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Late Events in T4 Bacteriophage Production

II. Giant Bacteriophages Contain Concatemers Generated by Recombination

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Analysis of "giant" phage, which package concatenated DNA into their capsids, shows that they are predominantly heterozygous. The results are compatible with the hypothesis that concatemers are generated by recombination.

The "headful" hypothesis suggested that a population of circularly permuted T4 DNA molecules could be produced by cutting a concatemer into pieces slightly longer than a full complement of phage genes (6). However, the mechanism which produces the DNA concatemers for phage maturation has remained in question. One hypothesis has been that concatemers are formed by recombination (5).

T4 DNA recombination begins with the introduction of single-stranded nicks into replicating DNA (3). The resulting double-stranded fragments mix and reassociate to make larger molecules by annealing homologous regions in their single-stranded ends (3). The nicks and gaps at the junctures of these joined fragments are repaired, restoring the integrity of the polynucleotide chain (4). A computer simulation has indicated that such a process could generate DNA chains longer than one phage equivalent. Moreover, concatemer formation and recombination can be inhibited by the addition of chloramphenicol to T4-infected cells at a time when replication is not inhibited (5).

If phage mature from bacteria simultaneously infected with two genetically distinguishable parents, it is quite likely that consecutive genomes of any one concatemer might bear markers from one or the other coinfecting mutants, as well as double mutants and wild-type recombinants, if recombination were responsible for generating the concatemer (see Fig. 1A). This possibility can be experimentally approached by utilizing a mutation in gene 23 which leads to the formation of "giant" phage (1). These phage exhibit elongated heads within which several genome lengths of DNA are packaged in concatenated form. Giants offer a unique system for examining the mechanism of concatemer formation.

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MATERIALS AND METHODS

Phage strains. T4 D *am*B17 (gene 23) and T4 D *am*E355 (gene 24) both have a common mutation in gene 23 (4) and were kindly provided by A. H. Doerman.

Bacterial strains. *Escherichia coli* strains B23 and CR63 were used in this study.

Media and growth conditions. Basic media and ^{32}P-labeling conditions have been described previously (9, 10). All experiments were performed at 37 C and at a cell density of 3×10^8/ml.

Phage purification. Infected cells were lysed with $CHCl_3$, and the lysate was incubated with DNase for 30 min at 37 C. Cellular debris was removed by sedimentation of the lysate at 5,000 rpm for 10 min. The supernatant was then sedimented twice at 17,000 rpm in a Beckman type 40 fixed-angle rotor with a final low-speed sedimentation. Complete and giant phages were separated by sedimentation of the purified phage and suspended in 0.01 M Tris (pH 7.4)-0.15 M NaCl in a sucrose density gradient. A 0.2-ml sample of the phage was layered on top of a 12 to 40% linear sucrose gradient prepared in 0.10 M NaCl-1.0 M $MgCl_2$-0.032 M PO_4 buffer (pH 5.2). Gradients were centrifuged at 19,500 rpm for 20 min at 20 C in a Beckman SW41 Ti rotor and fractionated by siphoning from 0.5 cm above the bottom of the tube using capillary tubing and a peristaltic pump.

RESULTS

The T4 phage used in this experiment bear a mutation in gene 23, *ptg*191, which leads to the formation of roughly three classes of phage (1): (i) "complete" phage containing a normal size genome, (ii) "petite" phage possessing a shorter head and shorter genome, incapable of producing progeny phage, and (iii) "giant" phage, possessing long heads, varying considerably in size, and containing from two to four genome

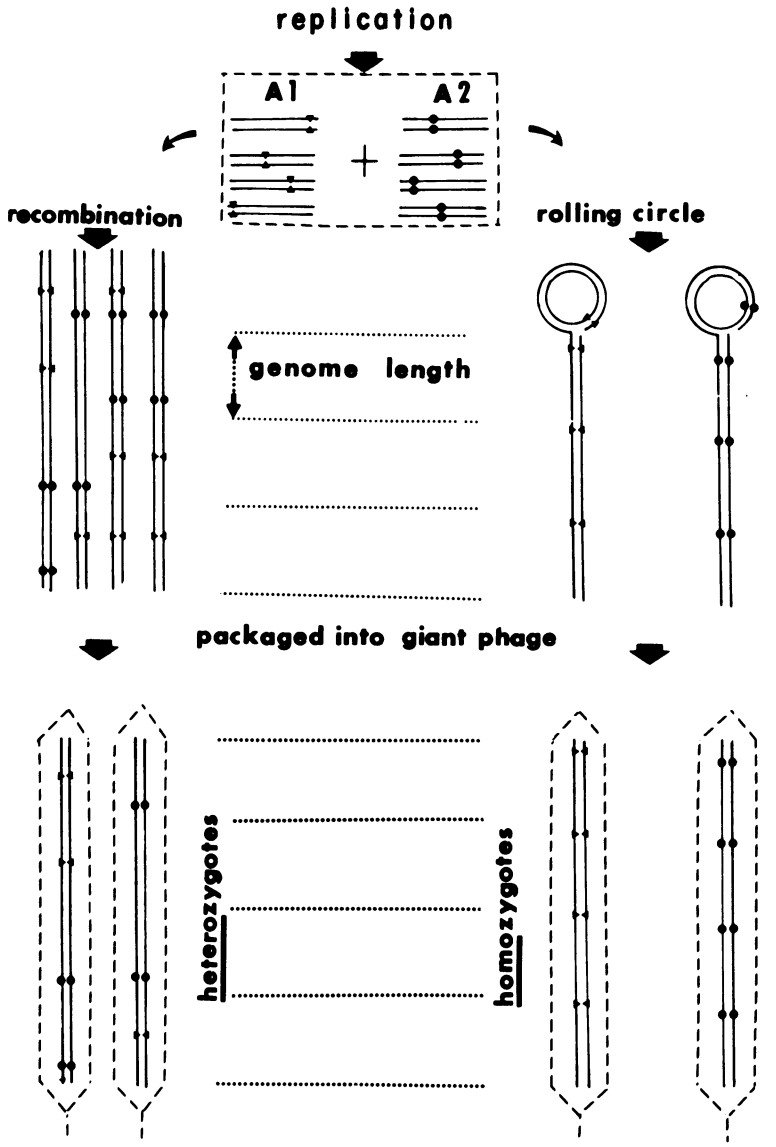

FIG. 1. *Two means of forming concatemers from the replicative pool of phage DNA in bacteria simultaneously infected with two genetically distinct parents, each containing an amber mutation in one of two genes, A1 or A2. Linear DNA molecules in the pool of replicating DNA can circularize and begin to replicate as rolling circles generating concatemeric tails (B), or the pool of replicating DNA can recombine to form concatemers (A). If concatemers produced by both models are immediately (that is, the concatemeric tails of the rolling circles molecules are not fragmented and reassembled by recombination before encapsidation) used for maturation of phage, the two models predict different results for the genetic character of giant phage produced. Concatemers produced by recombination (A) giant phage are likely to be heterozygous for genes A1 and/or A2, whereas concatemers produced by model B should be homozygous for both genes.*

lengths of DNA. Giant phage can easily be recognized in the electron microscope; they are resistant to UV irradiation and can be separated in a sucrose gradient well away from complete and petite phage (1).

In the experiment described below *E. coli* B23, grown in Tris-Casamino Acids-glucose medium containing $^{32}PO_4$, was infected with two different amber mutants, *am*B17 (gene 23) and *am*E355 (gene 24), bearing in common the *ptg*191 mutation in gene 23, each at a multiplicity of infection of 3. At 2 h after infection the

cells were lysed, and the total progeny phage were purified as described above. Sucrose gradient fractions corresponding to complete phage and giant phage were isolated, and the titer was measured by plating on CR63. Complete phage revealed 0.8 plating units for each phage equivalent (PE) of ^{32}P-labeled DNA. If it is assumed that giant particles have the same fraction of particles producing plaques, then the 0.2 plating units for each PE of ^{32}P-labeled DNA found for the pooled giant phage indicates that, on the average, each giant particle contained DNA four phage equivalents in length.

The genotypes of the purified complete and giant phage, with respect to genes 23 and 24, were analyzed in the laboratory of A. H. Doermann. As a control the UV inactivation of both phage preparations was determined. The results (Fig. 2) indicate that at least 85% of the giant phage particles are polygenomic (1).

To determine whether either phage preparation contained particles which were heterozygous for gene 23 (*am*B17) and/or gene 24 (*am*E355), a sample of each preparation was plated on CR63. Fifty clearly separated plaques were cut out and resuspended in broth. (In some ways these plaques are equivalent to 50 single-infection bursts of the original phage preparation.) Each resuspended plaque was plated on CR63 at a dilution assuring well-isolated plaques. Ten "progeny" plaques from each of the fifty resuspended plaques were tested by replica plating to determine whether each was *am*E355 or *am*E355+.

Thus each of the original 50 phage is classified, on the basis of 10 progeny phage, as to whether it was homozygous or heterozygous at one, the other, or both loci. For example, if all 10 progeny phage carried the *am*B17 marker then the parent phage would be considered homozygous for that locus. Otherwise, if some progeny phage were *am*B17 and others were *am*B17+, then the parent phage would be considered heterozygous for that locus. Sixteen giants and 49 completes were homozygous at both loci; 23 giants and 1 complete were heterozygous at both loci; 5 giants and no completes were homozygous in gene 23 but heterozygous in gene 24; 6 giants and no completes were homozygous in gene 24 but heterozygous in gene 23.

The data can be summarized by saying that in the sample of giant phage the majority (34 out of 50) was heterozygous, whereas in the sample of complete phage only 1 out of 50 was heterozygous. Most of the giant phages, being heterozygous, must have matured using DNA concatemers in which the process of recombination intervened between replication and encapsidation. The possible origin of the minority of phage which were homozygous will be elaborated below.

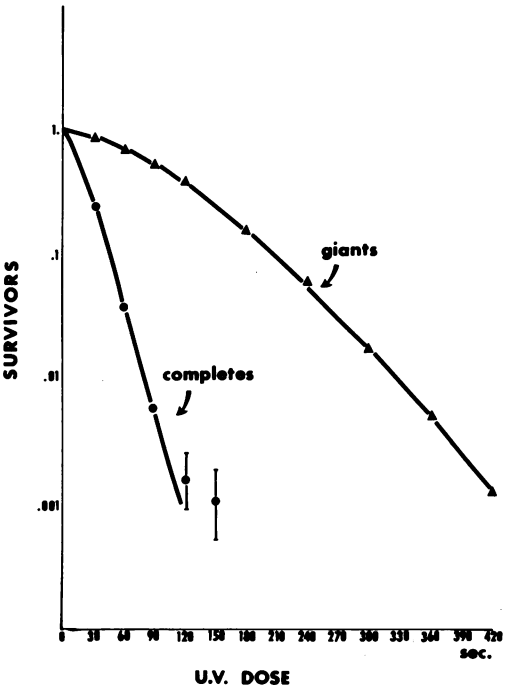

FIG. 2. *UV irradiation of complete and giant phages. The graph shows a semilog plot of the fraction of surviving phages versus the duration of irradiation in seconds.*

DISCUSSION

Recombination of T4 DNA proceeds by a process of breakage and rejoining (3, 4). Miller et al. suggested that recombination could form longer molecules from DNA fragments and showed that chloramphenicol can inhibit the formation of concatemers when added to T4-infected cells at a time that inhibits recombination but allows DNA synthesis to continue (5).

The experiment documented here shows that the majority of giant phage, resulting from a cross of two genetically distinct parent phage, was heterozygous. This result is compatible with the hypothesis that recombination generated the concatemers prior to their encapsultation.

Another mechanism that could produce concatemers is the repeated replication of a circular template (2). Although this experiment cannot rule out the possibility of rolling circle replication, the results eliminate the possibility that a concatemer could be elongated at one end by replication of a circular template while being packaged at the other end into phage heads,

without recombination intervening between the two processes (Fig. 1B). Such a mechanism would produce homozygous concatemers even if recombination preceded the formation of the circular template.

In this experiment 30% of the giant phage were homozygous. One might conclude that some concatemers could have been generated by the model of rolling circle replication mentioned above. However, other causes could account for the observed homozygous giant phage.

The survival curve of the UV-irradiated giant phage assured that at least 85% of the phage were polygenomic. The interpretation of the curve beyond this limit becomes difficult, and the possibility that about 10% of the giant phage preparation represent aggregated complete phage cannot be excluded. Of course, aggregated complete phage would be predominantly homozygous.

In addition, the size distribution of DNA extracted from giant phages ranges from slightly larger than 1 PE in length up to four times that size. Phages endowed with shorter concatenated DNA have a smaller chance of being heterozygous for any pairs of genes. For example, if recombination does not occur in the region between the two genes (if the genes are so closely linked that recombination between them is unlikely), a concatemer 2 PE in length has a 50% chance of being heterozygous (2 out of 4 possible permutations), whereas a concatemer 4 PE in length has an 88% chance (14 out of 16) of being heterozygous. If recombination does occur in the region between the two genes, then a concatemer 2 PE in length has a 75% chance (12 out of 16) of being heterozygous, whereas a concatemer 4 PE in length has more than a 98% chance (252 out of 256) of being heterozygous.

Lastly if all of the progeny genomes are randomly mixed within the cell, the probability of recombination for any pair of molecules is the same if the pair are replicas of the same parental template or if they are derived from two different parental molecules. However, if the replicating pool of DNA is not randomly mixed but centers geometrically around the original parental DNA, the probability of recombination between two genetically different DNA molecules increases toward the periphery of the pool of replicating DNA and decreases toward its core. One can visualize pools of replicative DNA partially overlapping at the peripheries of the pools, where genetic recombination leading to the formation of heterozygotes occurs with the greatest probability. Although our experiments cannot prove or disprove this last possibility, future experiments utilizing giant phages could be designed to directly attack that possibility. Any one of these three possibilities, or any combination of them, could easily account for a minimum of homozygous phages in the sucrose fraction isolated as giant phages; it is not necessary to invoke a second mechanism for concatemer formation.

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