THE GALACTOSE OPERON OF *E. COLI* K-12. II. A DELETION ANALYSIS OF OPERON STRUCTURE AND POLARITY

JAMES A. SHAPIRO[1] AND SANKAR L. ADHYA[2]

Medical Research Council, Microbial Genetics Research Unit, Hammersmith Hospital, Ducane Road, London W. 12 and Service de Génétique Cellulaire, Institut Pasteur, Paris XV, France; and Department of Biochemistry, University of Wisconsin, Madison.

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PREVIOUS genetic studies have conclusively established the order of the three structural genes in the galactose operon to be epimerase—transferase—kinase (see Figure 1) (ADLER and KAISER 1963; BUTTIN 1963b; ADLER and TEMPLETON 1963). The elegant work of ADLER and TEMPLETON (1963) and subsequently of KAYAJANIAN (1965), DAVISON, FRAME and BISHOP (1967), and PFEIFFER and OELLERMAN (1967) have demonstrated the feasibility of high-resolution deletion mapping of the kinase and transferase structural genes by the use of λ*dg* transducing particles which carry partial galactose operons. However, because only transducing particles carrying an intact, active epimerase gene can be isolated and purified, they cannot be used to map the epimerase end of the operon. Both biochemical (MICHAELIS and STARLINGER 1968) and genetic (ADHYA and SHAPIRO 1969) data suggest that reading of the galactose operon proceeds from the epimerase end and that there exist one or more non-structural, regulatory elements in which mutations can lead to reduction or abolition of the activity of all three genes of the galactose operon. Hence, the need for a complete and detailed map of the galactose operon, in particular the epimerase gene, is clear. In this communication we shall present such a map based on the use of transducing phage and bacterial deletions. Our results confirm our previous conclusion on the epimerase to kinase polarity of the galactose operon and show that essential and rate-limiting regulatory elements are located at the epimerase end.

MATERIALS AND METHODS

Bacterial strains: In addition to those strains of *Escherichia coli* K-12 described in the preceding communication (ADHYA and SHAPIRO 1969) we have used the strains listed in Table 1. We recall that HfrH derivatives carrying various mutant galactose operons have been constructed by P1 transduction of strain H80 (ADHYA and SHAPIRO 1968); these Hfr's and UV-induced *gal*$^-$ mutants of HfrH have been used as donor strains in the Hfr crosses described below. A series of F$^-$ [λ-*gal*]$_\Delta$ *his*$^-$ *str*^{r} recipient strains was constructed by crossing [λ-gal]$_\Delta$ mutants of Hfr strains B113-U7K and H50J with strain MSO (ADHYA and SHAPIRO 1969).

Bacteriophages: Active double lysogens for λ and the various λ*dg* transducing phages carrying

[1] Present address: Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts 02115.
[2] Present address: Department of Biological Science, Stanford University, Stanford California 94305.

partial galactose operons were obtained from Drs. KAYAJANIAN (1965), H. ECHOLS (unpublished), D. PFEIFFER (PFEIFFER and OELLERMANN 1967), and J. ADLER (ADLER and TEMPLETON 1963). These lysogens were induced to provide the HFT lysates used in mapping studies. λ*dg* 357 (DAVISON, FRAME and BISHOP 1967) was obtained from J. BISHOP as an HFT lysate. λ^{857} (SUSSMAN and JACOB 1962) comes from S. BRENNER. C600 lysogens of various λ*sus* mutants (CAMPBELL 1961) were obtained from Drs. R. THOMAS, E. SIGNER and H. EISEN and induced to provide the lysates used in marker-rescue experiments.

Media: As previously described (ADHYA and SHAPIRO 1969).

Marker-rescue experiments: Presence or absence of a given λ cistron in a deletion mutant was determined by spot test (CAMPBELL 1964). Approximately 10^6–10^7 particles of a λ*sus* mutant were spotted on top of an overlay of strain MSO on a T-broth agar plate where 0.05 ml of an exponential T-broth culture of a bacterial deletion mutant had already spotted. The phage particles and bacterial culture were mixed with a sterile platinum loop and allowed to dry into the soft-agar overlay. After 10 sec UV-irradiation (about ⅛ optimal inducing dose) the plate was incubated overnight at 37°C and scored. Strain MSO is non-suppressing and will not permit the growth of λ*sus* mutants. Thus, only wild-type recombinant phage will produce plaques in the overlay. The presence of a given λ cistron in a particular deletion mutant generally resulted in confluent lysis of the indicator strain; this response was scored +. Occasionally confluent lysis was not observed, but about 100 isolated plaques could be seen; this response was scored wk+. In no case was there any confusion between plaques produced by recombinant phage and plaques produced by revertants in the original λ*sus* lysate. An exponential culture of strain MSO was always used as a negative control.

Bacterial crosses: a) *Hfr* × *F⁻ crosses:* Single colonies of the strains to be crossed were picked off L-agar plates into Penassay broth, grown to approximately 10^9/ml at 37°C, and mixed so that there were approximately five times as many recipients as donors in the final mating mixture. The mating mixtures were incubated 150 to 250 min at 37°C without agitation before plating. In general, the cross was set up so that the recipient strain carried a non-reverting *gal⁻* mutation. Because all the recipient strains carried either a *pyrD⁻* or *his⁻* marker distal to the galactose operon, crosses could be scored quantitatively. In crosses essential for deducing the map produced below (Figure 4) those matings which did not yield at least 10^5 *his+* or *pyrD+* recombinants per ml were not considered. Quantitative results are presented in SHAPIRO (1967). In a given cross with a nonreverting recipient strain, all *gal+* recombinants could be detected by plating on EMBGal agar containing streptomycin. Best results were obtained when the Hfr strain was grown without agitation.

b) *F'*gal × *F⁻ crosses:* These were performed simply by cross-streaking or mixing the donor and recipient strains directly on selective medium and scoring the results qualitatively.

λdg *mapping:* This was performed by spot test as described by ADHYA (1966).

Assay of galactokinase activity: This was done as described for Table 4 of ADHYA and SHAPIRO (1969).

Isolation of λ-gal *deletion mutants by double selection:* Single colony cultures of strains B113-U7K and H50J were grown overnight at low temperature (32°C and 25°C for B113-U7K and H50J, respectively) to a density of approximately 10^9/ml and then plated to select galactose-resistant mutants able to grow at high temperature. Two methods of plating were used: (1) B113-U7K was plated on appropriately supplemented M9-galactose agar containing 5×10^{-3} M D-galactose, incubated overnight at 32°C, and then incubated 24 hrs at 42°C followed by 24 hrs at 37°C; (2) H50J was plated on GZ-agar, incubated at 42°C, and then 24 hrs at 37°C. GZ agar was used in the series of selections from H50J because large deletions of the galactose region of the chromosome leading to additional auxotrophic requirements would be able to grow on this complete medium; the large red galactose-resistant clones were readily distinguishable from small, white non-lysogenic *u⁻* clones. It should be pointed out that the pre-incubation of B113-U7K at low temperature increased the probability that galactose-resistant, thermo-resistant mutants had been derived from two independent events. However, no differences were observed in the properties of the mutants obtained from the two strains. Deletions, originating in B113-U7K are designated U7--; those originating in H50J, 5--. After the selection, deletions were crossed into

TABLE 1

E. coli *K-12 strains used*

Strain	Mating type	Genotype	Source
B113-U7K	HfrB11	*purB⁻ his⁻ met⁻ gal⁻* (u^-_{u106}) *str^r* λ^R (λ^{857})	B113-U7 (ADHYA and SHAPIRO 1969)
H50J	HfrH	*thi⁻ gal⁻* (u^-_{UV50}) *str^s* λ^R (λ^{857})	SHAPIRO (1967)
H138-1	HfrH	*thi⁻ gal⁻* (k^-_{138-1}) *str^s*	BUTTIN (1963b)
PL2	HfrH	*thi⁻ gal⁻* (e^-_{PL2}) *str^s*	BUTTIN (1963b)
PL5	HfrH	*thi⁻ gal⁻* (e^-_{PL5}) *str^s*	BUTTIN (1963b)
GA105	F⁻	*gal⁻*$_{\Delta GA105}$ *his⁻ str^r*	SHAPIRO (1967)
S1795	F⁻	*gal⁻*$_{\Delta S179}$ *pyrD⁻ str^r*	SHAPIRO (1967)
SF108	F′$_s$-*gal*	*gal⁻* (kte^-_{S108})/*gal⁻* (kte^-_{S108}) *his⁻ str^r*	SHAPIRO (1967)
SF148	F′$_s$-*gal*	*gal⁻* (kte^-_{S148})/*gal⁻* (kte^-_{S148}) *his⁻ str^r*	SHAPIRO (1967)

The terminology for *gal⁻* mutations is explained in the preceding communication (ADHYA and SHAPIRO 1969). Strains SF108 and SF148 each carry the same mutant galactose operon on the bacterial chromosome and on the F′$_s$-*gal* episome. The origins and directions of transfer of HfrH and B11 are shown in Figure 1, ADHYA and SHAPIRO, 1969).

strain MSO to construct a series of F⁻ [λ-*gal*]$_\Delta$ *his⁻ str^r* recipients. Marker-rescue experiments were also performed on these recipients because the original mutants were λ^r.

RESULTS

Mapping of the transferase gene with λdg *particles:* In order to have a detailed map of the transferase structural gene, we have used the λdg deletion mapping technique of ADLER and TEMPLETON (1963) to locate a large number of *t⁻* and *kt⁻* mutations described in the preceding communication (ADHYA and SHAPIRO 1969). The results are summarized in Figure 1. Although the various λdg particles contain different lengths of genetic material originating at the right-hand (epimerase) end of the galactose operon, we have drawn them as deletions entering the operon from the left-hand (kinase) end to facilitate comparison with results obtained using bacterial deletions. It can be seen that all *t⁻* and *kt⁻* mutations map between *k⁻* mutations on the left and *e⁻*, *e⁻* polar, and all *kte⁻* mutations on the right. (We have excluded two *kte⁻* mutations which will be shown later to be extended deletions of all three structural genes.) From this result we draw the following conclusions: (1) All *t⁻* and *kt⁻* mutations are in the transferase structural gene; (2) all non-deletion *kte⁻* mutations are either mutations of essential regulatory elements or polar mutations of the epimerase structural gene; and (3) no essential regulatory elements are located to the left of the transferase gene.

It should be noted that ten of the eleven spontaneous, extreme polar *kt⁻* mutations (S101 through S182) map between suppressible nonsense and non-polar mutations of the transferase gene. They do not, therefore, affect a special region of the *galT* gene. (We know that none of the nine non-reverting *kt⁻* mutations (S101, S114–S182) is a deletion extending to the right because all recombine with the gal_1 mutation.)

The isolation of bacterial deletions of the galactose operon: Because of the limi-

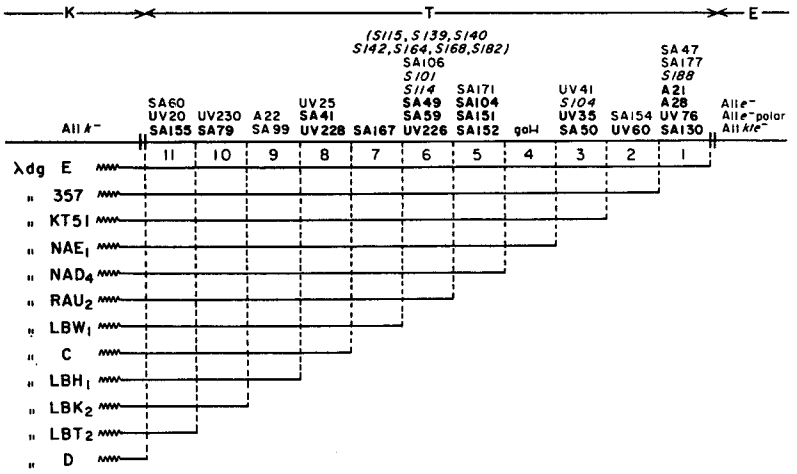


FIGURE 1.—A deletion map of the *galT* gene obtained with various λ*dg* transducing phages. *Amber* and *ochre* mutations are indicated by boldface characters; spontaneous *kt*⁻ mutations obtained by selection for galactose resistance (ADHYA and SHAPIRO 1969) are indicated by italic characters. Parentheses indicate the limits of map position of mutations whose precise locations have not been determined.

tations on the types of λ*dg* particles that can be isolated (see *Introduction*), it was imperative to isolate bacterial deletions of the galactose operon to construct a complete genetic map. The existence of an efficient positive selection system for galactose-negative mutations (ADHYA and SHAPIRO 1969) made the isolation of such deletions feasible. This could be done either by examining large numbers of spontaneous *gal*⁻ mutations or by a double selection scheme involving a second marker close to the galactose operon. The proximity of the λ prophage to the galactose operon and the existence of thermo-inducible mutants of λ which kill the host bacterium at high temperature (SUSSMAN and JACOB 1962) made it possible to select galactose-resistant, thermo-resistant mutants from a galactose-sensitive *u*⁻ mutant lysogenic for the thermo-inducible λ^{857} prophage. Providing no gene essential for the growth of the bacterium lay between the galactose operon and λ prophage, the majority of mutants isolated by this double selection should be deletions rendering the galactokinase gene inactive and the λ prophage non-lethal. We shall call such mutations λ-*gal* deletions.

In practice, we used both the single and double selections to obtain the deletions described below. The S165, S179, and GA105 deletions were isolated as galactose-resistant mutants of the non-lysogenic strain B113-U7 (see ADHYA and SHAPIRO 1969). S165 and S179 arose spontaneously; GA105 was induced by gamma irradiation. In the double selection experiment, 29 independent galactose-resistant, thermo-resistant mutants were isolated from two different lysogenic strains (H50J and B113-U7K) as described in MATERIALS AND METHODS. Of those mutants, 25 exhibit the expected *kte*⁻ phenotype, 21 of which are also non-reverting.

(It is worth mentioning here that one mutant isolated from strain H50J carries a leaky *k*⁻ mutation permitting 12% of normally inducible kinase activity. Hence,

our selection procedure for this strain does not require mutations which cause an absolute defect in kinase activity.)

The four reverting *kte*⁻ mutants obtained by double selection were studied and shown not to contain deletions between the galactose operon and λ prophage. Results presented elsewhere (SHAPIRO 1967) indicate that they resulted from complex mutational events involving curing of the λ^{857} prophage and aberrations in the λ-*gal* interval of the bacterial chromosome.

The remaining 21 non-reverting *kte*⁻ mutants obtained by double selection will be shown to carry λ-*gal* deletions. None requires biotin. Two grow poorly on both synthetic and complete media, but the majority grow normally. The two which grow poorly carry deletions (515 and 5141) extending beyond the galactose operon and the *aroG* locus on the left-hand side (Figure 4).

One of the mutants obtained by double selection (H506) was originally observed not to grow on any medium less complex than T-broth. We concluded that it carries an extensive deletion of the *gal* region of the chromosome. This strain and a derivative, H80, have been used to construct an otherwise isogenic series of strains carrying various *gal*⁻ mutations of the galactose operon (ADHYA and SHAPIRO 1969). Subsequent to our work, the requirements of strain H80 (carrying the 506 λ-*gal* deletion) were identified by DR. W. EPSTEIN as nicotinic acid and a non-specific amino acid auxotrophy (personal communication). The significance of this result for ordering markers in the *gal* region of the chromosome is discussed below.

To determine whether or not the deletion mutants we isolated were formed as a consequence of thermal induction of the λ^{857} prophage after plating, we carried out a fluctuation test on 25 independent cultures of strain H50J. It can be seen from Table 2 that our mutants were present before selection.

Prophage content of λ-gal *deletion mutants*: In order to establish rigorously that the mutants obtained by the double selection procedure carry deletions between the λ prophage and galactose operon, it was necessary to show that they had lost coherent sets of markers from both the operon and the prophage. The results of marker-rescue experiments with λ *sus* mutants defective in one of fifteen cistrons are shown in Table 3. The map of the λ prophage which we deduce from these results is presented in Figure 2. Clearly our mutants carry consistent deletions entering from the galactose operon end of the prophage, and the order of markers we obtain agrees with that previously determined by others (CAMPBELL 1962; ROTHMAN 1965; FRANKLIN, DOVE and YANOFSKY 1965). A notable feature of this map is that all our deletions extend at least into the *O* cistron. This result is consistent with the finding that functioning of the combined *O* and *P* genes of the thermo-inducible λ lysogen is lethal to the host bacterium at high temperature even in the absence of an active *N* gene (PEREIRA DA SILVA, EISEN and JACOB 1968).

The absence of any rescueable phage markers in several of these deletion mutants raised the possibility that they had lost the entire right hand λ attachment site. We have tested strains carrying the 502, 5141, U755, and U760 deletions for lysogenization by phage λ and find that they can be lysogenized, al-

TABLE 2

Isolation of λ-gal *deletion mutants from strain H50J*

Culture number	Aliquot A	B	C	D	E	Total mutants
1	0	0	0	0	0	0
2	0	0	0	1	0	1
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	1	1	0	0	0	2
6	7	5	8	7	10	37
7	3	5	3	5	3	19
8	21	1	3	1	6	32
9	7	11	10	10	7	45
10	7	7	11	9	12	46
11	174	173	184	158	149	838
12	0	0	0	0	0	0
13	0	0	0	0	0	0
14	0	0	0	0	0	0
15	0	0	0	0	0	0
16	0	0	0	0	0	0
17	0	0	0	0	0	0
18	0	0	0	0	0	0
19	0	0	0	0	0	0
20	0	0	0	0	0	0
21	3	3	8	6	11	31
22	0	0	0	0	0	0
23	133	(contam.)	99	117	124	476
24	0	0	0	0	0	0
25	0	0	0	0	0	0

Single colony cultures of strain H50J were grown to approximately 10^9 cells/ml at 25°C, a 1.0 ml sample taken of each culture, and 0.2 ml aliquots of this sample plated on each of five GZ-agar plates. These plates were then incubated as described in MATERIALS and METHODS and scored for the number of red galactose-resistant, thermo-resistant colonies.

though at a frequency about three times lower than a control strain. Transduction by λ*dg* is similarly depressed. (These results are comparable to those obtained with deletion mutants containing λ markers.) Furthermore, HFT lysates of λ*daroG* phage can be produced by *aroG*$^+$ transductants of strains carrying the 5141 and U760 deletions (see below), and *aroG*$^+$ transducing phage are found in increased frequency in induced lysates of lysogens carrying the 502 and U755 deletions compared to a wild-type lysogen. Hence, none of these four deletions appears to remove the entire λ attachment site. (We have not studied the lysogenization properties of strains carrying the U742 and U761 deletions.)

Galactose operon content of λ-gal *deletion mutants*: An extensive series of galactose operon mutations whose origins have been described in the preceding communication (ADHYA and SHAPIRO 1969) and elsewhere (BUTTIN 1963b; SAEDLER and STARLINGER 1967a) was crossed in bacterial mating experiments with the λ-*gal* deletions and the three deletions obtained from a non-lysogenic parent. In

TABLE 3

Prophage content of strains carrying λ-gal *deletions*

Deletion mutant	λ cistron *sus* (*amber*) marker	N 217	C_I	O 29	P 207	Q 203	R 216	A 11	B 1	C 20	F 96B	H 12	K 24	L 63	M 87	I 2	J 6
MS501		—	—	—	wk+	+	+	+	+	+	+	+	+	+	+	+	+
MS508		—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
MS5061		—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
MS509		—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
Su766		..	—	..	..	—	—	—	+	+	+	..	..	+	..	+	+
MS504		—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+
SU712		..	—	..	..	—	—	—	..	—	+	..	..	+	..	+	+
SU757		..	—	..	..	—	—	—	—	—	+	..	..	+	..	+	+
MS503		—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
MS512		—	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+
SU748		..	—	..	..	—	—	—	..	—	—	+	..	+	+	+	+
SU729		—	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
SU756		..	—	..	..	—	—	—	..	—	—	—	..	+	+	+	+
SU740		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	wk+
MS515		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	wk+
MS502		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MS5141		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SU742		..	—	..	..	—	—	—	..	—	—	..	..	—	..	—	—
SU755		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SU760		..	—	..	..	—	—	—	..	—	—	..	..	—	..	—	—
SU761		..	—	..	..	—	—	—	..	—	—	..	..	—	..	—	—

The absence of the c_I gene was determined by testing for immunity to phage λ^{857} at 30°C. The presence (+) or absence (—) of a given λ cistron was determined by spot test as described in MATERIALS and METHODS. MS-- strains carry the 5-- series of deletions isolated from strain H50J; SU--- strains carry the U7-- series of deletions isolated from strain B113-U7K.

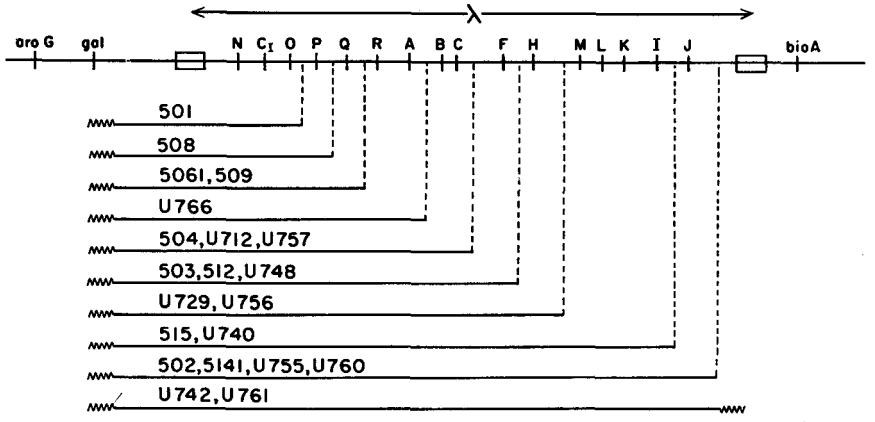
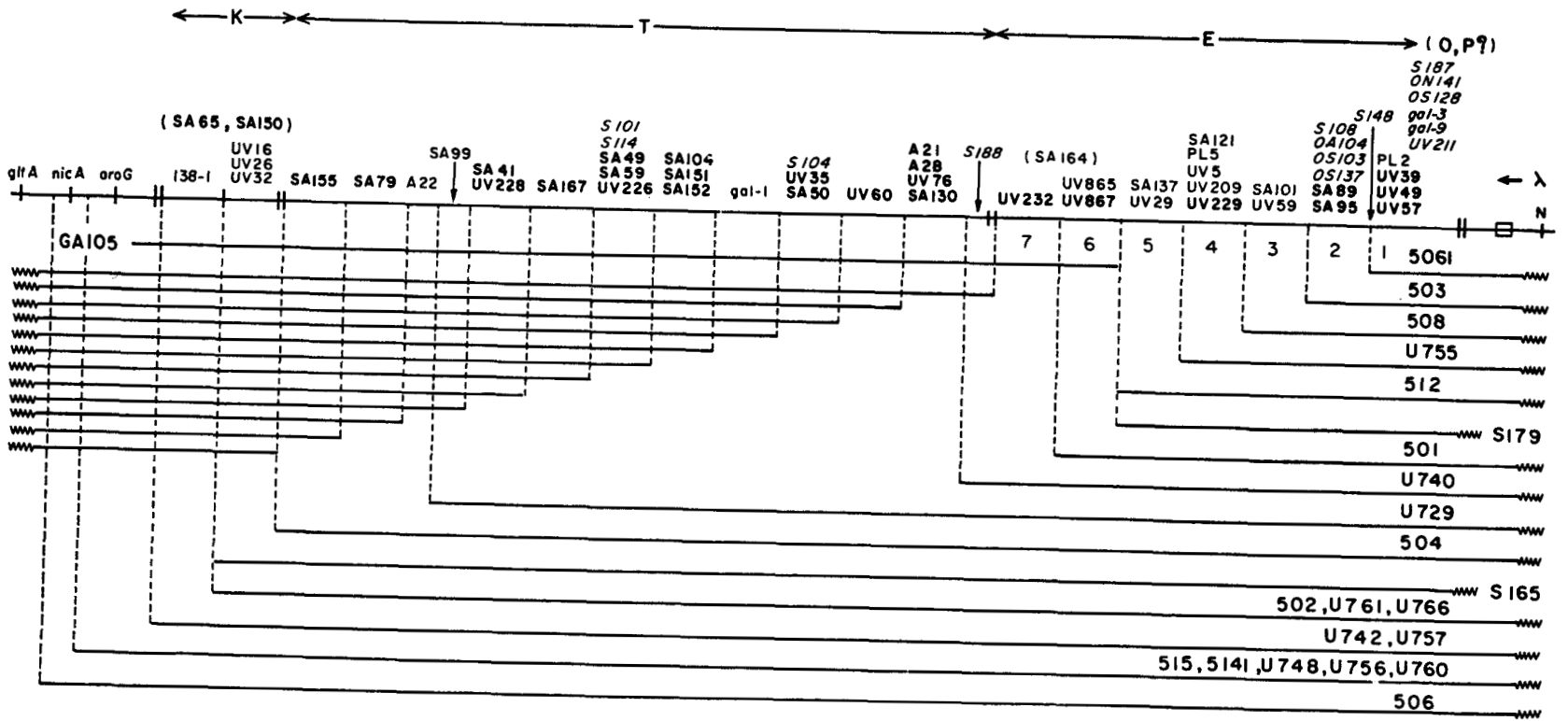


FIGURE 2.—Deletion map of the λ prophage. The order of markers within deletion segments has been taken from the work of others (CAMPBELL 1962; ROTHMAN 1965; FRANKLIN *et al.* 1965; KAYAJANIAN 1965).


K
T
E
(O,P?)
(SA65, SA150)
UV16
UV26
UV32
S101
S114
SA49
SA59
UV226
SA99
SA41
UV228
SA104
SA151
SA152
S104
UV35
SA50
A21
A28
UV76
SA130
S188
(SA164)
UV232
UV865
UV867
SA121
PL5
UV5
UV209
UV229
SA101
UV59
S148
S108
OA104
OS103
OS137
SA89
SA95
S187
ON141
OS128
gal-3
gal-9
UV211
PL2
UV39
UV49
UV57
λ
N
gltA
nicA
aroG
138-1
SA155
SA79
A22
SA167
gal-1
UV60
GA105
7
6
5
4
3
2
1
5061
503
508
U755
512
S179
501
U740
U729
504
S165
502,U761,U766
U742,U757
515,5141,U748,U756,U760
506

addition, the left-hand endpoints of the λ-*gal* deletions were verified by spot tests with the λ*dg*'s used to map the transferase gene. The results of all of these experiments and of our previous mapping of the transferase gene are summarized in Figure 3. As with the prophage markers, we see that the λ-*gal* mutations constitute a set of coherent deletions, in this case originating at the λ prophage end of the galactose operon. Two of the deletions isolated by single selection (S165 and S179) also enter the operon from the right-hand end, and one (GA105) from the left. Each of these three deletions probably extends beyond one of the extremities of the galactose operon because none will form homogenotes with the F_8-*gal* episome which carries the entire operon, including the operator (HILL and ECHOLS 1966). The S165 deletion does not remove the λ attachment site because it forms stable λ lysogens and is transduced by λ*dg* at normal frequency. (The S179 deletion has not been tested quantitatively for λ lysogenization.)

The first feature of this map to be noted is that all of the deletions entering from the right extend beyond at least one non-polar e^- mutation (PL2, BUTTIN 1963a) and three *amber* mutations of the epimerase gene (UV39, UV49, and UV57; ADHYA and SHAPIRO 1968). Thus, we cannot say definitely whether any of the mutations which fail to recombine with all of the λ-*gal* deletions is in the epimerase structural gene or in some non-structural genetic element to the right of it. On the other hand, all mutations which do recombine with at least the 5061 deletion are located within one of the operon's three structural genes and do not affect any genetic element between the epimerase gene and the λ prophage. Thus, two mutations which appear to abolish activity of all three genes (S148 and OS103; ADHYA and SHAPIRO 1969) are located within the epimerase structural gene and must be considered extreme polar mutations. Neither is an extended deletion nor contains a mutation of the transferase or kinase gene because both recombine with the GA105 deletion. Moreover, the OS103 mutation reverts spontaneously (SAEDLER and STARLINGER 1967a; ADHYA and SHAPIRO 1969). The S148 mutation is indicated as being at the end-point of the 5061 deletion because recombination between the two is very poor ($< 10^{-6}$ per zygote) but reproducible (neither mutation has ever been observed to revert).

The three possible regulatory mutations which lead to lowered but enzymatically or phenotypically detectable activity of the galactose enzymes (UV211, gal_3, and OS128; ADHYA and SHAPIRO 1969) all map under the 5061 deletion. This result is consistent with the hypothesis that they alter some non-structural genetic element at the right-hand end of the operon (cf. JACOB, ULLMAN and MONOD 1964; SCAIFE and BECKWITH 1966). It was of special interest to locate the gal_3 mutation because it displays unusual regulatory properties (HILL and ECHOLS 1966; MORSE 1967) and because earlier results of ADLER and KAISER (1963) indi-

FIGURE 3.—Deletion map of the galactose operon. Except for GA105, all deletions entering from the left represent the λ*dg* phages described in Figure 1. *Amber* and *ochre* mutations and all *kte*$^-$ mutations are indicated by italic characters. Parentheses indicate the limits of map position of mutations whose precise locations have not been determined. The mapping of the *gltA*, *nicA*, and *aroG* loci is based on arguments presented in the text.

cated that it might be located within the epimerase structural gene or even between the epimerase and transferase genes. Our results definitely exclude the second possibility. However, since we have not used the same e^- mutations as ADLER and KAISER, we cannot completely exclude their localization of gal_3 to the left of two sites in the epimerase gene, unlikely as it seems in view of the tentative nature of their three-point mapping technique.

Function of the epimerase terminus of the operon: Strains carrying the 501, 503, 508, 512, and 5061 deletions which terminate within the epimerase gene and a strain carrying the 502 deletion which terminates within the kinase gene were assayed for galactokinase activity. In all strains, less than 2% of fully induced wild-type activity could be detected, which is the limit of sensitivity of the assay in our hands. The activity we detected was not inducible by D-fucose. Moreover, none of the strains carrying the deletions ending within the epimerase gene is able to convert exogenous galactose to UDPGal via the kinase and transferase reactions *in vivo* because they all remain sensitive to phage C21 even after prolonged growth in the presence of galactose (see ADHYA and SHAPIRO 1969). From these results we conclude that deletion of the right-hand terminus of the galactose operon abolishes operon function.

The effect of location on polarity in the epimerase and transferase genes: The existence of a complete map of the galactose operon made it possible to determine the effect of genetic location on the degree of polarity of various polar mutations described in the preceding communication (ADHYA and SHAPIRO 1969). The results of plotting map position against fully induced galactokinase activity in strains carrying various suppressible nonsense and other polar mutations of the epimerase gene are summarized in Figure 4b. It can be seen that there is a fairly clear gradient of polarity among the suppressible nonsense mutations, kinase activity rising the closer a mutation is to the transferase gene. This result indicates that the epimerase gene is transcribed from right to left. The location of the non-reverting UV209 mutation on the gradient indicates that it might be polar by virtue of the same mechanism as *amber* and *ochre* mutations. This is consistent with the notion that UV209 is a small deletion that exerts a frameshift effect resulting in a nonsense codon in the new reading frame close to the mutated site. In contrast, the three spontaneous extreme polar mutations which definitely map within the epimerase structural gene and for which we have enzyme data (S108, S148, and OS137) do not fall on the gradient of polarity (Figure 4b).

The results of comparing map position of identified nonsense mutations in the transferase gene with their polar effects are shown in Figure 4a. The situation is more complex than in the epimerase gene. There appears to be a suggestion of a gradient of polarity, including all but three of the nonsense mutations. The direction of this gradient is consistent with the transferase gene's being transcribed from right to left. The three *amber* mutations which do not fall on this gradient (SA59, SA151, SA152) are located in deletion segments 5 and 6 of the *T* gene (Figure 1) and are clearly non-polar. Our mapping results suggest that these three mutations are clustered in a small region of the *T* gene. To account for this region of zero polarity in the middle of the *T* gene, we suggest that an efficient sequence

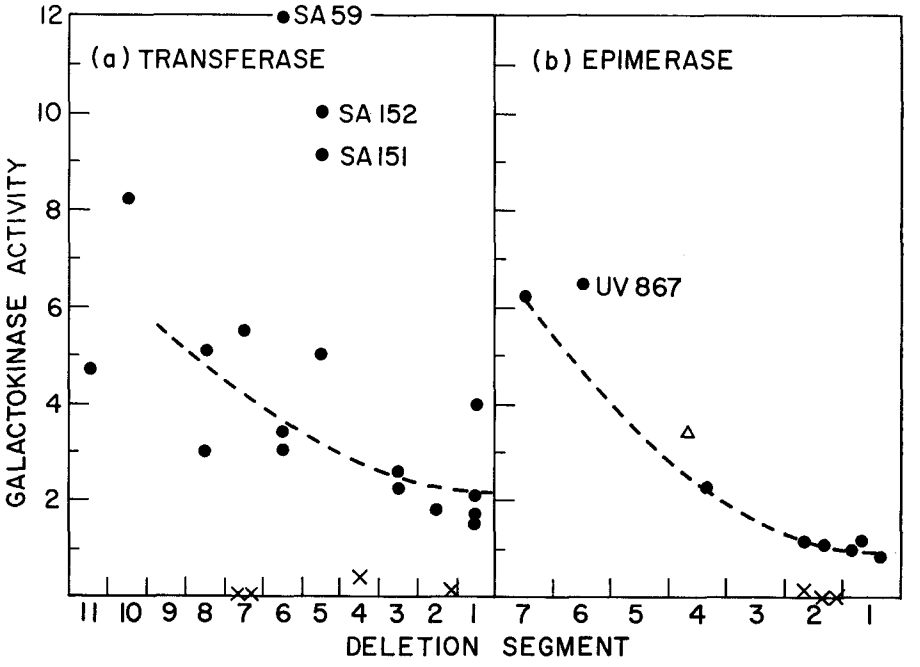


FIGURE 4.—Map position *versus* polarity in the epimerase and transferase genes. Deletion segments are as defined in Figure 1 for the transferase gene and Figure 3 for the epimerase gene. The mutations within each deletion segment of the *T* gene is arbitrarily placed in its middle; the order of mutations within deletion segments in the *E* gene is based on the results of two-factor crosses presented elsewhere (SHAPIRO 1967). Fully induced galactokinase activities are taken from Tables 4 and 5 of ADHYA and SHAPIRO (1969). (●), *amber* and *ochre* mutations (from left to right, and from top to bottom within a segment of the *T* gene—(a) t^- SA155, SA79, SA41, UV228, SA167, SA59, UV226, SA49, SA152, SA151, SA104, SA50, UV35, UV60, A28, UV76, SA130, A21; (b) e^- UV232, UV867, UV229, SA89, SA95, UV39, UV57, U49; (×), spontaneous extreme polar mutations (from left to right—(a) kt^- S101, S114, S104, S188); (b),kte^- S108, OS137, S148; (△), the UV209 non-reverting e^- mutation. We note that in (b) the level of UV867, which is an e^-o^c mutation, does not have a wild-type (gal^+o^c) control for normalization. The sensitivity of the kinase assay is described previously (ADHYA and ECHOLS 1966).

for polypeptide-chain initiation, in phase, is located very close to the sites of these non-polar *amber* mutations. The results of complementation tests (ADHYA and SHAPIRO 1969) rule out the possibility of two transferase cistrons.

As can be seen from Figure 4, the same contrast between the polarity of spontaneous mutations and *ambers* and *ochres* exists in the transferase gene as in the epimerase gene. Thus, it seems that the spontaneous extreme polar mutations of both genes display a kind of polarity qualitatively different from that of nonsense (and presumably frameshift) mutations.

Location of the aroG, nicA, *and* gltA *loci:* WALLACE and PITTARD (1967) have shown that mutations of the *aroG* locus which lead to a requirement for phenylalanine in the presence of tryptophan and tyrosine map close to the galactose operon and are transducible by phage λ. All deletion strains were consequently

tested for growth in the presence of tryptophan and tyrosine. Strains carrying the U748, U756, U760, 515, and 5141 deletions were inhibited by tryptophan and tyrosine on otherwise appropriately supplemented M9 agar. Hence, these deletions extend at least as far as the *aroG* locus. All delete the entire galactose operon. None of these *aroG*$^-$ deletions leads to a nicotinic acid requirement. As noted above, strain H80 carrying the 506 λ-*gal* deletion requires nicotinic acid and (nonspecifically) amino acids for growth on synthetic media. It does not require glutamate (W. EPSTEIN, personal communication). Thus, the order of loci to the left of *gal* must be *gltA—nicA—aroG—gal* as indicated in Figure 3.

DISCUSSION AND CONCLUSIONS

Structure and function of the galactose operon: From the data presented in this and the preceding communication (ADHYA and SHAPIRO 1969) we conclude that the galactose operon is a single polarized unit of transcription and translation governed by essential and regulatory genetic determinants located at the epimerase end of the operon. These determinants do not code for any diffusible product.

That transcription of the galactose operon begins at the end closest to the λ prophage is indicated by three observations on polarity: (1) polar mutations are found in the epimerase and transferase genes but not in the kinase gene (ADHYA and SHAPIRO 1969), (2) polarity is more or less coordinately expressed on genes distal to the λ prophage (ADHYA and SHAPIRO 1969), and (3) polarity of nonsense mutations within a given cistron seems to decrease with distance from the λ prophage (Figure 4).

That deletion of the lambda terminus of the operon abolishes the activity of intact kinase and transferase genes implies that a region essential for expression of the operon is located between the epimerase gene and lambda prophage. We cannot rule out polar effects in strains carrying those λ-*gal* deletions which do not enter the transferase gene, but this possibility seems highly unlikely in view of the fact that all five such strains tested cannot be distinguished enzymatically from a strain in which part of the kinase gene has been deleted. (We recall, moreover, that the selection procedure that yielded these five λ-*gal* deletions does not demand an absolute block of kinase activity.)

That all our deletions are *cis*-dominant in complementation tests and that none out of 21 leaves the three structural genes intact argues that no gene coding for a diffusible product essential to operon function is located between the epimerase gene and λ prophage. The mapping of three *cis*-dominant mutations which depress (but do not abolish) activity of all three structural genes (UV211, gal_3, and OS128) at the λ end of the operon suggests that at least one rate-limiting genetic element is located between the epimerase gene and λ prophage. This element may be equivalent to the "promoter" of the lactose operon (JACOB, ULLMAN and MONOD 1964). As we have mentioned previously (ADHYA and SHAPIRO 1969), our UV211 mutation is analogous to the promoter mutations of SCAIFE and BECKWITH (1966).

The location of the gal_3 mutation in this region of the operon (Figure 3) rein-

forces our conclusion that regulatory elements are at the λ end of the operon because HILL and ECHOLS (1966) and MORSE (1967) have shown that reversion of the gal_3 mutation often involves changes in inducibility of operon function and abnormalities in λ transduction of the structural genes. BUTTIN's mapping of an o^c mutation near the PL2 e^- mutation (1963b) is also in agreement with our results, for the PL2 mutation is in the λ-proximal deletion segment of the operon (Figure 3).

Negative control of the galactose operon has been confirmed by (1) the *cis*-dominance of all triply negative *kte*$^-$ mutations, and (2) the isolation of non-inducible mutations of the galactose regulator gene (*galR*^{s} mutations) which are dominant over the wild-type *galR*$^+$ allele (SHAPIRO and ADHYA, unpublished observations; SAEDLER, GULLAN, FIETHEN and STARLINGER, 1968). Strains carrying these mutations revert at high frequency to constitutive *gal*$^+$, yielding both recessive *galR*$^-$ and *cis*-dominant o^c type secondary mutations (ADHYA and SHAPIRO, unpublished observations).

Because all of our λ-*gal* deletions enter the epimerase gene, we cannot establish the order of regulatory elements with respect to the structural genes. We have attempted to resolve this problem by searching for operator-constitutive deletions extending into the λ prophage among *gal*$^+$ revertants of a lysogenic strain carrying a *galR*^{s} mutation. Our results were negative (less than 1 such o^c deletion mutant per 10^{10} bacteria; SHAPIRO, unpublished observations). This suggests two possibilities: (1) the operator is essential to the function of the galactose operon and cannot be deleted, or (2) the operator is located between the first structural gene and an element essential to operon function as found for the lactose operon (IPPEN, MILLER, SCAIFE and BECKWITH 1968).

Since λ transducing particles which only carry the epimerase end of the operon contain an active epimerase gene and can form complementation heterogenotes and since all the triply defective mutations we have mapped are located at the epimerase end of the operon, we conclude that no essential regulatory elements are situated to the left of the kinase gene.

The nature of the spontaneous extreme polar mutations: We have previously discussed our reasons for believing that our spontaneous extreme polar *kt*$^-$ and *kte*$^-$ mutations are not the result of either base-substitutions or frameshifts (ADHYA and SHAPIRO 1969). The results summarized in Figure 3 show further that these mutations are not extended deletions and do not necessarily affect special regions of the galactose operon. The contrast between the polar effects of these mutations and those of nonsense mutations (Figure 4; ADHYA and SHAPIRO 1969) shows that the simple model of chain termination at or near the mutated site will not account for their extreme polarity.

To explain our results we postulate that the spontaneous extreme polar mutations result from a unique kind of genetic event: the insertion of a large piece of foreign DNA into a structural gene. The insertion hypothesis predicts that mutant galactose operons containing spontaneous extreme polar mutations will have more DNA than the wild-type operon. In the following communication (SHAPIRO, 1969) we shall present the results of density gradient experiments which confirm

this prediction. We shall also present a detailed discussion of how the insertion hypothesis accounts for the properties of our spontaneous extreme polar mutations. Suffice it to say here that this hypothesis will explain the parallel results of SAEDLER and STARLINGER (1967a, b) and JORDAN, SAEDLER and STARLINGER (1967) on *gal*⁻ mutations and of MALAMY (1966) on *lac*⁻ mutations.

Lysogeny: The results summarized in Figures 2 and 3 prove that it is possible to isolate coherent deletions between the λ prophage and the galactose operon. The prophage map so obtained is a cyclic permutation of the vegetative λ map. These results thus agree with the analogous experiments of FRANKLIN, DOVE and YANOFSKY (1965) and confirm one of the fundamental predictions of the CAMPBELL model for prophage integration by linear insertion into the bacterial chromosome (CAMPBELL 1962).

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SUMMARY

A detailed genetic map of the galactose operon of *E. coli* K-12 has been constructed by deletion mapping of mutations described in the preceding communication (ADHYA and SHAPIRO 1969). Non-polar and polar mutations have been located in three groups corresponding to the kinase, transferase, and epimerase structural genes. As shown previously, the order of these genes on the bacterial chromosome is *K–T–E–*λ prophage. Three *gal*⁻ regulatory mutations all map at the λ end of the galactose operon. Comparison of map position *versus* polarity for nonsense mutations of the epimerase and transferase genes indicates that transcription of each gene begins at the end closest to the λ prophage. Analysis of strains carrying deletions shows that removal of the epimerase end of the galactose operon abolishes the activity of intact structural genes. Such pleiotropic negative deletions are all *cis*-dominant. From these results, we conclude that the galactose operon is a negatively controlled, polarized unit of transcription and translation governed by essential and rate-limiting genetic elements located at the end of the operon closest to the λ prophage.—Genetic analysis of spontaneous extreme polar mutations (ADHYA and SHAPIRO 1969) rules out the possibilities that they affect special regions of the operon or that they are multiple mutations or extended deletions. Comparison of map position *versus* polarity for these mutations shows that they exert qualitatively different polar effects from those of nonsense mutations. To account for their properties, we suggest that these spontaneous extreme polar mutations result from the insertion of large pieces of genetic material into structural genes.—In order to map the galactose operon, bacterial deletions ex-

tending between the operon and the λ prophage were isolated by a double selection technique. The deletion map of the λ prophage obtained with these mutants is consistent with that determined by other methods. The existence of a set of coherent deletions between the λ prophage and galactose operon supports the CAMPBELL model of prophage integration by linear insertion into the bacterial chromosome.

LITERATURE CITED

ADHYA, S., 1966 Control of synthesis of the galactose metabolizing enzymes of *Escherichia coli*. Ph.D. Thesis, University of Wisconsin.

ADHYA, S., and J. A. SHAPIRO, 1968 The galactose operon of *E. coli* K-12. I. Structural and pleiotropic mutations of the operon. Genetics **62**: 231–247.

ADLER, J., and A. D. KAISER, 1963 Mapping of the galactose genes of *Escherichia coli* by transduction with phage P1. Virology **19**: 117–126.

ADLER, J., and B. TEMPLETON, 1963 The amount of galactose genetic material in λ*dg* bacteriophage with different densities. J. Mol. Biol. **7**: 710–720.

BUTTIN, G., 1963 Mécanismes régulateurs dans la biosynthèse des enzymes du metabolisme du galactose chez *Escherichia coli* K-12. I. La biosynthèse induite de la galactokinase et l'induction simultanée de la sequence. J. Mol. Biol. **7**: 164–182. —— 1963b Mécanismes régulateurs dans le biosynthèse des enzymes du metabolisme du galactose chez *Escherichia coli* K-12. II. Le déterminisme génétique de la régulation. J. Mol. Biol. **7**: 183–205.

CAMPBELL, A., 1961 Sensitive mutants of bacteriophage λ. Virology **14**: 22–32. —— 1962 Episomes. Advan. Genet. **11**: 101–145. —— 1964 Transduction. pp. 49. In: *The Bacteria*, Vol. 5. Edited by I. GUNSALUS and R. STANIER.

DAVISON, J., R. FRAME, and J. BISHOP, 1967 A partial deletion map of the galactose operon in *E. coli* K-12. Genet. Res. **10**: 107–116.

FRANKLIN, N. C., W. F. DOVE, and C. YANOFSKY, 1965 The linear insertion of a prophage into the chromosome of *E. coli* shown by deletion mapping. Biochem. Biophys. Res. Commun. **18**: 910–923.

HILL, C. W., and H. ECHOLS, 1966 Properties of a mutant blocked in inducibility of mRNA for the galactose operon of *Escherichia coli*. J. Mol. Biol. **19**: 38–51.

IPPEN, K., J. H. MILLER, J. SCAIFE, and J. R. BECKWITH, 1968 A new initiation site in the *lac* operon of *E. coli*. Nature **217**: 825–827.

JACOB, F., A. ULLMAN, and J. MONOD, 1964 Le promoteur, élément génétique necessaire à l'expression d'un opéron. Compt. Rend. Acad. Sci. Paris **258**: 3125–3128.

JORDAN, E., H. SAEDLER, and P. STARLINGER, 1967 Strong-polar mutations in the transferase gene of the galactose operon in *E. coli*. Molec. Gen. Genetics **100**: 296–306.

KAYAJANIAN, G., 1965 Ph.D. Thesis, University of Rochester.

MALAMY, M., 1966 Frameshift mutations in the lactose operon of *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **31**: 189–201.

MORSE, M. L., 1967 Reversion instability of an extreme polar mutant of the galactose operon. Genetics **56**: 331–340.

PEREIRA DA SILVA, L. H., H. EISEN, and F. JACOB, 1968 Sur la replication du bacteriophage λ. Compt. Rend. Acad. Sci. Paris **266**: 926–928.

PFEIFFER, D., and R. OELLERMANN, 1967 Mapping of *gal*$^-$ mutants by transducing *dg* carrying deletions of the *gal*-region. Molec. Gen. Genetics **99**: 248–252.

ROTHMAN, J. L., 1965 Transduction studies on the relationship between prophage and host chromosome. J. Mol. Biol. **12**: 892–912.

SAEDLER, H., and A. GULLON, L. FIETHEN, and P. STARLINGER, 1968 Negative control of the galactose operon of *E. coli*. Molec. Gen. Genetics **102**: 79–88.

SAENDLER, H., and P. STARLINGER, 1967a O° mutations in the galactose operon in *E. coli*. I. Genetic Characterization. Molec. Gen. Genetics **100**: 178–189. —— 1967b O^{0} mutations in the galactose operon in *E. coli*. II. Physiological characterization. Molec. Gen. Genetics **100**: 190–202.

SCAIFE, J., and J. R. BECKWITH, 1966 Mutational alteration of the maximal level of *lac* operon expression. Cold Spring Harbor Symp. Quant. Biol. **31**: 403–408.

SHAPIRO, J. A., 1967 The structure of the galactose operon in *Escherichia coli* K-12. Ph.D. Thesis, University of Cambridge. —— 1969 Mutations caused by the insertion of genetic material into the *galactose* operon of *Escherichia coli*. J. Mol. Biol. **40**: 93–105.

SUSSMAN, R., and F. JACOB, 1962 Sur un système de répression thermosensible chez le bacteriophage λ d'*E. coli*. Compt. Rend. Acad. Sci. Paris **254**: 1517–1519.

TAYLOR, A. L., and C. D. TURNER, 1967 Revised linkage map of *Escherichia coli*. Bacteriol. Reviews **31**: 332–353.

WALLACE, B. J., and J. PITTARD, 1967 Genetic and biochemical analysis of the isoenzymes concerned in the first reaction of aromatic biosynthesis in *Escherichia coli*. J. Bacteriol. **93**: 237–244.