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A partial deletion map of the galactose operon in *E. coli* K12

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1. INTRODUCTION

The widely accepted Campbell model of λ lysogenization (Campbell, 1962) involves the insertion of the phage DNA in the bacterial chromosome by means of a recombinational event between homologous regions. The circular λ molecule comes to form a linear segment of the bacterial DNA. The release of the prophage after induction occurs by a reversal of this process, requiring a new recombinational event. The same model accounts for the formation of transducing particles at the time of induction, by supposing that, very rarely, recombination occurs between the 'wrong' parts of the prophage and the bacterial DNA. A DNA molecule is formed which contains only part of the λ DNA and carries some bacterial DNA (Fig. 1). Recently, Adler & Templeton (1963) found transducing particles carrying part but not all of the galactose operon. Effectively, such particles contain partial deletions of *gal*. We have now isolated nineteen phages of this type as HFT lysates. The *gal* deletions they carry enable us to divide the kinase and transferase cistrons into a number of genetic blocks. As a result, many galactose-negative mutations can be given an unambiguous order.

2. METHODS

(i) *Bacterial strains*

Galactose-negative strains were obtained from M. L. Morse, J. Adler and G. Buttin as shown in Table 1. Enzymic defects in these strains have been described by Kurahashi (1957), Kalckar *et al.* (1959), Soffer (1961), Morse (1962) and Buttin (1963*b*). Strain 261D (gal_8^-/F′ gal_8^-) was given by H. Echols (Echols, Reznichek & Adhya, 1963). Strains PL2A and PL5A were isolated from stabs of the epimerase – * mutants PL2 and PL5 (Buttin, 1963*b*) given by G. Buttin. Enzyme assays show these to have the properties of kinase-epimerase double mutants (Table 2). Kinase defects tend to arise spontaneously in epimerase mutant stocks due to their greater viability.

Strains with prefixes NR and NA were isolated after nitrosoguanidine treatment

* Abbreviations: *kinase*, galactokinase; *transferase*, galactose-1-phosphate uridyl transferase; *epimerase*, UDP-galactose 4-epimerase; *k*, *t* and *e*, cistrons specifying these enzymes; *gal*, galactose operon; λ *dg*, defective λ carrying the galactose operon; *LFT*, low frequency transduction; *HFT*, high frequency transduction.

Table 1. *Galactose negative strains*

Mutation	Strain	Source	Mutation	Strain	Source
		Kinase⁻			
2	W3102	Morse, Adler	13M	657A	Morse
8	W3108	Morse, Adler	15M	663A	Morse
10	W4666	Adler	20M	6916	Morse
12	W3748	Adler	21M	691H	Morse
13	W4673	Adler		112–19	Buttin
14	W3965	Adler		114	Buttin
20	W4251	Adler		138–1	Buttin
24	W4247	Adler			
		Transferase⁻			
4	W3104	Morse, Adler	17M	6885	Morse
10M	500–3	Morse	19M	691A	Morse
11M	633–1	Morse		112–21	Buttin
		Epimerase⁻			
16	W4221	Adler		PL2	Buttin
22	W3805	Adler		PL5	Buttin
		Triply defective			
3	W2236	Morse	9	W3109	Morse, Adler
3	W4611	Adler			

Table 2. *Enzymic defects of PL2A and PL5A*

	Units per mg protein		
Strain	Kinase	Transferase	Epimerase
K12(λ)	8·4	12·4	24·5
PL2A	0·1	15·7	0·5
PL5A	0·1	19·9	0·5

Bacteria were grown at 37°C. in Medium 63 (Buttin, 1963) supplemented with 0·1% casamino acids and 0·5% glycerol. The cells were induced for 1 hour with 0·2% galactose in mid log phase, chilled and washed, resuspended in Medium 63 containing 10^{-2} M GSH and 10^{-3} M Magnesium titriplex, broken by six 1-min. pulses with a Dawe sonicator, Type 1130A (20 Kc./sec.), with cooling to 0°C. between pulses, and clarified by centrifugation. The enzymes were assayed as described by Buttin (1962*a*) except as follows: In the kinase and transferase assays glycylglycine was replaced by *tris*-HCl buffer of the same pH and molarity. The specific activity of the C^{14}-galactose and C^{14}-galactose-1-phosphate substrates was 1 c./mole. To measure kinase activity galactose and galactose-1-phosphate were separated as described previously for argininosuccinic acid (Cohen & Bishop, 1966) except that electrophoresis was for 45 min. In the transferase assay galactose-1-phosphate and UDP-galactose were separated by 30 hours' paper chromatography in ethanol-Na acetate (pH 3·8) (Paladini & Leloir, 1952). One unit of enzyme activity represents the conversion of 1 μmole of substrate in 1 hour at 37°C. Protein was determined by the Lowry method (Lowry *et al.* 1951).

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of E1, which is a *try*$^-$ derivative of M2 (*F*$^-$*his*$^-$*Sm*^{r} λ-sensitive). M2 was given by S. Brenner.

The transducing phages λ *dg*A, *dg*B and *dg*C (Adler & Templeton, 1963) were kindly provided by J. Adler.

(ii) *Media*

Minimal medium contained 0·5% NH_4Cl, 0·1% NH_4NO_3, 0·2% Na_2SO_4, 0·3% K_2HPO_4, 0·1% KH_2PO_4 and 0·1% $MgSO_4$. Galactose or glucose was added to 0·2%, amino acid supplements to 0·002% or 0·01% (the higher level is required for the growth of transductant colonies of NR and NA strains). Difco Bacto agar was added to 0·7% and 1·2% to make top and bottom agar. MS broth is described by Davis & Sinsheimer (1963): 1% Tryptone, 0·8% NaCl, 0·1% yeast extract.

(iii) *Transduction tests*

Spot tests were carried out by making streaks (two loops) of an overnight MS broth culture onto minimal galactose. When the streaks were dry a loopful of each HFT lysate was spotted on top. When complementation occurs between transducing phage and bacterium, the spot is filled with confluent bacterial growth. Where recombination occurs individual papillae arise within the spot, and these may be few in number when recombination is infrequent.

Transduction frequencies were measured by incubating 0·2 ml. bacteria ($0·2–1 \times 10^8$) with 0·1 ml. of a suitable dilution of HFT lysate (multiplicity $\ll 1$), and 0·1 ml. helper (2×10^8) unless otherwise stated, for 20 min. at 37°C. 1·5 ml of liquid minimal-galactose top agar was added and the mixture poured over a minimal-galactose plate. The bacteria were subcultured from an overnight MS broth culture 1½ to 2 hours before use. The HFT lysates were diluted through phosphate buffer, pH 7, or MS broth each containing 0·2% $MgSO_4$. This method gave frequencies very similar to those obtained by the more time-consuming method of adsorbing to starved cells in 0·01 M $MgSO_4$.

3. RESULTS AND DISCUSSION

(i) *Isolation of incomplete* λ dg

An LFT lysate of K12(λ) was applied to W3805 which carries the epimerase mutation gal^-_{22}. Galactose-positive transductants were purified and HFT lysates made by ultra-violet induction. These lysates were then tested for transduction of W4247 which carries a mutation, gal^-_{24}, at the distal end of the kinase cistron (i.e. distal with respect to the attachment site). Of 327 lysates, 218 were HFT lysates transducing W3805, and, of these, 19 failed to transduce W4247. On further analysis each one of the latter proved to carry a proximal fragment of the galactose operon (see below).

Campbell's model (Campbell, 1962) predicts that all transducing particles should extend from the proximal end of the operon (Fig. 1). An attempt was made to isolate phages which would transduce W4247 but not W3805, that is, carrying a distal fragment of the galactose operon. Among 106 lysates of galactose-positive transductants of W4247, 79 were HFT lysates, transducing W4247. All of these also transduced W3805. That is, no proximal deletions were found, as predicted by the model.

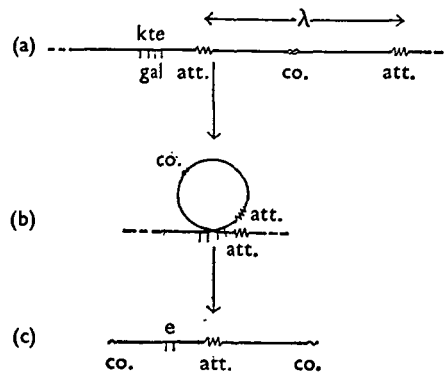

Fig. 1. Formation of incomplete λ *dg* according to the Campbell model. (a) Prophage inserted into the *E. coli* chromosome. (b) Following induction, recombination involving the galactose operon. (c) Vegetative incomplete λ *dg*.
att., λ-attachment site; *co*, cohesive ends; *k*, *t*, *e*, kinase, transferase and epimerase structural genes.

(ii) *Characterization*

The nineteen incomplete λ *dg*, and three others obtained from J. Adler (Adler and Templeton, 1963) were tested for transduction of a large number of *gal*⁻ mutants by spot tests. The termination point of each was accurately determined by transduction frequency tests. It is possible to arrange the *gal*⁻ mutants in such a way that those transduced by each phage stock form an unbroken sequence (Fig. 2). This operation brings together mutants with the same enzyme defect, and places the three cistrons in the order kinase-transferase-epimerase, in agreement with previous studies (Morse, 1962; Adler & Kaiser, 1963; Echols *et al.*, 1963; Adler & Templeton, 1963; Buttin, 1963*b*). The unbroken sequence of mutants transduced by each incomplete λ *dg* extends from the right-hand (epimerase) end of the operon. This is most clearly shown by the kinase-epimerase double mutants PL2A and PL5A which map, by this test, in the kinase cistron: each incomplete λ *dg* which does not cover their respective kinase defects necessarily fails to transduce these strains. Since the attachment site is to the right (Fig. 2) of the galactose operon, the formation of these transducing particles can readily be explained (Fig. 1) on the basis of the Campbell model (Campbell, 1962). If recombinational events within the galactose operon are indeed responsible, this indicates that λ DNA must contain a region or regions with some affinity for the galactose operon.

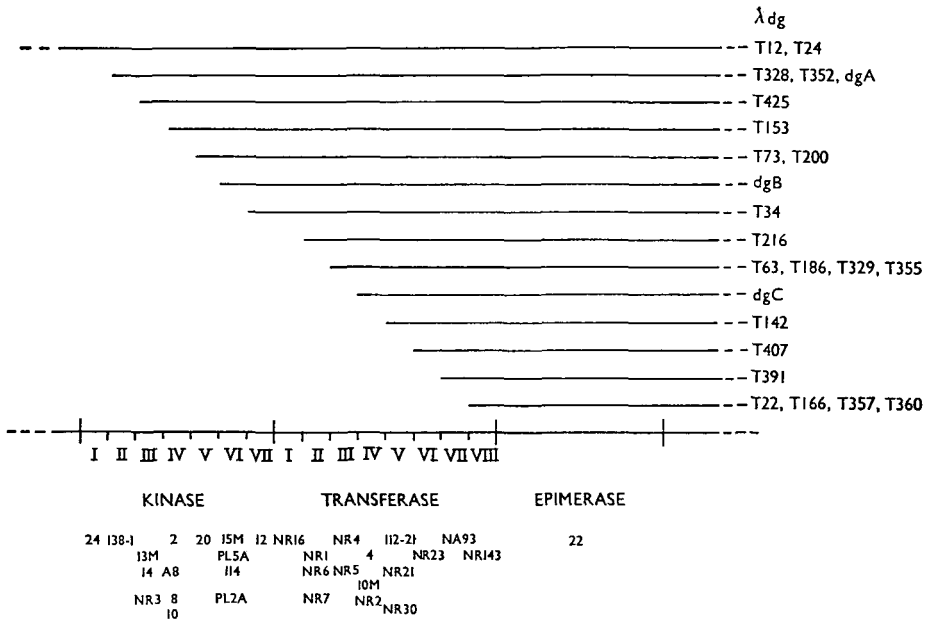

Fig. 2. Deletion map of kinase and transferase cistrons. The kinase cistron is shown divided into seven blocks, the transferase into eight. The identifying numbers of *gal*⁻ mutations are shown below the appropriate blocks, which are arbitrarily drawn to the same size.

(iii) *Transduction frequencies*

When a bacterium carrying a *gal*⁻ point mutation in a given cistron is transduced by a λ *dg* which carries the complete cistron, a galactose-positive may be produced by either recombination or complementation. We will call this C-type transduction. Where the λ *dg* carries only part of the cistron (incomplete λ *dg*) but overlapping the point mutation, a galactose-positive can be produced only by recombination between the two galactose regions. This we will call R-type transduction. If complementation is more frequent than recombination, C-type transduction should be more frequent than R-type, and this should be reflected in the frequencies with which different point mutations are transduced by a given incomplete λ *dg*.

To test this, the frequency of transduction of *gal*⁻ mutants by T73 was measured. Variation between bacterial strains, and other factors, lead to differences in lysogenization frequency. In order to minimize these we take the ratio of transduction by T73 to transduction by T12 (transduction ratio). Since T12 carries the complete *gal* operon, it should be able to transduce all bacterial strains without recombination within the operon. So that different deletion stocks can be compared directly we set the transduction ratio for W3805 (gal^-_{22}) arbitrarily to 1·0 and adjust the transduction ratios for the other strains accordingly (*relative transduction ratios or RTR*).†

† Thus RTR $= \dfrac{F_{(p\times b)}\cdot F_{(T12\times 22)}}{F_{(T12\times b)}\cdot F_{(p\times 22)}}$ where $F_{(p\times b)}$ etc. represent the four observed transduction frequencies: *p*, experimental HFT; *b*, experimental bacterium; *T12*, HFT lysate T12; *22*, W3805 (gal^-_{22}).

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Using T73, the *gal*⁻ mutants fell into three RTR classes (Fig. 3). In the region of the map not carried by T73 the RTR was always less than 5×10^{-5}. The few transductants observed are probably due to LFT particles originating during the induction of the HFT (Adler & Templeton, 1963). For the transferase⁻ and epimerase⁻ strains (but not for the triply defective gal_3^- and gal_9^-) transduction is C-type. The RTR of these strains was about 1. That is to say, after correction for differences in lysogenization frequency, the transferase⁻ and epimerase⁻ strains were transduced with the same frequency as the control epimerase-negative W3805 (gal_{22}^-). The transduction of mutants in the region of the kinase cistron carried by T73 is R-type. The RTR of these mutants was from 10^{-1} to 10^{-3}; that is, transduction was 10 to 1000 times less frequent than transduction of W3805 by the same

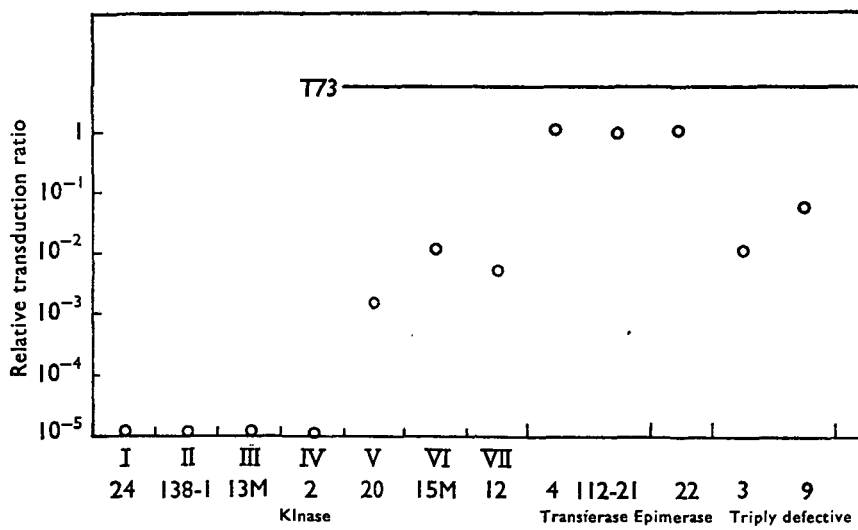

Fig. 3. Frequencies of transduction of galactose-negative mutants by incomplete λ *dg* T73.

lysate. The triply defective mutants (carrying gal_3^- and gal_9^-) fell into this same frequency class. Here again transduction is R-type. Recombination is obligatory because all three cistrons are defective in the bacterium. Thus R-type transduction is 10 to 1000 times less frequent than C-type transduction.

Transducing phage may be integrated either at the attachment site or at (or close to) the site of the bacterial gene which the phage carries (Signer & Beckwith, 1966). Integration at the attachment site will produce a C-type transductant by complementation in the heterogenote, but not an R-type transductant. This might explain the difference we observe in the frequencies of C-type and R-type transduction. Signer & Beckwith (1966) have shown that $\phi 80dlac$ tends to be integrated at the $\phi 80$ attachment site after infection in the presence of $\phi 80$-helper, but tends to be integrated at the *lac* site when helper is omitted. Thus, if our explanation of the difference in frequency between C-type and R-type transduction is correct, the

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difference ought to vanish if we omit the λ-helper. To test this, mutants NR3 (kinase⁻) and NR4 (transferase⁻) were transduced by T24 (complete) and T328 in the presence and absence of helper (Fig. 4 and Table 3). Omission of helper reduced

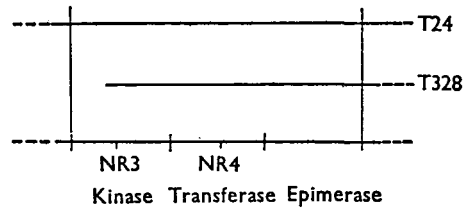

Fig. 4. Mapping relationships of T24, T328 and mutants NR3 and NR4.

the frequency of transduction of both recipient strains by T24 (C-type) by two orders of magnitude. In the transduction of NR3 by T328 recombination is obligatory (R-type). Here the frequency of transduction was low in the presence of helper and reduced very little by omission of helper. The transduction of NR4 by T328 is C-type. Here transduction was high in the presence of helper, and reduced, in the absence of helper, to about the level found with NR3.

Table 3. *Effect of helper on transduction frequencies* (*transductions per ml. HFT lysate* $\times 10^{-4}$)

	Transducing HFT			
	T24		T328	
Recipient	With helper	Without helper	With helper	Without helper
NR3	1500	6·2	15·2	5·5
NR4	1100	13·0	1300	12·0

Thus the frequency difference between C-type and R-type transduction is almost completely eliminated by omission of λ-helper. This suggests that most, though probably not all, C-type transduction results from the helper-mediated integration of the transducing phage at the λ-attachment site. If this interpretation is correct, our results also show a 100-fold difference between the frequency of integration at the λ site and the frequency of recombination at the *gal* site. These experiments do not allow us to distinguish between recombination at the *gal* site which leads to integration of the transducing phage, and recombination which repairs the *gal* operon without integration.

In view of these results our failure to find λ dg's terminating within the epimerase cistron is not surprising. The LFT lysates were screened for transducing particles by transduction of an epimerase⁻ mutant in the presence of λ-helper. A very large proportion of the LFT particles would be inserted at the attachment site. Of these,

only those which carried a complete epimerase cistron would produce galactose-positive colonies.

(iv) *Relationship between map position and recombination frequency*

Results of transduction frequency measurements with T73 and *dgA* in the kinase cistron are shown in Fig. 5, where the mutants are arranged in the unambiguous order previously shown. Within the kinase cistron, transduction is R-type and the RTR is a measure of recombination frequency. If an intact kinase cistron

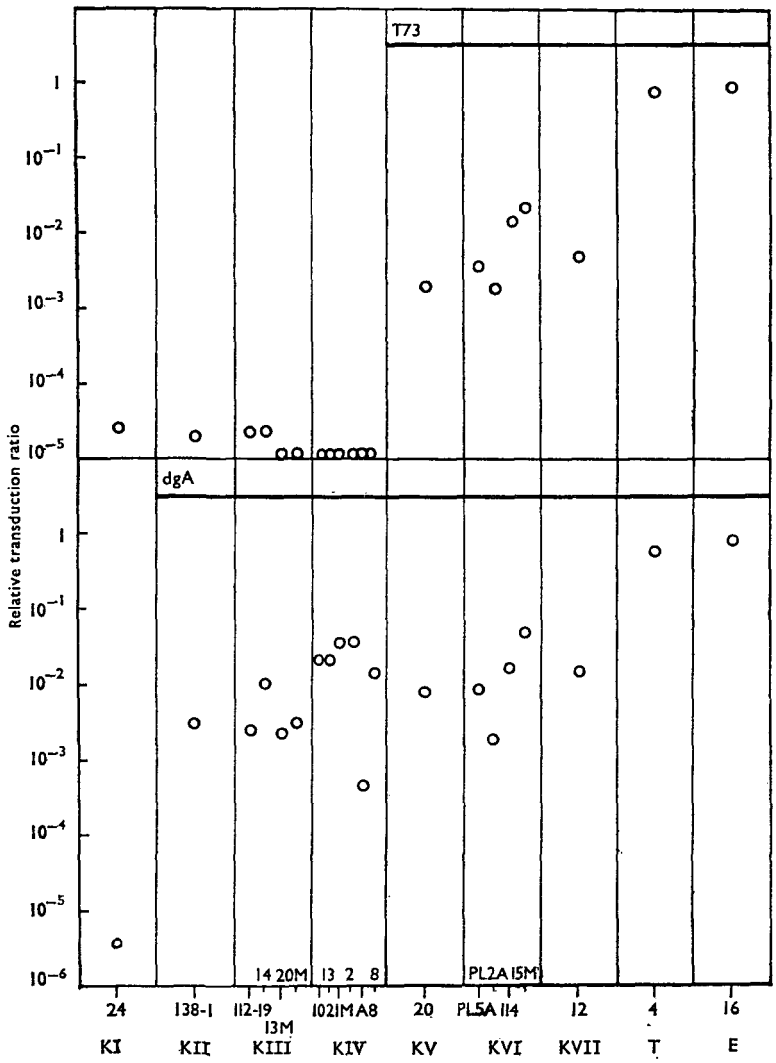

Fig. 5. Transduction frequencies of kinase mutants by T73 and *dgA*. Results with mutants gal_4^- (transferase) and gal_{16}^- (epimerase) are also shown. Individual mutants are shown as open circles. The relative positions of the mutants within each block are arbitrary.

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is to be produced, a recombinational event must occur *between* the mutant site and the end of the piece of the kinase cistron carried by the phage. Thus we should expect the RTR to be lower, the closer a mutant site is to this point, a relationship previously assumed by Adler & Templeton (1963). Figure 5 shows that this prediction is borne out in general. A linear regression of logRTR upon block number was calculated for the seventeen points representing positive transduction of kinase mutants by *dgA*, the block number being taken as an approximate measure of the position of each mutant. The regression was significant at the 5% level. The regression coefficient was $0{\cdot}26 \pm 0{\cdot}08$, indicating a ten-fold difference in recombination frequency between the extreme ends of the region.

It is evident however that individual mutants cannot be ordered by recombination frequency as measured by the RTR. Strain differences are a major source of variation within the recombination region. Note the similarity of the two patterns

Table 4. *Variation of transduction frequency with plating conditions*

Relative transduction frequencies ($RTR \times 10^4$)

	Plating conditions			
	Minimal-galactose	EMB-galactose		
Strain		Exp. 1	Exp. 2	Average
W3748 (gal_{12}^-)	14 ± 4	10	60	35
114 (gal_{114}^-)	107 ± 40	480	430	475
W4251 (gal_{20}^-)	63 ± 15	300	150	225

The strains shown above and W3805 (gal_{22}^-) which is the control for the calculation of RTR's, were transduced by T73 and T12 in the presence of helper λ.

(Fig. 5) in the region covered by both transducing phages. A striking example is strain W3108 (carrying gal_8^-) which we obtained both from M. L. Morse (designated 8) and J. Adler (A8). Neither could be F-duced by F'gal_8. Transduction spot tests placed both in the same region of the kinase cistron. However, in several tests their transduction frequencies by *dgA* were very different (Fig. 5) due presumably to divergence of the substrains of W3108.

In an attempt to eliminate strain differences, transduction tests were carried out using EMB-galactose plates instead of minimal-galactose. Galactose-negative strains grow on EMB-galactose, whereas only leaky strains grow significantly on minimal-galactose. Plating on EMB-galactose increased the transduction frequency significantly for all three strains tested (Table 4). However the RTR of strain 114 remained greater than that of W3748 (gal_{12}^-). These results suggest that the transduction frequencies of different bacterial strains are affected by differences in the processes of lysogenization and recombination rather than by variation in residual growth on galactose. If this is so, transduction of isogenic strains should give a more regular relationship between map position and transduction frequency.

SUMMARY

We have isolated a number of λ *dg* HFT lysates which carry proximal fragments of the galactose operon. Most of these have been shown to be different, and each terminates in either the kinase or transferase cistron. They divide the kinase cistron genetically into seven blocks of mutants, and the transferase into eight.

When a λ *dg* is used to transduce a bacterium which carries a mutation in a cistron which is intact in the λ *dg*, the transduction frequency is high in the presence of λ-helper. This is attributed to integration of the transducing fragment at the λ-attachment site and complementation between the two operons in the heterogenote. When the same λ *dg* transduces a mutation lying in the cistron in which the λ *dg* terminates, so that recombination within the galactose operon is obligatory, the transduction frequency is 10 to 1000 times less.

In such cases there is a general increase in transduction frequency between distal mutations (i.e. those lying near the termination of the deletion) and proximal mutations, but the relationship does not hold for many individual pairs of mutants, probably due to physiological differences between the bacterial strains.

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