THE ARRANGEMENTS OF NUCLEOTIDE SEQUENCES IN T2 AND T5 BACTERIOPHAGE DNA MOLECULES

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ABSTRACT The genetic map of T4 (and T2) bacteriophage is circular but the DNA molecule that is liberated by phenol extraction is a linear duplex of polynucleotide chains. If the genetic map is related to the physical structure of the DNA molecule, the problem arises as to how a linear molecule can give rise to a circular map. An explanation can be made on the basis that the bacteriophage liberate molecules which have nucleotide sequences which are circular permutations of each other. Thus, markers which are most distant on one molecule are closest together on another. To test this hypothesis, the middles of T2 and T5 DNA molecules were mechanically deleted and the absence of certain nucleotide sequences was tested by "renaturation" or "reannealing" experiments using columns containing denatured DNA immobilized in agar beads. The results indicate that when the middles are deleted from the T5 DNA molecule, some special sequences are removed; whereas, when the middles are deleted from the T2 DNA molecule, no special group of sequences is removed. This would indicate that T2 molecules begin at different points in their nucleotide sequence, while T5 molecules all begin at the same point. It is likely that this permutation of sequences of T2(T4) molecules is related to the circularity of their genetic map.

Genetic recombination studies on T2 or T4 bacteriophage have led to the conclusion that all markers can be placed on a single linkage map (1) which has recently been shown to have a circular form (2). In contrast to this, the genetic map of λ bacteriophage appears linear during virulent infection although there is a possibility that circularity may exist during the establishment of lysogeny (3). Thus it appears that phage genetic maps may have two forms—linear or circular. Physical and chemical studies of phage DNAs have led to the conclusion that there is a single linear duplex molecule in T2, T4, T5, and λ bacteriophage (4–18). The correlation of the genetic structure with the physical structure of these DNA molecules is of fundamental interest. An important experiment which bears on this subject has

been done with λ DNA (11, 12). If the λ molecule is sheared and broken near its middle, then the markers on opposite sides of the linkage map are separated from each other. Thus to the level of the first breakage, the map and molecule appear collinear.

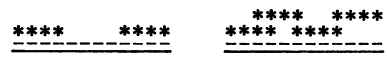
How can the notion of collinearity be preserved in the case of T4 (or T2) where the map is circular and the molecule is linear? Perhaps the nucleotide sequences of the molecules in a collection of T2 phage particles are circularly permuted. A circularly permuted collection of sequences can be generated by making a single random break in each of many circular molecules of identical nucleotide sequence. This suggestion first made by Steinberg and Stahl (33) has gained clarity and support from the genetic experiments of Streisinger *et al.* (2, 22, 29). We have attempted to obtain some direct physical evidence about the arrangements of nucleotide sequences in T2 DNA and in the unrelated T5 DNA molecule.

OUTLINE OF EXPERIMENTS

If all molecules have the same nucleotide sequence, then the mechanical deletion of the middle region of the molecule would remove that part of the nucleotide message which is in the middle of all the molecules. On the other hand, if the nucleotide message were circularly permuted and began at a different point in different molecules, then the mechanical deletion of the middle region would not remove a certain class of sequences, since all possible sequences would be represented in the remaining end fragments. In essence then the test was as follows:—the "middles" of T2 and T5 DNA molecules were mechanically deleted and the end fragments tested to see whether they contained all or just some of the sequences found in the whole molecule. The mechanical deletion of the middle region of the molecules was accomplished by: (*a*) passing a dilute solution of unbroken molecules through a capillary at gradients that cause a single break in some of the molecules and (*b*) fractionating by the methylated bovine serum–albumin column and collecting the smallest fragments. These fragments of less than half-size ("short halves") must come from the right ends of some molecules and the left ends of others; they are missing a fraction of the middle region of the whole molecule, the magnitude of which can be calculated from their measured fractional length.

To test whether these short halves have all possible nucleotide sequences or just some of them, we prepare a mixture of single polynucleotide chain segments from P^{32}-labeled short halves and C^{14}-labeled whole molecules and measure the relative fraction of each that will anneal to denatured whole molecules imbedded in agar beads. The polynucleotide chain segments (pncs) were prepared by sonicating duplex DNA to produce fragments of 200,000 daltons and then boiling to denature them. By annealing one means the reformation of the duplex structure which can take place between polynucleotide chains of complementary sequence (19, 25, 28). If we assume that complementary nucleotide sequence is a prerequisite for annealing,

then this implies that each segment has a specific address on the DNA molecule. The following ideograms represent the alternative situations resulting from the mechanical deletion of the middle region of a *unique* or *permuted* collection of molecules.



Unique Permuted

The solid line represents the denatured DNA immobilized in agar, and the broken line (–) represents C^{14}-labeled pncs derived from whole molecules, and (*) represents P^{32}-labeled pncs derived from short halves. If the molecule is unique, then the short halves will produce pncs which have addresses only belonging to the ends of the molecule. If the molecules have no special beginning point, then the short halves will produce pncs which have addresses found over the whole molecule and on the average C- and P-labeled pncs will be equally abundant for any and all addresses.

The ratio of C^{14} to P^{32} in the pncs which are annealed and complexed to the DNA in the agar is denoted $(C/P)_{II}$, while the same quantity measured in the uncomplexed material is $(C/P)_I$. The ratio of these two quantities, called R, is a useful measure of the preferential retention of C-labeled pncs. In the case of a unique nucleotide sequence, the magnitude of R may be calculated by assuming that the pncs have non-overlapping addresses:

$$R = \frac{(C/P)_{II}}{(C/P)_I} = \frac{1 + \frac{\bar{q}_m}{\bar{q}_e}\frac{\alpha}{1-\alpha}}{1 + \frac{(1-\bar{q}_m)}{(1-\bar{q}_e)}\frac{\alpha}{1-\alpha}}$$

where α is the fraction of the DNA molecule which has been mechanically deleted and the quantities $\bar{q}_m$ and $\bar{q}_e$ are the fraction of all pncs which are retained in the middle and end regions. In order to estimate the ratio $\bar{q}_m/\bar{q}_e$ the testable assumptions are made that competition among pncs is taking place and that sites on the ends do not have a greater average affinity for their complementary pncs than do sites in the middle of the molecule. In the unique case, since there are more pncs seeking end addresses than middle addresses, a smaller fraction of such pncs will be retained. This results in the ratio $\bar{q}_m/\bar{q}_e$ being greater than 1 and causes R to be greater than 1. If the short halves came from a permuted molecule, then the number of pncs for any address is the same, or $\alpha = 0$; thus they would all compete on an equal basis and the values of $\bar{q}_m/\bar{q}_e$ and R would be 1. The results reported below indicate that the T5 DNA molecules appear to be a collection of unique sequences, whereas T2 molecules appear to have no special beginning point.

METHODS

DNAs. Bacteriophage T2 *H* and T5 *st* (obtained from Dr. A. D. Hershey) were grown from stocks derived from single phage particles on *E. coli BB* or *H* respec-

tively in tris-glucose medium or 3 × D as previously described (7). P^{32}-labeled T2 or T5 phage was grown by adding P^{32} (final specific activity 0.1 to 0.2 c/gm) to a culture about 1 hour prior to infection. C^{14}-labeled phage were prepared by resuspending log-phase bacteria in a medium containing 0.2 per cent C^{14} glucose (initial specific activity 3.4 c/M) 1 hour prior to infection. These lysates were purified by differential centrifugation and the DNA phenol extracted (4, 23).

Fracture by Shear. The DNA solutions were diluted in 0.62 M (T5) or 0.64 M (T2) saline (containing 0.05 M PO_4) to 1.6 γ/ml as determined by OD (4) and cooled to 5°C (26). This solution was passed through four parallel stainless steel tubes with flared ends which were 10 cm long and 0.24 mm inside diameter at a rate of 0.038 (T2) or 0.052 (T5) ml/tube-second and then chromatographed to separate breakage products. These conditions routinely resulted in single breakage.

Methylated Serum–Albumin Column. For certain purposes it was necessary to scale up the normal size (23) by fourfold. These larger columns were equivalent to the smaller ones in all important aspects. Fractions were characterized by their elution position and by sedimentation coefficient. Tubes were selected which corresponded to the fractions desired for a particular experiment, dialyzed to adjust salts, and concentrated at room temperature under vacuum.

Sedimentation. Sedimentation constants were measured in an aluminum cell at 35,600 RPM, 10 to 15 γ DNA/ml, in 0.72 M NaCl, pH 6.7, at 22°C (4, 13).

DNA-Agar. Whole molecules of DNA were immobilized in agar beads following the procedures of Bolton and McCarthy (27). Enough DNA-agar was made for any one series of experiments according to multiples of the following recipe: 240 mg of Noble agar (Difco) was mixed with 4.0 ml of water and boiled for 15 minutes. Into this was poured an equal volume of DNA at 50 to 400 γ/ml in 0.01 × SSC which had been boiled for 5 minutes (SSC = 0.15 M NaCl + 0.015 M Nacitrate). The viscous solution was vigorously mixed for 30 seconds and then poured onto a chilled petri dish to cool for 15 minutes. The gel was pressed through a 200 mesh stainless steel screen and washed with 2 × SSC in a column held at 60°C. After draining the column thoroughly it was distributed into equal lots of about 1.8 gm each. The fraction of DNA actually immobilized in the agar ranged from 60 to 95 per cent depending on the concentration and molecular weight of the DNA.

Polynucleotide Chain Segments (pncs). The various mixtures of chromatographically purified labeled and unlabeled pncs to be annealed to the DNA-agar were prepared as a group. The solutions (2 to 4 ml) in 2 × SSC were bubbled with N_2 for 10 minutes and then sealed by the Mullard sonicator probe (¾ inch diameter) and rubber cap. The chilled solution was exposed to sonication for 2 minutes, full intensity. This procedure produces fragments of 6S corresponding to 200,000 to 300,000 daltons (10, 32) and little denaturation as judged by the constancy of optical density. 1 ml aliquots of the sonicated solutions were heated to 100° for 5 minutes to produce pncs, and mixed quickly with 1.8 gm of chilled DNA-agar beads, and incubated at 60° for 17 to 20 hours.

Agar Column Operation. The incubation mixture was then transferred to a column held at 60° and washed with about 30 ml of 2 × SSC which removed all of the pncs that were not specifically complexed to the DNA in the agar (peak I). The complexed material was removed by washing with 0.01 × SSC or by gradually reducing the salt concentration (peak II). Optical melting point determinations done on these DNAs indicate that each factor of two decrease in ionic strength, lowers the effective melting temperature by about 5° (20).

C^{14} — P^{32} Counting. Solutions in peak I and peak II were assayed in a scintillation counter and the C^{14}/P^{32} ratio was calculated. Aliquots of 2 to 10 ml were mixed with 250 γ of herring sperm DNA (CalBioChem) and precipitated with 0.60 M trichloracetic acid (TCA). After curing for 20 minutes in ice water the precipitate was filtered on a 1.2 μ millipore filter 25 mm diameter, washed with cold 0.30 M TCA, and dried for 1 hour at 60°C. In most cases there were no detectable counts in the filtrate. The dried filters were draped over a straight piece of cleaned nichrome wire in counting vials containing 12 ml of a toluene solution of 2, 5-diphenyloxazole (4.0 gm/liter) and *p-bis*-2-(5-phenyloxazolyl)-benzene (0.10 gm/liter). The Nuclear Chicago scintillation spectrometer was adjusted so that about 4 to 5 per cent of the P^{32} counts were in the "carbon" channel and less than 0.1 per cent of the C^{14} counts in the "phosphorus" channel.

EXPERIMENTS

The Mechanical Deletion of the Middle Region of a DNA Molecule. The conditions under which the dilute solutions of whole molecules were shared resulted in the breakage of 13 to 20 per cent of the T2 molecules and 20 to 50 per cent of the T5 molecules. Two fragments were produced ranging in size from 30 to 70 per cent (T2) or 40 to 60 per cent (T5) of the unbroken molecule (13, 30). This solution was then fractionated according to molecular weight of the methylated bovine serum–albumin column (13, 23). The smallest fragments which elute earliest from the column (short halves) must come from the right or left ends of the DNA molecule and are lacking the middle region of the unbroken molecule. Tubes containing short halves were pooled and the sedimentation constant measured on the solution in the next higher (and in some cases lower) tube. From this value of S, the fractional length, X, was calculated from the equation $X = (S/S_o)^{1.84}$ where S_o is 63 and 48.5 for whole T2 and T5 molecules respectively (4, 6). The fraction of the middle region deleted, α, is taken to be $1 - 2X$ assuming that both right and left ends are present in equal abundance. These values are listed in the captions to Figs. 2 and 3.

It is of importance that molecules are not broken more than once, otherwise some middle sections would be collected in the leading tubes along with the ends and invalidate the experiments. To be sure that single breakage was occurring under our shearing conditions, P^{32}-labeled "long halves" of $X \geq 0.7$ (T2) or $X \geq 0.6$ (T5) were mixed with unbroken whole molecules and then sheared and chromatographed. These chromatograms showed that the long halves were not broken by these shearing conditions whereas whole molecules were. Since the long halves are the most shear-sensitive of the breakage products (13), one may conclude that there is little or no multiple breakage. This result taken together with the fact that the leading tubes contain the smallest fragments, means that this procedure results in the deletion of the middle region of the molecule.

Some Properties of DNA-Agar Columns. Fig. 1 shows some of the im-

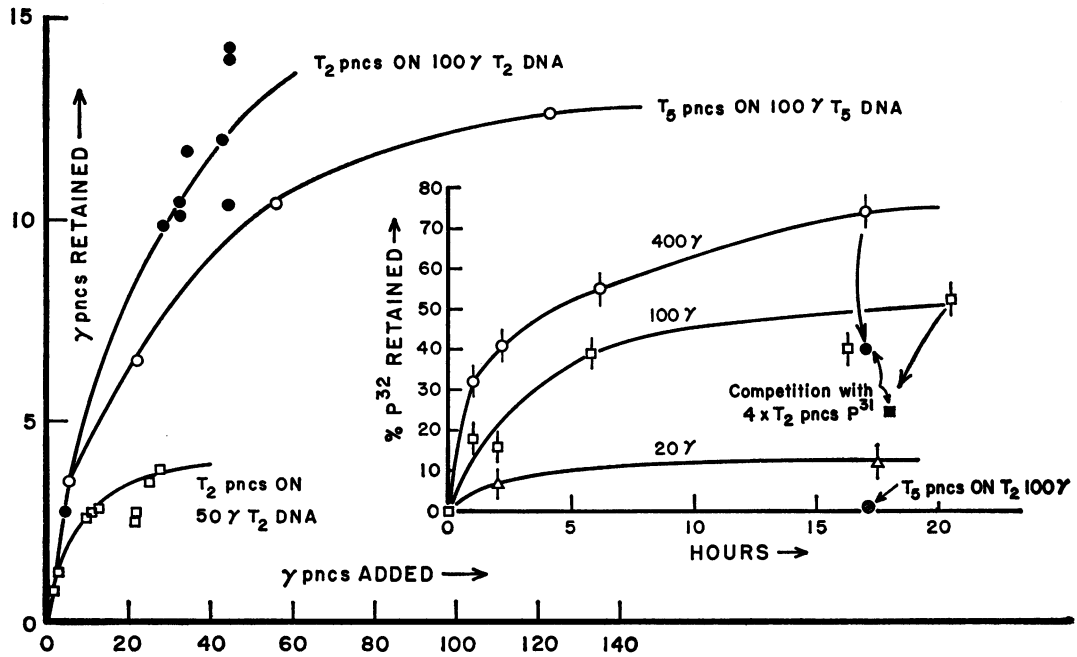


FIGURE 1 In this figure the amount of P^{32}-labeled, sonicated, denatured DNA (pncs) that is specifically complexed to DNA immobilized in agar beads is shown as a function of the weight of added pncs. The columns were maintained at 60°C for 20 hours and the specifically complexed segments were "melted" off by washing with 0.01 × SSC. Less than 1 per cent of label was left on the column after complete washing.

The inset shows the fraction of 7.2 γ of P^{32}-labeled pncs retained by columns containing 400, 100, and 20 γ of T2 DNA. Notice that no T5 pncs will be retained by the T2 column. The addition of unlabeled T2 pncs is seen to decrease, by competition, the fraction of labeled pncs that can be retained. In experiments not shown it was observed that the presence of heterologous T5 pncs has no effect on the fraction of T2 pncs retained.

portant properties of the DNA-agar columns: capacity, competition, specificity, and kinetics of annealing. The curves in the main graph show that the weight of pncs that can be complexed to the DNA-agar in 20 hours increases gradually and levels off as the concentration of added pncs is increased. Thus the apparent capacity of the column is seen to depend to some extent on the amount of added pncs. In all cases, however, the *fraction* of the added pncs that is retained becomes smaller at higher concentrations of added pncs. This effect also may be seen in the insert to Fig. 1. When unlabeled homologous pncs are added, the fraction of labeled pncs which is retained is reduced. Most experiments show a reduction of about twofold for each fourfold increase in the total amount of pncs added. The addition of heterologous T5 pncs results in no visible effect, in agreement with the finding that T5 pncs are not retained on a T2 column. Thus the greater the *abundance* of a

particular group of pncs, the smaller the fraction that will be specifically retained. In particular, if there are more pncs which have addresses belonging to the ends of the DNA molecule, then a smaller fraction of them will be retained and the ratio $\overline{q}_m/\overline{q}_e$ will be greater than 1. This reduction in the fraction of pncs that is retained is probably due to a number of competing reactions which can be demonstrated individually, but the relative rates of which are unknown: the pncs may anneal with themselves or with column DNA, the column DNA may anneal with itself if it has not already combined with pncs. Thus a kind of competition can take place, even though the number of sites in the column DNA is theoretically much greater than attained in these experiments.

The insert to Fig. 1 shows that the amount of pncs that can be complexed depends on the time allowed for annealing and the amount of DNA immobilized in the agar. The reaction apparently reaches a limit after 10 to 15 hours. In the experiments reported in Fig. 2, 10 to 60 γ of pncs were allowed to anneal to agar columns containing 50 γ of DNA for a period of 17 to 20 hours.

RESULTS

Fig. 2 shows the results of eight different experiments done with T2 and T5 DNA. The magnitude of R, which measures preferential retention of C^{14}, is shown as the ordinate and the different experiment numbers are located on the abscissa. In the caption to the figure the different experiments are depicted. If the nucleotide sequence is unique, then R values for Experiments 1, 7, 3, and 4 should be greater than 1. If the molecule is permuted, all R values should be 1. Experiments 2, 5, 6, and 8 should give R values of 1 in either case and represent controls.

Turning to Experiment 2, one may see that R is close to 1: there is no preferential retention of C- or P-labeled pncs either in the case of T2 or T5 DNA. This is as would be expected since there should be no difference in the abundance or affinities of C- and P-labeled pncs when both are derived from whole molecules. In the case of Experiment 1, however, the R value for T5 DNA is 1.16 to 1.30 while that for T2 is about 1. The implication is that Experiment 1 is equivalent to Experiment 2 for T2 DNA, but these two experiments are not equivalent for T5 DNA. We take this to mean that all of the nucleotide sequences are found in the pncs derived from the short halves of the T2 molecule, but that some sequences are missing from the pncs derived from the short halves of the T5 molecule.

The presence of a tenfold excess of cold heterologous DNA pncs (T5 in the case of T2 and T2 in the case of T5) does not alter these results as can be seen by the fact that Experiment 7 gives the same results as Experiment 1 in both cases. On the other hand, cold homologous pncs from whole molecules swamp the effect and R nearly returns to 1 (T5, Experiment 5). The same experiment with T2 DNA has no effect because R is close to 1 anyway.

The presence of an excess of unlabeled pncs from short halves should increase R

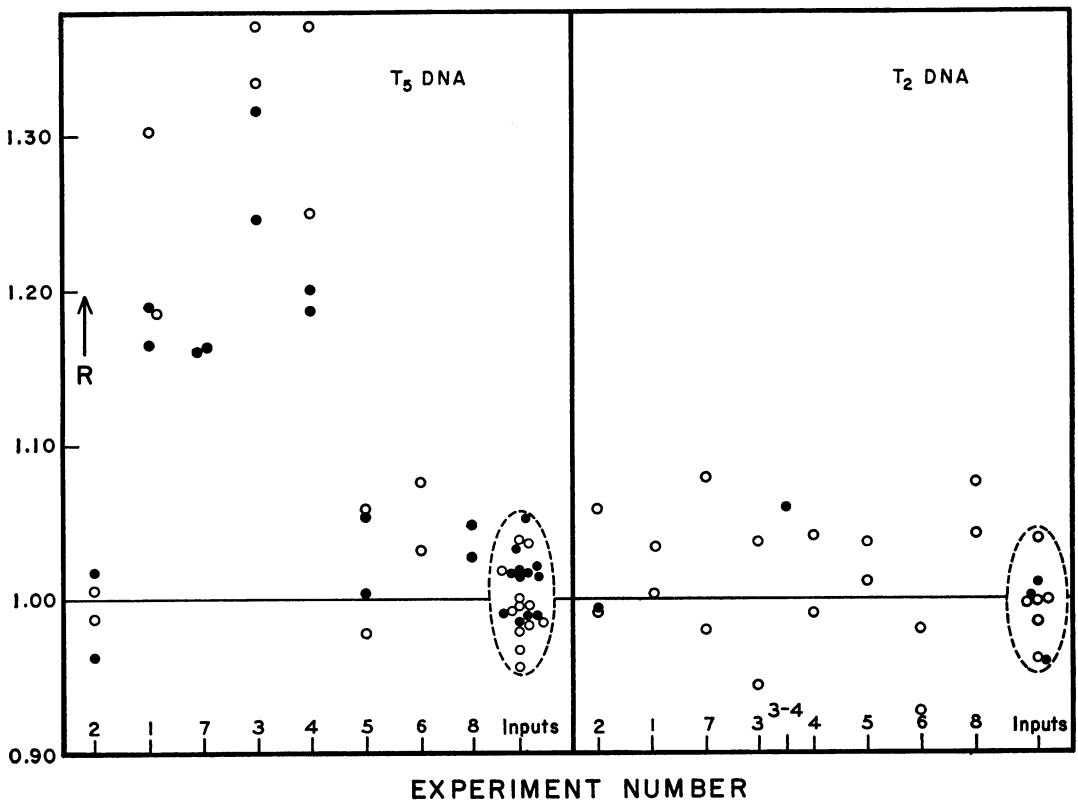
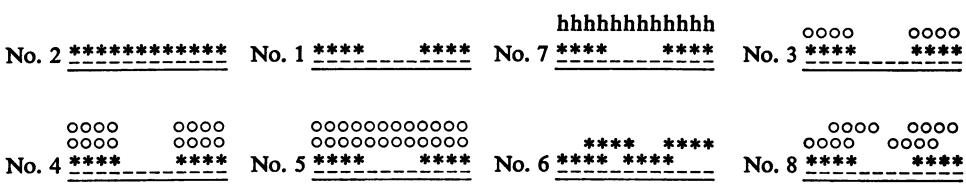


FIGURE 2 *Experiments*:



The above ideograms represent eight different experiments that were done as a group using T2 or T5 DNA. These are drawn as if the pncs from short halves were derived from unique molecules. The solid base lines depict schematically the 50 γ of DNA that is immobilized in the agar. The broken lines (–) represent 5 γ of C^{14} pncs, (*) represents 5 γ of P^{32} pncs, (○) and (8) represents 25 and 50 γ of unlabeled pncs. The *h* represents 50 γ of cold heterologous DNA (T5 in the case of T2, and T2 in the case of T5). Experiments 6 and 8 were done with pncs derived from fragments from doubly-broken molecules. In Experiment 6 the overlapping stars represent 5 γ of P-labeled pncs while in Experiment 8 the overlapping circles represent 50 γ of cold pncs. Experiment 3-4 (T2) was done with 35 γ of unlabeled pncs.

The DNA representing whole molecules, short halves, and doubly-broken fragments were taken from fractions eluting from the methylated serum albumin column and characterized by sedimentation coefficient, *S*. The fractional length, *X*, and in the case of singly-

because more pncs will be competing for the sites on the ends and thus increasing even more the ratio $\bar{q}_m/\bar{q}_e$. The addition of five times as many unlabeled end pncs is seen to further increase R (Experiment 3) in the case of T5 but not in the case of T2, even though competition is taking place in both cases as can be seen by the fact that the amount of C-labeled pncs specifically retained is reduced. A doubling of this amount of competition does not appear to increase appreciably the R value for T5 (Experiment 4) and still has no effect on R in the case of T2. This is understandable if one remembers that the effective capacity, and hence the effectiveness of competition, changes with the total amount of pncs that are added (Fig. 1). Secondly, the purity of the short halves from which the cold competing pncs were derived cannot be considered perfect, and contaminating middles would have the effect of reducing the value of R.

The short halves of the molecule are defined as the fragments eluting from the leading edge of a column run of a mixture of whole and broken molecules. If there were multiple breaks in the original molecule, and if the column discriminated with respect to molecular weight only, then the leading edge of the chromatogram should contain some middle as well as end segments. Thus one would expect the ratio, R, to be reduced to 1 when fragments from multiply-broken molecules are used in place of short halves. In Experiment 6, such an experiment was done with P-labeled pncs derived from the leading tubes from a chromatogram of doubly-broken fragments. In Experiment 8, cold pncs of similar origin were added to an

broken molecules the fraction of DNA mechanically deleted, α, was calculated. In Repetition A (○): T5 P^{32} short halves $20 \geq \alpha \geq 12$ per cent, T2 P^{32} short halves $\alpha \geq 30$ per cent, T5 cold short halves $20 \geq \alpha \geq 16$ per cent, T2 cold short halves $\alpha \geq 26$ per cent. In the second Repetition B (●): T5 P^{32} short halves $\alpha \geq 12$ per cent, T2 P^{32} short halves, same as Repetition A; T5 cold short halves $\alpha \geq 16$ per cent, T2 cold short halves $\alpha \geq 24$ per cent. The fragments derived from doubly-broken molecules were collected from tubes having a similar chromatographic position to short halves. Since α has no meaning in this case, the fractional length is reported. Repetition A: T5 P^{32} $0.33 \leq X \leq 0.40$, T2 P^{32} $X \leq 0.25$; T2 cold $X \leq 0.25$. Repetition B: T5 cold $X \leq 0.27$. Each point in the figure is the average of several recountings of the same scintillation vial; different points derived from different vials prepared from the same sample.

Aliquots from the same mixture of P^{32} short halves and C^{14} wholes were used in Experiments 1, 3, 4, 5, 7, and 8. In each case the mixture to be annealed to the agar column was sonicated and denatured together. The fraction of C^{14} pncs which are complexed is 28, 15, 11 per cent (T2) and 22, 10, 7 per cent (T5) for Experiments 1, 3, and 4 respectively, indicating that competition is taking place.

In an experiment similar to Experiment 2, P^{32} T5 pncs and C^{14} T2 pncs were annealed to a T2 agar column. Under these conditions R values of 15 to 18 were obtained, indicating that the species specificity for complex formation was very good. The reciprocal experiment using a T5 column and C^{14} T5 pncs gave similar results.

The clusters of points labeled "inputs" are repeated radioassays of different vials of the input mixture of C- and P-labeled pncs diluted into 2 × SSC and 0.01 × SSC which were made during the course of plating the other samples. The C/P ratio of the latter is divided by that of the former and the ratio plotted. The scatter of these points shows the precision of our measurements.

experiment which was similar to Experiment 1 in all other respects. One sees that in the case of T5, R is indeed reduced to a value close to 1 as expected, whereas no effect was to be expected in the case of T2 and none was found. The leading tubes of the chromatogram of doubly-broken T2 or T5 fragments were not able to provide a special group of sequences; this implies that the column is largely fractionating with respect to molecular weight independent of nucleotide sequence and composition. At the same time these results support the contention that the leading tubes of the chromatogram do contain the ends of the T2 and T5 molecules when there is a single breakage.

In the hopes of increasing the sensitivity of the test, a final series of experiments was done by melting off the complexed pncs gradually by decreasing the ionic strength stepwise by factors of about two. This results in the resolution of the complexed pncs into a number of separate fractions. This was done for experiments similar to Experiments 1 and 2 in the caption to Fig. 2 with increasing amounts of DNA immobilized in the agar. Fig 3*c* shows a characteristic bell-shaped distribution of C-labeled pncs eluted from a T2 column. The points in Fig. 3*c* show the R values obtained in each fraction for experiments with "wholes" and "short halves." These points can be seen to be identical, which indicates that there is no preferential retention or release of C^{14} and this is in accord with expectation for a permuted molecule. This pair of experiments was performed three times using agar columns containing different amounts of T2 DNA, and four times using columns containing different amounts of T5 DNA. In these experiments with T5, pncs derived from long halves were also tested. Rather than report many graphs, the R values are shown in Fig. 3 *a* and *b* for the three central fractions beginning at SSC/16. These fractions contain the most counts and give the best precision. There are significantly higher R values for T5 DNA when the P^{32}-labeled pncs are derived from the short halves than when they are derived from the whole molecule or from the long halves. These differences can be seen even though the deleted fraction of the T5 molecule is substantially smaller (about 10 per cent) than in the former experiments. In the case of T2 DNA the "whole" experiment gives R values equivalent to those obtained from the short halves experiment even though in the latter case the fraction of the molecule that was supposed to be deleted is greater than 22 per cent. Thus with more demanding test which is not only sensitive to any differences in the fraction of C- and P-labeled pncs which are complexed, but also to any differences in their melting profile, the T5 molecules appear unique, while those from T2 appear permuted. There is a general increase in R for the T2 experiments which is displayed by both the whole molecules and the short halves. This increase in R could be a result of the higher carbon content of the glucosylated hydroxymethyl cytosine residues which would be expected to melt off the column at lower ionic strengths. While a similar increase is apparent with T5 short halves, the whole molecules and long halves show no increase. This increasing trend shown by

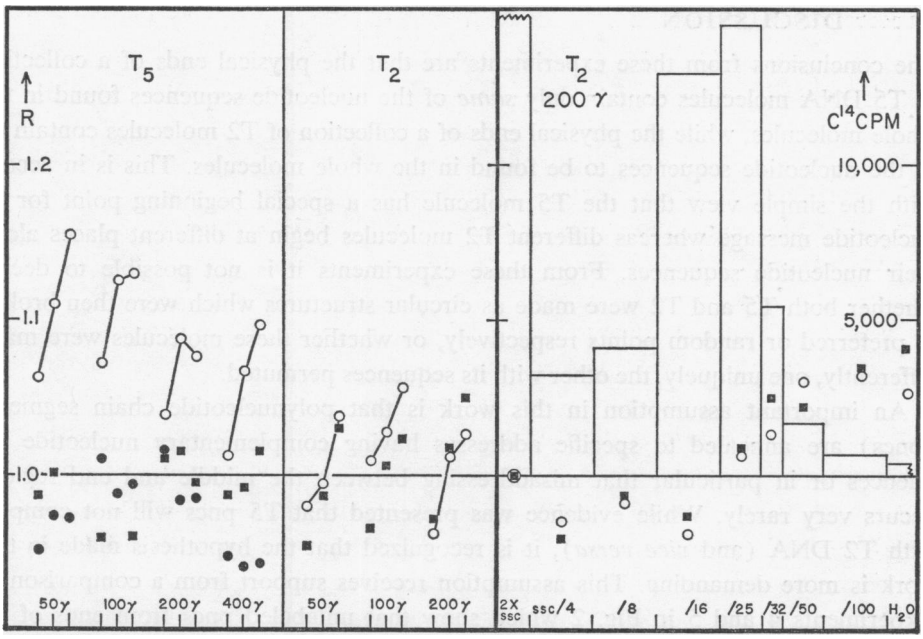


FIGURE 3

■ wholes ○ short halves ● long halves

The three types of experiments shown above were done with T5 DNA and the first two of them with T2 DNA. In each experiment a mixture of 5 γ each of C- and P-labeled pncs was annealed to agar columns containing various amounts of DNA. The per cents of T5 pncs (from whole molecules) that were specifically complexed were 31, 38, 50, and 59 per cent for columns containing 50, 100, 200, and 400 γ of T5 DNA. In the case of T2 pncs derived from whole molecules, 19, 28, and 66 per cent were retained by the 50, 100, and 200 γ columns. The fragments from which the pncs were derived had the following characteristics: T5 P^{32} short halves $20 \geq \alpha \geq 0$ per cent, T2 P^{32} short halves $\alpha \geq 22$ per cent, T5 P^{32} long halves $0.61 \leq X \leq 0.92$.

Frame *c* shows the result of the experiments with T2 pncs using a 200 γ column. The histogram shows the amount of C-labeled pncs removed by washing with solutions of decreasing ionic strength at a constant temperature of 60°. Superimposed on this "melting profile" are the values of *R* measured for each fraction. The *R* values for the whole and short half experiments are equivalent.

In frames *a* and *b* the *R* values obtained for the three central fractions are plotted for the experiments using different amounts of T5 and T2 DNA immobilized in the agar. For example, the *R* values obtained in the SSC/16, SSC/25, and SSC/32 fractions shown in *c* are replotted in *b* over 200 γ.

the T5 short halves should mean that later-melting segments have more carbon label. Since AT and GC pairs contain about the same number of carbon atoms, this implies that the middle region of the molecule is richer in GC pairs and produces pncs which melt off the column later at lower ionic strength.

DISCUSSION

The conclusions from these experiments are that the physical ends of a collection of T5 DNA molecules contain only *some* of the nucleotide sequences found in the whole molecules, while the physical ends of a collection of T2 molecules contain *all* of the nucleotide sequences to be found in the whole molecules. This is in accord with the simple view that the T5 molecule has a special beginning point for its nucleotide message whereas different T2 molecules begin at different places along their nucleotide sequences. From these experiments it is not possible to decide whether both T5 and T2 were made as circular structures which were then broken at preferred or random points respectively, or whether these molecules were made differently, one uniquely, the other with its sequences permuted.

An important assumption in this work is that polynucleotide chain segments (pncs) are annealed to specific addresses having complementary nucleotide sequences or in particular that misaddressing between the middle and end regions occurs very rarely. While evidence was presented that T5 pncs will not complex with T2 DNA (and *vice versa*), it is recognized that the hypothesis made in this work is more demanding. This assumption receives support from a comparison of Experiments 4 and 5 in Fig. 2 which show that unlabeled pncs from ends of T5 DNA enhance the R values indicating that "end-pncs" are not competing for middle sites. Whereas when unlabeled pncs derived from whole molecules are used, the R value is reduced to unity. Finally, if complementary nucleotide sequence is the basis for genetic recombination, this assumption is supported by the fact that genetic recombination is a very precise process.

Perhaps we have been misled to the conclusion that T2 molecules were permuted by the peculiar chance that the middle region of the hypothetically unique T2 molecule had a low affinity for complexing pncs; thus its deletion would scarcely be missed and the results presented would be seen. There is one consequence of this hypothetical objection that we attempted to test in the last group of experiments. If the middle region had a low affinity for pncs, then these would be washed out and appear in the uncomplexed peak I. In the short halves experiment these pncs would be largely C-labeled and would result in R values less than 1. This fractionation of middles would be greater and result in lower R values when the amount of DNA immobilized in the column becomes greater. On the other hand the compensating competition effect which has the tendency to raise R becomes less when the amount of DNA in the agar is increased as can be seen by the general downward drift in the R values for T5 short halves in Fig. 3*a*. Thus the R values for whole molecules and short halves for a 200 γ T2 column shown in Fig. 3*c* form our best test of this hypothesis. Here the fractionation effect would be maximal and the competition effect minimal. Since the R values for both whole and short half experiments are the same, we conclude that this objection is not a strong one.

Perhaps we have overestimated the sensitivity of the technique because the lead-

ing tubes of the T5 chromatogram preferentially contain only *one* of the two ends of the T5 molecule. Thus the fraction α of sequences that is missing would be greater than $1 - 2X$. This would not change any conclusion in regard to T5 or T2, but it would alter the confidence level in the case of T2. There is evidence that this is not so. The one-end hypothesis would demand that the long halves be missing a large fraction of sequences, which would result in R values slightly less than those seen for short halves in Fig. 3*a*. In fact they appear to scatter with the values obtained from whole molecules and imply that the long halves contain all sequences. This does not clearly rule out the possibility that there is a single preferred breakage point in T5; it merely suggests that if there is, it is not broken with great preference and the resulting left and right ends are not separated cleanly.

It is likely that the physically permuted T2 molecule is a consequence of the same process that results in the circular genetic map of T4 (2). On the basis of genetic studies, Streisinger *et al.* (2, 22, 29) have suggested that the T4 chromosome is made as an extended structure comprising many phage genomes from which a "headful" of DNA is accumulated by the maturation process. This process could result in different phage particles containing somewhat more than a complete genome and would result in a terminally located redundancy as well as each DNA molecule having a different starting point. This view is compatible with the studies of Frankel (24) which demonstrated the presence of DNA molecules in infected cells which were longer than those derived from mature phage. Our finding that the T2 molecule is physically permuted supports these ideas. A genetic analysis of T5 is urgently needed to parallel the more complete mapping experiments that have been done with T2 and T4. From the point of view of consistency, one would expect this phage to have a linear map similar to that of λ. There is evidence that the T5 molecule is arranged inside the head so that it is injected in a preferred order (21, 31). It would be interesting to know whether the same is not true in the case of T2.

The finding that some phage molecules are unique and others permuted leads to questions concerning the origin of permutation and its relationship to terminal redundancy and whether this differing arrangement of nucleotide sequences reflects some fundamental or trivial difference between these bacteriophage.

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