REGULATION OF NEWLY EVOLVED ENZYMES
II. THE *EBG* REPRESSOR

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ABSTRACT

The *ebg* (evolved β-galactosidase) regulatory locus has been mapped. The map order is *argG–ebgA–ebgR–tolC–metC*, and there is 1.6% recombination between *ebgR* and *ebgA*. Studies with *ebgR*⁻/*ebgR*+ merdiploids have shown that *ebgR*⁻ is recessive, and it is concluded that the synthesis of *ebg* enzyme is under negative control.

STUDIES on the evolution of a new lactase function in *E. coli* have shown that in strains of *E. coli* K-12 bearing a deletion of the *lacZ* gene there exists a locus called *ebg* which may, under sufficiently strong selective conditions, evolve so that its product permits growth on lactose as a sole carbon source (CAMPBELL, LENGYEL and LANGRIDGE 1973; HALL and HARTL 1974). In the first such *ebg*+ strain described, synthesis of *ebg* enzyme was constitutive (CAMPBELL, LENGYEL and LANGRIDGE 1973). We subsequently described the selection of 34 independent *ebg*+ strains; of these only three synthesized *ebg*+ enzyme constitutively. The remaining 31 isolates synthesized *ebg* enzyme only in the presence of lactose (HALL and HARTL 1974). Since in 31 of 34 isolates the *ebg*+ gene was regulated, we suggested that the progenitor gene was likewise regulated. Further studies showed this to be the case. Synthesis of the progenitor gene product is induced 200- to 500-fold by growth in the presence of lactose; however, these fully induced cells remain incapable of utilizing lactose as a sole carbon source (HARTL and HALL 1974). In this communication we report studies on the regulation of the *ebg* gene, and on the *ebg* regulatory gene which we call *ebgR*.

MATERIALS AND METHODS

Genetic nomenclature

ebgA is the structural gene specifying *ebg* enzyme. *ebgR* is the regulatory gene. An *ebgR*⁻ strain synthesizes *ebg* enzyme constitutively; an *ebgR*+ strain synthesizes *ebg* enzyme only when grown in the presence of lactose.

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The state of the *ebgA* allele is indicated by a superscript. *ebgA*^{0} is the original, wild-type, unevolved state and specifies an enzyme which does not hydrolyze lactose at a rate sufficient to permit utilization of lactose as a sole carbon source; *ebgA*+ is the evolved state and specifies an enzyme capable of hydrolyzing lactose at a rate sufficient to permit utilization of lactose as a sole carbon source. Enzyme specified by either the *ebgA*^{0} or the *ebgA*+ allele is capable of hydrolyzing ONPG. *ebgA*$^-$ alleles do not specify an active *ebg* enzyme as detected by ONPG hydrolysis.

E. coli K-12 strains are described in Table 1.

Phage P1: *P1 cml clr*^{ts}. This phage is heat-inducible and carries a chloramphenical-resistance gene.

Matings were carried out as described by MILLER (1972). In all cases streptomycin (100 μg/ml) was used as the counterselective agent.

Transductions were carried out as described in MILLER (1972) using phage P1 described above.

TABLE 1

Bacterial strains in E. coli K-12

Strain	Relevant genotype
DS4680A	*HfrC spc lacZ* (deletion W4680) *ebgA*^{0} *ebgR*+
A1	*ebgA*+ *ebgR*$^-$ mutant of DS4680A
A2	*ebgA*+ *ebgR*$^-$ mutant of DS4680A
A3	*ebgA*+ *ebgR*$^-$ mutant of DS4680A
A4	*ebgA*+ *ebgR*+ mutant of DS4680A
4A8	*ebgA*+ *ebgR*$^-$ mutant of DS4680A
4A9	*ebgA*+ *ebgR*$^-$ mutant of DS4680A
5A1	*ebgA*+ *ebgR*+ mutant of DS4680A
5A2	*ebgA*+ *ebgR*$^-$ mutant of DS4680A
10A2	*ebgA*+ *ebgR*$^-$ mutant of DS4680A
1B1	*ebgA*^{0} *ebgR*$^-$ mutant of DS4680A obtained by U.V. mutagenesis.
DLH-18	F$^-$ *tolC str metC lacZ* (deletion W4680)
DLH-20	F$^-$ *tolC argG metC ebgA*+ *ebgR*+ *lacZ* (deletion W4680)
a-lY+	*HfrC spc ebgA*$^-$ *ebgR*$^-$ *lacZ* (deletion W4680). Obtained by nitroso-guanidine mutagenesis of strain A2. *ebgA*$^-$ reverts at low frequency.
SJ-1	F$^-$ *argG his tolC str lacZ* (deletion W4680)
SJ-7	F$^-$ *argG tolC metC str lacZ* (deletion W4680)
SJ-5	*ebgA*+ *ebgR*$^-$ *tolC*+ transductant of SJ-7. Donor was strain A3
SJ-6	*ebgA*+ *ebgR*$^-$ *tolC*+ transductant of SJ-7. Donor was strain 10A2
SJ-8	*ebgA*+ *ebgR*$^-$ *tolC*+ transductant of SJ-7. Donor was strain A2
SJ-9	*ebgA*+ *ebgR*$^-$ *tolC*+ transductant of SJ-7. Donor was strain A1
SJ-10	*ebgA*+ *ebgR*$^-$ *tolC*+ transductant of SJ-7. Donor was strain 4A8
SJ-11	*ebgA*+ *ebgR*$^-$ *tolC*+ transductant of SJ-7. Donor was strain 4A9
SJ-12	*ebgA*+ *ebgR*$^-$ *tolC*+ transductant of SJ-7. Donor was strain 5A2
SJ-13	*ebgA*+ *ebgR*+ *tolC*+ transductant of SJ-7. Donor was strain 5A1
SJ-14	*ebgA*+ *ebgR*+ *tolC*+ transductant of SJ-7. Donor was strain A4
KLF22/KL110	F'122 (PO12 covering *argG* to *thyA*)/argG6 metB1 his-1 leu-6 *thy-23 recA1 mtl-2 xyl-7 malA1 lacY1* or Z4 *gal-6 str-104 sup-59 tonA2* λ^r λ^-

All strains are *ebgA*^{0} *ebgR*+ unless otherwise noted. The *ebgA*+ alleles are the result of an unknown number of spontaneous mutations, and were selected as described in CAMPBELL, LENGYEL and LANGRIDGE (1973). Strains SJ-5, SJ-6 and SJ-8 through SJ-14 were prepared by P1 transduction from the specified donor. TolC+ was selected by plating on Mac-Lac +IPTG plates, and the ebgA+ allele scored by red colony color.

Media

Minimal medium was described in HALL and HARTL (1974). All media containing lactose or phenylgalactoside also contained 2×10^{-4} M IPTG. Amino acids were added to a final concentration of 100 μg/ml. Mac-lac medium: MacConkey-Lactose medium prepared from MacConkey Agar Base (Difco) according to instructions on bottle, but containing 2×10^{-4} M IPTG. Lactose-fermenting colonies appear red or pink on this medium, while nonfermenters appear white. X-GAL medium: glucose minimal medium containing 40 μg/ml of X-GAL (5-bromo-4-chloro-3-indolyl-β-D-galactoside) $+2 \times 10^{-4}$ M IPTG.

Abbreviations

IPTG: isopropyl-β-D-galactopyranoside
ONP: Ortho-nitrophenol
ONPG: ortho-Nitrophenyl-β-D-galactopyranoside
Phenylgalactoside: phenyl-β-D-galactopyranoside

Preparation of cell extracts was described in HALL and HARTL (1974). Extracts contain only soluble proteins; nucleic acids and nucleoproteins having been removed by streptomycin precipitation,

Measurement of protein concentrations

Absorbance of protein solutions at 225 nM in a 1 cm lightpath was determined in a Beckman U. V. spectrophotometer. The A_{225} of a 1 mg/ml protein solution is 9.17. The assay is linear from 2 to 100 μg/ml of protein, and is thus very useful in assaying dilute solutions so often encountered in enzyme purification procedures.

Since the protein concentrations of our extracts range from 20 to 40 mg/ml, our standard procedure is to make an initial 40-fold dilution into distilled water, followed by a final 21-fold dilution accomplished by pipetting 50 μl into 1.0 ml of water in the measuring cuvette. This procedure was adopted because: (1) it is some 40-fold more sensitive than measurement at 280 nM; (2) it is extremely convenient and requires very little material for accurate determinations; (3) the assay does not prohibit further use of the sample; and (4) potassium salts present in our buffers interfere strongly with the Folin method of LOWRY *et al.* (1951), thus prohibiting use of the Folin method at later stages of enzyme purification.

Our method yields the same values as the Folin-Lowry method or the spectrophotometric method of GROVES, DAVIS and SELLS (1968) when applied to our extracts. Since the GROVES method, which eliminates contributions due to nucleic acids, yields the same values as our method, there is no significant interference from nucleic acids in our extracts. The presence of streptomycin sulfate in our extracts does not interfere with this assay. Protein standards were prepared to a concentration of 40 mg/ml in H_2O and in a 25 mg/ml solution of streptomycin sulfate. Standard curves of protein concentration *vs.* A_{225} using the standards with streptomycin were indistinguishable from those using the standards without streptomycin.

In all cases the protein standard used was the Sigma Protein Standard Solution (50 mg/ml Human Albumin, 30 mg/ml Human Globin).

Ebg enzyme essay

Fifty microliters of extract was mixed with 1.0 ml of substrate solution (5 mM ONPG in 125 mM potassium phosphate buffer, pH 7.5 containing 5 mM $MgSO_4$) at 37°. The change in A_{420} was monitored in a Beckman 24 recording spectrophotometer in which the cuvette temperature was maintained at 37°. One unit of activity equals the release of one nanamole of ONP per minute. Specific activities of extracts are reported in units of activity per milligram of total protein.

Genotype scoring

Arginine and methionine markers were distinguished on minimal glucose medium containing or lacking the amino acid. *tolC*+ cells were distinguished by their ability to grow on MacConkey's medium.

ebgA+ cells were detected by (1) their ability to grow on lactose-IPTG minimal medium and (2) in the case of *tolC*+ cells, by their appearance on Mac-lac plates (red colonies).

ebgR- cells were distinguished by several methods: (1) *ebgR*-*ebgA*+ cells form colonies on lactose-IPTG minimal medium at 42°, while *ebgR*+*ebgA*+ cells do not; (2) *ebgR*- (*ebgA*+ or *ebgA*°) cells form colonies on phenylgalactoside-IPTG minimal medium, while *ebgR*+ cells do not; *ebgR*- (*ebgA*+ or *ebgA*°) cells form blue colonies on X-GAL plates; *ebgR*+ cells form white colonies.

RESULTS

Mapping the ebgA *and* ebgR *loci*

Preliminary mapping to establish the order of *ebgA*, *tolC*, and *metC* was carried out by phage P1 transduction. Our earlier study had shown that *ebgA* was linked to *tolC*, giving 19.7% recombination between *ebgA* and *tolC* (HALL and HARTL 1974). The donor strain was A2; the recipient was DLH-18. The *tolC*+ allele was selected by plating on Mac-lac. Among the *tolC*+ progeny 11 colonies were *ebgA*+ *metC*+, 72 were *ebgA*+ *metC*-, 38 were *ebgA*° *metC*+, and 71 were *ebgA*° *metC*-.

When the *metC*+ allele was selected and *ebgA* and *tolC* alleles scored, there were, among the *metC*+ progeny, 4 *tolC*+ *ebgA*+, 15 *tolC*+ *ebgA*°, 1 *tolC*- *ebgA*+, and 49 *tolC*- *ebgA*°. From these data we obtained the following cotransduction frequencies: *tolC* with *metC* = 26%, *tolC* with *ebgA* = 43.3%, *metC* with *ebgA* = 7.2%. This established the order as *ebgA*–*tolC*–*metC*.

More extensive mapping data was obtained from analysis of conjugation experiments. Table 2 shows the results of several experiments mapping the *argG* to *metC* region. Pooling of the data from the four experiments yields the following genetic distances between adjacent markers: *argG* to *ebgA*: 48.5% recombination; *ebgA* to *tolC*: 24.8% recombination; *tolC* to *metC*: 11.4% recombination. The distance between *tolC* and *metC* is very close to that reported by WHITNEY (1970), 13% to 15% recombination.

Preliminary experiments (unpublished) had indicated that *ebgR* is closely linked to *ebgA*. The experiment shown in Table 3 was designed to map the position of *ebgR*. The male strain, 1B1, was *ebgR*- *ebgA*°, while the female strain was *ebgR*+ *ebgA*+. The distal marker *metC*+ was selected, and all *metC*+ recombinants were scored for *argG*, *tolC*, *ebgA*, and *ebgR*. The *ebgR* allele was double-checked by utilizing both the phenylgalactoside medium and the X-GAL medium methods for detecting *ebgR*- colonies. Of 2411 *metC*+ recombinants only 40, or 1.65%, were recombinant in the region between *ebgA* and *ebgR* (classes 3, 7, 9, 11, 12, 14, 15 and 16 in Table 3). There are two possible orders of the *ebgA*, *ebgR*, and *tolC* genes. If the order is *ebgA*–*ebgR*–*tolC*, then there are 1629 colonies which are apparently nonrecombinant between *ebgA* and *tolC*. Among these we would predict that 13 ($0.0165 \times 0.342 \times 2411$) would have resulted from double recombinations between *ebgR* and *ebgA*, and between *ebgR* and *tolC*. There are in fact 16 such double recombinants (classes 9, 12, and 14 in Table 3). Similarly, of the 1647 colonies which are not recombinant between *ebgR* and *tolC* we would predict that 27.8 colonies (0.016×1647) would be recombinant between *ebgR* and *ebgA*. There are 24 such colonies (classes 3 and 11 in Table 3). The alterna-

TABLE 2

Hfr × F⁻	Selected marker	Unselected markers				Total progeny
A2 × DLH-18	*metC*+	*ebgA*+ *tolC*+	*ebgA*⁰ *tolC*⁻	*ebgA*+ *tolC*⁻	*ebgA*⁰ *tolC*⁻	
experiment #1		211	55	3	31	300
experiment #2		275	76	1	45	397
A2 × SJ-1	*tolC*+	*ebgA*+ *argG*+	*ebgA*+ *argG*⁻	*ebgA*⁰ *argG*+	*ebgA*⁰ *argG*⁻	
experiment #3		69	173	3	85	330
al-Y+ × DLH-20	*tolC*+	*ebgA*+ *argG*+	*ebgA*+ *argG*⁻	*ebgA*⁻ *argG*+	*ebgA*⁻ *argG*⁻	
experiment #4		77	111	225	208	621

TABLE 3

Mating between strain 1B1 (Hfr) and SJ15 (F^-)

Class	Genotype	No. of colonies
1.	*argG*$^-$ *ebgA*$^+$ *ebgR*$^+$ *tolC*$^-$	194
2.	*argG*$^-$ *ebgA*$^+$ *ebgR*$^+$ *tolC*$^+$	553
3.	*argG*$^-$ *ebgA*$^+$ *ebgR*$^-$ *tolC*$^+$	14
4.	*argG*$^-$ *ebgA*^{0} *ebgR*$^-$ *tolC*$^+$	686
5.	*argG*$^+$ *ebgA*^{0} *ebgR*$^-$ *tolC*$^+$	661
6.	*argG*$^+$ *ebgA*^{0} *ebgR*$^-$ *tolC*$^-$	8
7.	*argG*$^+$ *ebgA*^{0} *ebgR*$^+$ *tolC*$^-$	0
8.	*argG*$^+$ *ebgA*$^+$ *ebgR*$^+$ *tolC*$^-$	72
9.	*argG*$^+$ *ebgA*^{0} *ebgR*$^+$ *tolC*$^+$	4
10.	*argG*$^+$ *ebgA*$^+$ *ebgR*$^+$ *tolC*$^+$	187
11.	*argG*$^+$ *ebgA*$^+$ *ebgR*$^-$ *tolC*$^+$	10
12.	*argG*$^-$ *ebgA*$^+$ *ebgR*$^-$ *tolC*$^-$	3
13.	*argG*$^-$ *ebgA*^{0} *ebgR*$^-$ *tolC*$^-$	10
14.	*argG*$^-$ *ebgA*^{0} *ebgR*$^+$ *tolC*$^+$	9
15.	*argG*$^+$ *ebgA*$^+$ *ebgR*$^-$ *tolC*$^-$	0
16.	*argG*$^-$ *ebgA*^{0} *ebgR*$^+$ *tolC*$^-$	0
Total metC$^+$ colonies scored		2411

tive order predicts 12.7 doubles and 27 singles, but given the order *ebgR–ebgA–tolC*, classes 9, 12, and 14 would be singles, and classes 3 and 11 would be doubles, resulting in 16 observed singles and 24 observed doubles. The data are clearly consistent with the order *argG–ebgA–tolC–metC*.

The gene order was further tested by transductions. Phage P1 was grown in strain A3 (*ebgA*$^+$ *ebgR*$^-$ *tolC*$^+$) and used to transduce strain SJ-7 (*ebgA*^{0} *ebgR*$^+$ *tolC*$^-$). The *ebgA*$^+$ allele was selected by plating on lactose-IPTG-methionine-arginine minimal medium at 30°. *EbgA*$^+$ transductants were scored for *ebgR* and *tolC* by replicate plating to X-GAL and Mac-lac medium respectively. If the order is *ebgR–ebgA–tolC*, at least 70% of the *ebgA*$^+$–*ebgR*$^+$ transductants will be *tolC*$^+$; if the order is *ebgA–ebgR–tolC* all *ebgA*$^+$–*ebgR*$^+$ transductants will be *tolC*$^-$. Of 1540 *ebgA*$^+$ transductants 181, or 11.75%, were *tolC*$^+$. Only 5 were *ebgR*$^+$, giving a cotransduction frequency between *ebgR* and *ebgA* of 99.67%. All five *ebgA*$^+$–*ebgR*$^+$ were *tolC*$^-$. The probability of obtaining these data if the order were *ebgR–ebgA–tolC* is $(0.3)^5$ or 0.0024. These data thus support the order *ebgA–ebgR–tolC*.

Pooling the data in Table 2 with those in Table 3, we obtain the following lengths for the regions defined by our markers: *argG* to *ebgA*, 42.7%; *ebgA* to *ebgR*; 1.6%; *ebgR* to *tolC*, 29.0%; and *tolC* to *metC*, 11.9%. Figure 1 shows the map generated by these data.

Functioning of the ebg *repressor*

The symbology of the previous section tacitly assumes that the *ebgR* locus specifies a repressor. We have tested this assumption by constructing a series of *ebgR*$^-$/*ebgR*$^+$ merodiploids, and comparing the amount of *ebg* enzyme synthe-

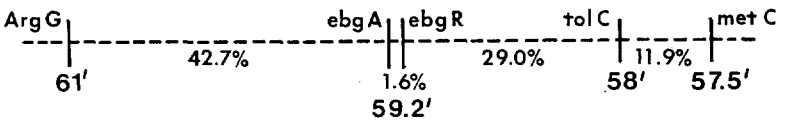

FIGURE 1.—Map of the argG–metC of *E. coli*. Number below the vertical lines indicate gene positions corresponding to the position (in minutes on the *E. coli* map). We have taken the positions of *argG* and *metC* from TAYLOR and TROTTER (1972) and calculated the positions of *ebg* and *tolC* from the percent recombination.

sized in the merodiploid with that synthesized in the parental haploid strain in the absence of lactose.

In each case the haploid strain had the genotype *argG*$^-$ *ebgR*$^-$ *ebgA*$^+$ *metC*$^-$ F$^-$. Haploid strains (designated F$^-$ in Table 4) were grown in succinate minimal medium containing methionine and arginine and extracts were prepared from log phase cultures as described in MATERIALS AND METHODS.

Merodiploid derivatives of each haploid strain were prepared by introducing F′122 from strain KLF22/KL110. KLF22/KL110 and the F$^-$ strain were cross streaked on medium lacking methionine and arginine. Since the donor strain requires both histidine and leucine, and the recipient requires methionine and arginine, only the merodiploids derived from the F$^-$ strain could form colonies. F′122 includes the region from *argG* to *metC*, and thus carries *ebgR*$^+$ *ebgA*^{0}. Thus the merodiploids were F′ *argG*$^+$ *ebgA*^{0} *ebgR*$^+$ *metC*$^+$/*argG*$^-$ *ebgA*$^+$ *ebgR*$^-$ *metC*$^-$. Merodiploid strains were always grown in succinate minimal medium in the absence of methionine and arginine in order to enhance retention of the F′ episome.

Cultures were inoculated from the resulting colonies the day after cross streaking and harvested the following day. Table 4, lines 1–7, shows the specific activity of extracts prepared from haploid and merodiploid strains. It is clear that strains of the genotype *ebgR*$^+$/*ebgR*$^-$ synthesize at least twentyfold less ebg enzyme than their parental *ebgR*$^-$ strains. Thus it would appear that the *ebgR*$^+$ allele carried on the F′ episome specifies a diffusable gene product, i.e. a repressor.

TABLE 4

Specific ebg enzyme activities of uninduced cultures

Line	Strain	F$^-$ strain	F′122 merodiploid derivative
1.	SJ-5	120 Units/mg	5.7 Units/mg
2.	SJ-6	282 Units/mg	7.2 Units/mg
3.	SJ-8	193 Units/mg	6.6 Units/mg
4.	SJ-9	257 Units/mg	7.2 Units/mg
5.	SJ-10	392 Units/mg	15.0 Units/mg
6.	SJ-11	444 Units/mg	7.8 Units/mg
7.	SJ-12	341 Units/mg	7.4 Units/mg
8.	SJ-13	< 0.1 Units/mg	———
9.	SJ-14	0.1 Units/mg	———

Strains SJ-13 and SJ-14 are *ebgR*+ haploids.

However, lines 8 and 9 of Table 4 show that the amount of the *ebg* enzyme synthesized by a haploid *ebgR*$^+$ strain is almost two orders of magnitude less than the amount synthesized by an *ebgR*$^+$/*ebgR*$^-$ merodiploid. This does not necessarily indicate that the repressor acts more strongly in *cis* than in *trans*. If the *ebgR*$^+$ allele were lost (either by loss of episome or by non-reciprocal recombination) in a fraction of the population, then that fraction would be *ebgR*$^-$, and would synthesize *ebg* enzyme at the constitutive rate. If it is assumed that the *ebgR*$^+$/*ebgR*$^-$ fraction of the population synthesizes 0.1 units/mg of *ebg* activity, while the *ebgR*$^-$ fraction synthesizes 193 units/mg in the case of SJ-8 or 341 units/mg in the case of SJ-12, then the constitutive fraction would be 0.034 in the case of SJ-8/F'122 and 0.021 in the case of SJ-12/F'122.

The proposition that population heterogeneity accounted for the high basal level of *ebg* enzyme synthesis in *ebgR*$^+$/*ebgR*$^-$ merodiploids was tested by directly measuring the fraction of the population which was *ebgR*$^-$.

Fresh merodiploid strains were prepared by reintroducing F'122 into strains SJ-8 and SJ-12. Instead of preparing extracts from the resulting merodiploid cultures, the cultures were plated on glycerol minimal medium containing IPTG. The resulting colonies have fully induced levels of lactose permease (*lacY* gene product). The following day the resulting colonies were individually incubated in buffer containing ONPG. Only fully constitutive *ebg* colonies result in visible yellowing within five hours. Of 270 *SJ-12/F'122* colonies, 7 were constitutive (i.e. *ebgR*$^-$) *vs.* a predicted number (0.021×270) of 5.7; and of 270 *SJ-8-F'122* colonies, 10 were constitutive *vs.* a predicted number (0.034×270) of 9.2. Thus population heterogeneity accounts well for the observed high level of ebg enzyme synthesis is repressed *ebgR*$^+$/*ebgR*$^-$ merodiploids. Based upon these observations, we suggest that *ebg* regulation is very similar to that of other loci under negative control. *EbgR*$^+$ is dominant to *ebgR*$^-$, and the repressor acts as strongly in *trans* as in *cis*.

DISCUSSION

We have shown that the *ebgR* locus is very tightly linked to the structural locus *ebgA*. The map order presented with respect to *ebgR* and *ebgA* is not unambiguous; however, a more reliable determination would require the use of additional markers located nearer to ebg than either *tolC* or *argG*.

We have shown that the *ebgR* locus is apparently a normal repressor locus present in strains of *E. coli* K-12. Constitutive (*ebgR*$^-$) mutations are recessive and the *ebgR* locus is very close to the structural locus it regulates, *ebgA*. The repressor responds to lactose or to one of its metabolic products. The above properties are common to the *ebg* repressor and the *lac* repressor. A major difference is that the *lac* repressor responds to IPTG, while the ebg repressor is completely insensitive to IPTG (HALL and HARTL 1974).

The function of *ebg*^{o} enzyme remains unknown. The operon is sensitive to catabolite repression (ARRAJ 1973), thus it appears likely that its normal function is catabolic. It is not at all apparent why the *ebg* repressor should respond to lactose, since the *ebg*^{o} enzyme is virtually inactive on the substrate. The obser-

vation that fully induced *ebgR*$^+$ cells synthesize only 10–20% as much enzyme as *ebgR*$^-$ cells (HALL and HARTL 1974 and unpublished results (HALL) suggests that lactose is in fact a rather poor inducer and may well not be the "normal" inducer of *ebg*.

Although some *ebgR*$^-$ strains are found among the *ebgA*$^+$ strains selected, we have no evidence that "evolution" has favored any other changes in *ebg* regulatory functions.

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