*BIOSYNTHESIS OF β-D-GALACTOSIDASE CONTROLLED BY PHAGE-CARRIED GENES. II. THE BEHAVIOR OF PHAGE-TRANSDUCED z^+ GENES TOWARD REGULATORY MECHANISMS**

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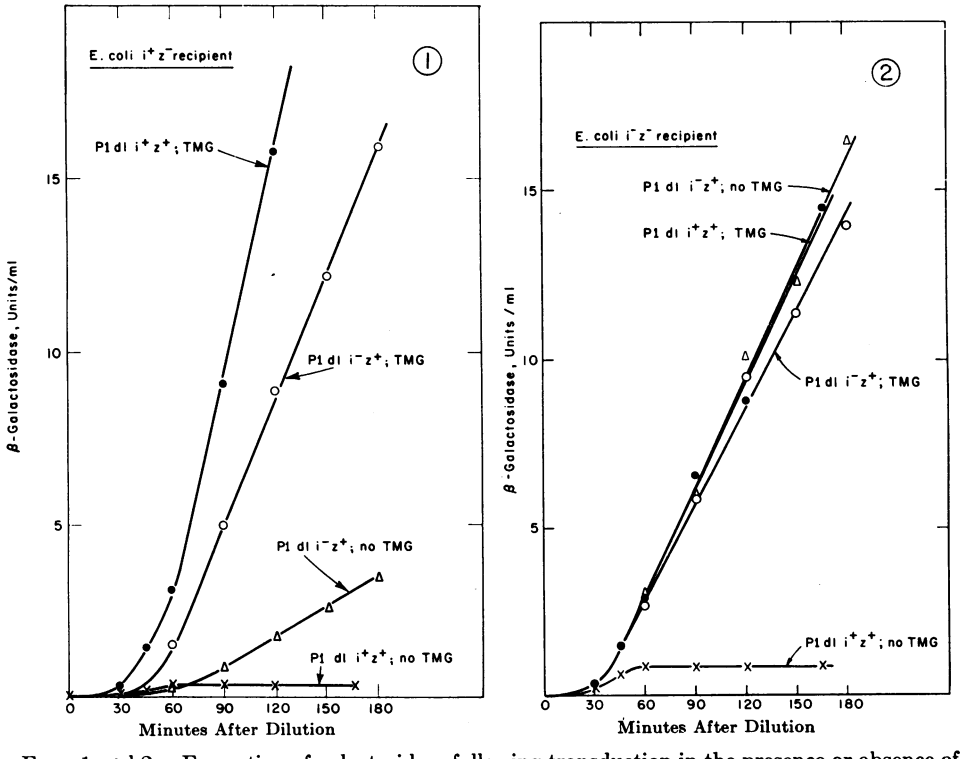
The preceding paper[1] described the kinetics of induced β-D-galactosidase production in z^- bacteria following transduction of the z^+ gene by the defective phage P1*dl*. It was concluded that, in the presence of an inducer, the newly entered z^+ genes begin to function shortly after having gained access to the recipient cells and continue to function at a constant rate, even though they do not multiply and are not integrated into the recipient cell chromosome. The present paper is concerned with the function of the *i* locus and with the functional interplay between the specific repressors for galactosidase and for phage P1 on the expression of newly entered z^+ genes. The results reveal some novel features of the control of gene function in bacterial cells.

Materials and methods were the same as in the preceding paper.[1]

Results.—Synthesis of galactosidase after transduction in the absence of inducer: Figures 1 and 2 show the results of infection of *E. coli* i^-z^- or i^+z^- with high fre-



FIGS. 1 and 2.—Formation of galactosidase following transduction in the presence or absence of an inducer. Bacteria, infected in TGA medium for 15 min, were diluted at time 0 in medium with or without 10^{-3} *M* TMG. P1*dlw* i^+z^+ and P1*dl* i^-z^+ were used as donor lysates.

quency transducing (= HFT) lysates containing P1*dl* particles that carry either i^-z^+ or i^+z^+ genes in the absence of an external inducer; results obtained in the presence of inducer[1] are included for purposes of comparison. The results can be summarized as follows:

1. When no i^+ gene is present, galactosidase synthesis proceeds as it would in the presence of an inducer.

2. When the i^+ gene is present in the phage only, synthesis of the enzyme begins as usual, then slows down and ceases at about 60 min. This finding is similar to that obtained in mating experiments[2] and, as in the case of mating, can be ascribed to a delayed expression of the i^+ gene in producing the specific repressor for the *lac* genes.

3. When the recipient is i^+z^- and the transducing phage carries i^-z^+, there is a constitutive synthesis of galactosidase at about 20 per cent the rate observed in the presence of inducer. This synthesis continues at a linear rate for at least 3 hours.

4. When both the recipient bacterium and the transducing phage carry the i^+ gene, there is an early constitutive enzyme production ceasing at about 60 min. If the amounts of enzyme produced in cases 2 and 4 are normalized to the corresponding rates of synthesis by these two systems in the presence of inducer, the value for case 4 is 20 to 25 per cent that for case 2.

The results of cases 3 and 4 are unexpected. According to current views, based mainly on the study of heterogenotes, in which the exogenote is part of a male chromosome or of a fertility factor,[3] one copy of the i^+ gene should be enough to saturate with repressor all the *lac* operons present in a cell. Instead, we observe that a z^+ gene in the phage is only incompletely repressed after entering an i^+ recipient, although it soon becomes completely repressed by the activity of an i^+ gene carried in the phage itself.

Similar results were obtained with other i^+ bacterial recipients, such as *S. dysenteriae* strain Sh, and with another type of HFT lysate containing a P1*dl* i^-z^+ phage of different origin and with different properties.

Before proceeding to further analysis of these results, we should point out that the experiment analogous to case 3, namely, the introduction of an i^-z^+ set of genes into an i^+z^- recipient, could not have been done in mating experiments,[2] because the enzyme in the donor cells would preclude detection of enzyme made by the zygotes (barring studies with labeled protein). Likewise, in a mating analogous to case 4, that is, the introduction of an i^+z^+ gene into an i^+z^- recipient, it would be difficult to detect the small amounts of enzyme made by the zygotes over the background level of enzyme in the uninduced i^+z^+ parent cells. In phage transduction, of course, there are no limitations of the sort.

The mechanism of constitutive galactosidase synthesis after transduction: Several trivial explanations of the constitutive synthesis observed in case 3 were tested and excluded by simple experiments. Thus, heterogeneity in the recipient z^- populations with respect to $i^+ \rightarrow i^-$ mutation was excluded by using as recipients several single colony isolates from an i^+z^- strain; all gave results like those of Figure 2. The possibility that the P1*dl* phages that were supposedly $i^-o^+z^+$ (P1*dl* type 13-4) had instead a *lac* region with the genetic composition $i^+o^cz^+$ or $i^-o^cz^+$ (dominant constitutive) was excluded, among other reasons, because the heterogenote strains with i^+z^- in their chromosome and a P1*dl* 13-4 prophage behaved as fully

or almost fully repressed (see below), whereas heterogenotes i^+z^- carrying P1*dl* $i^-o^cz^+$ behaved as typical o^c strains (constitutive synthesis 6–7 per cent of the induced level[3,4]).

Another interpretation, that the i^+ gene in the i^+z^- recipient cells may not be fully functional because the adjacent z portion of the *lac* region is inactive, was excluded by the observation that constitutive synthesis, as in case 3, was also observed when phage with i^-z^+ was used to infect i^+z^+ bacteria in the absence of inducer, as shown in Figure 3. This experiment also indicated that the escape of a

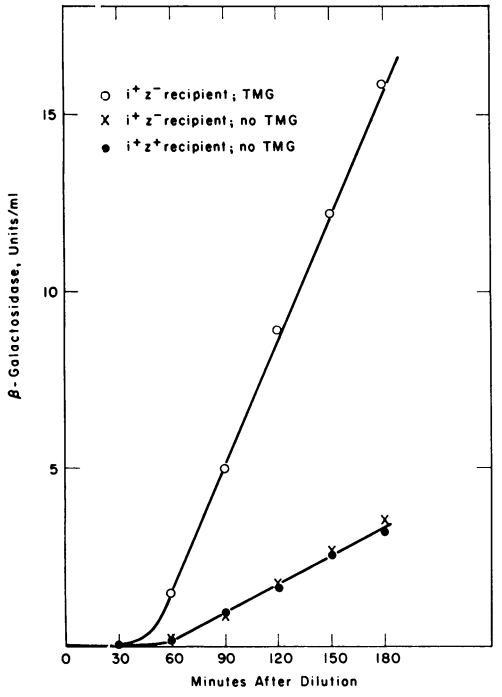


FIG. 3.—Formation of galactosidase following transduction of *E. coli* i^+z^- or i^+z^+ by phage P1*dl* i^-z^+. The values for the i^+z^+ recipient were corrected by subtracting the small amounts of β-galactosidase formed by a control culture without phage.

phage-carried gene z^+ from repression is probably not shared by a z^+ gene in the recipient, because the rate of constitutive synthesis was not higher than in the case of a z^- recipient.

In addition, it was easy to show that escape from repression was not due to phage infection *per se* since infection of i^+z^+ bacteria with normal phage P1 does not lead to constitutive galactosidase synthesis; nor does mixed infection of i^+z^- cells with P1*dl* i^-z^+ and normal P1 increase the level of constitutive synthesis.

The escape phenomenon is related to the *entry* of z^+ genes as part of a phage and not simply to their *location* within a phage element. In fact, the study of several strains of different genetic constitutions, listed in Table 1, showed that once a stable (or quasi-stable) lysogenic relation is established, repression of the z^+ gene in phage P1*dl* by an i^+ gene in the host chromosome, that is, in "trans" position, is quite effective, although not always as complete as repression by an i^+ gene in "cis" position.[5] Also, an i^+ gene in an established prophage represses a y^+ gene in the host chromosome and vice versa, as shown by permease measurement on strains $i^-z^-y^+$ (P1*dl* $i^+z^+y^-$) and $i^+z^+y^-$ (P1*dl* $i^-z^-y^+$).[4] The repression of z^+ or y^+

TABLE 1

EFFECTIVENESS OF REPRESSION BY I^+ IN VARIOUS LAC^+ STRAINS

Strain	Genotype: Chromosome	Genotype: P1*dl*	Enzyme units/O.D. units: (*a*) −TMG	Enzyme units/O.D. units: (*b*) +TMG	Ratio (*b*)/(*a*)
3.000	i^+z^+	...	1.4	1540	1100
3.300	i^-z^+	...	1500	1500	1
W4032-W-5	lac^{del}	i^+z^+	1.2	1700	1400
W4032-W-1	lac^{del}	i^+z^+	1.5	1800	1250
Sh I-4	i^+z^-	i^+z^+	1.0	900	900
Sh 31-20	i^+z^-	i^+z^+	1.6	2500	1600
3.0S0-13-4-1	i^+z^-	i^-z^+	1.4	800	600
3.0S0-13-4-2	i^+z^-	i^-z^+	17.3	875	50
Sh 125-13-4	i^+z^-	i^-z^+	3.9	900	250

The bacterial strains were grown in TGA medium in the presence or absence of 10^{-3} *M* TMG. Aliquots were removed at intervals for measurement of optical density and of galactosidase.

genes by an i^+ gene located in trans is analogous, of course, to the repression observed in similar situations between genes carried in two chromosomal fragments in mating and in F-duction heterogenotes.[2, 3]

It seemed possible that the z^+ gene in a newly entered phage escaped repression because of an insufficient cytoplasmic level of the product of the internal i^+ gene. If so, the z^+ gene might be brought under control by addition of glucose, which antagonizes external inducers, supposedly by increasing production of internal repressors.[6] The results shown in Table 2 indicate, however, that the escape syn-

TABLE 2

EFFECT OF GLUCOSE ON ENZYME SYNTHESIS

Carbon source in growth medium	Enzyme synthesis after transduction i^-z^+P1*dl* → i^+z^- cells: (1) Escape (−TMG)	(2) Total (+TMG)	(3) Induced (2-1)	Enzyme synthesis 3.300 i^-z^+ Constitutive (−TMG)	3.000 i^+z^+ Induced (+TMG)
	units per ml per hour			units per 1 unit O.D.	
Glycerol	0.85	3.7	2.85	1270	1050
Glucose	0.5	0.7	0.2	516	110
% Inhibition by glucose	40%	...	93%	59%	89%

thesis, like the constitutive synthesis in i^-z^+ bacteria, is reduced only to about one-half by glucose, whereas the TMG-induced synthesis is reduced 10- to 20-fold. Thus, if the escape phenomenon is due to an insufficient amount of repressor, this insufficiency is not remedied by glucose; it may be an insufficiency of aporepressor only. An insufficient amount of repressor might be a reflection of early multiplication of the i^+ gene copies introduced with phage. If so, when these gene copies become diluted in the course of cell division, one might expect escape synthesis of enzyme to cease. For at least 3 hours this was not observed.

Another explanation of the escape phenomenon would be an early interaction between the newly entered genes and the host genes, leading to inactivation of the host gene i^+ in some of the bacteria, presumably by recombination. Apart from the fact that most recipient cells probably have several nuclei, hence several i^+ copies, we have obtained no evidence to suggest such an early recombination between exogenote and endogenote. If integration of newly entered genes could occur almost immediately, some of the lac^+ transductants may be expected to start to multiply quite early; instead, the number of lac^+ transductant colonies remains constant for hours. In addition, several experiments were done in which HFT

lysates containing P1*dl* phage with the genes o^+z^- were used to infect either another o^+z^- or an o^0z^+ recipient. In these cases, recombination within the *o-z* region is required to give a functional z^+ locus, and the transduction frequencies are correspondingly lower than with o^+z^+ donors. If a substantial part of the recombinational events could occur very early after infection, some galactosidase activity should be found; none was observed (beyond the very low levels corresponding to the uninfected levels of the recipient cells in the cases of two leaky mutant strains, *E. coli* 3.320 and 3.0U0).

In summary, we conclude that the escape phenomenon is not due to failure or displacement of the i^+ gene of the host, but reflects a peculiar behavior of a *lac* region that has newly entered a bacterial cell as part of a phage.

Transduction to P1-lysogenic recipients: Another peculiarity of the behavior of phage-transduced z^+ genes toward repression is revealed by a comparison of P1-sensitive and P1-lysogenic recipient strains. The course of enzyme production in the presence of an inducer is qualitatively similar to both cases; but the rate of enzyme production is much lower with the lysogenic recipients (see ref. 1, Fig. 5). Table 3 gives values for several recipients and for a variety of phage

TABLE 3

SYNTHESIS OF GALACTOSIDASE AFTER TRANSDUCTION TO P1-SENSITIVE AND P1-LYSOGENIC RECIPIENTS

Recipient strain	Donor phage P1*dlw* i^+z^+ Enzyme, units/ml/hr	Ratio	P1*dl* i^+z^+ Enzyme, units/ml/hr	Ratio	P1*dl* $i^+o^cz^+$ Enzyme, units/ml/hr	Ratio
E. coli i^+z^-	5.2		4.5		0.6	
		8		*6.5*		*2.3*
E. coli i^+z^-(P1)	0.65		0.67		0.26	
E. coli i^-z^-	2.7		1.1			
		67		*5.5*		
E. coli i^-z^-(P1)	0.04		0.2			
Shigella i^+z^-	8.4		4.2		1.3	
		34		*3.8*		*7.2*
Shigella i^+z^-(P1)	0.25		1.1		0.18	

The values for enzyme correspond to the linear phase of synthesis in the presence of 10^{-3} *M* TMG. The values in italics are the ratios between the values for P1-sensitive and for P1-lysogenic recipient strains. The rates of P1 adsorption to lysogenic and sensitive recipients are identical.

types. The ratios between rates of synthesis in lysogenic vs sensitive cells range from 1:2.3 to 1:67. It is noteworthy that the repression by the P1 prophage is more effective with those types of P1*dl* phages, such as P1*dlw*, that are known to be themselves less effective in phage-immunity functions and less able to compete with a normal P1 prophage.[7]

Discussion.—Two main findings emerge from these results: the partial escape of newly transduced z^+ genes from repression by the i^+ genes of the recipient cells, and the inhibition by a P1 prophage of the expression of a z^+ gene newly entered as part of a phage.

The escape phenomenon indicates that repression by i^+, which is fully effective on a z^+ gene in cis position, is not always so in trans. Repression appears to work effectively in trans between genes in the chromosome and in an F′ factor;[3] it is almost as effective, although often incomplete, between chromosome and P1*dl* prophage.[5] Yet, internally produced repressor is only about 80 per cent effective in repressing z^+ genes that have recently entered with a phage. Individual-cell ex-

periments with fluorogenic substrate[1] will be needed to decide whether the escape is due to full enzyme production in 20 per cent of the cells or to enzyme production at 20 per cent the maximal rate in all the i^+z^- cells that have received an i^-z^+ phage.

It remains to be seen whether the escape phenomenon is peculiar to *lac*$^+$ genes that have entered with a phage or occurs with any "newcomer" genes. Experiments with F′ elements carrying i^-z^+ genes are in progress.

The lower level of galactosidase synthesis after transduction to recipient bacteria that are P1-lysogenic might reflect either a suppression of multiplication of the newly-entered phage element or an effect of the prophage, probably through the immunity repressor, on the function of the *lac*$^+$ genes. Arguments against extensive multiplication of the recently entered P1*dl* phage have been given.[1] Also, a phase of accelerated enzyme synthesis is still observed with P1-immune recipients (see ref. 1, Fig. 5) indicating that phage multiplication is probably not the explanation of the accelerated phase. It seems more likely that the effect of lysogeny on galactosidase production reflects a submission of the z^+ gene in P1*dl* to the restraining effect of the immunity repressor: the genes of the *lac* operon, being part of a (defective) P1 phage, become part of a "superoperon" controlled by the P1-immunity repressor. It is known that the functions of most phage genes are repressed by immunity.[3] The *lac* genes in the phage are apparently subject to this control; more or less so, depending on the complement of P1 genes with which they are associated in different P1*dl* types. In fact, specific phage genes are responsible, not only for production of the immunity repressor, but also for sensitivity to it (immunity-operator genes[3]).

It is remarkable that immunity should repress the function of z^+ genes in a newly entered P1*dl* phage and not in an established P1*dl* prophage of the same type (see Table 1). Clearly, with regard both to repression by the i^+ gene and to the effects of immunity, the recently entered genes behave differently from genes in stabilized components of the cellular genome. The differences may reflect either differences in intracellular topography or differences in status of the genetic elements with regard to multiplication. This question may be discussed more profitably after considering the experiments on derepression to be reported in the following paper.[5]

Summary.—Introduction of z^+ genes into i^+z^- bacteria by transduction, in the absence of an external inducer, results in a constitutive synthesis of β-D-galactosidase at about one-fifth the maximum induced rate. This "escape phenomenon" is attributed to a partial failure of phage-associated z^+ genes to respond to the chromosomally-controlled repressor when they first enter the recipient cell, but not when they are part of an established prophage. In addition, the z^+ gene in a newly entered phage element, but not in an established prophage, is subject to partial repression by the specific phage-immunity repressor.

The z^+ gene in the phage, however, is fully subject to repression by an i^+ gene in the same phage element.

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*BIOSYNTHESIS OF β-D-GALACTOSIDASE CONTROLLED BY PHAGE-CARRIED GENES. III: DEREPRESSION OF β-D-GALACTOSIDASE SYNTHESIS FOLLOWING INDUCTION OF PHAGE DEVELOPMENT IN LYSOGENIC BACTERIA**

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When *E. coli* bacteria carrying the prophage λ*dg*, which contains the *gal* loci for galactose utilization, are treated with doses of ultraviolet (UV) light sufficient to induce phage production in λ-lysogenic bacteria, the *gal* genes become derepressed.[1] That is, synthesis of galactokinase in the absence of an external inducer increases 20- to 30-fold over the constitutive level. A similar result has been reported for galactose-1-phosphate uridyl transferase.[2] Following UV induction, even *gal*$^+$ bacteria that carry a normal λ prophage show a marked constitutive synthesis of these enzymes,[1] as though the induction of prophage λ, which is attached in the immediate vicinity of the chromosomal *gal* genes,[3] caused derepression of these genes.

A similar derepression by z^+ genes in prophage P1*dl* was independently observed two years ago in our laboratory. In the course of the production of high-frequency-transducing (= HFT) lysates by UV treatment of heterogenote *E. coli* lac^{del} (P1*dl* $i^+ z^+$) it was noted that the crude lysates, which were prepared in media without external inducers, contained significant amounts of β-D-galactosidase, while the unirradiated bacteria had much less. A systematic study of this phenomenon was undertaken following the discovery of the escape phenomenon described in the preceding paper,[4] and the results are reported here.

Materials and methods were as described in a previous paper.[5] UV treatment was done on bacteria collected from growing cultures and resuspended in saline. After irradiation the bacteria were diluted in TGA medium.

Results.—Derepression following UV irradiation: Figure 1 illustrates the production of β-D-galactosidase and its release in extracellular form by a culture of *E. coli* $i^+ z^- y^+$ (P1*dl* $i^- z^+$) following UV treatment and P1-superinfection. Also shown is the production of transducing activity assayed in samples from the same culture. There is a close parallelism between release of transducing phage and of galactosidase; both are presumably freed by bacterial lysis. A delay occurs between synthesis and release of enzyme, so that the total amounts of enzyme are at first in excess over the cell-free enzyme. Ultimately, however, practically all the enzyme formed is released as free enzyme. This is true even with UV treatment alone without superinfection with P1, and indicates that all bacteria in which enzyme is produced as a response to UV are ultimately lysed.