Fusion of the *lac* Genes to the Proximal Promoters of the *deo* Operon of *Escherichia coli* K12

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Fusions of the *lac* structural genes to the proximal promoters *deoP* and *cytP* of the *deo* operon have been isolated using a λ (*lac*, Mu') phage. This phage was inserted into a heat-inducible Mu prophage which had itself been inserted into the *deoA* gene. Selection was made for heat-resistant derivatives of this lysogen which maximally expressed the *lac* genes when an inducer of the *deo* operon was present; these were shown to have the *lac* genes fused to the *deo* operon – in some cases with a concomitant deletion of the *deoC* gene, whilst in others *deoC* was retained. The level of β -galactosidase activity in these strains was induced by growth in the presence of deoxyadenosine or cytidine, both of which lead to induction of the *deo* enzymes – deoxyadenosine through the *deoOP* system and cytidine through the *cytOP* system. The level of induction of β -galactosidase was of the same order as the level of induction of deoxyriboaldolase, the *deoC* gene product, in those strains retaining an intact *deoC*⁺ gene. Plaque-forming λ phages carrying the *deo-lac* fusions were isolated by inducing the lysogens with mitomycin C.

INTRODUCTION

The expression of the four genes comprising the *deo* operon (*deoCABD*), which code for enzymes involved in the catabolism of nucleosides and deoxynucleosides (Fig. 1), is controlled by two repressor molecules, coded by the *deoR* and *cytR* genes (Ahmad & Pritchard, 1971; Munch-Petersen *et al.*, 1972). These molecules have been shown, both *in vivo* (Hammer-Jespersen & Munch-Petersen, 1975) and *in vitro* (Valentin-Hansen *et al.*, 1978), to negatively control the production of a messenger RNA from all four genes. They bind to two operator sites, *deoO* and *cytO*, located adjacent to one another, thus inhibiting initiations of transcription from two promoter sites, *deoP* and *cytP* (Hammer-Jespersen & Munch-Petersen, 1975). These two control regions are topologically different, since *deoOP* is cleaved by the R. *EcoRI* restriction enzyme whilst *cytOP* is left functionally intact (Jørgensen *et al.*, 1977). The order thus deduced is *deo(OP)*, *cyt(OP)*, *deoC*.

The two control regions are also functionally distinct, since the effectors for the CytR system are cytidine and adenosine (Hammer-Jespersen & Munch-Petersen, 1973; Munch-Petersen *et al.*, 1972; Valentin-Hansen *et al.*, 1978), whereas deoxyribose 5-phosphate is the inducer for the DeoR system (Breitman & Bradford, 1968; Svenningsen, 1975; Valentin-Hansen *et al.*, 1978). Initiations at *cytP* are also dependent upon the presence of the cyclic AMP–CRP complex (Hammer-Jespersen & Nygaard, 1976). *In vitro* experiments (P. Valentin-Hansen, K. Hammer-Jespersen & R. S. Buxton, unpublished results) have revealed that initiations of transcription at *cytP* start immediately, whereas there is a lag in initiation at *deoP*.

Superimposed upon this control system is another system controlling the two distal genes *deoB* and *deoD*; thus the *deo* operon is composed of two transcriptional units (Baumanis *et*

Fig. 1. Reactions catalysed by the deo enzymes. The enzymes are represented by the three-letter code used to designate their respective genes: *deoA* codes for thymidine phosphorylase, *deoB* for phosphodeoxyribomutase, *deoC* for deoxyriboaldolase and *deoD* for purine-nucleoside phosphorylase.

Fig. 2. Regulation of the *deo* operon. *DeoOP*, *cytOP* and OP3 designate the operator-promoter regions where the RNA polymerase initiates the transcription of mRNA1 and mRNA2, respectively. The transcription of mRNA1 from *deoOP* is regulated by the *deoR* repressor, the effector being deoxyribose 5-phosphate, whereas initiation at *cytOP* is regulated by the *cytR* repressor, the effector being either adenosine or cytidine. Initiation at *cytP* requires the cyclic AMP-CRP complex. The protein(s) involved in regulation of mRNA2, coding for *deoB* and *deoD*, has not been identified. *In vivo*, inosine and guanosine give rise to an induction of *deoB* and *deoD*. The *deoR* and *cytR* genes are not linked to the *deo* operon. P4 and P5 represent 'open', i.e. non-inducible, promoters.

al., 1974; Buxton, 1975) transcribed in an overlapping manner (Albrechtsen *et al.*, 1976; Buxton *et al.*, 1977) (Fig. 2). This short message, mRNA2, is not controlled by DeoR or CytR, but *in vivo* experiments have shown it to be inducible, the inducers apparently being inosine and guanosine (Buxton *et al.*, 1977). A third message is also probably transcribed from 'open', i.e. non-inducible, promoters in the *deoB* gene (Buxton, 1975; Buxton *et al.*, 1978).

No promoter mutations have yet been found. Although regulatory mutants, *deoR* and

Table 1. *Escherichia coli* and phage strains used

Strain	Genotype	Origin or reference
<i>E. coli</i> K12		
MC4100	F ⁻ <i>thi araD139</i> Δ (<i>lac</i>) <i>U169 strA</i>	M. J. Casadaban
RB548	MC4100, <i>upp</i>	5-FU ^R mutant of MC4100
RB549	RB548, <i>deoA</i> ::- <i>Mucts62</i>	(FU+AdR) ^R mutant of RB548
RB653	RB548, <i>deoA</i> ::- <i>Mucts62</i> :: λ p1(209)	λ p1(209) lysogen of RB549
S ϕ 863	F ⁻ <i>lac</i> (Am) <i>trp</i> (Am) <i>rpsL tyrT</i> ϕ 80 ^R (P2)	Buxton <i>et al.</i> (1977)
EC601.9	F ['] <i>ts lacI</i> ^S ::+ <i>Mucts62</i> /F ⁻ Δ (<i>lac</i>) <i>U169 melB</i> ? <i>glp</i> ? <i>thi</i>	M. J. Casadaban
EC601.14	EC601.9 with <i>lacI</i> ^S ::- <i>Mucts62</i>	M. J. Casadaban
RB138	F ⁻ <i>deoC thyA</i>	I. B. Holland
Phage		
Mu	<i>cts62</i>	M. Howe
λ p1(209)	<i>lacA</i> ? <i>YZO'</i> - Δ <i>W209-trp'AB'</i> ::+ Mu'	M. J. Casadaban
λ h80 <i>cdeI9</i>		M. E. Gottesman
λ b2 <i>c</i>		M. E. Gottesman
P1 <i>clm clr100</i>		J. Neuhaud

cytR, have been isolated (Ahmad & Pritchard, 1971; Munch-Petersen *et al.*, 1972), only one *deo* operator constitutive mutant has been obtained (Ahmad & Pritchard, 1973). Unfortunately, because of the method of selection employed, the strain also acquired a *deoB* mutation.

A procedure has therefore been sought to facilitate the isolation of operator constitutives, amongst other regulatory mutants, promoter mutants and super-repressor mutants, in order to study the inter-relationships between the regulatory proteins and their respective operator regions, and the interaction between RNA polymerase and the promoter sites of the *deo* operon. This has been done by fusing the regulatory regions of the *deo* operon with the structural genes of the lactose operon, so that the *lac* genes are transcribed from the *deo* promoters and are only maximally expressed in the presence of effectors for the *deo* operon. This opens up the possibility of using the many techniques for isolating mutations which alter the level of expression of the *lac* genes (Beckwith, 1970; Hopkins, 1974). This paper describes the fusion of the *lac* genes to the proximal promoters *deoP* and *cytP* of the *deo* operon, using the technique developed by Casadaban (1976).

METHODS

Bacterial and phage strains. The bacterial strains, all derivatives of *E. coli* K12, and phage strains used are listed in Table 1.

Media. Complex media used were tryptone broth (TB) and L-broth (LB) described previously (Buxton *et al.*, 1978). Minimal medium was M9 salts. Xgal minimal medium contained glucose (0.1 %, w/v) and 5-bromo-4-chloro-3-indolyl- β -D-galactoside [Xgal; Sigma; 20 μ g ml⁻¹; stock solution (8 mg ml⁻¹) dissolved in *N,N*-dimethylformamide]. When cleaved by β -galactosidase, Xgal yields a blue dye. Lactose-MacConkey agar was as described in the *Difco Manual*.

Mutagenesis with phage Mucts62 has been described previously (Buxton, 1975).

Selection of *upp* and *deoA* mutants has been described previously (Buxton *et al.*, 1977).

Selection of λ p1(209) lysogens was made as described by Casadaban (1976), using λ b2*c* and λ h80*cdeI9* phage.

Determination of phage Mu orientation was performed as described by Zeldis *et al.* (1973) using the F-prime strains EC601.9 and EC601.14.

Selection for *deo-lac* fusions. Strain RB653 was grown overnight at 30 °C in LB, centrifuged, washed and resuspended in phosphate buffer. Samples (0.1 ml) were plated on lactose minimal plates containing deoxyadenosine (1 mg ml⁻¹) and incubated overnight at 42 °C and then overnight at 37 °C.

P1 transduction was carried out using P1*clm clr100* as described by Rosner (1972).

Preparations of λ lysates. These were prepared from *deo-lac* fusion strains by adding mitomycin C (1 μ g ml⁻¹) to an exponentially growing culture of a lysogen in TB medium at 37 °C. After lysis of the bacteria, chloroform was added and the debris was removed by centrifugation; MgSO₄ (0.01 M) was then added.

λ lysates prepared by lytic growth starting with a phage preparation were made by infecting an expo-

nentially growing bacterial culture with phage at a multiplicity of infection of approximately 1.0 in the presence of mitomycin C ($1 \mu\text{g ml}^{-1}$), and the lysate produced was treated as above.

Strain RB548 (Δlac) was always used to grow λ phages carrying the *deo-lac* fusions.

Test of Spi⁻ phenotype of λ phages. This was performed by streaking lysates on the P2 lysogen S ϕ 863 as described previously (Buxton *et al.*, 1978).

Enzyme assays. Extracts were prepared from bacteria grown in M9 plus glycerol (0.2 %, w/v). These were grown at 37 °C to an absorbance of 1.0 at 436 nm, centrifuged, resuspended in 0.025 vol. Tris/EDTA buffer (0.1 M-Tris/HCl, pH 7.6, 2 mM-EDTA) and disrupted by sonication for 1 min at 2 °C. The debris was removed by centrifugation and the supernatant was used in assays.

For induction with nucleosides and deoxynucleosides, the bacteria were usually grown to an absorbance of 1.0 and then diluted to an absorbance of 0.25 into fresh medium containing the inducing compound (at 1 mg ml^{-1} , final concentration) and grown for approximately two generations (about 140 min) before harvesting.

β -Galactosidase (β -D-galactoside galactohydrolase; EC 3.2.1.23) was assayed essentially as described by Miller (1972), except that Tris/EDTA buffer was used instead of BB buffer. No difference in activity was detected between extracts prepared in these two different buffers. One unit of activity is defined as the amount of enzyme that will hydrolyse 1 nmol *o*-nitrophenyl- β -D-galactoside min^{-1} at 28 °C.

Assay mixtures for the determination of deoxyriboaldolase (2-deoxy-D-ribose-5-phosphate acetaldehyde-lyase; EC 4.1.2.4) contained: Tris/EDTA buffer (10 mM-Tris/HCl, pH 7.3, 2 mM-EDTA), 125 μl ; enzyme (5 to 30 units), 10 μl ; dehydrogenase mix, 100 μl . These were mixed at 4 °C, then transferred to 37 °C; after 2 min the reaction was initiated by adding 15 μl deoxyribose 5-phosphate (20 mM). Samples (50 μl) were taken at intervals into 400 μl 7 % (w/v) perchloric acid, and deoxyribose 5-phosphate was determined with diphenylamine (Burton, 1956). The dehydrogenase mix contained 5 μl alcohol dehydrogenase (30 mg ml^{-1}) and 2.5 mg NADH, made up to 1 ml with Tris/HCl (10 mM, pH 7.3), EDTA (2 mM). One unit of activity is defined as the amount of enzyme which will degrade 1 nmol substrate min^{-1} at 37 °C.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

RESULTS

Essentially the procedure devised by Casadaban (1976) for the isolation of gene fusions consists of transposing the *lac* genes near to the promoters of interest and then selecting deletions which join the *lac* genes to these promoters. With the *deo* operon, this has been done by infecting a *deoA* mutant, caused by insertion of bacteriophage Mu, with a λ phage containing the *lac* genes and a part of the Mu genome [$\lambda(\text{lac}, \text{Mu}')$]. Since the bacterial strain used lacks the chromosomal *lac* region, and because the λ phage is defective for normal λ integration, the genome of this λ phage frequently integrates by homologous recombination between the Mu DNA sequences. Because of the particular structure of this λ phage, this results in Mu lying between the *lac* genes and the *deo* promoters. These bacteria are still Lac⁻ because of a deletion of the *lac* promoter. Thus deletions of the Mu insertion should often result in the fusion of the *lac* genes to the *deo* promoters (Fig. 3).

Isolation of Mu-induced deoA mutants

Bacteriophage Mu was inserted into the *deoA* gene of a Δlac strain in order to provide a region of homology on the bacterial chromosome close to the *deo* promoters for the $\lambda(\text{lac}, \text{Mu}')$ phage. This was accomplished by lysogenizing strain RB548, a *upp* derivative of strain MC4100 (Δlac), with phage Mu cts62 and selecting for resistance to fluorouracil and deoxyadenosine; *deoA* mutants were distinguished from *deoD* mutants by the former's inability to grow on thymidine as carbon source, and by the latter's inability to use adenosine as carbon source [see Buxton *et al.* (1977) for an explanation of this selection]. Eighteen independent Mu-induced *deoA* mutants were isolated. These were Mu lysogens since they were immune to Mu, and they were heat-sensitive, because Mu cts62 is a heat-inducible mutant.

To establish that Mu was only inserted in the *deo* operon and not elsewhere on the bacterial chromosome, the mutants were transduced to *deoA*⁺ with P1 clm clr100 grown on a *deo*⁺ strain, by selecting for growth on thymidine as carbon source, and the transductants

Fig. 3. Isolation of *deo-lac* fusions and a λ phage carrying such a fusion. (a) Lysogenization of the *deoA::Mucts* mutant RB549 with the hybrid λ phage $\lambda p1(209)$ by homologous crossing-over within the Mu DNA leads to strain RB653. (b) Selection for deletions of *Mucts* produces fusions of *lac* genes to *deo* promoters, e.g. in strain RB666. Induction of this strain with mitomycin C leads to the isolation of a specialized transducing phage carrying the *deo-lac* fusion.

Not drawn to scale. Note that the amount of *trp* DNA retained in the fusion strain has not been determined. The arrows represent the direction of transcription.

were tested for their sensitivity to Mu. Of the 18 mutants, 12 could be transduced to Mu sensitivity in this way and were therefore presumed to have Mu inserted only in the *deo* operon.

Since Mu can insert itself into the bacterial chromosome in either of two orientations, it was desirable to determine this orientation in order to choose a suitable $\lambda(lac, Mu')$ so that the *lac* genes would be transcribed in the same orientation. Using $F' lac$ episomes containing Mu insertions of known orientations (Zeldis *et al.*, 1973), the orientation of Mu in the *deoA* mutants was determined. Of five mutants tested, one had Mu in a + orientation, one a - orientation, whilst the orientation was indeterminate in the other three.

Isolation of λ p1(209) lysogens

Since the *deo* and *lac* operons are transcribed in opposite orientations on the chromosome, it was necessary to choose a $\lambda(lac, Mu')$ phage with Mu' in the opposite orientation to the *deoA*-inserted Mu . Thus strain RB549 with Mu in the $-$ orientation was lysogenized with λ p1(209) having Mu' in the $+$ orientation. The $\lambda(lac, Mu')$ phage had lysogenized most probably by utilizing the homology provided by the Mu phage DNA, since *deoA*⁺ transductants of the lysogen obtained with phage P1 grown on a *deo*⁺ strain and selecting for growth on thymidine as carbon source, were sensitive to $\lambda b2c$.

*Selection for *deo-lac* fusions*

The presumed structure of the λ p1(209) lysogen of strain RB549 (*deoA*:: Mu) is shown in Fig. 3(a). The Mu prophage, carrying the heat-inducible mutation *cts62*, lies between the *deo* promoters controlling the *deoC* gene and the *lacZ* and *lacY* genes, which are not transcribed since the *lac* promoter is deleted in λ p1(209). In addition there is some *trp* DNA, since the W209 deletion of the *lac* promoter was isolated as a fusion which expressed the *lac* genes from the *trp* promoter. Thus there is no large block to transcription at the join between *trp* DNA, which replaces the *lac* promoter, and the *lac* genes. Deletions of Mu which fuse the *lac* genes to another promoter can therefore end anywhere within the *trp* DNA on the phage.

Since the Mu phage was heat-inducible, selection for such deletions was performed by plating the bacteria at 42 °C on lactose minimal plates containing deoxyadenosine (1 mg ml⁻¹); the latter, when degraded by the *deoD* and *deoB* gene products, gives rise to deoxyribose 5-phosphate, an inducer of the *deo* operon. This procedure was made possible because the *deoB* and *deoD* genes are still transcribed from the internal 'P3' promoter. Although the λ p1(209) lysogens can still utilize deoxyadenosine as carbon source, Lac⁺ mutants grew much faster than the background of Lac⁻, but deoxyadenosine-utilizing, bacteria.

Heat-resistant, Lac⁺ mutants isolated in this way arose at a frequency of approximately 3×10^{-9} . Lac⁺ colonies did appear on minimal lactose plates lacking deoxyadenosine, but only at a 10-fold lower frequency. It seemed likely, therefore, that the majority of these mutants were *deo-lac* fusions, and this was confirmed in the following way.

Heat-resistant derivatives were tested for their ability to ferment lactose on MacConkey agar in the presence or absence of deoxyadenosine (1 mg ml⁻¹). Isolates shown to be Lac⁺ only in the presence of deoxyadenosine were retained for further study. Confirmation was obtained on lactose minimal plates in the presence or absence of deoxyadenosine. On this basis six out of 44 Lac⁺ colonies (from two selections) had *lac* fused to the *deo* promoters, although more may have done so but were not identified as such. All six gave a blue colour on Xgal plates in the presence or absence of deoxyadenosine, presumably because they have a relatively high basal level of β -galactosidase (see later).

*Enzyme levels and induction patterns in the *deo-lac* fusions*

If the *lac* genes had been fused to the *deo* regulatory region, β -galactosidase should be induced by compounds which also induce the *deo* enzymes. β -Galactosidase and deoxyriboaldolase, the *deoC* gene product, were therefore assayed in extracts of cultures grown for two generations with deoxyadenosine (AdR) or cytidine (CR) at 1 mg ml⁻¹. The results (Table 2) show that the six mutants tested fell into a number of groups. In strains RB662, RB664 and RB666, deoxyriboaldolase was induced approximately 18- to 20-fold by AdR and 12- to 14-fold by CR. This compares with induction ratios of 31 with AdR and 12 with CR in the parental strain MC4100. The lower induction ratio of the mutants with AdR probably arises because AdR catabolism to deoxyribose 5-phosphate, the actual inducer, is

Table 2. Enzyme activities of deo-lac fusion strains under different inducing conditions

The strains were grown in M9 plus glycerol, as described in Methods, and induced for two generations as indicated (AdR, deoxyadenosine; CR, cytidine). Specific activities are expressed as units (mg protein)⁻¹. The figures in parentheses are the induction ratios. Strain MC4100 is the *deo*⁺ Δ *lac* parent of the *deo-lac* fusion strains RB661 to RB666. Strains RB661 and RB662 are from one mutant selection; the rest are from another selection.

Strain	Inducer present	Specific activities	
		Deoxyriboaldolase	β -Galactosidase
MC4100	None	9.9	
	AdR	309 (31.2)	ND
	CR	119 (12.0)	ND
RB661	None	12.3	5.7
	AdR	134 (10.9)	50 (8.8)
	CR	118 (9.6)	62 (10.9)
RB662	None	8.7	23.3
	AdR	175 (20.1)	299 (12.8)
	CR	120 (13.8)	347 (14.9)
RB663	None	ND	75.5
	AdR	ND	4291 (56.8)
	CR	ND	1493 (19.8)
RB664	None	8.1	22.4
	AdR	152 (18.8)	265 (11.8)
	CR	98 (12.1)	299 (13.3)
RB665	None	ND	4.8
	AdR	ND	295 (61.5)
	CR	ND	127 (26.5)
RB666	None	7.9	26
	AdR	147 (18.6)	338 (13.0)
	CR	114 (14.4)	372 (14.3)

ND, Not detectable.

curtailed, since transcription of *deoB* and *deoD* originates only from P3 when phage DNA is inserted in *deoA*. The induction ratio of β -galactosidase by CR (13 to 15) was similar to that of deoxyriboaldolase. The induction by AdR was much lower, however, being only 12- to 13-fold compared with 18- to 20-fold with deoxyriboaldolase. No direct inhibition of β -galactosidase activity by AdR could be detected when AdR was added directly to a bacterial extract, and AdR did not inhibit the induction of β -galactosidase when *lacZ* was transcribed from the *lac* promoters (unpublished data). It is possible that AdR or deoxyribose 5-phosphate could be directly inhibiting transcription between *deoC* and *lacZ*. The actual enzyme specific activities were similar in the three strains.

Strain RB661 was generally similar to the above strains, except that the induction ratios of the two enzymes were somewhat lower and the absolute level of β -galactosidase activity was approximately 24 % of that in the other three strains. It is possible that in this strain the fusion has resulted in the production of a transcription termination sequence or a translational termination resulting in the termination of transcription.

Strains RB663 and RB665 which failed to grow on AdR as carbon source had no detectable deoxyriboaldolase activity. Thus the deletion of the Mu phage had removed all or part of the *deoC* gene. In both strains, induction of β -galactosidase by CR was somewhat higher than that in strains RB662, RB664 and RB666 (20 to 26 compared with 12 to 14); induction by AdR was much higher, however (56 to 61 compared with approximately 12). This is most probably because in RB663 and RB665 deoxyribose-5-phosphate was not degraded, as the bacteria lack *deoC* activity, and hence a very high level of induction occurs. This also probably accounts for the high basal level of β -galactosidase in strain RB663. It therefore

seems to rule out deoxyribose 5-phosphate itself inhibiting transcription of *lacZ* in RB661 as mentioned previously. In strain RB663 the absolute β -galactosidase levels were 10 to 15 times higher than the corresponding levels in RB665. This may again be a property of the fusion generating a termination signal.

In all six strains, β -galactosidase activity was, therefore, induced by the two compounds known to induce the *deo* enzymes – one, deoxyribose 5-phosphate, through inactivation of the *deoR* repressor, and the other, cytidine, through inactivation of the *cytR* repressor.

Both prophage λ and the *lac* genes were shown to be linked to the *deo* operon in strain RB666, i.e. *deoA*⁺ transductants of RB666 obtained with P1 grown on a λ -*deo*⁺ strain were λ b2c sensitive and Lac[−] (on Xgal plates).

Isolation of λ phages carrying the deo-lac fusion

Prophage λ should not be affected by the deletions causing the fusion, so it should be possible to obtain λ transducing phages which carry the fusion.

Lysates of fusion strains RB661 and RB666 were prepared by mitomycin C induction (see Methods) and plated for single plaques on strain RB548 using TB plates containing Xgal. Both gave low titres indicative of the low level of homology (4×10^4 p.f.u. ml^{−1} for RB661 and 1×10^5 p.f.u. ml^{−1} for RB666). Approximately 20 % of the plaques from RB661 and 30 % from RB666 were blue. Both were equally blue on plates either with or without deoxyadenosine. As mentioned previously, this is probably because the basal level of β -galactosidase in the fusion strains is sufficient to give the blue colour on Xgal plates. Transcriptional read-through starting from the P_L promoter of λ could also be contributing to this activity in the absence of deoxyadenosine; this could be expected because of the structure of the phage (Fig. 3*b*). In both lysates the frequency of Spi[−] phages, which can form plaques on a phage P2 lysogen (Lindahl *et al.*, 1970), having lost the λ genes *red α* , *red β* , δ and γ from the left end of the prophage map (Zissler *et al.*, 1971), was approximately 1.9×10^{-2} . Two single blue plaques from each lysate were picked and purified: none were Spi[−]. Because of the orientation of the λ prophage it is presumed the λ (*deo-lac*) phages were of the λ bio⁺ type, i.e. the lost phage genes had been replaced by bacterial DNA adjacent to the right prophage end. When these plaques were grown up again, all of the plaques from the new lysates were blue. Thus the *deo-lac* fusion was stably carried by the λ phage.

The λ (*deo-lac*) phage from RB666 was shown to carry an intact *deoC*⁺ gene since it could transduce strain RB138 (*deoC*) to *deoC*⁺ by selecting for growth on thymidine as sole carbon source.

Strain RB548 (Δ *lac*) was lysogenized with λ (*deo-lac*) phage from strains RB661 and RB666. The lysogens were Lac⁺ (i.e. blue on Xgal plates) but segregated Lac[−] colonies very rapidly; an overnight LB culture picked from a colony on lactose minimal medium gave only about 5 % blue colonies, but when four of these from RB666 were tested, the Lac⁺ phenotype was retained more stably, there being only 5 to 17 % white Lac[−] colonies after overnight growth in LB.

The excision event that is assumed to have led to the formation of λ (*deo-lac*) is shown in Fig. 3(*b*).

DISCUSSION

The *deo-lac* fusions which have been isolated and described in this paper should enable use to be made of the very powerful techniques for regulatory mutant isolation developed for the *lac* operon, and the isolation and study of these mutants is in progress. The fusion also makes possible the study of the regulatory region of the *deo* operon independently of the *deo* gene products. The procedure developed by Casadaban (1976) has the advantage that any mutations generated can easily be incorporated into phage λ transducing particles, and can be used subsequently in DNA-directed *in vitro* protein-synthesizing systems and for DNA sequencing studies. Since the assay for β -galactosidase activity is so simple and sensitive, it

is easy to detect small differences in *deo* operon activity in *in vivo* experiments. In many of the *deo-lac* fusions isolated, the induction of β -galactosidase was of the same order as the induction of the *deoC* gene product, except for the case of induction by AdR already mentioned. This gives confidence that the fusion has led to the *lac* genes being expressed in a similar manner to the *deo* genes.

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