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# The Role of Recombination in the Formation of Circular Oligomers of the λ*dv*1 Plasmid

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The λ*dv*1 plasmid forms an extensive oligomeric series of circular DNA molecules in recombination-proficient (*rec*$^+$) *Escherichia coli*. These *rec*$^+$[λ*dv*1]$^+$ strains can be typed into the following four classes according to which member of the oligomeric series is most frequent: monomer, dimer, trimer, and tetramer strains. Each of these strains forms a set of circular λ*dv*1 DNA molecules in which most members belong to the series $l$, $2l$, $3l$, $4l$, . . . , where $l$ is the length of the most frequent circular DNA that characterizes the strain—i.e. $l$ equals the length of the most frequent oligomer in the respective strain. In a given strain, the frequency of a molecular species decreases as its length becomes a larger multiple of $l$. For example, the dimer strains produce dimers, tetramers, hexamers, octomers, etc., in decreasing frequencies, which reach the limits of detection at about the hexadecamer.

When *recA*$^-$ mutations that are absolutely defective for host recombination are introduced into each of these four strains, $l$ retains the same values as in the parent *rec*$^+$ strain, but oligomers larger than $2l$ are not formed, and the frequency of the $2l$ oligomer is much reduced. The introduction of *recB*$^-$ or *recC*$^-$ mutations, which are only partially defective for host recombination, produces a much smaller perturbation of the *rec*$^+$ distributions, and *rec*$^+$/*recA*$^-$ merodiploids exhibit the *rec*$^+$ phenotype with respect to both oligomerization and host recombination.

The effects of *rec*$^-$ mutations on the distribution of λ*dv*1 oligomers and the nature of the oligomeric series produced in *rec*$^+$ cells all indicate that an intermolecular reciprocal recombination between two circular λ*dv*1 DNAs is the principal reaction responsible for oligomerization. It is suggested that the small residual oligomerization that yields $2l$ oligomers in *recA*$^-$ cells results from aberrant segregation of the DNA strands at the termination of the replication of $l$-sized molecules.

The inactivation of *recA*, but not of *recB* or *C*, also results in a marked reduction in the frequency of spontaneous curing which in *recA*$^+$[λ*dv*1]$^+$ hosts leads to the segregation of [λ*dv*]$^-$ cells. However, spontaneous curing does not appear to be dependent upon the recombination reactions that yield the λ*dv*1 oligomers, since the frequency of oligomerization in *recA*$^+$ hosts decreases with increasing $l$, whereas the frequency of curing increases with increasing $l$.

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## 1. Introduction †

Many of the circular plasmid DNAs exhibit oligomerization in which the genome of the plasmid is represented an integral number of times in a single closed duplex: e.g. the colcinogenic factor *col* E1 in *Proteus mirabilis* (Bazaral & Helinski, 1968; Goebel & Helinski, 1968), mitochondrial DNA in leukemic leukocytes (Clayton & Vinograd, 1969), and polyoma DNA formed from transformed cells (Cuzin *et al.*, 1970). Two mechanisms have been proposed for the generation of these circular oligomers: reciprocal recombination between the circular DNA molecules, or errors in their replication (Hudson *et al.*, 1968; Goebel & Helinski, 1968). However, the experimental evidence has been generally insufficient to identify the mechanism of oligomerization for any circular plasmid.

The *λdv* plasmids provide a good system for the study of this process. These circular plasmids are deletion mutants of bacteriophage λ, which replicate autonomously in *Escherichia coli*. The small fraction of the λ genome retained in the *λdv* plasmids

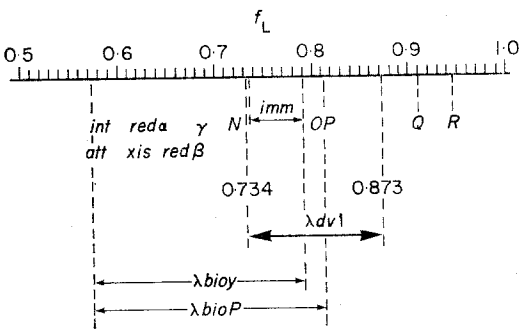


FIG. 1. Map of the right-hand half of λ DNA.

The scale gives the values of $f_L$, the fraction of the molecular length of λ DNA measured from the left-hand end of the molecule. The *int*, *xis*, *red* α, *red* β and γ genes affect recombination processes; the *O* and *P* genes are required for DNA replication. The positions of these genes, the attachment site (*att*), the immunity region of λ (*imm* is defined as that segment which is substituted in *λimm*$^{434}$ phage) and genes *N*, *Q* and *R* are taken from Davidson & Szybalski (1971) and Egan & Hogness (1972). The position of *λdv*1 is taken from Davidson & Szybalski (1971); Chow *et al.* (1974) place it at a slightly different position (i.e. $f_L$ = 0·737 to 0·886). The boundaries of the *λbioy* deletion were determined by heteroduplex mapping ($f_L$ = 0·573 and 0·791; M. Fiandt & W. Szybalski, personal communication). The right boundary of the *λbioP* deletion intersects gene *P* and is placed at the position of $P_{80}$ locus (Egan & Hogness, 1972; see also Davidson & Szybalski, 1971).

includes the genes required for the replication of λ DNA, but is missing the λ genes that are known to affect recombination (Matsubara & Kaiser, 1968; Chow *et al.*, 1974). This fraction is indicated in Figure 1 for *λdv*1, the prototype isolated by Matsubara & Kaiser (1968). We have observed that *λdv*1 forms an extensive series of circular oligomers, extending at least to the hexadecamer, in *rec*$^+$ bacteria, i.e. in cells that contain the complete recombination system of *E. coli*. This recombination system is sensitive to mutation in each of several known genes, e.g. *recA*, *recB*, and *recC*

† *Terminology:* covalently closed double-stranded circular DNA molecules are termed closed circles or closed circular DNA; closed circles that have received one or more breaks in either strand but retain the circular configuration are termed nicked circles, or nicked circular DNA; when no distinction is made between these two states, the term circular DNA or circle is used.

(Willetts *et al.*, 1969; Willetts & Clark, 1969; Willetts & Mount, 1969). Since the λ*dv*1 genome lacks the recombination genes of λ, it should therefore be possible to determine the relevance of recombination processes to the oligomerization of this plasmid by determining the effect of such *rec*$^-$ mutations on the oligomeric distribution.

In this paper we examine the distribution of λ*dv*1 oligomers in *rec*$^+$, *recA*$^-$, *recB*$^-$ and *recC*$^-$ cells and come to the conclusion that oligomerization in *rec*$^+$ cell is primarily due to intermolecular reciprocal recombination events. A low level of highly restricted oligomerization appears to be recombination-independent, and may result from infrequent errors in the replication process.

## 2. Materials and Methods

### (a) *Bacteria*

The properties, origins and construction of the bacterial strains are given in Table 1.

### (b) *Bacteriophages and their DNAs*

The purification of λ$^+$ (Kaiser, 1957), λ*imm*$^{434}$*c* (the 434 hy of Kaiser & Jacob, 1957, carrying a *cI*-type mutation), λ*imm*$^{434}$*N*am 7, λ*imm*$^{434}$*O*am 29, λ*imm*$^{434}$*P*am 80 (Matsubara & Kaiser, 1968), λ*imm*$^{21}$ (Liedke-Kulke & Kaiser, 1967), λ*vir* (Jacob & Wollman, 1954), λ*virh*$^{434}$, λ*virh*$^{\phi 80}$, and of λ*imm*$^{\phi 80}$ (Matsubara & Kaiser, 1968) was accomplished according to the procedures of Doerfler & Hogness (1968). The purification of λ*bioP* and λ*bioy* (the M38-5 and M58-2, respectively, of Kayajanian, 1968,1970) has been described by Champoux & Hogness (1972), and the deleted segments in their DNAs are indicated in Fig. 1. The deletion in the λ*bio*, M55-3 (Kayajanian, 1968) extends rightward from *att* past *R* (Fig. 1).

DNA was isolated from phage as described by Egan & Hogness (1972).

### (c) *Isolation of covalently closed circular* λdv*1 DNA*

#### (i) *Growth and* $^3$*H-labeling*

Owing to the instability of *rec*$^+$[λ*dv*1]$^+$, each preparation was initiated by inoculating 20 ml of K medium (Young & Sinsheimer, 1967*a*,*b*) containing 20 μg thymidine/ml with a single [λ*dv*1]$^+$ colony. After overnight growth, a portion was diluted tenfold into 20 ml of the same medium and growth continued until the absorbance at 600 nm ($A_{600}$) equalled 0·6, when the cells were washed and resuspended in 100 ml K medium containing 0·25 mg deoxyadenosine/ml and a total of 0·2 mCi of [$^3$H]methyl-labeled thymidine (> 15 Ci/mmol, New England Nuclear). After growth to $A_{600}$ = 0·8, the cells were chilled and harvested by centrifugation. About 50% of the [$^3$H]thymidine was incorporated into DNA. The temperature of growth of each experiment is given under Results.

#### (ii) *Cell lysis*

Cell lysates were prepared by the following modifications of the Brij extraction procedure of Godson & Sinsheimer (1967) and of the Sarkosyl extraction procedure of Young & Sinsheimer (1967*a*,*b*).

*Brij extraction.* Washed cells were resuspended in 8 ml of 25% (w/v) sucrose in 0·01 M-Tris·HCl, pH 8·0 at 0°C, and 2 ml each of cold lysozyme solution (0·85 mg/ml in 0·25 M-Tris·HCl, pH 8·0) and of 0·008 M-EDTA were mixed and added to the suspension. After 5 min at 0°C, 2 ml each of 5% (w/v) Brij 58 (Atlas Chemical Co.) and of 0·1 M-$MgSO_4$ were added with shaking, and 5 min later the partially cleared but not viscous suspension was centrifuged at 8000 revs/min for 10 min. The supernatant solution was extracted with phenol once, or twice and, after bringing the upper aqueous phase to 1·0 M-NaCl, the nucleic acids were precipitated by addition of 2·5 vol. ethanol. This precipitate was prepared for ethidium bromide/CsCl centrifugation by dissolving it in 5 ml of 0·5 mM-EDTA, 10 mM-Tris·HCl (pH 8·0), plus 0·3 ml of an 8·0 mg/ml solution of ethidium bromide, plus 5·0 g of CsCl.

TABLE 1

*Properties and origins of the bacterial strains*

| Strain[1] | Properties | | Origin | |
|---|---|---|---|---|
| | $\lambda dv1$[2] | *rec*[3] | Source | Reference |
| (a) JC5072 | — | $A_{67}^-$ | A. J. Clark | Clark (1967) |
| JC5088 | — | $A_{56}^-$ | A. J. Clark | Clark (1967) |
| JC4668 | — | $B_{21}^-$ | A. J. Clark | — |
| JC5426 | — | $C_{22}^-$ | A. J. Clark | Willetts & Mount (1969) |
| (b) KLF8/MA50 | — | $+/A^-$ | B. Low | Low (1968) |
| (c) KM314 | II | + | K. Matsubara | Matsubara & Kaiser (1968) |
| (d) A46 | II | $A_{56}^-$ | JC5088 × KM314 | This paper |
| A08 | II | $A_{67}^-$ | JC5072 × KM314 | This paper |
| B10 | II | $B_{21}^-$ | JC4668 × KM314 | This paper |
| C22 | II | $C_{22}^-$ | JC5426 × KM314 | This paper |
| (e) KLF8/A46 | II | $+/A_{56}^-$ | KLF8/MA50 × A46 | This paper |
| (f) M31-m | I | + | JC5072 × KM314 | This paper |
| M31-d | II | + | Segregant of M31-m | This paper |
| M31-t | III | + | Segregant of M31-m | This paper |
| M31-tet | IV | + | Segregant of M31-m | This paper |
| (g) A31-m | I | $A_{56}^-$ | JC5088 × M31-m | This paper |
| A31-d | II | $A_{56}^-$ | JC5088 × M31-d | This paper |
| A31-t | III | $A_{56}^-$ | JC5088 × M31-t | This paper |
| A31-tet | IV | $A_{56}^-$ | JC5088 × M31-tet | This paper |

[1] Additional characteristics and methods of isolation are given below for each group.

(a) These strains were derived from and retain the properties of JC5029, an Hfr, *str*$^S$, *spc*$^R$, *ilv*$^-$, *thr*$^-$ strain formed from Hfr KL16 by mutation (Clark, 1967). The *recA*$^-$ mutations were added by nitrosoguanidine mutagenesis (Clark, 1967); *recB*$_{21}^-$ and *recC*$_{22}^-$ were added by cotransduction with *thy*$^+$ to a *thy*$^-$ derivative of JC5029 (JC5401) (Clark, 1967; Willetts & Mount, 1969). JC4668 has not been referred to previously; it was formed in the same manner and has the same properties as JC5142 (Willetts & Mount, 1969; A. J. Clark, personal communication).

(b) The F′, KLF8, was formed from Hfr KL16 (Low, 1968) and is plus for all three *rec* genes. The KLF8/MA50 is *lys*$^+$, *cys*$^+$/*lys*$^-$, *cys*$^-$.

(c) KM314 is *str*$^R$, *his*$^-$, *su*$^-$, *ade*$^-$ (K. Matsubara, personal communication). It was obtained from the original [$\lambda dv1$]$^+$ isolate, KM195, by mating with Hfr H and selecting for *gal*$^+$ transfer (Matsubara & Kaiser, 1968).

(d) *his*$^+$(*str*$^R$) recombinants that exhibit the sensitivity to ultraviolet light of the *rec*$^-$ parent and restricted the growth of $\lambda vir$ (see Table 3) were selected from the indicated matings (Adelberg & Burns, 1960). A46 is one of 30 such recombinants isolated from JC5088 × KM314 and A08 is one of 15 recombinants isolated from JC5072 × KM314.

(e) KLF8/A46 was isolated as an ultraviolet-resistant, $\lambda vir$-immune (Table 3) colony after F-duction of A46 with KLF8/M50.

(f) M31-m is a *his*$^+$(*str*$^R$) recombinant which is $\lambda vir$-immune (Table 3); however, its sensitivity to ultraviolet light is much less than the *recA*$^-$ parent, though more than wild-type carrying $\lambda dv$. The same unusual u.v.-sensitivity curve was exhibited by M1-m, another monomeric $\lambda dv$ strain isolated as a *his*$^+$(*str*$^R$) recombinant from the mating JC5426 × KM314. These strains and their indicated segregants are classified *rec*$^+$ because of their lesser u.v.-sensitivity, which can be increased by introduction of *recA*$_{56}^-$ (see (g) below), and because of the *rec*$^+$ characteristics given in Table 3.

(g) These strains were isolated from the indicated matings as recombinants carrying $\lambda dv1$ of the M31 parent ($\lambda vir$-immunity) and exhibiting the u.v.-sensitivity of the *recA*$^-$ parent.

[2] The roman numerals indicate the most frequent circular form of $\lambda dv$: I, monomer; II, dimer; III, trimer; IV, tetramer.

[3] *A*, *B* and *C* indicate genes *recA*, *recB* and *recC*, the subscript referring to the number originally assigned to the *rec*$^-$ mutation; + indicates all three genes are wild-type.

This rapid Brij extraction was used for screening colonies for their distribution of λdv oligomers; 40 to 70% of the covalently closed λdv1 molecules are extracted by this procedure, the larger oligomers suffering disproportionate losses. For the quantitative distributions shown in the text Figures, either the precipitate from the above 8000 revs/min centrifugation was subjected to the Sarkosyl extraction and combined with the nucleic acids obtained by the above procedure, or the cells were directly extracted with Sarkosyl.

*Sarkosyl extraction.* The washed cells (or the residue from the Brij extraction) were resuspended in 3·0 ml of 12·5% (w/v) sucrose, and 1·5 ml each of a 10 mg/ml solution of lysozyme in 0·25 M-Tris·HCl (pH 8·0), and of 0·20 M-EDTA were added. After 10 min at 0°C, 0·30 ml Sarkosyl NL30 (Geigy Chemicals) and 2·25 ml Pronase solution (1·0 mg/ml, pretreated as described by Young & Sinsheimer (1967*a*,*b*)) were added, and the mixture shaken at 20°C for 60 min. Ethidium bromide (0·50 ml of an 8 mg/ml solution) and 8·25 g of CsCl were then added prior to the following centrifugation.

(iii) *Centrifugation in ethidium bromide/CsCl gradients (Radloff* et al., *1967)*

The above mixtures were centrifuged in the Spinco model 40 rotor for 36 to 60 h at 38,000 revs/min and 5°C. The bands of DNA were observed by their fluorescence at 360 nm and collected under this visual control; the relevant fractions were analyzed for trichloroacetic acid-precipitable radioactivity. The band of closed circular DNA from the Sarkosyl preparations was recentrifuged under the conditions indicated above. If this DNA was derived from Sarkosyl extraction of the Brij residue, it was combined with the band of closed circular DNA obtained from the Brij extraction and centrifuged a third time.

Ethidium bromide was removed from the closed circular DNA by 6 to 8 extractions with isopropanol presaturated with 3·0 M-CsCl and then dialyzed against 0·5 mM-EDTA, 10 mM-Tris·HCl (pH 8·0).

No band of closed circular DNA could be detected from any of the $[\lambda dv]^-$ strains in Table 1 or from the cured colonies derived from all $[\lambda dv1]^+$ strains except A31-m and A31-t, which were not tested.

(d) *Zone sedimentation*

Zone sedimentation was performed at 5°C in tubes of the Spinco model SW40 rotor containing a linear 5% to 20% (w/v) sucrose gradient in 25 mM-NaCl, 0·5 mM-EDTA, 10 mM-Tris·HCl (pH 8·0) (total volume = 11·2 ml) rotating at 40,000 revs/min for 3 h, except as noted in Fig. 2(b).

Computation indicates that the sedimentation behavior of DNA molecules under these conditions is essentially isokinetic; i.e. the ratio, $[s_{20,w}]_i/d_i$, is constant to within 1·5% for $[s_{20,w}]_i$ in the range 15 to 80 S, where $d_i$ is the distance sedimented by $DNA_i$. This conclusion is confirmed by the fact that no significant differences were observed in the relative distances sedimented by different DNAs in the above gradient and in a 5-ml linear 5% to 20% sucrose gradient in an SW39 rotor, where isokinetic behavior has been demonstrated (Doerfler & Hogness, 1968; Egan & Hogness, 1972). Sedimentation coefficients for the different oligomers of λdv1 DNA were therefore computed from $[s_{20,w}]_i/[s_{20,w}]_R = d_i/d_R$, where $i$ refers to the λdv1 DNA and $R$ denotes a reference molecule whose sedimentation coefficient is known (see Figs 2 and 3).

(e) *Electron microscopy*

DNA samples were prepared for electron microscopy by a modification of the Kleinschmidt technique (Vasquez & Kleinschmidt, 1968; Lang *et al.*, 1967). The mean contour lengths reported in this paper result from the measurement of 24 to 120 molecules of the different size classes; only the relaxed configurations typical of nicked circles were measured.

(f) *DNA/DNA hybridization on nitrocellulose filters*

Following a modification of Denhardt's (1966) procedure, $^3$H-labeled circular λdv1 DNA was sonicated to produce fragments much smaller than the λdv1 monomer. These fragments were denatured by a 10-min exposure to 100°C, immediately chilled, and then hybridized in 0·50 M-KCl, 0·01 M-Tris·HCl (pH 7·3) for 14 h at 65°C to filters containing

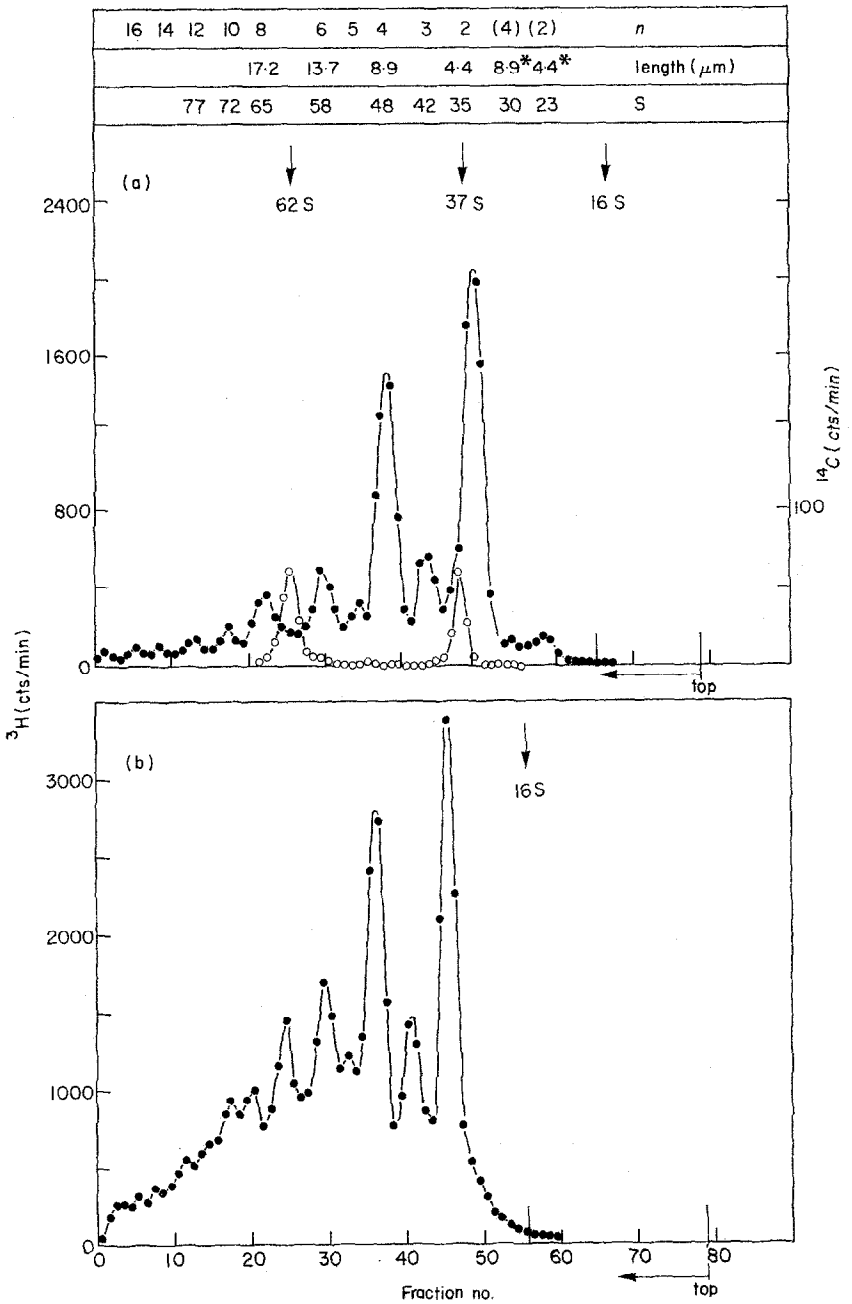


FIG. 2. Distribution of λ*dv*1 oligomers in KM314 grown at 22°C.

(a) $^{3}$H-Labeled closed circles were isolated from the combined Brij and Sarkosyl treatments by centrifugation in ethidium bromide/CsCl gradients and then subjected to zone sedimentation after addition of the sedimentation reference, *E. coli* β-galactosidase ($s$ = 16 S; see legend to Fig. 3), according to procedures described in Materials and Methods. The two $^{14}$C-labeled peaks are λ DNA in the form of nicked ($s$ = 37 S) and closed ($s$ = 62 S) circles and were provided by infection of the KM314 cells with $^{14}$C-labeled phage prior to lysis. Portions of 10-drop fractions were used for counting the [$^{3}$H]- and [$^{14}$C]DNA. See text for determination of the indicated sedimentation coefficients ($s$), contour lengths, and values of $n$; values of $n$ appearing in parentheses refer

0·225 μg denatured DNA from $\lambda^+$, or one of the *λbio* variants. After hybridization, the filters were washed in 1·0 mM-Tris·HCl, pH 9·2, and assayed for radioactivity. Blank filters which accompanied the DNA filter in each hybridization experiment contained 1·0 to 1·5% of the counts on the hybridization filter in the plateau region.

## 3. Results

### (a) *Distribution of circular oligomers from the* rec$^+$[λdv*1*]$^+$ *parent strain, KM314*

#### (i) *Analysis of closed circles*

Matsubara & Kaiser (1968) examined the closed circular DNA isolated from their original [*λdv*1]$^+$ strain (KM195) in the electron microscope and observed that most molecules had a contour length of 4·4±0·1 μm, with some molecules exhibiting lengths twice this value. When we examined the size distribution of $^3$H-labeled closed circles from KM314, a derivative of KM195 (Table 1), by zone sedimentation, the results shown in Figure 2(a) were obtained. While compatible with the observations of Matsubara & Kaiser, this sedimentation pattern indicates a much more extensive range of circular oligomers than was anticipated.

The sedimentation coefficients of the peak fractions were determined from their distances of sedimentation relative to that for a reference molecule (see Materials and Methods). The resulting values are given in Figure 2(a), and with the exception of the two very small peaks nearest the top of the centrifuge tube (23 and 30 S), represent the sedimentation coefficients of different size classes of closed circles (see legend, Fig. 2(a)). The contour lengths of molecules in those peak fractions, which exhibit sedimentation coefficients of 35, 48, 58 and 65 S, were measured in the electron microscope (see Materials and Methods), and mean values of 4·4, 8·9, 13·7 and 17·2 μm, respectively, were obtained. These lengths conform to the series *l*, 2*l*, 3*l*, 4*l*, where *l* is 4·4 μm, the length of the most frequent circle (abbreviated, *mfc*) in the distribution.

As will become apparent, the sedimentation coefficients of the closed circles in peaks further down the centrifuge tube (i.e. 72 S, 77 S, etc.) are consistent with molecular lengths that are even larger integral multiples of *l*. However, the small but significant peak at 42 S between the larger two peaks containing molecules of lengths *l* and 2*l* does not conform to this series. The contour length of closed circles exhibiting this sedimentation coefficient is 7·0 μm (determined from *λdv*1 circles in strain M31-t, which is described later; see Fig. 5(c)), or about three-halves *l*. This suggests that the *λdv*1 genome is represented twice in molecules of length 4·4 μm.

Circles containing only one copy of the *λdv* genome should then have contour lengths of about 2·2 μm. No molecules of this size were found in the KM314 distribution. However, we have obtained [*λdv*1]$^+$ strains in which the most frequent circles have a mean contour length of 2·25 μm (e.g. M31-m, to be described later; see Fig. 5(a)). We therefore presume that the six classes of circles for which we have obtained mean contour lengths are members of an oligomeric series containing 1, 2, 3, 4, 6 and 8 copies of the *λdv*1 genome.

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to nicked circles. The starred length values are taken from molecules in the peaks of (b) that exhibit the same sedimentation coefficient.

—○—○—, $^{14}$C; —●—●—, $^3$H.

(b) Nicked circles were prepared from the closed circles as described in the text. Conditions of zone sedimentation are those described in Materials and Methods except that the time of sedimentation was 5 instead of 3 h.

If one plots the logarithm of the sedimentation coefficient of the closed circles (see Table 2) against the logarithm of the respective number of copies, $n$, the upper straight line shown in Figure 3 is obtained. Analysis by least-squares yields the following equivalent equations for this line:

$$S_{cc} = 25{\cdot}9\, n^{0{\cdot}440} \tag{1}$$

and

$$n = 6{\cdot}13 \times 10^{-4}\, S_{cc}^{2{\cdot}273}, \tag{2}$$

where $S_{cc}$ is the sedimentation coefficient of the closed circles. The values of $n$ computed from equation (2) and the $S_{cc}$ values given in Figure 2(a) are 1·96, 3·00, 4·06, 4·88, 6·24, 8·10, 10·2, 11·9, and 13·7, all within $\pm 4\%$ of the closest integer, and indicating that the sedimentation coefficients provide a sufficient measure of $n$ for values up to about 14. We have added the points for which $n$ was determined only by computation from equation (2) (i.e. contour lengths were not determined for $n > 8$) to the plots in Figure 3 as open circles, using the closest integral value for $n$.

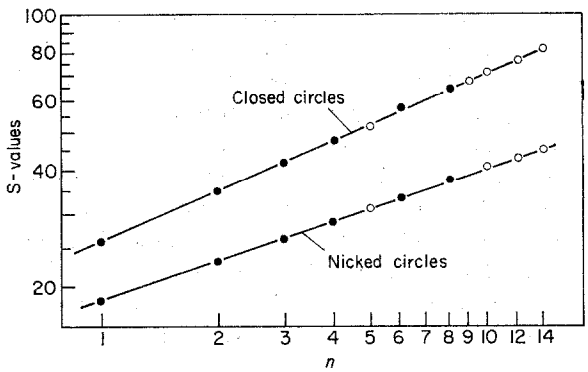


FIG. 3. Sedimentation coefficients of closed and nicked circles of λ*dv*1 oligomers as a function of $n$.

The filled circles in the Figure represent those instances where $n$ was determined from measurements of contour lengths (Table 2); the values of $n$ represented by the unfilled circles were determined from equation (2) for the upper curve (see text). The sedimentation coefficients are taken from zone sedimentation profiles such as those shown in Figures 2, 4 and 5, and are the average of 5 to 30 values. *E. coli* β-galactosidase with $s^{\circ}_{20,w} = 16{\cdot}0$ S (Sund & Weber, 1963) was used as the primary sedimentation reference; $\lambda^+$ DNAs in the form of linear molecules and nicked and closed circles were sometimes used as secondary references, which exhibit sedimentation coefficients of 33, 37 and 62 S, respectively, relative to the preceding primary value.

The value of $n$ computed for closed circular $\lambda^+$ DNA ($s = 62$ S) from equation (2) is 7·27, indicating that the length of the λ*dv*1 monomer circle is 13·8% of the $\lambda^+$ DNA. This value is in good agreement with the ratio of contour lengths of the λ*dv*1 monomer circle (2·25 μm) to that of $\lambda^+$ DNA measured under the same conditions (16·2 μm, Table 2), namely, 0·139. Electron microscopic mapping of heteroduplexes formed between λ and λ*dv*1 DNAs demonstrate that the λ*dv*1 genome exhibits the same length as the λ*dv*1 monomer (Fig. 1); i.e. in one heteroduplex experiment, λ*dv*1 was observed to retain 13·9% of the base sequences in λ DNA (Davidson & Szybalski, 1971) and in another, 14·9% (Chow *et al.*, 1974). Hence, we infer that $n$ equals the number of times the λ*dv*1 genome is repeated in a given oligomer, an inference that is confirmed by the hybridization experiments given in section (d) below.

The mean of the values for the ratio of monomer to λ DNA lengths is 14·0% (the included values are the above four plus the two given in sections (a)(ii) and (d) below). The molecular weight of the λdv1 monomer calculated from that for λ DNA ($30{\cdot}8\times10^6$; Davidson & Szybalski, 1971) is $4{\cdot}3\times10^6$; hence $n = M/(4{\cdot}3\times10^6)$, and substitution in equation (1) yields

$$S_{cc} = 0{\cdot}0312\, M^{0{\cdot}440}, \tag{3}$$

where $M$ is the molecular weight of the closed circular DNA.

This equation has the general form, $S_{cc} = KM^a$. Since the evaluation of $a$ depends only on the *relative* values of $M$ and $S_{cc}$, the extensive oligomeric series used here is ideally suited to its determination—both because the relative values of $M$ are accurately fixed by the series, and because factors that may affect the relative values of $S_{cc}$ (e.g. supercoil density, temperature, ionic strength (Wang, 1970)) are not varied. Furthermore, the determination of $K$ depends on the absolute value for the molecular weight of only λ DNA, a value that has been determined with considerable accuracy (Davidson & Szybalski, 1971). The only other case† where an oligomeric series has been used to determine the correlation between $S_{cc}$ and $M$ is that for the closed circular monomer, dimer and trimer of *col* E1 given by Bazaral & Helinski (1968), who found $S_{cc} = 0{\cdot}034\, M^{0{\cdot}428}$. Though these values for $a$ and $K$ are close to those in equation (3), they are considered less accurate (the values of $M$ calculated from this equation are too large) because they derive from a smaller number of data points and a less accurate absolute molecular weight.

Other correlations between $S_{cc}$ and $M$ that have been reported derive from a collection of data points for unrelated DNAs taken from the literature, in which case the relative values of $M$ are inherently less accurate, the supercoil density may vary, and the $S_{cc}$ are often determined under different sedimentation conditions. Clayton & Vinograd (1967) found that $S_{cc}$ was linearly dependent on $M^{0{\cdot}38}$ for seven data points. This was later changed to the correlation, $S_{cc} = 7{\cdot}44+2{\cdot}43\times10^{-3}\, M^{0{\cdot}58}$, by increasing the number of data points to 17 (Hudson & Vinograd, 1969). And using 19 data points, most of which overlap with the collection used by Hudson & Vinograd (1969), Böttger *et al.* (1971) found $S_{cc} = 5{\cdot}16+4{\cdot}39\times10^{-3}\, M^{0{\cdot}553}$. The equation used in the last two cases, $S_{cc} = b + KM^a$, will give a higher value of $a$ than the equation $S_{cc} = KM^a$, when fitted to the same data. For example, when the data points used by Böttger *et al.* (1971) are fitted by least-squares to the latter equation, we find $a = 0{\cdot}438$. The agreement between this value and that in equation (3) is probably fortuitous, since the elimination of the most divergent of the 19 data points changes the value to 0·467. We conclude that the equations that have been reported previously differ from equation (3) because of some combination of the factors enumerated above, and that equation (3) is the most satisfactory of these empirical correlations.

† The $S_{cc}$ values for the oligomeric series formed by the closed circular monomer and dimer of human mitochondrial DNA can be extended to the trimer if one assumes that the $S_{cc}$ observed for the triply-closed catenated trimer equals that for the closed circular trimer (Hudson & Vinograd, 1969; also see Wang, 1970, for the validity of this assumption). A least-squares evaluation of the data given by Hudson & Vinograd (1969) for this three-membered series yields $S_{cc} = (37{\cdot}1)n^{0{\cdot}472}$, or $S_{cc} = (0{\cdot}0185)M^{0{\cdot}472}$, if one takes $9{\cdot}9\times10^6$ for the molecular weight of the monomer (D. Clayton, personal communication). This equation yields a molecular weight that is 10% higher than that given by equation (3) at $M = 3\times10^6$, about the same at $M = 10\times10^6$, 7% lower at $M = 30\times10^6$ and 11% lower at $M = 60\times10^6$.

(ii) *Analysis of nicked circles*

Nicked circles were obtained from the $^3$H-labeled preparation of closed circles used in Figure 2(a) by allowing radioactive decay to convert 40% of the DNA to nicked circles, which were then isolated in an ethidium bromide/CsCl gradient (see Materials and Methods). Zone sedimentation of these nicked circles yielded the distribution given in Figure 2(b). Except for the expected increase in the proportion of higher oligomers due to the proportionality between length and the probability of a single-strand scission, the distribution in Figure 2(b) parallels that in Figure 2(a). The correspondence of the dimer and tetramer peaks between the two distributions was directly established by additional measurements of the contour lengths of molecules in the two peaks of Figure 2(b), mean values of 4·4 and 8·9 $\mu$m again being obtained.

A log/log plot of the sedimentation coefficients of the nicked circles (Table 2) against the $n$ values determined in the preceding section yields the lower straight line in Figure 3 and the least-squares equations:

$$S_{nc} = 18{\cdot}3\, n^{0{\cdot}348} \tag{4}$$

and

$$n = 2{\cdot}36 \times 10^{-4}\, S_{nc}^{2{\cdot}87} \tag{5}$$

where $S_{nc}$ is the sedimentation coefficient of the nicked circles. Calculation of $n$ from equation (5) and the sedimentation coefficients for the nicked $\lambda dv1$ circles yields values which are all within $\pm 5\%$ of the integrals assigned in the preceding section and given in Table 2. The length of the $\lambda dv1$ monomer is 13·4% of the length of $\lambda$ DNA according to equation (5) and the $S_{nc}$ for $\lambda$ DNA (37 S).

The two small peaks nearest the top of the centrifuge tube depicted in Figure 2(a) have sedimentation coefficients (23 and 30 S) that yield values of $n$ equal to 1·92 and 4·09, according to equation (5), and are therefore taken to represent nicked dimers and tetramers derived from the two major peaks of closed circles. Both this correlation and the lack of any peak at 18·4 S in Figure 2(b) indicate that strain KM314 does not contain a significant amount of monomers.

Substitution of $M/(4{\cdot}3 \times 10^6)$ for $n$ in equation (4) yields $S_{nc} = 0{\cdot}090\, M^{0{\cdot}348}$. (6)

The exponent in equation (6) does not differ significantly from that found for *linear* DNA when $S$ and $M$ are similarly fitted to $S = KM^a$; e.g. values of $a$ determined for linear DNA are: 0·35 by Burgi & Hershey (1963), 0·346 by Studier (1965), 0·350 by Leighton & Rubinstein (1969), and 0·350 by Egan & Hogness (1972). When $S$ and $M$ are plotted according to $S = b + KM^a$, a single value for $a$ has also been observed to satisfy both linear and nicked circular DNAs (Hudson & Vinograd, 1969; Böttger *et al.*, 1971), and there is some theoretical expectation for this result (Gray *et al.*, 1967). In this case, the value of $a$ is in the range $0{\cdot}440 \pm 0{\cdot}005$ for values of $b$ in the range $2{\cdot}6 \pm 0{\cdot}1$.

This difference in $a$ is not entirely due to the two equations to which the data are fitted, since our results for nicked circles give values of $a$ in the range $0{\cdot}387 \pm 0{\cdot}003$ for the above range of $b$ values, and these values must be doubled for the exponent to approach 0·44. We attribute this difference to the greater accuracy in the relative molecular weights that is inherent in an oligomeric series, as emphasized in the preceding section. Furthermore, the fit of our data to the equation with $b = 0$ is sufficiently good that we have found little if any empirical advantage in attempting to further optimize the fit by varying $b$.

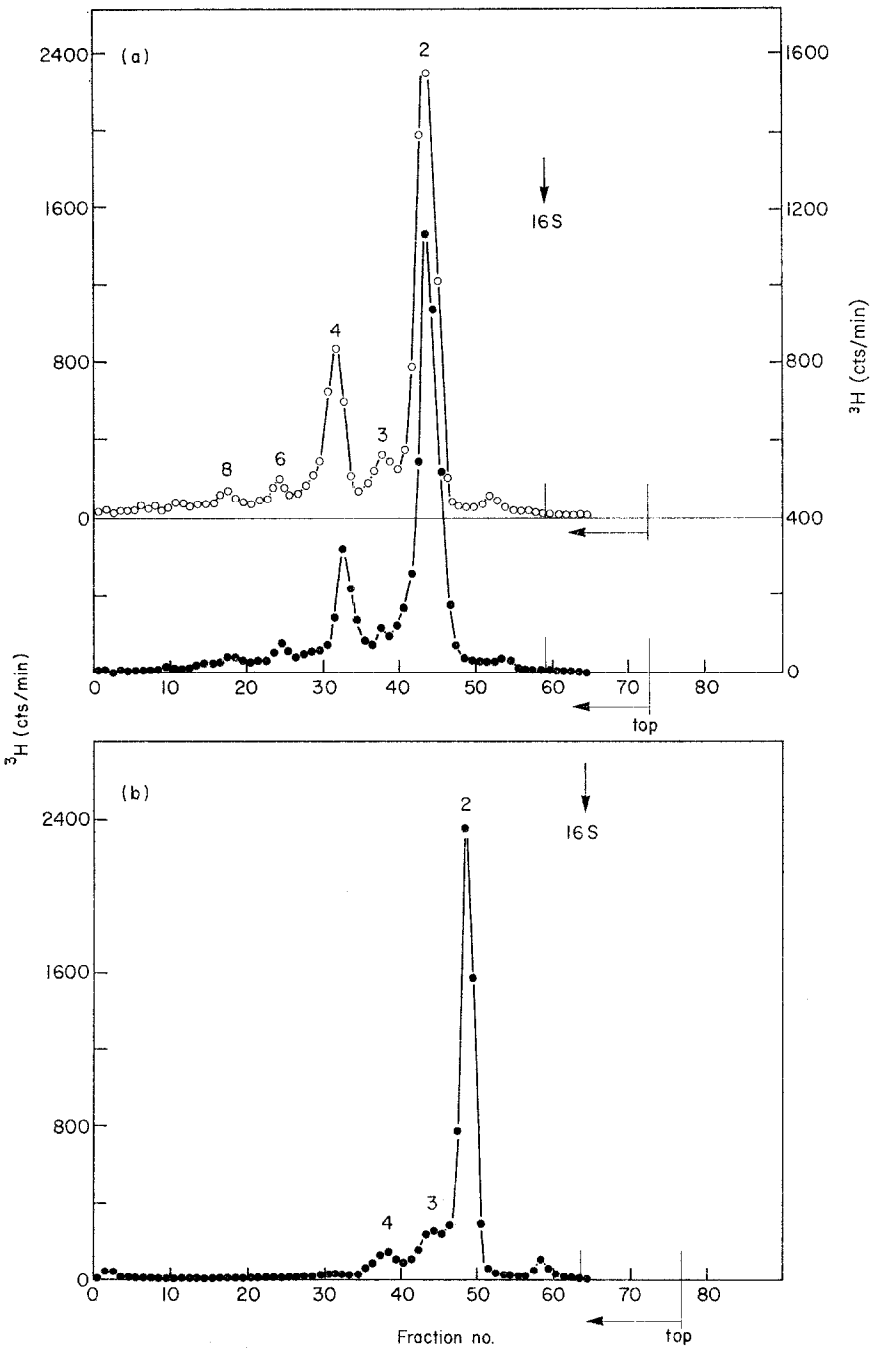


FIG. 4. Distributions of λdv1 oligomers from *rec*$^+$ and *recA*$^-$ dimer strains grown at 37°C. Preparation of the closed circles and their zone sedimentation were carried out as described in the legend to Fig. 2(a). Values of $n$ for the predominant peaks of closed circles are indicated by the numbers above the peaks. (a) Upper curve, KM314; lower curve, KLF8/A46. (b) A46.

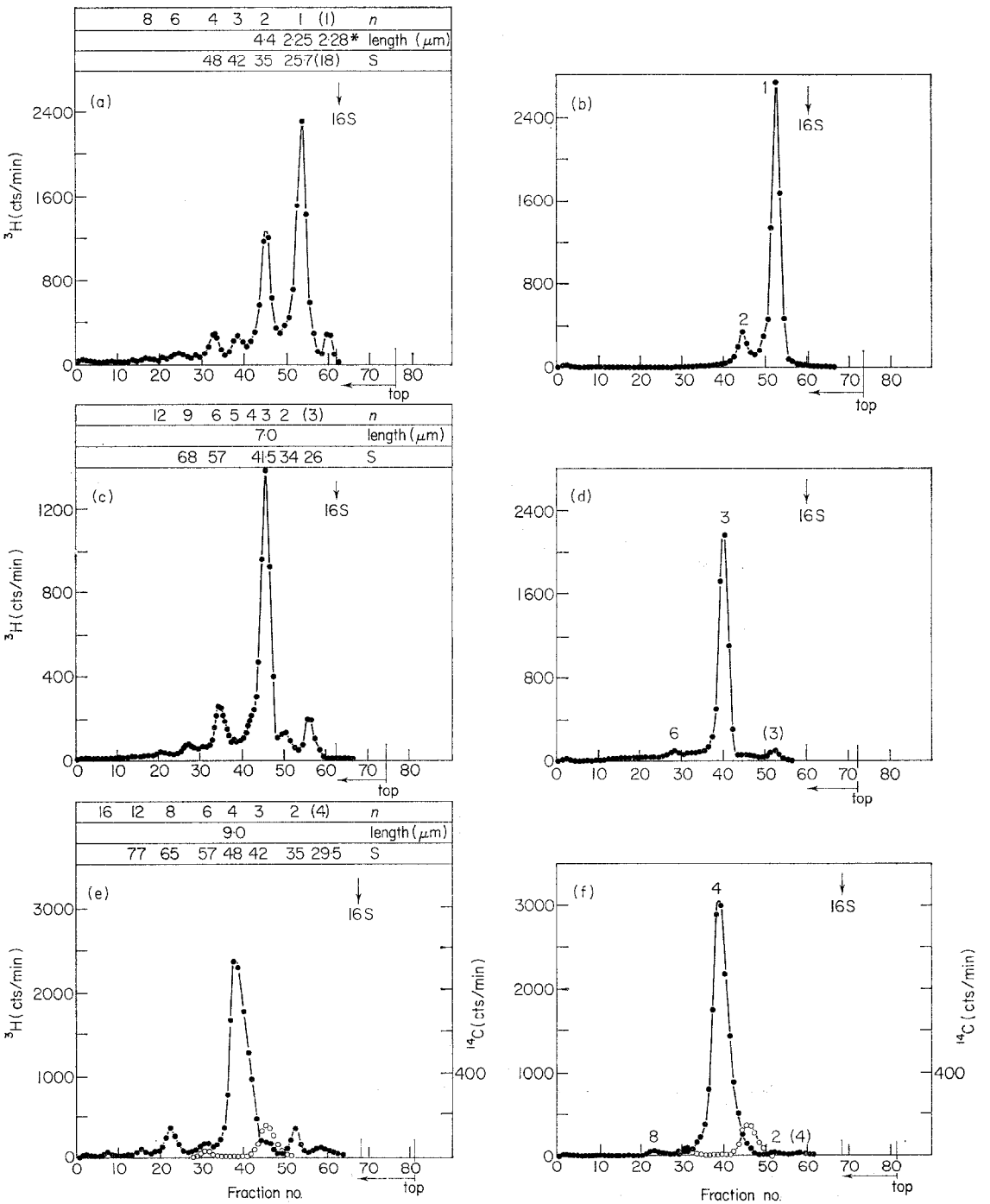


FIG. 5. Distributions of *λdv*1 oligomers from *rec*$^+$ and *recA*$^-$ monomer, trimer and tetramer strains grown at 37°C.

Preparation of closed circles and their zone sedimentation were carried out as described in

(iii) *Effect of temperature on the oligomer distribution*

The distributions given in Figure 2 were obtained from cells grown at 22°C. When the temperature of growth is raised to 37°C, the distribution of closed λ*dv*1 circles is given by the upper curve in Figure 4(a). Although the fraction of λ*dv*1 DNA in the form of higher oligomers is decreased from 58% at 22°C to 34% at 37°C (Table 2), the general features of the distribution remain the same: most oligomers conform to the series *l*, 2*l*, 3*l*, . . . . *il*, . . . , where $n = 2$ for *l*, the length of the most frequent circle; and the frequencies of these oligomers decrease with increasing *i*.

(b) *The distribution of* λdv*1 closed circles in* rec$^-$ *dimer strains*

(i) recA$^-$

Both $recA^-_{56}[\lambda dv1]^+$ and $recA^-_{67}[\lambda dv1]^+$ strains were obtained by mating KM314, which is $[\lambda dv1]^+$, $rec^+$, $his^-$, $str^R$, with derivatives of HfrKL16 (JC5088 and JC5072), which are $[\lambda dv]^-$, $recA^-$, $his^+$, $str^S$ (Table 1), and selecting $his^+$, $str^R$ recombinants that are ultraviolet-sensitive (i.e. $recA^-$) and refractory to λ*vir* (i.e. $[\lambda dv1]^+$). The distributions of closed circular λ*dv*1 DNA in 30 $recA^-_{56}[\lambda dv1]^+$ recombinants and 15 $recA_{67}[\lambda dv1]^+$ recombinants have been examined. Each of the 45 distributions is essentially the same as that exhibited by A46 (one of the $recA^-_{56}[\lambda dv1]^+$ recombinants) and given in Figure 4(b). Although the length of the most frequent circle is unchanged (i.e. $n = 2$ for *l*), the fraction of λ*dv*1 DNA appearing in the form of higher oligomers is dramatically reduced—from 58 to 6% at 22°C, and from 34 to 5% at 37°C (Table 2). Furthermore, no closed circular DNA (<0·03%) larger than 2*l* could be found in the $recA^-$ bacteria.

To demonstrate that this loss of higher oligomers is specific to the $recA^-$ state, a $rec^+/recA^-_{56}$ merodiploid, KLF8/A46, was constructed from A46 by F-duction with the F′,KLF8 (Table 1) and the distribution of closed circular DNA in this strain examined. It can be seen that this distribution is the same as that for KM314, the $rec^+$ parent of A46 (Fig. 4(a), lower curve; Table 2).

(ii) recB$^-$ *and* recC$^-$

Both $recB^-_{21}$ $[\lambda dv1]^+$ and $recC^-_{22}$ $[\lambda dv1]^+$ recombinants were isolated by the method described above for the $recA^-$ $[\lambda dv]^+$ recombinants (see Table 1). It is evident from the distributions of closed circles obtained from representatives of these two types of recombinants (B10 and C22, Table 2) that inactivation of the *recB* or the *recC* gene results in only a small perturbation of the $rec^+$ distribution. The value of *l* is unchanged, and although the higher oligomers represent a significantly smaller fraction of the DNA (40% in $recB^-_{21}$ and 42% in $recC^-$, as compared to 58% in the $rec^+$ parent), they conform to the kind of series present in $rec^+$ bacteria.

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the legend to Fig. 2(a). The values of *n*, contour length, and S for the distributions from the $rec^+$ strains (left-hand column of figures) are given above each distribution, as in Fig. 2(a). The starred contour length in (a) refers to molecules taken from the corresponding peak in a distribution of nicked circles prepared as described in the legend to Fig. 2(b). The *n*-values for peaks in the distributions from $recA^-$ strains (right-hand column of figures) are indicated by the numbers associated with each peak, the numbers in parentheses referring to nicked circles. The $^{14}$C-labeled DNA in (e) and (f) (—○—○—) are closed circles with $n = 3$ and 6, which were added as additional reference markers. (a) M31-m; (b) A31-m; (c) M31-t; (d) A31-t; (e) M31-tet; (f) A31-tet.

### (c) *Strains in which the most frequent circle is the monomer, trimer or tetramer of* λdv*1*

#### (i) *Monomer strains*

In the above matings, two unusual $[\lambda dv1]^+$, $his^+$, $str^R$ recombinants were isolated, Ml-m from JC5426 × KM314 and M31-m from JC5072 × KM314 (Table 1). Since they have the same characteristics, only the M31-m strain is described here. Although the Hfr JC5072 donor is $recA^-_{67}$, M31-m is considered to be $rec^+$ for the reasons given in Table 1, footnote [1](f). It is unusual because its ultraviolet-sensitivity, while much less than that of $rec^-$ strains, is greater than that of wild-type $[\lambda dv1]^+$ cells†, and because the most frequent circle (*mfc*) of λ*dv*1 in M31-m is the monomer (Fig. 5(a)). The values of $n$ assigned to the peaks in this distribution are not only consistent with values calculated from the sedimentation coefficients of closed circles and equation (2), but also with values calculated from the sedimentation coefficients of the corresponding nicked circles (prepared as described for the population in Fig. 2(b)) and equation (5). Furthermore, contour lengths of the monomers and dimers were measured, to yield the values given in the Figure.

This distribution is not unlike that for the dimer strains except that $n = 1$ for $l$, as if the *mfc* is the basic unit from which the higher oligomers are derived. The oligomerization in monomer strains exhibits the same sensitivity to temperature as shown previously for the dimer strains, and is similarly dependent upon the $recA^+$ gene. The latter point is demonstrated in Figure 5(b), which shows the distribution of closed circles obtained from strain A31-m, a $recA^-_{56}$ $[\lambda dv1]^+$ recombinant formed by mating M31-m with the Hfr, $recA^-_{56}$, strain JC5088 (Table 1). As with the dimer strain, inactivation of the *recA* gene causes a drastic reduction of oligomers larger than the *mfc* and limits the maximum oligomer to $2l$, in this case the dimer.

#### (ii) *Trimer and tetramer strains*

M31-m spontaneously segregates clones which yield distributions of closed λ*dv*1 circles in which the *mfc* is the dimer, trimer or tetramer (Table 1). The distribution of the dimer strain, M31-d, and its $recA^-_{56}$ derivative, A31-d, are essentially the same as those shown in Figure 4. The distributions for the trimer strain, M31-t, and its $recA^-_{56}$ derivative, A31-t, are given in Figures 5(c) and (d), while the distributions exhibited by the corresponding tetramer strains, M31-tet and A31-tet, are shown in Figures 5(e) and (f). The size of the oligomers was determined from their sedimentation coefficients and in each case $l$, the length of the *mfc*, was confirmed by electron microscopy. Clearly, $l$ corresponds to $n = 3$ for the trimer strain and to $n = 4$ for the tetramer strain.

The distribution of the circular oligomers in M31-t and M31-tet conform to the same qualitative rules defined for the $rec^+$ monomer and dimer strains. The predominant oligomers are represented by the series $l$, $2l$, $3l$ . . . . $il$, . . . . , and the frequencies decrease with increasing $i$ (Table 2). As with the dimer strain, some intermediate

† The unusual ultraviolet-sensitivity curves characteristic of M1-m and M31-m (G. Hobom unpublished results) will be given in a later paper. This characteristic does not appear to be due to another mutation induced in the Hfr parent of M31-m, JC5072, by nitrosoguanidine, which was used to form the $recA^-_{67}$ mutation in that strain, since the Hfr parent of M1-m, JC5426, was not subject to such mutagenesis, the $recC^-_{22}$ mutation being introduced by transduction (Table 1). Furthermore, other strains having this characteristic can be formed by mating of KM314 with donor strains quite different from these two (B. Hobom & G. Hobom, unpublished results).

oligomers between $l$ and $2l$ are observed in significant frequency; however, this frequency is always less than that for the $2l$ oligomer. In addition, all oligomers smaller than $l$, with the exception of the monomer, appear in both strains. The absence of the monomer from all strains other than the monomer strain itself has also been demonstrated by alkaline sucrose sedimentation, and appears to be another general characteristic of these distributions.

The distributions of λdv1 circular DNA obtained from the $recA_{56}^{-}$ strains, A31-t and A31-tet, also conform to the qualitative rules describing the effect of the inactivation of the *recA* gene: the frequency of oligomers with lengths greater than $l$ is much reduced; and oligomers with lengths greater than $2l$ are eliminated.

### (d) *Equivalence of the base sequence in monomers and in oligomers*

We have previously inferred from the length correlations given in section 3(a) that the $n$th oligomer contains $n$ repeats of the sequence of bases in the monomer, and that the monomer sequence is the minimum sequence containing the entire λdv1 genome. The following hybridization experiments provide additional support for these conclusions.

Denatured, sonicated preparations of $^{3}$H-labeled λdv1 DNA obtained from monomers (isolated from A31-m), dimers (isolated from A46), and the mixture of oligomers provided by KM314 were separately hybridized to each of three phage DNAs that contain different fractions of the λdv1 genome. The phage DNAs were isolated from $\lambda^{+}$, λbioy and λbioP phages, which contain respectively 100%, 59 to 64%, and 43 to 48% of the λdv1 genome (see Fig. 1 and its legend). The hybridization curves obtained by exposing filters containing a constant amount of the denatured phage DNA (0·225 μg per filter) to increasing concentrations of small denatured

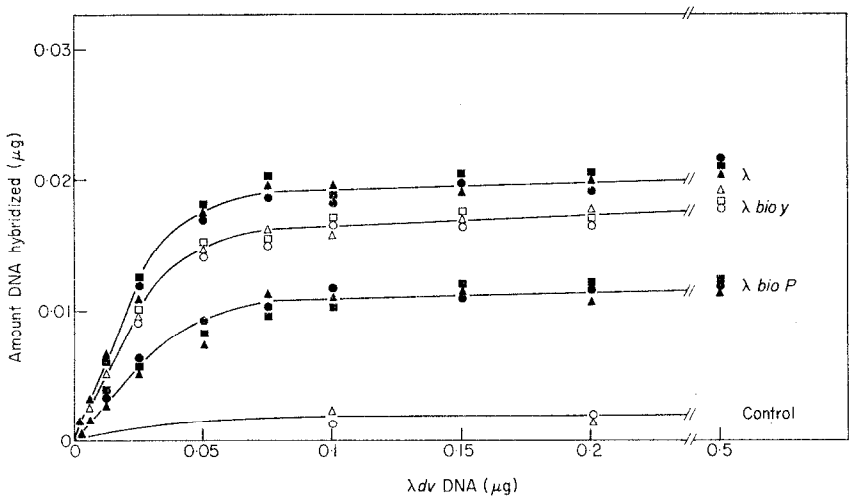


FIG. 6. Hybridization of different preparations of λdv1 DNA to DNA from $\lambda^{+}$, λbioy and λbioP phages.

The procedure is described in Materials and Methods. The control filters contained DNA from the λbio, M55-3, which contains no DNA from the λdv1 region, since the deleted segment extends from *att* to a point to the right of gene *R* (Fig. 1). Symbols: circles, monomers; triangles, dimers; squares, complete set of oligomers isolated from KM314. The saturation levels for λbioP and λbioy DNAs are approximately 50 and 80% of that for $\lambda^{+}$ DNA, after correcting for the control.

TABLE 2

*Properties of λdv1 oligomers and of their distributions in* rec$^+$ *and* rec$^-$ *strains*

| (a) Properties | *n* 1 | 2 | 3 | 4 | 5 | 6 | 8 | 9 | 10 | 12 | 14 | $\lambda^+$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $S_{cc}$ | 26 | 34·8 | 42 | 48 | 52 | 58 | 65 | 68·5 | 72 | 77 | 82 | 62 |
| $S_{nc}$ | 18·4 | 23·2 | 26·5 | 29·5 | 32 | 34 | 38 | · | 41 | 43 | 45 | 37 |
| Length (μm) | 2·25 | 4·4 | 7·0 | 8·9 | · | 13·7 | 17·2 | · | · | · | · | 16·2 |
| $f^{(1)}$ | 0·140 | 0·280 | 0·42 | 0·56 | 0·70 | 0·84 | 1·12 | 1·26 | 1·40 | 1·68 | 1·96 | 1·00 |

| (b) Distributions | Percentage of total *λdv* DNA in oligomers with *n* equal to:[2] 1 | 2 | 3 | 4 | 5 | 6 | 8 | 9 | 10 | 12 | 14 | (16) | Monomer-equivalents per cell[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22°C | | | | | | | | | | | | | *(M)* |
| *rec*$^+$, KM314 | <0·1 | 42 | 6 | 28 | 2 | 10 | 8 | · | 2 | 1·5 | 0·5 | 0·3 | 100 | –– |
| *recA*$^-$, A46 | <0·01 | 94 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 92 | –– |
| *recB*$^-$, B10 | <0·1 | 60 | 5 | 23 | · | 6 | 5 | · | + | + | · | · | 90 | –– |
| *recC*$^-$, C22 | <0·1 | 58 | 5 | 25 | · | 6 | 5 | · | + | + | · | · | 100 | –– |
| 37°C | | | | | | | | | | | | | | |
| *rec*$^+$ | | | | | | | | | | | | | | |
| M31-m | 48 | 32 | 7 | 8 | · | 2 | 2 | · | + | + | · | · | 88 | (93) |
| KM314 | <0·1 | 66 | 4 | 21 | · | 4 | 3·5 | · | + | + | · | · | 90 | (112) |
| M31-t | <0·1 | 7 | 72 | + | + | 14 | + | 4 | · | 2 | · | · | 62 | (103) |
| M31-tet | <0·1 | 7 | 1·5 | 76 | · | 5 | 8 | · | 1 | 1·5 | · | 1 | 75 | (125) |
| *recA*$^-$ | | | | | | | | | | | | | | |
| A31-m | 90 | 8 | · | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 88 | (88) |
| A46 | <0·1 | 95 | 2·5 | 2·5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 85 | (85) |
| A31-t | <0·1 | + | 95 | + | + | 2·5 | 0 | 0 | 0 | 0 | 0 | 0 | 75 | (77) |
| A31-tet | <0·1 | 1 | + | 97 | + | + | 1 | 0 | 0 | 0 | 0 | 0 | 88 | (90) |
| *rec*$^+$/*recA*$^-$ | | | | | | | | | | | | | | |
| KLF8/A46 | <0·1 | 66 | 4 | 19 | · | 4 | 3·5 | · | · | · | · | · | 90 | (112) |

[1] $f$ is the length (or molecular weight) relative to that of $\lambda^+$ DNA. The $f$-value of the monomer is the average given in the text (Results section (a)(i)); other $f$-values are the indicated integral multiples of this value.

[2] These are mass percentages, often the average of several sedimentation profiles. The values for $l$ are underlined. (+) = small amount definitely present, but value could not be accurately determined; (○) = absent; (·) = presence or absence could not be determined.

[3] See text for computation of these values. The values in the first column represent the mean for all cells in the population; those given in parentheses under ($M$) represent the mean for the [*λdv*1]$^+$ cells, and were corrected for the frequency of [*λdv*]$^-$ cells in each population (Table 3).

fragments of the λdv1 DNAs are given in Figure 6, along with a control series in which the DNA fixed to the filter was obtained from a λbio phage that lacks all of the λdv1 genome (see legend to Fig. 6).

There is no significant difference in the behavior of the three preparations of λdv1 DNA fragments, regardless of the phage DNA used in the hybridization. This observation indicates that the same base sequences are represented in the same proportion in each λdv1 DNA preparation, and is the result required if the monomeric DNA is repeated an integral number of times in each oligomer.

The maximum amount of λdv1 DNA that was observed to hybridize to the $\lambda^+$ DNA filters is 0·018 μg, after correction for the control filters. In comparable experiments (not shown in Fig. 6), the maximum amount of $^3$H-labeled $\lambda^+$ DNA fragments observed to hybridize to the same $\lambda^+$ DNA filters is 0·13 μg. The ratio of these two saturation maxima is 0·14 and is equal to the ratio of the length of the λdv1 monomer to that of $\lambda^+$ DNA (see section (a) above). This observation indicates that the monomer sequence is the minimum sequence containing the entire λdv1 genome.

Finally, we note that the saturation values observed for the λbio*P* and λbio*y* DNAs relative to that for $\lambda^+$ DMA are approximately those expected from the fraction of the λdv1 genome that they contain (Fig. 6).

### (e) *Monomer-equivalents per cell*

Table 2 contains the mean number of λdv1 monomer-equivalents per cell that appear in the closed circular fraction obtained from each strain. This number is simply calculated from (a) the ratio of λdv1 to *E. coli* DNA (obtained from the two bands of DNA in the ethidium bromide/CsCl gradients), (b) the ratio of the contour length of the *E. coli* chromosome (Cairns, 1963) to that for the λdv1 monomer, and (c) the number of chromosomes per exponentially growing bacterium, i.e. about 2·5 (Matsubara & Kaiser, 1968).

There is little consistent variation in these numbers, except perhaps for the lower values for the trimer and tetramer *rec*$^+$ strains. This is probably due to the lower stability of these strains and the consequently higher frequency of [λdv]$^-$ segregants in their cell populations (see section (f) below). When corrected for the frequency of [λdv]$^-$ segregants in the populations of each strain (Table 3), the values in the parentheses are obtained and represent the mean number of monomer-equivalents per [λdv1]$^+$ cell in such populations. The corrected values for *rec*$^+$ strains do not appear to vary significantly among themselves, but the mean value for these strains (108 monomer-equivalents per [λdv1]$^+$ cell) appears to be significantly higher than the mean of the corresponding *recA*$^-$ strains (85), with the *rec*$^+$/*recA*$^-$ strain conforming to the first group. The populations grown at 22°C have not been similarly corrected, since frequencies of [λdv]$^-$ segregants have not been determined at this temperature.

### (f) *Stability of* λdv1 *in* rec$^+$ *and* recA$^-$ *strains*

All [λdv1]$^+$ strains reported here are refractory to infection by $\lambda^+$, λ*vir*, and λ*imm*$^{434}$, but are sensitive to λ*imm*$^{21}$ and λ*imm*$^{\varphi 80}$ (Table 3), similar results having been found by Kumar & Szybalski (1970). The fact that some plaques are formed by the first three phages on certain of these [λdv1]$^+$ strains (as indicated by the + symbols in Table 3) is explained by their different stabilities, i.e. by the frequency of [λdv]$^-$ segregants arising from spontaneous curing in the [λdv1]$^+$ populations.

TABLE 3

*Sensitivity to infection and stability of* [λdv*1*]$^+$ *strains*

| Strains | Sensitivity to infection by[1]: $\lambda^+$ | $\lambda vir$ | $\lambda imm^{434}$ | $\lambda imm^{21}$ | $\lambda imm^{\phi 80}$ | Frequency of $\lambda dv^-$ cells[2] (%) |
|---|---|---|---|---|---|---|
| *rec*$^+$ | | | | | | |
| M31-m | — | + | — | c | c | 5 |
| M31-d | — | ++ | (+) | c | c | 20 |
| KM314 | — | ++ | (+) | c | c | 20 |
| KLF8/A46 | — | ++ | (+) | c | c | 20 |
| M31-t | (+) | +++ | + | c | c | 40 |
| M31-tet | (+) | +++ | + | c | c | 40 |
| *recA*$^-$ | | | | | | |
| A31-m | — | — | — | c | c | <0·2 |
| A31-d | — | — | — | c | c | <0·2 |
| A46 | — | — | — | c | c | <0·2 |
| A31-t | — | (+) | — | c | c | 2 |
| A31-tet | — | (+) | — | c | c | 2 |

[1] Assignments of sensitivity levels were based on results of cross-streaking and of drops of phage placed on a lawn of bacteria. Minus indicates no lysis; (+), +, ++ and +++ indicates increasing degrees of partial lysis and therefore of increasing sensitivity to infection; c, indicates complete lysis.

[2] These values are for populations formed by inoculating 1 ml Tryptone medium with a single colony and growing at 37°C to a density of $3 \times 10^8$ cells/ml. See text for the determination of frequency.

Such cured cells are [λ*dv*]$^-$, as shown by their lack of ability to form closed circular DNA (see Materials and Methods, section (c)(iii)), and are sensitive to infection by all five phages.

The frequency of cured [λ*dv*]$^-$ cells in the [λ*dv*1]$^+$ populations was determined by isolating many colonies from each population and cross-streaking exponentially growing cells from those colonies against a mixture of $\lambda virh^{\lambda}$, $\lambda virh^{434}$, and $\lambda virh^{\varphi 80}$. The results are given in the last column of Table 3.

Clearly, the *recA*$^-$ cells afford a much more stable environment than do their *rec*$^+$ counterparts. Within the *rec*$^+$ set, the stability decreases with increasing size of the *mfc*, a characteristic which is reflected to some extent in the *recA*$^-$ set. The stability of λ*dv*1 in the *recB*$^-$ and *recC*$^-$ cells did not differ from that in the corresponding *rec*$^+$ cells.

## 4. Discussion

### (a) *Oligomerization by intermolecular reciprocal recombination in* rec$^+$ *strains*

In all cases tested here, the *recA*$^-$ mutations cause a five- to tenfold reduction in the amount of λ*dv*1 DNA in oligomers larger than *l*, the length of the most frequent circle, and eliminate oligomers larger than 2*l*. By contrast, inactivation of the *recB* or *recC* genes results in a reduction of only about 1·5-fold in these higher oligomers, with no cut-off at 2*l*. The greater effect of *recA*$^-$ mutations on oligomerization conforms qualitatively to the relative effects of these *rec*$^-$ mutations on genetic

recombination; i.e. *recA*$^-$ is far more effective than *recB*$^-$ or *recC*$^-$ in decreasing the recombination frequency, whether of the host (Willetts & Clark, 1969) or of infecting phages (Tessman, 1968; Schulman *et al.*, 1970). Furthermore, a comparison of the distribution of λ*dv*1 oligomers in the *rec*$^+$/*recA*$^-$ merodiploid with the distributions exhibited by *rec*$^+$ and *recA*$^-$ bacteria (Table 2) indicates that *recA*$^+$ is dominant to *recA*$^-$ with respect to oligomerization, just as it is with respect to recombination. We therefore suppose that most higher λ*dv*1 oligomers in *rec*$^+$ cells are formed by recombination mechanisms.

The length distributions of the circular λ*dv*1 DNA molecules isolated from *rec*$^+$ strains support this supposition. Most oligomers from a given strain conform to the series, $l$, $2l$, $3l$, . . . . , where the frequencies decrease with increasing length and $l$ may correspond to $n = 1$, 2, 3 or 4, i.e. to the monomer, dimer, trimer or tetramer strains. A reasonable explanation for the fact that the members of this series exhibit lengths that are successive integral multiples of $l$ is that circular molecules larger than $l$ are formed by intermolecular reciprocal recombination between two smaller circular molecules. Thus $2l$ molecules would be formed by $l + l \rightarrow 2l$; $3l$ molecules by $l + 2l \rightarrow 3l$; $4l$ molecules by $l + 3l \rightarrow 4l$, or by $2l + 2l \rightarrow 4l$, etc. That the *rec*$^+$ pathway can produce reciprocal recombinants has been shown by both Meselson (1967) and Herman (1965,1968) for exchanges between an F′ episome and the bacterial chromosome; it is also indicated by the *rec*$^+$-controlled insertion of λ*red*$^-$ DNA into pre-existing prophage (Gottesman & Yarmolinsky, 1968).

However, these reciprocal recombination reactions do not, *a priori*, explain the observed decrease in frequency with increasing size, nor the relative stability of the oligomeric distribution in a given strain—e.g. different clones from the dimer strain generally exhibit distributions in which $l$ corresponds to $n = 2$. The explanation for these two observations that comes most easily to our minds is that the replication of λ*dv*1 DNA is largely restricted to molecules of the length, $l$.

Such a restricted system of replication would provide the driving force that determines and maintains the specificity of the distribution that characterizes each strain. For example, if most of the replication sites in an exponentially expanding population of bacteria are occupied by dimers, and if the rate of replication of these molecules is greater than the rate they undergo intermolecular reciprocal recombination, then the most frequent circles in the population are expected to be the dimers; i.e. $l$ will correspond to $n = 2$, the characteristic of the dimer strain. Furthermore, successively lower frequencies are expected for the $2l$ molecules (tetramers), the $3l$ molecules (hexamers), etc., that are formed only by the postulated recombination reactions, providing that the probability of each recombination event is proportional to the product of the concentrations of the two reactants, and that *intra*molecular recombination events are ignored†.

† This expectation is based on the following formulation. Since the $l$ molecules are assumed to be formed by replication and lost by intermolecular reciprocal recombination, then in the steady-state of exponential growth, $(l) = (M)/i - [k_{11}(l)^2 + k_{12}(l)(2l) + k_{13}(l)(3l) + \ldots]$, where $(M)/i$ is the replication rate of $l$ per cell per generation, $(M)$ being the monomer-equivalents per cell and $i$ the number of monomer-equivalents per $l$; $(l)$ is the concentration of the $l$ molecules per cell, $(2l)$ the concentration of $2l$, etc.; and the $k_{ab}$ are the reaction constants for the recombination, $al + bl \rightarrow (a + b)l$, in (molecule)$^{-1}$(cell). Assuming that $k_{ab} = abk_{11}$, the preceding equation reduces to $(l) = (M)/(i + k_{11}(M))$. If the higher oligomers ($2l$, $3l$, etc.) are formed and lost only by intermolecular reciprocal recombination, then

*continued*

Another indication that replication is largely restricted to molecules of size $l$ is provided by the experiments in which *recA*$^-$ mutations were introduced into the *rec*$^+$ [$\lambda dv1$]$^+$ dimer strain, KM314, by mating with Hfr *recA*$^-$ [$\lambda dv$]$^-$ strains (Results, section (b)). Each of 45 independently isolated *recA*$^-$ [$\lambda dv1$]$^+$ recombinants from this mating yielded the dimer distribution shown in Figure 4(b). This is the result that is expected if the replication sites in the parent dimer strain are fully occupied with dimer molecules. By contrast, if the $2l$ molecules, or tetramers, in the parent occupied a fraction of these replications sites equal to their frequency in the isolated $\lambda dv1$ DNA (12 to 21%, depending on the growth temperature), then some of the 45 recombinants should have been tetramer strains, or yielded distributions in which the tetramers appeared in a much higher frequency than the 1 to 2% observed.

Certain of the $\lambda dv1$ circular oligomers that appear in low frequency in the *rec*$^+$ strains are not integral multiples of $l$; e.g. the trimer in the dimer strain, the dimer in the trimer strain, and the dimer, trimer and hexamer in the tetramer strain (Table 2). We imagine that these oligomers are also formed by recombination processes, since their frequencies are also reduced by *recA*$^-$ mutations. One possibility is that they are produced from the other oligomers by *intra*molecular recombination events, either reciprocal or non-reciprocal. If this were the case, then one might expect that the proportion of these atypical oligomers would increase with increasing $l$; i.e. as $l$ increases and the number of molecules per cell decreases, the ratio of intramolecular to intermolecular reactions should increase. This appears to be the case, since the number frequency of the atypical oligomers increases from about 3·3% in the dimer strain to 11·5 and 23% in the trimer and tetramer strains, respectively.

However, our data provide little further information regarding the formation of these oligomers, except to indicate that there are difficulties in a simple intramolecular recombination model. Thus the puzzling absence of monomers in all but the monomer strains does not fit such a simple scheme.

### (b) *Distribution of circular* λdv*1 DNA in* recA$^-$ *strains: oligomerization without recombination?*

A small fraction of the circular $\lambda dv1$ DNA isolated from *recA*$^-$ cells exhibits lengths different from $l$ (Table 2), even though the *recA*$^-$ mutations used here are absolutely defective for recombination in bacterial crosses (Willetts & Clark, 1969). We therefore suppose that a different and less efficient mechanism of oligomerization is operative in *recA*$^-$ cells, a supposition that receives additional support from the observation that the oligomeric distributions in *recA*$^-$ and *recA*$^+$ are differently sensitive to the temperature of growth (B. Hobom & G. Hobom, unpublished results).

A striking aspect of the oligomeric distributions from *recA*$^-$ cells is that the $2l$ oligomer is formed in all strains but that oligomers larger than $2l$ are not. A model that is compatible with this observation is depicted in Figure 7. Here, the $2l$ molecules are formed by aberrant segregation of the DNA strands upon termination of replication to form head-to-tail linkages between the daughter molecules by covalently

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$$(jl) = [i\, k_{11}/(i + jk_{11}(M))]\cdot[\sum_{a=1}^{a=j/2} a(j-a)(al)((j-a)l)]$$

for $j$ = even integral; if $j$ = odd integral, then the same equation applies except that the sum is taken to $a = (j-1)/2$. Using these equations, it can then be shown that $((j+1)l)/(jl)$ is less than one.

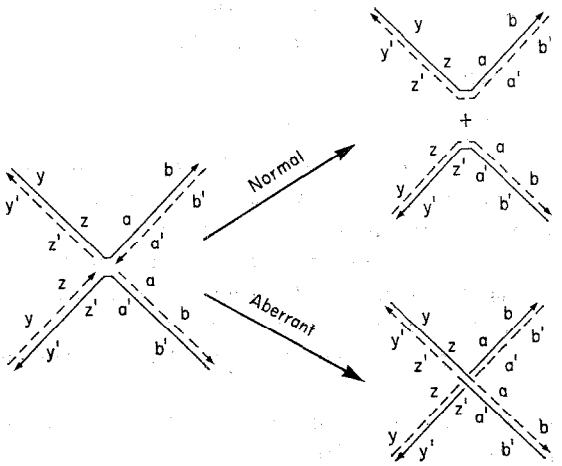


FIG. 7. A mechanism for generating circles of length $2l$ by aberrant segregation of the strands at the termination of the replication of a circle with length, $l$.

The letters ab . . . yz represent the sequence of bases in one strand, and the primed letters represent the sequence in the complementary strand.

joining the termini of the newly synthesized strands to the equivalent parental strands. This mechanism could therefore produce the same head-to-tail form for the oligomers as that expected from recombination and observed for λdv1 dimers (Champoux & Hobom, unpublished results; Chow *et al.*, 1974).

If such aberrant products of replication could in turn be replicated within the same cell population in which they are formed, then a geometric series of oligomers ($l$, $2l$, $4l$, $8l$, . . . .) would be expected. However, if replication of λdv1 DNA is restricted to the $l$ molecules in *recA*$^-$ cells, just as we suppose that it is in *recA*$^+$ cells, then the upper limit for such a series would be the $2l$ molecules.

Circular oligomers, which are not multiples of $l$, also appear in the λdv1 DNA isolated from *recA*$^-$ strains (Table 2). These can also be imagined to result from the aberrant breakage and joining of strands during replication. However, in this case the aberrant events would have to be induced when the two opposing replication forks within the same circular molecule simultaneously pass through identical sequences, rather than when they meet at the termination of replication.

It is of interest to note that the distribution of circular oligomers in the mitochondria from human leukemic leukocytes and other malignant cells is like that in the *recA*$^-$ monomer strain in that dimer circles provide the upper limit in both cases (Clayton & Vinograd, 1969; D. Clayton, personal communication). This analogy suggests that mitochondria in these cells and in their normal counterparts (which rarely if ever form even the circular dimers) lack an active mechanism for reciprocal recombination.

DNA from mitochondria in both normal and malignant cells is different from λdv1 DNA in containing catenated oligomers in which two circles are interlocked as links in a chain. We have looked assiduously for catenated λdv1 oligomers from both *recA*$^-$ and *recA*$^+$ cells in ethidium bromide/CsCl gradients after 20, 40 or 70% conversion of isolated $^3$H-labeled closed circles to nicked circles, and not detected significant amounts of catenated DNA in the region between the bands of closed and nicked circles (Hudson & Vinograd, 1967), even after rebanding fractions from

this region three or four times. We have in this manner placed an upper limit of about 0·01% on the amount of λ*dv*1 DNA in the form of catenated oligomers in either *recA*$^+$ or *recA*$^-$ cells.

(c) *Frequency of spontaneous curing of* λdv*1 in* recA$^+$ *and* recA$^-$ *cells*

The spontaneous curing of λ*dv*1 in *recA*$^+$ cells does not appear to result from the reciprocal recombination events that lead to oligomerization, even though both are reduced or eliminated by inactivation of *recA*. One indication of this lack of dependence is that the frequency of oligomerization decreases with increasing *l* in *recA*$^+$ cells (Table 2), whereas the frequency of curing increases (Table 3). Furthermore, a rough estimate of the frequency of curing per cell per generation in *recA*$^+$ cells indicates that it is several orders of magnitude less than the frequency of oligomerization.

Curing represents the end result of the inability of the λ*dv*1 DNA replication system to keep up with the multiplication of the host cells, even though our data indicate that an average of 90 monomer molecules are produced per cell per generation in both the *rec*$^+$ and *recA*$^-$ monomer strains (Table 2). Both the curing phenomenon and the previously postulated restriction of replication to *l*-sized molecules can be accounted for if it is assumed that λ*dv*1 DNA replication occurs at a small number of replication sites per cell, perhaps only one.

Since the number of sites is assumed to be far fewer than the number of λ*dv*1 DNA molecules, termination of replication would usually result in the creation of at least one "free" daughter molecule; we suppose that the other daughter remains fixed to the site. When however a new site is created, the daughter molecule that would otherwise have been liberated is assumed to occupy it. If the site is not occupied, then we presume that it is, in effect, permanently lost to the λ*dv*1 DNA replication system, either because the free λ*dv*1 DNA molecules cannot associate with it, or because it decays rapidly when unoccupied. Curing would then result from such rare losses of the replication sites, and the replication of λ*dv*1 DNA in a given clone would be restricted to the *l*-sized molecules.

According to this view of curing, the effect of the *recA* alleles would be to alter the probability for the loss of replication sites, *recA*$^+$ increasing that probability and *recA*$^-$ decreasing it. This would place curing in the set of phenotypic characteristics of the *recA* alleles that are not dependent on recombination *per se*. Another of these is the low yield exhibited by λ*fec*$^-$ in *recA*$^-$ hosts, whereas normal yields are obtained in *rec*$^+$ and *recB*$^-$ or *C*$^-$ mutants. The *fec*$^-$ state results from the inactivation of two λ genes, γ and either *red* α or *red* β (Zissler *et al.*, 1971), and since these three genes are missing in λ*dv*1 (Fig. 1), the effect of the *recA* alleles on λ*fec*$^-$ growth and on curing of λ*dv*1 may be related through some common aspect of DNA replication.

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## REFERENCES

Adelberg, E. A. & Burns, S. N. (1960). *J. Bacteriol.* **79**, 321–330.
Bazaral, M. & Helinski, D. R. (1968). *Biochemistry*, **7**, 3513–3519.
Böttger, M., Bierwolf, D., Wunderlick, V. & Graffi, A. (1971). *Biochim. Biophys. Acta*, **232**, 21–31.

Burgi, E. & Hershey, A. D. (1963). *Biophys. J.* **3**, 309–321.
Cairns, J. (1963). *Cold Spring Harbor Symp. Quant. Biol.* **28**, 43–46.
Champoux, J. J. & Hogness, D. S. (1972). *J. Mol. Biol.* **71**, 383–405.
Chow, L. T., Davidson, N. & Berg, D. (1974). *J. Mol. Biol.* **86**, 69–90.
Clark, A. J. (1967). *J. Cell. Comp. Physiol.* **70** (Suppl. 1), 165–180.
Clayton, D. A. & Vinograd, J. (1967). *Nature (London)*, **216**, 652–657.
Clayton, D. A. & Vinograd, J. (1969). *Proc. Nat. Acad. Sci., U.S.A.* **62**, 1077–1084.
Cuzin, F., Vogt, M., Dieckmann, M. & Berg, P. (1970). *J. Mol. Biol.* **47**, 317–333.
Davidson, N. & Szybalski, W. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 45–82, Cold Spring Harbor Laboratories, New York.
Denhardt, D. T. (1966). *Biochem. Biophys. Res. Commun.* **23**, 641–646.
Doerfler, W. & Hogness, D. S. (1968). *J. Mol. Biol.* **33**, 635–659.
Egan, J. B. & Hogness, D. S. (1972). *J. Mol. Biol.* **71**, 363–381.
Godson, G. N. & Sinsheimer, R. L. (1967). *Biochim. Biophys. Acta*, **149**, 476–488.
Goebel, W. & Helinski, D. R. (1968). *Proc. Nat. Acad. Sci., U.S.A.* **61**, 1406–1413.
Gottesman, M. & Yarmolinsky, M. (1968). *J. Mol. Biol.* **31**, 487.
Gray, H. B., Bloomfield, V. A. & Hearst, J. E. (1967). *J. Chem. Phys.* **46**, 1493–1498.
Herman, R. K. (1965). *J. Bacteriol.* **90**, 1664–1668.
Herman, R. K. (1968). *J. Bacteriol.* **96**, 173–179.
Hudson, B. & Vinograd, J. (1967). *Nature (London)*, **216**, 647–652.
Hudson, B. & Vinograd, J. (1969). *Nature (London)*, **221**, 332–337.
Hudson, B., Clayton, D. A. & Vinograd, J. (1968). *Cold Spring Harbor Symp. Quant. Biol.* **33**, 435–442.
Jacob, F. & Wollman, E. (1954). *Ann. Inst. Pasteur*, **87**, 653–673.
Kaiser, A. D. (1957). *Virology*, **3**, 42–61.
Kaiser, A. D. & Jacob, F. (1957). *Virology*, **4**, 509–521.
Kayajanian, G. (1968). *Virology*, **36**, 30–41.
Kayajanian, G. (1970). *Virology*, **41**, 170–174.
Kumar, S. & Szybalski, W. (1970). *Virology*, **41**, 665–679.
Lang, D., Bujard, H., Wolf, B. & Russell, D. (1967). *J. Mol. Biol.* **23**, 163–181.
Leighton, S. B. & Rubinstein, I. (1969). *J. Mol. Biol.* **46**, 313–328.
Liedke-Kulke, M. & Kaiser, A. D. (1967). *Virology*, **32**, 465–474.
Low, B. (1968). *Proc. Nat. Acad. Sci., U.S.A.* **60**, 160–167.
Matsubara, K. & Kaiser, A. D. (1968). *Cold Spring Harbor Symp. Quant. Biol.* **33**, 769–775.
Meselson, M. (1967). *J. Cell. Physiol.* **70** (Suppl. 1), 113–118.
Radloff, R., Bauer, W. & Vinograd, J. (1967). *Proc. Nat. Acad. Sci., U.S.A.* **57**, 1514–1521.
Schulman, M. J., Hallick, L. M., Echols, H. & Signer, E. R. (1970). *J. Mol. Biol.* **52**, 501–520.
Studier, F. W. (1965). *J. Mol. Biol.* **11**, 373–390.
Sund, H. & Weber, K. (1963). *Biochem. Z.* **337**, 24–34.
Tessman, I. (1968). *Science*, **161**, 481–482.
Vasquez, C. & Kleinschmidt, A. K. (1968). *J. Mol. Biol.* **34**, 137–147.
Wang, J. C. (1970). *Biopolymers*, **9**, 489–502.
Willetts, N. S. & Clark, A. J. (1969). *J. Bacteriol.* **100**, 231–239.
Willetts, N. S. & Mount, D. W. (1969). *J. Bacteriol.* **100**, 923–934.
Willetts, N. S., Clark, A. J. & Low, B. (1969). *J. Bacteriol.* **97**, 244–249.
Young, E. T. & Sinsheimer, R. L. (1967*a*). *J. Mol. Biol.* **30**, 147–164.
Young, E. T. & Sinsheimer, R. L. (1967*b*). *J. Mol. Biol.* **30**, 165–200.
Zissler, J., Signer, E. & Schaefer, F. (1971). In *The Bacteriophage Lambda* (Hershey, A. D., ed.), pp. 455–475, Cold Spring Harbor Laboratories, New York.