JOURNAL OF BACTERIOLOGY, Nov. 1978, p. 668-681
0021-9193/78/0136-0668$02.00/0

Vol. 136, No. 2
Printed in U.S.A.

Insertion of Bacteriophage Lambda into the *deo* Operon of *Escherichia coli* K-12 and Isolation of Plaque-Forming λ*deo*$^+$ Transducing Bacteriophages

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Received for publication 26 May 1978

A procedure has been devised to isolate plaque-forming λ*c*I857*S*7 transducing bacteriophage which carry the internal promoter, P3, of the *deo* operon of *Escherichia coli* and the *deoB* and *deoD* genes, while lacking the *deoP* and *cytP* promoters of the same operon, in order to study, specifically, regulation at the P3 site. This has been accomplished by selecting for the insertion of bacteriophage lambda into the *deoA* gene in a strain deleted for the normal lambda attachment site (Δ*att*λ) and isolating from this lysogen λ*spi*$^-$ and λEDTA^r phage. Among these, λp*deoB*$^+$*D*$^+$ phage were identified by their transducing abilities. From in vivo enzyme induction experiments performed on a Δ*deo* strain lysogenized with such phage, they were shown to carry the P3 promoter while lacking the *deoP* and *cytP* promoters. A λp*deoB*$^+$*D*$^+$ phage was used to lysogenize a *deo*$^+$ Δ*att*λ strain, integration of λ occurring within the region of homology, and, from a heat-induced lysate of this strain, a plaque-forming λ$^+$ phage carrying the complete *deo* operon was obtained. Phage lambda was also inserted into the *deoB* and *deoD* genes and into the *tdk* gene. By isolating λ*spi*$^-$ and λEDTA^r phage from the *deo*::(λ) mutants and determining which bacterial genes they carried and whether they retained the *int* gene of λ, it was found that λ had inserted into *deoD* with the same orientation as λ inserted into *att*λ, whereas λ inserted into *deoA* and *deoB* had the opposite orientation. Deletions extending from the site of λ insertion into the bacterial chromosome were isolated by selecting for heat-resistant revertants. These confirmed the order of markers to be *deo-serB-trpR-thr* and also placed a locus, *msp*, determining sensitivity or resistance of male strains to male-specific phages, between *trp*R and *thr*. For some reason unknown, but which may be related to the orientation of the λ prophages, short deletions rendering the bacterium Ser$^-$ Thr$^+$ were of much lower frequency from the *deo*D::(λ) lysogen than from the other two lysogens. From an examination of the residual *deoD* enzyme levels in *deoB*::(λ) mutants, it was deduced that there may be two promoter sites within the *deoB* gene, transcription from one of these being sufficient to account for the noncoordinate nature of the induction of *deoB* and *deoD* gene products.

The *deo* operon of *Escherichia coli*, which codes for four genes involved in nucleoside and deoxynucleoside catabolism (Fig. 1), namely, deoxyriboaldolase (*deoC*), thymidine phosphorylase (*deoA*), phosphodeoxyribomutase (*deoB*), and purine nucleoside phosphorylase (*deoD*) (1), is composed of two transcriptional units (6, 10), transcribed in an overlapping manner (3, 11) (Fig. 2). Thus, a long message, mRNA1, is transcribed from all four genes (41), while *deoB* and *deoD* are also transcribed into a short message, mRNA2, and there is some evidence for a third message transcribed only from *deoD* (10). The long message, mRNA1, has been shown, both in vivo (17) and in vitro (42), to be negatively controlled by two repressor proteins, the *cyt* repressor and *deo* repressor coded by the *cytR* and *deoR* genes, respectively (2, 31). Cytidine and adenosine act as inducers in the *cytR* system (16, 31, 42), whereas deoxyribose-5-phosphate is the inducer for the *deoR* system (9, 41, 42). mRNA2, in contrast, is not controlled by these proteins (11), but in vivo experiments have shown it to be inducible, the inducers apparently being inosine and guanosine (18). This "internal" control region, P3, therefore differs from the internal promoter in the *trp* operon (e.g., see 5, 30), not only because it is

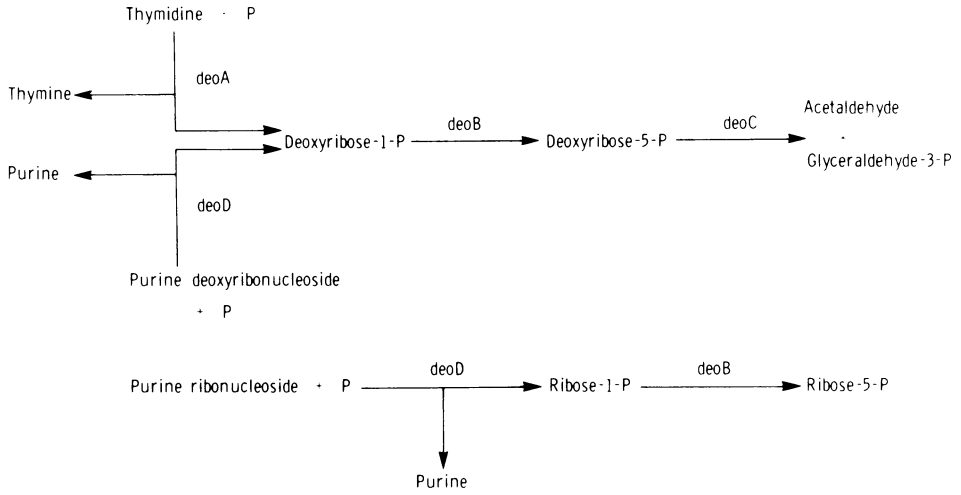

FIG. 1. *Reactions catalyzed by the Deo enzymes. The enzymes are represented by the three-letter code used to designate their respective genes. deoA, -B, -C, and -D code for thymidine phosphorylase, phosphodeoxyribomutase, deoxyriboaldolase, and purine nucleoside phosphorylase, respectively.*

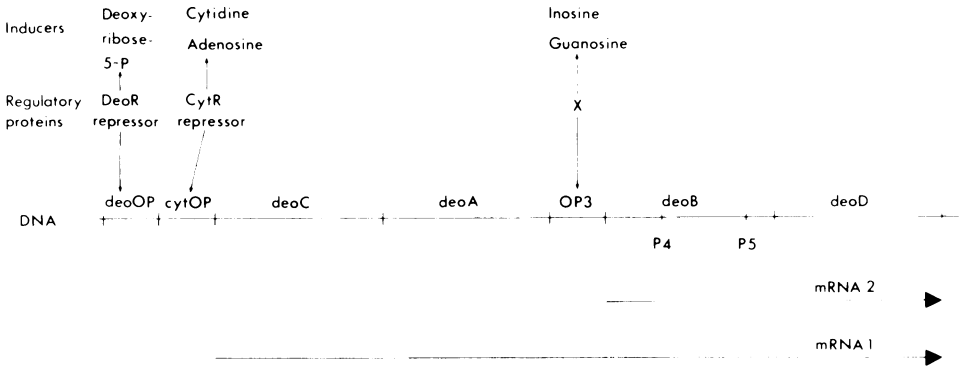

FIG. 2. *Model for regulation of the deo operon. deoOP, cytOP, and OP3 designate the operator-promoter regions where the RNA polymerase initiates the transcription of mRNA1 and mRNA2, respectively. The transcription of mRNA1 from the deoOP is regulated by the deo repressor, whereas initiation at the cytOP is regulated by both the cyt repressor and the cyclic AMP-CRP complex. Deoxyribose-5-P has been identified as effector for the deo repressor, whereas either cytidine or adenosine can act as effector for the cyt repressor. The protein(s) involved in regulation of mRNA2, coding only 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., noninducible) promoters (see text).*

inducible, but also because it is active enough to be of significance in vivo (11). The physiological meaning behind this is probably related to the fact that mRNA2 codes for the two enzymes involved in ribonucleoside catabolism. The synthesis of mRNA3 from *deoD* may be initiated at a weak "open" (i.e., noninducible) promoter similar to the internal *trp* promoter (10).

mRNA1 synthesis has been studied in vitro (41, 42), using as DNA template a λd*deo*⁺ phage (24). From this work, and from restriction endonuclease analysis of λd*deo*⁺ (24), it has been shown that synthesis of mRNA1 is initiated at two promoters, *deoP* and *cytP*. Because the synthesis of mRNA1 is greater than that of mRNA2, it is difficult to study the synthesis of the latter using λd*deo*⁺. We have therefore sought to isolate phage lambda transducing particles carrying OP3, *deoB*⁺, and *deoD*⁺, but lacking *deoP* and *cytP*, for use in an in vitro protein-synthesizing system (see 45) to study mRNA2 synthesis. We have used the method of Shimada et al. (39) to direct the integration of phage lambda into the *deo* operon by lysogenizing an *E. coli* strain deleted for the normal lambda attachment site (i.e., Δ*att*λ). From such stable secondary-site lysogens carrying the heat-inducible, lysis-defective lambda λ*c*I857*S*7, we have

isolated the plaque-forming *deo* transducing phages required for the in vitro experiments.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains, all derivatives of *E. coli* K-12, are listed in Table 1. The phage strains used were: λ*c*I857*S7* from G. Smith; λ*b2c*, λh80*cdel9*, and λ*c*I90*c*17 from M. E. Gottesman (39); and P1*clm clr*100 from J. Neuhard (34).

Media. Complex media were tryptone broth (36), supplemented with 10^{-2} M $MgSO_4$ and 0.2% (wt/vol) maltose (TBMM), and L-broth (36). Lambda top agar was tryptone broth plus 0.7% (wt/vol) Difco agar. Minimal selective plates were made with M9 salts (36). For enzyme assays, bacteria were grown in phosphate-buffered minimal medium containing, per liter: $(NH_4)_2SO_4$, 2 g; $Na_2HPO_4 \cdot 2H_2O$, 7.5 g; KH_2PO_4, 3 g; NaCl, 3 g; $MgCl_2 \cdot 6H_2O$, 0.4 g; $CaCl_2 \cdot 6H_2O$, 0.014 g; $FeCl_3$, 0.0008 g (29). Lambda buffer for the dilution of λ phage contained, per liter of distilled water: 1 M tris(hydroxymethyl)aminomethane (Tris; pH 7.2), 6.0 ml; $MgSO_4 \cdot 7H_2O$, 2.46 g; gelatin, 0.05 g.

Tests for resistance to fluorodeoxyuridine (FUdR) were performed on minimal agar plates containing 10 μg of FUdR and 30 μg of uridine per ml.

Growth of bacteria for λ infection and preparation of λ phage stocks. Growth of bacteria for λ infection and preparation of λ phage stocks were performed as described by Schrenk and Weisberg (36). The usual host for titering λ*c*I857*S7* was Sφ862, which carries *tyrT* necessary to suppress *S7*.

Isolation of λ lysogens. Overnight shaken cultures grown in TBMM at 32°C were starved by incubation in 10^{-2} M $MgSO_4$ for 45 min at 32°C, infected with λ*c*I857*S7* at a multiplicity of infection of about 10 for 15 min at 32°C, and then diluted 100-fold into 10 flasks, each containing fresh TBMM and grown overnight at 32°C with shaking. These cultures were also starved in 10^{-2} M $MgSO_4$. The frequency of λ lysogens was estimated by plating 0.1-ml samples on TBMM plates together with about 10^9 λ*b2c* and 10^9 λ*h80cdel9*. Two phages were used to reduce the possibility of isolating λ- or φ80-resistant mutants. In a typical experiment using a Δ*att*λ strain, the frequency of λ lysogens was 0.003.

To select for thymidine (TdR)-resistant λ lysogens from a Δ*att*λ *deoC* strain, the infected, starved cultures were plated on minimal agar containing glucose (0.4%, wt/vol) as carbon source and TdR (1 mg/ml), together with λ clear phage.

To select for λ lysogens resistant to fluorouracil (FU) and deoxyadenosine (AdR) from a Δ*att*λ *upp* strain, the infected, starved cultures were plated on minimal agar containing glucose as carbon source, plus FU (2.5 μg/ml) and AdR (150 μg/ml) together with λ non-lysogenizing phage. It was found that these selections were improved by using λ*b2c* and λ*h80cdel9* phage preparations which had been dialyzed against λ buffer.

Properties of λ lysogens. (i) Measurement of spontaneous and heat-pulse curing frequencies. These measurements were performed as described by Shimada et al. (39). Since the λ*c*I857*S7* phage was lysis defective in HfrH (*tyrT*$^+$), it was not necessary to isolate λ*vir*-resistant derivatives for the curing experiments.

(ii) Measurement of phage release. Measurement of phage release was carried out as described by Shimada et al. (39). Cultures were aerated by bubbling with air, although later it was found that higher titers were obtained from shaken cultures.

(iii) Determination of the number of prophages. The number of prophages was determined by cross-streaking exponentially growing TBMM cultures against 0.03 ml of λ*c*I90*c*17 phage at 3×10^8 plaque-forming units/ml on TBMM agar plates and incubating these for 24 h at 32°C, as described by Shimada et al. (39).

TABLE 1. *E. coli K-12 strains*[a]

Strain	Mating type	Genotype	Origin/reference
Sφ862	F$^-$	*lac*(Am) *trp*(Am) *rpsL tyrT* φ80^r	φ80^r mutant of M72 *tyrT* (G. Smith)
Sφ863	F$^-$	*lac*(Am) *trp*(Am) *rpsL tyrT* φ80^r (P2)	φ80^r mutant of M72 *tyrT* (P2) (G. Smith)
RB258	HfrH	*thi tyrT*$^+$ Δ*(gal-att*λ*-bio-uvrB-deoR)*	M. E. Gottesman (39)
RB595	HfrH	*thi tyrT*$^+$ Δ*(gal-att*λ*-bio-uvrB-deoR) upp*	5-FU^r mutant of RB258
RW599	HfrH	*thi galE tyrT*$^+$ Δ*(att*λ*-bio-uvrB)*	M. E. Gottesman (39)
Sφ1056	HfrH	*thi galE tyrT*$^+$ Δ*(att*λ*-bio-uvrB) deoC*	*deoC* derivative of RW599
RB522	F$^-$	*thi thr leu trp his arg(ECBH) upp rpsL*	5-FU^r mutant of RB49 (12)
RB446	HfrH	*thi galE tyrT*$^+$ Δ*(att*λ*-bio-uvrB) deoC* Δ*(deoB-deoD-serB)*	Heat-resistant derivative of RB425 (this paper)
RB609	F$^-$	*thi metB leu trp* Nal^r P1^r *deoA*::(Mucts62)	11
Sφ801	F$^-$	*thi thr leu deoB thyA*	C600 derivative
Sφ540	F$^-$	*thi leu* Δ*deo-11 rpsL*	C600 derivative, Δ*deo-11* from strain VS419 by conjugation (V. Sukhodolets; see 41)
RB391	HfrH	*thi upp deoD*	(FU+AdR)^r mutant of RB590 (11)
Sφ928	HfrH	*thi ton upp udp* Δ*lac* Δ*deo-11*	42
Sφ003	F$^-$	*metB1 rpsL relA1*(λ$^+$) λ^r	= Strain 58-161 (see 4)
LE284	HfrH	*thi tyrT* Δ*(pgl-att*λ*-bio) galT*::[λΔ*(int-G)*]	L. Enquist (14)

[a] The positions of some of the genetic markers referred to are shown in Fig. 3.

Selection of potential λ transducing phage. (i) λspi⁻ phage. A 0.1-ml amount of λ lysate was mixed with 0.1 ml of the P2 lysogen Sφ863, to give a ratio of bacteria to phage of at least 20:1. This avoids the killing of a large fraction of the P2 lysogens by wild-type λ phage. This was incubated for 10 min at 37°C, 2.5 ml of λ top agar was added, and the mixture was poured on a TBMM agar plate. Very small *spi*⁻ plaques could be seen after overnight incubation at 37°C. Single plaques were picked and streaked out with a very fine platinum loop on top-layer lawns, which were cooled to 4°C for about 30 min to allow the agar to set firmly. This purification was sometimes repeated for λ*spi*⁻ plaques in order to pick larger plaques. After treating the plates with chloroform vapor, single plaques were picked with a Pasteur pipette and used to make lysates.

(ii) EDTA-resistant plaques. The inactivation rate of λ phage at low ionic strength in the absence of Mg^{2+} ions is largely dependent on the DNA content of the lambda head, and deletion mutants of λ can be selected, therefore, by resistance to chelating agents (33). A λ phage lysate (approximately 10^5 to 10^6 plaque-forming units/ml; 0.02 ml) was added to 0.5 ml of 10 mM ethylenediaminetetraacetate (EDTA)–10 mM Tris-hydrochloride, pH 7.4, and incubated for 20 min at 37°C. One drop of 1 M $MgSO_4$ was added and then 0.1 ml of strain Sφ862. After 10 min of incubation at 37°C, 3.0 ml of λ top agar was added, and the whole mixture was poured on a TBMM plate and incubated overnight at 37°C. EDTA-resistant plaques were of normal λ plaque size. They were streaked out to give single plaques at least once to purify them. To test EDTA resistance, lysates were first diluted 100-fold into phosphate buffer, and then 0.02 ml was added to 0.5 ml of 10 mM EDTA–10 mM Tris.

Detection of *int* function of λ. Detection of the *int* function of λ was performed on strain LE284, using the red plaque test exactly as described by Enquist and Weisberg (14).

Detection of residual phage markers. Detection of residual phage markers in a prophage deletion mutant was performed as described by Shimada et al. (39).

Genetic crosses. (i) Transduction with P1*clm clr*100. Transduction with P1*clm clr*100 was carried out as described by Rosner (34).

(ii) Transduction with phage λ. A sample of λ lysate (titer of 10^7 to 10^9 plaque-forming units/ml; see Table 5), usually 0.2 or 0.4 ml, was added to 0.1 ml of starved cells and incubated for 20 min at 32°C, and 0.5 ml of phosphate buffer was added. The cells were sedimented by centrifugation at room temperature, resuspended in phosphate buffer, plated out, and incubated at 32°C.

(iii) Bacterial matings. Bacterial matings were performed as described by Miller (28).

Enzyme assays. Preparation of dialyzed cell extracts as well as procedures for most of the enzyme assays have been described (18). Thymidine kinase was assayed essentially as described by Voytek et al. (43). Cells growing exponentially in minimal medium containing glycerol (0.2%, wt/vol) and Casamino Acids (0.2%, wt/vol) were harvested by centrifugation, resuspended in 100 mM Tris-hydrochloride (pH 7.8), containing 2 mM EDTA, and used to prepare sonic extracts. These were centrifuged, and the supernatants (containing approximately 6 mg of protein per ml) were dialyzed overnight against 50 mM Tris-hydrochloride, pH 7.8, containing 0.5 mM EDTA and 9 ml mercaptoethanol. The assay mixtures contained, in a final volume of 0.135 ml: 100 mM Tris-hydrochloride, pH 7.8; 5 mM mercaptoethanol; 0.3 mM EDTA; 6.7 mM ATP; 3.3 mM $MgCl_2$; 0.82 mM [2-^{14}C]TdR (1 μCi/μmol; Radiochemical Centre, Amersham, England); dialyzed cell extract, 0.075 ml. The assay was performed at 37°C. At 3, 30, and 90 min, 0.03-ml samples were removed and mixed with 0.005 ml of marker mixture (5 mM TdR plus 5 mM thymidine monophosphate) and applied to polyethyleneimine plates. After drying, the plates were developed in water. TdR and thymine run at the solvent front whereas the TdR nucleotides stay bound at the origin. The amount of radioactivity in TdR nucleotides remaining at the origin was counted in a Packard Tri-Carb liquid scintillation spectrometer.

One enzyme unit is defined as the amount of enzyme which at 37°C degrades 1 nmol of substrate per min. Protein determination was performed by the method of Lowry et al. (27), with bovine serum albumin as the standard.

The enzymes assayed were: purine nucleoside-phosphorylase or purine nucleoside:orthophosphate (deoxy) ribosyltransferase (EC 2.4.2.1); uridine phosphorylase or uridine:orthophosphate ribosyltransferase (EC 2.4.2.3); thymidine phosphorylase or thymidine:orthophosphate deoxyribosyltransferase (EC 2.4.2.4); thymidine kinase or ATP:thymidine 5′-phosphotransferase (EC 2.7.1.75); phosphodeoxyribomutase (EC 2.7.5.6); deoxyriboaldolase or 2-deoxy-D-ribose-5-phosphate acetaldehyde-lyase (EC 4.1.2.4).

RESULTS

Selections for insertion of λ into *deo*. Shimada et al. (39) have shown that stable secondary-site λ lysogens can be isolated if an *E. coli* strain deleted for the normal lambda attachment site (i.e., Δ*att*λ) is infected with λ. We have used this procedure to isolate such lysogens, and among these we have identified those with λ inserted into the *deo* operon by performing two types of selection: (i) mutants resistant to FU and AdR were selected from secondary-site λ lysogens of a *upp* derivative of HfrH Δ*att*λ (RB595). This selection (see 11) should give at least three types of mutants, namely, *deoA*, *deoD*, and *tdk* (thymidine kinase; this is not linked to *deo*).

(ii) Mutants resistant to TdR were selected among λ lysogens of a *deoC* derivative of HfrH Δ*att*λ (Sφ1056). *deoC* mutants are sensitive to TdR because the catabolism of this compound leads to the accumulation of deoxyribose-5-phosphate, which is toxic to the cell. TdR-resistant mutants of *deoC* strains have been shown to be either *deoB* or *deoA*, since in these mutants breakdown of TdR to deoxyribose-5-phosphate

is blocked (7). (Polar *deoC* mutations would also be phenotypically DeoA; 3, 6, 10.)

The results of these selections are shown in Table 2. Selection for (FU + AdR) resistance unfortunately gave only eight strains which had phenotypes indicating that they were Deo^-, i.e., TdR^- AR^+ (*deoA*) or TdR^+ AR^- (*deoD*), and of these only one (*deoD*) was shown by heat-pulse curing experiments to be caused by λ insertion; many had λ inserted apparently into *tdk* (i.e., they were TdR^+ AR^+ and were resistant to FUdR [10 μg/ml]).

Selection for TdR resistance in a *deoC* mutant produced 13 AR^+ (presumably *deoA* or polar *deoC*) and 12 AR^- (presumably *deoB*) mutants caused by the insertion of phage λ (Table 2).

Properties of the *deo*::(λ) and *tdk*::(λ) mutants. Enzyme assays were performed on the λ lysogens to determine if the mutant designations given to them on the basis of phenotypic classification were correct. The results are shown in Table 3. (The symbol :: indicates that the λ prophage given in parentheses is inserted in the gene preceding the symbol [20].)

Mutants in class I (TdR^- AR^+) all had low levels of thymidine phosphorylase (*deoA* gene product) when compared with their parent, strain Sφ1056. The *deo* enzyme levels in Sφ1056 are raised when compared with another strain, Sφ003 (Table 3); this is probably due to internal induction by deoxyribose-5-phosphate, since degradation of this compound is blocked by a *deoC* mutation. The low thymidine phosphorylase levels found in the class I mutants are consistent with λ being inserted into the *deoC* or *deoA* gene. The residual low thymidine phosphorylase activity of 3 U/mg of protein found in some of the mutants is probably due to uridine phosphorylase (8, 10, 25). Two explanations are offered for the higher thymidine phosphorylase activity (24 U/mg of protein) in strains RB414 and RB428: (i) λ could be inserted in the distal end of the *deoA* gene so that a partially active *deoA* gene product is still transcribed from the *cytP* and *deoP* promoters. (ii) Alternatively, λ could be inserted into the *deoC* gene, and the residual *deoA* gene product could be due to transcription from a low-efficiency promoter in the distal end of *deoC* or from a λ promoter. Shimada and Campbell (38) have localized a λ promoter, pI, within the *xis* gene, giving rise to synthesis of the *int* and *trpB* gene products. If the *deoA* gene product formed in RB414 and RB428 originates from the λpI promoter, then in these strains λ must have been inserted into the *deoC* gene in an opposite orientation to that in the normal *att*λ site. It is of interest that the frequency of heat-pulse curing of λ in strains RB419 and RN428 was markedly lower than in other class I mutants; this may be a reflection of the different position of the λ insertion in these mutants.

Assays on class II mutants (Td^{r-} AR^-) demonstrated that they had all lost phosphodeoxyribomutase (*deoB* gene product) activity. Although the parental strain Sφ1056 has a raised level of *deoA* gene product, class II mutants have wild-type *deoA* enzyme levels. This is indicative that *deoB* gene product is missing also in vivo (a *deoB* mutation prohibits formation of deoxyribose-5-phosphate and thus prevents the internal induction). These results strongly indicate that λ is inserted into the *deoB* gene in all class II mutants.

TABLE 2. *Selections for the insertion of λ into deo and tdk*[a]

Parental strain	Selection	Phenotype of mutants	Presumed genotype	No. of λ lysogens isolated	No. of strains where mutation was caused by λ insertion
Δ*att*λ *upp*	$(FU+AdR)^r$	$FUdR^s$ TdR^- AR^+	*deoA*	2	0
		$FUdR^s$ TdR^+ AR^-	*deoD*	6	1
		$FUdR^r$ TdR^+ AR^+	*tdk*	97	15/44[b]
Δ*att*λ *deoC*	TdR^r	AR^+	polar *deoC* or *deoA*	14	13
		AR^-	*deoB*	54	12

[a] Strains RB595 (Δ*att*λ *upp*) and Sφ1056 (Δ*att*λ *deoC*) were each infected with λ*c*I857*S*7, and either $(FU+AdR)^r$ or TdR^r mutants were isolated from among the λ lysogens, as described in the text. After purification by single-colony isolation, these mutants were tested for their ability to grow on TdR or adenosine (AR) as carbon source. To test whether the mutation was caused by the insertion of λ, heat-pulsed cured strains which could grow at 42°C were isolated and tested for restoration of the parental phenotype. In both types of selection, the mutants were derived from 10 cultures obtained by diluting the bacteria infected with λ*c*I857*S*7, so many of the mutants will be of independent origin.

[b] Only 44 of the mutants were tested.

TABLE 3. *Properties of the secondary-site λ lysogens*[a]

Class	λ inserted into:	Parent	Selection used	Strain no.	Frequency of:		λcI90c17 test	Burst size	Utilization as carbon sources		Uninduced enzyme activity (U/mg of protein)			
					Spontaneous curing	Heat-pulse curing			TdR	AR	*deoA*	*deoB*	*deoD*	*tdk*
I	*deoC* or *deoA*	Sφ1056 [Δ(*attλ*) *deoC*]	TdR^r	RB401	$<10^{-8}$	1.5×10^{-4}	Resistant	359	−	+	3			
				RB414	$<10^{-8}$	7.0×10^{-7}	Resistant	192	−	+	23		138	
				RB417	1.5×10^{-7}	5.4×10^{-4}	Sensitive	0.094	−	+	3			
				RB418	1.3×10^{-8}	1.5×10^{-4}		126	−	+				
				RB421	6.1×10^{-8}	2.5×10^{-4}	Sensitive	0.031	−	+	3			
				RB428	6.5×10^{-8}	2.4×10^{-6}	Resistant	264	−	+	24			
II	*deoB*	Sφ1056 [Δ(*attλ*) *deoC*]	TdR^r	RB407	$<10^{-8}$	8.0×10^{-5}	Sensitive	0.019	−	−	60	<1	39	
				RB411	1.4×10^{-8}	2.2×10^{-5}	Resistant	302	−	−	54	<1	400	
				RB413	5.4×10^{-7}	2.1×10^{-5}	Sensitive	0.0096	−	−	60	<1	379	
				RB420	1.3×10^{-7}	3.3×10^{-5}	Resistant	251	−	−	78	<1	28	
				RB425	9.3×10^{-8}	5.7×10^{-5}		0.0067	−	−				
				RB427	1.5×10^{-7}	2.2×10^{-5}	Sensitive	0.011	−	−	64	<1	278	
III	*deoD*	RB595 [Δ(*attλ*) *upp*]	(FU+AdR)^r	RB400	4.4×10^{-7}	1.5×10^{-5}		0.00058	+	−			<20	
IV	*tdk*	RB595 [Δ(*attλ*) *upp*]	(FU+AdR)^r	RB429	4.2×10^{-7}			>200	+	+	3,390			0.02
				RB432	1.0×10^{-5}			>200	+	+	3,012			0.03
				RB435				190	+	+	3,320			0.03
				RB436	4.2×10^{-8}	2.2×10^{-5}		0.0031	+	+	3,580		1,260	0.06
				RB440	2.5×10^{-7}	1.1×10^{-6}		0.0019	+	+	3,220		869	0.06
Control strains				Sφ1056					+	+	470	182	203	
				RB595					+	+	3,200		1,450	2.0
				Sφ003					+	+	60	80	160	2.5

[a] Heat-induced λ lysates were made from the lysogens and selections were made for λ*spi*$^-$ and EDTA^r phage, as described in the text.

Strain RB400 was the only representative of class III (TdR^+ AR^-), and enzyme assays revealed it to be a *deoD* mutant. The class IV mutants ($FUdR^r$ TdR^+ AR^+), as well as RB400, were derived from RB595, which has a *deoR* mutation, and thus had constitutive amounts of the *deo* enzymes. Enzyme analyses verified that thymidine kinase was affected in these strains and not the *deo* enzymes. Additionally, class IV mutants showed no incorporation of ^{14}C-labeled TdR into trichloroacetic acid-precipitable material, consistent with their having very low levels of thymidine kinase activity (unpublished data).

The frequency of spontaneous curing of λ and of heat-pulse curing were estimated, together with the burst sizes of the secondary lysogens (see Materials and Methods). As reported by Shimada et al. (39), the heat-pulse curing frequencies were from 10 to 10^3 times higher than the spontaneous curing frequencies, which were usually of the order of 10^{-7} to 10^{-8}, one (RB432) being 10^{-5} (Table 3).

From the results of Shimada et al. (39), we assumed that the lysogens giving a low burst size (i.e., few free phage in the culture) were single lysogens, reflecting a structural excision defect, and multiple lysogens were those producing a high burst of phage through excision by formation of cohesive ends. This was confirmed for the three lysogens chosen for further study, RB400 (*deoD*), RB417 (*deoA* or polar *deoC*), and RB427 (*deoB*), since these strains were sensitive to phage λ*c*I90*c*17, whereas a known multiple lysogen was resistant and a known single lysogen was sensitive. It is known that multiple lysogens are immune to this phage, whereas single lysogens are sensitive (39, 40).

The position on the chromosome of the λ insertions chosen for further study was tested by screening heat-resistant bacteria for the acquisition of auxotrophy, i.e., where the deletion extends from the prophage into the adjacent bacterial genes. Strains RB400, RB417, and RB427 each gave rise to serine-requiring mutants, presumably *serB*, and also to threonine-requiring mutants, and we therefore have confidence that λ in these strains is inserted into the *deo* operon at 99 min (4).

No auxotrophs were isolated from the heat-resistant derivatives of *tdk*::(λ) strains. To map the *tdk* mutation in one of these strains, RB440, it was necessary to isolate a heat-resistant derivative (RB532) lacking all or part of the λ prophage, since otherwise zygotic induction would occur if this strain was used as a donor. Phage P1 was grown on RB532 and used to transduce a *trp upp* strain (RB422) to Trp^+. Three out of 21 Trp^+ transductants tested were resistant to

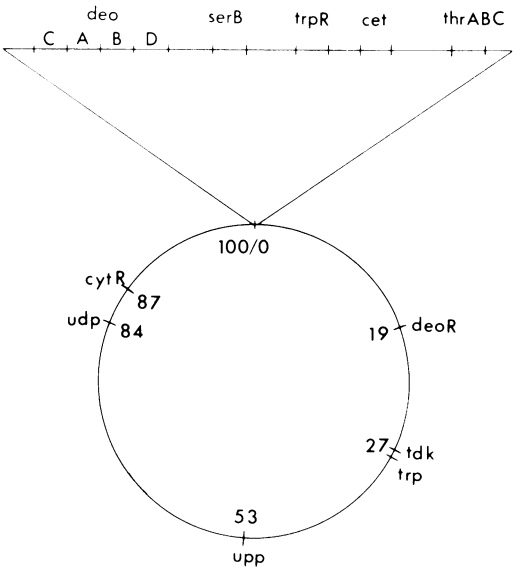

FIG. 3. *Diagram of the genetic map of E. coli showing some of the genes mentioned in the text. The part covering the deo region is shown expanded. Not to scale. Numbers refer to minutes (see 4).*

FUdR and were thus Tdk. This is approximately 14% cotransduction, comparable to the 27% cotransduction obtained by Igarashi et al. (21). In a similar type of experiment with a heat-resistant derivative of strain RB432 (RB521), this was used as an Hfr donor in a cross with the F^- *trp upp* strain RB522, selecting for Trp^+ Str^r recombinants; 8 out of 12 of these were FUdR resistant. These results therefore indicate close linkage between FUdR resistance and *trp* (at min 27), compatible with the known position of *tdk*.

Isolation and properties of plaque-forming λ*deo*$^+$ transducing phages. Single lysogens, one having λ inserted into *deoD* (RB400), one into *deoB* (RB427), and one into *deoA* or *deoC* (RB417), were chosen for the isolation of transducing phages. λ*spi*$^-$ and $\lambda EDTA^r$ phages were isolated as described in Materials and Methods. λ*spi*$^-$ variants, which can form plaques on a phage P2 lysogen, have lost the phage genes *redα redβ*, δ, and γ from the left end of the prophage map (44). When these are isolated from lysates prepared by induction of a lysogen, the lost phage genes have usually been replaced by bacterial DNA adjacent to the right prophage end, i.e., λ*bio*$^+$ type (26). $\lambda EDTA^r$ mutants, in contrast, have usually, but not always, lost phage genes from the right end of the prophage map, replacing them with bacterial DNA adjacent to the left end, i.e., λ*gal*$^+$ type (M. E. Gottesman, personal communication).

The frequencies of λ*spi*$^-$ and $\lambda EDTA^r$ phages are shown in Table 4. The frequencies of both

types of phage were higher than from a normal λ lysogen, probably indicative of the higher frequency of aberrant excision. Single plaques were picked and streaked out, and lysates were made from single plaques.

These lysates were then used to test for the transduction of nearby genes, to determine the orientation of λ in the original lysogens. Many of the lysates failed to give rise to any transductants, but this may be because the titers were too low. In Table 5 are listed the results with λ phages having higher titers. Since λ*spi*⁻ are transducing phages of the λ*bio*⁺ type and λEDTA^r phages are normally transducing phages of the possible orientation (λ*gal*⁺ type), this should determine the orientation of the λ prophage in the original lysogen as well as the plaque-forming transducing phages. The λ*gal*⁺ type of the λEDTA^r phages was shown by the presence of the *int*⁺ gene on indicator plates (see Materials and Methods). It can be seen that all of the λEDTA^r phages were *int*⁺ except for BP48; all of the λ*spi*⁻ phages, as expected, were *int*⁻.

From these results, the orientation of the three λ prophages in the *deo* operon are shown in Fig. 4. The λ inserted into the *deoD* gene had the same orientation as λ when present in the normal attachment site; the other two λ prophages were inserted with the opposite orientation. Confirmation of the orientation of the λ prophage in strain RB427 (*deoB*) was obtained from a heat-resistant revertant which had a deletion extending into *serB* and which had lost the λ genes *A*, *Q*, *O*, and *N*, but retained the λ *J* gene.

λ*deo*⁺ transducing phages from a λRB400 lysate were also selected directly on TdR (e.g., KT21 in Table 5). One out of three of these λ*deoC*⁺*A*⁺*B*⁺ were found to be plaque forming. Thus, with genes which are very close to the site of insertion of the prophage, it is probably not necessary to select for EDTA^r or *spi*⁻ phages, because many of the transducing phages will be plaque formers.

Properties of Δ*deo* strains lysogenized with λp*deoB*⁺*D*⁺. For the in vitro experiments

TABLE 4. *Frequency of λspi⁻ and λEDTA^r phage from deo::(λ) lysogens*[a]

Lysate	λ inserted into:	Frequency of:	
		λ*spi*⁻	λEDTA^r
λRB400	*deoD*	3.7×10^{-2}	3.8×10^{-3}
λRB417	*deoA*	3.4×10^{-5}	1.2×10^{-3}
λRB427	*deoB*	1.2×10^{-3}	4.3×10^{-3}
λcI857S7	*attλ*	1.0×10^{-6}	1.7×10^{-4}

[a] Heat-induced λ lysates were made from the lysogens and selections were made for λ*spi*⁻ and λEDTA^r phage, as described in the text.

TABLE 5. *Transducing ability of λspi⁻ and λEDTA^r phage derived from deo::(λ) lysogens*[a]

Phage strain	Isolated from strain with λ inserted into:	Selected as:	Titer (PFU/ml)	EDTA^r frequency	*int* genotype	Transduction (transductants/ml of lysate) of:				
						deo⁺	*deoA*⁺	*deoB*⁺	*deoD*⁺	*serB*⁺
BP45	*deoA*	EDTA^r	7.9×10^9	1.5×10^{-1}	+		2.1×10^3	0	0	0
BP46	*deoA*		5.8×10^9	1.0×10^{-1}	+		1.5×10^5	$>5 \times 10^6$	7.1×10^5	0
BP47	*deoA*		4.2×10^9	6.4×10^{-2}	+		2.6×10^5	$>5 \times 10^6$	9.4×10^5	0
BP48	*deoA*		6.9×10^9	3.0×10^{-1}	−		0	0	0	0
BP49	*deoB*	*spi*⁻	2.5×10^7	$<2.0 \times 10^{-3}$	−		2.8×10^2		0	
BP50	*deoB*		1.7×10^9	2.7×10^{-1}	−		0		0	
BP51	*deoB*		2.6×10^7	$<2.0 \times 10^{-3}$	−		1.3×10^2		0	
BP52	*deoB*		$<1 \times 10^5$		−		0		0	
BP53	*deoB*	EDTA^r	1.6×10^8	6.8×10^{-3}	+		0		2.4×10^5	5.9×10^3
BP54	*deoB*		4.3×10^9	2.0×10^{-3}	+		0		2.6×10^4	0
BP55	*deoD*		6.3×10^8	c1.0	+	0	0	$>1 \times 10^6$		
KT21	*deoD*	TdR⁺ transductant								
λRB417	*deoA*		1.8×10^8	1.8×10^{-3}				$>2.5 \times 10^3$		4.9×10^2
λRB427	*deoB*			4.4×10^{-3}						
λRB400	*deoD*		1.0×10^6	7.7×10^{-2}		1.0×10^2				
λcI857S7	*attλ*			1.7×10^{-4}	+					

[a] Lysates made by infecting Sφ862 with the appropriate phage (except the last four lysates, which were heat-induced lysates) were used to transduce the appropriate recipient, as described in the text. The recipients for the various markers were: *deo*⁺, Sφ540 (Δ*deo*); *deoA*⁺, RB609; *deoB*⁺, Sφ801; *deoD*⁺, RB391; *serB*⁺, RB446 (Δ*serB*). Selection for *deo*⁺ and *deoA*⁺ was on TdR as the carbon source; for *deoB*⁺ and *deoD*⁺ it was on inosine as the carbon source. Where no number is given, the experiment was not performed. 0 = 0 to 10 transductants/ml of lysate.

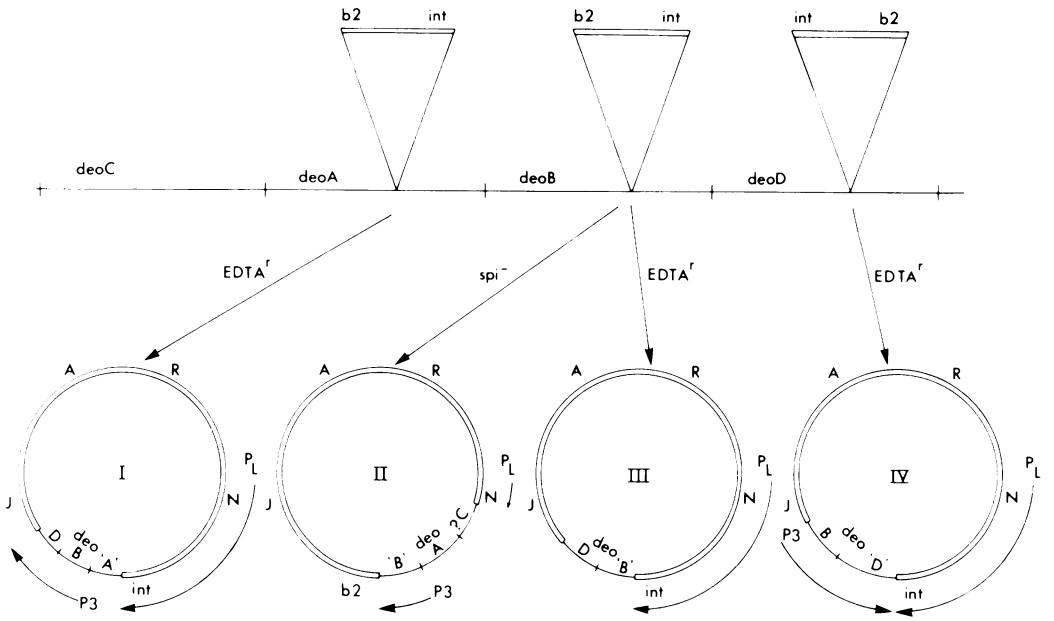

FIG. 4. *Diagram showing orientation of* λ *insertions within the deoA (strain RB417), deoB (strain RB427), and deoD (strain RB400) genes and some of the types of* λ*pdeo transducing phages derived from these lysogens. Phage BP46 is an example of type I, BP49 of type II, BP53 of type III, and BP55 of type IV.*

to study mRNA2 synthesis, phages were required which carry OP3 while lacking *deoOP* and *cytOP*. The λp*deoB*$^+$*D*$^+$ phages BP46 and BP47 were of this type. Both phages carried all of *deoD*$^+$ since they could transduce a strain (RB446) with a deletion extending through *deoB*, *deoD*, and *serB* to IR$^+$ (growth on inosine as carbon source, requiring both *deoB*$^+$ and *deoD*$^+$ activities). They could also transduce a Δ*deo* strain, Sφ540, which has a deletion extending through *deoA* and *deoB* and into *deoD*, to IR$^+$, and they therefore carried OP3.

To confirm that the *deoB* and *deoD* gene products produced from these phages were solely under the control of OP3 rather than *deoOP cytOP*, the induction of these enzymes was tested, in vivo, using a Δ*deo* strain lysogenized with BP46 (RB536) and BP47 (RB538). The results (Table 6) showed that inosine and guanosine, thought to act through the OP3 system, could induce the *deoB* and *deoD* gene products, whereas cytidine, acting through the *cytOP* system, could not. AdR acting through the *deoOP* system would, in a *deoC deoB*$^+$ *deoD*$^+$ background like RB536 but with an intact *deoOP* region, induce the *deoB* gene expression approximately eightfold (18). In RB536 and RB538 a small rise (1.7-fold) in *deoB* gene product was seen, but no effect on *deoD* expression was seen. The combined results from the induction experiments therefore confirm that the *deoOP* and *cytOP* regions were missing from both phages but that the OP3 region was present. Both strains RB536 and RB538 were resistant to λ*c*I90*c*17; since the level of *deoB* and *deoD* products in the latter strain were approximately twice that in strain RB536, this latter may be a double lysogen and strain RB538 may be a quadruple lysogen.

The level of thymidine phosphorylase activity was low in these strains, but still detectable, and was inducible by cytidine. Since this compound also induces uridine phosphorylase (coded by the *udp* gene, unlinked to *deo*), and since this enzyme is known to have a low activity towards TdR, i.e., like thymidine phosphorylase (8, 10, 25), this low level of activity was probably due to the action of uridine phorphorylase. This was confirmed by the inability to detect *deoA* activity in the *udp* strain lysogenized with BP47 (Table 6).

The enzyme levels found after heat induction of BP47 in strain Sφ1059 also clearly demonstrate that the *deoOP* and *cytOP* regions were missing from this phage, since no *deoA* gene product was detected in contrast to the very high levels of *deoB* and *deoD* gene products. Moreover, if an intact *deoA* gene was present in BP47 it would be transcribed in the same direction as the λ leftward transcription. Since there is no evidence for termination of normal λ transcription between the *int* gene and PP′ (15), we

TABLE 6. *Enzyme induction in deo strains lysogenized with λpdeoB⁺ deoD⁺ phage*[a]

Expt	Parent	Strain	Lysogenized with:	Addition (1 mg/ml)	Enzyme activity (U/mg of protein)				
					deoC	*deoA*	*deoB*	*deoD*	*udp*
1	Sφ540 (Δ*deo*)	RB536	BP46	None	≤4	7	72 (1.0)	298 (1.0)	
				AdR	≤6	2	128 (1.7)	286 (0.9)	
				Cytidine		15	79 (1.1)	256 (0.8)	609
				Inosine		2	275 (3.8)	553 (1.9)	44
				Guanosine		1	164 (2.3)	422 (1.4)	33
2	Sφ540 (Δ*deo*)	RB538	BP47	None	≤4	4	146 (1.0)	613 (1.0)	56
				AdR	≤3	2	247 (1.7)	555 (0.9)	94
				Cytidine		15	108 (0.7)	539 (0.9)	677
				Inosine		1	307 (2.1)	993 (1.6)	53
				Guanosine		1	293 (2.0)	819 (1.3)	59
3	Sφ928 (Δ*deo udp*)	Sφ1059	BP47	None		<1	95	397	<5
				Heat induced		<1	4,000	12,467	<5

[a] Cells were grown at 32°C with glycerol (experiments 1 and 2) or glucose (experiment 3) as carbon source. Induction with nucleosides or deoxynucleosides was performed for two generations. The numbers in parentheses indicate the induction ratio. All three strains were resistant to λ*c*I90*c*17 and are therefore probably multiple lysogens. Heat induction of exponentially growing cultures was for 10 min at 43°C and then for 90 min at 38°C.

would expect *deoA* gene expression from λP_L. The absence of any *deoA* gene product after induction is therefore evidence for the absence of an intact *deoA* gene on BP47. This implies that RB417 contains a *deoA*::(λ) mutation and not a *deoC*::(λ). The fact that BP46 and BP47 could transduce a *deoA* mutant to *deoA*⁺ (Table 5) may merely indicate that they carry the portion of the wild type in which that particular *deoA* mutation is located.

Selection of a plaque-forming λ*deoC*⁺*A*⁺*B*⁺*D*⁺. The only phage carrying the complete *deo* operon (24) is unfortunately defective. To allow a more convenient isolation of *deo*-enriched DNA, we have isolated a plaque-forming λ*deoC*⁺*A*⁺*B*⁺*D*⁺. An HfrH *deo*⁺ Δ*att*λ strain (Sφ716) was infected with BP46, and a λ lysogen, RB516, was isolated (lysogenization frequency, 8.2×10^{-3}). BP46 had most probably inserted in or near the *deo* operon, since zygotic induction experiments indicated a lowering of *thr*⁺ transfer into an F⁻ λ⁻ λʳ strain compared with a λ⁺ strain (unpublished data). Since the original phage was EDTAʳ and *int*⁺, selection for λ*spi*⁻ phages from a heat-induced lysate of strain RB516 should have been a method of obtaining λ phages carrying the whole *deo* operon (Fig. 5). Unfortunately, none of the λ*spi*⁻ phages were of this type (unpublished data).

Selection was made for phages able to transduce a Δ*deo* strain to TdR⁺. By using 7×10^{10} plaque-forming units of λRB516 phage with 0.1 ml of starved bacteria (strain Sφ540), one TdR⁺ transductant was obtained. This was a λ lysogen, and, when λ plaques were isolated from a heat-induced lysate, these could again transduce a Δ*deo* strain to TdR⁺ and IR⁺. This phage was therefore a plaque-forming λ*deo*⁺.

Isolation of bacterial deletions from the *deo* secondary-site λ lysogens. The rare heat-resistant survivors from a λ*c*I857 lysogen plated at 41°C often derive from cells in which the phage genes involved in the lethal functions have been deleted (32, 37), and the deletion may extend into an adjacent bacterial region (37). The analysis of such deletions can be used to order adjacent bacterial markers, providing that none of these involve essential functions of the bacterium.

From each of the three lysogens mentioned earlier, i.e., RB400 (*deoD*), RB417 (*deoA*), and RB427 (*deoB*), heat-resistant bacteria were isolated on L-broth agar plates at 41°C, and their auxotrophic requirements and other properties were tested. The results from RB427 (*deoB*) are shown in Table 7. Assuming that the deletions remove a continuous linear segment of DNA (37), examination of the types of deletions revealed the order of markers to be: *deoB-serB-trpR-msp-thr*. *msp* is the gene symbol given to a locus (or loci) which determines resistance to the male-specific phages R17 and μ2. Thus, strain RB427, being an Hfr, is sensitive to male-specific phages; deletions of the *msp* locus rendered the cells resistant to these phages.

The frequency of long or short deletions into the bacterial chromosome varied depending upon which lysogen was used, Thus, the number of *ser*⁻ *thr*⁺ deletions from the *deoD* lysogen was very low compared with the other two lysogens

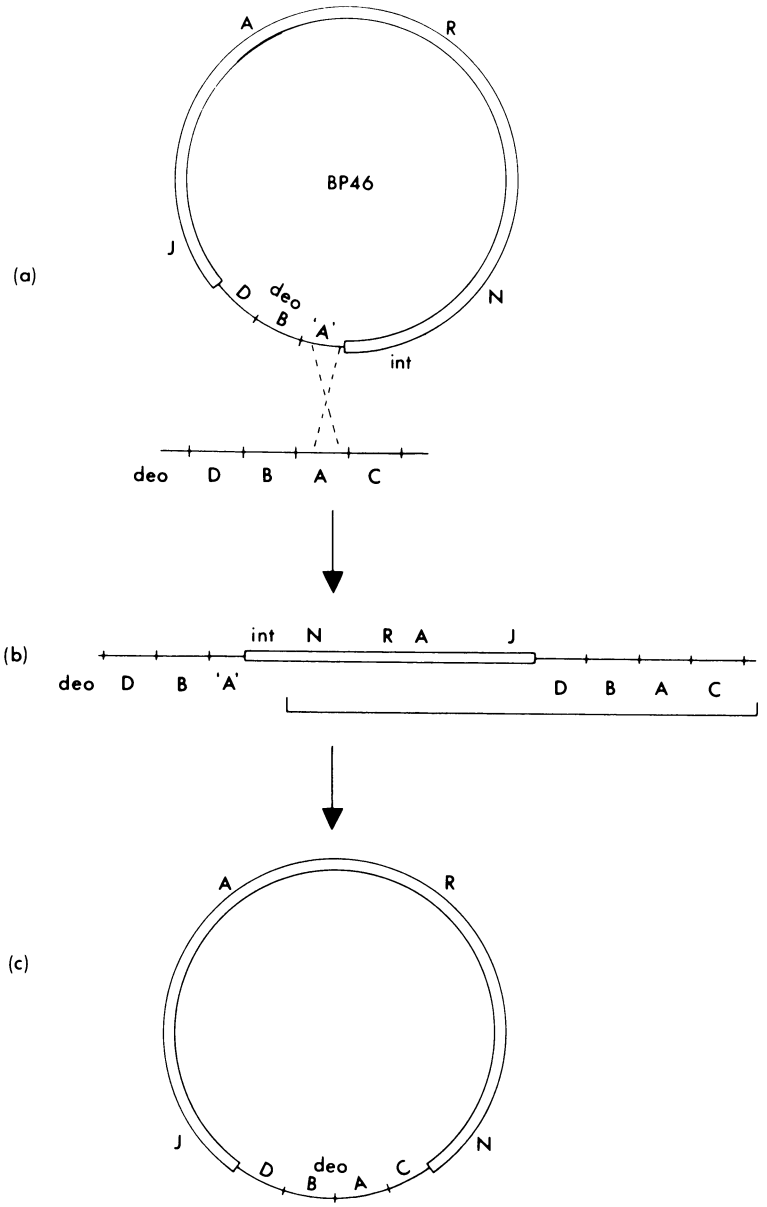

FIG. 5. *Suggested mechanism of production of λpdeoC⁺A⁺B⁺D⁺. BP46 was integrated into the deo operon of Sφ716 (a), and a λpdeoC⁺A⁺B⁺D⁺ phage was isolated (b) from a heat-induced lysate of this strain by transduction (c) (see text).*

(Table 8).

DISCUSSION

The isolation of a λ phage carrying only OP3, *deoB*⁺, and *deoD*⁺ while lacking *deoOP cytOP* has made possible the in vitro study of the OP3 control region. The fact that the phage is plaque forming has made the extraction of *deoB*⁺ *deoD*⁺-enriched DNA much easier than that from a defective phage. In vitro experiments have been performed with BP47 to investigate the regulation occurring at OP3 (P. Valentin-Hansen, K. Hammer-Jespersen, and R. S. Buxton, unpublished data). In future studies the range of phages which we have isolated, carrying either the whole *deo* operon, *deoB*⁺ *deoD*⁺, *deoA*⁺, *deoB*⁺, or *deoD*⁺, should enable us to estimate the amounts of mRNA produced from

TABLE 7. *Deletion mapping of the serB-thr region*[a]

Genotype of heat-resistant derivatives of RB427				No. of colonies
serB	*trpR*	*msp*	*thr*	
+	+	+	+	53[b]
−	+	+	+	26
−	−	+	+	4
−	−	−	+	2
−	−	−	−	14

[a] Strain RB427 [*deoB*::(λcI857*S*7)] was grown as 10 independent cultures (i.e., from 10 colonies) in L-broth (LB), plated out on LB agar plates, and incubated overnight at 41°C. Colonies were picked, purified by single-colony isolation, and tested for their auxotrophic requirements. The presence of the *trpR* allele was tested by streaking out colonies on minimal plates containing 5-methyltryptophan (100 μg/ml); strains carrying *trpR* are resistant to 5-methyltryptophan (13). Msp strains are resistant to the male-specific phage R17 (it is a male strain); this was tested on LB plates supplemented with 0.005 M $CaCl_2$.

[b] Thirteen of these were still λ*b*2*c*^{r} and were presumably *c*I$^+$ revertants of λcI857*S*7. All the others, including the auxotrophs, were λ*b*2*c*^{s}.

TABLE 8. *Frequency of long and short deletions from deo::(λ) lysogens*[a]

Mutation	Orientation of λ	No. (%) of deletions of phenotype:		
		Ser$^+$ Thr$^+$	Ser$^-$ Thr$^+$	Ser$^-$ Thr$^-$
deoA::(λ)	Opposite to *att*λ	86 (73)	23 (20)	8 (7)
deoB::(λ)	Opposite to *att*λ	53 (54)	32 (32)	14 (14)
deoD::(λ)	Same as *att*λ	42 (30)	3 (2)	93 (67)

[a] Heat-resistant derivatives were isolated and tested as described in Table 7. The bacteria were isolated from a number of independent cultures; for *deoA*::(λ) it was 16 cultures, for *deoB*::(λ) it was 10 cultures, and for *deoD*::(λ) it was 13 cultures.

each gene under varying inducing conditions, using DNA-RNA hybridization. They should also be useful for DNA sequencing studies.

The isolation of λ transducing phages by initially isolating the secondary-site lysogens has the advantage over the Schrenk and Weisberg (36) method, which omits this step, of being able to specifically isolate plaque-forming phages, and also of allowing the isolation of phages carrying certain genes but not others. Our experience with the transducing phages has been that the EDTA^r phages were much easier to work with than the *spi*$^-$ phages. The latter invariably gave rather low titers, and when transductants were isolated using these phages, they often turned out not to be λ lysogens (unpublished data). The use of the red-plaque test, however, to isolate *int*$^-$ phage (14) is a very useful method for isolating transducing phage of the λ*bio*$^+$ type, which still grow well.

Previous results (10) have shown that when phage Mu was inserted into *deoB* there was still a residual level of *deoD* gene product formed, which could originate either from a phage promoter or from a promoter (P5) in front of the *deoD* gene. Certain of the *deoB*::(λ) mutants had a similar level of *deoD* gene product (strains RB407 and RB420, Table 3) (35 U/mg of protein). This, however, may not be sufficient to account for the noncoordinate induction of the *deoB* and *deoD* products observed both from transcription originating in the *deoP* and *cytP* regions and from the P3 promoter (18). It is therefore very interesting that other *deoB*::(λ) mutants (strains RB411, RB413, and RB427, Table 3) had higher *deoD* enzyme levels (300 U/mg of protein). We suggest that this may be accounted for if λ were inserted in the proximal part of the *deoB* gene, leaving intact another promoter, P4, in the λ distal part of the *deoB* gene. The P4 promoter could donate approximately 120 U/mg of protein of the 180 U seen in an uninduced wild-type *deo*$^+$ strain, and this would be sufficient to explain the noncoordinate induction of the *deoB* and *deoD* products. The reason why these *deoD* enzyme levels are higher than 120 U may be due to a stimulation of P4 by the λ insertion. Spontaneous polar *deoB* mutants with similar high *deoD* levels have also been isolated (10).

From the heat-resistant derivatives of the λ lysogens we obtained no other auxotrophs except for one which required cysteine and serine. The cysteine requirement was not, however, linked to serine in an Hfr × F$^-$ cross, and its ability to grow on sulfide but not on sulfite led us to believe that it is blocked in *cysI*, *-J*, or *-G* (see 4, 23). Thus, one should be wary of ascribing linkage where only a single deletion mutant has been obtained. Perhaps the Cys$^-$ strain arose by some sort of reintegration of λ and subsequent deletions.

The frequency of short (i.e., Ser$^-$ Thr$^+$) deletions, compared with long (Ser$^-$ Thr$^-$) deletions, was very low in the *deoD* lysogen compared with the others (Table 8). The *deoD* lysogen has λ in the same orientation as λ inserted into *att*λ; the other lysogens have λ in the opposite orientation. Since such deletions extend into the O cistron (37) (genes O and N may be present in some cases, according to marker tests, but not able to complement a superinfecting phage [32]), it may be that most deletions in the *deoD*

lysogen which extend rightwards to *serB* from gene O, through most of the λ prophage, are long deletions. If the mechanism of deletion production is base specific, this may be one factor affecting the frequency of different classes of deletions.

The deletion map of the *serB-trpR-thr* genes is compatible with our previous mapping data using P1 transduction and conjugation (12). The *cet* locus, mutation of which renders *E. coli* tolerant to colicin E2, also lies between *trpR* and *thr*. None of the *serB-thr* deletions tested in the present study were tolerant to colicin E2 (unpublished data), but since *cet* is dominant to *cet*$^+$ (12) and may therefore be an operator mutation, it is not entirely unexpected that strains with deletions of this part of the chromosome are still colicin E2 sensitive. We have found, however, that a locus conferring resistance or sensitivity to male-specific phages, termed *msp*, lies between *trpR* and *thr*. Jamieson and Bergquist (22) have mapped, also between *serB* and *thr*, *seg* mutations which affect the replication of F-prime factors. *msp* mutants differ from *seg* mutants in two respects: first, *seg* mutants could be integratively suppressed to form Hfrs (19), whereas *msp* mutants were isolated in Hfr strains; and second, *seg* mutants were λ resistant (22), whereas *msp* mutants were λ sensitive (unpublished data). Since *msp* and *cet* both lie between *trpR* and *thr*, it is interesting to speculate that *msp* and *cet* may be related, both affecting cell envelope proteins, for example. A change in the cell envelope proteins has been found for *cet* mutants (35).

The majority, at least, of mutations formed by phage integration are nonleaky and do not revert easily (Table 3). We have made use of this fact to isolate secondary-site revertants of the *deoD*::(λ) strain by selecting for growth on nucleosides such as adenosine. These revertants are still λ lysogens, and the site of the reversion is not linked to *deoD*. The nature of these mutants, which appear to have an altered phosphorolytic activity of some other enzyme, is under investigation (Buxton and Hammer-Jespersen, unpublished data).

ACKNOWLEDGMENTS

K. H.-J. gratefully acknowledges the receipt of a European Molecular Biology Organisation short-term fellowship and a grant from the Danish Natural Science Research Council.

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