Genetic Control of Catabolite Repression of the Lac Operon in Escherichia coli

William F. Loomis, Jr. and Boris Magasanik
Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

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Catabolite repression exerted by glucose reduces the differential rate of β-galactosidase synthesis in fully induced or constitutive cells to about 50% of the rate observed in a glycerol medium. When the rate of anabolism is reduced, the degree of repression by catabolites is greatly enhanced (Mandelstam, 1961). Catabolite repression has been shown to control the rate of synthesis of M-RNA specific for β-galactosidase (Nakada and Magasanik, 1964). The regulator gene that determines the inducer-sensitive repression of the lac operon is not required for catabolite repression of the operon (Mandelstam, 1962; Loomis and Magasanik, 1964). Another regulator gene, therefore, should exist that determines the catabolite-sensitive repression of the lac operon. We have been able to demonstrate such a gene specific for the lac operon.

MATERIALS AND METHODS

L-(1-^{14}C) leucine and β-methyl-^{14}C-thiogalactoside (^{14}C-TMG) were purchased from New England Nuclear Corp., Boston, Mass. 50 mg of crystalline 4-O-β-D-galactopyranosyl-N-acetyl glucosamine (N-acetyl lactosamine) was the generous gift of Dr. R. Tomarelli of the Wyeth Laboratories, Philadelphia, Pa.

β-Galactosidase was induced by 10^{-3}M isopropylthio-β-D-galactoside (IPTG) and assayed as in Loomis and Magasanik (1964). Tryptophanase was induced by 1 mg/ml L-tryptophan and assayed as in Pardee and Prestidge (1961). Galactokinase was induced by 0.4% galactose and assayed as in Sherman and Adler (1963) and Sherman (1963). β-Galactoside permease was induced by 10^{-3}M IPTG and estimated by measuring the amount of ^{14}C-TMG in cells after incubation at 37° for 20 minutes in the presence of 5 x 10^{-4}M ^{14}C-TMG and 100 μg/ml chloramphenicol. Amylomaltose was induced by 0.4% maltose and assayed by measuring with Glucostat reagents (Worthington Biochemical Corp., Freehold, N. J.) the amount of glucose formed in 30 minutes at 37° from a solution 1% maltose in 0.1M potassium phosphate buffer pH 7.0 by sonic extracts.

Phage lysates and transduction were carried out as in Luria et al. (1960). Strains 3.000 (Hfr lac^+ (i^+ z^+ y^+), C600 (F^-lac^- (i^+ z^+ y^-), A-221 (lac^- (i^- z^- y^+), 2340 (F^- lac^- (i^- z^- y^+), W4132 (F^- lac^{del}) and phage $P1_{kc}$ were from the stock of Dr. S. E. Luria.

RESULTS

We found that 0.05% N-acetyl lactosamine can serve as sole source of carbon and nitrogen (mass doubling time = 40 hrs) for a lac^+ strain, 3.000, but not for a lac^- strain, A-221. Strain 3.000 did not grow in minimal medium with N-acetyl lactosamine as sole source of nitrogen when 0.4% glucose was also present in the medium. Strain 3.000 was mutagenized with ethyl methane sulfonate (EMS) (Lin et al., 1962) and grown up in glucose-minimal medium. We inoculated 2 x 10^7 mutagenized bacteria into glucose, minimal medium with 0.05% N-acetyl lactosamine as sole source of nitrogen and 10^{-3}M IPTG. After 12 days at 37° the culture was found to contain 2 x 10^8 bacteria by viable count. Six individual colonies were picked and tested for catabolite repression of β-galactosidase in glucose minimal medium. Three isolates were found to be altered in this control system. Further work was per-

formed on one of these strains, LA-12.

The growth rate of strain LA-12 was found to be about 20% less on glucose, gluconate or glycerol than that of the parent strain 3.000. Therefore, strain LA-12 was mutagenized with EMS and plated on glucose minimal agar plates. A large colony was isolated and termed LA-12G. This strain grew at the same rate or somewhat faster than the parental strain 3.000 on all these carbon sources and on such compounds as maltose, galactose, D- and L-lactate.

We found that minimal agar plates containing 1% glucose and 0.2% lactose would differentiate between cells of strain 3.000 and those of strains LA-12 and LA-12G. When uninduced cells of these strains were plated on such a medium, incubated for 20 hours at 37°, treated with toluene vapor for 5 minutes, and layered with a 10^{-2}M solution of o-nitrophenyl-β-D galactoside, the colonies of strain 3.000 remained colorless for more than an hour while the colonies of strains LA-12 and LA-12G turned yellow within 5 minutes.

The response of various normally catabolite sensitive enzymes to glucose was determined in strains 3.000, LA-12 and LA-12G (Table I).

TABLE I

Enzyme Synthesis in Glucose Medium

Strain	β-Gal*	Permease+	Maltase+	T' ase*	Gal' kinase+
3.000	36	51	64	20	12
LA-12	117	115	56	29	--
LA-12G	120	--	--	10	22

*The values are the differential rates of enzyme synthesis in glucose minimal medium. The value in glycerol minimal medium is taken as 100. and was similar in all three strains. The rate of growth was estimated by observing the optical density at 530 mμ in a Klett-Summerson photoelectric colorimeter. β-Gal = β-galactosidase; T' ase = tryptophanase.

+The values are the relative specific activities of the enzymes of cultures grown in glucose minimal medium. The values of cultures grown in glycerol minimal medium are taken as 100 and were similar in all three strains. Galactokinase was assayed by Dr. M. Stodolsky. Permease = β-galactoside permease; Maltase = amylomaltase; Gal' kinase = galactokinase.

It is apparent that in strains LA-12 and LA-12G, in contrast to the parent strain 3.000, glucose fails to repress β-galactosidase and permease. The susceptibility of the other enzymes to glucose is the same in all strains.

When anabolism is reduced by omission of a source of nitrogen, the presence of any carbon source to which the cells are adapted will greatly decrease the differential rate of β-galactosidase formation (Mandelstam, 1961). It can be seen (Table II) that during nitrogen starvation, the five carbon sources tested almost completely inhibited β-galactosidase synthesis in the wild type strain, C600, but had very little effect on the synthesis of this enzyme in strain LA-12G.

TABLE II

β-Galactosidase Synthesis during Nitrogen Starvation

Strain	Glucose	Gluconate	Glycerol	L-Lactate	D-Lactate
C600	3	6	7	3	3
LA-12G	110	70	128	30	30

The cells were grown in minimal media containing the carbon source to be tested. They were collected on membrane filters and suspended in media free of a source of nitrogen containing inducer and ^{14}C-leucine, 10 μg/ml, with and without the appropriate source of carbon. The differential rate of enzyme synthesis was determined using the uptake of ^{14}C-leucine as measure of protein synthesis (Nakada and Magasanik, 1964). The rate in a medium lacking both a source of carbon and of nitrogen is taken as 100.

The results show that strain LA-12 and its derivative LA-12G carry a mutation, CR^-, that affects specifically the control of the lac operon by catabolite repression. The formation of the products of the lac operon, β-galactosidase and permease, in these strains is highly insensitive to repression by catabolites derived from diverse carbon sources.

Using the plate assay for the CR^- phenotype, we found that the CR^- gene is less than 1% co-transduced with the structural gene for β-galactosidase by phage P1 into strains W4132 and 2340. We conclude that the

CR^- gene is not closely linked to the lac operon. Results to be reported elsewhere indicate that the CR^- gene maps near the tryptophan locus and that the wild type allele is dominant over the CR^- gene in trans position in a stable merodiploid for this gene.

We suggest that the CR gene determines a cytoplasmic molecule involved in catabolite repression of the lac operon. The function of the CR gene product in catabolite repression may be analogous to that of the i gene product in induction (Pardee et al., 1959). Further characterization of the control mechanisms of the lac operon, using the simplifying properties of the CR^- strains, are in progress.

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