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THE ROLE OF THE REGULATOR-GENE PRODUCT (REPRESSOR) IN CATABOLITE REPRESSION OF β-GALACTOSIDASE SYNTHESIS IN *Escherichia coli*

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The Role of the Regulator-Gene Product (Repressor) in Catabolite Repression of β-Galactosidase Synthesis in *Escherichia coli*

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ABSTRACT

The specific involvement of the *lac* repressor (i-gene product) in acute transient catabolite repression has been investigated using temperature sentitive control mutants of the i-gene (1). These mutants, while normally inducible at low temperatures (32 C), become increasingly phenotypically constitutive as the growth temperature is raised towards 42 C. One mutant (i^{TSS}) was believed to have an impaired synthesis of the repressor molecule at high temperatures; such cells continue to show acute transient catabolite repression. In a second mutant (i^{TL}) the repressor itself is unstable at 42 C; at this temperature the cells are about 25% constitutive, and fail to show the transient repression phenomenon with glucose.

Further examination of the status of repressor in these strains was carried out using o-nitrophenyl-β-D-fucoside (ONPF), a substance which represses induced enzyme synthesis in the *lac* system. The i^{TSS} mutant at 42 C showed repression with ONPF, and this was partly reversible by isopropyl-thio-β-D-galactoside (IPTG), a powerful inducer. This strain thus

contains a repressor which fails to repress β-galactosidase synthesis unless activated by ONPF or by catabolite repression. The i^{TL} mutant, on the other hand, could be further induced by IPTG at 42 C, but was unaffected by ONPF either in the presence or absence of IPTG. Such cells therefore possess an appreciable concentration of an altered repressor, capable of preventing 75% of β-galactosidase synthesis in the absence of IPTG, but incapable of being activated by ONPF or catabolite repression.

A model is presented for a trivalent repressor, possessing sites for interaction with inducer, operator and catabolite repressor. A number of possible genotypes and phenotypes are predicted, and examples are given for known categories. The experimental results are compared with the model, and the nature of the alteration in the repressor caused by the mutation in each strain is discussed.

It has previously been shown (J. Palmer and V. Moses, Biochem. J., in press) that acute transient catabolite repression of β-galactosidase synthesis, observed when glucose is added to glycerol-grown cells of Escherichia coli (11), requires the presence of a functional operator gene (o). The specific involvement of the i-gene product (repressor) was not then tested since a regulator gene deletion mutant (i^{del}) was not available to us. While such a mutant has still not been isolated to the best of our knowledge, the characterization of certain temperature-sensitive control mutants by Sadler and Novick (13) has made possible another approach to the investigation of the role of repressor in acute transient catabolite repression.

Two types of temperature-sensitive regulatory mutants have been obtained (13). In one series repressor itself appeared to be increasingly unstable as the temperature was raised, so that growth at 42 C produced a cell largely constitutive in character, while the same strain cultured at 30 C was typically inducible. This mutation has been designated i^{TL}. In the other series (i^{TSS}) it was believed that the synthesis of repressor at high temperature was prevented, although repressor, once formed, was not destroyed by heat. Thus investigation of the catabolite repression behavior of these strains at high (42 C) and low (32 C) temperatures and comparison with their wild type parent strains should yield information on the relation between the functional state of the repressor and the phenomenon of transient catabolite repression.

MATERIALS AND METHODS

Organisms. The strains of *E. coli* used are listed with their relevant genetic characters: E 103 (i^{TL} o^+ z^+); E 102 (i^+ parent of E 103); E 321 (i^{TSS} o^+ z^+); WI-4 (i^+ parent of E 321); 230 U (i^- o^+ z^+) 112 - 12 - A - 84 (i^{--sus} [suppressible i^- constitutive] o^+ z^+ y^+ su^-). The first four strains were obtained from A. Novick, and their derivatives and properties are described in references 13 and 5. Strains 230 U and 112 - 12 - A - 84 were gifts from J. Monod and B. Müller-Hill, respectively.

Growth conditions. The cells were grown at 32 C, 37 C, or 42 C in M63 medium (12) containing 22 mM glycerol and supplemented with the necessary specific nutrients. Temperatures were controlled to ± 0.5 C in a water bath. Aeration was provided by shaking or by magnetic stirring. Growth was measured as described previously (11); at an extinction value of 1.0 the cultures contain 225 μg bacterial protein/ml. Glucose was introduced to the cultures where indicated by adding 1 M glucose solution to give a final concentration of 10 mM.

Enzyme induction and assay. β-Galactosidase synthesis was induced, when necessary, with 0.5 mM isopropyl-thio-β-D-galactopyranoside (IPTG). Since many measurements were made of constitutive enzyme synthesis, particular care was exercised to minimize errors in sampling volume. Samples (0.05 to 0.20 ml) of culture were added to clean weighed 10 ml shell vials containing 0.2 ml of chloramphenical solution (1 mg/ml), mixed and reweighed to correct for volume errors. Before assay, one drop (about 6 μl) of toluene was added to each sample, and these were stoppered and shaken for 30 min at 37 C. The stoppers were then

removed and the toluene allowed to evaporate for 30 min at 37 C. Enzyme activity was measured by the rate of hydrolysis of o-nitrophenyl-β-D-galactoside (ONPG) as described by Kepes (8), using 0.8 ml of 3.3 mM substrate solution, and 0.8 ml of 0.75 M Na_2CO_3 to stop the reaction. About 100 mg of solid $BaCO_3$ was added to each vial, and after mixing the vials were centrifuged for 1 min at 1400 x g in a bench-top centrifuge to clarify the solution and remove turbidity due to the bacteria. The amount of enzyme catalyzing the hydrolysis of 1 mμmole of substrate/min at 37 C is defined as one unit (EU).

Effect of o-nitrophenyl-β-D-fucoside (ONPF). For these experiments cultures of each strain were grown overnight with shaking at 42 C for E 103 and E 321, and at 37 C for 230 U. The cultures were then diluted to an extinction at 650 mμ of about 0.01 (equal to 2.25 μg of bacterial protein/ml), and 10 ml quantities of each culture were incubated at the above temperatures in separate flasks containing either no additions, 0.5 mM IPTG, 1 mM ONPF, or both IPTG and ONPF in the previous amounts. The cells were allowed to grow through five or six doublings; during one further doubling samples for enzyme assay were withdrawn and growth was closely followed.

Chemicals. IPTG and ONPG were purchased from Calbiochem, Los Angeles, Calif., chloramphenicol was obtained from Parke, Davis and Co., Detroit, Mich., and ONPF was a gift from Dr. K. Paigen.

RESULTS

Effect of temperature on transient repression. The effect on β-galactosidase synthesis of adding glucose to cells growing on glycerol at 32 C and 42 C is shown for the i^+ strain E 102 (Fig. 1) and

the i^{TL} strain E 103 (Fig. 2). Both strains have identical growth responses to addition of glucose; no change in doubling time when glucose is added at 32 C, but a 6-7% decrease in the doubling time when glucose is added at 42 C. For strain E 102 a transient repression of enzyme synthesis was observed at both temperatures, lasting about the same time in each case. A similar experiment performed with E 103 (i^{TL}), however, shows that transient repression, while still present at 32 C, is no longer evident at 42 C (Fig. 2). The mutation from i^+ to i^{TL} thus abolished acute transient repression and simultaneously permitted a partially constitutive synthesis of β-galactosidase when these cells were grown at 42 C.

The i^{TSS} strain and its wild-type i^+ parent exhibited a different pattern of behavior. Both strains showed an erratic growth response to the addition of glucose at both temperatures. Instead of a sharp increase in growth rate of 20-40%, which is typically found with many strains of E. coli under such conditions, WI-4 sometimes showed a fall in growth rate when glucose was added, and E 321 invariably showed a slowing down of growth from about 0.7 to 0.1 generations/hour, which became apparent some 45-60 min after adding glucose.

The wild-type strain WI-4 showed no transient repression of β-galactosidase synthesis at low temperature, although transient repression was observed at 42 C (Fig. 3). Strain E 321 (i^{TSS}), on the other hand, showed severe transient repression at both temperatures (Fig. 4).

Effect of glucose on an amber-suppressor-sensitive i^- strain. Strain 112 - 12 - A - 84, carrying a suppressible mutation of the

i-gene which confers a phenotypic constitutive character, offers a further opportunity of investigating the behavior of a strain bearing the equivalent of a partial deletion in the i-gene. Addition of glucose to these cells growing on glycerol increased the growth rate by 11%. No transient repression of β-galactosidase synthesis was observed, and the steady differential rate of enzyme synthesis immediately fell to 61% of the rate in the absence of glucose.

Effect of IPTG and ONPF on the rates of β-galactosidase synthesis. Jayaraman, Müller-Hill and Rickenberg (7) have used ONPF to study repressor and modified repressor function in a variety of regulator mutants. We have made use of their techniques in order to clarify the condition of the repressor in the temperature-sensitive strains E 103 and E 321, which show varying transient catabolite repression responses in the apparent absence of repressor in both cases. These strains were compared with 230 U, an i^- constitutive known to exhibit transient repression (11).

Table 1 gives values for the specific content of β-galactosidase in cells grown exponentially for 6-7 generations under constant conditions. Each value is the average of three samples taken at different extinctions ranging over about one doubling of cell mass. Experiments 1 and 2 were performed on different days.

Table 1 shows that E 103 cultured at 42 C contained some repressor (it is only 24% constitutive), but that while this repressor was sensitive to the presence of IPTG it was unaffected by ONPF. Strain E 321 grown for several generations at 42 C also contained repressor; even though it was 92% constitutive under these conditions it was severely

repressed by ONPF, and this repression was partially reversible by IPTG. Strain 230 U, as would be predicted from the findings of Jayaraman et al., contained no repressor that was significantly affected by either IPTG or ONPF.

DISCUSSION

It was pointed out elsewhere (J. Palmer and V. Moses, Biochem. J., in press) that arguments excluding repressor from a role in transient repression are not acceptable when based on the fact that i^- constitutive mutants exhibit this sort of repression. It is possible that i^- point mutants possess an altered repressor which, while having lost its normal capacity to bind to the operator, may be activated to do so under metabolic conditions giving rise to catabolite repression. Similarly, the observation of repression by glucose of β-galactosidase synthesis during the one-hour period of constitutive synthesis of the enzyme following transfer by conjugation of i^+ z^+ into lac-deletion recipients (9), also does not eliminate repressor from the mechanism of catabolite repression. One might argue that repressor is effective at lower concentrations in the presence of glucose metabolites. In a very recent paper Barbour and Pardee (1) have indeed obtained evidence that repressor or a precursor of repressor is present in low concentration from a very early period during mating in these experiments.

The observation that transient repression is linked to a functional operator gene (J. Palmer and V. Moses, Biochem. J., in press) strongly suggests the likelihood of repressor also being involved. A formal test of this possibility requires a comparison of the glucose effect in related strains, one of which contains repressor while in the

other it is absent. Such a comparison cannot yet be made with a deletion mutant, but the temperature-sensitive regulator strains described by Sadler and Novick (13) and the i^{-} ^{sus} mutant offer a possible opportunity of testing repressor involvement.

Sadler and Novick (13) interpret their findings to mean that the i^{TL} mutant (E 103) makes an i-gene product which is unstable at high temperature. The i^{TSS} mutant (E 321), on the other hand, fails entirely to synthesize repressor at high temperature, while pre-existing repressor molecules are eventually destroyed in metabolism. A correlation between appearance of constitutive character in these strains at high temperature with a disappearance of transient catabolite repression would be strong evidence in favor of both functions being mediated by repressor. An absence of correlation, however, would be inconclusive, since it could be argued that only a part of the repressor function is absent at 42 C. A further investigation of the status of repressor in these strains has therefore been performed.

ONPF is known to repress uninduced leaky i^{-} mutants (7) and thus appears to be an activator of repressor as distinct from an antagonist of inducer. It has no effect on tight i^{-} mutants because these presumably possess no repressor for ONPF to activate. Strain E 103 at 42 C is 24% constitutive; it therefore contains either a low concentration of repressor, or an altered repressor, or both. This strain may be further induced with IPTG, but induced or uninduced it shows no response to ONPF (Table 1). We conclude that it does have an altered repressor, and this repressor may also be present in lower concentrations than normal. E 103 has also lost the transient catabolite repression response at 42 C.

In the case of E.321, the cells are effectively completely constitutive at 42 C. They therefore either contain an altered repressor or none at all. However, these cells are repressed by ONPF, and this repression is at least partly reversible by IPTG, so that the binding sites for inducer and operator have not been lost. This leads us to suppose that a form of repressor is present which has no repressing activity unless it is first activated by ONPF. E 321 also demonstrates transient catabolite repression at 42 C, suggesting that repressor may also be activated by glucose metabolites. These results indicate that changes in susceptibility to ONPF repression and to glucose repression are both due to mutation in the i-gene locus and support indications from earlier studies (J. Palmer and V. Moses, Biochem. J., in press) that transient catabolite repression is effected through repressor-operator interaction.

Strain 230 U is an i^- point mutant and may be presumed to contain an altered repressor molecule. Cells of this strain are fully constitutive and do not respond to ONPF. They do, however, exhibit transient catabolite repression (11), and as this appears to be effected via repressor we may conclude that glucose metabolites are more powerful than ONPF in promoting the binding of repressor to the operator.

The absence of transient repression in strain 112 - 12 - A - 84 supports the idea of the involvement of repressor in this phenomenon. Repressor in this strain has presumably lost affinity for operator, and this has resulted in constitutive character. Gilbert and Müller-Hill (4) have shown that this repressor has also lost affinity for inducer. This argues strongly for a large functional deletion in the i-gene and is thus consistent with transient catabolite repression being mediated by the i-gene product.

The observed relation of transient catabolite repression to various mutations in the i-gene may be explained by a model in which repressor is a trivalent molecule, rather than the bivalent entity originally suggested by Jacob and Monod (6). This concept has previously been briefly discussed by Clarke and Brammar (3), and was also considered by Loomis and Magasanik (9) but was rejected by them for reasons which were subsequently criticized by Clarke and Brammar (3) and by Palmer and Moses (Biochem. J., in press). The three postulated sites on the repressor would be an inducer-interacting site (I), an operator-interacting site (O) and a catabolite corepressor-interacting site (CR). According to this model, the O-site interacts with the operator in the absence of inducer and prevents transcription. In the presence of inducer the I-site binds to the latter and the O-site is allosterically modified in such a way that it no longer has affinity for the operator and transcription proceeds (10). The CR-site interacts with the catabolite corepressor and allosterically increases the affinity of the O-site for the operator. Thus the CR- and I-sites have opposite effects on the degree of binding of the repressor to the operator and can be viewed as being competitive. Evidence has been published indicating that induction and catabolite repression may be competitive phenomena (2). Whether or not ONPF also interacts at the CR-site, or at yet another site on the repressor, is not clear. Although the identity of the catabolite corepressor is not yet definitely known, the chemical nature of the possible candidates for this role proposed by Prevost and Moses (C. Prevost and V. Moses, Biochem. J., in press)

does not make it seem likely that the catabolite corepressor and ONPF do act at the same place.

Each of the proposed three repressor sites may be conceived as existing in several allelic forms giving rise to the following genotypes:

I^+: repressor is capable of interacting reversibly with inducer and thus losing affinity for the operator;

I^-: repressor is incapable of interacting with inducer;

I^{++}: repressor binds to inducer more tightly than normal;

CR^+: repressor is capable of interacting reversibly with the catabolite corepressor and thus increasing affinity for the operator;

CR^-: repressor is incapable of interacting with the catabolite corepressor;

CR^{++}: repressor binds to the catabolite corepressor more tightly than normal;

O^+: repressor is capable of interacting reversibly with the operator;

O^-: repressor is incapable of interacting with the operator;

O^{++}: repressor binds to operator more tightly than usual.

In order to account for the presence of transient catabolite repression in i^- constitutive mutants, it is also necessary to postulate a further allele of the O-site:

O^{CR}: repressor interacts with the operator only when the CR-site is occupied by the catabolite corepressor.

These genotypes considered in isolation would confer the following properties: I^+ would be wild-type inducible, I^- would exhibit superrepression (i^s in the usual notation), while I^{++} would respond to

lower concentrations of inducer than I^+. A mutant of the latter variety has recently been reported by Gilbert and Müller-Hill (4), and given the designation i^T (for "tight" binding of inducer). Among alleles of the O-site, O^+ represents wild-type repressible, O^- the common constitutive (i^-) and O^{++} is another mutation giving rise to a super-repressed condition. In a strain with the O^{CR} allele, repression would occur only under conditions of catabolite repression: in the complete absence of catabolite repression (if that is ever possible) the cells would be phenotypically constitutive. Such a strain may or may not be inducible in the presence of catabolite repression, depending on the relative competitive effects on the O-site of interaction at the I- and CR-sites. In the CR-site, CR^+ is wild type showing transient catabolite repression, CR^- is not sensitive to such repression, while CR^{++} would be very sensitive and possibly permanently repressed, (yet another form of i^S). In diploids the expected dominance relationships would be $I^- > I^+ > I^{++}$; $CR^{++} > CR^+ > CR^-$; $O^{++} > O^+ > O^{CR} > O^-$.

Independent variation of these alleles gives 36 possible genotypes, falling into nine phenotypic groups; Table 2 lists these possibilities. In making assignments to phenotypes C and E, we assume from our present results that ONPF and the catabolite corepressor both bind at the same (CR) site. Examples are presently known fitting into seven of these nine categories, and future work in this laboratory is being directed to a closer investigation of phenotypic and genetic consequences of mutation in the i-gene.

ACKNOWLEDGMENTS

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Table 1. Effect of IPTG and ONPF on the differential rates of β-galactosidase synthesis[a]

Strain	Additions	FU/extinction Expt. 1	FU/extinction Expt. 2	As percentages Expt. 1	As percentages Expt. 2	As percentages Average
E 103 (42 C)	None	865	806	100	100	100
	IPTG	3720	3360	430	417	424
	ONPF	920	830	106	103	105
	IPTG & ONPF	-	3110	-	386	386
E 321 (42 C)	None	1255	1180	100	100	100
	IPTG	1225	1410	98	119	109
	ONPF	650	645	52	55	54
	IPTG & ONPF	-	765	-	65	65
230 U (37 C)	None	-	2400	-	100	
	IPTG	-	2680	-	112	
	ONPF	-	2360	-	98	
	IPTG & ONPF	-	2320	-	97	

[a]Separate cultures of the indicated strains of *E. coli* were grown for 6-7 generations in glycerol-minimal salts with the indicated additions (see Materials and Methods). Each value shown is the average of three determinations ranging over about one cell generation.

Table 2. Possible phenotypic classes and different genotypes in each class based on a trivalent repressor model[a]

	A	B	C	D	E	F	G	H	I
Possible phenotypes	$i^+ cr^+$	$i^- cr^+$	i^- when no cr; i^+ when cr.	i^- when no cr; $hypi^+$ when cr.	$i^+ cr^-$	$i^- cr^-$	i^S	$hypi^+ cr^+$	$hypi^+ cr^-$
Different genotypes	$I^+O^+CR^+$	$I^-O^{CR}CR^+$	$I^+O^{CR}CR^+$	$I^{++}O^{CR}CR^+$	$I^+O^+CR^-$	$I^+O^-CR^-$ $I^-O^-CR^-$ $I^{++}O^-CR^-$ $I^+O^-CR^+$ $I^-O^-CR^+$ $I^{++}O^-CR^+$ $I^+O^-CR^{++}$ $I^-O^-CR^{++}$ $I^{++}O^-CR^{++}$ $I^+O^{CR}CR^-$ $I^-O^{CR}CR^-$ $I^{++}O^{CR}CR^-$	$I^-O^+CR^+$ $I^-O^+CR^-$ $I^+O^+CR^{++}$ $I^-O^+CR^{++}$ $I^{++}O^+CR^{++}$ $I^+O^{++}CR^+$ $I^-O^{++}CR^+$ $I^{++}O^{++}CR^+$ $I^+O^{++}CR^-$ $I^-O^{++}CR^-$ $I^{++}O^{++}CR^-$ $I^+O^{++}CR^{++}$ $I^-O^{++}CR^{++}$ $I^{++}O^{++}CR^{++}$ $I^+O^{CR}CR^{++}$ $I^-O^{CR}CR^{++}$ $I^{++}O^{CR}CR^{++}$	$I^{++}O^+CR^+$	$I^{++}O^+CR^-$
Examples and references	wild type	230 U 3300	E 321, on basis of ONPF at 42 C	not known	E 103 at 42 C	E 103 at 42 C, uninduced	i^S, reported by Willson et al. (14)	i^T Ref. (7)	not known

[a]Phenotypes are represented with lower case letters: i^+ is wild-type inducible, i^- is a regulator constitutive, i^S is superrepressed, $hypi^+$ is induced by lower than normal levels of inducer, cr^+ shows transient catabolite repression, cr^- shows no transient repression. Genotypes are indicated with capital letters and are explained in the text.

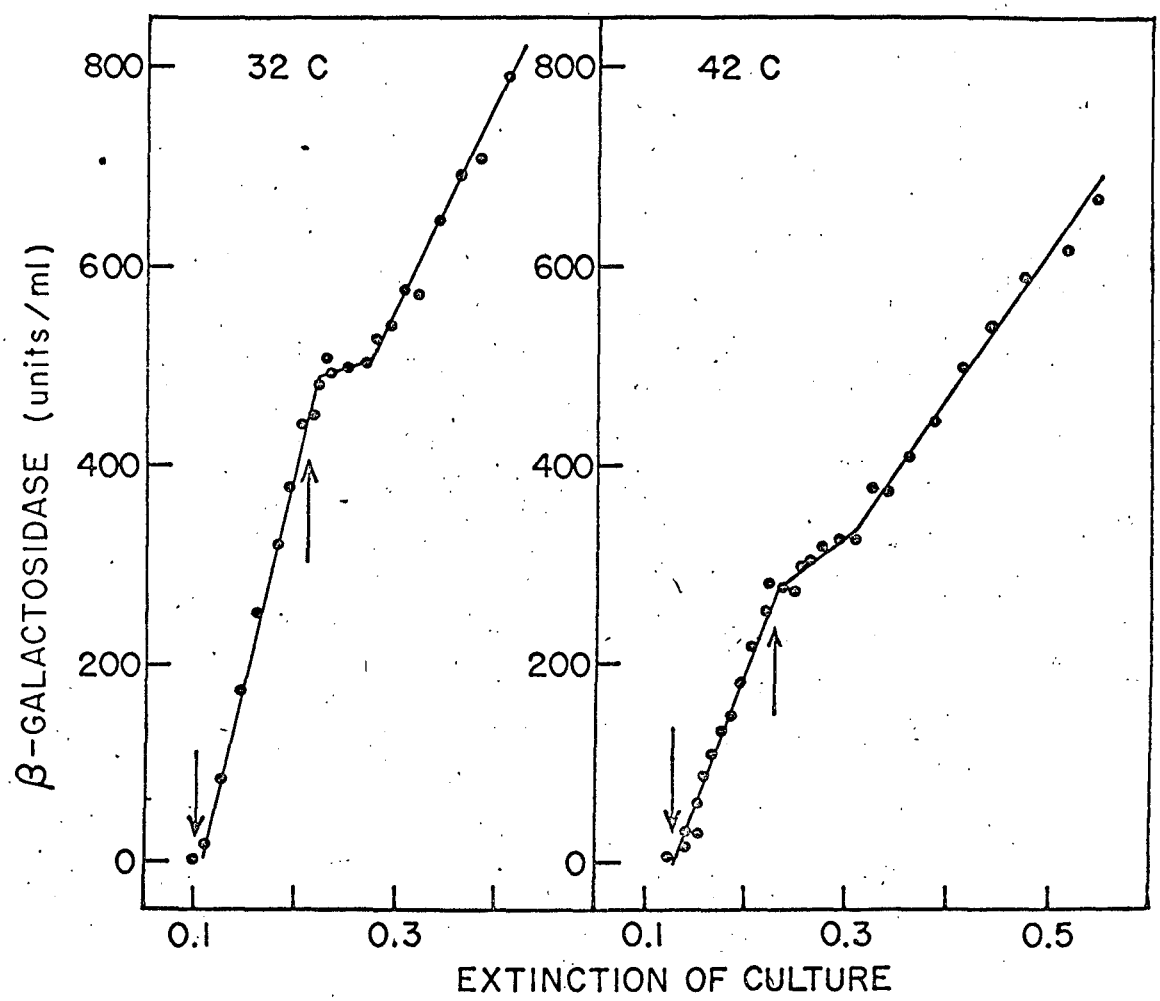
FIGURE CAPTIONS

Fig. 1. Repression by glucose of β-galactosidase synthesis in strain E 102 at 32 C and 42 C. IPTG to 0.5 mM was added at downward arrows and glucose to 10 mM, at upward arrows. Mass doubling times (min) before and after glucose addition and length of transient repression, respectively: at 32 C, 78, 77, 25; at 42 C, 70, 66, 29.

Fig. 2. Repression by glucose of β-galactosidase synthesis in strain E 103 at 32 C and 42 C. IPTG to 0.5 mM was added at downward arrows and glucose to 10 mM at upward arrows. Mass doubling times (min) before and after glucose addition and length of transient repression, respectively: at 32 C, 70, 72, 23; at 42 C, 73, 68, no transient.

Fig. 3. Repression by glucose of β-galactosidase synthesis in strain W1-4 at 32 C and 42 C. IPTG to 0.5 mM was added at downward arrows and glucose to 10 mM at upward arrows. Mass doubling times (min) before and after glucose addition and length of transient repression, respectively: at 32 C, 86, 74, no transient; at 42 C, 93, 56, 28.

Fig. 4. Repression by glucose of β-galactosidase synthesis in strain E 321 at 32 C and 42 C. IPTG to 0.5 mM was added at downward arrows and glucose to 10 mM at upward arrows. Mass doubling time (min) before and after glucose addition and length of transient repression, respectively: at 32 C, 78, 73 with large increase beginning after 45 min, 27; at 42 C, 120, 90 with large increase beginning after 60 min, 22.



XBL671-419

Fig. 1

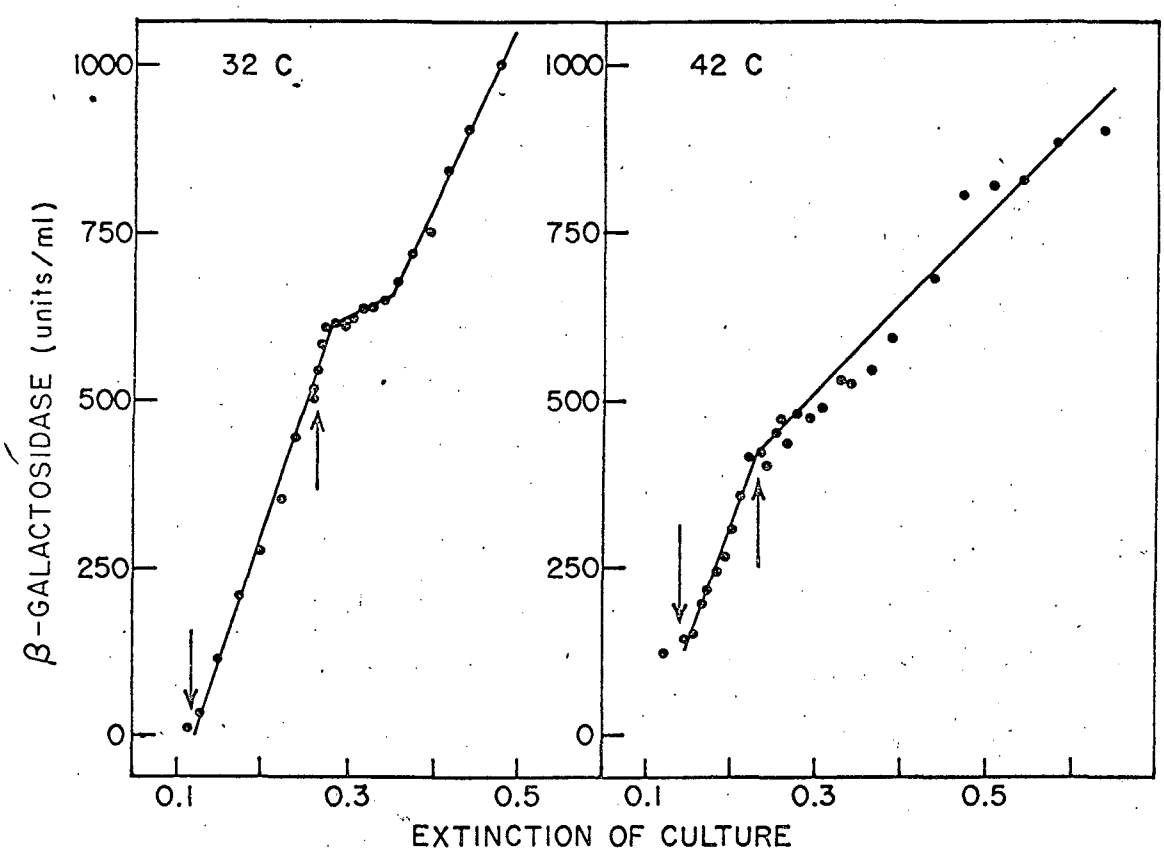


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Fig. 2

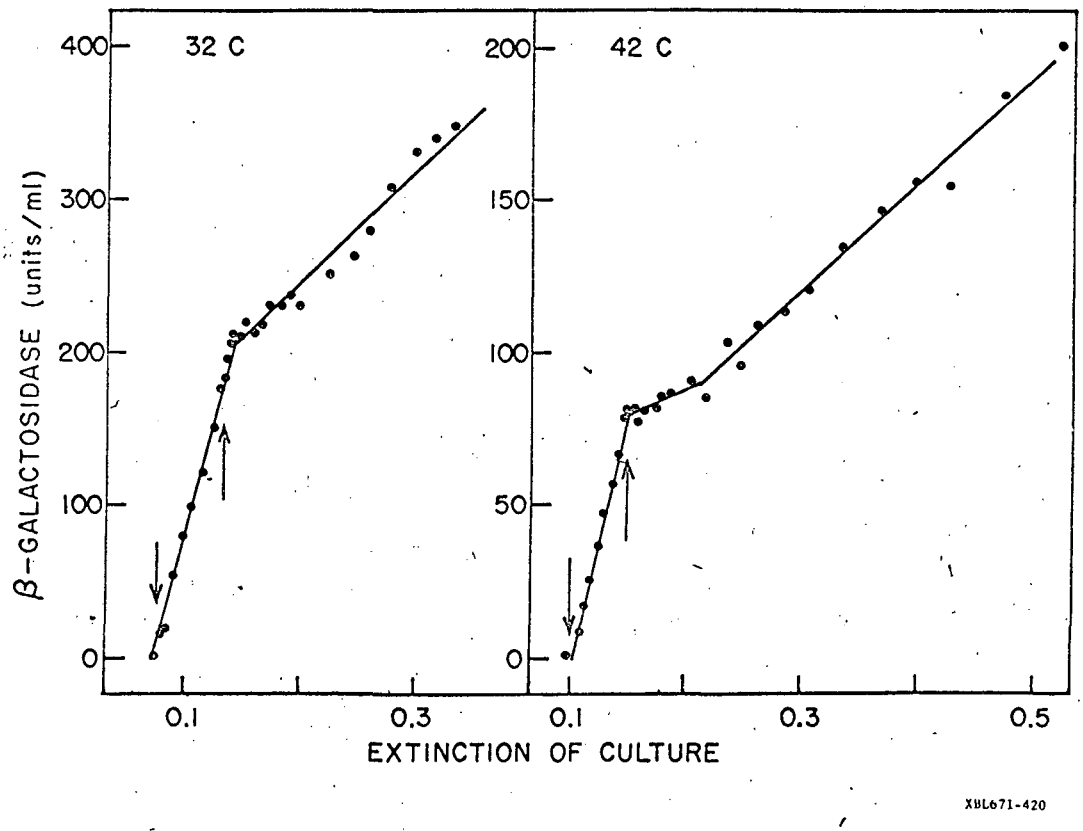
32 C
42 C
β-GALACTOSIDASE (units/ml)
400
300
200
100
0
200
150
100
50
0
0.1
0.3
0.1
0.3
0.5
EXTINCTION OF CULTURE
XBL671-420

Fig. 3

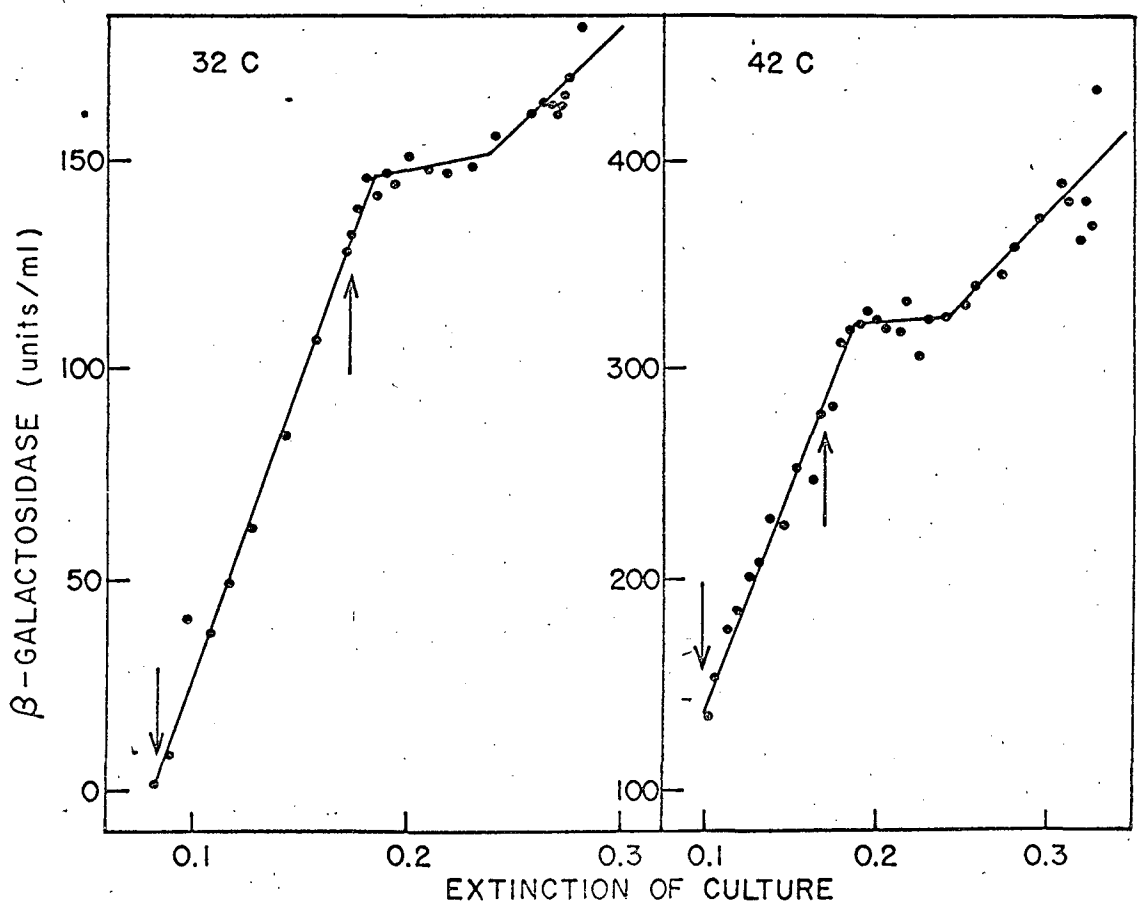

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Fig. 4

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