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Physiological Regulation of a Decontrolled *lac* Operon

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The expression of the *lac* operon was studied under a variety of growth conditions in induced and in constitutive cells of *Escherichia coli* that carried different catabolite-insensitive *lac* promoters. Use of such "decontrolled" *lac* operons permitted a study of the expression of an operon that was presumably subject only to passive control. Since the use of toluenized cells was demonstrated not to be completely reliable, all enzyme assays were performed on sonic supernatant fluids. The cells contained different catabolite-insensitive promoters, which included the *L1* and *UV5 lac* promoters, as well as others isolated in this study. There were three major observations. First, small but real carbon source effects were seen. Second, there was only a small change in β-galactosidase specific activity with changes in the growth rate. This result implies a limited transcription and/or translation capacity within the cell. Third, at rapid growth rates, most promoters exhibited a decreased expression. The *UV5* promoter, which was the "strongest" promoter, was an exception. A mechanism to explain this promoter-dependent control is discussed.

The primary purpose of this work was to describe the behavior of an operon for which no specific controlling element was operative. Such an operon would be under passive control. A model describing the behavior of genes dependent solely upon such passive factors has been proposed by Maaløe (19). His model actually illustrates a mechanism whereby the regulation observed for ribosome biosynthesis could be explained by passive control. In brief, his model proposes that at faster growth rates a greater proportion of repressible operons, e.g., biosynthetic ones, are shut down, and ribonucleic acid (RNA) polymerases are available for passive partitioning to constitutive (ribosomal protein?) operons. According to his model, such partitioning of RNA polymerase molecules could explain how ribosomal protein synthesis is coordinated with the growth rate without invoking any specific control on ribosomal protein genes. A study of an operon subject to passive control would provide information on Maaløe's model for ribosome control. Knowing the behavior of a passively controlled operon would also be helpful in assessing the regulation of other operons. Assuming that the behavior of all deregulated operons is qualitatively similar, one could expect this behavior in any operon of interest under conditions that remove known controls. Finding some other pattern of expression would argue for the existence of a hitherto unknown controlling element on this operon.

Unfortunately, no wild-type gene is known to be solely under passive control. Therefore, we chose to investigate the regulation pattern of a passively controlled gene product by a study of variants of the *lac* operon. The wild-type *lac* operon in *Escherichia coli* is negatively controlled by the *lacI* gene repressor and positively controlled by catabolite repression; both of these regulatory mechanisms can be circumvented. The *lacI* gene repression can be abolished either by the gratuitous inducer, isopropyl-β-D-thiogalactoside (IPTG), or by mutation in the *lacI* structural gene; catabolite-repression control can be removed by a mutation(s) within the *lac* promoter region (6). By eliminating both of these controls, we presumably have created a "decontrolled" system, where the *lac* genes are subject only to passive control.

(A preliminary report of part of this work was presented at the Annual Meeting of the American Society of Microbiology held in New York, May, 1975).

(The results reported here are taken from a thesis presented by Barry L. Wanner to the University of Michigan in partial fulfillment of the requirements for the Ph.D. degree.)

MATERIALS AND METHODS

Reagents. Morpholinopropane sulfonic acid (MOPS), tricine [*N*-tris(hydroxymethyl)methyl

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glycine], glycyl-L-proline, 5-bromo-4-chloro-3-indolyl-β-D-fucoside, cyclic adenosine 3',5'-monophosphate (cAMP), and IPTG were purchased from Sigma Chemical Co. Radioactive amino acids were obtained from New England Nuclear Corp. or Schwarz/Mann. *o*-Nitrophenyl-β-D-thiogalactoside was purchased from Cyclo Chemical. cAMP was also kindly donated by Asahi Chemical Co. (Japan).

Analytical methods. The protein concentration of cell extracts was determined colorimetrically by the method of Lowry et al. (18).

The RNA concentration was determined colorimetrically by the orcinol procedure of Schneider (25), with yeast RNA (type XI) as standard.

Media. MOPS medium was used for all growth studies and was prepared as described previously (22). Carbon sources and supplements were added as listed in Table 1. All components were filter sterilized.

Bacterial strains. All strains received from other laboratories are listed in Table 2. All measurements were made by using derivatives of *E. coli* B/r that were isogenic to wild type. All lesions were introduced into the reference strain by transduction with a linked marker by using bacteriophage P1*kc*.

The *E. coli* B/r derivative, NC3, was used as a standard strain to permit quantitative studies to be directly compared between different laboratories and to eliminate problems caused by strain differences (F. Neidhardt and B. Wanner, unpublished data). NC3 has the following advantages, since it was derived from *E. coli* B/r: (i) it can be used in synchronous cell studies employing the Helmstetter and Cummings technique (12); (ii) it is now being used extensively for studies in the regulation of macromolecular synthesis (cf. 8); and (iii) its growth rate on glucose minimal medium is more rapid than that of most *E. coli* K-12 strains. The only known difference between strain NC3 and strain B/r is the presence of a mutation in the *hsr* locus of strain NC3, which eliminates the *E. coli* B-type host restriction system. This permits greater efficiency in genetic transfers involving donor deoxyribonucleic acid lacking the strain B-type modification(s). Therefore, a large number of lesions can be introduced into strain NC3 that have been originally isolated in other *E. coli* strains, particularly the K-12 strain.

TABLE 1. *Composition of media*

Medium no.[a]	Carbon source(s)[b]	Amino acids[c]	Bases[d]	Vitamins[e]
1	Glu	–[f]	–	–
2	Glu	Complete	–	–
3	Glu	Ser	+	+
4	Glu	Complete	+	+
5	Glu, Rib, Fru	–	+	–
6	Glu, Rib, Fru	Complete	+	+
7	Glu, Pyr	–	–	–
8	Glu	Gly, Leu, Ser (only)	–	–
9	Gly	–	–	–
10	Gly	Ser	+	+
11	Gly	Complete	+	+
12	Gly, Pyr	–	–	–
13	Gly, Gal	–	–	–
14	L-Ser	–	–	–
15	L-Ser	Complete	+	+
16	Ace	–	–	–
17	Ace	Ser	+	+
18	Ace	Complete	+	+
19	L-Asp	–	–	–
20	Pyr	–	–	–
21	D-Ser	–	–	–
22	Suc	–	–	–
23	D-Ala	–	–	–

[a] MOPS medium was used and prepared according to Neidhardt et al. (22).

[b] The final concentrations (and abbreviations) for the carbon sources were: acetate (Ace, potassium salt), 0.2% (wt/vol); D-alanine (Ala), 40 mM; L-aspartate (Asp, potassium salt), 40 mM; β-D-fructose (Fru), 25 mM; D-galactose (Gal), 25 mM; D-glucose (Glu), 25 mM; glycerol (Gly), 0.4% (wt/vol); pyruvate (Pyr, sodium salt), 40 mM; D-ribose (Rib), 40 mM; D-serine (Ser), 40 mM; L-serine, 40 mM (total concentration); and succinate (Suc, sodium salt), 0.4% (wt/vol).

[c] All amino acids were the L-isomer and included: 0.8 mM Ala, 0.4 mM Arg, 0.4 mM Asn, 0.4 mM Asp, 0.1 mM Cys, 0.6 mM Glu, 0.6 mM Gln, 0.8 mM Gly, 0.2 mM His, 0.4 mM Ile, 0.8 mM Leu, 0.4 mM Lys, 0.2 mM Met, 0.4 mM Phe, 0.4 mM Pro, 10.0 mM Ser, 0.4 mM Thr, 0.1 mM Trp, 0.2 mM Tyr, and 0.6 mM Val.

[d] All four bases were present at 0.2 mM each and included: adenine, guanine, cytosine, and uracil as the free bases.

[e] All vitamins were added at 0.01 mM each and included: *p*-aminobenzoic acid, *p*-dihydroxybenzoic acid, *p*-hydroxybenzoic acid, pantothenate (calcium salt), and thiamine.

[f] Dash indicates absence of the indicated component.

TABLE 2. *Bacterial strains received*

Strain	Genotype[a]	Source[b]
Bc251	*E. coli* B *gal hsr mal*(K-12)[c] *met*	(a)
B/r	*E. coli* B/r	(b)
CA8.001	HrfH *lacL1 thi*	(c)
CSH23	Δ (*lac-pro*) *supE thi*/F′ *lac*$^+$ *proA*$^+$,*B*$^+$	(d)
UV5	HfrH *lacL37UV5 thi*	(e)
X8068	*LacL1UV5 strA*	(c)

[a] Symbols used are taken from Bachmann et al. (4). All known markers are listed. Unless indicated otherwise, all strains are derivatives of *E. coli* K-12.

[b] (a) H. Boyer; (b) S. Cooper; (c) J. Beckwith; (d) R. Olsen; (e) B. Magasanik.

[c] *mal*(K-12) indicates that this strain carries the *mal* allele from *E. coli* strain K-12.

Derivation of the reference strain *E. coli* NC3. The *hsr* lesion was introduced into our reference strain according to the following protocol: initially, a spontaneous *thyA* mutant was isolated by trimethoprim selection (28). A *thyA thyR* double mutant was then found by selecting a low thymine requirer in the presence of trimethoprim (23). After transduction of this strain to thymine independence, the *hsr* lesion was introduced by transduction with the *thyR*$^+$ allele by selecting for the utilization of thymidine as the sole carbon source. Transductants were tested for their *hsr* allele by the plating efficiency of P1*kc* grown on a K-12 strain. Twelve out of 100 transductants were found to carry the mutant *hsr* allele. After careful examination of their growth properties, one was designated strain NC3.

Isolation of *lac* promoter-like mutants. Several independent Lac$^+$ revertants of a NC3 derivative carrying the *lacL1* promoter were selected on lactose minimal plates. Derivatives carrying stable Lac$^+$ mutations were assayed and tested for their linkage to *proC* and *lacY* by P1 transduction. No instability of the level of *lac* expression has been observed for any of the strains reported, either during cloning or during transduction into other strains. One mutation, *lacL1M1*, was isolated after transduction of a *proC* recipient to proline independence by using a donor carrying the *LacL1* lesion. We propose that this promoter arose as an error in recombination during transduction. This new promoter was also shown to be linked to *lac* and was stable.

Characterization of bacteria with *lac* promoter-like mutations. β-Galactosidase levels were measured in the new promoter-like mutants and in the original strains under fully induced and noninduced conditions (Table 3). The derepression ratio for the *L1* strain had a value of only 2.6 compared with 1,500 for the wild-type strain. The partial constitutivity of this strain was one consequence of the *L1* deletion, which removes both the CAP interaction site and that portion of the *LacI* gene that codes for the carboxyl terminal part of the repressor (3). The derepression ratio for the *L1UV5*-bearing strain was similar to that for the *L1* strain. Of the seven promoter or promoter-like mutants examined that contained the *L1* deletion, only two, *L1R4* and *L1R6*, have significantly altered derepression ratios in glucose minimal medium.

Isolation and characterization of *pro* auxotrophs. Penicillin enrichments were all conducted at 37°C without any mutagenesis by the following method: after logarithmic growth in glucose minimal medium with the amino acid added, the culture was filtered and resuspended in glucose minimal medium. One generation later, penicillin was added at 10,000 U/ml for about one-half of a generation. The culture was again filtered, suspended, and grown fully with the amino acid readded. Auxotrophs were obtained by replica plating after two penicillin enrichments. The proline lesions isolated were tested for their linkage to *lac* by P1 transduction. On the basis of their linkage and their complementation by the F′ in strain CSH23, they were assigned as either *proB* or *proC*.

Transductions. Bacteriophage P1 transductions were performed essentially according to the procedure of Lennox (16).

Frequently, a *lacY* allele was transduced into a strain that (prior to the transduction) carried a *lacZ* polar mutation. Selections were performed for the utilization of phenyl-β-galactoside as sole carbon source in the presence of IPTG and *o*-nitrophenyl-β-D-thiogalactoside. Occasionally, this selection was used to transduce a linked proline auxotrophic mutation into the recipient. In these cases, it became necessary to substitute glycyl-L-proline for proline, since proline can be used also as a carbon source.

Matings. Bacterial conjugations were all performed in liquid media according to Miller (20).

Selection of spontaneous lactose negative and lactose constitutive strains. Mutants unable to ferment lactose were selected by the *o*-nitrophenyl-β-D-thiogalactoside method described by Smith and Sadler (27), except all media used were based on MOPS

TABLE 3. *Level of β-galactosidase in strains with different promoters*

Promoter	β-Galactosidase (U/mg of protein)		Derepression ratio[a]
	Basal[b]	Induced[c]	
lacL1	0.0889 (3)[d]	0.248 (6)	2.63
lacL1R3	1.33 (2)	3.60 (5)	2.71
lacL1R4	0.370 (2)	6.08 (5)	16.4
lacL1R6	0.462 (2)	1.83 (4)	3.97
lacL1R11	0.307 (2)	0.842 (4)	2.74
lacL1M1	0.0568 (3)	0.170 (4)	2.99
lacL1UV5	4.67 (3)	12.4 (20)	2.65
lacL37UV5	0.0413 (3)	12.0 (15)	290
lac wild type	0.00501 (3)	7.49 (15)	1,500

[a] Ratios of induced-to-basal level of β-galactosidase are shown.

[b] Cultures were maintained in log-phase in glucose minimal medium at 37°C for at least 16 generations before assay.

[c] IPTG was present at 1 mM during growth.

[d] Numbers in parentheses represent total number of cultures assayed. Averages are shown.

medium (22). The phenyl-β-D-galactoside selection (27) was used to select mutants that expressed β-galactosidase constitutively. Since most lactose constitutive mutations result in only partial constitutivity, the galactoside analogue *o*-2-nitrophenyl-β-D-fucoside (an inhibitor of induction that binds to the *lacI* repressor [131]) was added at 1 mM, in some cases, and was found to enhance the proportion of mutants that were fully constitutive.

Bacterial growth and cell harvesting. The bacterial cultures were grown aerobically in baffled flasks with a culture-to-flask volume of 1:5 or less. Bacterial mass was monitored at an optical density at 420 nm (OD_{420}). All growth rates were determined between a culture OD_{420} of 0.1 and 2.0 (by suitably diluting the culture prior to OD measurement). Growth rates are expressed in terms of the specific growth rate constant k, as calculated from the expression $k = (\ln 2)/(\text{mass doubling time [hours]})$.

Culture samples were taken and added to prechilled tubes in an ice water bath containing chloramphenicol, at a final concentration of 100 μg/ml. The cells were pelleted, washed once, and suspended in the appropriate buffer. The cells were sonically disrupted for 0.5 to 2.0 min at a power setting of 4.0 with the standard tip (Heat Systems Ultrasonics, Inc., Plainview, N. Y.). Throughout sonic treatment, the samples were chilled in an ice water bath. For total sonic treatment times greater than 0.75 min, the samples were sonified for 0.5-min periods, with 0.5-min intervals for cooling. This procedure prevented the sample temperature from rising above room temperature. Debris was removed by centrifugation at 27,000 × *g* for 20 min at 4°C.

Enzyme assays. The enzyme activity of thiogalactoside transacetylase was determined in crude extracts of sonically treated samples by the method of chloramphenicol per ml, and 10% (wt/vol) glyc- were calculated by using a molar absorbtivity, ϵ_{420}, for thionitrobenzoic acid of 15,700. One unit is equivalent to 1 nmol of free coenzyme A produced per min at 28°C.

Glucose-6-phosphate dehydrogenase activity was measured by the rate of production of nicotinamide adenine dinucleotide phosphate, reduced form, with the assay system described by Lessie and Neidhardt (17). The units of enzyme activity were calculated by using a ϵ_{340} for nicotinamide adenine dinucleotide, reduced form, of 6,200. One enzyme unit equals 1 nmol of nicotinamide adenine dinucleotide, reduced form, formed per min at room temperature.

β-Galactosidase was assayed by the hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside. Sonic supernatant fluids were prepared in extract buffer that contained: 0.2 M sodium hydrogen phosphate (pH 7.0), 0.01 M KCl, 0.14 M β-mercaptoethanol, 100 μg of chloramphenicol per ml, and 10% (wt/vol) of glycerol. Assays were performed in assay buffer that, in addition, contained 0.001 M $MgSO_4$, but lacked glycerol. Portions of the extract were added so that the final concentration of glycerol was 1% or less. The assay was started by adding 0.6 ml of *o*-nitrophenyl-β-D-galactopyranoside (2 mg/ml in assay buffer) to the extract in 1.0 ml of assay buffer. After sufficient yellow color developed, 1.0 ml of 1 M Na_2CO_3 was added to stop the reaction. The ϵ_{420} of 4,500 was used to calculate all data shown. Units are micromoles of *o*-nitrophenol produced per minute at 28°C.

During preliminary experiments, different protocols were used to prepare samples for β-galactosidase assay. These included the use of toluene without detergent, according to Monod et al. (21), and with deoxycholate, according to Revel et al. (24). Chloroform was also used with and without detergent, according to Miller (20). In the Brij method, all buffers contained 1% (wt/vol) Brij 58. The results are shown in Table 4. Since the highest specific activity for β-galactosidase was obtained when sonic treatment was used, and since other methods showed differing recoveries, sonic supernatant fluids were used to obtain all quantitative data presented.

Controls were performed to determine the amount of β-galactosidase activity and protein recovered by sonic treatment. Generally, the amount of protein recovered for a given sonic treatment time decreased with decreasing growth rate, but no effect on the β-galactosidase specific activity (units per milligram of protein recovered) was observed. Sonic treatment times for particular samples were chosen to yield 85% or greater recovery of the sample protein.

RESULTS

The expression of an operon subject only to passive control should reflect only those parameters that define the metabolic state of the cell. Cells grown in media of different composition usually differ both in their growth rate and in the pattern of flow of metabolites through am-

TABLE 4. *Comparison of various protocols for the assay of β-galactosidase*

Treatment	β-Galactosidase[a] (U/mg of protein)	
	Slow cell[b]	Fast cell[c]
Toluene	3.1 (39)[d]	3.4 (72)
Toluene + 1% DOC[e]	5.3 (66)	3.6 (77)
1% DOC alone	2.0 (25)	3.0 (64)
Chloroform	4.5 (56)	3.2 (68)
Chloroform + 0.1% SDS[f]	4.7 (59)	3.5 (75)
Brij	0.95 (12)	4.2 (89)
Sonic	8.0 (100)	4.7 (100)

[a] All values shown are the averages of triplicate samples.

[b] The specific growth rate of this culture was 0.231 (generation time equal to 180 min).

[c] The specific growth rate of this culture was 1.04 (generation time, 40 min).

[d] The number in parentheses refers to the relative activity based upon a value of 100 for the sonically treated sample.

[e] DOC, Deoxycholate.

[f] SDS, Sodium dodecyl sulfate.

phibolic and biosynthetic pathways in the cell. To reveal both growth rate-related responses and media-specific responses of a decontrolled *lac* operon, it was desirable to choose a large number of different media. The behavior of an operon might also be dependent on the absolute level of its expression. Therefore, isogenic strains that carry promoter mutations that result in widely different frequencies of transcription initiation were examined.

Expression of *lac* in the *lacL1* strain. Measurements of the specific activity of β-galactosidase in wild-type bacteria carrying the *lac* promoter deletion *L1* are shown in Fig. 1. The function of the weak *lac* promoter in this strain was independent of the CAP site. Presumably, a low initiation rate independent of catabolite repression occurred at the normal RNA polymerase interaction site. It is this low rate of transcription that remained when the CAP site within the *lac* promoter was deleted. A quantitatively similar low rate of transcription also occurs in a *crp* or *cya* background (5).

The variation in *lac* expression controlled by the *lacL1* promoter was approximately twofold over the entire range of growth rates examined, and the relative rate of β-galactosidase synthesis tended to decrease inversely with increasing growth rates, at specific growth rates greater than 1.0. With the exception of pyruvate and D-serine minimal media (media *20* and *21*), the expression at growth rates slower than that in glucose minimal medium (k = 1.0) may be described as invariant.

Expression of *lac* in the *UV5* promoter strain. To check for an effect that the frequency of transcription initiation might have on the behavior of a passively controlled operon, the high level *UV5* promoter was used. The level of expression in an isogenic *E. coli* NC3 derivative carrying the catabolite-insensitive *UV5* promoter mutation is about 50-fold higher than the level of expression from the *L1* promoter (26). (Actually, all strains examined containing the *UV5* promoter had an additional mutation in the catabolite-sensitive site, which either deleted this site [such as in *L1UV5* strains] or made it insignificant [such as in *L37UV5*].) Since no detectable differences could be seen between the fully induced levels of β-galactosidase in the *L1UV5* and *L37UV5* strains used in this study, all data for induced cultures of all *UV5* strains were combined and are shown in Fig. 2. In contrast to the data for the *L1* strains, the specific activities for the *UV5* strains appeared largely independent of growth rate. They did exhibit some media-specific differences at growth rates below 1.0.

Expression of *lac* in additional promoter strains. The relative levels of expression for the different promoters varied dramatically. If one assigns the value of 1.0 to the *L1* strain, then the relative level of expression for *L1R3* is 15.0; for *L1R4*, 25.0; for *L1R6*, 7.4; for *L1R11*, 3.4; for *L1M1*, 0.69; and for *L1UV5*, 50.0. The maximum variation (between *L1M1* and *L1UV5*) was 73-fold. Therefore, the study of these mutants should permit evaluation of the effect that the initiation efficiency of an operon might have on the expression of that operon. Data for

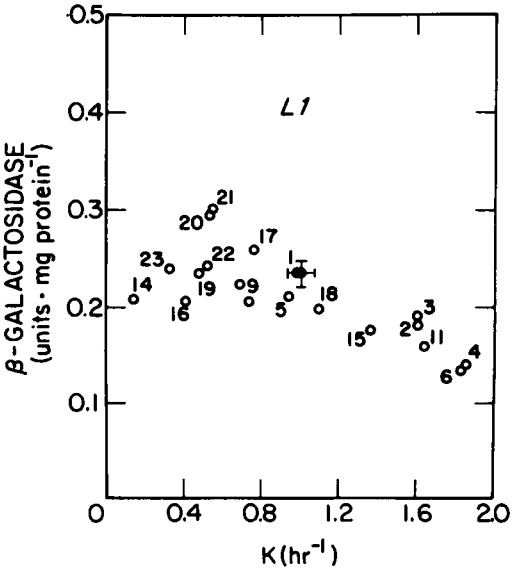


FIG. 1. *β-Galactosidase activity in E. coli NC3 harboring the lacL1 promoter mutation. The bacteria were maintained in logarithmic growth at 37°C in the various media for at least 10 generations prior to removing portions for assays. Inducible strains were grown in media containing 1 mM IPTG. The media are identified by number in the body of the Fig. and are described in Table 1. At an OD_{420} of approximately 0.3, 0.6, and 1.0, samples were taken into prechilled tubes containing sufficient chloramphenicol to give a final concentration of 100 μg/ml in an ice water bath. After washing by pelleting and suspending the cells, the samples were sonically treated, and the supernatant fluids were assayed for protein and β-galactosidase activity as described in the text. Each point represents the average value for three samples. Reproducibility of results was always within 10%. Open circles represent fully induced level of β-galactosidase activity from the Lac promoter in a cell with wild-type background grown in media without cAMP; closed symbols are for those strains grown in the presence of 5 mM cAMP. When a particular medium was used more than three independent times, the standard deviation is represented by a vertical or horizontal bar drawn through each appropriate point. Medium 1 was used for six cultures.*

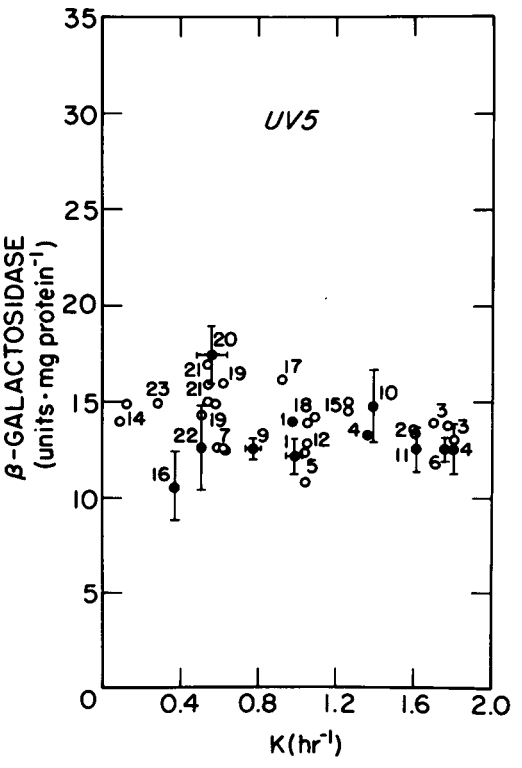


FIG. 2. *β-Galactosidase activity in E. coli NC3 with the L1UV5 and the L37UV5 lac promoters. See legend to Fig. 1 for key to the media, methods, and symbols used. Medium 1 was used for 35 cultures; medium 4, for seven cultures; medium 6, for four cultures; medium 9, for nine cultures; medium 10, for five cultures; medium 11, for seven cultures; medium 16, for five cultures; medium 20, for eight cultures; and medium 22, for four cultures.*

the *L1* and *UV5* strains have already been presented in Fig. 1 and 2, respectively. Measurements of the specific activities of β-galactosidase, under various growth conditions, for strains carrying the *L1R3*, *L1R4*, *L1R11*, and *L1M1 lac* promoters are shown in Fig. 3A through E, respectively. In each case, the ordinate scale has been changed to permit direct comparisons between the various figures. The similarity between these figures (3A through E) and Fig. 1 was striking. Therefore, the normalized data for all these strains were calculated and plotted in Fig. 4. Similar to the results shown in Fig. 1, there was a definite tendency for a decrease in the specific activity with increasing growth rates. This is unlike what was found for the *UV5* promoter strains.

Comparison of Fig. 2 and 4 reveals the existence of media-specific effects, particularly at k values less than 1.0. In particular, note the relative values for media *9*, *14*, *16*, *20*, *21*, *22*, and *23*. (Whether or not media *7* and *19* are exceptions is unclear, since these media were used relatively infrequently.)

Effect of media on transacetylase activity. To examine the media-specific effects further, the levels of transacetylase (product of the *lacA* gene) and β-galactosidase were assayed simultaneously in a constitutive strain carrying the *L37UV5* promoter. The value obtained for transacetylase in a glucose-grown culture was 110 U/mg of protein. The ratios of specific activities of transacetylase to β-galactosidase were approximately the same in glucose minimal (*1*), glucose rich (*4*), glycerol minimal (*9*), acetate minimal (*16*), and pyruvate minimal (*20*) media. The result implies that the relative efficiency of translation is the same in these cases. (Of course, corresponding changes may exist in the efficiencies of translation for both cistrons.) The ratio was about 30% higher in succinate minimal (*20*) medium. This higher ratio in succinate resulted from a similar specific activity for transacetylase in the succinate and pyruvate minimal media cultures, implying that the same number of RNA transcripts were synthesized in both the pyruvate- and succinate-grown cultures, but the level of translation of active β-galactosidase was adversely affected in succinate minimal medium.

Effect of media on glucose-6-phosphate dehydrogenase activity. According to the literature (10), the synthesis of glucose-6-phosphate dehydrogenase is constitutive, i.e., its relative rate of synthesis does not vary during growth in different media. This is similar to the behavior of β-galactosidase in the *UV5* strain. The specific activity for glucose-6-phosphate dehydrogenase was, therefore, assayed in an NC3 derivative carrying the *lacI L37UV5* mutations. The results are shown in Fig. 5 for several culture conditions. Importantly, the levels of β-galactosidase, transacetylase, and glucose-6-phosphate dehydrogenase were assayed in the same cultures. At least in the case of medium *16*, and perhaps in the case of medium *9*, media-specific effects on this enzyme could be observed that were similar to those seen in the catabolite-insensitive *lac* promoter mutants (cf. Fig. 5 with Fig. 2 and 4).

DISCUSSION

The study of the expression of catabolite-insensitive promoters (*L1*, *UV5*, as well as the mutants of *L1*) was undertaken to elucidate the pattern of regulation for a passively controlled operon and, thereby, to probe the physiological state of the cell with respect to its transcription

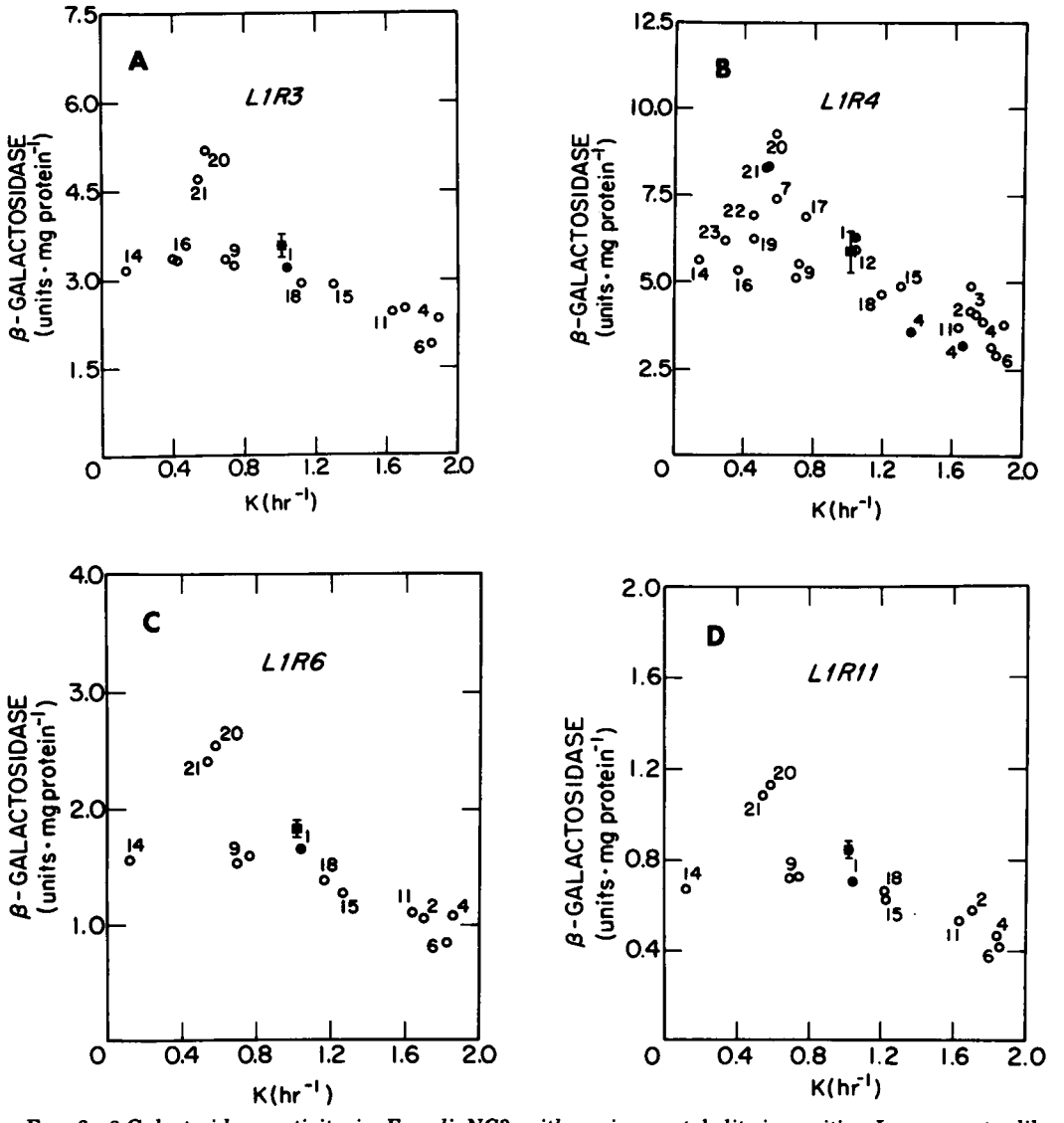
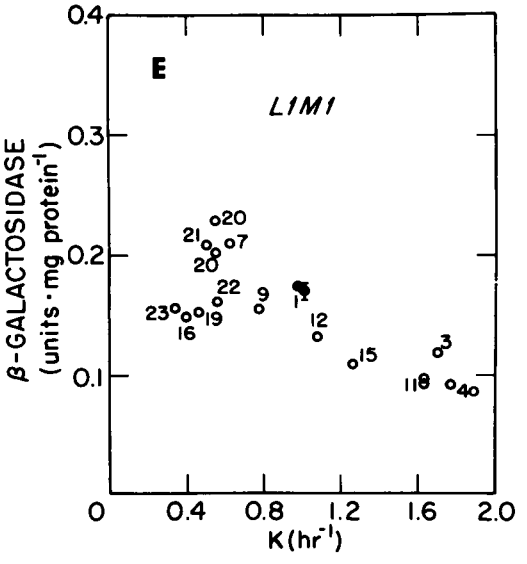


FIG. 3. *β-Galactosidase activity in E. coli NC3 with various catabolite-insensitive Lac promoter-like mutations. See legend to Fig. 1 for key to the media, methods, and symbols used. (A) NC3 derivatives that carry the lacL1R3 promoter; (B) NC3 derivatives that carry the lacL1R4 promoter; (C) NC3 derivatives that carry the lacL1R6 promoter; (D) NC3 derivatives that carry the lacL1R11 promoter; (E) NC3 derivatives that carry the LacL1M1 promoter.*

and translation capability. Three different effects were seen: (i) media-specific effects occurred with all the catabolite-insensitive *lac* promoters; (ii) aside from the media-specific effects, there was a fairly constant specific activity for all the catabolite-insensitive *lac* promoters examined at growth rates slower than those in glucose minimal medium; and (iii) with growth rates increasingly more rapid than that in glucose minimal medium, there was either a constant specific activity or a decreasing specific activity, depending on the promoter.

While the media-specific effects were relatively small, there is little doubt that they are experimentally, if not physiologically, significant. The media-specific effects could result from stimulatory or inhibitory effects on gene expression brought about by certain media. For instance, the level of guanosine 5'-diphosphate



3'-diphosphate (ppGpp) in a cell may have an indirect effect on the control of a passively controlled operon. By increasing or decreasing transcription of other operons, the level of free RNA polymerase inside the cell could change. Depending upon the gene (promoter) examined, ppGpp stimulates, inhibits, or has no effect on gene expression (29). Furthermore, the level of this nucleotide may be dependent upon both the media composition (14) and the growth rate (15), as seems to be true for the media-specific effects observed.

Another explanation for the media-specific behavior could involve an effect of media composition on the frequency or fidelity of messenger RNA(mRNA) translation. (This would be affected by a change in the mRNA half-life.) For example, it has been shown that abortion of nascent polypeptide chains may occur under growth conditions that restrict protein chain elongation (7, 11). Whether this occurs during growth in certain media has not been determined. However, a preferential effect could occur on an enzyme such as β-galactosidase, because its monomer molecular weight is much higher than the average molecular weight for *E. coli* protein monomers. Certainly, the order of magnitude of the media-specific effects is consistent with their being caused by subtle differences in the translation of a gene. Nevertheless, the constant ratio of specific activities for transacetylase to β-galactosidase in most media examined (but not all) suggests that the mechanism(s) responsible for the media-specific effects may vary.

Over the entire range of growth rates examined, there is, at most, a twofold change in the expression of the *UV5* promoter. But what does this mean? If there were no restriction on the expression of the *UV5* promoter, the rate of enzyme synthesis would be invariant with growth rate. The specific activity of an enzyme made at a constant rate decreases with increasing growth rate, simply by dilution resulting from the increased rate of synthesis of bulk protein. (A halving of the generation time would lead to a halving of the specific activity [relative rate of synthesis] of an enzyme made at a constant rate, and so forth.) Conversely, the reasonably constant specific activities of β-galactosidase observed for cells that carry the *UV5* and *L1*-type promoters implies that the rate of expression from these promoters must be restricted. This leads to the interesting view that there is a limited transcription and/or translation capacity within the cell.

The third major observation of the behavior of the catabolite-insensitive promoters concerned their expression at rapid growth rates. A large number of catabolite-insensitive promoters, with over a 36-fold range in level of expression, exhibit decreasing expression with increasing growth rates, whereas the *UV5* promoter does not. Three hypotheses can be offered.

(i) Only the *UV5* promoter is insensitive to some unidentified factor(s) that specifically affects expression of the *lac* operon. In vitro studies have demonstrated that transcription from both the *L1* and *UV5* promoters occurs in a purified transcription system that is dependent upon deoxyribonucleic acid, nucleoside triphosphates, RNA polymerase, and σ factor (9). While the nucleotide, ppGpp, does stimulate expression of the wild-type *lac* promoter in such a system (1), it does not appear to affect the *UV5* promoter (P. Primakoff, personal communication). No studies on the effect of ppGpp on *L1* or *L1*-type promoters have been reported, but one might postulate that they are still subject to ppGpp control. (The *UV5* mutation does consist of two adjacent base-pair changes in the promoter region [J. Gralla, personal communication].)

(ii) The *UV5* promoter may be unique because of some physical parameter of its structure independent of its very high level of expression. For example, several RNA polymerases may be bound simultaneously to the *UV5* promoter region, but only one to the *L1*-type promoters. A decrease in the number of free RNA polymerase molecules at faster growth might, therefore, affect transcription initiation at the *L1*-type promoters, while sim-

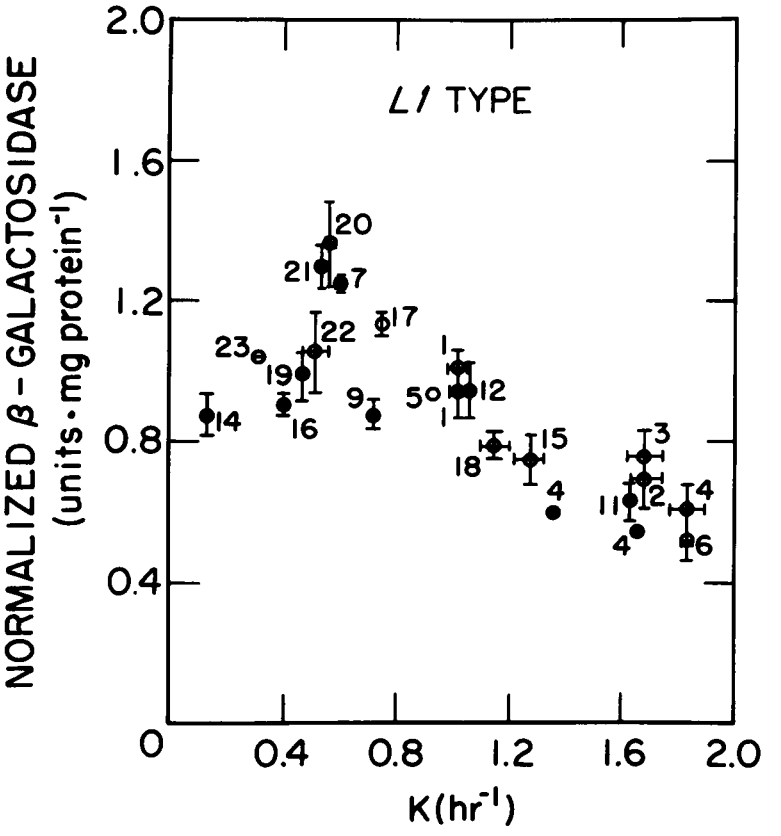


FIG. 4. *The normalized level of β-galactosidase activity in E. coli NC3 with the L1-type lac promoters. All specific activities are relative to an assigned glucose specific activity of 1.00. After normalizing and collating the data for all strains with the L1, L1R3, L1R4, L1R6, L1R11, or L1M1 lac promoters, the standard deviations were calculated. See Table 1 for the key to the media used. Data and symbols are taken from Fig. 1 and 3.*

ply decreasing the number of simultaneously bound polymerase molecules at the *UV5* promoter without decreasing the frequency of transcription initiation.

(iii) The *UV5* promoter may be unique because of its high level of catabolite-insensitive initiation, and the less efficient promoters may be preferentially affected at fast growth rates, perhaps because strong promoters compete well against ribosomal protein and ribosomal RNA promoters for free polymerases.

The latter two hypotheses (ii and iii) assume a decrease in the number of free RNA polymerase molecules (or transcription initiation factors). This decrease might be expected from the increased rate of ribosome biosynthesis in cells growing rapidly. If decreased expression of a passively controlled operon at rapid growth rates were caused by a depletion of free RNA polymerase resulting from the increased ribosome biosynthesis, a high-level promoter (e.g., one with a rate of expression very similar to that for a ribosomal protein mRNA promoter) might better compete for free RNA polymerases and, thereby, prevent a decreased expression. From a knowledge of the number of ribosomes inside a cell, and the assumption that ribosomal protein mRNA and *UV5*-initiated mRNA are translated at the same frequency (assuming similar half-lives for these mRNA species), it can be calculated that these two species of mRNA are synthesized in roughly the same molar proportion in rich media. This could explain the behavior of the *UV5* promoter. Furthermore, the existence of a frequency of initiation threshold very near to that for ribosomal protein mRNA seems reasonable.

Our results show that the product of an operon presumably freed of active controls represents a relatively constant fraction of total cellular protein under a large variety of growth conditions. Since ribosomal protein synthesis

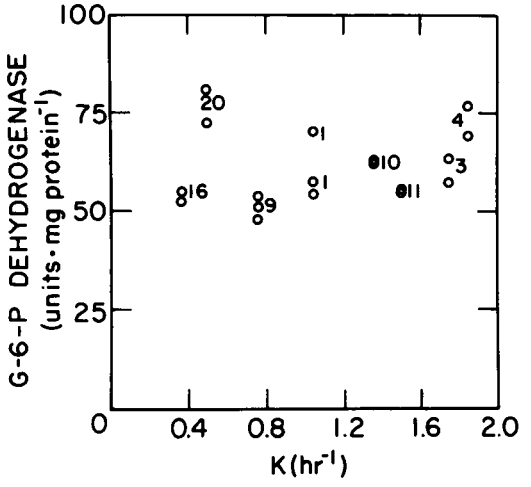


FIG. 5. *Glucose-6-phosphate dehydrogenase activity in E. coli NC3 grown on different carbon sources. An NC3 derivative carrying lacIL37UV5 was maintained in log-phase growth for at least 16 generations. At an OD_{420} of approximately 1.0, chloramphenicol was added at 100 μg/ml final concentration, and the cultures were rapidly chilled in an ice water bath. The cells were pelleted, washed once, and suspended in a small volume of 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.4, buffer. After sonic treatment and removal of debris, portions of the sonic supernatant were diluted 10-fold into either extract buffer or a buffer containing 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride, 0.01 M ethylenediaminetetraacetate (pH 7.9) for assay of β-galactosidase or transacetylase activity, respectively. β-Galactosidase, transacetylase, and glucose-6-phosphate dehydrogenase activity assays were performed on each culture as presented in the text. Data for β-galactosidase activity were collected as a control and are included in Fig. 2 along with the figure symbols. See text for discussion of the transacetylase values. The legend to the media used is given in Table 1.*

increases preferentially with increasing growth rate, these results do not support the model (20) for passive control of ribosomal protein genes. On the other hand, the high degree of similarity in the behavior of the *UV5* promoter and that of the gene for glucose-6-phosphate dehydrogenase suggests that the latter gene may be subject to passive control. Interestingly, the addition of exogenous cAMP to fully induced cells carrying the wild-type *lac* promoter does not result in *UV5*-like behavior, suggesting the existence of additional controls on the wild-type *lac* operon (B. Wanner, R. Kodaira, and F. Neidhardt, manuscript in preparation).

In summary, the expression of a decontrolled *lac* operon, which is presumed to be passively controlled (i.e., one not subject to any known specific or general control), appears to be generally constant with respect to growth rate, to be somewhat dependent upon media composition, and to be dependent upon the actual promoter structure. Two different types of promoter-dependent control were shown to be possible, the *L1* type and the *UV5* type. Whether or not particular promoter structures can result in different patterns of regulation must await a more thorough understanding of the process of transcription initiation. Since there was no detectable difference in the pattern of regulation from the two classes of promoters at slow growth rates, the difference in control seems to reflect the physiological state of the cell and does not strongly suggest a new type of promoter-dependent control.

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