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Method for the Isolation of Bacteriophage T4 Mutants That Produce Particles with Giant Capsids

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An efficient procedure is described for isolating bacteriophage T4 mutants that produce particles with giant capsids. The selective parameters of the technique rely primarily on the giants' characteristic density in cesium gradients and on their resistance to UV radiation.

Giant T4 phage, which are characterized by elongated capsids and concatenated multiple genomes, have been useful for investigations into capsid structure and the nature of capsid length control (1). Such phage could be of further use as an experimental probe if significant numbers of different mutants that produce them could be isolated. For example, using a genetic approach, such mutants could be used to identify, first, those genes to which the giant phenotype is restricted and, second, whether such mutations are limited to certain areas within those genes (clustering).

Two methods have thus far been used for obtaining mutants that produce giant phage. Doermann et al. found some giant-producing mutants among those selected for production of petite T4 particles (4). Bijlenga et al. (in press) found others by growing certain temperature-sensitive *(ts)* mutants of gene 24 at semipermissive temperatures. These methods, though effective, have limitations when considering the above-mentioned uses of giant-producing mutants, because there is no a priori reason why giant producers should be restricted to mutations that also yield petite particles and/or to gene 24 *ts* mutants. The following method, by selecting on the basis of the giant phenotype, should provide a way to obtain mutants different from those already isolated.

Phage stocks to be mutagenized were grown with aeration on *Escherichia coli* B, infecting separate cultures at 37 C with single plaques of T4D*os* (4). These stocks, each originating from a different phage particle, were subjected to the entire procedure separately, thus assuring that the mutations obtained from the individually treated stocks will have arisen from independent mutational events. The phage stocks were mutagenized as follows. Using H-broth (2) supplemented with 20 μg of adenine per ml, *E. coli* B was grown to a density of 3×10^8 cells/ml. After chilling, the bacteria were pelleted and resuspended at a concentration of 1.5×10^8 cells/ml in M9+ medium (4) supplemented with 20 μg of uracil, 20 μg of adenine, and 200 μg of 5-bromodeoxyuridine per ml. A 30-ml amount of the bacterial suspension was distributed to separate tubes, and 2×10^9 phage particles from the T4D*os* stocks were added. The cultures were aerated at 37 C until clearing occurred, and 1 ml of $CHCl_3$ was added to each of the lysates. All work with the bromodeoxyuridine-mutagenized stocks was done in yellow light.

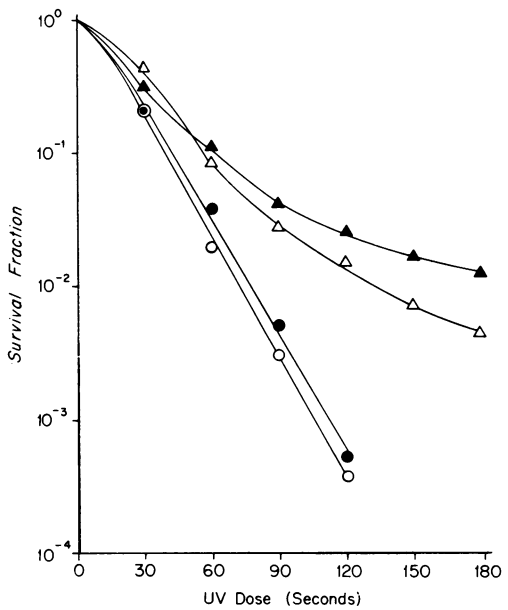

FIG. 1. *UV survival curves for samples from two of the four cesium formate gradients. Samples from the other two gradients gave similar results. Combined high-density (hd) fractions and normal-density (nd) control samples are represented as:* △ = *hd,* ○ = *nd for one gradient;* ▲ = *hd,* ● = *nd for the other gradient. The high survival shown in both hd curves is diagnostic for the presence of giant T4 particles.*

Under usual conditions of phage stock preparation, the terminal round of phage multiplication involves high multiplicity of infection (MOI). Because mutations yielding giant phage are expected to be infrequent, they will invariably be outnumbered in multiple-infected cells, and it was anticipated that the giant phenotype would rarely be expressed due to phenotypic mixing (5). To avoid such losses of giant particles and to assure homozygosity among the several genomes encapsidated in giant particles, subcultures from the mutagenized stocks were prepared in which the multiplicity of infection was 0.5 phage/cell. *E. coli* B cultures were grown at 37 C in 1,000 ml of M9+ to a density of 4×10^8 cells/ml and 2×10^{11} mutagenized phages were then added to each culture. Seven minutes after infection, 2×10^{12} T1 phage particles were added to each culture together with Ca^{2+} at a concentration of 10^{-3} M. The T1, with its short latent period, eliminates the bacteria not infected with T4 but has no noticeable effect on the T4-infected cells. This step effectively prevents a second round of T4 infection and consequent giant losses due to phenotypic mixing. Seven minutes after T1 was added, 1.2×10^{12} T4D*am*B17-*am*E355 phage particles were added to each culture. The superinfection with T4 effects lysis inhibition (3) of the initially T4-infected bacteria. Finally, 90 min after the original infection, the cultures, not yet undergoing significant lysis, were chilled and centrifuged. The pellets were resuspended in 25 ml of M9+ medium lacking glucose and Casamino Acids, and lysis was induced by shaking with 2 ml of $CHCl_3$. The lysate was then incubated for 30 min at 37 C with 20 μg of DNase per ml and supplementary Mg^{2+} at 10^{-3} M. Thereafter it was centrifuged at low speed for 10 min to sediment the larger debris.

Ten milliliters of a cesium formate stock solution (53 g of cesium formate, 48 ml of D_2O, 3.2 ml of Tris buffer [2 M, pH 7.6], 0.32 ml of $MgCl_2$ [1 M]) was mixed with 2.5 ml of each low MOI stock. After incubation at 45 C for 1 h, the mixtures were placed in an ice bath for 30 min. The precipitate that formed was sedimented by centrifugation, and the supernatants were spun in the SB-283 rotor of an IEC B-60 ultracentri-

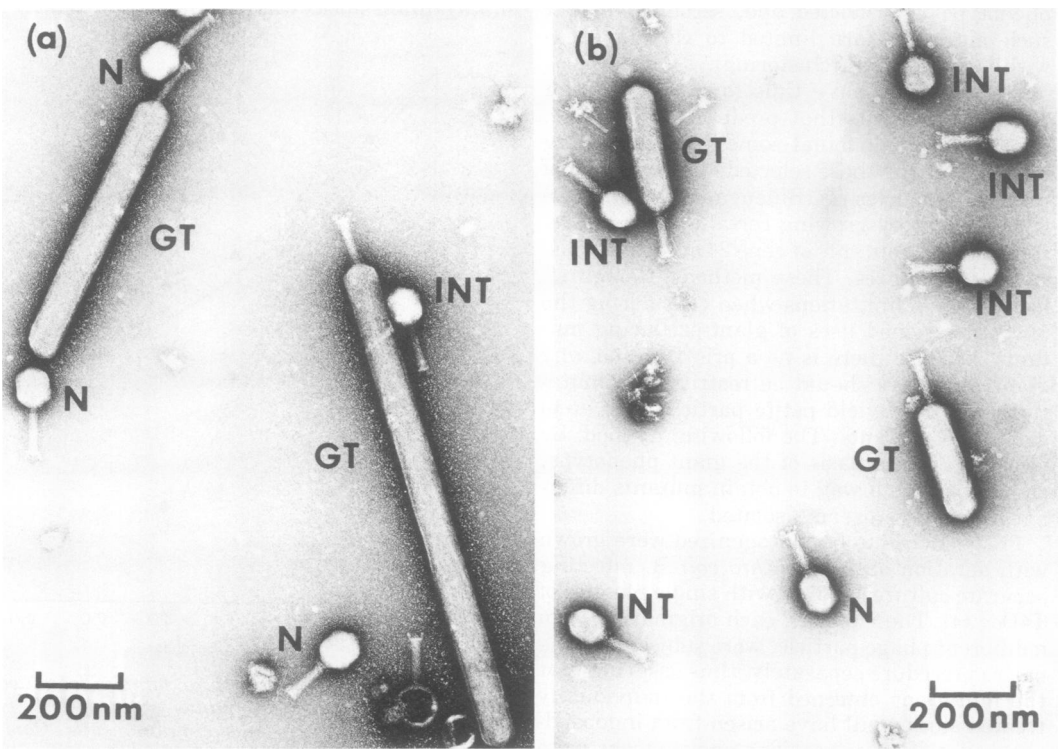

FIG. 2. *Electron micrographs of phage particles from two mutants (a and b) isolated from a single gradient using the described procedure. Capsid size classification follows that of Doermann et al. (4): INT, intermediate petite; N, normal; GT, giant. The phenotypes of the two mutants differ in the average lengths of the giant particles produced. Note the typically long giants in (a) in contrast to the shorter ones which characterize (b).*

fuge at 30,000 rpm for 60 h. Eight-drop fractions were collected by puncturing the bottom of the centrifuge tubes. (Inspection of the gradients at 12 to 24 h may reveal that the phages are banding too low in the tubes as a result of excessive loss of salts during the preliminary centrifugation. Additional cesium formate may have to be introduced to alter the position of the main band.)

Although only one band was seen in each of the gradients, plaque-former assays of the fractions revealed the presence of some phage with higher than normal density. The fractions containing this phage were combined, and a UV survival test was run using phage from the major band as a control. The conditions used were similar to those used by Doermann et al., and the shapes of the survival curves for combined high-density fractions (Fig. 1) are similar to the curves they found with mixtures of giant and normal phages (4). The frequencies of giants appeared to be in the range of 2 to 10% of the total plaque-former phages present in the combined high-density fractions. Because giant particles have greater UV resistance than normal phage, UV exposure of the high-density samples is expected to increase greatly the proportion of giants among the surviving plaque-formers. Platings of the low MOI stocks were made on *E. coli* B/1,5, on which neither the T1 nor the double-amber phage used in the original lysate preparation can multiply.

All giant-producing mutants found previously make smaller than normal plaques. Small plaques were therefore selected and subcultured from platings of the high-density fractions that had been exposed to UV for 180 s. Samples from each subculture were negatively stained with uranyl acetate (4) and examined in the electron microscope. The majority of isolates from each of the four stocks run through the described procedure proved to be giant-producing mutants. Figure 2 shows particles from two of those mutants. Numerous other mutants showing a wide range of phenotypic variation were isolated but have not yet been studied in detail.

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