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The Strands of DNA from Lambda and Related Bacteriophages: Isolation and Characterization

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Each of the intact complementary strands of DNA from bacteriophage λ has been isolated by virtue of the different buoyant densities they exhibit in alkaline CsCl. Preparations of a given strand are free (< 1%) of the other strand and do not contain detectable amounts of fragments. Individual strands exhibit no significant activity for the genes of λ in the helper-phage assay of Kaiser & Hogness (1960), but renaturation of equimolar mixtures of the two isolated strands yields duplex molecules active for genes which span the λ genome.

The difference in density observed in alkaline CsCl ($\Delta\rho_{alk}$) is not seen in neutral CsCl and is presumed due to a difference between strands in the sum of the frequencies of the titratable bases, G and T (ΔGT). This ΔGT has been estimated to involve about 1500 out of 50,000 base pairs in λ DNA, and essentially all of these have been located in its left half. Comparison of these results with the binding of poly rG to the strands in whole and half molecules of λ DNA indicate that in a given segment of this DNA the strand with the lesser GT content binds the greater amount of poly rG.

The DNA's from a λdg variant and from the λ-related phage, 424, exhibit values of $\Delta\rho_{alk}$ sufficient for the isolation of their strands. Four different types of 424 phage particles were observed on the basis of their buoyant densities in neutral CsCl. The most and least dense of these contain DNA that is 97 and 83% the size of λ DNA, respectively, and the estimated number of base pairs responsible for the ΔGT in each is about 1900. Heteroduplex molecules can be formed between strands of DNA from λ and the most dense 424 particle, providing the strand of highest GT content from one phage is combined with that of lowest GT content from the other. Such heteroduplexes exhibit densities in neutral CsCl midway between the densities of native and denatured DNA, indicating an extent of homology of about 50% between the two phages.

1. Introduction

We are interested in determining the direction of transcription for the various genes in λ DNA (Hogness, Doerfler, Egan & Black, 1966). This direction can be inferred from a knowledge of which strand in the DNA acts as template during the transcription of a given gene. The different methods for identifying the template strand generally depend upon the isolation of the strands. In this paper we first describe a method for the isolation of the complementary strands in the DNA of λ and two related phages, a λdg and 424, reserving the description of a method for identifying the template strand of a λ gene to the succeeding paper (Doerfler & Hogness, 1968).

During the development of this isolation procedure we discovered certain properties of the strands which are described in the second and major part of this paper. One of

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these concerns the difference between complementary strands in the sum of the frequencies of guanine and thymine residues (ΔGT). In alkaline CsCl, these residues each lose a proton, causing the association of additional cesium ions and thereby increasing the buoyant density of the strands (Vinograd, Morris, Davidson & Dove, 1963; Baldwin & Shooter, 1963). One can therefore estimate the ΔGT by examining the differences in buoyant density in alkaline CsCl that do not exist at neutrality, such density differences forming the basis of the isolation procedure described here. We have found that the ΔGT of whole λ DNA is not distributed uniformly along the linear molecule. Rather the left half exhibits a ΔGT almost twice that of the whole molecule, whereas that of the right half is little, if at all, different from zero. Comparison of this distribution with that of the binding sites for poly rG indicates that for a given segment of λ DNA, the strand with the lower GT content binds the greater amount of poly rG.

Finally, we describe here some experiments on the formation of heteroduplex DNA from pairs of isolated strands in which one member derives from λ and the other from 424, experiments which are indicative of the extent of homology existing between these two phages. Of the four heterologous pairs, only the two in which the strand of lowest GT content from one phage is paired with that of highest GT content from the other, form heteroduplex molecules. However, these heteroduplexes exhibit buoyant densities in neutral CsCl which are intermediate between the density of the respective homoduplexes and that of the single strands, indicating a lack of homology over extensive regions of these two phage DNA's of similar size.

2. Experimental Procedures

(a) *Materials*

(i) *Media*

The HCA medium consists of 650 ml. of H-1 medium to which is added 350 ml. of 3% Bacto Casamino acids (Difco) previously treated with Norit A (Pfanstiehl) to remove amino acids absorbing ultraviolet light and 16·5 ml. of a solution containing 10 mg each of L-threonine and L-leucine/ml. and 1 mg of thiamine/ml. The H-1 and other media have been described by Kaiser & Hogness (1960). TM is 0·01 M-Tris–HCl buffer (pH 7·1 at 25°C) and 0·01 M-$MgSO_4$, and TCM is TM plus 0·01 M-$CaCl_2$. TE is 0·01 M-Tris–HCl buffer (pH 7·1 at 25°C) and 0·001 M-EDTA.

(ii) *Bacteriophages*

Wild-type λ (Kaiser, 1957) is implied when the symbol λ is written without qualification. The λdg variant (Kaiser & Hogness, 1960) used here contains all the genes of the galactose operon (Hogness & Simmons, 1964). When tested by the method of Campbell (1959), the deletion in this λdg includes genes *G* through *J* but not *A* through *F* in the left arm of λ. Its DNA is 90% the length of λ DNA (Hogness, 1966). $\lambda i^{434}mi$ has been described (Kaiser, 1962), as has $\lambda ind^{-}cI_{857}$ (Sussman & Jacob, 1962). $\lambda A_{32}J_{27}i^{434}cQ_{21}R_{60}$ was isolated by A. D. Kaiser. It contains the immunity region of phage 434, which includes *c*, a *cI* mutation of λi^{434}; the rest of the genome is from λ carrying the four amber mutations *sus32*, *sus27*, *sus21* and *sus60* in genes *A*, *J*, *Q* and *R* (Campbell, 1961).

$\lambda N_{7,53}$ contains two amber mutations in the *N* gene and was isolated by crossing λN_7 and λN_{53} (Campbell, 1961) in C600 irradiated with ultraviolet light 15 min after infection in λ-dil with a multiplicity of infection of 5 for each parent. Those progeny which exhibited smaller than average plaques on C600, were isolated and backcrossed to both λN_7 and λN_{53}. The $\lambda N_{7,53}$ failed to give rise to wild-type phage capable of plaque formation on W3350 in either backcross.

The density variants of 424 ($424d_1$, $424d_2$, $424d_3$ and $424d_4$) derive from 424c (Baldwin, Barrand, Fritsch, Goldthwait & Jacob, 1966), a clear-plaque mutant of 424. Their properties are described in the section on preparation and purification of phages.

(iii) *Special reagents*

The alternating dAT (Schachman, Adler, Radding, Lehman & Kornberg, 1960) was in 0·15 M-NaCl, 0·015 M-sodium citrate (pH 7) at an O.D.$_{260}$ of 2·6 and is referred to as dAT-solution. The DNA of phage M13 and the β-galactosidase of *E. coli* were provided in purified form by Z. Lucas and M. Cohn, respectively. CsCl was obtained from the Harshaw Chemical Company and subjected to spectrographic analysis; only those preparations containing less than 0·001% as oxides of Al, Ba, Ca, Cu, Mg and Fe were used. A 30% (w/v) stock solution of sucrose (Mann, enzyme grade) in water was treated twice with Norit A before use in sucrose gradients.

(b) *Methods*

(i) *Preparation and purification of phages*

The phages in the following lysogens were prepared by ultraviolet induction of W3104 (λ), W3104 (λ,λdg) (Kaiser & Hogness, 1960), W3104 ($\lambda i^{434} mi$) (Hogness & Simmons, 1964), C600 (λN_7), C600 (λN_{53}) (Campbell, 1961) and C600 ($\lambda N_{7,53}$) as described by Hogness & Simmons (1964) with the exception that growth occurred in HCA rather than H-1 medium prior to irradiation. $\lambda ind^- cI_{857}$ was prepared by heat induction of C600 ($\lambda ind^- cI_{857}$); cells growing exponentially in TB medium at 37°C were heated to 45°C for 10 min when at a concentration of 5×10^8 cells/ml. and then returned to 37°C until lysis. $\lambda A_{32} J_{27} i^{434} c Q_{21} R_{60}$ was prepared from plate stocks.

All of the above phages were purified from their lysates as described by Hogness & Simmons (1964), except for the following modifications which serve to reduce the number of single-strand breaks in the phage DNA. Phage pellets formed by centrifugation at 8000 rev./min in the Sorvall GSA rotor for 7 hr were resuspended in 0·1 M-$MgSO_4$, 0·05 M-Tris–HCl buffer (pH 7·5), the buffer concentration and pH being raised over the previous procedure. CsCl was added as before except that the pH was measured afterwards to guarantee a value in the range 7·1 to 7·5. Banding of the phage in CsCl was accomplished by sedimentation at 4 to 6°C for 7 to 14 hr at 43,000 rev./min in the Spinco model 50 angle rotor.

The preparation of the d_1, d_2, d_3 and d_4 density variants of phage 424 requires detailed comment. In attempts to produce 1-liter lysates of 424c (Baldwin *et al.*, 1966), plate stocks were prepared from plaques and used to infect a 20-ml. culture of C600 according to the method of Baldwin *et al.* (1966), with the exception that the starvation of the bacteria in 0·01 M-$MgSO_4$ for 1 hr prior to the phage infection was omitted. The 20-ml. lysates were used to infect a 1-liter culture of C600 according to the method of Baldwin *et al.* (1966) without modification. After concentration of the phage by centrifugation, resuspension in TM, addition of CsCl and sedimentation to equilibrium according to the standard procedure given above, four clear bands of phage were observed visually and as peaks in the curve of O.D.$_{260}$ *versus* fraction number, a curve similar to that given in Fig. 1. The phage in each band exhibited approximately the same efficiency of plating on C600 ($4 \pm 1 \times 10^{11}$ plaque-forming units/ml. of suspension with O.D.$_{260} = 1$), will not generate plaques on C600 (424), but will do so on C600 (λ) and C600 (434). The phage in the four peaks were termed $424d_1$, $424d_2$, $424d_3$ and $424d_4$, in order to increasing density.

Once-banded phage were recentrifuged and a fraction on the dense side of the $424d_4$ peak used to initiate the two-stage multiplication procedure given in the preceding paragraph. The phage in the final lysate yielded the tracing shown in Fig. 1 after centrifugation in CsCl for 15 hr at 44,700 rev./min and 24°C; hence four density variants are generated from $424d_4$. Using λ phage labeled with 5-bromouracil and having a density of 1·541 g ml.$^{-1}$ as a reference (gift of A. Fritsch), the four peak densities are: $424d_1$, 1·485; $424d_2$, 1·488; $424d_3$, 1·493; and $424d_4$, 1·502 g ml.$^{-1}$.

Since $424d_4$ was obtained almost free of the other density variants when the procedure of Baldwin *et al.* (1966) was used without modification, we infer that $424d_4$ and the 424c used by these workers are equivalent. The $424d_4$ used in the experiments reported here

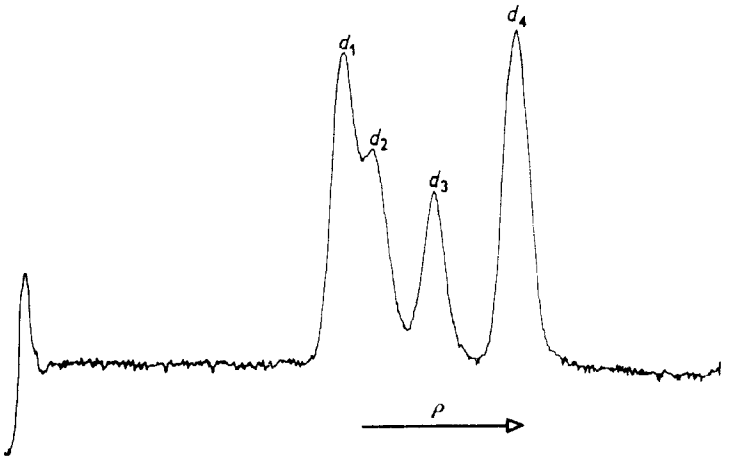

FIG. 1. Equilibrium sedimentation of the density variants of 424 phage in neutral CsCl. The conditions of centrifugation and the definition of the symbols are given in Experimental Procedures section (b)(i).

has been purified by at least one centrifugation in CsCl to remove the lower density variants. While phage from the $424d_1$ peak propagate successfully, attempts to concentrate and band these progeny phage were frustrated by their instability; hence it is not known if the other variants can be formed from $424d_1$. The $424d_1$ referred to under Results are from the lower-density half of the $424d_1$ band. The $424d_2$ and $424d_3$ have not been further investigated.

(ii) *Preparation of DNA*

Phage DNA was prepared according to Kaiser & Hogness (1960) except that the phenol was saturated with 1 M-Tris–HCl buffer (pH 7·1).

(iii) *Assays*

Infective phage were assayed as plaque-forming units on W3104 or C600 as described by Hogness & Simmons (1964). Activities of gal^+, i^λ and i^{424} in phage DNA were assayed according to the method described by Hogness & Simmons (1964), the i^{424} assay being identical to the i^λ assay. The helper phage were $\lambda i^{434} mi$ propagated in W3104. The activity of N and the combined activities of A, J, Q and R in phage DNA were assayed as described by Kaiser & Inman (1965), using W3350 as both recipient and plating bacteria and $\lambda N_{7,53}$ and $\lambda A_{32} J_{27} i^{434} c Q_{21} R_{60}$ as the respective helper phages. The efficiency of the assay was 10^{-3} to 10^{-2} infectious units per phage gene assayed.

β-Galactosidase was assayed according to Pardee, Jacob & Monod (1959) with the modification that 0·001 M-$MgSO_4$ and 0·1 M-β-mercaptoethanol were added to the 0·04 M-sodium phosphate buffer.

(iv) *Analytical centrifugations of DNA*

Sedimentation equilibrium and velocity measurements were performed in the Spinco model E ultracentrifuge using ultraviolet absorption photographs scanned with a Joyce–Loebl recording microdensitometer for measurement of DNA concentrations.

Boundary sedimentation velocity in alkaline NaCl. The DNA (as isolated DNA or as phage) was taken up in 0·9 M-NaCl, 0·1 M-NaOH, 0·001 M-EDTA to a concentration between 5 and 10 μg/ml., and introduced into a 4°, 30 mm, Kel-F cell with a polypropylene pipet inserted through an enlarged filling hole. No breakage of strands resulted from this procedure (see Fig. 3(b)). Photographs were taken every 2 to 4 min, after reaching 25,980 rev./min at 23 to 26°C.

Sedimentation equilibrium in alkaline CsCl. DNA (0·5 to 2·5 μg) in 0·45 or 0·55 ml. TE was mixed with 45 μl. of a freshly prepared solution consisting of: 5 parts 0·50 M-

sodium phosphate buffer (pH 11·7); 2 parts 0·10 M-neutral EDTA; 1 part 1 M-NaOH; and 1 part dAT solution (see Experimental Procedures section (a)). CsCl was added to $\rho_{25} = 1{\cdot}74$ to 1·75 g ml.$^{-1}$ and the solution transferred to a 4°, 12 mm, Kel-F cell as above. Equilibrium was reached after 15 hr at 44,770 rev./min and 23 to 26°C.

Sedimentation equilibrium in neutral CsCl. To 0·5 to 1·0 μg of DNA in 0·45 ml. TE were added 3 to 5 μl. of dAT solution and enough CsCl to yield a density of 1·705 g ml.$^{-1}$. This solution was added to the cell and centrifuged as described above.

Band sedimentation velocity in neutral NaCl. The procedure described by Studier (1965) was followed, the speed and temperature being the same as for the boundary sedimentation.

Calculations. Density differences between the DNA of interest and the reference dAT were calculated according to Ifft, Voet & Vinograd (1961). While the value of ρ for dAT in neutral CsCl was taken as 1·679 g ml.$^{-1}$ (Schildkraut, Marmur & Doty, 1962), we use the symbol ρ_{dAT} in alkaline CsCl, rather than a numerical value since the considerable effects of Na^+ in the buffered solution (Vinograd *et al.*, 1963; Baldwin & Shooter, 1963) and of pressure were not determined.

(v) *Preparative zone centrifugation*

Zone sedimentation was carried out at 2 to 5°C in 5 to 20% (w/v) constant sucrose gradients (1·0 M-NaCl; 0·001 M-EDTA, 0·01 M-Tris–HCl buffer, pH 7·1) centrifuged in Spinco SW25.1 and SW39 rotors at 23,000 and 38,000 rev./min respectively.

(vi) *Alkaline denaturation and renaturation*

Isolated DNA in TE and with an O.D.$_{260}$ as high as 10 was denatured by adding NaOH to 0·03 to 0·05 M. It was kept 5 min at 25°C, then diluted in cold water (0°C) to an O.D.$_{260}$ of 1 to 2, and, at 0°C, rapidly adjusted to pH 7 to 8 by addition of HCl with stirring. The dilution, low temperature and rapidity of neutralization are necessary to avoid renaturation, which is negligible in our hands under these conditions.

All DNA samples (O.D.$_{260}$ = 0·3 to 1·2) were first made 0·05 M in NaOH and then renatured by dialysis in a closed container for 15 to 24 hr at 37°C against 0·1 M-NaCl, 0·001 M-EDTA adjusted to pH 10·5 by addition of NaOH. The dialysate was changed every hour for the first 3 hr to maintain constant pH, after which the pH remained constant (± 0·05) without change of dialysate. Increasing the time interval above 24 hr did not increase the biological activity characteristic of duplexes (see Results section (b)(v)) and no significant breakage of intact strands occurred during the 24 hr.

(vii) *Miscellany*

The pH was measured with a Radiometer type G222B glass electrode and type K130 calomel electrode. Densities of CsCl solutions were calculated from the refractive index according to $\rho_{25} = 10{\cdot}8601\ (n_D^{25}) - 13{\cdot}4974$. Absorbance was measured with a Zeiss PMQII spectrophotometer using a 1-cm light path.

3. Results

(a) *Isolation of the intact strands*

The following procedure results in the isolation of each of the two strands of duplex DNA from λ, λdg or 424. The details are given below and the results of a typical fractionation of λ DNA in Table 1. Unless otherwise stated, manipulations were performed at 0 to 5°C, with equipment treated to sterilize the surfaces coming in contact with the sterile solutions used throughout.

(i) *Sedimentation equilibrium in alkaline cesium chloride*

When either isolated duplex DNA or complete phage particles of λ, λdg or 424 are introduced into solutions of CsCl at pH 12 and centrifuged to equilibrium, the DNA exhibits a bimodal distribution. This was first observed by Baldwin & Fritsch (personal communication) for λ and 424 and is shown for all three phage

TABLE 1

Purification of the H and L strands of λ DNA

Procedure	Fraction	DNA, μμmoles of single strands†	Yield
Starting material	Purified phage		
	H strands	48·	100
	L strands	48·	100
Sedimentation equilibrium in alkaline CsCl	H-i	24·	50
	L-i	27·	56
First zone sedimentation	H-ii	9·6	20
	L-ii	6·7	14
Renaturation	H-iii	6·7	14
	L-iii	5·8	12
Second zone sedimentation	H-iv	1·5	3·1
	L-iv	1·4	2·9

† In computing the μμmoles of strands from the O.D.$_{260}$, it was assumed that each intact strand contains 5×10^4 phosphate residues (Thomas, 1966) and that the extinction coefficients at 260 mμ for phages and for free strands are $7{\cdot}6 \times 10^3$ M^{-1} cm^{-1} and 9×10^3 M^{-1} cm^{-1}, respectively, where M refers to molarity of the phosphate residues (Kaiser & Hogness, 1960).

DNA's in Figure 2. The two modes result from the two strands in each duplex. Discernment of the modes can be obscured by single-strand breaks in the DNA (see Results section (b)(iii)). Hence the DNA or phage used in this first step should have at least 75% of the strands intact. The DNA in the purified phage (Experimental Procedures section (b)) generally has 85 to 100% of the strands intact when examined shortly after purification by the method illustrated in Figure 3. Prior fractionation to remove strand fragments is therefore unnecessary, though it can be accomplished by the method given in Results section (a)(ii) and illustrated in Figure 3.

Preparative banding of the DNA strands was accomplished by centrifugation at 48,000 rev./min for 25 to 37 hr at 5 to 7°C in a Spinco model 50 angle head rotor. To decrease the time of centrifugation the volume per centrifuge tube was reduced to 2·5 ml. and this was introduced in three distinct layers having the same pH (12·1 ± 0·1) but differing in density, the middle layer containing the phage.

The bottom layer consisted of 0·72 ml. per tube of a solution made by mixing 0·11 ml. of 0·10 M neutral EDTA, 0·080 ml. of 1·0 M-NaOH, 0·60 ml. of 0·50 M-sodium phosphate buffer (pH 11·7), 3·05 ml. of TE and enough CsCl to bring the density to 1·77 g ml.$^{-1}$. The middle layer consisted of 0·60 ml. per tube of a solution prepared as follows: 0·85 ml. of a purified phage suspension in 0·05 M-Tris–HCl (pH 7·1), 0·01 M-$MgSO_4$ with O.D.$_{260}$ equal to about 50 were added to 0·55 ml. of 0·10 M neutral EDTA; this mixture was held at 25°C for 10 minutes prior to the addition of 0·11 ml. of 1·0 M-NaOH, 0·48 ml. of 0·50 M-sodium phosphate buffer (pH 11·7), 1·20 ml. of TE and sufficient CsCl to obtain a density of 1·73 g ml.$^{-1}$. The components should be added in the given order. The top layer consisted of 1·21 ml. per tube of a solution made by mixing 0·18 ml. of 0·10 M neutral EDTA, 0·15 ml. of 1·0 M-NaOH, 0·99 ml. of 0·50 M-sodium phosphate (pH 11·7), 5·02 ml. of TE and CsCl to a density of

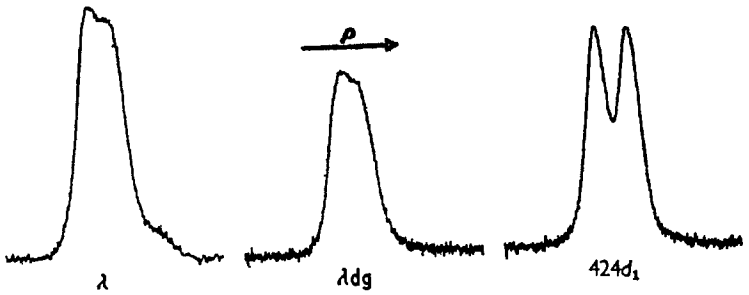

FIG. 2. Equilibrium sedimentation of DNA from λ, λdg and $424d_1$ phages in alkaline CsCl. The conditions of centrifugation of λ and $424d_1$ DNA are the standard ones given under Experimental Procedures section (b). The 2·5 μg of λ DNA was added to the alkaline CsCl as 2 μl. of a suspension of *λind⁻ cI*$_{857}$ phage in the TM, CsCl solution used in its purification. The 2 μg of $424d_1$ DNA was added as 3 μl. of purified $424d_1$ phage in TM, CsCl solution (see Experimental Procedures section (b)). The conditions for the centrifugation of the λdg DNA were different from standard in speed, time and solvent: 1·7 μg of λdg DNA purified from strand fragments as described in Fig. 3(b) and 0·6 μg of dAT in 0·6 ml. of 0·01 M-NaOH, 0·0006 M-EDTA, 0·0016 M-Tris, CsCl ($\rho = 1{\cdot}76$ g ml.$^{-1}$), pH 11·9, were centrifuged for 31 hr at 39,460 rev./min and 22°C. All cells contained dAT (not shown) as a density marker.

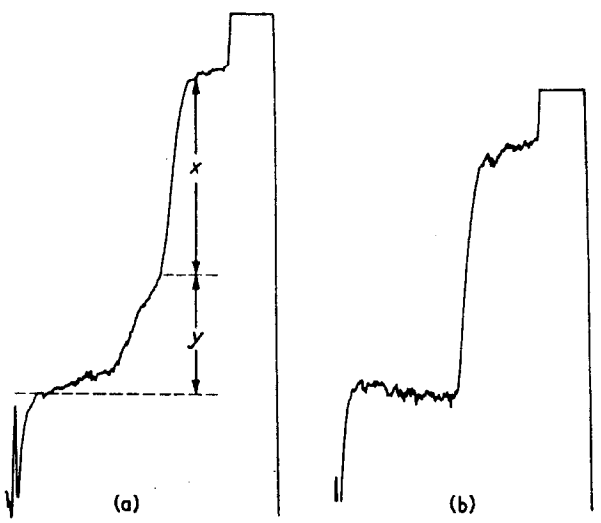

FIG. 3. Boundary sedimentation of DNA in alkaline NaCl before and after removal of strand fragments. The conditions of sedimentation are given under Experimental Procedures section (b). The meniscus is at the extreme left of each Figure.

(a) The λdg DNA used here was isolated by Hogness & Simmons (1964) and had been kept in TE for 2 years in a freezer (−15 to −20°C). To estimate the fraction of intact strands, the heights, x and y, are measured and the ratio, $x/(x + y)$, taken as the desired fraction. For the above tracing, the fraction is 0·62. The average value for all tracings (taken at 2-min intervals after reaching speed) in which it is feasible to measure x and y is 0·59, with a range of 0·56 to 0·62. Freshly prepared λdg DNA gives a value of 0·8 to 1·0.

(b) The λdg DNA used in (a) was subjected to alkaline denaturation and neutralized (Experimental Procedures section (b)). After zone sedimentation as described in Results section (a)(ii), the leading peak was dialyzed against TE and centrifuged to give the above tracing. No significant amount of strand fragments can be detected in this and the other tracings taken at different times. Artificial contamination of these intact strands by addition of halves of λdg DNA (Hogness & Simmons, 1964) was easily detected by boundary sedimentation when the halves constituted 10% (w/w) or more of the DNA, but detection was questionable at 5%.

The $S_{20,w}$ of the DNA in (b) and that represented by x in (a) have the same value, 40 S, in agreement with the value obtained by Studier (1965) using band sedimentation in alkaline NaCl.

1·70 g ml.$^{-1}$. Thick-walled 9-ml. polypropylene centrifuge tubes (Spinco-Beckman) were used to avoid tube collapse during centrifugation of this small volume; thin-walled Polyallomer tubes, if filled with an overlay of mineral oil, will not collapse but have not been tested in this procedure.

The tubes were punctured with a hot insect needle and the O.D.$_{260}$ of the fractions (25 to 75 μl.) determined after appropriate dilution with TE to obtain band profiles equivalent to those shown in Figure 2. Fractions in the heavy half of the distribution were combined, as were those in the light half. The first is enriched for the strand exhibiting the higher density in alkaline CsCl (called H), and the second for the strand with lower density (called L). Each contains about one-half the input DNA for a given strand. The center fractions were discarded because of the overlap in the distribution of the intact strands, and those at the extremes because they are enriched in strand fragments.

The fractions were dialyzed against 5×10^{-3} M-Tris–HCl (pH 7·1), 5×10^{-4} M-EDTA and concentrated by evaporation at 34°C until the O.D.$_{260}$ was about 8, yielding fractions H-i and L-i. No significant losses are encountered in the dialysis and concentration steps.

(ii) *First zone sedimentation: removal of single-strand fragments*

Fractions H-i and L-i were made 0·05 M in NaOH and kept at 25°C for five minutes to reverse any renaturation that may have occurred in the previous steps. After quickly cooling to 0°C and neutralizing by addition of 1·0 M-HCl, the solution was layered onto sucrose gradients and centrifuged in the SW25.1 rotor for 350 ± 10 minutes as described under Experimental Procedures section (b).

After measuring the O.D.$_{260}$ of 0·5-ml. fractions, those constituting the leading and largest peak were pooled to yield fractions H-ii and L-ii. This peak is located about two-thirds of the column volume from the meniscus in the same position as the leading peak shown in Figure 4. It contains intact strands with $S_{20,w} = 96$ S.

The total DNA recovered after centrifugation is somewhat variable, losses of as much as 50% being encountered. The fraction of the recovered DNA selected as fraction H-ii or L-ii is about two-thirds.

(iii) *Renaturation*

The H-ii and L-ii fractions are each contaminated with complementary strands. These are eliminated by renaturation to form duplex DNA, and removal of these duplexes from the desired strands by zone sedimentation. Fractions H-ii and L-ii were first dialyzed against TE for at least four hours, then subjected to the renaturation conditions given under Experimental Procedures section (b), after which they were again dialyzed against TE for at least four hours. Prior to centrifugation (Results section (a)(iv)), the fractions were concentrated by evaporation until the O.D.$_{260}$ was between 0·5 and 2·5, yielding fractions H-iii and L-iii.

(iv) *Second zone sedimentation: separation of the purified intact strands from duplexes*

Fractions H-iii and L-iii were sedimented through sucrose gradients as described in Results section (a)(ii) (see Fig. 4). The fractions making up the leading peak were pooled and dialyzed for at least four hours against TE to yield fractions H-iv and L-iv. These contain the purified intact H and L strands, the characteristics of which are described in the succeeding sections.

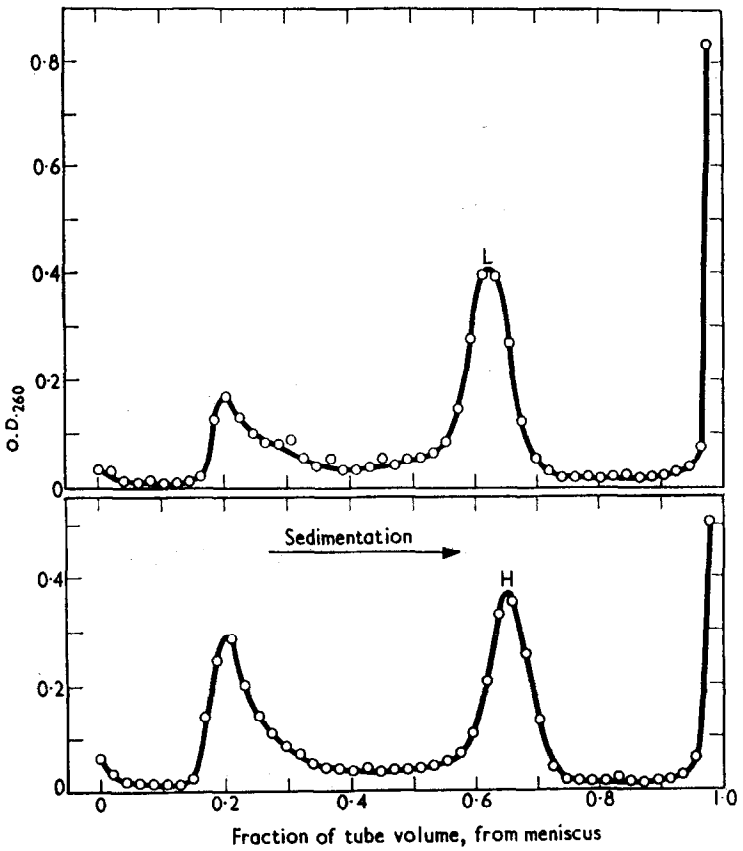

FIG. 4. Zone sedimentation of fractions L-iii and H-iii to yield purified L and H strands of λ DNA. The conditions are given in the text.

The DNA at the maximum of the trailing peak shown in Figure 4 apparently consists of the whole λ duplexes formed from the contaminating strands by renaturation. Band sedimentation in neutral 1 M-NaCl of H-iii in the analytical ultracentrifuge (see Experimental Procedures section (b)) yielded two peaks, similar in relative size to those shown in Figure 4 and exhibiting $S_{20,w}$ values of 35 and 96 S. These values do not differ significantly from those reported for whole duplexes and single strands of λ DNA, respectively (Burgi & Hershey, 1963; Studier, 1965).

(b) *Characterization of the individual strands of λ DNA*

(i) *Sedimentation velocity*

The distribution of the DNA in the H-iv and L-iv fractions during band centrifugation in neutral 1 M-NaCl is shown in Figure 5. The sharp trailing edge with no significant shoulder indicates freedom from contaminating fragments. The $S_{20,w}$ values calculated from these data are 95·6 S and 95·4 S for the H and L strands, respectively. These values are consistent with values we have obtained for unfractionated single strands and are within 4% of the value expected from Studier's (1965) data.

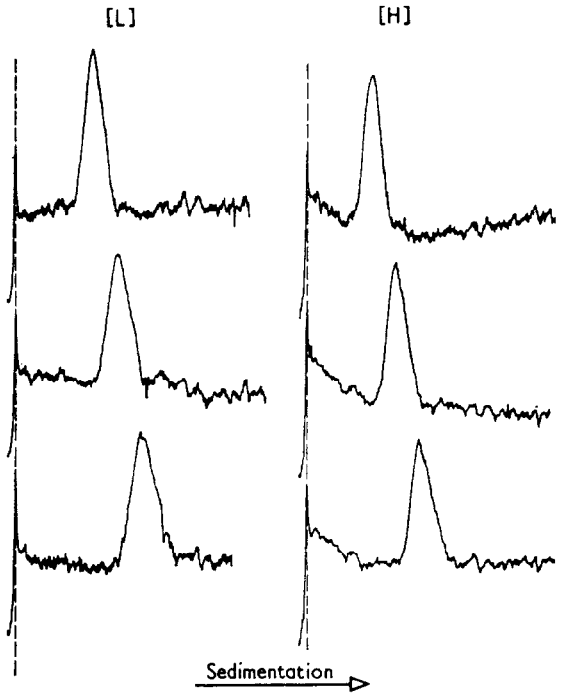

FIG. 5. Band sedimentation of purified L and H strands of λ DNA in neutral NaCl.
After concentration of the L-iv and H-iv fractions to an O.D.$_{260}$ of about 1, they were subject to band sedimentation as described under Experimental Procedures section (b) to yield the above tracings of photographs taken 11, 15 and 19 min after reaching speed for L and 9, 13 and 17 min for H.

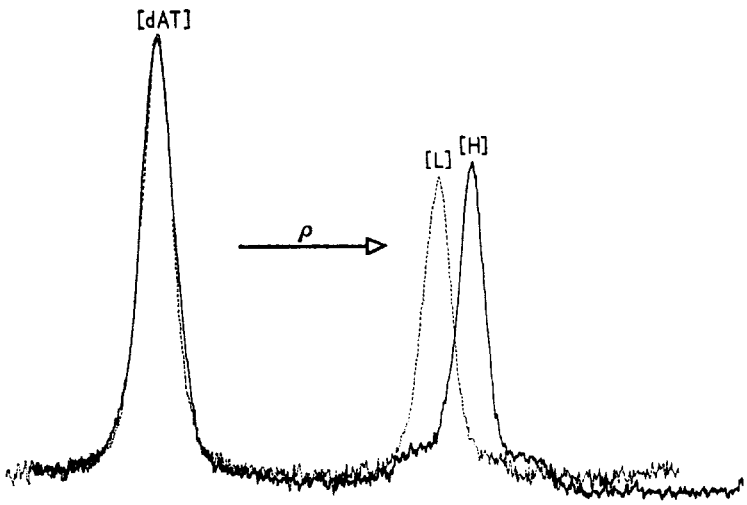

FIG. 6. Equilibrium sedimentation of L and H strands of λ DNA in alkaline CsCl.
Fractions L-iv and H-iv were sedimented according to the procedure given under Experimental Procedures section (b), using identical liquid column heights and densities in each of the two cells centrifuged simultaneously in the same rotor. The tracing of L was superimposed on that of H to yield the above Figure.

(ii) *Equilibrium sedimentation in alkaline cesium chloride gradients*

The distributions of DNA in fractions H-iv and L-iv after 20 hours of centrifugation in alkaline CsCl are shown in Figure 6. The densities of the maxima for the H and L distributions differ by 0·0041 g ml.$^{-1}$. When the same method and calculation were applied to the isolated strands of λdg and 424d$_4$ DNA, the densities listed in Table 2 were found. The densities for the two peaks exhibited by 424d$_1$ DNA in alkaline CsCl (Fig. 2) are also included.

TABLE 2

Densities of H and L strands in alkaline CsCl

Phage	$(\rho_L - \rho_{dAT})$† (g ml.$^{-1}$)	$(\rho_H - \rho_L)$† (g ml.$^{-1}$)
λdg	$0{\cdot}032_0$	$0{\cdot}002_9$
λ	$0{\cdot}032_0$	$0{\cdot}004_1$
424d$_4$	$0{\cdot}032_2$	$0{\cdot}005_1$
424d$_1$	$0{\cdot}032_4$	$0{\cdot}006_0$

† The symbols ρ_H, ρ_L and ρ_{dAT} are the buoyant densities in alkaline CsCl of the H and L strands of each phage DNA, and that of dAT, respectively. The values for λ, λdg and 424d$_4$ derive from individual experiments with each isolated strand. The values for 424d$_1$ were calculated from the unfractionated mixture shown in Fig. 2 after correcting for overlap between the peaks.

(iii) *Cleavage of single strands in alkaline cesium chloride*

We have observed a very slow rate of breakage of the strands of λ DNA in alkaline CsCl. The effects of such breaks on the density distribution of the DNA in each strand is indicated in Figure 7, where the distributions obtained after exposure to alkaline CsCl for 76, 136·5, 478·5 and 890 hours are given.

By 890 hours, or about 37 days, the fraction of the DNA from the H strands which still exhibits the original density is small, indicating that most of the strands have suffered at least one break. From the rate of loss of DNA with this density we estimate that the half-life of the H-strand under these conditions is about 250 hours, and that by 890 hours only 8% of the molecules are intact. In the next section we show that the change in distribution of the L strands is also consistent with this rate of breakage. This rate is between two and three times that which we have measured at lower temperature (4°C) under the conditions used in the first step of the purification. In this case, the fraction of intact strands from λ DNA remaining after various times up to 734 hours was estimated by boundary sedimentation measurements under the conditions given in Figure 3. It should, however, be noted that the loss of intact strands during the 25 to 37 hours of centrifugation in the first step is estimated to be closer to 20% than to the few per cent predicted from these rates, because of a rapid loss of 10 to 15% during the first 10 hours of exposure of unfractionated phage DNA to the cold alkaline conditions. This rapid small loss followed by the slower rate given above was observed in two independent experiments. While we have not investigated this phenomenon further, we presume it to indicate that some 10 to 15% of the strands in a population of mature phage contain a bond, or bonds, that are very much more

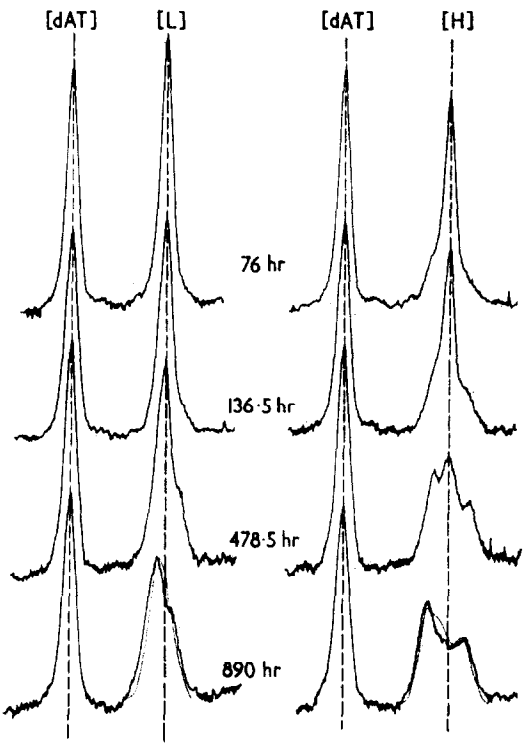

FIG. 7. Cleavage of L and H strands of λ DNA upon long exposure to alkaline CsCl.

The L and H strands in the solvent standardly used for analytical centrifugation in alkaline CsCl were placed in Kel-F cells at time zero and kept at room temperature. At various times thereafter, the cells were simultaneoulsy centrifuged for at least 20 hr under the standard conditions (Experimental Procedures section (b)). The times given in the Figure equal the interval from time zero until the completion of each centrifugation.

The dotted lines superimposed on the 890 hr tracing represent the expected curves if each strand has the structure described in the text and receives an average of 2·5 breaks randomly distributed along its length. These curves were constructed by approximating the 50,000 possible breakpoints with nine equally spaced "breakpoints" and assuming a Poisson distribution of the number of breaks per strand. The fraction of strands receiving one break is 0·205 and as the approximation allows nine different ways of making a single break, each of the two fragments resulting from a given break occurs with a frequency of 0·0228. Multiplication of this value by the length of the fragment relative to the total length gives the desired weight or mass frequency, while the density of the fragment is known from the model given in the text.

This procedure was extended to the 81 ways of receiving two breaks and the 729 ways for three breaks, it being assumed that a given "breakpoint" could encompass multiple breaks. Strands receiving four and more breaks account for only 24% of the total and were more simply estimated by reducing the number of density classes that were considered. The mass frequencies were then summed for each density class. The radial distribution of each class was assumed to be Gaussian, with a mean radius at that position where solvent density equals class density and with a standard deviation determined by molecular weight (Meselson, Stahl & Vinograd, 1957). For all density classes except that for intact strands, the molecular weight was taken as the number-average resulting from 2·5 breaks. The class distributions were graphed approximately with areas proportional to mass frequencies. They were then summed graphically and the resulting curves superimposed on the above tracings after normalization of peak heights, no correction for radial dilution in the sectored cells being made.

sensitive to alkali than are those forming the standard link between subunits. There should, of course, be a strong selection against such strands in our purification procedure.

(iv) *Localization of the difference in GT content between the strands to the left half of λ DNA*

If the 890-hour distributions of H and L DNA, shown in Figure 7, are considered in first approximation to be bimodal, four classes of DNA can be defined: the lower-density fragments from each strand (L-*r* and H-*r*) and the higher-density fragments from each strand (L-*l* and H-*l*); the *r* and *l* designations represent the right and left arms of the strands, respectively, as will become apparent. The calculated differences in the density between the peak for each class and that for dAT are: L-*r*, $0{\cdot}029_3$; H-*r*, $0{\cdot}029_1$; L-*l*, $0{\cdot}034_6$; H-*l*, $0{\cdot}041_8$ g ml.$^{-1}$. The L-*r* and H-*r* classes have the same density, while the L-*l* and H-*l* exhibit greater densities, their average being $0{\cdot}009_0$g ml.$^{-1}$ larger than that for the L-*r* and H-*r* classes. This difference of 0·009 g ml.$^{-1}$ equals the amount by which the buoyant density of left halves of the λ duplex exceeds that for the right halves in neutral CsCl (Hershey & Burgi, 1965). The equivalence suggests that the L-*l* and H-*l* fragments derive from the left halves of the respective strands, and the L-*r* and H-*r* fragments from the right halves.

This interpretation is based on the finding of Vinograd *et al.* (1963) that the increase in buoyant density attendant upon raising the pH of CsCl solutions of duplex DNA above that necessary for denaturation is the same for all duplexes they tested, covering a range of GC contents from 0·34 to 0·72. Consequently, one can anticipate that in alkaline CsCl the mean densities of the strands derived from the left half should exceed that for the strands from the right half by the same 0·009 g ml.$^{-1}$ observed for the difference in density between left and right duplex halves in neutral CsCl.

Why then do the *r* and *l* modes of the H strands exhibit a larger density difference than 0·009 g ml.$^{-1}$, namely, $0{\cdot}012_7$ g ml.$^{-1}$, while the modes of the L strand exhibit a smaller difference of $0{\cdot}005_3$ g ml.$^{-1}$? At this level of first approximation, the answer lies in the observation that the H-*r* and L-*r* modes exhibit essentially the same density, indicating that the complementary strands in the right half of λ DNA have the same GT content. The corollary of this conclusion is that the complementary strands of the left half have a ΔGT value twice that of whole λ DNA and hence should exhibit a density difference twice that found for intact strands, or 0·008 g ml.$^{-1}$. The difference in density between H-*l* and L-*l* of $0{\cdot}007_2$ g ml.$^{-1}$ is in substantial agreement with this expectation. Hence we expect the GT content of the right halves of both H and L strands to be 0·5, while the GT content of the left halves of H and L should be greater and less than 0·5, respectively. The difference in density between the left and right halves of the H strand in alkaline CsCl must then be *greater* than 0·009 g ml.$^{-1}$ by 0·004 g ml.$^{-1}$, i.e. by one-half the expected density difference between the complementary strands in the left half. Similarly, the density difference between left and right halves of the L strand must be *less* than 0·009 by the same 0·004 g ml.$^{-1}$. These expected differences of 0·013 and 0·005 g ml.$^{-1}$ compare favorably with the above observed values of $0{\cdot}012_7$ and $0{\cdot}005_3$ g ml.$^{-1}$.

The 890-hour distributions given in Figure 7 also agree with the above model localizing ΔGT to the left half at a second, somewhat more sophisticated level of approximation. We assume that breakage of the strands in the alkaline CsCl occurs randomly along

each chain, with a probability per phosphodiester bond that is the same for each strand and remains constant with time. From the rate of loss of the intact H strands (Results section (b)(iii)) and the assumption that the distribution of the number of breaks per strand is Poisson, we find that by 890 hours the average number of breaks per strand is 2·5. If we now assign densities to the DNA of various regions in each strand, we can then compute the distribution of the densities of the fragments that would result if populations of such strands were subject to an average of 2·5 random breaks per strand, comparing the computed distributions to the observed distributions given in Figure 7.

For the assignment of densities, we make use of the recent data of Hershey & Burgi (1966) and of Hirschman, Gellert, Falkow & Felsenfeld (1967) which indicate a small region (10 to 20% of the molecule) at the center of linear λ DNA which has a GC content of 0·41, while the remaining left- and right-hand regions have GC contents of 0·56 and 0·46, respectively. We assume that these three regions are of uniform composition and that the center region occupies the middle 10% of the molecule. Assigning the densities of the H-*l* and L-*l* modes to the left-hand regions of the H and L strands, respectively, we can calculate the expected densities for the central region from the values for the GC content if we assume $\Delta GT = 0$ for this region†. Assigning the densities of the H-*r* and L-*r* modes to the respective right-hand regions, we obtain the following model, where the numbers associated with each strand indicate the assigned densities, expressed as the difference between the densities of λ DNA and dAT in alkaline CsCl:

H strand:	0·042	0·023	0·029
L strand:	0·035	0·023	0·029
Fraction of total length	← 0·45 →	← 0·1 →	← 0·45 →

The computed density distributions resulting from these assignments are given as the dotted curves accompanying the 890-hour tracings in Figure 7, the legend of which contains an explanation of the computation. The fit is sufficiently good to indicate consistency of the model with the observed distributions.

This model predicts that the density distribution of isolated right halves in alkaline CsCl will be considerably narrower than that for isolated left halves. The results from such an experiment are shown in Figure 8; they confirm the expectations of the model in some detail. First, the mean densities for the right and left halves are $0{\cdot}029_2$ and $0{\cdot}037_5$ g ml.$^{-1}$ greater than ρ_{dAT}, respectively. These values are to be compared with $0{\cdot}028_4$ and $0{\cdot}037_0$ calculated for exact half-molecules from the above

† $\Delta\rho = 0{\cdot}10\ \Delta GC$ where $\Delta\rho$ is the density difference between two duplexes in neutral CsCl and ΔGC is the difference in GC-content (Schildkraut, Marmur & Doty, 1962). This equation is also valid in alkaline CsCl if $\Delta\rho$ now refers to the difference in mean density of the strands from each duplex (see text). Densities in alkaline CsCl assigned to the left-hand regions are those of H-*l* ($\rho_{dAT} + 0{\cdot}041_8$) and L-*l* ($\rho_{dAT} + 0{\cdot}034_6$), whose mean is $\rho_{dA} + 0{\cdot}038_2$. The ΔGC between the left-hand and central region is 0·15; hence the mean density for the central region is $\rho_{dAT} + 0{\cdot}023_2$, which is the density for this region in each strand on the assumption that its $\Delta GT = 0$.

density assignments. Next, the band-width of right halves measured at half the maximum amplitude is $0{\cdot}006_7$ g ml.$^{-1}$, about one-half the value of $0{\cdot}013_2$ g ml.$^{-1}$ found for left halves (Fig. 8). It can be shown that the distribution for left halves is almost identical to the sum of two distributions, each having the same shape as that for right halves when normalized to one-half the area under the distribution of left halves, and with their means located $0{\cdot}006_6$ g ml.$^{-1}$ apart and equidistant from the mean of left halves. Supposing each of these two distributions to represent one of the strands in the left half, this difference between their mean densities should be compared with $0{\cdot}006_3$ g ml.$^{-1}$, the density difference between strands in the left half expected from the density assignments.

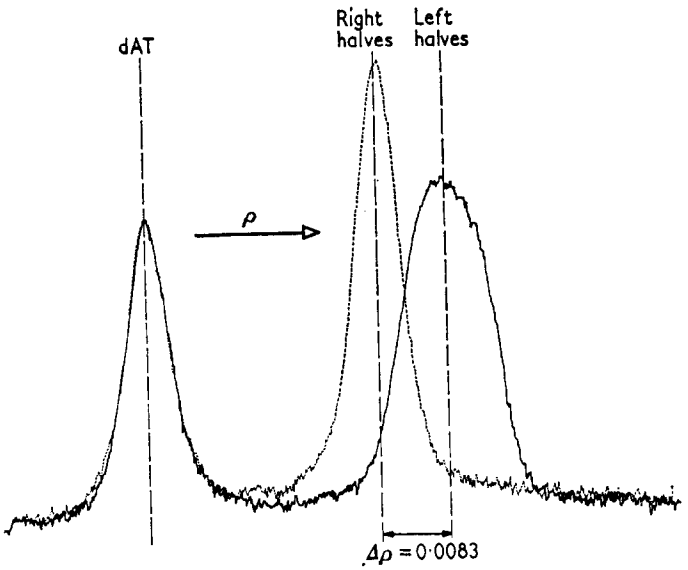

FIG. 8. Equilibrium sedimentation of right and left halves of λ DNA in alkaline CsCl. The conditions of centrifugation are standard (see Experimental Procedures section (b)) except that the dAT was smaller than that used in the other Figures. The halves were isolated by J. B. Egan according to the procedure of Wang, Nandi, Hogness & Davidson (1965).

It should be pointed out that the failure to observe two distinct modes in the distribution of left halves is not unexpected. The distribution of molecular lengths in preparations of halves is known to be quite broad, extending up to 0·65 the length of whole molecules (Hogness, 1966), and strands of different lengths will exhibit different densities due to heterogeneity in the GC content of λ DNA. The maximum range of densities for strands of different lengths in preparations of left halves predicted from the density assignments is $\pm\, 0{\cdot}001_8$ g ml.$^{-1}$ for H and two-thirds this value for L, these ranges being expressed as differences from the density of exact half-strands.

(v) *Formation of duplexes from isolated strands of λ DNA*

When equimolar amounts of isolated L and H strands are mixed at pH 10·5 and 37°C under the conditions for renaturation described in Experimental Procedures section (b), intact duplex molecules are formed. This conclusion derives from the changes which occur in buoyant density in neutral CsCl, in biological activity and in sedimentation coefficient.

Changes in density. The density distribution of an equimolar mixture of isolated H and L strands after renaturation is shown in Figure 9, along with the controls showing distributions of the DNA in the L-iv and H-iv fractions after these were subject to the same renaturation conditions. When treated individually, these conditions do not affect the buoyant density of the DNA in either fraction; it remains at 1·724 g ml.$^{-1}$. The fact that no DNA is detectable at the position of duplex λ DNA ($\rho = 1{\cdot}709$ g ml.$^{-1}$) indicates that there is very little, if any, cross-contamination. When equal amounts of DNA from the L-iv and H-iv fractions are mixed, essentially all of the DNA exhibits a shift to the duplex density. The new band exhibits a slight asymmetry when compared to native λ DNA, there being an excess of 15 ± 5% on the more dense side.

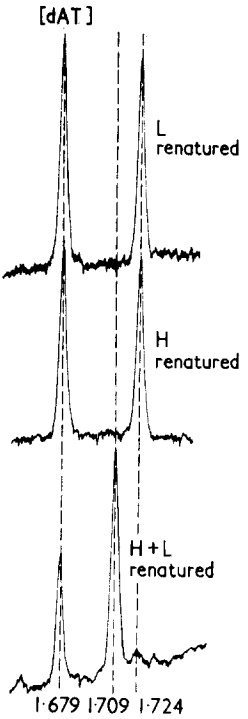

FIG. 9. Equilibrium sedimentation in neutral CsCl of isolated strands of λ DNA, separately, and in equimolar mixture after renaturation. The conditions of renaturation and of centrifugation are described under Experimental Procedures section (b).

Changes in gene activity. The activity of individual genes in λ DNA can be measured with an efficiency between 10^{-3} and 10^{-2} plaque-forming unit per gene when the recipient cells are made competent by a previous infection with helper phage defective with regard to the gene activity to be assayed (see Experimental Procedures section (b)). The data in Table 3 indicate that the isolated single strands of λ exhibit negligible activity ($< 10^{-4}$ that of native λ DNA) in this helper-assay.

Gene activity can be regenerated when equal amounts of H and L strands are subject to renaturation conditions (Table 3). The specific activity (plaque-forming units per ml. of DNA solution with O.D.$_{260} = 1{\cdot}0$) of the renatured material is

TABLE 3

Biological activity of λ single strands and duplexes reconstituted from them

DNA	Relative activity of the *N* gene† (%)
Native λ DNA	100
H + L, renatured‡	46
L, renatured‡	0·07
H, renatured‡	0·11
L, denatured just prior to assay§	0·002
H, denatured just prior to assay§	0·01

† These are specific activity (plaque-forming units/ml. of solution with $O.D._{260} = 1$; see Experimental Procedures section (b)) normalized to the value for native λ DNA (2×10^9). All DNA samples were heated in TE for 10 min at 65°C prior to assay to relieve any cohesion at the molecular ends.

‡ These samples were subjected to the renaturation conditions given under Experimental Procedures section (b).

§ These samples were subjected to the alkaline denaturation and neutralization procedure given under Experimental Procedures section (b).

generally about one-half that of native λ DNA ($0{\cdot}5 \pm 0{\cdot}15$) when renaturation takes place at the lower DNA concentrations of 1·5 to 5 μg ml.$^{-1}$. The relative specific activity appears to be slightly higher ($0{\cdot}7 \pm 0{\cdot}2$) when the DNA concentration is greater (5 to 15 μg ml.$^{-1}$). The activities of reconstituted λdg and $424d_4$ DNA fall within these ranges, as do the activities found when native DNA from all three phages is denatured and renatured without strand fractionation.

The activity measured in Table 3 is that of the *N* gene, located about 12,000 base pairs from the right end of this molecule of 50,000 base pairs (Hogness *et al.*, 1966). We expect that the molecules active for *N* also contain the other λ genes, since the data in the previous sections indicate that the isolated strands are intact. This has been confirmed in the case of genes *A*, *J*, *Q* and *R*. The *A* gene is located a few thousand base pairs from the left end (Jordan & Meselson, 1965), while *Q* and *R* have been located 4000 to 5000 and less than 3000 base pairs, respectively, from the right end (Hogness *et al.*, 1966). The *J* gene is in the left half of the DNA (Radding & Kaiser, 1963), about one-third from the end (Kayajanian & Campbell, 1966). When the activity of all four genes is demanded of the same DNA molecule by using the A^-, J^-, Q^-, R^- quadruple mutant as the helper phage (see Experimental Procedures section (b)), the ratio of the specific activity exhibited by a given renatured mixture of L and H strands to that of native λ DNA is the same ($\pm$ 10%) as the ratio found for *N* activity alone.

When either the L-iv or H-iv fractions are individually subjected to the renaturation conditions, they exhibit activities which are about 0·1% of that for native λ DNA (Table 3). These values are significantly higher than those found if the fractions have been denatured just prior to assay (Table 3), and we suppose they arise from a very small contamination (0·2%) of intact complementary strands in the L-iv and H-iv fractions.

Changes in sedimentation coefficient. When native λ DNA and that formed by renaturation of H and L were separately sedimented as a zone through a sucrose gradient in 1 M-NaCl, the distribution of biological activities (*N* gene) for the two

DNA's was indistinguishable (Fig. 10). In each case, the peak of *N* activity corresponds to an $S_{20,w}$ of 34 to 35 s when referred to M13 DNA present in the initial zone and used as a standard. β-Galactosidase from *E. coli* was also present in the initial zone as another standard, both standards giving consistent results (see the legend of Fig. 10). The $S_{20,w}$ found for *N* activity is equal to that for whole linear duplexes of λ DNA (Burgi & Hershey, 1963; Studier, 1965).

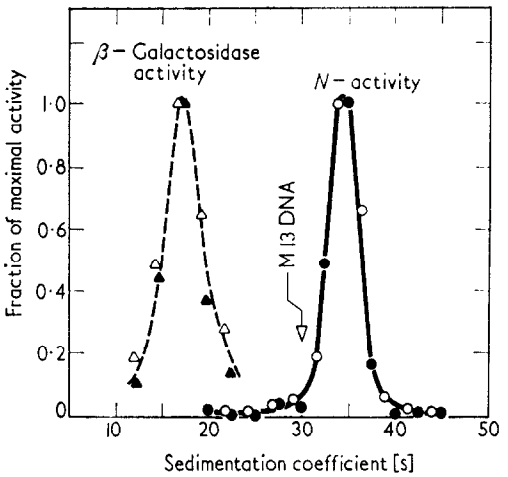

FIG. 10. Zone sedimentation of active duplex λ DNA reconstituted from isolated single strands. Except for time (222 min), the conditions of centrifugation are those given under Experimental Procedures section (b). The results from two SW39 centrifuge tubes are combined in this Figure. In the control tube (open symbols), the reference materials, β-galactosidase (75 units) and M13 DNA (8 μg), were mixed with 0·1 μg of native λ DNA, and the resulting 0·2 ml. layered on the gradient. The other tube (filled symbols) was prepared similarly except that the native λ DNA of the control was replaced by 0·03 μg of DNA formed by renaturation of isolated L and H strands and used in Fig. 9. After centrifugation, 3-drop fractions were assayed for O.D.$_{260}$ (representing the M13 DNA), β-galactosidase and the activity of the *N* gene. The abscissa represents the distance from meniscus to each fraction when normalized to a value of 30 s for the position of the peak fraction of O.D.$_{260}$, this being the value of the $S_{20,w}$ for M13 DNA (Mitra & Kornberg, 1966). The position of the other peaks should equal their $S_{20,w}$ values (Martin & Ames, 1961). The peak of β-galactosidase is at 17 s, in reasonable agreement with the value of 16 s found by Craven, Steers & Anfinsen (1965).

These results and those given in the previous sections indicate that the preparations of isolated strands contain no more than a fraction of one per cent of the complementary strand, and that the isolated strands are intact, even to the extent of containing most of the twenty 5′-terminal residues of the cohesive sites (Wu & Kaiser, 1967, and personal communication) which are necessary for the biological activity assayed here (Kaiser & Inman, 1965).

(c) *The homology between the strands of λ and 424d$_4$ DNA*

The phages of λ and 424 exhibit a certain relatedness (Jacob & Wollman, 1956,1961). They can infect the same host to yield recombinants. Like λ, the 424 phage can be induced by ultraviolet light or by conjugation and is inactivated by anti-λ sera. Baldwin *et al.* (1966) indicate that the DNA from 424 is only 3% smaller than that of λ and has an almost identical buoyant density and, therefore, base composition; they also found that 424 DNA contains two complementary cohesive sites which can cohere with those of the λ DNA. These characteristics indicate homology between

the two DNA's but do not define its extent. Clearly it is not 100%, since the two phages exhibit different immunities, attachment sites, host ranges and efficiencies of induction (Jacob & Wollman, 1956,1961); furthermore, their DNA's exhibit significant differences when sedimented to equilibrium in alkaline CsCl (Fig. 2, Table 2).

One criterion for the extent of the homology between two DNA's of similar length suggested by the preceding experiments would be a measure of the extent of ordered, probably bihelical, structure that could be formed between the four possible heterologous pairs of strands: L^{λ}/L^{424}, L^{λ}/H^{424}, H^{λ}/L^{424}, H^{λ}/H^{424}. Measures defining the extent of ordered regions which come to mind are: observation of duplex and disordered segments in the electron microscope (Inman, 1966), changes in optical density and changes in buoyant density. We have measured only the density changes.

We have used $424d_4$ because its DNA is closer to λ DNA in size than the DNA of the other density variants of 424. We find that the value for $S_{20,w}$ of the single strands of $424d_4$ sedimenting in neutral 1 M-NaCl is 94 s, only slightly less than that found for λ and consistent with the 3% lower molecular weight found by Baldwin *et al.* (1966). The denatured DNA of $424d_1$, the least dense variant, exhibits a sedimentation coefficient 92% that of $424d_4$, indicating that the length of the DNA in $424d_1$ is 86% that in $424d_4$ (Studier, 1965). Since this is in substantial agreement with the density difference of 0·017 g ml.$^{-1}$ that we observe between the two phage particles (Weigle, Meselson & Paigen, 1959), we assume that the DNA in the other two density variants is also smaller than that in $424d_4$.

The mixtures of isolated strands indicated in Table 4 were subject to renaturation conditions, centrifuged to equilibrium in neutral CsCl and the distribution of the DNA determined. The densities of the peaks in the distribution which result from the renaturation are given in Table 4. The new peak of density 1·716 to 1·717 g ml.$^{-1}$

TABLE 4

Formation of heteroduplexes between strands of λ and $424d_4$ DNA

Pairs of strands†	Density in neutral CsCl of new band resulting from renaturation‡ (g ml.$^{-1}$)
$L^{\lambda} + H^{424}$	1·716
$H^{\lambda} + L^{424}$	1·717
$L^{\lambda} + L^{424}$	No new band
$H^{\lambda} + H^{424}$	No new band
$L^{\lambda} + H^{\lambda}$	1·709
$L^{424} + H^{424}$	1·710

† The L^{λ} and H^{λ} are the L-iv and H-iv fractions described in Results (section (a)). The L^{424} and H^{424} were isolated by the same procedure from $424d_4$ DNA except that the renaturation step (Results section (a)(iii)) was omitted. Assay for the activity of the immunity region of 424 (i^{424}) after renaturation of the individual strand preparations and their mixture as described in Results section (b)(v) indicated less than 10% cross-contamination.

‡ The renaturation procedure and the density determinations are described under Experimental Procedures section (b). Each of the single strands when individually subject to this procedure yields a density of 1·724 ± 0·001 g ml.$^{-1}$. In the case of the $L^{\lambda} + L^{424}$ and $H^{\lambda} + H^{424}$, a single band at this density was the only one present. In the case of $L^{\lambda} + H^{424}$ and $H^{\lambda} + L^{424}$, two bands were visible, one at the density of denatured DNA and one at the density given above. The other two pairs yielded only one band at the density given above.

given by the L^{λ}/H^{424} and H^{λ}/L^{424} mixtures, and the lack of any new peak in the density distribution for the L^{λ}/L^{424} and H^{λ}/H^{424} mixtures, indicate a correspondence of the H and L strands in the two phage. If we let the decrease in density of 0·015 g ml.$^{-1}$ that occurs in the formation of the L^{λ}/H^{λ} duplex represent 100% homology, then the drop of 0·007 to 0·008 g ml.$^{-1}$ exhibited by the H and L mixtures of λ and 424 DNA can be taken to indicate a homology between these phage DNA's of about 50%.

It is tempting to adopt the simplest interpretation that, for pairs of strands of essentially equivalent length, this measure of percentage homology corresponds to the formation of heteroduplex molecules in which the standard bihelical structure exists over that same percentage of their length, with no specific strand–strand interaction over the remainder.

4. Discussion

(a) *Differences in GT content between strands*

The complementary strands of λ DNA exhibit a density difference in alkaline CsCl ($\Delta\rho_{alk}$) of $0{\cdot}004_1$ g ml.$^{-1}$ which we presume results from a difference in GT content between the strands. To estimate the value of this ΔGT, we suppose that the density of a strand in alkaline CsCl can be formulated as $\rho_{alk} = \Sigma f_x \rho_x$, where f_x is the frequency of base x and ρ_x is a coefficient specific to base x and having the units of density. Defining ΔG as $(f_G)_H - (f_G)_L$, ΔT as $(f_T)_H - (f_T)_L$, and ΔGT as ΔG + ΔT, where H and L refer to the strands of highest and lowest density in alkaline CsCl, we have:

$$\Delta\rho_{alk} = \Delta T(\rho_T - \rho_A) + \Delta G(\rho_G - \rho_C).$$

Since

$$\Delta T = \Delta GT - \Delta G,$$

then

$$\Delta\rho_{alk} = \Delta GT(\rho_T - \rho_A) + \Delta G[(\rho_G - \rho_C) - (\rho_T - \rho_A)],$$

which becomes,

$$\Delta GT = \frac{\Delta\rho_{alk} - \Delta G[(\rho_G - \rho_C) - (\rho_T - \rho_A)]}{(\rho_T - \rho_A)}. \quad (1)$$

The values of $(\rho_G - \rho_C)$ and $(\rho_T - \rho_A)$ can be calculated from equation (1) and the known density differences for the complementary strands of certain synthetic polydeoxynucleotides. From the $\Delta\rho_{alk}$ for each member of the pair, poly dTC : dGA and poly dTG : dCA, we can calculate† that $(\rho_T - \rho_A) = 0{\cdot}16_4$ g ml.$^{-1}$ and $(\rho_G - \rho_C) = 0{\cdot}11_2$ g ml.$^{-1}$. The value of $(\rho_T - \rho_A)$ can be calculated† independently from the $\Delta\rho_{alk}$ of poly dT : dA to be $0{\cdot}14_9$ g ml.$^{-1}$, in fair agreement with the previous

† Alternating poly dTC is 0·026 g ml.$^{-1}$ more dense than its complement, poly dGA in alkaline CsCl (data of R. D. Wells quoted by Szybalski, 1968), while alternating poly dTG is 0·138 g ml.$^{-1}$ more dense than its complement, poly dCA (Doerfler & Hogness, 1965). Substituting these values into equation (1), one obtains $(\rho_T - \rho_A) = 0{\cdot}276 - (\rho_G - \rho_C)$, and $(\rho_T - \rho_A) = 0{\cdot}052 + (\rho_G - \rho_C)$, respectively, from which $(\rho_T - \rho_A) = 0{\cdot}16_4$ g ml.$^{-1}$ and $(\rho_G - \rho_C) = 0{\cdot}11_2$ g ml.$^{-1}$. Poly dT is 0·149 g ml.$^{-1}$ more dense than poly dA in alkaline CsCl (Wells & Blair, 1967), giving $(\rho_T - \rho_A) = 0{\cdot}14_9$ g ml.$^{-1}$.

value. Substituting the single value for $(\rho_G - \rho_C)$ and the mean of the two values for $(\rho_T - \rho_A)$ in equation (1), we obtain:

$$\Delta GT = 6{\cdot}_4\,\Delta\rho_{alk} + 0{\cdot}2_8\,\Delta G. \quad (2)$$

The ΔGT for λ DNA calculated from equation (2) is $0{\cdot}031 \pm 0{\cdot}005$, where the range of values results from allowing ΔG to vary between 0 and ΔGT (the range will be greater if ΔG and ΔT are allowed to have opposite signs; e.g. if ΔG is varied between $-0{\cdot}5$ ΔGT and 1·5 ΔGT, the calculated values lie within the range $0{\cdot}034 \pm 0{\cdot}011$). This result is in rough agreement with the value of 0·04 estimated by assuming that the increase in density of single strands in CsCl resulting from alkaline titration is proportional to the GT content†.

The ΔGT calculated for the left halves of λ DNA ($\Delta\rho_{alk} = 0{\cdot}007$ g ml.$^{-1}$, Results section (b)(iv) from equation (2) is $0{\cdot}054 \pm 0{\cdot}009$, again allowing ΔG to vary between 0 and ΔGT. Such a bias in GT content would involve some 1350 ± 225 base pairs out of the 25,000 pairs in a half-molecule of λ DNA. For whole molecules, the calculated number of base pairs accounting for their ΔGT of $0{\cdot}031 \pm 0{\cdot}005$ is 1550 ± 250. That these two values are essentially the same re-emphasizes the previous conclusion that the ΔGT of λ DNA is concentrated in the left half.

The values of ΔGT calculated in a similar manner from the $\Delta\rho_{alk}$ of the DNA from the λdg variant used here, and from $424d_4$ and $424d_1$ (Table 2) are $0{\cdot}022 \pm 0{\cdot}004$, $0{\cdot}039 \pm 0{\cdot}006$ and $0{\cdot}046 \pm 0{\cdot}008$, respectively. Taking into account the fact that the λdg DNA is 90% of the length of λ DNA (Hogness, 1966), and that $424d_4$ and $424d_1$ are 97 and 83% the length of λ DNA (Baldwin *et al.*, 1966; Results section (c)), the number of base pairs accounting for these ΔGT's are: λdg, 990 ± 180; $424d_4$, 1890 ± 270; and $424d_1$, 1910 ± 330. Since the last two values are essentially the same, we may suppose that the DNA lost from $424d_4$ in the creation of $424d_1$ has a ΔGT very close to zero. Implicit in this supposition is the assumption that $424d_1$ is a simple deletion variant which contains no DNA foreign to $424d_4$. This is the apparent relationship between the λb_2 deletion variant and λ.

The DNA of λdg, on the other hand, has acquired some *E. coli* DNA into its left half (Hogness & Simmons, 1964) while deleting a segment of λ DNA. The deleted λ DNA extends from a region between genes *F* and *G* (see Experimental Procedures section (a)) located 10 to 15% of the molecular length from the left end of λ DNA (Kayajanian & Campbell, 1966) to a point at least 54% of the molecular length from the left end (Skalka & Burgi, 1967). In losing at least 40% of the λ DNA, or about 20,000 base pairs, this λdg must have gained at least 15,000 base pairs of *E. coli* DNA, since the net loss of DNA in forming this λdg is about 10% (Hogness, 1966) or 5000 base pairs. In this exchange, the calculated ΔGT decreases from 0·031 to 0·022, indicating the relation between the ΔGT of the deleted λ DNA (ΔGT_d) and that of the acquired *E. coli* DNA (ΔGT_g) is approximated by $\Delta GT_g = (4/3)\,\Delta GT_d - 0{\cdot}04$. The value of

† The difference in mean density between denatured natural DNA's in alkaline and neutral CsCl is roughly 0·05 g ml.$^{-1}$ (0·047 and 0·050 g ml.$^{-1}$ for the DNA's of *E. coli* and *M. lysodeikticus*; Vinograd *et al.*, 1963), which is taken as the density difference for strands with a GT content of 0·5 since this is the mean GT content of denatured bihelical DNA. If such density differences are proportional to GT content, we have:

$$\Delta GT = (0{\cdot}5)[(\rho_{alk_H} - \rho_N)/0{\cdot}05] - (0{\cdot}5)[(\rho_{alk_L} - \rho_N)/0{\cdot}05],$$

where ρ_{alk_H} and ρ_{alk_L} are the densities of the H and L strands in alkaline CsCl, and ρ_N is their common density in neutral CsCl. This becomes $\Delta GT = (\rho_{alk_H} - \rho_{alk_L})/0{\cdot}1 = 10\,\Delta\rho_{alk}$, this equation applying only where there is no difference in density between the strands in neutral CsCl.

ΔGT_g may therefore be positive, zero, or negative depending upon whether ΔGT_d is greater than, equal to, or less than 0·03. Because the ΔGT of λ DNA is markedly different for the two halves, an estimate of ΔGT_d on the basis of existing data would be excessively hazardous, and consequently we cannot yet restrict the sign of ΔGT_g.

(b) *Correlation between GT content and binding of poly rG*

The strands of λ DNA can also be isolated by equilibrium sedimentation in neutral CsCl following reaction with poly rG, poly rIG, or poly rUG (Hradecna & Szybalski, 1967). One strand (called C) binds more of these synthetic polymers than the other (called W), and hence exhibits the greater buoyant density. We find that the L strand, the one with the lesser GT content, binds the greater amount of poly rG (unpublished experiments of J. Champoux, this laboratory); similarly, Hradecna & Szybalski (1967) find that the C strand exhibits the lesser buoyant density in alkaline CsCl. Hence the equalities, L = C and H = W, relate the two nomenclatures.

The correlation of lower GT content with greater capacity to bind poly rG (or rIG) that exists at the level of intact strands extends to the level of halves. Thus, the L strand of the left halves, which contains a considerably lower GT content than its complement, binds almost two-thirds as much poly rIG as the intact strand, whereas its complement exhibits little or no binding. Similarly, the strands of the right halves, exhibiting no significant ΔGT, manifest no difference in binding of poly rIG (Hradecna & Szybalski, 1967). The same qualitative results have been observed in our laboratory using poly rG as the reagent (J. Champoux, personal communication).

One can imagine that the correlation results from a *direct* relationship between GT content and ability to bind poly rG. Szybalski and his associates (Szybalski, Kubinski & Sheldrick, 1966) have proposed that binding of poly rG results from clusters of C in the DNA strand. If this simple notion is correct and were there no other causes for a GT bias, then there should be more such clusters of C in the L than in the H strand. Indeed, since the left half of the H strand binds little if any poly rG, these assumptions lead to the conclusion that the total number of C's in the binding sites of the left half of L would equal the estimated 1575 base pairs making up the ΔGT. One would like to compare this number with the number of binding sites for poly rG in the left half of L. Hradecna & Szybalski (1967) assign 12 binding sites to this region, yielding an average value of 130 C's per site. While this value is quite large, we need a more adequate determination of the composition and number of binding sites before considering restrictive comments.

One may suppose on the other hand that the correlation is *indirect*, particularly as the lower GT content and binding of poly rG appear to be characteristics of the strand that is transcribed (see Doerfler & Hogness, 1968). Were the codons used in λ to exhibit a bias in favor of G and/or U, then the transcribed strand would have the lesser GT content. The preferential binding of poly rG to the transcribed strand might then be representative of special signals involved either in transcription or translation, and thereby yield the indirect correlation.

If the correlation which exists between the GC content and binding of poly rG for the strands of λ DNA applies to all natural bihelical DNA's, then wherever complementary strands can be isolated by virtue of the $\Delta\rho_{alk}$ they exhibit, they should

be isolatable by reaction with poly rG. If this correlation is not general, then each method may be expected to be uniquely applicable to some DNA. In the case of λ DNA, where both methods are applicable, the more recently developed procedure involving poly rG binding gives the greater separation of the complementary strands in the CsCl gradient, and would therefore appear to be preferred, providing the subsequent removal of the poly rG does not present special problems.

(c) *Stability in alkaline cesium chloride*

The stability of DNA in alkaline solutions has been variously reported (Berns & Thomas, 1961; Vinograd *et al.*, 1963; Davison, Freifelder & Holloway, 1964; Tomizawa & Anraku, 1965). Tomizawa & Anraku (1965) observed the high rate of 0·03 break per hour per strand of λ DNA in 3·7 M-CsCl (pH 12·1), 12°C, whereas the other reports indicate a greater stability.

We observe a breakage rate that is more than an order of magnitude less than that reported by Tomizawa & Anraku (1965) and suspect that the difference depends upon the level of contamination of the CsCl by heavy metals. Barium hydroxide is known to increase the rate of alkaline hydrolysis of DNA (Helleiner & Butler, 1955), and other heavy metals (e.g. Ca) may do the same. We have, for quite independent reasons, consistently used CsCl which contained less than 10 parts per million of Ba, Ca or Mg (as their oxides), and EDTA has always been present in our alkaline CsCl solutions. We have also observed a considerable variation in contamination of various commercial preparations of CsCl with these metals and have noted that recrystallization from hot aqueous solutions in glass containers increases the contamination. With these facts in mind, it seems reasonable to suppose that the greater rates of breakage observed by Tomizawa & Anraku (1965) result from greater amounts of heavy-metal contaminants. They did not use EDTA, and although they did recrystallize the CsCl, they do not comment on heavy-metal contamination. Furthermore, they note that the DNA is considerably more stable under alkaline conditions if the CsCl is absent.

The rapid initial breakage of 10 to 15% of the strands in the first ten hours of exposure to the alkaline CsCl conditions used in our purification procedure, followed by the very slow breakage of the remaining intact strands (half-life = 600 to 700 hours), indicates a heterogeneity in which 10 to 15% of the strands contain at least one alkaline-sensitive bond in the DNA chain. Two possible explanations for this sensitivity come easily to mind. It might result from the infrequent substitution of a ribonucleotide for a deoxyribonucleotide during DNA synthesis (Berg, Fancher & Chamberlin, 1963), the frequency of substitution necessary to account for the heterogeneity being only 2 to 3×10^{-6}. The sensitive bond might also result from a similarly infrequent subunit in which no base is linked to the deoxyribose residue (e.g. as a result of depurination), allowing alkaline-induced chain cleavage by β-elimination. If such possibilities as these exist, then the frequencies of occurrence might be influenced by the phage, bacterium and environment, and variations in these factors may account for the different rates of alkaline cleavage that have been reported. Thus Vinograd *et al.* (1963) report that both φX174 DNA and the single strands of T4 DNA in alkaline CsCl suffer one hit per strand in about the same time interval (about two days), though the T4 strand is 35-times the size of the strand in φX174.

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