GALACTOSE MUTATIONS OF ESCHERICHIA COLI K-12 NOT AMENABLE TO LAMBDA TRANSDUCTION¹

M. L. MORSE²

*Webb-Waring Institute for Medical Research, and the Department of Biophysics,
University of Colorado Medical Center, Denver 20, Colorado*

Received February 15, 1963

OF 17 mutations to inability to use galactose (*Gal*⁻) obtained after ultraviolet treatment of *E. coli* K-12 cultures, three were found not subject to transduction by the temperate bacteriophage lambda (MORSE 1955). Attempts to transduce to these mutants with a variety of lambda preparations giving a high frequency of transduction (HFT) failed to produce *Gal*⁺, even when the donor source was *Gal*⁺. All three of the cultures were lambda sensitive, so that inability to adsorb lambda was ruled out as an explanation. All the cultures fermented glucose so that a lesion in the glycolytic pathway was also ruled out. One of the mutants (W3142) was analysed biochemically (KURAHASHI 1957) and found to have a galactokinase defect. W3142 and another mutation also not subject to lambda transduction have been placed in a single group (Group E) by E. LEDERBERG (1960).

The purpose here is to present an analysis of W3142 and three other independently isolated *Gal*⁻ mutants with the same characteristic behavior. Each culture has been found not to yield galactose positive clones after exposure to a variety of HFT lambda preparations, including one derived from a *Gal*⁺ culture.

MATERIALS AND METHODS

All the *Gal*⁻ mutant cultures are *E. coli* K-12 derivatives. The genotypes (*met*, methionine; *T*, threonine; *L*, leucine; *thi*, thiamine) and history of the cultures are as follows: (1) Mutant W3142 was isolated after exposure of a lambda sensitive *Gal*⁺ culture (W1503) to HFT lambda derived from a *Gal*₄⁻ homogenote. The initial *Gal*⁻ isolated (W2733) was lambda sensitive (*Lp*^{*}), gave no *Gal*⁺ when exposed to HFT lambda preparations, and grew slowly in several different media. The cross, *F*⁺ *met* *Gal*₄⁻ *Lp*^{*} by W2733, which is *T*⁻ *L*⁻ *thi*⁻, gave in 1954 (MORSE, unpublished) prototrophic recombinants of which 11.4 percent were *Gal*⁺. The statements of KURAHASHI (1957) and FUKASAWA and NIKAIDO (1961) concerning the closeness of this mutation to the *Gal*⁻ amenable to transductional analysis are based on this observation. A derivative of W2733, W2734 was selected by E. LEDERBERG on the basis of better growth. W2734 was crossed with an Hfr

¹ Supported by Public Health Service Grant E-1345(C5) and National Science Foundation Grant 14210. Contribution No. 205, Department of Biophysics.

² Career Development Awardee, Public Health Service.

Gal⁺ *Lp*⁺ (W1895) to yield the prototrophic, glucose-utilizing lambda lysogenic (*Lp*⁺) derivative, W3142. (2) Mutant 655A was isolated after treatment of the F⁻ *Gal*⁺ *Lp*⁺ culture W3100 with nitrous acid. 655A is lysogenic for lambda, but a phage sensitive derivative has been prepared from it. (3) Mutant 687K was isolated after treatment of the F⁻ *Gal*⁺ *Lp*^s culture, W3110, with ultraviolet radiation. A lysogenic derivative of this culture has been prepared. (4) Mutant 781 was obtained after treatment of the auxotrophic (proline⁻, serine or glycine⁻) F⁺ *Lp*^s culture (W1673) with ultraviolet radiation. A *Gal*⁻ *Lp*^s derivative was initially obtained, but this culture is no longer in our culture collection. The crosses in 1953, of W2312 by F⁻ *T-L-thi*⁻ *Gal*₄⁻ *Lp*⁺ yielded 0.5 percent *Gal*⁺ prototrophic recombinants, while W2312 by F⁻ *T-L-thi*⁻ *Gal*₂⁻ *Lp*⁺ yielded 0.005 percent *Gal*⁺ prototrophic recombinants. Mutant 781 is an *Lp*⁺ derivative of W2312.

Methods of culture handling are as previously given (MORSE, LEDERBERG and LEDERBERG 1956a,b) except that instead of Eosin Methylene Blue agar a dehydrocholic acid, neutral red medium (C medium) (MORSE and ALIRE 1958) was employed for scoring ability to use galactose. Penassay broth was used for routine small cultures, nutrient broth (2×) for larger cultures. All cultures were incubated at 37°C. Special technics will be detailed below at the appropriate place.

RESULTS

In addition to the principal cultures listed above, W2734 (the female parent of W3142) was also carried through many of the analyses. It was in no way different from its derivative W3142 with respect to these tests, which included assay for kinase and transferase.

The mutants were first examined for ability to revert to the *Gal*⁺ phenotype, using the methods of LURIA and DELBRÜCK (1943) and ARMITAGE (1953). Each of the mutants was noted to revert (Table 1), eliminating the possibility that a deletion of the *Gal* region was the cause of the failure to give wild type in transduction.

TABLE 1
Reverse mutation rates of the Gal⁻ mutants

Mutant	<i>Gal</i> ⁻ → <i>Gal</i> ⁺ Probability/bacterium/division	Method of measurement
W3142	$5.3 \pm 2.7 \times 10^{-11}$	Median
781	$< 10^{-12}$	P ₀
655A	$4.5 \pm 0.8 \times 10^{-12}$	P ₀
687K	$7.1 \pm 0.3 \times 10^{-10}$	P ₀

With the exception of 781, a series of ten 5 ml penassay cultures, started with small inocula from separate colonies, was analysed after 16 hours growth for total number of cells (=total number of divisions) and total number of *Gal*⁺ revertants. Ascertainment of the number of revertants was made on cholate medium without galactose, on which the revertants were able, and the *Gal*⁻ unable, to grow. Reconstruction experiments gave 100 percent recovery of inserted *Gal*⁺ on the cholate galactose minimal medium. Mutant 781, because of its low revertibility, was studied by using independent cultures grown on the surface of tryptose agar plates. Cells from the plate cultures were washed and concentrated before plating on minimal agar. The 781 experiments performed to quantitate the reversion rate of 781 did not yield any reversions, but other qualitative experiments have indicated revertibility. The value given for 781 is, therefore, a maximum value for revertibility rate.

The mutants were examined biochemically, by means of extracts, to ascertain if they had lesions of the known pathway of galactose metabolism. Extracts were made by freezing cells (optical density 50.0) in the presence of lysozyme (333 $\mu\text{g/ml}$) to -80°C and then thawing. The frozen cells may also be stored at -35°C for some time without loss of enzyme activity. Thawing at 37°C yields a viscous solution (about 90 percent cell lysis) whose viscosity is quickly destroyed by deoxyribonuclease. After thawing the solutions are centrifuged to remove cell debris. The cells used for the extracts were grown in double strength nutrient broth for 16 hours, at which time an equivalent amount of broth and galactose (final concentration 0.1 percent) was added. The shaking rate of the cultures was increased and the cultures incubated for 2.5 hours for adaptation to galactose.

Galactokinase assay was performed colorimetrically using bromthymol blue to measure the acidity produced in the phosphorylation of galactose; measurement was made at 610 millimicrons in a Beckman DB spectrophotometer (1.0 ml cells, initial pH 7.2, at 23°C) with a photovolt recording attachment. The rate of color change appears linear for the first two minutes of the reaction. The reaction was not calibrated absolutely and the rates are expressed relative to the reference wild-type culture (W3110). The other enzymes were assayed according to the methods given by KURAHASHI (1957) and KALCKAR, KURAHASHI and JORDAN (1959).

The results of the biochemical analysis of the mutants and the mutant *Gal*⁻ recovered from them are shown in Table 2. Two of the mutants (W3142, 781) have kinase defects, being devoid of kinase activity, while a third mutant, 687K, has reduced amounts of transferase activity. These findings confirm earlier observations on the kinase and transferase activity of these mutants using C¹⁴-labelled substrates and chromatographic separation of substrate and end product. The fourth mutant appears to have all enzymes in amounts comparable to the wild-type standard. The kinase defect noted in W3142 confirms the earlier observation of KURAHASHI (1957).

The mutants were also examined genetically. While they cannot serve as

TABLE 2
Biochemical analysis of the mutants

Mutant*	Galactokinase†	Transferase‡	Epimerase‡	Genotype§		
				K	T	E
W3110 (<i>Gal</i> ⁺)	1.0	4.2	7.0	+	+	+
W3142	< 0.01	7.6	37.5	—	+	+
W3142 (<i>Gal</i> ⁻)	< 0.01	11.4	48.0	—	+	+
781	< 0.01	17.6	54.2	—	+	+
781 (<i>Gal</i> ⁻)	< 0.01	19.1	33.0	—	+	+
687K	1.1	1.4	14.5	+	±	+
655A	0.83	6.3	9.9	+	+	+

* W3110 is *Gal*⁺ wild type reference. W3142 *Gal*⁻ and 781 *Gal*⁻ are the *Gal*⁻ recovered after transduction, recombination and segregation.

† Rates of change of color of bromthymol blue relative to the rate of change of W3110 per equivalent amounts of protein.

‡ Micromoles of substrate changed per milligram protein per hour.

§ K, kinase; T, transferase; E, epimerase.

recipients in transduction, they may serve as donors, and information on the nature of the genetic constitution of each mutant may be obtained from a study of the heterogenotes formed by transductions from them.

LFT lysates were prepared by UV induction of each mutant (or a lysogenic derivative) and these lysates applied to recipient cultures of known genetic and biochemical constitution. Two mutants (W3142 and 781) formed *Gal*⁺ clones poorly with kinase defective recipients, indicating genetically that they possess kinase defects; the other two mutants donated more poorly to kinaseless recipients than to transferase, but with a higher efficiency than W3142 and 781.

Heterogenotes formed between the mutants and *Gal*₁⁻ recipients were analysed further by selecting segregants from them (one segregant per heterogenote) and testing them for *Gal* genotype with HFT lambda preparations from *Gal*₁⁻ and *Gal*₂⁻ homogenotes. This classification serves to identify the source of the *Gal*⁻ mutation in the segregants: (1) if the segregant *Gal*⁻ forms *Gal*⁺ with HFT *Gal*₂⁻, but not with HFT *Gal*₁⁻, the *Gal*⁻ is the endogenotic *Gal*⁻ and derived from the test recipient *Gal*₁⁻ culture; (2) if it does not form *Gal*⁺ with HFT *Gal*₁⁻ and *Gal*₂⁻, it presumably is the nonamenable *Gal*⁻ from the donor mutant, recovered intact after transduction and recombination; (3) if it forms *Gal*⁺ with both *Gal*₁⁻ and *Gal*₂⁻ HFT's, it is presumably the nonamenable *Gal*⁻ recovered after transduction and recombination, but separated now from some modifying factor (second mutation) with which it was coupled in the parent donor strain. The result of the analysis of segregants is given in Table 3. As expected, most of the segregants resemble the *Gal*₁⁻ recipients. From two of the mutants (W3142 and 781) it was possible to transduce and recover a mutation which was now *amenable* to lambda transduction.

Sixteen of 49 segregants from heterogenotes formed from W3142 were of this type, four of 40 segregants from the heterogenotes made from 781 were of a similar type. One representative from each group was examined biochemically and shown to be identical to its donor parent (Table 2). It was also shown to be genetically identical to its donor source by exposing it to phage from this source and failing to obtain transductions (Table 4).

TABLE 3

Analysis of the segregants from heterogenotes formed by transduction from cultures nonamenable to lambda transduction

Donor	Recipient	Segregant phenotypes		
		<i>Gal</i> ₁ ⁻	Nonamenable <i>Gal</i> ⁻	Amenable <i>Gal</i> _x ⁻
655A	<i>Gal</i> ₁ ⁻	141	0	0
687K	<i>Gal</i> ₁ ⁻	121	0	0
781	<i>Gal</i> ₁ ⁻	40	0	4
W3142	<i>Gal</i> ₁ ⁻	49	0	16

Transductions using LFT phage were made from the mutants to *Gal*₁⁻ recipients. Segregants were collected, one per heterogenote, and tested for *Gal* genotype against HFT *Gal*₁⁻ and HFT *Gal*₂⁻. Amenable signifies ability to serve as a recipient in transduction. The amenable *Gal*_x⁻ gave fewer *Gal*⁺ heterogenotes with HFT *Gal*₂⁻, indicating that they probably had a defect in the kinase cistron.

TABLE 4

Identification of Gal⁻ recovered with their source cultures

Donor	Recipient	Control (spontaneous reversions)	Gal ⁺ per 0.1 ml lysate (reversions plus transductions)
781	Gal ₁ ⁻	0	300
	781 Gal ⁻	7	9
W3142	Gal ₁ ⁻	0	240
	W3142 Gal ⁻	59	57

The applications of phage to Gal₁⁻ recipients serve as a control, indicating that the LFT lysates are active. Failure to observe augmentation in the presence of phage indicates genetic homology, i.e., Gal_x⁻ —× Gal_x⁻ yields no Gal⁺. The recipients designated 781 Gal⁻, W3142 Gal⁻ are the Gal⁻ recovered from 781 and W3142, respectively.

It was not possible to transduce mutations from the other mutants (655A, 687K) to Gal₁⁻ and recover them after recombination and segregation. Thus the genetic constitution of these strains remains unknown. It perhaps would have been better to transduce from 687K to a kinaseless mutant, rather than a transferaseless, as was done, since 687K appears to have a modified transferase, and the heterogenotes studied between it and Gal₁⁻ may already be recombinants in the transferase region. The defect of 655A remains obscure, but could be of galactose permease or of pyrophosphorylase.

DISCUSSION

The recovery in a form now *amenable* to transduction, of mutations genetically and biochemically identical to the mutations detected biochemically in mutants W3142 and 781, indicates that these two mutants are double mutants, once for a gene of a known step in the metabolism of galactose, and once at some other locus. The function of the second locus is not known and since it was not recovered following transduction to Gal₁⁻ (assuming the tests to be extensive enough) it may be: (1) not recognizable on the basis of galactose phenotype; (2) not in the region transduced by lambda. This may also be the case for 687K, which has transferase activity approximately one third that of the wild-type culture used as reference. Presumably 687K also possesses the second mutational change, or a similar one, to those possessed by W3142 and 781. Whether the second locus changes are identical is not known, since it has not been possible to study them. The nature of the change (or changes) in mutant 655A is not known, but it must involve biochemical steps other than those studied above, since 655A has a normal complement of the known enzymes.

The report by FUKASAWA, JOKURA and KURAHASHI (1962), that W3142 and another mutant of Group E studied by them were defective in pyrophosphorylase, appeared after it was known that W3142 and 781 were doubly mutant. It is therefore likely that the second mutation in these cultures is the defect reported by them, as are also the defects of 655A and 687K, although 687K in addition has a partial transferase defect. Pyrophosphorylase assays were performed on sonicates of the above mutants, but we were unable to obtain satisfactory results

with this enzyme assay procedure. The failure to recover the pyrophosphorylase defect after transduction and recombination indicates that the gene for this enzyme is not associated with the *Gal* operon, and therefore not in the region transduced by lambda.

SUMMARY

Four galactose mutants not amenable to lambda transduction were analysed genetically and biochemically. Two of the mutants, and possibly a third, are double mutants for a gene in the *Gal* operon and for a gene whose function was not identified. The fourth mutant did not have a defect in kinase, transferase, or epimerase, and must therefore have a genetic lesion outside the *Gal* operon. According to results of FUKASAWA, JOKURA and KURAHASHI (1962), the gene not identified may be for the enzyme pyrophosphorylase. This gene is not transduced by lambda.

LITERATURE CITED

- ARMITAGE, P., 1953 Statistical concepts in the theory of bacterial mutation. *J. Hyg.* **51**: 162-184.
- FUKASAWA, T., K. JOKURA, and K. KURAHASHI, 1962 A new enzymatic defect of galactose metabolism in *Escherichia coli* K-12 mutants. *Biochem. Biophys. Research Comm.* **7**: 121-125.
- FUKASAWA, T., and H. NIKAIIDO, 1961 Galactose mutants of *Salmonella typhimurium*. *Genetics* **46**: 1295-1303.
- 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.
- KURAHASHI, K., 1957 Enzyme formation in galactose-negative mutants of *Escherichia coli*. *Science* **125**: 114-115.
- LEDERBERG, E., 1960 Genetic and functional aspects of galactose metabolism in *Escherichia coli* K-12. Pp. 115-130. *Microbial Genetics*. Tenth Symposium, Society for General Microbiology. Cambridge University Press, New York.
- LURIA, S. E., and M. DELBRÜCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* **28**: 491-511.
- MORSE, M. L., 1955 Genetic transduction in *Escherichia coli*. Doctoral dissertation, University of Wisconsin, Madison, Wisconsin.
- MORSE, M. L., E. M. LEDERBERG, and J. LEDERBERG, 1956a Transduction in *Escherichia coli* K-12. *Genetics* **41**: 142-156.
- 1956b Transductional heterogenotes in *Escherichia coli*. *Genetics* **41**: 758-779.
- MORSE, M. L., and M. L. ALIRE, 1958 An agar medium indicating acid production. *J. Bacteriol.* **76**: 270-271.