THE GALACTOSE OPERON OF *E. COLI* K-12. I. STRUCTURAL AND PLEIOTROPIC MUTATIONS OF THE OPERON

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Received October 18, 1968

Escherichia coli K-12 utilizes D-galactose as sole source of carbon and energy by the the Leloir pathway (KALCKAR, KURAHASHI and JORDAN 1959). Galactose can be taken up from the growth medium by several different permeation systems (GANESAN and ROTMAN 1966). The three enzymatic reactions specific to metabolism of endogenous galactose are:

$$(1)\ \text{Gal}^3 + \text{ATP} \xrightarrow{\text{kinase}} \text{Gal-1-P} + \text{ADP}$$

$$(2)\ \text{Gal-1-P} + \text{UDPG} \xrightleftharpoons{\text{transferase}} \text{UDPGal} + \text{Glu-1-P}$$

$$(3)\ \text{UDPGal} \xrightleftharpoons{\text{epimerase}} \text{UDPG}$$

The sum of these three reactions is:

$$\text{Gal} + \text{ATP} \longrightarrow \text{Glu-1-P} + \text{ADP}$$

Glu-1-P is subsequently converted by phosphoglucomutase to Glu-6-P, a glycolytic intermediate, for the complete catabolism of galactose. Each reaction of the Leloir pathway is catalyzed by a well-characterized enzyme: (1) galactokinase (kinase); (2) galactose-1-phosphate uridyl transferase (transferase); and (3) uridinediphosphogalactose-4-epimerase (epimerase). A mutational defect in the structural gene for any one of these enzymes (k^-, t^-, or e^- mutations) results in the loss of ability to utilize galactose as a growth substrate. Since UDPG is essential for reaction (2), even though it is regenerated, a mutation in the structural gene for uridinediphosphoglucose pyrophosphorylase also results in a galactose-negative phenotype. We shall call this gene *GalU* (TAYLOR 1967) and mutants unable to synthesize UDPG u^-.

Previous biochemical studies have shown that synthesis of the three Leloir enzymes in *E. coli* is coordinately induced from 10- to 25-fold by D-galactose or its non-metabolizable analogue, D-fucose (KALCKAR *et al.* 1959; BUTTIN 1963a). Genetic studies have established definitively that the structural genes for these

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[3] Abbreviations used: Gal, D-galactose; Gal-1-P, galactose-1-phosphate; UDPG, uridine diphosphoglucose; UDPGal, uridine diphosphogalactose; Glu-1-P, glucose-1-phosphate; Glu-6-P, glucose-6-phosphate; 2AP: 2-aminopurine; NTG, N-methyl-N′-nitro-N-nitrosoguanidine.

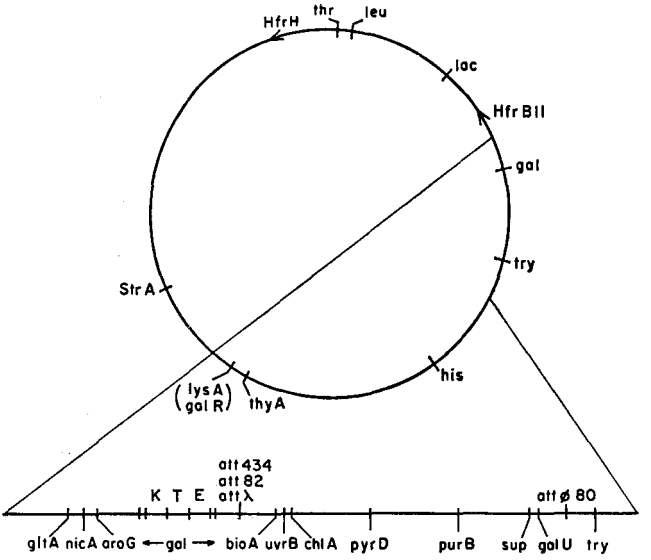

FIGURE 1.—The circular linkage group of *Escherichia coli* K-12 with the *gal-try* region expanded. The symbols used are those defined by TAYLOR (1967). Note that the order of some markers in the interval between *gltA* and *chlA* has been corrected from that of TAYLOR (1967) (SHAPIRO and ADHYA 1968; ADHYA, CLEARY and CAMPBELL 1968; W. EPSTEIN, personal communication).

three enzymes (GalK, T, and E) are tightly clustered on the bacterial chromosome in a region subject to specialized transduction by phage λ and that their order is as shown in Figure 1 (ADLER and TEMPLETON 1963). *GalU* is located near the tryptophan locus in a region subject to specialized transduction by phage φ80 (SHAPIRO 1966; ADHYA 1966) and synthesis of UDPG pyrophosphorylase appears to be constitutive (SUNDARARAJAN, RAPIN and KALCKAR 1962). In addition to these loci, BUTTIN (1963b) identified two regulatory sites. One near the lysine and thymine loci on the bacterial chromosome (see Figure 1) contains constitutive mutations which BUTTIN identified as lesions of the R_{gal} gene coding for a cytoplasmic repressor; the other contains a *cis*-dominant constitutive mutation of the o^c type (JACOB and MONOD 1961) located near the epimerase end of the galactose gene cluster. On the basis of all these results, BUTTIN concluded that the three genes coding for the galactose enzymes are part of a negatively controlled polarized operon of the type specified by the JACOB-MONOD theory. This conclusion has since been supported by results showing that a constitutive mutation of the R_{gal} locus is recessive to the wild-type allele (ADHYA and ECHOLS 1966), and that the three galactose enzymes are synthesized sequentially in the order epimerase-transferase-kinase (MICHAELIS and STARLINGER 1967).

The purpose of this and the following communication is to establish the polarity of the galactose operon and describe the properties of many new galactose-negative mutations, in particular polar and regulatory mutations of the galactose operon. Our results fully agree with BUTTIN's conclusion and demonstrate that,

as far as we can tell, the regulation of the galactose operon is analogous to that of the lactose operon (see BECKWITH 1967). On the basis of enzymatic and phenotypic studies, we have distinguished three *cis*-dominant mutations which appear to affect regulatory elements essential, or rate-limiting, for operon function.

Moreover, we have identified a class of spontaneous mutations that appear to be the results of genetic changes different from base substitution, frameshift, or extended deletion. Recently SAEDLER and STARLINGER (1967a, b) and JORDAN, SAEDLER and STARLINGER (1967) have independently isolated a similar set of spontaneous *gal*$^-$ mutants, with almost identical properties.

MATERIALS AND METHODS

Bacterial strains: The bacterial strains from which *gal*$^-$ derivatives were isolated either by mutagenesis and selection or by recombination are listed in Table 1. Also listed are four *gal* deletion strains used in suppression tests. Strains carrying the gal_3 and gal_9 mutations were obtained from J. ADLER. The derivatives of HfrH (carrying the OS103, OS137, OS128, OA104, and ON141 *kte*$^-$ mutations were obtained from H. SAEDLER. Strains carrying all but one of the wild-type and mutant F_8-*gal* episomes used in complementation tests have been described in ECHOLS, REZNICHEK, and ADHYA (1963). Strain EM1 carries an F_8-*gal* episome and is homogenotic for the PL2 *e*$^-$ mutation of BUTTIN (1963b). Strains WK1 (from H. SAEDLER) and WB2 (from S. BRENNER) are, respectively, lysogens for λ*dg*+ and λ*dg*$^-$ (carrying the gal_1 (*t*$^-$) mutation). Active double lysogens for φ80 and φ80 transducing phages carrying the su_c, su_4 and su_{III} suppressors were obtained from E. SIGNER, E. GALLUCCI, and S. BRENNER.

Bacteriophages: Strains lysogenic for various transducing phage were induced to provide HFT lysates used in the suppression and complementation tests described below. We note that $su^+{}_3$ and $su^+{}_{III}$ are the same suppressor (GAREN 1968). Derivatives of phage P1kc were obtained from W. DOVE and L. CARO. Phage C21 comes from S. BRENNER.

Media:

1) *T broth:* 10 g Bactotryptone and 5 g NaCl per liter.

TABLE 1

E. coli *K-12 strains used*

Strain	Mating type	Genotype	Source
W3100	F$^-$	*gal*+ *str*^{s}(λ)	J. ADLER
B78A	F$^-$	*gal*+ *thr*$^-$ *leu*$^-$ *thi*$^-$ $su^+{}_{II}$ *galR*$^-$ (λ)	G. BUTTIN (1963b)
HfrH	HfrH	*gal*+ *thi*$^-$ *str*^{s}	W. HAYES
H81–2	HfrH	*gal*+ *O*^{c} *thi*$^-$ *str*^{s}	G. BUTTIN (1963b)
B113-U7	HfrB11	*purB*$^-$ *his*$^-$ *met*$^-$ *gal*$^-$ ($u^-{}_{u106}$) *str*^{r}	J. A. SHAPIRO (1967)
MS0	F$^-$	*gal*+ *pyrD*$^-$ *his*$^-$ *thi*$^-$ *str*^{r}	M25U of J. A. SHAPIRO (1966)
H80	HfrH	$[\lambda\text{-}gal]_{\Delta 506}$ *thi*$^-$ *T1*^{r} λ^r *str*^{s}	J. A. SHAPIRO and S. ADHYA (1969)
S165	F$^-$	$gal^-_{\Delta S165}$ *his*$^-$ *str*^{r}	J. A. SHAPIRO and S. ADHYA (1969)
S1652	F$^-$	$gal^-_{\Delta S165}$ $su^+{}_I$ *str*^{r}	J. A. SHAPIRO (1967)
S1654	F$^-$	$gal^-_{\Delta S165}$ *his*$^-$ $su^+{}_{II}$ *str*^{r}	S. ADHYA (unpublished)
S1656	F$^-$	$gal^-_{\Delta S165}$ *his*$^-$ $su^+{}_B$ *str*^{r}	S. ADHYA (unpublished)

In addition to standard terminology we have adopted the convention of indicating deletions by a subscript Δ followed by the marker number. The origins and directions of transfer of HfrH and B11 are shown in Figure 1. Suppressor nomenclature is the same as that of BRENNER and BECKWITH (1965) and GALLUCCI and GAREN (1966).

2) *EMBGal agar:* 10 g Bactotryptone, 1 g yeast extract, 5 g NaCl, 2.5 g eosin methylene blue dye powder, 15 g agar per liter, 100 ml of sterile 10% galactose added after autoclaving.

3) *GZ agar:* 25.5 g Difco Antibiotic Medium No. 2, 50 mg 2,3,5-triphenyltetrazolium chloride per liter. Sterile galactose added to 1% after autoclaving. Note that the tetrazolium salt is sterilized with the agar base and that isolated *gal*$^-$ colonies are red, isolated *gal*+ colonies white on this medium.

4) *Penassay broth:* 17.5 g Penassay broth (Difco) per liter.

5) *7.OP Medium:* 13.6 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4 \cdot 7H_2O$, and 0.5 mg $FeSO_4 \cdot 7H_2O$ per liter. The pH was adjusted to 7.0 before sterilization. Carbon sources were added at 0.3% concentration. Amino acids and thiamin when needed for growth were supplied at concentrations of 20 and 1 μg/ml, respectively.

6) *M9 Medium:* 7.0 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl, and 0.2 g $MgSO_4$ per liter. Carbon sources added at 0.25% concentration. Amino acids and thiamin added as above; purine and pyrimidine bases added at 25 μg/ml final concentration when required.

7) *L broth:* 5 g yeast extract, 10 g Bactotryptone, and 10 g NaCl per liter.

Liquid media were solidified with 15 g agar per liter for plating bacteria and either 12 or 10 g per liter for plating phage. Soft agar contained 7 g agar per liter. When required, streptomycin was added at 125 μg/ml.

Isolation of gal$^-$ *mutants:*

1) *Mutagenesis:* Exponential cultures of the *gal*+ strains W3100, B78A, HfrH, or H81–2 in 7.OP or M9 medium were treated with 2AP (0.5 mg/ml for 24 hrs), NTG (0.8 mg/ml for 6 hrs), or ultraviolet light (various doses).

2) *Penicillin selection:* Non-mutagenized or mutagenized cultures of the various *gal*+ strains which had been allowed to express *gal*$^-$ mutations were centrifuged and resuspended in galactose medium containing either 2000 or 3000 units of penicillin/ml and incubated for 2 or 20 hrs at 37°C with aeration. This treatment generally increased the frequency of *gal*$^-$ mutants in a culture by at least 1000-fold.

3) *Isolation of galactose-resistant mutants from strain B113-U7:* Small inocula of the galactose-sensitive *u*$^-$ mutant Hfr B113-U7 (approximately 100 cells) were put into tubes of Penassay broth and allowed to grow overnight at 37°C. An aliquot of each of these cultures was then plated on appropriately supplemented M9-glycerol agar containing 5×10^{-3} M D-galactose and incubated 72 hrs at 37°C. About 50 to 100 galactose-resistant colonies appeared per 10^7 to 10^8 cells plated. This low frequency of resistant clones precluded the possibility that any of the inocula contained pre-existing galactose-resistant mutants. One colony from each culture was picked, repurified, and crossed to strain MSO to obtain *F*$^-$*gal*$^-$*his*$^-$*str*$^-$ recombinants which were then further studied. This rather laborious technique ensured that all of the mutations studied were of independent origin.

Construction of isogenic gal$^-$ *strains:* To avoid variations in enzyme levels due to differences of genetic background, all the *gal*$^-$ mutations not isolated from HfrH or H81–2 were introduced into strains descended from HfrH by P1 transduction (LENNOX 1955). This task was facilitated by the isolation of a derivative of HfrH (strain H80) which carries a large deletion of the galactose region of the bacterial chromosome leading to a nicotinic acid requirement (see SHAPIRO and ADHYA 1969). When H80 is transduced to prototrophy by P1, it invariably inherits the galactose operon of the donor strain, which can then be crossed into strain MSO (likewise descended from HfrH) by conjugation. In this way, a series of F$^-$*gal*$^-$*his*$^-$*str*^{r} strains practically isogenic with one another and closely related to HfrH was built up. Only enzyme assays performed on these strains or on UV-induced mutants of HfrH or H81–2 are reported here.

Suppression tests: 1) *By* λdg *transduction: gal*$^-$ mutants lysogenic for phage λ were induced to produce LFT lysates. The presence of λ*dg* phage in these lysates was confirmed by transduction of appropriate *gal*$^-$ recipients. They were then assayed for transduction of strains carrying both an *amber* or *ochre* suppressor and the large S165 deletion which removes the entire epimerase and transferase genes and most of the kinase gene (SHAPIRO and ADHYA 1969). Positive transduction results were interpreted as the result of suppression of the *gal*$^-$ mutation by the corresponding suppressor. The non-suppressing deletion strain S165 was used as a control.

2) *By φ80 transduction:* About 5×10^8 cells were plated on 7.OP or M9 galactose agar and spotted with a drop of an HFT lysate of either $\phi 80 dsu^+_c$, $\phi 80 dsu^+_3$, $\phi 80 dsu^+_4$, or $\phi 80 dsu^+_{III}$. Heavy growth in the region of the spot after 96 hrs incubation at 30°C was scored as a positive suppression result. The results of tests with the su^+_3 and su^+_{III} phage have been presented together.

3) *By conjugation:* HfrH str^s strains carrying various gal^- mutations (either those isolated from HfrH or introduced into strain H80 by P1 transduction) were cross-streaked against a lawn of $F^- str^r$ strains carrying an *amber* or *ochre* suppressor and the S165 deletion on 7.OP- or M9-galactose agar containing histidine and streptomycin. These plates were then incubated 96 hrs at 30°C; the presence of $gal^+ str^r$ recombinants was scored as a positive suppression result. The non-suppressing strain S165 was used as a control recipient.

Reversion tests: Mutagen-induced reversion was determined by the method of WHITFIELD, MARTIN and AMES (1966), using 7.OP- or M9-galactose agar as a test medium. The acridine mustards ICR170 and ICR191A were a gift of Dr. HUGH J. CREECH of the Institute for Cancer Research, Philadelphia. Because strains responded identically to these two compounds, we have pooled the results presented in Table 3. Strains which showed no spontaneous or mutagen-induced reversion on 7.OP- or M9-galactose agar were also tested on EMBGal agar because the background growth of gal^- cells on this medium permits the detection of very rare reversion events as black papillae. Those mutations which we have scored as completely non-reverting do not show reversion on either synthetic or complete indicator media.

Enzyme assays: 1) The assays of kinase, transferase, and epimerase reported in Table 4 were done as described previously (ECHOLS, REZNICHEK, and ADHYA 1963). For these assays cells were grown for 4–5 generations at 37°C by rotary shaking in a 1000 ml Erlenmeyer flask containing 200 ml of 7.OP medium (supplemented with 0.1% casamino acids) and 10^{-3} M D-fucose. The cells were then chilled, centrifuged, washed, resuspended and sonicated in potassium phosphate mercaptoacetic acid buffer (SHERMAN and ADLER 1963). Sonic treatment was carried out for 2 min in a Raytheon 10-KC oscillator, and the lysate centrifuged at $10{,}000 \times g$ for 10 min. The supernatant fluid was stored frozen and used for enzyme assays within 24 hrs. The protein concentrations in the extracts were determined by the method of LOWRY, ROSEBROUGH, FARR and RANDALL (1951).

2) The assays of galactokinase reported in Table 5 were performed by the method of BUTTIN (1963a) following toluene extraction. Cultures were grown with aeration at 37°C in M9-glycerol medium (supplemented with 0.4% casamino acids); induced cultures were grown in the presence of 2×10^{-3} M D-fucose for at least five generations before extraction. Protein concentrations were determined by O.D. readings of each culture before extraction on an EEL "Unigalvo" nephelometer.

RESULTS

Classification of gal^- *mutants by complementation and phenotype tests:* Approximately 400 gal^- mutants were isolated by two methods from various parent strains carrying a wild-type *gal* operon. The first method consisted of penicillin selection of the gal^+ strains W3100, B78A, HfrH or H81–2 following (in all but a few cases) mutagenesis with NTG, 2AP, or UV irradiation. The second procedure involved selecting spontaneous galactose-resistant mutants from the galactose-sensitive Hfr strain B113-U7 and crossing the resulting mutant *gal* operons into an appropriate recipient as described in MATERIALS and METHODS. The first selection procedure is non-specific and was intended to provide a complete spectrum of all possible mutations causing a gal^- phenotype. The second selection procedure depends on the fact that strains carrying u^- mutations cannot carry out the second reaction of the Leloir pathway and are prevented from growing in the presence of galactose by the resultant accumulation of gal-1-P (SUNDARA-

RAJAN, RAPIN and KALCKAR 1962). Because any mutation in the galactose operon resulting in the loss of significant kinase activity prevents the formation of high levels of gal-1-P, this procedure specifically selects mutations of the kinase gene and polar and regulatory mutations which strongly depress expression of the kinase gene. Although these two selection procedures were initially employed with no other *a priori* assumptions as to what sort of mutations they would yield, we shall see that in fact the two groups of mutations present some striking differences which it will be useful to consider as due to their different origins.

In addition to our newly isolated *gal*$^-$ mutations, we have also included the gal_3 and gal_9 mutations from the LEDERBERG collection (see HILL and ECHOLS 1966) and several mutations independently isolated by H. SAEDLER (SAEDLER and STARLINGER 1967a) using a technique analogous to the second procedure outlined above.

gal$^-$ mutations in the galactose operon were detected by F-duction with F_8-*gal*$^+$ episome carrying an intact, wild-type galactose operon. Of these, 285 were further classified by standard complementation tests with λdg or F_8-*gal* episomes carrying known non-polar mutations in each of the three structural genes (cf. ECHOLS, REZNICHEK and ADHYA 1963). We also tested many of the mutants for sensitivity to galactose (mutants defective in either transferase or epimerase but not kinase activity accumulate phosphorylated intermediates which inhibit growth in the presence of galactose; NIKAIDO 1961; SUNDARARAJAN 1963) and to phage C21 (which only grows on *E. coli* strains defective in the *galE* or *galU* genes; SHEDLOVSKY and BRENNER 1963). The nature of our classification scheme is summarized in Table 2.

Those strains which clearly behaved as e^- in the complementation tests and were resistant to phage C21 appear to be more or less leaky with respect to epimerase activity; hence it seems that an absolute block in the epimerase gene

TABLE 2

Classification of gal$^-$ *mutants*

Complementation with episomes carrying mutations			Sensitivity to		
k^-	t^-	e^-	Phage C21	Galactose	Classification
—	+	+	r	r	k^-
+	—	+	r	s	t^-
+	+	—	s	s	e^-
—	—	+	r	r	kt^-
—	—	—	s	s	e^- polar
—	—	—	s	r	kte^-
—	—	—	r	r	kte^-

Complementation tests were performed as described previously (ECHOLS, REZNICHEK, and ADHYA 1963). Sensitivity to phage C21 was tested by spotting cultures on L-broth plates with a phage lysate. Sensitivity to galactose was determined by replica-plating to appropriately supplemented glycerol-M9 agar containing 5×10^{-3} M D-galactose. It should be noted that the distinction between "e^- polar" and "kte^-" is empirical; galactose-sensitive, phage C21^s strains were assumed to have significant levels of kinase (and transferase) activity, but to be completely defective in epimerase activity.

is needed to confer sensitivity to phage C21 (cf. SAEDLER and STARLINGER 1967b). It should be mentioned that no *gal*⁻ mutant we have tested complemented episomes mutant in each of the three structural genes. These three genes appear to be, therefore, monocistronic (BENZER 1955).

Suppression and reversion tests: To determine the nature of the various *gal*⁻ mutations, in particular, of those with possible polar or pleiotropic effects, suppression and reversion tests were carried out as described in MATERIALS and METHODS. An extract of the results of these tests is given in Table 3; more complete data have been presented elsewhere (ADHYA 1966; SHAPIRO 1967). We have not included *k*⁻ mutations.

Among the penicillin-selected mutants (Table 3a), at least twenty-six are suppressible by one or more of the *amber* and *ochre* suppressors we have used. Eighteen of these are of the *t*⁻ or *kt*⁻ type. Since all these mutations have been mapped in the *galT* gene (SHAPIRO and ADHYA 1969), we conclude that they are nonsense mutations of the structural gene for the transferase enzyme. According to the classification of BRENNER and BECKWITH (1965),[4] sixteen are *amber* and two *ochre* mutations. The remaining eight suppressible mutations are either *e*⁻ or *e*⁻ polar mutations. All have been mapped in the *galE* gene (SHAPIRO and ADHYA 1969) and are, therefore, considered to be nonsense mutations of the structural gene for the epimerase enzyme. Five are *amber* and three *ochre* mutations. The enzyme assays of strains carrying these suppressible mutations reported below (Table 4a) are in agreement with the above assignments.

None of the spontaneous mutations obtained from the selection for galactose resistance is sensitive to suppression by either the su^{+}_{III} *amber* or su^{+}_{c} *ochre* suppressor. Although we have not systematically tested the other suppressors, this result makes it doubtful that the spontaneous mutations are *ambers* or *ochres*, for it can be seen that all but three of our identifiable nonsense mutations are suppressible by one of these two suppressors. The possibility remains that these mutations represent UGA nonsense codons (BRENNER, BARNETT, KATZ and CRICK 1967). This too appears unlikely because of the negative results on mutagen-induced reversion of these mutations discussed below. This confirms the results of JORDAN, SAEDLER and STARLINGER (1967).

The results of reversion tests given in Table 3a show that seven of the mutations tested (SA164, SA118, SA154, A28, SA41, SA50, and SA108) can be induced to revert by at least one of the acridine mustards, ICR170 and ICR191A. These compounds have previously been reported to revert frameshift mutations of the Salmonella histidine operon (WHITFIELD, MARTIN and AMES 1966). However, it is unlikely that any of these seven mutations is a frameshift because all but one (A28) have been observed to revert in response to base-substitution mutagens (2AP and/or NTG), and the A28 mutation which we do not find revertible by NTG, together with SA41 and SA50, have been identified as *ambers*. Hence, we have no direct evidence that any of our mutations is a frameshift, nor do we find any correlation between the mutagen's induced reversion pattern and the nature of mutations (Table 3, cf. WHITFIELD *et al.* 1966).

[4] *Viz. amber* suppressors suppress only *amber* mutations; *ochre* suppressors suppress both *amber* and *ochre* mutations.

TABLE 3

Genetic characterization of gal⁻ *mutations*

Mutation	Classification	Phage C21	Reversion				Suppression					
							Amber			Ochre		
			Spon.	2AP	NTG	ICR	su_I	su_{II}	su_{III}	su_B	su_e	su_4
(a) Mutations obtained after penicillin-selection												
SA101	e^-	s	+	+	+	—	—	—	—	—	—	—
SA157	e^-	s	—	..	—	—	—	—	—	—	—	—
UV209	e^-	s	—	..	—	—	—	..	—	..	—	..
UV232	e^-	s	+	..	..	..	+	+	+	+	+	+
UV867	e^- (o^c)	s	+	..	..	..	—	—	—	—	+	+
SA89	e^- polar	s	+	+	—	—	—	—	—	—	+	+
SA95	e^- polar	s	+	+	+	—	—	—	—	—	+	+
SA121	e^- polar	s	+	+	+	..	—	—	—	—	—	—
SA164	e^- polar	s	—	+	+	+	—	—	—	—	—	—
UV39	e^- polar	s	+	..	+	..	—	+	—	+	—	—
UV49	e^- polar	s	+	..	+	..	—	+	—	+	—	—
UV57	e^- polar	s	+	..	+	..	—	+	—	+	—	—
UV229	e^- polar	s	+	..	..	..	+	+	+	+	+	+
A22	t^-	r	—	..	—	—	—	—	..	..	..	..
UV25	t^-	r	..	..	..	..	—	—	—	—	—	—
SA59	t^-	r	+	+	+	—	—	+	+	+	+	+
SA79	t^-	r	+	+	+	—	+	+	+	+	+	+
SA104	t^-	r	+	+	+	—	—	+	+	—	+	+
SA118	t^-	r	+	+	+	+	—	—	—	—	—	—
SA133	t^-	r	+	+	+	—	—	—	—	—	—	—
SA146	t^-	r	+	+	+	—	—	—	—	—	—	—
SA151	t^-	r	+	+	+	—	—	+	+	—	+	+
SA152	t^-	r	+	+	+	—	—	+	+	—	+	+
SA154	t^-	r	+	+	+	+	—	—	—	—	—	—
SA155	t^-	r	+	+	+	—	—	—	+	—	+	+
SA177	t^-	r	+	+	+	—	—	—	—	—	—	—
UV226	t^-	r	+	..	..	..	+	+	+	—	+	+
UV228	t^-	r	+	..	..	..	+	+	+	—	+	+
A21	kt^-	r	+	..	+	—	—	—	+	—	+	+
A28	kt^-	r	+	..	—	+	—	—	+	—	+	+
SA41	kt^-	r	+	+	+	+	—	+	+	—	+	+
SA49	kt^-	r	+	—	—	—	—	+	+	—	+	+
SA50	kt^-	r	+	—	+	+	—	—	+	—	+	+
SA60	kt^-	r	—	—	—	—	—	—	—	—	—	—
SA99	kt^-	r	+	—	+	—	—	—	—	—	—	—
SA106	kt^-	r	+	+	+	—	—	—	—	—	—	—
SA107	kt^-	r	+	+	+	—	—	—	—	—	—	—
SA108	kt^-	r	+	+	—	+	—	—	—	—	—	—
SA130	kt^-	r	+	—	—	—	—	—	+	—	+	+
SA167	kt^-	r	+	+	+	—	—	+	+	—	+	+
SA171	kt^-	r	+	+	+	—	—	—	—	—	—	—
UV30	kt^-	r	..	..	..	..	—	—	—	—	—	—
UV35	kt^-	r	+	..	..	..	—	—	—	—	+	+
UV41	kt^-	r	..	..	..	..	—	—	—	—	—	—
UV60	kt^-	r	+	+	..	..	—	—	—	—	+	+

TABLE 3—Continued

Mutation	Classification	Phage C21	Reversion				Suppression					
							Amber			Ochre		
			Spon.	2AP	NTG	ICR	su_I	su_{II}	su_{III}	su_B	su_c	su_4
UV76	*kt*⁻	r	+	..	..	..	+	+	+	—	+	+
UV222	*kt*⁻	r	..	..	..	..	—	—	—	—	—	—
UV230	*kt*⁻	r	..	..	..	..	—	—	—	—	—	—
UV211	*kte*⁻	r	+	..	..	..	—	—	—	—	—	—
		(b) Mutations obtained after selection for galactose resistance										
S101	*kt*⁻	r	—	—	—	—	..	..	—	..	—	—
S104	*kt*⁻	r	+	—	—	—	..	..	—	..	—	—
S114	*kt*⁻	r	—	—	—	—	..	..	—	..	—	..
S115	*kt*⁻	r	—	—	—	—	..	..	—	..	—	..
S139	*kt*⁻	r	—	—	—	—	..	..	—	..	—	..
S140	*kt*⁻	r	—	—	—	—	..	..	—	..	—	..
S142	*kt*⁻	r	—	—	—	—	..	..	—	..	—	..
S161	*kt*⁻	r	—	—	—	—	..	..	—	..	—	..
S168	*kt*⁻	r	—	—	—	—	..	..	—	..	—	..
S182	*kt*⁻	r	—	—	—	—	..	..	—	..	—	..
S188	*kt*⁻	r	+	—	—	—	..	..	—	..	—	..
S108	*kte*⁻	s	+	—	—	—	..	..	—	..	—	..
S148	*kte*⁻	s	—	—	—	—	..	..	—	..	—	..
S187	*kte*⁻	s	+	—	—	—	..	..	—	..	—	—
OS103*	*kte*⁻	s	+	..	..	..	..	..	..	..	..	..
OS137*	*kte*⁻	s	+	..	..	..	..	..	..	..	..	..
OS128*	*kte*⁻	r	+	..	..	..	..	..	..	..	..	..
OA104*	*kte*⁻	s	+	..	..	..	..	..	..	..	..	..
ON141*	*kte*⁻	s	+	..	..	..	..	..	..	..	..	..
		(c) Mutations from the LEDERBERG collection										
gal_3	*kte*⁻	r	+	—	—	—	—	—	—	—	—	—
gal_9	*kte*⁻	s	+	—	—	—	—	—	—	—	—	—

Suppression and reversion tests were performed as described in MATERIALS AND METHODS. Sensitivity to phage C21 was tested as described in the legend to TABLE 2. Mutations labeled *UV*-- were obtained from HfrH and UV mutagenesis, with the exception of UV 867 which comes from H81–2. Mutations labelled SA-- were obtained from W3100 after NTG or 2AP mutagenesis. Mutations A21 and A28 arose spontaneously in B78A. Mutations labelled S--- come from independent spontaneous galactose-resistant mutants of strain B113-U7. The OS---, OA---, and ON--- mutations come from galactose-resistant mutants of a *u*⁻ mutant of HfrH (SAEDLER and STARLINGER, 1967a).

* Reversion and suppression data given in SAEDLER and STARLINGER (1967a).

Four of the penicillin-selected mutations did not revert spontaneously or in the presence of any of the mutagens tested. It is possible that these are the results of deletions larger than a few bases. One of these has been shown by enzyme assay (UV209, Table 5a) and one by complementation tests (SA60, Table 3a) to have polar or pleiotropic effects. They could, therefore, be deletions of $3n + 1$ nucleotide pairs and have frameshift effects. We will pursue this point further with respect to the UV209 mutation in the following communication (SHAPIRO and ADHYA 1969).

TABLE 4

Enzyme levels in induced cultures

Mutation	Classification	Suppression	Induced enzyme activity		
			Kinase	Transferase	Epimerase
Wild type	*gal*+	.....	10	10	10
(a) Mutations obtained after penicillin-selection					
SA101	*e*⁻	.....	4.2	6.2	0.1
UV232	*e*⁻	amber	5.1	5.0	0.1
UV867*	*e*⁻ *o*^{c}	ochre	"5.9"	"6.5"	"0.2"
SA89	*e*⁻ polar	ochre	1.0	1.2	0.1
SA95	*e*⁻ polar	ochre	0.7	0.6	0.1
SA121	*e*⁻ polar	.....	2.0	2.4	0.1
UV39	*e*⁻ polar	amber	1.2	1.5	0.1
UV49	*e*⁻ polar	amber	0.8	1.0	0.1
UV57	*e*⁻ polar	amber	1.7	1.9	0.1
UV229	*e*⁻ polar	amber	1.6	1.2	0.1
UV25	*t*⁻	.....	7.9	0.4	10
SA59	*t*⁻	amber	12	0.5	10
SA79	*t*⁻	amber	8.2	0.2	11
SA104	*t*⁻	amber	5.0	0.5	11
SA133	*t*⁻	.....	8.9	0.6	9.2
SA146	*t*⁻	.....	8.9	0.4	9.2
SA151	*t*⁻	amber	9.1	0.7	9.2
SA152	*t*⁻	amber	10	0.6	9.2
SA155	*t*⁻	amber	4.7	0.2	9.2
SA177	*t*⁻	.....	10	0.6	8.5
UV226	*kt*⁻	amber	2.8	0.5	8.5
UV228	*kt*⁻	amber	1.8	0.4	11.5
UV35	*kt*⁻	ochre	2.7	0.5	9.2
UV76	*kt*⁻	amber	2.5	0.4	7.7
UV60	*kt*⁻	ochre	2.5	0.2	6.2
A28	*kt*⁻	amber	4.0	0.2	7.7
A21	*kt*⁻	amber	1.5	0.2	10
SA41	*kt*⁻	amber	5.1	0.4	10
SA49	*kt*⁻	amber	3.0	0.2	8.5
SA50	*kt*⁻	amber	2.6	0.4	8.5
SA130	*kt*⁻	amber	1.7	0.5	10
SA167	*kt*⁻	amber	5.5	0.6	9.2
UV24	*kte*⁻ (C21^r)	.....	1.4	2.5	1.9
(b) gal_3 and mutations obtained after selection for resistance to galactose					
S101	*kt*⁻	.....	0.4	0.9	14
S114	*kt*⁻	.....	0.5	0.5	11.5
S187	*kte*⁻ (C21^s)	.....	0.6	0.6	0.1
OA104	*kte*⁻ (C21^s)	.....	0.5	0.6	0.1
gal_3	*kte*⁻ (C21^r)	.....	0.1	0.2	0.1

Assays were performed as described in MATERIALS and METHODS. Results for each enzyme were normalized to the specific activity of a strain carrying the *gal*+ operon of W3100. We have not subtracted background from these data. The values for the strain carrying the UV 867 *ochre* mutation are in inverted commas because we have not normalized these figures to a strain carrying the parental *gal*+ *o*^{c} operon of H81–2. All assays were repeated at least once.

* N. B. No *gal*+ *o*^{c} control in these experiments.

The spontaneous mutations obtained from the selection for galactose-resistance (Table 3b) are again noteworthy because none of them is induced to revert by either NTG or the acridine mustards. Many of them do not revert at all, while those which revert do so at high frequency (on the order of 10^{-7} to 10^{-6} or higher). Hence, we cannot directly identify any of these mutations as a possible base-substitution or frameshift. Our results confirm those of SAEDLER and STARLINGER (1967a) and JORDAN, SAEDLER and STARLINGER (1967).

The gal_3 and gal_9 *kts*⁻ mutations, both of which revert spontaneously (HILL and ECHOLS 1966; MORSE 1967), behaved in suppression and mutagen-induced reversion tests as did the spontaneous mutations (Table 3a).

Enzyme assays: Otherwise isogenic strains carrying all of the suppressible nonsense mutations, some of the other penicillin-selected mutations, and some of the spontaneous mutations selected for galactose resistance were screened for their induced levels of the three galactose enzymes. The results are given in Table 4.

All but three of the suppressible nonsense mutations have polar effects of varying degrees (Table 4a). Those which we have concluded to be mutations of the transferase gene have reduced levels of kinase activity but normal levels of epimerase activity, while nonsense mutations of the epimerase gene have reduced levels of both kinase and transferase activities. It can be seen, moreover, that the polar effects of *e*⁻ nonsense mutations are manifested to approximately the same extent on both activities.

In a different experiment, strains carrying eleven nonsense mutations isolated from wild-type, inducible parent strains were assayed for induction of galactokinase activity by D-fucose. The results are presented in Table 5a. The polar effect of these nonsense mutations on kinase activity is again clear, and the strains are inducible by the normal 10- to 15-fold with one exception (that carrying the UV49 *e*⁻ *amber* mutation). Thus, polar nonsense mutations do not interfere with normal induction of the galactose operon. The results of assaying kinase activity in strains carrying the UV867 *e*⁻ *ochre* mutation isolated from the operator-constitutive strain H81–2 (BUTTIN 1963b) and the completely non-reverting UV209 *e*⁻ mutation are also presented. Both mutations are polar, and kinase activity is clearly constitutive in the *e*⁻ o^c strain. It can also be seen that kinase activity in a strain carrying the UV211 *kte*⁻ mutation is normally inducible.

The spontaneous mutations obtained from the selection for galactose-resistance are again distinct, their polar or pleiotropic effects on kinase activity being uniformly much more severely negative than those of penicillin-selected polar mutations (Tables 4b and 5b). The results of tests for inducibility of kinase activity in strains carrying some of these spontaneous mutations are given in Table 5b. Those mutations which permit detectable levels of kinase activity (the S108, OS114, and OS128 *kte*⁻ mutations and the S104 and S188 *kt*⁻ mutations) do not seem to eliminate inducibility of this enzyme. It is interesting to note that all five of these mutations revert spontaneously (Table 3b). However, the results of JORDAN, SAEDLER and STARLINGER (1967) on inducibility of such a mutation (strain OS128) differ from ours.

Sensitivity of kte⁻ *mutants to phage C21:* Among the classes of *kte*⁻ mutations

TABLE 5

Inducibility of galactokinase activity

Mutation	Classification	Suppression	Galactokinase activity –FU	Galactokinase activity +FU
wild type	*gal*+	.....	0.8	10
	(a) Mutations obtained following penicillin-selection			
UV39	*e*⁻ polar	amber	<0.1 (2)	0.8 (2)
UV49	*e*⁻ polar	amber	0.3 (1)	0.85(2)
UV57	*e*⁻ polar	amber	<0.1 (1)	0.78(2)
SA89	*e*⁻ polar	ochre	0.1 (2)	1.3 (2)
SA95	*e*⁻ polar	ochre	<0.1 (2)	1.5 (2)
UV229	*e*⁻ polar	amber	0.18(2)	3.0 (2)
UV232	*e*⁻	amber	0.56(3)	7.2 (3)
UV867	*e*⁻ *o*ᶜ	ochre	7.3 (2)	7.1 (1)
UV209	*e*⁻	.....	0.3 (1)	3.4 (1)
UV35	*kt*⁻	ochre	0.2 (1)	1.8 (1)
UV76	*kt*⁻	amber	0.12(1)	1.7 (1)
UV60	*kt*⁻	ochre	0.11(2)	1.35(2)
UV226	*kt*⁻	amber	0.25(1)	4.0 (1)
UV228	*t*⁻	amber	0.3 (1)	4.2 (1)
UV211	*kte*⁻ (C21ʳ)	.....	0.22(1)	2.1 (1)
	(b) Mutations obtained following selection of spontaneous galactose-resistant mutants			
S101	*kt*⁻	.....	<0.1 (1)	<0.1 (1)
S104	*kt*⁻	.....	<0.1 (2)	0.45(2)
S114	*kt*⁻	.....	<0.1 (2)	<0.1 (2)
S188	*kt*⁻	.....	<0.1 (2)	0.21(2)
S108	*kte*⁻ (C21ˢ)	.....	<0.1 (2)	0.15(1)
S148	*kte*⁻ (C21ˢ)	.....	<0.1 (2)	<0.1 (2)
S187	*kte*⁻ (C21ˢ)	.....	<0.1 (1)	<0.1 (1)
OS128	*kte*⁻ (C21ʳ)	.....	<0.1 (1)	0.7 (1)
OS137	*kte*⁻ (C21ˢ)	.....	<0.1 (1)	<0.1 (1)
ON141	*kte*⁻	.....	<0.1 (1)	<0.1 (1)

Assays performed as described in MATERIALS and METHODS. (—Fu): cultures grown without induction. (+Fu): cultures grown in presence of 2×10^{-3} M D-fucose (Sigma Chemical Co.) for at least five generations. All values have been normalized to fully induced kinase activity in parental *gal*+ strains. The numbers in parentheses indicate how many separate determinations were averaged to derive the activity reported. The background of our procedure was determined by assay of a strain carrying a deletion of the kinase gene and has been subtracted. In general, background was approximately 2–3% of fully induced wild-type activity.

selected by both procedures, two types can be readily distinguished, those which confer sensitivity to phage C21 and those which do not (Table 3). This distinction is significant because sensitivity to phage C21 appears to be a more sensitive indicator of very low levels of epimerase activity than the *in vitro* assay of the enzyme (cf. SAEDLER and STARLINGER 1967b). Thus, *kte*⁻ mutations conferring C21 sensitivity can either be very strong polar mutations of the epimerase gene or negative regulatory mutations reducing activity of all three structural genes to virtually nothing. In order to distinguish between these two possibilities we car-

TABLE 6

Resistance to phage C21 after growth in the presence of galactose

Mutation	Sensitivity to phage C21	
	−Gal	+Gal
S108	s	r
S148	s	s
S187	s	s
OS103	s	s
OA104	s	r
ON141	s	r

Single colony cultures of strains carrying the *kte*⁻ mutations to be tested were grown at 37°C in L-broth and L-broth containing 0.2% galactose. When the cultures had reached saturation, they were streaked onto either L-agar or L-agar containing 0.2% galactose as appropriate, spotted with a lysate of phage C21, and incubated overnight at 37°C, after which time lysis was scored. Results were unequivocal and reproducible in different genetic backgrounds.

ried out an *in vivo* test for coupled kinase and transferase activities in some of the *C21*^{s} *kte*⁻ strains. This test depends on the facts that UDPGal can be synthesized from exogenous galactose by the kinase and transferase reactions without the epimerase reaction and that the presence of UDPGal renders the bacterium resistant to phage C21 (SHEDLOVSKY and BRENNER 1963). Polar mutants with some remaining kinase and transferase activities should become resistant to phage C21 after growth in the presence of galactose. The results of such a test are shown in Table 6. From these results we conclude that the S108, OA104, and ON141 mutations are definitely polar mutations of the epimerase gene; the S148, S187, and OS103 mutations are either completely negative regulatory mutations or extremely strong polar mutations. It should be noted that of these seven mutations, only S148 does not revert spontaneously.

Mutations of the *kte*⁻ class which do not confer sensitivity to phage C21 are likely to have low levels of epimerase activity and to be, therefore, regulatory mutations which coordinately reduce the activities of all three genes. Among the mutations described here, UV211, gal_3, and OS128 are of this type (Table 3). As can be seen from Table 4a, the UV211 mutation does indeed lead to reduced but clearly detectable levels of all three galactose enzymes which are normally inducible (Table 5a; ADHYA, unpublished observations).

DISCUSSION AND CONCLUSIONS

Structure of the enzymes of the Leloir pathway in E. coli: Since every mutant carrying a mutation in the galactose operon we have tested could be assigned to a definite complementation class, we conclude that none of the structural genes corresponding to the kinase, transferase, and epimerase enzymes contains more than one cistron and that these enzymes are, therefore, composed either of single polypeptide chains or of identical polypeptide subunits. Mutants defective in only one of these enzymes have not been found to carry mutations outside the galactose operon.

Polarity of the galactose operon: From the results presented in Table 4 it can be seen that suppressible nonsense mutations of the transferase and epimerase genes could have polar effects. Because such polar mutations of the transferase gene are polar only for kinase activity while those of the epimerase gene are polar for both transferase and kinase activities, we confirm the biochemical observation of MICHAELIS and STARLINGER (1967) that reading of the galactose operon proceeds in the order epimerase—transferase—kinase, or as we have drawn the map of the galactose operon in Figure 1, from right to left. Thus, the orientation of the galactose operon is the same as that of the lactose operon.

This conclusion is also supported by the isolation of both *kt*$^-$ and *kte*$^-$ polar mutations after selection for loss of kinase activity, for if reading of the operon proceeded from the kinase gene to the epimerase gene, we should only find the *kte*$^-$ class of polar mutation. Moreover, an *amber* mutation (SA150) and an *ochre* mutation (SA65) of the kinase gene have each been shown not to reduce the levels of epimerase and transferase activities in strain W3100 (ADHYA, unpublished observations).

Possible regulatory sites in the galactose operon: Among the mutations classified as *kte*$^-$ by complementation and phenotypic tests, many are clearly polar mutations of the epimerase gene (see SHAPIRO and ADHYA 1969). Strains carrying these are all sensitive to phage C21. However, three *kte*$^-$ mutations—UV211, OS128, and *gal*$_3$—do not confer sensitivity to phage C21 and therefore permit some expression of the epimerase gene. They are all *cis*-dominant as shown by their complementation behavior; thus, they are not lesions of a structural gene coding for a diffusible product. The results presented in Table 4 show that UV211 and OS128 lead to a more or less coordinate reduction of all three enzyme activities. Evidence for the regulatory nature of the *gal*$_3$ mutation has been presented by HILL and ECHOLS (1966) and by MORSE (1967) who have shown that some of the spontaneous revertants of strains carrying the *gal*$_3$ mutation are constitutive for synthesis of the galactose enzymes and may mutate back to the original *gal*$_3$ type at high frequency. On the basis of these results, we conclude that these three mutations define one or more regulatory sites of the galactose operon which are not part of a structural gene. The UV211 mutation is, by virtue of its leakiness, normal inducibility, UV-induced origin, and insensitivity to nonsense suppression, analogous to the "promoter" mutations of SCAIFE and BECKWITH (1966).

Nature of the spontaneous extreme polar mutations: We have seen that the spontaneous mutations isolated by selecting for galactose resistance differ from the penicillin-selected mutations by virtue of their origin and in three other respects: (1) none is susceptible to mutagen-induced reversion (Table 3b); (2) none can be suppressed by the *su*$^+_{III}$ *amber* or *su*^+_c *ochre* suppressor (Table 3b); and (3) the polar or pleiotropic negative effects of the spontaneous mutations on kinase activity are uniformly stronger than those of the penicillin-selected mutations (Tables 4 and 5). We note, in particular, that this last difference applies to spontaneous mutations which we can classify as polar mutations of the epimerase and transferase genes. These results, although they do not permit us to rule out other explanations, argue strongly that the spontaneous extreme polar mutations

are fundamentally different from the mutations obtained by penicillin selection. Our negative reversion results and the finding in other operons that frameshift and suppressible nonsense mutations have approximately the same polar effects (MARTIN, SILBERT, SMITH and WHITFIELD 1966; IMAMOTO, ITO and YANOFSKY 1966) suggest, in particular, that these spontaneous mutations are not the result of either base substitutions or frameshifts. Although many of the spontaneous mutations described above never revert, the possibility that they are necessarily extended deletions can be excluded by genetic studies (SHAPIRO and ADHYA 1969) and by the observation that reverting spontaneous mutations display the same strong polarity and insensitivity to mutagens and suppressors. We must conclude, therefore, that the extreme polar spontaneous mutations are either the result of a special type of genetic change or that they affect certain special regions of the galactose operon. In the following communication (SHAPIRO and ADHYA 1969) we shall show that the spontaneous mutations do not necessarily affect any special region of the galactose operon and propose a specific type of chromosomal change to explain both their extreme polarity and insensitivity to mutagen-induced reversion and nonsense suppression.

We wish to thank HARRISON ECHOLS for his advice, encouragement, and active assistance in this work and GÉRARD BUTTIN, WILLIAM HAYES, SYDNEY BRENNER, HEINZ SAEDLER, JULIUS ADLER and ALLAN CAMPBELL for strains and many helpful discussions. This research was supported in part by U.S. Public Health Service Grant GM08407 and AI08573, and by a grant from the British Empire Cancer Campaign to T. ALDERSON. S. L. A. was a Stauffer Chemical Research Fellow, and J. A. S. was the recipient of scholarships from the Marshall Aid Commemoration Commission and the Wellcome Trust.

SUMMARY

Several hundred newly isolated *gal*$^-$ mutations of the galactose operon have been classified and studied by phenotypic, complementation, reversion and suppression tests and by enzyme assays. No mutations complement known defects in all three structural genes. Hence, there appear to be only three cistrons coding for diffusible products in the galactose operon; these correspond to the three enzymes of the Leloir pathway.—Polar mutations are found in the epimerase and transferase genes but not in the kinase gene. These polar mutations include *ambers* and *ochres*. From the properties of polar mutations we deduce that reading of the galactose operon proceeds in the order epimerase—transferase—kinase. Three mutations appear to reduce the activity of all three structural genes but not to abolish the activity of any one. They are all *cis*-dominant. We conclude that they identify one or more genetic elements essential or rate-limiting for operon function.—A distinct class of spontaneous extreme polar mutations has been identified. None of these mutations appears to result from a base-substitution, frameshift, or extended deletion.

LITERATURE CITED

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

ADHYA, S., P. CLEARY, and A. CAMPBELL, 1968 A deletion analysis of prophage λ and adjacent genetic region. Proc. Natl. Acad. Sci. U.S. **61**: 956–962.

ADHYA, S., and H. ECHOLS, 1966 Glucose effect and the galactose enzymes of *Escherichia coli:* correlation between glucose inhibition of induction and inducer transport. J. Bacteriol. **92**: 601–608.

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.

BECKWITH, J. R., 1967 Regulation of the *lac* operon. Science **156**: 597–604.

BENZER, S., 1955 Fine structure of a genetic region in bacteriophage. Proc. Natl. Acad. Sci. U.S. **41**: 344–354.

BRENNER, S., L. BARNETT, E. R. KATZ, and F. H. C. CRICK, 1967 UGA: a third nonsense triplet in the genetic code. Nature **213**: 449–450.

BRENNER, S., and J. R. BECKWITH, 1965 Ochre mutants, a new class of suppressible nonsense mutants. J. Mol. Biol. **13**: 629–637.

BUTTIN, G., 1963a Mecanismes 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 séquence enzymatique. J. Mol. Biol. **7**: 164–182. —— 1963b Mecanismes 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.

ECHOLS, H., J. REZNICHEK, and S. ADHYA, 1963 Complementation, recombination, and suppression in galactose negative mutants of *E. coli*. Proc. Natl. Acad. Sci. U.S. **50**: 286–293.

GALLUCCI, E., and A. GAREN, 1966 Suppressor genes for nonsense mutations. J. Mol. Biol. **15**: 193–200.

GANESAN, A. K., and B. ROTMAN, 1966 Transport systems for galactose and galactosides in *Escherichia coli*. I. Genetic determination and regulation of methyl-galactoside permease. J. Mol. Biol. **16**: 42–50.

GAREN, A., 1968 Sense and nonsense in genetic code. Science **160**: 149–159.

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.

IMAMOTO, F., J. ITO, and C. YANOFSKY, 1966 Polarity in the tryptophan operon of *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **31**: 235–249.

JACOB, F., and J. MONOD, 1961 Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. **3**: 318–356.

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

KALCKAR, H. M., K. KURAHASHI, and E. JORDAN, 1959 Hereditary defects in galactose metabolism in *Escherichia coli* mutants. I. Determination of enzyme activities. Proc. Natl. Acad. Sci. U.S. **45**: 1776–1786.

LENNOX, E. S., 1955 Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**: 190–206.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL, 1951 Protein measurement with the phenol reagent. J. Biol. Chem. **193**: 265–275.

MARTIN, R. G., D. F. SILBERT, D. W. E. SMITH, and H. J. WHITFIELD, 1966 Polarity in the histidine operon. J. Mol. Biol. **21**: 357–369.

MICHAELIS, G., and P. STARLINGER, 1967 Sequential appearance of the galactose enzymes in *E. coli*. Molec. Gen. Genetics **100**: 210–215.

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

NIKAIDO, H., 1961 Galactose-sensitive mutants of Salmonella. II. Bacteriolysis induced by galactose. Biochim. Biophys. Acta **48**: 460–469.

SAEDLER, 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° mutations in the galactose operon in *E. coli*. II. Physiological characterization. Molec. Gen. Genetics **100**: 190–202.

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

SHAPIRO, J. A., 1966 Chromosomal location of the gene determining uridine diphosphoglucose formation in *Escherichia coli* K-12. J. Bacteriol. **92**: 518–520.—— 1967 The structure of the galactose operon in *Escherichia coli* K-12. Ph.D. Thesis, University of Cambridge.

SHAPIRO, J. A., and S. ADHYA, 1969 The galactose operon of *E. coli* K-12. II. A deletion analysis of operon structure and polarity. Genetics **62**: 249–264.

SHEDLOVSKY, A., and S. BRENNER, 1963 A chemical basis for the host-induced modification of T-even bacteriophage. Proc. Natl. Acad. Sci. U.S. **50**: 300–305.

SHERMAN, J. R., and J. ADLER, 1963 Galactokinase from *E. coli*. J. Biol. Chem. **238**: 873–878.

SUNDARARAJAN, T. A., 1963 Interference with glycerokinase induction in mutants of *E. coli* accumulating gal-1-P. Proc. Natl. Acad. Sci. U.S. **50**: 463–469.

SUNDARARAJAN, T. A., A. M. C. RAPIN, and H. M. KALCKAR, 1962 Biochemical observations on *E. coli* mutants defective in uridine diphosphoglucose. Proc. Natl. Acad. Sci. U.S. **48**: 2187–2193.

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

WHITFIELD, H. J., R. G. MARTIN, and B. N. AMES, 1966 Classification of aminotransferase (C gene) mutants in the histidine operon. J. Mol. Biol. **21**: 335–355.