. An example of such a strain is the donor used in the transduction experiments, strain LA12G. This organism is the product of two selections: first for the Cat$^-$ phenotype, that is, insensitivity of β-galactosidase to catabolite repression, and then for rapid growth on glucose (11). Judging from the results of the transduction experiments reported here, the first mutation has simultaneously affected the ability of glucose to support rapid growth and to exert catabolite repression; the second mutation has restored the ability of glucose to support rapid growth but not its ability to exert catabolite repression. This second mutation, as shown in an earlier paper, has enhanced the ability of glucose to exert transient repression (19). This ability appears to be related to the transport of glucose through the cell membrane,

TABLE 6. *Cell-associated glucose-6-phosphate*

Strain	Carbon sources in medium	Glucose-6-phosphate (nmoles/4 × 10^8 cells)	Relative rates of β-galactosidase synthesis[a]
DF2000	Gluconate	<0.75	100
	Gluconate + glucose	7.1	100
3,000	Gluconate	<0.75	70
	Gluconate + glucose	1.5	30

[a] The rates are relative to that in a culture of strain DF2000 growing on gluconate-minimal medium. Glucose-6-phosphate was measured as described in Materials and Methods.

TABLE 7. *Catabolite repression in strain DF2000*

Carbon source present during nitrogen starvation	Relative differential rate of β-galactosidase synthesis[a]
None	100
Gluconate[b]	13
Glycerol[c]	5
Glucose[b]	100

[a] The rates were determined as in Materials and Methods. The values are relative to the differential rate of synthesis in a control culture similarly pregrown and incubated in the absence of both carbon and nitrogen sources. ^{14}C-leucine (0.1 μc per 10 μg per ml) was present in all cultures for determination of the differential rate.

[b] Cells pregrown in medium containing glucose and gluconate.

[c] Cells pregrown in medium containing glycerol.

and it is therefore possible that the rapid growth on glucose of strain LA12G reflects an enhanced uptake mechanism for this sugar. The second mutation is not linked closely to *cat-1*, but can be transferred in matings in which selection is for markers distant from *cat-1* (*see* Table 3); we have not attempted to map it. It is of further interest that in some of the strains that carry the *cat-1* allele as well as the gene restoring rapid growth on glucose, tryptophanase is completely insensitive to catabolite repression (*see* Table 4, strain T1), whereas in others it is partially sensitive (strain T6) or almost fully sensitive (strain LA12G). [In the wild strain, tryptophanase is repressed 90% by glucose; in strain LA12G, Loomis and Magasanik (11) reported 90% repression, Rickenberg et al. (17) reported 75% repression, and in the present study we found 75% repression.] Apparently, the second mutation, although it fails to restore the ability of glucose to repress β-galactosidase, an enzyme not very sensitive to catabolite repression, may restore its ability to repress tryptophanase, an enzyme very sensitive to catabolite repression.

We showed earlier that in strain LA12G β-galactosidase cannot be repressed by glucose or by glycerol when the rate of anabolism has been reduced by starvation for nitrogen (11). We have now found, using appropriate auxotrophs carrying the *cat-1* allele, that glucose or glycerol represses β-galactosidase when anabolism is restricted by uracil or leucine starvation. Thus, under appropriate conditions the *cat-1* mutants do exhibit sensitivity to catabolite repression. This finding is in agreement with the observation by Rickenberg et al. (17), which we have confirmed here, that the *cat-1* strains are as sensitive to repression of β-galactosidase by glucose-6-phosphate as are cat^+ strains.

On the basis of the evidence presented in this paper, it appears that the *cat-1* mutation causes an impairment in glucose metabolism. This impairment decreases the production of a general effector of catabolite repression from glucose. A separate second mutation restores glucose metabolism so that rapid growth on glucose becomes possible in spite of the defect determined by the *cat-1* allele. A cell carrying this second mutation as well as *cat-1* does not produce that general effector from glucose in sufficient amount to repress β-galactosidase, but may produce enough to repress tryptophanase.

The nature of the defect in glucose metabolism caused by the *cat-1* mutation is not known (3). The fact that a *cat-1* mutant is as sensitive to repression by glucose-6-phosphate as a cat^+ strain suggested the possibility that the mutant is unable to produce glucose-6-phosphate from glucose at a rate sufficient for rapid growth and catabolite repression (17). However, Hsie et al. (9) have recently found that the levels of glucose-6-phosphate in glucose-grown cells of strain LA12G are as high as those in glucose-grown cells of the wild strain. Moreover, glucose-6-phosphate is clearly not the effector of catabolite repression as suggested earlier by Hsie and Rickenberg (8). We find that a mutant that lacks the enzymes necessary to metabolize glucose-6-phosphate accumulates this compound intracellularly during growth in a glucose-containing medium; nevertheless, in these cells β-galactosidase is insensitive to repression by glucose.

ACKNOWLEDGMENTS

This investigation was supported by grant GB-265 from the National Science Foundation and by Public Health Service grants GM-07446 and CA-05210 from the National Institutes of Health. Bonnie Tyler was a Predoctoral Fellow of the National Institutes of Health under fellowship 5-F1-GM-25,882-03.

LITERATURE CITED

1. Bilezekian, J. P., R. O. R. Kaempfer, and B. Magasanik. 1967. Mechanism of tryptophanase induction in *Escherichia coli*. J. Mol. Biol. 27:495–506.
2. Demerec, M., E. Adelberg, A. J. Clark, and P. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics 54:61–76.
3. Dobrogosz, W. J. 1968. Effect of amino sugars on catabolite repression in *Escherichia coli*. J. Bacteriol. 95:578–584.
4. Fraenkel, D. G. 1968. Selection of *Escherichia coli* mutants lacking glucose-6-phosphate dehydrogenase or gluconate-6-phosphate dehydrogenase. J. Bacteriol. 95:1267–1271.
5. Fraenkel, D. G., and S. R. Levisohn. 1967. Glucose and gluconate metabolism in an *Escherichia coli* mutant lacking phosphoglucose isomerase. J. Bacteriol. 93:1571–1578.
6. Hirota, Y. 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 46:57–64.
7. Horecker, B. L., and W. A. Wood. 1957. *In* Methods in enzymology, S. Colowick and N. Kaplan, ed., p. 153–154. Academic Press Inc., New York.
8. Hsie, A. W., and H. V. Rickenberg. 1967. Catabolite repression in *Escherichia coli*: The role of glucose-6-phosphate. Biochem. Biophys. Res. Commun. 29:303–310.
9. Hsie, A. W., H. V. Rickenberg, D. W. Schulz, and W. M. Kirsch. 1969. Steady-state concentrations of glucose-6-phosphate, 6-phosphogluconate, and reduced nicotinamide adenine dinucleotide phosphate in strains of *Escherichia coli* sensitive and resistant to catabolite repression. J. Bacteriol. 98:1407–1408.
10. Loomis, W. F., Jr., and B. Magasanik. 1964. The relation of catabolite repression to the induction system for β-galactosidase in *Escherichia coli*. J. Mol. Biol. 8:417–426.
11. Loomis, W. F., Jr., and B. Magasanik. 1965. Genetic control of catabolite repression of the *lac* operon in *Escherichia coli*. Biochem. Biophys. Res. Commun. 20:230–234.
12. Loomis, W. F., Jr., and B. Magasanik. 1966. Nature of the effector of catabolite repression of β-galactosidase in *Escherichia coli*. J. Bacteriol. 92:170–177.
13. Loomis, W. F., Jr., and B. Magasanik. 1967. The catabolite

repression gene of the *lac* operon in *Escherichia coli*. J. Mol. Biol. 23:487–595.
14. Magasanik, B. 1961. Catabolite repression. Cold Spring Harbor Symp. Quant. Biol. 26:249–256.
15. Mandelstam, J. 1962. The repression of constitutive β-galactosidase in *Escherichia coli* by glucose and other carbon sources. Biochem. J. 82:489–493.
16. Nakada, D., and B. Magasanik. 1964. The roles of inducer and catabolite repressor in the synthesis of β-galactosidase by *Escherichia coli*. J. Mol. Biol. 8:105–127.
17. Rickenberg, H. V., A. W. Hsie, and J. Janecek. 1968. The CR mutation and catabolite repression in *Escherichia coli*. Biochem. Biophys. Res. Commun. 31:603–608.
18. Signer, E. R., J. R. Beckwith, and S. Brenner. 1965. Mapping of suppressor loci in *Escherichia coli*. J. Mol. Biol. 14:153–166.
19. Tyler, B., W. F. Loomis, Jr., and B. Magasanik. 1967. Transient repression of the *lac* operon. J. Bacteriol. 94:2001–2011.