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Breakage of λdg DNA: Chemical and Genetic Characterization of Each Isolated Half-molecule

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Molecules of DNA isolated from bacteriophage λdg can be broken in half when exposed to hydrodynamic shear. There are two types of half-molecules as demonstrated by their biological activity: those that contain the gal^+ and m_6^+ genes (gal^+-halves), and those that contain the i^λ and mi^+ genes (i^λ-halves). This is the result expected if the linkage map for vegetative λdg phage were also cleaved at its center to form two linkage groups.

Although the two types of half-molecules co-sediment in a sucrose gradient, they can be separated from each other by chromatography on methylated serum albumin columns, 50% of the total DNA being associated with each activity. The buoyant densities of the isolated half-molecules differ by 0·006 g/cm^3, suggesting that the guanine–cytosine content of the gal^+-half is 0·52 while that of the i^λ-half is 0·46. The thermal denaturation curves of each half-molecule as well as the whole molecule are compatible with this suggestion.

1. Introduction

The DNA isolated from λdg, a variant of coliphage λ, can transform galactose-negative strains of *Escherichia coli* K12 to the galactose-positive state (Kaiser & Hogness, 1960; Hogness, 1962). As is the case for transduction with complete λdg phage (Morse, Lederberg & Lederberg, 1956), the transformed cell is a heterogenote in which the mutant gal^- gene of the recipient cell continues to occupy its position in the bacterial chromosome, while the unblemished gal^+ genes of λdg remain in the phage chromosome as prophage.

The *gal* genes that are integrated into the λdg chromosome form an operon that controls the synthesis of three enzymes necessary for galactose metabolism: galactokinase, galactose-1-phosphate uridyl transferase, and uridine diphosphogalactose 4-epimerase (Kurahashi, 1957; Kalckar, Kurahashi & Jordan, 1959; Lederberg, E., 1960). These genes are closely linked in the *E. coli* chromosome (Morse, 1962; Buttin, 1963; Adler & Kaiser, 1963). It is presumed that they are similarly restricted in λdg and occupy the dg-region of the chromosome map (Fig. 1); a coherent region defined by the absence in λdg of phage genes present in ordinary λ (Arber, 1958; Campbell, 1959,1961).

Except for this defective dg-region, λdg contains the remainder of the λ genes: the immunity-specific gene, i^λ, and a plaque-size determining locus, *mi*, are examples (Fig. 1). Since the λdg DNA molecules that are active in galactose transformation also carry i^λ and *mi* into the transformed cells, it appears that such DNA molecules

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include the entire λdg chromosome (Kaiser & Hogness, 1960), and in this paper will be referred to as whole molecules.

The molecular weight of these whole molecules† is about 3×10^7. Consequently one should be able to induce single breaks near the midpoint of the whole molecules by introducing relatively mild velocity gradients into its solutions, using the same procedures as those employed in the breakage of phage T2 DNA into half- and quarter-molecules (Burgi & Hershey, 1961; Rubinstein, Thomas & Hershey, 1961). Such breakage procedures have been described for λ DNA (Kaiser, 1962) and their application to λdg DNA is described in this paper.

Two related aspects of midpoint breakage of λdg DNA by hydrodynamic shear are examined here. The first concerns the change in the distribution of genes resulting from half-molecule information. It is to be expected that a single break in the whole molecule will result in the formation of two linkage groups from one. Indeed, if the extremes on the map (m_6 and *mi*, Fig. 1) are the true extremes for the complete genome, and if colinearity exists between the sequence of genes on the map and the sequence of base pairs in the DNA, then the simplest expectation of a single break at the molecular midpoint is a break in linkage at the center of the map. The experimental results to be reported here are consistent with a single interruption of linkage to the right of the dg-region and to the left of i^λ; i.e. somewhere near the center of the map (Fig. 1). In this connection, a more detailed analysis of the point of dissociation on the map resulting from half-molecule formation of λ DNA has been presented and is also consistent with the above expectation (Kaiser, 1962; Radding & Kaiser, 1963).

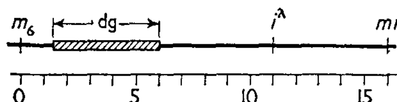


FIG. 1. Linkage map of vegetative phage λdg according to the data of Arber (1958). Distances are in recombination units. The dg region is defined as that part of λ which is not recoverable in active phage when λdg is crossed to normal λ. The m_6 (Jacob & Wollman, 1954) and *mi* (Kaiser, 1955) mutations lead to decreased plaque size, while *i* represents the immunity gene and may be either that of λ phage, i^λ, or that of another phage, e.g. i^{434} (Kaiser & Jacob, 1957).

The second aspect of breakage that is emphasized here concerns the gross distribution of base pairs within λdg DNA. The whole molecule contains some 5×10^4 base pairs, of which 49% are GC,‡ and the remainder are AT (Kaiser & Hogness, 1960). Although a half-molecule will therefore represent a large sample of base pairs, there is at present no reliable basis for predicting whether the base-pair frequencies of the individual half-molecules will be those of the whole molecule. In the case of λdg DNA, an experimental answer to this question is facilitated by the ability to distinguish the two halves in terms of their gene content. For purposes of reference,

† The 16 ± 1-μ contour length of λ DNA has been measured in the electron microscope (Ris & Chandler, 1963; Inman, personal communication; MacHattie & Thomas, personal communication). Preliminary contour measurements with the λdg DNA used here indicate a length that is 80 to 90% that of λ (Inman, personal communication); a lower value was expected, since CsCl buoyant density of this λdg phage is about 0·01 g/cm³ less than that of λ (Kaiser & Hogness, 1960). The size of λ DNA computed by other methods is consistent with the contour measurements (Cairns, 1962; Burgi & Hershey, 1963).

‡ Abbreviations used: G, guanine; C, cytosine; A, adenine; T, thymine; TB, tryptone broth; TCM, 0·01 M-tris–HCl buffer, pH 7·1, 0·01 M-$CaCl_2$, 0·01 M-$MgSO_4$.

the half-molecules containing the *gal* genes are termed *gal*-halves, while those containing i^λ and *mi* are termed i^λ-halves. Employing these genetic labels as an assay, it has been possible in the experiments to be reported here to effect the isolation of the *gal*- and i^λ-halves by chromatography on methylated serum albumin columns (Lerman, 1955; Mandell & Hershey, 1960). The properties of the isolated half-molecules are consistent with an assignment of a 46% GC-content for the i^λ-halves and a value of 52% for the *gal*-halves. The whole molecules thus contain a considerable gross heterogeneity in the distribution of base pairs. This condition is probably responsible for the fractionation of the half-molecules, since their chromatographic behavior is that predicted from their base content (Sueoka & Cheng, 1962).

Two dividends result from this heterogeneity and consequent isolation of the halves. One of these consists in obtaining more critical evidence than heretofore existed that the two types of first-break fragment have an equivalent average size and are therefore half-molecules. The other consists in a twofold purification of any gene in the λdg genome. In this connection it should be noted that the ratio of *gal* genes to DNA is about 100-fold greater for the DNA isolated from λdg phage than for DNA isolated from *E. coli*. With the isolation of the half-molecules of λdg DNA this ratio is doubled, and thus the isolated *gal*-half can be viewed as 200-fold more pure with respect to the *gal* genes than is *E. coli* DNA, their original source.

2. Materials and Methods

(a) *Media*

(1) The TB, TB soft agar, TB plate agar, EMB plate agar, H and P media and the diluent for phage stocks (λ-dil) have been described previously (Kaiser & Hogness, 1960).

(2) The TTGal agar consists of TB plate agar, to which is added 0·2% galactose and 0·05% 2,3,5-triphenyl tetrazolium chloride.

(3) The HTGal agar consists of H medium to which is added 1·2% agar, 1% galactose, and 0·05% 2,3,5-triphenyl tetrazolium chloride.

(4) The AA-G soft agar contains the same salts as H medium except that the potassium phosphate buffer, pH 7·0, is 0·001 M, and 0·05 M-tris–HCl buffer, pH 7·1, has been added. In addition, it contains 120 mg glucose/l. and a mixture of 18 L-amino acids in the following concentrations (mg/l.): 2·5 alanine, 3·0 arginine–HCl, 3·4 aspartic acid, 0·66 cysteine–HCl,$2H_2O$, 4·1 glutamic acid, 1·6 glycine, 0·62 histidine–HCl, 1·8 isoleucine, 2·7 leucine, 3·2 lysine–HCl, 1·4 methionine, 1·7 phenylalanine, 1·4 proline, 1·4 serine, 1·6 threonine, 0·57 tryptophan, 1·1 tyrosine and 2·1 valine.

(5) Freezing medium is the high-glycerol storage medium of McFall, Pardee & Stent (1958) modified as follows: (a) concentrations of all salts lowered by 10%; (b) concentration of glycerol increased to 44 g/l.; and (c) 1·5 g NaCl/l. added.

Bacteriophage stocks were stored in 0·01 M-$MgSO_4$ that was buffered with 0·01 M-potassium phosphate, pH 7·0, or tris–HCl, pH 7·1.

The DNA for assay was diluted in TCM.

(b) *Bacteria*

Except for *E. coli* K12 strain C600 (Appleyard, 1954) all bacteria used here were derived from galactose-negative mutants of *E. coli* K12 obtained through the courtesy of Dr E. Lederberg. They have been described by Morse *et al.* (1956) and Lederberg (1960). Mutants W3102 (gal_{K2}^-) and W3108 (gal_{K8}^-) are defective in the synthesis of galactokinase; mutants W3101 (gal_{t1}^-) and W3104 (gal_{t4}^-) are defective in galactose-1-phosphate uridyl transferase; and mutant W3805 (gal_{e22}^-) is defective in uridine diphosphogalactose 4-epimerase. The double mutant $gal_{K2}^-\ gal_{t1}^-$ was isolated by J. Weigle and the triple mutant W4960 ($gal_{K2}^-\ gal_{t1}^-\ gal_{e22}^-$) by E. Lederberg. Activities of the above enzymes were determined in crude extracts from induced cultures of each strain that was used. The results

were in agreement with the above designations, which in turn result from activity determinations of others (Kurahashi, 1957; Kalckar *et al.*, 1959; Soffer, 1961).

The lysogenic derivatives of these strains were isolated from the survivors of phage infection and are indicated by the bacterial genotype followed, in parenthesis, by the prophage genotype, e.g. gal^-_{t4} ($\lambda i^{434}mi$).

(c) *Bacteriophages*

The λdg phage used here is described in a previous publication (Kaiser & Hogness, 1960), as are the phages $\lambda i^{434}mi$ and $\lambda m_6 i^{434}$ (Kaiser, 1962). The source for preparation of the λdg is gal^-_{t4} (λ, λdg)/λ, a bacterium that is doubly lysogenic for wild type λ and for λdg, and which is unable to absorb λ (Kaiser & Hogness, 1960).

The $\lambda i^{434}mi$ was prepared from either gal^-_{t4} ($\lambda i^{434}mi$) or $gal^-_{K2}\, gal^-_{t1}\, gal^-_{e22}$ ($\lambda i^{434}mi$) to yield stocks t⁻ $\lambda i^{434}mi$ and kte⁻ $\lambda i^{434}mi$, respectively. The $\lambda m_6 i^{434}$ was prepared from gal^-_{t1} ($\lambda m_6 i^{434}$).

(d) *Purification of bacteriophage*

(i) *25-liter cultures*

The appropriate lysogenic strain, growing exponentially at 37°C in H medium (1% glucose), was irradiated with u.v. light after achieving a concentration of $1\cdot3 \times 10^9$ cells/ml. and cooling to 25°C. A Biogen (American Sterilizer Corporation, Erie, Pa., U.S.A.) was used for these purposes. After irradiation, 2·5 l. of 10% Difco bactotryptone was added with a small amount of antifoam A (Dow-Corning Corporation) and the temperature returned to 37°C. After maximum lysis had occurred (about 1×10^{11} phage/ml.), the culture was quickly cooled to 0°C and freed of some bacterial debris by centrifugation at 18,750 rev./min in a Spinco model 170 continuous centrifuge at a flow rate of 0·3 l./min, a radial sedimentation path-length of 1·2 cm and a maximum radial distance of 7·0 cm. In some cases the lysate was stored overnight at 4°C before this centrifugation.

The phage were sedimented from the supernatant solution by centrifugation as above except that the flow rate was 0·01 l./min and the radial sedimentation path-length was 0·25 cm. The pellet was suspended in 0·01 M-$MgSO_4$, 0·01 M-buffer, pH 7·0 (tris–HCl or potassium phosphate) and CsCl added until the density was 1·46 g/cm³ at 25°C. The yield of phage at this point is 80 to 100%.

After preliminary centrifugation at 23,000 ***g*** (max) for 30 min to remove fast-sedimenting impurities that form a pellet and a floating gel, the suspension was centrifuged for at least 40 hr at 27,000 rev./min in a Spinco model L no. 30 angle rotor. Fractions were collected as drops flowing through a hole pierced in the bottom of the tube. In the case of λ, $\lambda i^{434}mi$ or $\lambda m_6 i^{434}$ phage, the band fractions were pooled and dialysed at 4°C against 0·01 M-$MgSO_4$, 0·01 M-buffer, pH 7·0 (tris–HCl or potassium phosphate). The yield at this point is 60 to 70%. In the case of the lysates from gal^-_{t4} (λ, λdg)/λ, the band fractions with λdg/λ ratios either greater or less than that of the lysate were separately re-banded in CsCl solution until contamination of the λdg by λ (and *vice versa*) was less than or equal to 1% (Kaiser & Hogness, 1960). Such fractions were then dialysed as above.

(ii) *1-liter cultures*

Small lysates (about 1 l.) of λ, $\lambda i^{434}mi$ and $\lambda m_6 i^{434}$ were prepared and purified according to Kaiser (1962).

(e) *Preparation of DNA*

λdg and λ DNA were prepared by extraction of the phage suspensions with water-saturated phenol as previously described (Kaiser & Hogness, 1960). *Micrococcus lysodeikticus* DNA was prepared by J. Josse as described by Josse, Kaiser & Kornberg (1961).

(f) *Biological assays*

(i) *Bacteriophage*

Non-defective λ phage were assayed by mixing, at 0°C, 0·1 ml. of phage in λ-dil with 0·2 ml. of a suspension of gal^-_{t4} in TB obtained by chilling an exponentially growing culture

when it contained 1×10^9 cells/ml. The mixture is placed at 37°C for 20 min and then plated with 2 ml. of TB soft agar on TB plate agar. A plaque-forming unit (p.f.u.) is that amount of phage yielding one plaque by this assay. The most highly purified λ yield 5×10^{11} p.f.u./ml. for a suspension with an absorbancy at 260 mμ (A_{260}) of 1·0 (uncorrected for scattered light). The maximum possible value is 8×10^{11} p.f.u./ml., assuming a molar absorbancy index at 260 mμ of $7 \cdot 6 \times 10^3$ (uncorrected for scattered light, Kaiser & Hogness, 1960), and a DNA of 32 million mol. wt/particle.[2]

The λdg phage are assayed by mixing, at 0°C, 0·1 ml. of the suspension to be assayed with 0·1 ml. of t^- $\lambda i^{434}mi$ helper phage at 1×10^{10} p.f.u./ml. (both in λ-dil medium) and 0·2 ml. of an exponentially growing culture of gal^-_{t4} ($\lambda i^{434}mi$) in TB containing 1×10^9 cells/ml. After 20 min incubation at 37°C, the mixture is plated with 2 ml. TB soft agar on TTGal agar. Plates are counted for galactose-positive (Gal^+) papillae that are surrounded by a zone of lysis after 24 to 30 hr incubation at 37°C. The purified λdg yield $2 \cdot 7 \times 10^{11}$ Gal^+/ml. for $A_{260} = 1 \cdot 00$, but $\leqslant 6 \times 10^9$ p.f.u./ml. by the λ assay. Assuming both λ and λdg suffer the same degree of inactivation during purification, the λdg transduction efficiency in this assay is about one-half the p.f.u. efficiency of λ. When this was tested by isolating both λ and λdg from the same lysate, keeping the procedures as nearly identical as possible, the ratio of λdg Gal^+ transductions to λ p.f.u. (testing each at the same A_{260}) was 0·5[1], which is the value used to calculate the proportion of λ and λdg in a given suspension.

When other gal^- bacteria were used as recipients (e.g. Tables, 2, 3 and 4), the kte^- $\lambda i^{434}mi$ stock of helper phage was used. It should be noted that when the λdg contained the same immunity gene as the helper phage, the Gal^+ transduction efficiency was 0·5[4] that when the immunity genes were different, only the combinations of i^λ and i^{434} having been tested.

(ii) *Activity of* gal$^+$ *and* i$^\lambda$ *genes in DNA*

Method A. This assay is similar to that described previously (Kaiser & Hogness, 1960). The gal^-_{t4} ($\lambda i^{434}mi$) were grown in P medium (0·15% glucose) at 37°C under high aeration to $1 \cdot 0 \times 10^9$ cells/ml. without allowing the pH to drop below 6·7. After cooling to 0°C, the bacteria were sedimented and resuspended in the same volume of 0·01 M-$MgSO_4$, 0·01 M-tris–HCl buffer, pH 7·1, 0·5 mg glucose/ml. and placed at 37°C for 15 min (no agitation). The t^- $\lambda i^{434}mi$ helper phage at 4×10^{11} p.f.u./ml. were then added to a final concentration of 1×10^{10} p.f.u./ml. After 5 more minutes at 37°C, the mixture was cooled to 0°C, the infected bacteria sedimented and resuspended (a) in TCM to 1×10^9 cells/ml. if the bacteria are to be used for assay within 4 hr, or (b) in ½ vol. of freezing medium and are then frozen as 1-ml. portions in liquid nitrogen for later use. If frozen, the bacteria are thawed by 1·5 min exposure to 37°C, quickly cooled to 0°C, sedimented and resuspended in 1·5 vol. of TCM. After another sedimentation, they are suspended in TCM to 1×10^9 cells/ml. Cells frozen in this manner show little, if any, loss in competence after storage for one year.

For assay, 0·1 ml. of DNA in TCM and 0·1 ml. of the above bacteria were mixed at 0°C and placed at 30°C for 50 min. Then 0·1 ml. of 30 μg pancreatic DNase/ml. in 0·01 M-$MgSO_4$, 0·01 M-tris–HCl buffer, pH 7·1, was added. 5 min later, 2 ml. TB soft agar were added and the total poured on TTGal agar plates. The plates were scored after 24 to 30 hr incubation at 37°C.

As in the case of the λdg phage assay, Gal^+ transformants register as papillae which, because of the triphenyl tetrazolium dye, are red against a white confluent background of recipient bacteria. The i^λ activity is detected by the formation of plaques within this same background. These plaques are due to active i^λ-bearing phage which result from recombination between the $\lambda i^{434}mi$ helper phage (to which the recipient bacteria are immune) and λdg DNA which contains i^λ.

Examination of the results in Table 1 reveals that with unstirred whole λdg DNA, essentially all ($\geqslant$ 98%) of the Gal^+ transformants occur as red centers of plaques. This is also the result of the previously described λdg phage assay. With plaques the focus of selective interest, it is seen (Table 1) that both in this assay for whole λdg DNA and in the previous assay for λdg phage, about 80% of them contain Gal^+ centers. These results

TABLE 1

The assay of gal, i^λ *and their linkage*

Agent assayed	gal_{t4}^- ($\lambda i^{434}mi$) recipient† i^λ Plaques per μg DNA $\times 10^{-6}$	i^λ Fraction with Gal⁺ centers	gal^+ Gal⁺ per μg DNA $\times 10^{-6}$	gal^+ Fraction as plaque-centers	gal_{t4}^- recipient‡ i^λ Plaques per μg DNA $\times 10^{-6}$	gal^+ Gal⁺ per μg DNA $\times 10^{-6}$
(A) λdg phage§	5000·0	0·85	4300·0	0·98	4200·0	3200·0
(B) λdg DNA treated as follows:						
(a) no stirring	10·0	0·79	8·0	0·99	9·7	6·0
(b) 900 rev./min for 250 min	10·2	0·80	8·2	1·00	9·6	6·0
(c) 1200 rev./min for 250 min	0·06	0·007	0·002	0·16	5·2	0·72

† The assay of DNA is method A, while that of phage is the standard assay for λdg (Materials and Methods). 500 to 1000 plaques or Gal⁺ were counted in each assay except for the gal^+ assay of DNA stirred at 1200 rev./min, in which case 126 Gal⁺ were examined.

‡ The DNA was assayed by method B in which plaques were formed on TB agar and Gal⁺ on HTGal agar (Materials and Methods). The phage assay was identical to the standard assay except that gal_{t4}^- bacteria were substituted for gal_{t4}^- ($\lambda i^{434}mi$) and separate portions of the assay mixture were plated on TB agar (for plaques) and on TTGal agar (for Gal⁺). 500 to 1000 plaques or Gal⁺ were counted in each assay..

§ The preparation of λdg phage used here contained 0·4% ordinary λ. Its activity had dropped to 85% of the original value determined after isolation.

indicate complete linkage between the gal^+ and i^λ genes in whole λdg DNA and this assay (method A) is used for measuring the gal^+–i^λ linked activity.

Method B. The gal_{t4}^- sensitive bacteria were grown to 1×10^9 cells/ml. as were the bacteria in method A.† These bacteria were sedimented at 0°C and resuspended in the same volume of a modified P medium in which the pH was altered to 7·2, and glucose added to 0·6 mg/ml. Helper phage stock t⁻ $\lambda i^{434}mi$ at 8×10^{11} p.f.u./ml. was added at 0°C to a final concentration of 2×10^{10} p.f.u./ml., and the mixture incubated at 37°C for 15 min, at which time it was cooled to 0°C and centrifuged. The helper infected bacteria were suspended in TCM to a concentration of $1{\cdot}5 \times 10^9$ cells/ml.

1 vol. bacteria was mixed at 0°C with 1 vol. DNA, incubated at 30°C for 50 min, and 1 vol. pancreatic DNase (30 μg/ml., as above) added. 5 min later, 0·3 ml. of this suspension is mixed with (a) 2 ml. of TB soft agar and poured on to TB plate agar, or (b) 2 ml. of AA-G soft agar and poured on to HTGal agar plates. The TB agar plates are incubated at 37°C overnight and scored for plaques (i^λ activity), while the HTGal agar plates are scored for red Gal⁺ colonies (gal^+ activity) after about 48 hr incubation at 37°C.

The results in Table 1 indicate that the efficiency of detection of individual gal^+ and i^λ activities in whole λdg DNA is about the same when the non-lysogenic gal_{t4}^- recipients are used (method B) as when the lysogenic recipients are used (method A). However, the results of method B do differ from those of A in two important particulars; there is a decrease in the efficiency of detection of cotransfer of gal^+ and i^λ; there is an increase in the efficiency of detection of the individual activities for fragments of whole λdg DNA.

If TTGal agar is used with gal_{t4}^- recipients, in place of HTGal agar, the fraction of Gal⁺ papillae which are plaque-centers is low and variable (50 to 80% in more than 100 experi-

† We have recently found that growing the gal_{t4}^- bacteria to $1{\cdot}2 \times 10^9$ cells/ml. in H medium (0·15% glucose) increases the efficiency of the i^λ assay by method B about fivefold over the results reported in Table 1.

ments of 100 or more papillae per experiment).† In one experiment 651 Gal$^+$ papillae were observed of which 315 (57%) were plaque-centers. The i^λ content of 25 papillae that were not plaque-centers was examined (by the method of Table 5) and 17 (68%) contained this gene. Consequently only 86% (57 + 0·68 × 43) of the Gal$^+$ transformants can be demonstrated to contain i^λ, as compared to the 98 to 100% found with method A. There is a similar decrease in the fraction of plaques containing Gal$^+$ transformants; 65% contained Gal$^+$ papillae with the gal^-_{t4} recipient of method B (Table 6), while 80% was found in method A (Table 1).

Since λdg phage also exhibit a reduction of cotransfer of gal^+ and i^λ when assayed on non-lysogenic as compared to lysogenic recipients, it appears that this reduction represents an increase in the fraction of infective events in which one of the two entering markers is lost. One mechanism for this loss would be recombination between λdg and $\lambda i^{434}mi$ helper phage in which the segments not forming the recombinant are lost (Hershey & Rotman, 1949). If this is the case, then the probability of recombination should be higher in the non-lysogenic than in the lysogenic recipient. This is a reasonable condition, since we should expect the number of copies of helper phage chromosome to be greater in the non-lysogenic strain where there is less immunity restriction.

This same plausibility argument can be used to predict that the efficiency of detection of gal^+ and i^λ activities in fragments of λdg DNA should be higher in method B than in method A. In the assay of fragments (but presumably not in the assay of whole molecules) recombination between helper phage and fragment is a necessary primary event. If this recombination is limiting, then the greater its probability, the greater the efficiency. The fact is that the fragments produced by stirring at 1200 rev./min (half-molecules) are far more active in method B than in method A; gal^+ activity is about 400-fold greater; i^λ activity is about 90-fold greater (Table 1).

Consequently, the method B assay is used to measure the individual gal^+ and i^λ activities (particularly of fragments), while method A is used for the gal^+–i^λ linked activity peculiar to whole molecules.

All assays (method A or B) were performed in duplicate at DNA concentrations ($\leqslant$ 0·02 μg/ml.) such that the number scored (plaques, Gal$^+$ or their combination) was proportional to the DNA concentration.

(g) *Shearing*

Solutions of DNA were stirred in a Virtis stainless steel cup (4·5 cm in diameter, 6·5 cm in height) at a volume of 22 ml., using a propeller consisting of two crossed (90°) Virtis 4·0-cm blades having razor-edges on one side. The axis of the stirring shaft is aligned with the axis of the cup and the bottom of the shaft placed 1/32 inch from the bottom of the cup. This shaft is driven by a Heller GT21 motor the speed of which can be continuously varied (to about 5000 rev./min) and held constant to ± 3%. The temperature was kept near 0°C by surrounding the cup with ice-water and employing a room temperature of 4°C.

(h) *Chromatography of DNA on columns of methylated serum albumin*

The method for chromatography of λdg DNA and its fragments (Figs 4 and 5) is a modification of the procedure of Mandell & Hershey (1960) and differs only in the following aspects. All column operations were carried out at 4 ± 1°C. The NaCl solutions referred to by Mandell & Hershey were modified here to contain 0·05 M-tris–HCl buffer, pH 6·7 (25°C) instead of phosphate buffer; except that in the preparation of the protein-coated kieselguhr for the second layer, 0·05 M-potassium phosphate buffer, pH 6·7, was used. In the second layer itself, 15 rather than 10 ml. of the protein-coated kieselguhr was added to the 40-ml. suspension of 6·0 g kieselguhr in 0·40 M-NaCl, 0·05 M-tris–HCl buffer, pH 6·7 (25°C). 15 hr before use, the column was poured and washed with 250 ml. of the solvent

† Plaques form when using the non-lysogenic recipient, since most bacteria which survive the helper infection to form a confluent layer on TB or TTGal agar are lysogenic for the helper phage and consequently immune to i^{434}-bearing phage but not to i^λ-bearing phage. The HTGal plate agar overlayed with 2 ml. of AA-G soft agar does not contain sufficient non-galactose carbon source for the formation of a significant confluent layer of gal^- bacteria.

used for the DNA solution. Just prior to loading the column with the DNA solution, it was again washed with the same solvent until the A_{260} of the eluant was equal to or less than 0·002.

The DNA to be loaded on the column is first dialysed to equilibrate it to a known NaCl concentration (e.g. 0·520 M-NaCl is used in Fig. 5) in 0·050 M-tris–HCl, pH 6·7 (25°C). All salt concentrations in loading and eluting of the column were controlled to within $\pm$ 0·2% by conductivity measurements. The DNA solutions (40 to 50 ml.) were loaded on the column at a flow rate of 30 ml./hr. The DNA was then eluted from the column using either (a) an exponential gradient of NaCl concentration (holding the buffer concentration and pH constant) that employs a constant volume mixing vessel of 260 ml. capacity (Fig. 4), or (b) a constant NaCl concentration equal to that of the DNA solution loaded on the column, followed by such an exponential gradient (Fig. 5). The flow rate is held constant at 30 ml./hr regardless of the elution program and 4·5 to 5·0-ml. fractions are collected by siphon and assayed for (a) NaCl concentration by conductivity, (b) DNA concentration by absorbance at 260 mμ and (c) biological activity by the above assays.

(i) *Physical determinations*

Absorbancy at a single wavelength was determined in a Zeiss PMQ II spectrophotometer using a 1·00-cm light-path. The viscosity of DNA solutions was determined at 25·000 $\pm$ 0·005°C with a capillary viscometer at concentrations of between 10 and 25 μg/ml. (Schachman, 1957). Sedimentation equilibrium (CsCl solutions) and sedimentation velocity measurements were performed in the Spinco model E ultracentrifuge at 25°C using ultraviolet absorption photographs scanned with a Joyce–Loebl recording microdensitometer for measurement of DNA concentrations. The original tracings, made with a thick pen, are shown in Fig. 7.

Results

(a) *Effect of stirring on the viscosity of λdg DNA*

As the hydrodynamic shear applied to λdg DNA is increased, the viscosity of the DNA decreases in discrete steps. The kinetics of this viscosity change at different stirring speeds are shown in Fig. 2.

There exists a critical speed of stirring which must be achieved before any change in viscosity occurs. This speed is greater than 900 rev./min and less than 1200 rev./min for the geometry, temperature and solution environment used here. At 1200 rev./min the viscosity decreases to a stable value called the first plateau. This value is 72% of the initial viscosity; and it indicates that a reduction in average molecular weight of the DNA has occurred.

As the stirring speed is increased above 1200 rev./min, two effects are observed: there is an increase in the rate of transition from the initial to the first plateau value (e.g. 1500 rev./min), and a new critical speed is defined. Consider the condition at 1800 rev./min. Stirring the solution of whole molecules at this speed (upper 1800 rev./min curve, Fig. 2), yields a biphasic curve consistent with two sequential breakage processes: a rapid initial breakage to yield first-plateau material, followed by a second and slower breakage. When first-plateau material (stirred at 1200 rev./min for 180 min, Fig. 2) is subject to 1800 rev./min stirring (lower 1800 rev./min curve), the kinetics of viscosity change are the same as the second phase of the previous curve, and establish a second plateau at a viscosity that is 60% that of the first-plateau value. Increasing the stirring speed above 1800 rev./min increases the rate of transition from the first to the second plateau, but does not significantly alter the viscosity at the second plateau.

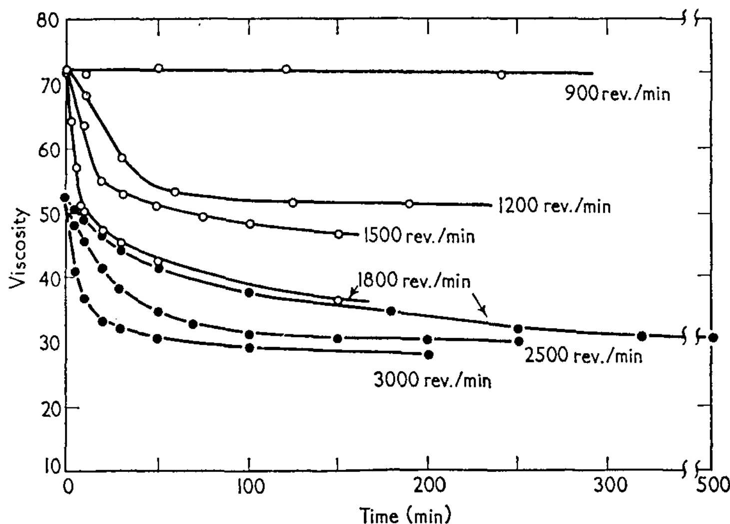


FIG. 2. Change in viscosity of λdg DNA caused by stirring. The solvent for the DNA (25 μg/ml.) was 0·01 M-$MgSO_4$, 0·015 M-NaCl, 0·01 M-tris–HCl buffer, pH 6·7 (25°C). The units for the reduced viscosity ordinate are dl./g. Reduced viscosities of unstirred λdg DNA in 0·001 M-EDTA, 0·01 M-tris–HCl buffer, pH 6·7 (25°C) are $1{\cdot}2_7$-fold greater than in the above solvent, the effect being reversible to changes in solvent by dialysis. The conditions of stirring are given in Materials and Methods; (—○—○—) effects of stirring of untreated λdg DNA; (—●—●—) effects of stirring DNA which had previously been stirred at 1200 rev./min for 180 min.

These results indicate that there are three states of the λdg DNA which are stable to some stirring speed in the range 0 to 1800 rev./min. These are: (1) the whole molecules, stable up to 900 rev./min; (2) the first-plateau material, stable up to 1200 rev./min; and (3) the second-plateau material, stable to 1800 rev./min. The existence of these states, and the lack of other stable states throughout the range of shear forces applied, indicate a quantized process of degradation in which the transition from one state to the next lower state results from only one break per DNA molecule degraded in the transition.

If an analogy is drawn between the shear breakage of DNA in the system employed here and that for a long flexible rod exposed to a linear velocity gradient, mechanical considerations lead one to expect that the breakage point for each transition will occur at the middle of the DNA molecule (Frenkel, 1944; Levinthal & Davison, 1961). The viscosity results in Fig. 2 are consistent with this expectation, but do not offer a very precise confirmation. The viscosities given here were measured with a capillary viscometer with a shear rate of about 10^2 sec^{-1}, and are significantly different from intrinsic viscosities, which involve an extrapolation to zero shear rates.† The relative increase in viscosity attendant upon such an extrapolation appears to be greater the larger the molecular weight of the DNA (Doty, McGill & Rice, 1958; Eigner, 1959; Burgi & Hershey, 1961). Consequently one would expect the ratio of the final to initial measured viscosities to be greater than the corresponding ratio of intrinsic viscosities, and to approach the intrinsic ratio as the molecular weight of the initial DNA in the

† Using our viscometer, we have determined the reduced viscosity of λ DNA to be 100 dl./g in 0·001 M-EDTA, 0·01 M-tris–HCl, pH 6·7 (it is 75 dl./g in the solvent used in Fig. 2). When measured at very low shear rates (1 sec^{-1}) by the method of Zimm & Crothers (1962), the reduced viscosity is about double this value (Kaiser, personal communication).

transition decreases. Since the measured viscosity ratios are 0·72 and 0·60 for the first and second transitions, respectively, the intrinsic ratio should be less than 0·6. If one utilizes the empiric relationship between intrinsic viscosity and molecular weight employed by Doty, Marmur, Eigner & Schildkraut (1960), the ratio of final to initial molecular weights per transition would be less than 0·63.

(b) *Effect of stirring on the genetic activity of λdg DNA*

The transition from whole λdg DNA molecules to the first-plateau fragments defined by the viscosity decrement can be correlated with a break in the linkage between the gal^+ and i^λ genes. If λdg DNA is stirred at speeds just below that critical for the first break (e.g. 900 rev./min) there is no change in activity of the gal^+ and i^λ genes, nor in their linkage relationship (Table 1, Fig. 3). However, when the speed of stirring exceeds the critical value (e.g. 1200 rev./min), there is a pronounced change in the activities (Table 1, Fig. 3).

Consider first the change in the individual i^λ and gal^+ activities assayed with the non-lysogenic gal^-_{t4} (method B, Materials and Methods). From Fig. 3 one observes

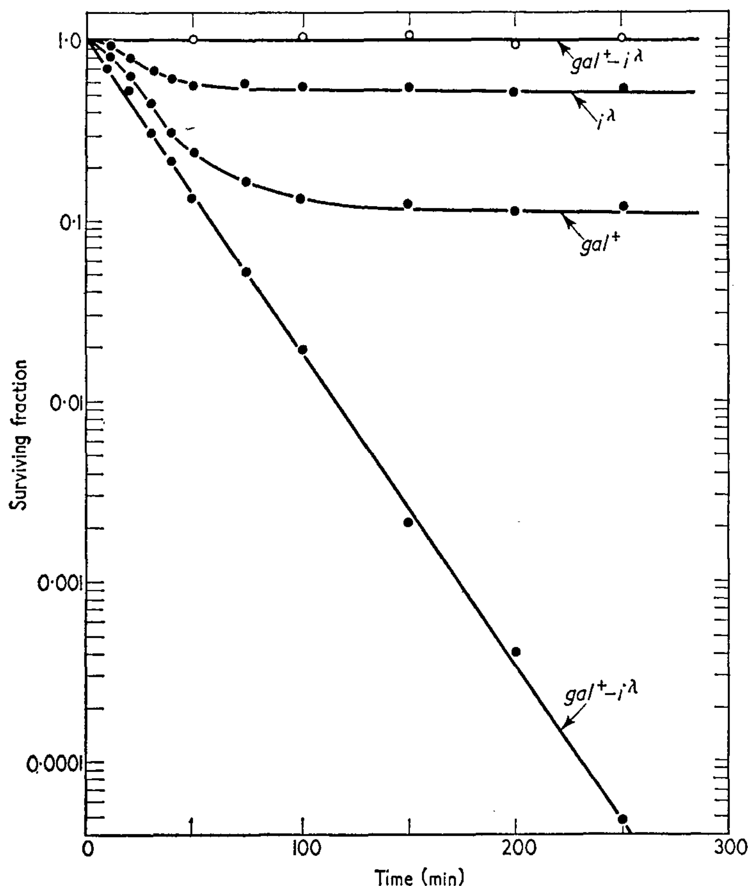


FIG. 3. The sensitivity of gal^+–i^λ linkage to stirring. The experiment is that given in Fig. 2 for 900 and 1200 rev./min. The gene assays on dilutions of 0·1-ml. portions removed at the times indicated are described in the Materials and Methods (method A being used for gal^+–i^λ linked activity and method B for the gal^+ and i^λ individual activities). The initial specific activities are those given in Table 1. —○—○—, 900 rev./min; —●—●—, 1200 rev./min.

that there is a decrease in both these activities, which, like the decrease in viscosity under these conditions, reaches a stable plateau value by 150 min. The gal^+ and i^λ activities in the first plateau material are 12 and 54%, respectively, of the corresponding activities of the whole DNA (Table 1).

When the lysogenic gal^-_{t4} ($\lambda i^{434}mi$) recipient is used (method A, Materials and Methods), there is a much more drastic decrease in the total gal^+ and i^λ activities (Table 1). Although not shown in Fig. 3, the decrease in these total activities with time also occurs at a diminishing rate, tending to a plateau which is about 0·02 and 0·6% of the initial value of the total gal^+ and i^λ activities, respectively (Table 1). On the other hand, the gal^+–i^λ linked activity (see method A, Materials and Methods) after a small lag, decreases with first-order kinetics which show no signs of abating (Fig. 3).

The model that is adopted here to explain these effects and which will be confirmed in the succeeding sections is the following. The break in whole λdg DNA that leads to first-plateau material effects a separation of the gal^+ and i^λ genes such that, of the two fragments produced, one contains gal^+ and the other i^λ. It is assumed that under the conditions of assay used, the efficiency of gene expression for either fragment relative to that for whole molecules is equal to the ratio of the plateau activity to the initial activity. The observed kinetics are consistent with the model: (1) the linked gal^+–i^λ activity decreases exponentially in accord with a one-hit mechanism; and (2) the individual gal^+ and i^λ activities decrease until the fraction of unbroken molecules is small compared to the ratio of fragment to whole efficiencies, at which time the gene activity is stable to further stirring. Thus the apparent time of attainment of a plateau varies with the ratio of fragment to whole efficiencies: the order is i^λ (non-lysogenic), gal^+ (non-lysogenic), i^λ (lysogenic) and finally gal^+ (lysogenic); and the efficiency ratios are 0·54, 0·12, 0·006 and 0·0002, respectively.

(c) *Chromatographic separation of the* gal$^+$- *and* i$^\lambda$-*bearing fragments*

The gal^+-bearing fragments in the material of the first plateau can be separated from the i^λ-bearing fragments by chromatography on columns of methylated serum albumin. This is shown in Figs 4 and 5.

A comparison of the elution profiles for whole λdg DNA (Fig. 4(a)) and the first-plateau material (Fig. 4(b)), as determined by the adsorption at 260 mμ (A_{260}) of the eluant, indicates that the fragments are eluted at lower salt concentrations than the whole molecules, this being qualitatively consistent with the chromatographic behavior of phage T2 DNA and its first-break fragments (Hershey & Burgi, 1960). In fact, the fragments first appear at $0{\cdot}520 \pm 0{\cdot}005$ M-NaCl, whereas the wholes do not appear until the concentration is $0{\cdot}545 \pm 0{\cdot}005$ M.

When the various gene activities are examined, a more detailed analysis is possible. In the case of whole molecules, the elution profile of A_{260} is identical, within experimental error, to that of gene activity, whether that activity is the linked gal^+–i^λ or the individual gal^+ and i^λ activities. This "co-chromatography" of gene activity and of DNA mass is consistent with homogeneity of the whole λdg DNA preparation.

When the first-plateau fragments eluting from the column are assayed for gal^+ and i^λ activity, a clear separation of activities results (Fig. 4(b)). Because of the trailing character of the gradient elution profiles typical for these columns, the leading gal^+ components can be isolated free of i^λ activity, whereas the enriched i^λ fractions all contain some gal^+ activity.

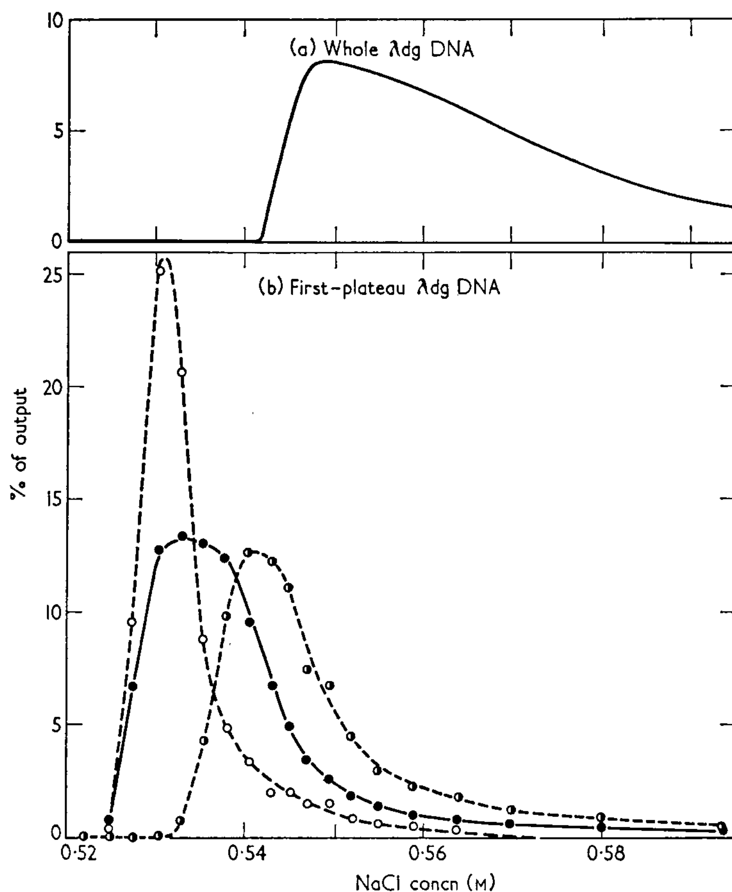


FIG. 4. Gradient elution of whole and first-plateau λdg DNA from methylated serum albumin columns. Except where stated below, the column procedures are those given in Materials and Methods. The ordinate in each case is the percentage of the total activity eluting from the column that is contained in the fraction analysed.

(a) Whole λdg DNA at a concentration of 11 μg/ml. (final salt concentrations were 0·400 M-NaCl, 0·050 M-tris–HCl, pH 6·7, 25°C) and 50 ml. volume was loaded on to the column at a flow rate of 60 ml./hr. It was eluted at 30 ml./hr with an exponential gradient running from 0·500 M-NaCl in the mixing vessel to 0·700 M-NaCl in the reservoir, both at 0·050 M-tris–HCl, pH 6·7 (25°C). Fractions (vol. = 5 ml., Δ[NaCl] = 0·0025 to 0·003 M) were assayed for A_{260} values as well as for *gal*$^{+}$–*i*λ linked activity (method A), and the individual *gal*$^{+}$ and *i*λ activities (method B, modified as in Table 5 legend). When plotted as percentage of output, each of the four values for every fraction falls within ± 15% of the value indicated by the line in the graph, with an average deviation of ± 8%. The yields were as follows: A_{260}, 74%; *gal*$^{+}$–*i*λ, 58%; *gal*$^{+}$, 55%; *i*λ, 61%.

(b) First-plateau λdg DNA that resulted from stirring for 30 min at 1500 rev./min (Fig. 2) was diluted to 10 μg/ml. (final salt concentrations were 0·492 M-NaCl, 0·0037 M-$MgSO_4$, 0·050 M-tris–HCl, pH 6·7, 25°C) and 50 ml. loaded on to the column at a flow rate of 30 ml./hr. It was eluted under the same conditions as in (a) above and fractions assayed for A_{260} (—●—●—) as well as individual *gal*$^{+}$ (– – ○ – – – ○ – –) and *i*λ (– – ◑ – – – ◑ – –) activities (method B, modified as in Table 5 legend). Although the A_{260} distribution has only one peak, the continuous absorbance trace provided by a Gilson ultraviolet transmittance recorder indicated two peaks, the trough between them appearing at 0·535 M-NaCl. The yields were as follows: A_{260}, 73%; *gal*$^{+}$, 61%; *i*λ, 70%.

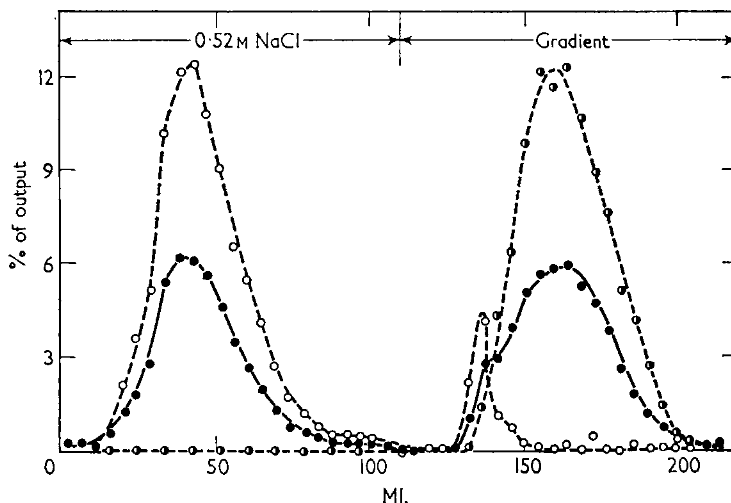


FIG. 5. Stepwise elution of first-plateau λdg DNA from methylated serum albumin columns. After λdg DNA was stirred for 250 min at 1200 rev./min according to conditions given in Fig. 2, it was dialysed first against 100 times its volume of 0·520 M-NaCl, 0·050 M-tris–HCl, 0·010 M-EDTA, pH 6·7 (25°C), then similarly dialysed against 0·520 M-NaCl, 0·050 M-tris–HCl, pH 6·7 (25°C) (with one change of dialysate), and finally diluted to 12 μg/ml. This DNA (43 ml.) was loaded on and eluted from the column exactly as described in the Materials and Methods. The gradient applied at the point indicated was from 0·520 M-NaCl in the mixing vessel to 0·600 M in the reservoir. The assays performed on each fraction, and the symbols used, are the same as those for Fig. 4(b). The yields were as follows: A_{260}, 87%; gal^+, 77%; i^λ, 80%.

A better separation of the two types of fragments can be effected by introducing the first-plateau material to the column in a solution having the NaCl concentration at which the gal^+-bearing fragments first appear in the gradient elution (0·520 ± 0·005 M). In this case, most of the gal^+ activity passes through the column and all of the i^λ activity is retained (Fig. 5). If a gradient in NaCl concentration is then applied to the column, the remaining portion of the gal^+ activity (9%) appears, followed by the i^λ activity, most of which is free of gal^+.

If one assumes that the small fraction of gal^+ fragments which are eluted after the gradient is applied have the same specific activity (gal^+ transformations per μg DNA) as the majority which are eluted earlier, then one can calculate that 51% of the DNA released from the column is associated with gal^+ activity, and 49% is associated with i^λ activity. This 1 : 1 mass ratio of the two fragment populations indicates that the mean of the distribution of breaking points along the whole molecule is at the center.

(d) *Gene content of the isolated fragments*

(i) *The* gal$^+$*-bearing fragments*

The gal^+ activity in the DNA has thus far referred only to the gal_t^+, the gene which specifically controls the formation of galactose-1-phosphate uridyl transferase (transferase). We should like to know the fate of two other genes of the gal operon, gal_k^+ and gal_e^+, that specifically control the formation of galactokinase (kinase) and uridine diphosphogalactose 4-epimerase (epimerase), respectively. For this reason the λdg phage, whole λdg DNA and the isolated gal^+ fragments were each tested for

ability to transform recipients individually mutant for kinase, transferase or epimerase. A kinaseless-transferaseless double mutant and a kinaseless-transferaseless-epimeraseless triple mutant were also tested. The results are given in Table 2.

TABLE 2

Transformation of different gal⁻ *recipients*

Recipient	Gal$^+$/μg DNA × 10^{-6} from:			Ratio of fragment activity to whole activity
	λdg phage†	whole DNA‡	isolated gal^+ fragments §	
gal^-_{k8}	2400	6·5	1·16	0·18
gal^-_{t4}	2500	6·0	0·96	0·16
gal^-_{e22}	2100	3·4	0·48	0·14
gal^-_{k2} gal^-_{t1}	2100	4·6	0·66	0·14
gal^-_{k2} gal^-_{t1} gal^-_{e22}	2000	3·2	0·36	0·11

† The λdg phage contained 0·5% ordinary λ. When used here, its transducing activity (determined by the standard assay) had decreased to 63% of the activity found on isolation. The assay used with the above non-lysogenic recipients was identical to the standard assay except that the given recipient replaced the gal^-_{t4} ($\lambda i^{434}mi$).

‡ The whole DNA was assayed by method B on HTGal agar plates using the given recipients.

§ The gal^+ fragments were isolated according to the procedure given in Fig. 5 and were assayed by the same method as used for wholes.

More than 500 galactose-positive (Gal$^+$) papillae were counted in each assay.

The relatively constant value for the activity of the λdg phage among the various recipients indicates that the particular λdg phage used in this and previous work (Kaiser & Hogness, 1960) is of the common type that includes the three gal^+ genes in its genome, and is not of the rare type that fails to transduce one or two of these genes (Calef, 1961; Adler & Templeton, 1963; Hirota & Lederberg, personal communication).

A comparison of the results from whole λdg DNA and from isolated gal^+ fragments suggests that breakage has not altered the linkage among the three gal^+ genes, since the ratio of fragment to whole activity does not vary greatly among the recipients. Indeed, the transformation of the triple mutant by gal^+ fragments at an efficiency similar to that for the transformation of single mutants clearly indicates that some molecules in the gal^+ fragment population contain all three gal^+ genes. The question we now ask is whether the fragment molecules responsible for single mutant transformations also contain the three genes; i.e. whether the molecules that transform the single mutants are the same as those that transform the triple mutant? The experimental answer to this question depends upon the state of the gal^+ genes in the transformant.

It has previously been shown that the large majority of galactose-positive bacteria resulting from λdg phage transduction (Morse *et al.*, 1956; Campbell, 1957; Arber, 1958), or from whole λdg DNA transformation (Kaiser & Hogness, 1960) are lysogenic for λdg. They are heterogenotes with respect to the relevant *gal* gene; the gal^+ from the infecting agent remains in the λdg prophage, and the gal^- is retained in the host chromosome. Such heterogenotes are unstable. When the λdg prophage is lost, which occurs about once in 10^3 cell generations (Morse *et al.*, 1956; Campbell, 1957), the host bacterium becomes galactose-negative. Consequently when the galactose-positive

transformants from whole λdg DNA (or from λdg phage) are picked and streaked on EMB plates, one finds that about 90% of them (Kaiser & Hogness, 1960) yield one galactose-negative colony per several hundred galactose-positive colonies.

The transformants resulting from the action of the gal^+ fragments on the single- and double-mutant recipients were similarly tested for stability of the galactose-positive phenotype. The results are given in Table 3. It can be seen that the majority (68 to 84% among the four types) are unstable in the above sense.

TABLE 3

Stability and λdg formation in gal⁺ *fragment transformants*

Recipient transformed	Stability		λdg Formation	
	No. tested	Fraction segregating Gal⁻	No. tested	Fraction having λdg/λ ratio greater than 0·1
gal^-_{k8}	44	0·84	10	1·0
gal^-_{t4}	44	0·68	10	1·0
gal^-_{e22}	41	0·71	10	1·0
gal^-_{k2} gal^-_{t1}	44	0·84	10	1·0

Using the assay and gal^+ fragment DNA given in Table 2, galactose-positive papillae were picked and streaked out on EMB plates. These streaks were examined for colonies showing Gal⁻ sectors (Morse *et al.*, 1956). A single Gal⁺ colony was picked from those plates not showing any obvious sectored colonies (includes all of the gal^-_{e22} group) and re-streaked on EMB plates to obtain 500 or more isolated colonies. These streaks were examined for Gal⁻ colonies. The "fraction segregating Gal⁻" includes those giving sectored colonies or giving Gal⁻ colonies on the second streak.

A sectored colony or a colony which gave Gal⁻ on the second streak (all from the first EMB plate) was picked, grown in H medium (0·3% galactose) to 10^9/ml., and a lysate prepared by u.v. irradiation. The lysates were assayed for plaque-forming (λ) and transducing (λdg) activities by the standard assays (Materials and Methods) except that in the transducing assay the recipient was the gal^- mutant used in each transformation. The sum of the λ and λdg activities was in the range 1 to 6×10^{10}/ml. for all lysates.

Another characteristic of the whole of the λdg DNA transformants is that they produce lysates after u.v. irradiation which contain λdg and active λ in roughly equal amounts (Kaiser & Hogness, 1960). This is a characteristic of bacteria doubly lysogenic for λdg and active λ. The unstable transformants resulting from gal^+ fragment infection were also tested for this characteristic; and from the results given in Table 3, one can see that such is the case.

We interpret these results to mean that the preferred path for transformation by gal^+ fragments involves a recombinational event between the infecting fragment and the helper phage to form a λdg chromosome which, by becoming prophage, yields the transformed clone. It should be mentioned here that these lysates contained only $i^{434}mi$ active phage; the i^λ and mi^+ contained in whole λdg DNA were not detected. This indicates that the more complete genetic designation of λdg prophage in the unstable transformants is λdg $i^{434}mi$. One would expect that the assay of λdg $i^{434}mi$ phage in the transformant lysates when using $\lambda i^{434}mi$ helper phage would yield galactose-positive papillae on TTGal agar that were not plaque-centers. This indeed was the case for all 40 lysates. On the other hand, if the helper phage are $\lambda i^\lambda mi^+$, then one would expect the galactose-positive papillae to be plaque-centers. This again was the case in the lysates that were examined with the $\lambda i^\lambda mi^+$ helper (the 20 lysates

derived from the gal^-_{t4} and the gal^-_{k2} gal^-_{t1} recipients). All of these results confirm the above model for the transformation reaction with the gal^+ fragments.

Accepting this model, it should then be possible to measure the number and type of gal^+ genes in the infecting fragment of any recipient by examining the gal^+ gene content of the λdg phage in the lysates of the unstable transformants. This was done by testing each lysate for its transducing activity for the gal^-_{k2} gal^-_{t1} gal^-_{e22} triple mutant and for the recipient from which the particular transformant was derived. The ratio of these two activities is then compared to the identical ratio when the transduction is done with the λdg phage that serves as the original source of the transforming DNA. The results of these assays are shown in Table 4. With only two exceptions

TABLE 4

Test for λdg containing gal_k gal_t gal_e *in the lysates of* gal^+ *fragment transformants*

Recipient transformed	No. of lysates	Ratio of activity for gal^-_{k2} gal^-_{t1} gal^-_{e22} to activity for original recipient: Lysates	Original λdg
gal^-_{k8}	9	0·95 ± 0·14	0·83
	1	0·02	
gal^-_{t4}	10	0·75 ± 0·23	0·80
gal^-_{e22}	9	0·89 ± 0·12	0·95
	1	0·04	
gal^-_{k2} gal^-_{t1}	10	1·01 ± 0·13	0·95

The 10 lysates examined for each class of transformants are those described in Table 3. The assay for transduction was the standard assay (Materials and Methods) except that either the gal^-_{k2} gal^-_{t4} gal^-_{e22} triple mutant or the strains shown in the first column were substituted for gal^-_{t4} ($\lambda i^{434}mi$). The values given in the last two columns equal the ratio of transducing activity for the triple mutant to that for the recipient shown in the first column. The values given under lysates are the averages for the number of lysates given in the preceding column; the range indicated by the ± value includes the values for all of those lysates. Original λdg refers to the isolated λdg preparations used as the source of transforming DNA (Materials and Methods).

among the 40 transformant lysates, the ratios of the two transducing activities are within experimental error of the ratio for the original λdg phage. Thus essentially all of the λdg phage in the 38 lysates contain the three gal^+ genes.

This condition can arise only if the infecting gal^+ fragment contains these three gal^+ genes. While it is true that the single- and double-mutant recipients contain two and one gal^+ gene(s), respectively, if these recipient chromosomes served as the only source of these gal^+ genes, then only a small fraction of the total λdg in the lysate would contain all three gal^+ genes; the vast majority would contain only those gal^+ which the recipient cell lacks. This is because recombination between λdg prophage and the gal^+ operon in the host chromosome is a rare event (Morse *et al.*, 1956; Campbell, 1957; Arber, 1958; Lederberg, 1960). Indeed, were it not so, the two exceptional lysates would not be detectable. In these two lysates, the majority of λdg apparently do not contain all three gal^+ genes, but are lacking in one or two of them. It is possible that these exceptions arise from some gal^+ fragments which lack one or two of the gal^+ genes. Alternatively, they may result from loss of gal^+ genes in the recombination event between the fragment and the helper phage.

The data in section (b) and the absence of i^{λ} and mi^{+} in lysates of the unstable transformants from gal^{+} fragments indicate that the linkage between the gal^{+} genes and i^{λ} or mi^{+} has been interrupted. The latter results suffer from the fact that the lysates examined were from a selected fraction of the transformants, namely, those that are unstable. In order to avoid any ambiguity that might be caused by this selection, galactose-positive transformants formed by whole λdg DNA and by the gal^{+} fragments were picked at random and their lysates examined for i^{λ} and mi^{+} phage (Table 5). It is clear that the transformants from gal^{+} fragments do not contain

TABLE 5

i^{λ} *and* mi^{+} *phage in lysates of transformants*

Agent	No. of transformants examined	No. of lysates containing: i^{λ} and mi^{+}	i^{λ} only	mi^{+} only	neither
Whole λdg DNA	48	40	1	1	6
gal^{+} fragments	53	0	0	0	53

The recipient for the transformation was gal_{t4}^{-} assayed by method B modified in that: (a) the bacteria were infected in the P medium of growth after raising the $MgSO_4$ concentration to 0·01 M; (b) the ratio of helper phage to bacteria was 10; and (c) TTGal agar plates were used. To avoid bias in the selection of Gal^{+} papillae, all papillae in arbitrary sectors were picked. After growing to a total of 10^9 viable cells in 1 ml. H medium (0·3% galactose), these cells were induced to produce phage by u.v. irradiation. The active phage produced were examined by plating on C600 and gal_{t4}^{-} ($\lambda i^{434}mi$) in order to detect mi^{+} and i^{λ} phage, respectively At least 500 plaques were examined on C600 and the equivalent amount plated on gal_{t4}^{-} ($\lambda i^{434}mi$). The mi^{+} phage are also observable on gal_{t4}^{-} ($\lambda i^{434}mi$) if they contain, in addition, i^{λ}.

The gal^{+} fragments were those used in Table 2.

i^{λ} or mi^{+}, whereas the large majority (83%) of those from whole λdg DNA contain both. This confirms the previous results; and we therefore conclude that whereas the linkage among the three gal^{+} genes is not destroyed by the first break in the whole λdg DNA, the linkage between these gal^{+} genes and i^{λ} or mi^{+} is.

(ii) *The* i^{λ}*-bearing fragment*

From the previous results one would expect that the isolated i^{λ} fragments, while not carrying the gal^{+} genes, should carry the mi^{+} gene absent in the gal^{+} fragment. To test this expectation, plaques formed by exposing gal_{t4}^{-} recipients to whole λdg DNA or to isolated i^{λ} fragments were picked and the resulting phage tested for mi^{+}. To confirm the destruction of linkage between i^{λ} and gal^{+}, the same turbid plaques were extracted from the plate and streaked on EMB agar to detect the presence of galactose-positive bacteria within the plaque. The results are given in Table 6.

The lack of galactose-positive bacteria in the plaques caused by i^{λ} fragments and their presence in 65% of the plaques from whole λdg DNA clearly confirm the previous results indicating interruption of linkage between these gal^{+} and i^{λ}. The presence of mi^{+} in greater than 90% of both types of plaques means that the i^{λ}–mi^{+} linkage is not disturbed by the first break. All is consistent with the first break in whole λdg DNA causing a single break in the linkage map somewhere between the dg region and i^{λ} (Fig. 1).

TABLE 6

Gal$^+$ *bacteria and* mi$^+$ *phage in* i$^\lambda$ *plaques*

Agent	No. of plaques examined	No. giving Gal$^+$ bacteria	No. giving mi^+ phage
Whole λdg DNA	200	130	195
i^λ fragments	200	0	189

The i^λ fragments were isolated by the procedure indicated in Fig. 5. The particular fractions used here gave one galactose-positive transformant for every 500 plaques when assayed by method B using TB and HTGal agar plates, respectively. The plaques from both whole λdg DNA and i^λ fragments were formed according to method B. Individual plaques were picked at random and tested for mi^+ phage by plating with C600 on TB plates. More than 500 plaques were examined on these latter plates. The same plaques on the original plate were extracted with a loop and streaked on EMB agar plates. Either more than 100 galactose-positive colonies or papillae (Gal$^+$) or from none to a few are observed. The former group is classified as Gal$^+$ present and the latter as Gal$^+$ absent.

(e) *Physical properties of the* gal$^+$ *and* i$^\lambda$ *fragments*

We now consider the properties which permit the chromatographic separation of the two types of fragments. Although the fact that each type of fragment constitutes one half of the DNA being eluted from the column indicates the mean breaking point is the center of the whole molecule, it does not necessitate that the size distributions of each type of fragment be identical (e.g. the breaking point distribution could be asymmetric). Thus it is possible that the difference in chromatographic behavior is due to a difference in size. This was tested by comparing their sedimentation behavior.

(i) *Zone sedimentation of fragments and whole λdg DNA*

The DNA from the peak gal^+ and i^λ fractions shown in Fig. 4 were mixed with whole molecules and the mixture floated on top of a sucrose gradient column contained in a centrifuge tube. After centrifugation, fractions from this tube were collected by allowing drops to pass through a hole pierced in the bottom of the tube. These fractions were assayed for gal^+–i^λ activity emanating from whole molecules, and for the individual gal^+ and i^λ activities emanating from both wholes and fragments. The results are given in Fig. 6. The gal^+–i^λ activity forms one peak (II), which identifies the position of the zone of sedimenting whole molecules. The individual gal^+ and i^λ activities form two peaks, one of which coincides with the gal^+–i^λ peak and therefore represents the activity of whole molecules. The slower-moving peak (I) of gal^+ and of i^λ therefore represents the zone of the respective fragments. The gal^+ fragment peak fraction and the i^λ fragment peak fraction from the chromatographic column have the same sedimentation coefficients to within 5%. This fact renders unlikely the supposition that their different chromatographic behavior depends upon a difference in size. It also increases the precision of the term "halves" as applied to the gal^+ and i^λ fragments. We therefore adopt this term for the remainder of the paper.

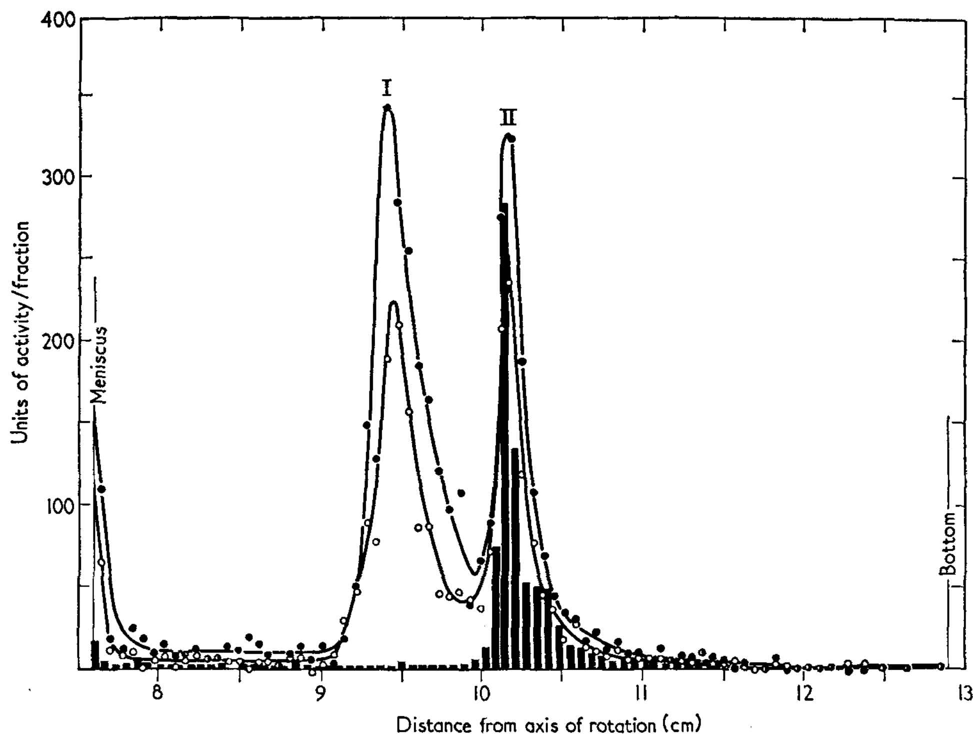


FIG. 6. Zone sedimentation of a mixture of whole λdg DNA and each half-molecule. Portions of the peak fractions of gal^+ and i^λ activity shown in Fig. 4(b) were mixed with unstirred λdg DNA such that the gene activity from each source was approximately equal and the total DNA concentration was 2·3 μg/ml. in a solvent that was equivalent to TCM with the addition of 0·106 M-NaCl. 0·5 ml. of this solution was floated on top of 24 ml. of TCM containing an exponential gradient of sucrose formed by having a constant-volume mixing vessel (60 ml.). The sucrose solution was introduced from the mixing vessel to the bottom of the centrifuge tube by a capillary at an initial concentration of 65 g/l. The sucrose concentration in the reservoirs was 500 g/l., so that the final concentration at the bottom of the tube was 209 g/l. The solution was centrifuged for 7 hr 14 min at 24,800 rev./min (average speed) at 6°C in the Spinco model L no. SW25·1 rotor. After stopping without braking, 150 two-drop fractions were collected through a hole pierced in the bottom of the tube. Alternate fractions were assayed for gal^+–i^λ linked activity (method A) or individual gal^+ and i^λ activities (method B modified according to Table 5 legend). The yields were as follows: gal^+–i^λ, 75%; gal^+, 68%; i^λ, 72%. gal^+–i^λ, ▮▮; i^λ, —●—●—; gal^+, —○—○—.

(ii) *Buoyant densities of halves and wholes in CsCl solutions*

The chromatographic separation of gal^+- and i^λ-halves may be due to a difference in their base-pair composition. If this is the case, then the gal^+-half should be richer in GC pairs than is the i^λ-half, since amongst DNA's of approximately the same size but of different GC content, Sueoka & Cheng (1962) have shown that the higher the GC content the lower the salt concentration necessary for elution from the methylated serum albumin column. Two physical properties of the DNA are associated with GC content: (1) the buoyant density in CsCl solutions (Rolfe & Meselson, 1959; Sueoka, Marmur & Doty, 1959); and (2) the temperature of "melting", i.e. the temperature at which the transition from an ordered double-helical structure to a disordered structure occurs (Marmur & Doty, 1959).

The buoyant densities of whole- and half-molecules in CsCl gradients created by equilibrium centrifugation are given in Fig. 7. The density of the gal^+-halves is seen to be 0·006 g/cm^3 greater than that of the i^λ-halves. Since $\Delta(\text{GC}) = 10 \times \Delta\rho$

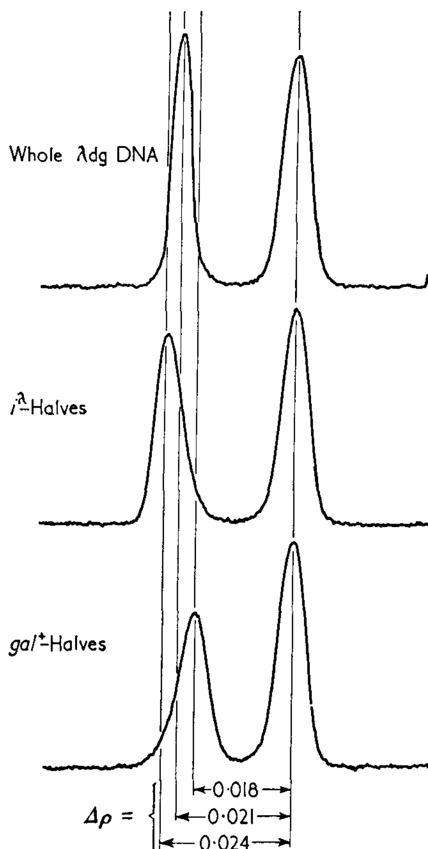


FIG. 7. Sedimentation equilibrium of whole and isolated half-molecules of λdg DNA in CsCl density-gradient. The DNA samples (gal^+ and i^λ half-molecules fractionated according to Fig. 5, whole λdg DNA and *M. lysodeikticus* DNA) were separately dialysed against a solution of $\rho_{25} = 1{\cdot}722$ g/cm^3 made by adding CsCl (Maywood Chemical Co., Maywood, N.J., U.S.A., optical grade) to 0·01 M-tris–HCl, 0·001 M-EDTA and having a final pH of 8·6 at 25°C. Mixtures of dialysed *M. lysodeikticus* DNA with dialysed whole λdg DNA, i^λ-halves, or gal^+-halves were made such that the DNA concentration of each member of the pair was 4 ± 1 μg/ml., CsCl solution equivalent to the above dialysate being added as necessary. After filling standard cells (Kel-F centerpieces) with 0·61 ml. of the mixtures, they were centrifuged 20 to 24 hr at 24·7°C and 44,720 rev./min before the photographs were taken. In the above tracings the density increases toward the right, and the *M. lysodeikticus* DNA is always represented by the right-hand peak. The difference in density between the two peaks of any pair was determined from the distance between them, and the density gradient was calculated from the equation ($d\rho/dr = \omega^2 r/\beta$) given by Ifft, Voet & Vinograd (1961), in which β was taken to be $1{\cdot}20 \times 10^9$, this being the mean of the values given by the above authors and by Trautman (1960) at 25°C for the density region 1·65 to 1·78 g/cm^3.

(where GC is the fraction of base-pairs that are guanine–cytosine, and ρ is the buoyant density in CsCl solution; Rolfe & Meselson, 1959; Sueoka *et al.*, 1959), then the gal^+-half should have a GC-value 0·06 higher than that of the i^λ-half. This is qualitatively consistent with the behavior of these two fractions on the column.

The GC-value for whole λdg DNA is 0·49 (Kaiser & Hogness, 1960). The GC-value for the i^{λ}- and gal^{+}-halves should then be 0·46 and 0·52, respectively, providing these fractions represent true half-molecules. The fact that the same GC-values are derived from a comparison of the difference in buoyant density between either half and the whole molecules indicates that these results are also consistent with the half-molecule conclusion.

(iii) *Heat denaturation of halves and wholes*

The effect of temperature on the gal^{+} and i^{λ} activities of halves and wholes is shown in Fig. 8. The technique here is to examine the activity of either halves or wholes at various times after exposure to a given temperature. At temperatures below 93°C, a plot of logarithm of the surviving fraction against time yields a straight line of near-zero slope over a 15-minute interval. However, as the temperature is increased above this value, temperatures are found at which these curves become biphasic, having an initial, fast inactivation phase followed by a slower inactivating phase (Fig. 8(a), (b) and (c)). Bi-phasic curves similar to these have been found for the heat inactivation of transforming activity of bacterial DNA (Lerman & Tolmach, 1959; Ginoza & Zimm, 1961; Roger & Hotchkiss, 1961). A measure of the degree of first-phase inactivation at a given temperature is provided by extrapolation of the second-phase line to determine its intercept with the ordinate. These intercept values are then plotted against temperature to yield the "melting" curves shown in Fig. 8(d). A collapse of the double-helical DNA structure which is not reversed by quick cooling is thus equated to the rapid inactivation process. The second, slower inactivation is assumed to be caused by such reactions as depurinization or phosphodiester bond cleavage (Doty *et al.*, 1960; Greer & Zamenhof, 1962).

An examination of the "melting" curves in Fig. 8(d) reveals that the gal^{+}-half activity is more stable than the i^{λ}-half activity by 3·2°C. Under similar conditions of high ionic strength, Marmur & Doty (1959) found the empiric relationship, $\Delta(\mathrm{GC}) = 0{\cdot}024 \times \Delta T_{\mathrm{m}}$, and Dove & Davidson (1962*a*) find $\Delta(\mathrm{GC}) = 0{\cdot}019 \times \Delta T_{\mathrm{m}}$ (where T_{m} is the temperature at which 50% of the hyperchromic effect at 260 mμ attendant upon heat denaturation has occurred). A ΔT_{m} of 3·2°C is therefore correlated with a $\Delta(\mathrm{GC})$ of 0·06 to 0·08. Should the activity melting curves for the halves exhibit the same correlation with GC content, one would predict that the GC value for gal^{+}-halves is higher than that for the i^{λ}-halves by $0{\cdot}07 \pm 0{\cdot}01$. This is in good agreement with the $\Delta(\mathrm{GC})$ value derived from the buoyant densities in the previous experiment.

The melting curve for the activity of whole molecules is broader and lies between those of the two halves. Three activities associated with the whole molecules are plotted here: the gal^{+}–i^{λ} activity and the total gal^{+} and i^{λ} activities. It can be seen that these activities all yield the same melting curve within experimental error. This coincidence of gal^{+} and i^{λ} inactivation provides further evidence that both are contained on a single molecule in the whole λdg DNA.

Discussion

The viscosity measurements on stirred λdg DNA (Fig. 2) indicate three states: (1) whole molecules stable to stirring at 900 rev./min but unstable at 1200 rev./min; (2) half-molecules stable at 1200 rev./min but unstable at 1800 rev./min; and (3) mole-

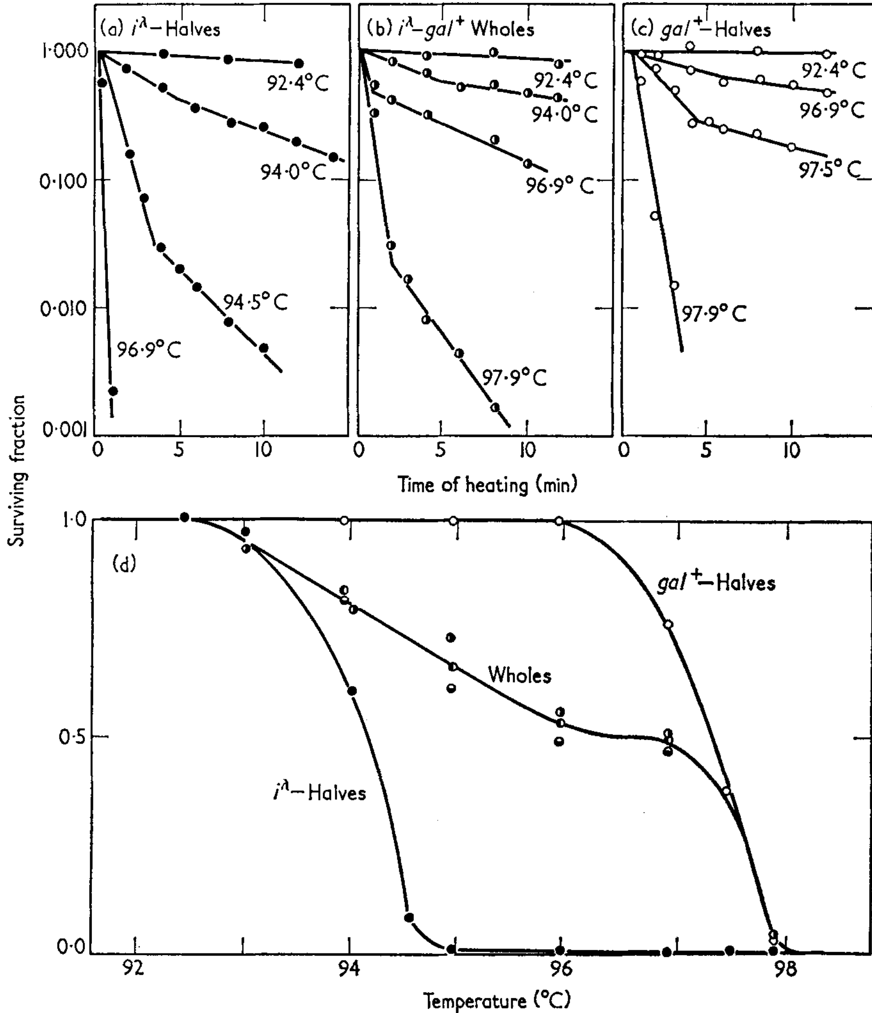


FIG. 8. The heat-denaturation of the wholes and halves of λdg DNA. The peak fractions of gal^+ and i^λ activity shown in Fig. 4(b) and whole λdg DNA were diluted so that the final concentrations were as follows: gal^+-half, 0·45 μg/ml.; i^λ-half, 0·05 μg/ml.; wholes, 0·50 μg/ml.; 0·15 M-NaCl; 0·030 M-sodium citrate; 0·005 M-tris–HCl; pH 7·6 (25°C). The gal^+- and i^λ-halves were mixed during the dilution, while the wholes were kept by themselves. Small glass tubes of 3 mm inside diameter and 5 cm long were filled with 0·050 ml. of either of the two solutions, sealed with a small flame, and were completely submerged in an oil bath having the temperature indicated at zero time. The sealed tubes were removed at the times indicated (Fig. 8(a), (b) and (c)) and quickly cooled in ice water. They were then opened, diluted in TCM and assayed; the tubes containing both the gal^+- and i^λ-halves were assayed by method B; the tubes containing the whole λdg DNA were assayed by method A (i^λ–gal^+ linked activity) as well as by method B for the individual gal^+ and i^λ activities. The method of determining the points in Fig. 8(d) from the kinetic data in Fig. 8(a), (b) and (c) is described in the text, only representative examples of the kinetic data being given in these graphs for the sake of clarity. Fig. 8(d), wholes: i^λ–gal^+, ◑; total gal^+, ◐; total i^λ, ◒.

cules, presumed to be quarters, stable to 1800 rev./min stirring. We have restricted our attention to the first two of these states.

We consider the first state to consist of linear monomeric λdg DNA molecules which contain the entire λdg phage genome. The reasons for considering the molecular units of the first state to contain the entire λdg genome have been given in detail above and in a previous paper (Kaiser & Hogness, 1960). The following arguments indicate that these units are linear monomers, as opposed to either the closed monomers in which the two ends are joined or the end-to-end aggregates reported for λ DNA (Hershey, Burgi & Ingraham, 1963; Ris & Chandler, 1963; Inman, personal communication).

(1) Aggregates comparable to those observed with λ DNA would not be expected to be stable to stirring at 900 rev./min in a system the geometry of which allows formation of half-molecules at 1200 rev./min (Hershey *et al.*, 1963).

(2) Stirring λdg DNA for 30 minutes at 1000 and 1100 rev./min did not significantly alter the viscosity. Were closed λdg DNA monomers present, one would expect to see a rise in viscosity, since (a) the closed monomer of λ DNA is converted to the linear monomer at stirring speeds less than critical for half-molecule formation (Hershey *et al.*, 1963), and (b) the closed monomer should have a lower viscosity. It is probable that the closed λdg monomer can form very slowly under the conditions of solvent, concentration and temperature used in the stirring experiments. Thus after storage of λdg DNA under such conditions for 50, 67, 102 and 144 hours, the viscosity dropped continuously from 71·5 to 69·7, 69·1, 67·0 and 66·0 dl. g^{-1}. However, when subject to 900 rev./min stirring for 50 minutes, each of these solutions yielded a viscosity of $71{\cdot}5 \pm 0{\cdot}4$ dl. g^{-1}. This is consistent with the slow formation of closed monomers from the linear, and rapid reversal of the process by 900 rev./min stirring. Closed λdg DNA monomers have been observed in the electron microscope after storage of the DNA in 2 M-ammonium acetate buffer at 2° to 4°C for a few days (Inman, personal communication).

(3) The λdg DNA used here exhibits an $S_{20,\,w}$ of 32 s at 10 μg/ml. Under comparable conditions of measurements, Hershey *et al.* (1963) observe an $S_{20,\,w}$ of 32 s for linear monomers of λ DNA, 37 s for the closed monomers, and $\geqslant 40$ s for the aggregates.

The molecules in the second state result from center-directed breakage of the linear monomers and have an average size one-half that of the monomers. The major lines of evidence used in the derivation of this conclusion are based on the ability to recognize two classes of molecules. We recognize these classes first of all by their gene activity: one containing the three structural genes of the galactose operon, and the other containing the i^{λ} and mi^{+} phage genes. The column separation of these two classes allows the measurement of the weight fraction of each, which is one-half (Fig. 5). The yield (0·87) was less than unity; but in other experiments the fraction was found to be independent of yield which varied between 0·6 and 0·9, making it unlikely that selection on the column is distorting the true frequency of each class. Hence we conclude that the mean of the breaking point distribution is at the center of the linear monomer.

The second line of evidence indicating equality of size for the two classes of molecules comes from the equivalence of sedimentation properties of the two peak fractions from the chromatographic column (Fig. 6). These peak fractions should represent the most frequent molecular type (on a weight basis) in each class, when the characteristic

of the distribution is the relative binding strength of the DNA to the methylated serum albumin fixed to the column. If we assume that this characteristic is primarily based on DNA size, then the molecules of most frequent size of each class co-sediment (Fig. 6), and therefore have the same size. This would be the result expected for a symmetrical distribution of breaking point, which if unimodal, would have both mean and maximum at the center of the linear monomer. Since the characteristic of the column distribution for either of the two classes may depend upon parameters other than size (e.g. GC-content), this symmetry condition is consistent with, but not demanded by, these results.

Combination of the results of Doty *et al.* (1958) for DNA in the range 0·3 to 7 million molecular weight with that of Burgi & Hershey (1963) for DNA from 15 to 33 million yields the equation $S_2/S_1 = (M_2/M_1)^{0\cdot36}$, where S represents the sedimentation coefficient and M the molecular weight of two DNA molecules. If this relation is correct, the ratio of sedimentation coefficients of half-molecules to whole molecules would be 0·78. Indeed, Burgi & Hershey (1963) found ratios between 0·76 and 0·80 for presumed half and whole molecules of λ DNA. The ratio for sedimentation coefficients of the first-plateau material (Fig. 2) to whole molecules of λdg DNA is 0·78, with a variation of $\pm$ 4% when measured in 1 M-NaCl by the band-sedimentation method of Vinograd, Bruner, Kent & Weigle (1963), using a peak DNA concentration of about 5 μg/ml. (Studier, personal communication). These results agree with those of the zone-centrifugation shown in Fig. 6 if a comparable error is assigned to this type of centrifugation. Thus the ratio of the sedimentation coefficients of the fragments to the wholes computed from the movement of the two peaks is 0·74. A small correction to account for the gradients of viscosity and density in the tube was incorporated into the computation, following the method of Nomura, Hall & Spiegelman (1960), except that account was taken of the non-linear nature of these gradients (the uncorrected ratio is 0·77).

The two classes of half-molecules differ in base composition. This is indicated by differences in their behavior on the column (Figs 4 and 5), their buoyant density in CsCl solution (Fig. 7) and their stability to heat (Fig. 8). The magnitude of the latter two differences is consistent with a difference in GC-content of 0·06 to 0·07. This value and the known GC-content of whole λdg DNA molecules ($0{\cdot}48_6$; Kaiser & Hogness, 1960) leads to an assignment of 0·46 ($\pm$ 0·01) and 0·52 ($\pm$ 0·01) for the GC-content of i^λ and gal^+ halves, respectively.

There are several reasons for believing that this difference is a characteristic of λ DNA rather than a result of the dg insertion (Fig. 1). For example, λ DNA has the same GC-content as λdg DNA ($0{\cdot}48_1$; Meyer, Mackal, Tao & Evans, 1961). Were there no difference in GC content between the two halves of λ DNA, then the resulting difference in GC-content of the i^λ-containing half from λ DNA compared to that from λdg DNA would define a region (about 10% of the half-molecule) of abnormally high GC-content (about 0·75) at the broken end of the λ DNA half-molecule.

We have, in fact, investigated the chromatographic properties of the half-molecules of λ DNA which were prepared under the same conditions of solvent, temperature and stirring speed used for breakage of λdg DNA into halves (Fig. 2). When the elution profiles for the total DNA and for i^λ activity were determined for the λ DNA halves, it was found that the i^λ activity was associated only with the terminal part of the DNA profile, the relationship between the profiles of i^λ activity and DNA being essentially the same as that found for λdg DNA (Figs 4 and 5). This indicates that the

two halves from λ DNA differ in GC-content and that the half with the lower value is the i^{λ}-containing half. Hershey (personal communication) has recently confirmed this supposition by finding that the leading fractions of the elution profile of λ DNA halves contain molecules having a GC-content about 0·07 higher than that found in the trailing fractions of the profile.

The shapes of the curves for the heat inactivation of gene activity (Fig. 8(d)) deserve comment in relation to the heterogeneity of GC-content within the whole λdg DNA molecule. The curve for whole molecules clearly covers a greater temperature range than that for either half-molecule, and it is apparently bimodal, whereas the half-molecule curves appear to be unimodal. Neither of these differences can be accounted for on the basis of a difference in molecular weight, but both can be attributed to intramolecular heterogeneity of GC-content.

To a first approximation, we imagine that the whole λdg DNA molecule denatures with increasing temperatures, as would a simple block polymer consisting of two different double-stranded helical polynucleotides of equal length but different uniform composition that are tied together with phospho-diester bonds. As the temperature is increased, denaturation will first occur in that half which has the lower GC-content (i^{λ}-containing half), causing collapse of the helix and perhaps unwinding of the strands, whereas the half with the higher GC-content (gal^{+}-containing half) remains helical. If such a partially denatured molecule is rapidly cooled, it might zip-up and return to the completely helical configuration (Geiduschek, 1962). However, if this occurred with high probability, then quick cooling would not yield denatured molecules until temperatures sufficient to collapse the high GC-content half were attained. Consequently we should not expect the inactivation of whole molecules to precede (in temperature) the inactivation of isolated gal^{+} half-molecules. Since such a precedence is observed (Fig. 8(d)), we suppose that rapid cooling of the partially denatured molecules leads (a) to metastable partially denatured molecules with gene activities less than those of native molecules, or (b) to molecules of low or zero activity that have lost segments of one strand due to single-strand breaks in the region of denaturation, along with native molecules that have not suffered such breaks, or (c) to both (Lerman & Tolmach, 1959; Rownd, Lanyi & Doty, 1961; Dove & Davidson, 1962*b*). We prefer alternative (b) because of its simplicity, and because such single-stranded breaks appear to exist in whole λdg DNA prior to heating.†

This simple model accounts for the bimodality and width of the heat denaturation curves for the whole molecule as compared to the narrow unimodal curves observed for the half-molecules. It is likely, however, that the assumption of uniform composition in the half-molecules approaches over-simplification, particularly if such heat denaturations were examined at lower ionic strengths, where the form of the transition would be expected to be more sensitive to intramolecular variations in GC-content over smaller regions (Dove & Davidson, 1962*a*,*b*).

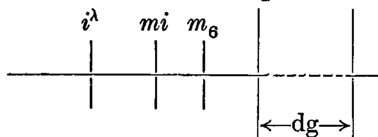
† Whole molecules of λdg DNA, which sediment with a single boundary at pH 7 (1 M-NaCl), exhibit a complex sedimentation boundary in alkaline solutions (0·1 M-NaOH, 0·9 M-NaCl) which indicates that 65 to 80% of the DNA (the percentage depending upon the particular preparation of DNA) moves as a single component, whereas the remaining molecules sediment at a variety of lower rates. Since the pH of the latter solvent is high enough to cause strand separation (Vinograd *et al.*, 1963; Baldwin & Shooter, 1963), this indicates that whereas most single strands in native whole λdg DNA contain no breaks, some strands contain one or more such breaks (or one or more alkaline-sensitive bonds). Similar observations have previously been made with λ DNA (Freifelder & Davison, personal communication).

The distribution of the λdg genes in the half-molecules is consistent with co-linearity between gene sequence on the linkage map for vegetative phage (Fig. 1) and chemical sequence along the DNA molecule. Thus breakage of the DNA molecule at its center causes an interruption of the gal^+ to i^λ or mi^+ linkage without interfering with the i^λ to mi^+ linkage or the linkage among the gal^+ genes. These are the results expected if the linkage map in Fig. 1 were broken at its center (or anywhere between dg and i^λ) to form two independent maps.

Further information consistent with this proposition has been obtained from recent experiments in which we (in co-operation with A. D. Kaiser) tested for the presence of m_6^+ (Fig. 1) in the gal^+ and i^λ half-molecules. Ten lysates of galactose-positive heterogenotes resulting from transformation of gal_{t1}^- bacteria (infected with $\lambda m_6 i^{434}$ helper phage) by isolated gal^+ half-molecules were prepared by the procedures given in Tables 3 and 5 and examined for m_6^+ phage. Seven out of ten of these lysates yielded m_6^+ phage, and all ten yielded normal dg phage which could transduce the triple mutant $gal_{k2}^- \, gal_{t1}^- \, gal_{e22}^-$ with about the same efficiency as they could the original single mutant, gal_{t1}^-. On the other hand, examination of phage picked from 20 plaques that resulted from infection of the above bacteria with isolated i^λ half-molecules revealed no m_6^+ phage in any of the 20 cases (scanning about 100 to 400 plaques in each case). We therefore conclude that the gal^+ halves contain m_6^+ linked to the gal^+ genes, while the i^λ halves do not contain m_6^+.

Our results are also consistent with the gene distributions previously found for λ DNA and its halves (Kaiser, 1962; Radding & Kaiser, 1963). Indeed, a more profitable use of our results might be to accept the argument of co-linearity given by these other experimental results with λ DNA, and to use the gene distributions obtained from λdg DNA to place the gal^+ genes on the same half of the linkage map that contains the dg-region; this region has been mapped only in respect to the normal λ phage genes that it does not contain, and not in respect to the genes that it does contain (Arber, 1958; Campbell, 1959,1961).

To emphasize that the present results give no indication of the distribution of genes within each half-molecule, imagine the map in Fig. 1 to be cut somewhere between the dg-region and i^λ, the two fragments inverted so that mi and m_6 are adjacent to each other, and sealed at this point to form a new map that would have the order:



This map would be consistent with our breakage data and the co-linearity concept, the breaking point now being restricted to a position between mi and m_6. The interesting and cautionary aspect of this manipulation is that the above sequence is that predicted for the λdg prophage on the basis of recent prophage maps constructed for λ (Calef & Licciardello, 1960; Rothman, personal communication) and the suggestion by Campbell (1962,1963) that the genetic map of λ (or λdg) prophage is a circular permutation of the vegetative phage map (Fig. 1). Thus it is possible that the linear monomers of λ or λdg DNA contain the gene sequence found in prophage rather than that found in vegetative phage.

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