*CHROMOSOME STRUCTURE IN PHAGE T4, III. TERMINAL REDUNDANCY AND LENGTH DETERMINATION**

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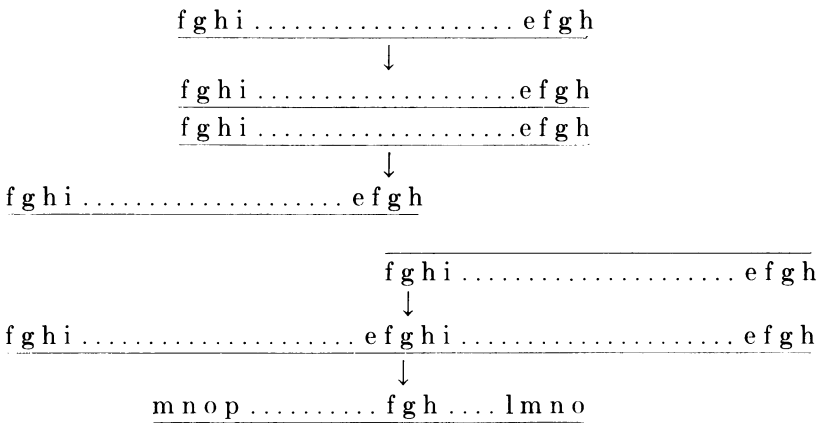
Communicated by A. D. Hershey, December 14, 1966

In previous communications it was suggested that chromosomes of phage T4 are circularly permuted and terminally redundant.[1, 2] Heterozygotes formed by deletion r_{II} mutants and r^+, and by the host range alleles h^{2+} and h^{4+}, were shown to behave as if they were due to this terminal redundancy.[2]

After infection and replication the chromosome of any one phage particle is assumed to become circularly permuted so that the genetic location of the beginning of any one progeny chromosome is randomly distributed over the genome. This could take place through the formation of recombinants by whole chromosomes or by fragments of chromosomes:



It is difficult to imagine, under this model, what factor *intrinsic to the chromosome itself* would determine its total length. We assume, therefore, that the mean length of the chromosome of a mature phage particle is determined by some extrinsic factor, such as, for instance, the amount of DNA that can be contained in a phage head. The mean length of the chromosome would therefore correspond to a "headful" of DNA.

If this notion is correct, we can expect that the length of the chromosome will not be affected by deletion of a part of it. Deletions should be compensated for by a lengthening of the region of terminal redundancy by an amount equal to the length of the deletion. A test of this prediction forms the substance of this communication.

The materials and the methods have been described previously,[2] except for the T4B mutant strains *r*196b, *r*H88, and *r*1272, which were obtained from Dr. S. Benzer.

Results.—The frequency of h^{2+}/h^{4+} heterozygotes among progeny phage carrying deletions: The frequency of heterozygosis for a given marker will depend on the

mean length of the heterozygous region in which that marker can be included. In order to compare the length of the region of terminal redundancy in chromosomes carrying deletions with those carrying point mutations, the crosses rH23 $h^{2+} \times$ rH23 h^{4+} and $r607$ $h^{2+} \times r607$ h^{4+} were performed. The mutant $r607$ is an r_{II} point mutant, whereas rH23 is a deletion covering the entire r_{II} region. The markers h^{2+} and h^{4+} are allelic and can form heterozygotes only within the region of terminal redundancy.[2] As shown by the results presented in Table 1, the frequency of plaques containing both h^{2+} and h^{4+} phage (h^{2+}/h^{4+} plaques) was higher among the progeny of crosses involving a long deletion than among the progeny of crosses involving a point mutation. Clumps which may have contributed to the formation of h^{2+}/h^{4+} plaques[2] were not eliminated in this experiment. The results nevertheless suggest that the deletion of a long section of the genome increases the length of the region of terminal redundancy.

TABLE 1

THE FREQUENCY OF h^{2+}/h^{4+} PLAQUES IN THE PRESENCE OF A DELETION

Cross	Frequency of h^{2+}/h^{4+} progeny	Number of h^{2+}/h^{4+} plaques counted
rH23 $h^{2+} \times$ rH23 h^{4+}	10×10^{-3}	75
$r607$ $h^{2+} \times r607$ h^{4+}	5×10^{-3}	146

Bacteria of strain B were grown to a concentration of 10^8 cells per milliliter in aerated broth at 37°C, and were chilled and concentrated by centrifugation. The bacteria were infected at a concentration of 5×10^8 per milliliter with an average of about five phage particles of each parental type. After 5 min at 37°C they were diluted into broth at 37°C and incubated at that temperature; chloroform was added to the cultures at 35 min after infection. The lysates were plated on B/2 + B/4, and the frequency of clear plaques was determined after overnight incubation. The average number of plaques per plate was less than 70.

It was expected that the frequency of h^{2+}/h^{4+} heterozygotes would depend on the length of the deletion carried by the parental stocks. Three crosses using deletions of various lengths were therefore performed: rH23 $h^{2+} \times$ rH23 h^{4+}, rH88 $h^{2+} \times$ rH88 h^{4+}, and $r168$ $h^{2+} \times r168$ h^{4+}, where rH23 is the longest deletion, rH88 is shorter, and $r168$ is shorter still. In order to eliminate clumps from among the progeny of crosses the progeny phage of each cross were partially sedimented in a deuterium gradient and the phage from the peak drop of the gradient were examined. This procedure eliminates most of the clumps.[2]

Since the frequency of heterozygotes may vary from cross to cross, and since the scoring of h^{2+}/h^{4+} heterozygotes is sensitive to the conditions of plating, two independent sets of the three crosses were performed. The progeny of the three

TABLE 2

THE FREQUENCY OF h^{2+}/h^{4+} PLAQUES IN THE PRESENCE OF DELETIONS OF VARIOUS LENGTHS

Set	Cross	Frequency of h^{2+}/h^{4+} progeny	Number of h^{2+}/h^{4+} plaques counted
1	rH23 $h^{2+} \times$ rH23 h^{4+}	9.0×10^{-3}	99
	rH88 $h^{2+} \times$ rH88 h^{4+}	4.0×10^{-3}	67
	$r168$ $h^{2+} \times r168$ h^{4+}	2.6×10^{-3}	60
2	rH23 $h^{2+} \times$ rH23 h^{4+}	4.1×10^{-3}	26
	rH88 $h^{2+} \times$ rH88 h^{4+}	2.7×10^{-3}	27
	$r168$ $h^{2+} \times r168$ h^{4+}	2.0×10^{-3}	51

Bacteria of strain B were grown to a concentration of 10^8 cells per milliliter in aerated broth at 37°C and were chilled and concentrated by centrifugation. The bacteria were infected at a concentration of 10^9 per milliliter with an average of about five phage particles of each parental type. After 5 min at 37°C they were diluted into broth at 37°C and incubated at that temperature; chloroform was added to the culture 30 min after infection. An aliquot of the progeny of each cross was partially sedimented in a deuterium gradient.[2] The peak drop was plated on B/2 + B/4 and the frequency of clear plaques was determined after overnight incubation. The average number of plaques per plate was less than 30.

crosses of any one set were plated and scored at the same time. As shown in Table 2, the frequency of h^{2+}/h^{4+} heterozygotes was greatest among the progeny of the cross with the longest deletion and decreased as the length of the deletion decreased.

The frequency of r and r⁺ markers among h^{2+}/h^{4+} heterozygous progeny of $r^+h^{2+} \times rh^{4+}$ crosses: Progeny of crosses of $r^+h^{2+} \times rh^{4+}$ will contain h^{2+}/h^{4+} heterozygotes. Some of these will be r, others will be r^+, and some may be r/r^+ heterozygotes. One would expect the frequencies of the r and r^+ types to be equal among the h^{2+}/h^{4+} heterozygotes when the r genotype is a point mutation or a short deletion. When it is a long deletion, more of the h^{2+}/h^{4+} heterozygotes should be r than r^+, owing to the longer terminal redundancy of the r chromosomes. The results presented in Table 3 indicate that progeny of crosses involving long deletions did indeed contain more rh^{2+}/h^{4+} heterozygotes than r^+h^{2+}/h^{4+} heterozygotes.

TABLE 3

THE EFFECT OF DELETIONS ON THE FREQUENCY OF r TYPES AMONG h^{2+}/h^{4+} PROGENY

Cross	Frequency of h^{2+}/h^{4+} plaques	Number of h^{2+}/h^{4+} plaques tested	Fraction r	Fraction r^+	Fraction mixed	Ratio $r:r^+$
		(*a*) Unfractionated Progeny				
rH23 $h^{4+} \times r^+h^{2+}$	11.0×10^{-3}	173	0.48	0.17	0.35	2.8
rH23 $h^{2+} \times r^+h^{4+}$	7.5×10^{-3}	70	0.72	0.09	0.20	8.3
r1272 $h^{4+} \times r^+h^{2+}$	13.0×10^{-3}	119	0.45	0.14	0.40	3.2
r196b $h^{4+} \times r^+h^{2+}$	7.0×10^{-3}	82	0.33	0.18	0.49	1.8
r607 $h^{4+} \times r^+h^{2+}$	6.0×10^{-3}	87	0.30	0.25	0.45	1.2
r607 $h^{2+} \times r^+h^{4+}$	3.4×10^{-3}	96	0.31	0.42	0.27	0.8
		(*b*) Peak Fraction of Partially Sedimented Progeny				
r1272 $h^{4+} \times r^+h^{2+}$	5.1×10^{-3}	206	0.81	0.16	0.03	4.9
r168 $h^{4+} \times r^+h^{2+}$	2.2×10^{-3}	76	0.39	0.52	0.09	0.8
r168 $h^{4+} \times r^+h^{2+}$	2.0×10^{-3}	90	0.48	0.45	0.07	1.1

The crosses and platings were performed as described for Tables 1 and 2; the average number of plaques per plate was less than 35.

The progeny of the crosses in part (*b*) were partially sedimented in sucrose gradient[3] or a deuterium gradient[2] and the peak drop was plated.

rH23 and r1272 are long deletions covering all of the r_{II} region; r196b and r168 are very short deletions and r607 is a point mutation.

The h^{2+}/h^{4+} plaques that contained only r or only r^+ phage were probably formed by heterozygotes rather than by clumps, since most h^{2+}/h^{4+} plaques formed by clumps of phage would be expected to contain both r and r^+ phage. A fraction of the h^{2+}/h^{4+} plaques (Table 3A) *did* contain both r and r^+ phage and this fraction probably originated from clumps.

In order to eliminate clumps, the phage from the progeny of three crosses were partially sedimented in a deuterium (or else a sucrose) gradient, and phage from the peak drop of the gradient were plated. Since only a small fraction of the h^{2+}/h^{4+} plaques from the peak drop contained both r and r^+ phage, most of the clumps were in fact eliminated. As shown in Table 3B, the progeny of crosses involving long deletions again contained more rh^{2+}/h^{4+} heterozygotes than r^+h^{2+}/h^{4+} heterozygotes, whereas control crosses involving point r mutants contained approximately equal frequencies of the two types.

In the crosses described in Table 3 it is noteworthy that the total frequency of h^{2+}/h^{4+} heterozygotes was greater in crosses involving long deletions than in crosses involving point mutants, confirming the results presented in Tables 1 and 2.

Discussion.—The results presented here confirm a rather bizarre prediction of the terminal redundancy model of the chromosome of phage T4. We have been

unable to devise any other model that would incorporate this feature and the others that have been presented in previous papers.[1, 2]

The essential features of the model suggested by our experiments are: (1) The chromosome of mature phage particles is linear, with a terminal redundancy. (2) The chromosomes of progeny phage represent circular permutations of the chromosome of the parent. (3) A mature phage particle contains, on the average, a "headful" of DNA.

The model of the T4 chromosome that has been presented here has a number of implications concerning the mechanism of recombination in phage T4. These implications will be discussed in a subsequent communication.

Summary.—Crosses of phage carrying long deletions yield a greater frequency of terminal redundancy heterozygotes than crosses of phage carrying short deletions or point mutations. This suggests that each particle of phage T4 contains, on the average, a "headful" of DNA.

The experiment reported in this communication was conceived during the course of a conversation with Dr. M. Fox. We are grateful to Dr. F. W. Stahl for many helpful discussions.

* This investigation was supported by research grants from the National Science Foundation (G14055 and 466-GB-2261) and from the National Institute of Allergy and Infectious Diseases (E3892).

[1] Streisinger, G., R. S. Edgar, and G. H. Denhardt, these PROCEEDINGS, **51**, 775 (1964).

[2] Séchaud, J., G. Streisinger, J. Emrich, J. Newton, H. Lanford, H. Reinhold, and M. M. Stahl, these PROCEEDINGS, **54**, 1333 (1965).

[3] A continuous gradient of sucrose was produced in a centrifuge tube by mixing continuously increasing amounts of broth containing 0.5% sucrose with continuously decreasing amounts of broth containing 20% sucrose. Partial sedimentation in a sucrose gradient could not be performed reproducibly in subsequent experiments and further sedimentations were therefore performed with a deuterium gradient.