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Suppression of DNA Arrest Mutants in Bacteriophage T4

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A mutation in gene 49 of phage T4 was not able to restore DNA synthesis in a gene 46 mutant.

Mutants in genes 46 or 47 of phage T4 which belong to the class of DNA arrest (DA) mutants stop DNA synthesis when the DNA is prematurely dissociated from a fast-sedimenting, membrane-containing DNA complex (10, 14, 15). This phenotype can be suppressed by secondary mutations in either gene 55 or 33. As a consequence, a double mutant in genes 46 and 55 (or 46 and 33) shows normal DNA synthesis, and no premature release of the DNA from the fast-sedimenting complex can be observed (10, 14, 15). Genes 55 and 33 have regulatory functions during the development of phage T4. Mutations in any of these genes are maturation defective (MD) and do not allow the expression of late functions (2, 5). In MD mutants, the rate of DNA synthesis is normal. The release of the newly synthesized DNA from the fast-sedimenting complex, however, is blocked, and no maturation of the DNA can be detected (3, 4, 6).

It is possible that the observed suppression of the DA mutants in gene 46 or 47 is not directly caused by mutations in gene 55 or 33. The true reason could be the concomitant loss of one (or more) late function(s) (7).

Gene 49 represents a late function of T4 that in the defective form does not allow the production of viable phage, although normal DNA synthesis occurs. The analysis of the replication products shows that the DNA is permanently bound to a fast-sedimenting complex (4). In this respect, the phenotype of gene 49 mutants is very similar to the phenotype of mutants in genes 55 and 33 (4, 6). Although it has not yet been proven that the fast-sedimenting DNAs are identical in gene 55 and 49 mutants, we wanted to investigate the influence of a mutation in gene 49 on the DA phenotype of a mutation in gene 46. The results showed that the gene 55 mutation in a gene 55-46 double mutant could not be replaced by a mutation in gene 49 without losing the suppression effect.

In Fig. 1, thymidine incorporation of T4-infected cells is plotted versus time after infection. In agreement with others (10, 14, 15), we found that the DNA synthesis of a gene 46 mutant was stopped by 25 min after infection, whereas wild-type phage, a gene 49 mutant, and a gene 55 mutant continued the incorporation of labeled thymidine until at least 60 min. When the mutation in gene 46 was combined with the mutation in gene 55 to give a double mutant, the DNA synthesis did not prematurely stop but continued beyond the point where the gene 46 mutant normally turns off its DNA synthesis (Fig. 1b). However, the DNA synthesis of a double mutant in genes 46 and 49 was not significantly altered when compared with the gene 46 single mutant.

To investigate the fate of the newly synthesized DNA, the cells infected with the different T4 mutant strains were opened at different times after infection by the lysozyme-Brij 58 technique (8). Samples of the lysates were analyzed on neutral sucrose gradients as described by Shah and Berger (14) (Fig. 2).

At 30 min after infection, a DNA structure that sedimented faster than the 1,000*S* marker phage developed in cells infected with a gene 49 or a gene 55 mutant (Fig. 2a). In contrast, the newly synthesized DNA in T4 wild-type-in-infected cells at 30 min after infection appeared in two positions. One of them was characteristic for mature phage, and the second peak marked the position of approximately 200*S* (Fig. 2a).

Figure 2b shows the sedimentation behavior of newly synthesized DNA in cells infected with fected cells at 30 min after infection appeared most of the DNA was in the fast-sedimenting complex. At 20 and 30 min, however, these complexes disappeared and the DNA accumulated at the top of the gradient. The situation was very different in gene 46-55 double-mutant-infected cells (Fig. 2c). The majority of the DNA remained in the fast-sedimenting complex, and at 30 min after infection 38% of the label appeared outside in the 200*S* position, although no production of viable phage could be

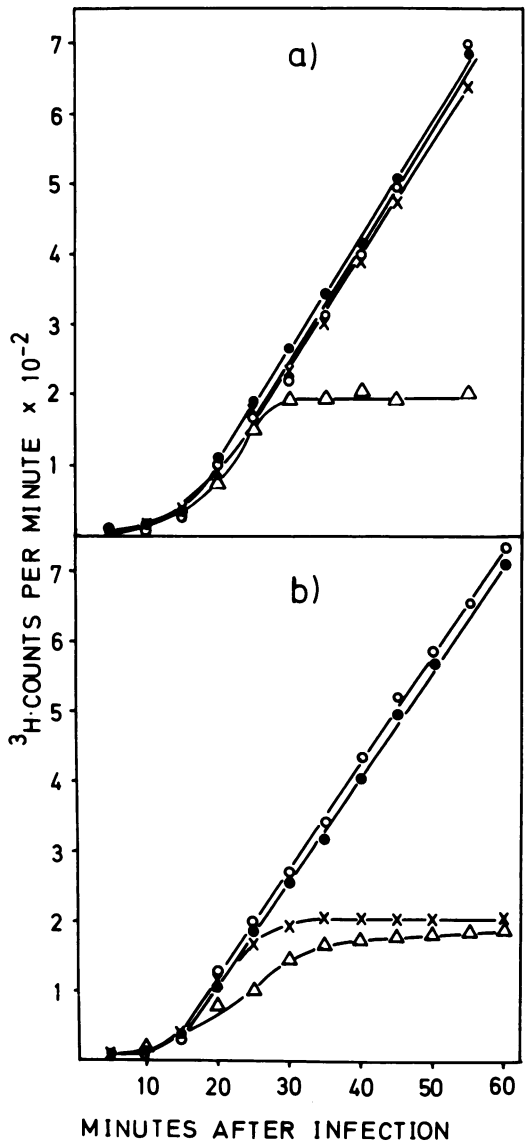

FIG. 1. *Incorporation of [^{3}H]thymidine into trichloroacetic acid-insoluble material in T4-infected cells. E. coli B23 (nonpermissive host for T4 amber mutants) was grown at 37 C in a minimal glucose-containing medium. When the cell growth reached the log phase, 4 × 10^8 cells were harvested by centrifugation and resuspended in 1 ml of phosphate buffer. The cells were starved by incubation for 15 min at 37 C before 20 μg of L-tryptophan per ml (final concentration) and 5 phage per cell were added. Adsorption was allowed for 5 min at 37 C, and nonadsorbed phage were removed by centrifugation. (Under these conditions, more than 98% of the cells were infected as checked by counting viable cells before and after the addition of phage.) The phage-infected cells were resuspended in 10 ml of 37 C prewarmed minimal medium containing 200 μg of D-adenosine per ml. This moment was considered to be the start of the experiment and represents "0 min after infection". Four minutes later, 1 μCi of [methyl-^{3}H]-thymidine (specific activity, 21 Ci/mmol; Amersham) per ml was added. At the times indicated, 50-μliter samples were removed and immediately placed into 5 ml of 5% ice-cold trichloroacetic acid. Trichloroacetic acid-insoluble material was collected on glass fiber filters (GF/C, Whatman), washed twice with 1% trichloroacetic acid, dried, and counted in a toluene-based scintillation fluid in a Packard scintillation counter. T4 wild-type and T4 mutant strains were kindly provided by W. B. Wood. Double mutants were constructed by standard phage crosses and tested as described by Hercules and Wiberg (9). To exclude the possibility that the gene 49 mutation suffered from some uncontrolled change during the construction procedure of the gene 46-49 double mutant, the original mutations were reisolated from the double-mutant strain. Their phenotypes were tested with respect to DNA synthesis as described above, and the production of fast-sedimenting DNA complexes was investigated as described in the legend of Fig. 2. No difference between the original and the reisolated mutants could be detected. Symbols for (a): T4$^+$ (wild type; ●), T4amBL292 (gene 55; ○), T4amE727 (gene 49; ×), and T4amB14 (gene 46; △). Symbols for (b): reisolated T4amE727 (gene 49; ●), T4amB14 + T4amBL292 (gene 46 + gene 55; ○), reisolated T4amB14 (gene 46; ×), and T4amB14 + T4amE727 (gene 46 + gene 49; △).*

detected. When the same experiment was done with a gene 46–49 double mutant, the release of the DNA from the fast-sedimenting complex was not prevented, although the degradation seemed less extensive than in cells infected with mutant phage in gene 46 alone. This possibly reflects the lack of a nuclease(s) in gene 49 mutant phage (7).

The suppression of DA mutants in gene 46 or 47 by the addition of MD mutants in genes 55 and 33 may be explained in two different ways. The first explanation is based on the assumption that the product(s) of genes 46 and 47 is involved in DNA synthesis by protecting the DNA from being degraded. Most likely, nucleases are responsible for this degradation process. If they are late functions, the elimination of such nuclease(s) could be achieved by MD mutants, and it would make the gene 46–47 product(s) dispensable for replication. Evidence has been accumulated that genes 46 and 47 themselves are responsible for the production of a nuclease(s) (see for discussion ref. 13). The situation would then be comparable with the *recB/C* system in *Escherichia coli*, where the lack of the *recB/C*-controlled nuclease can be

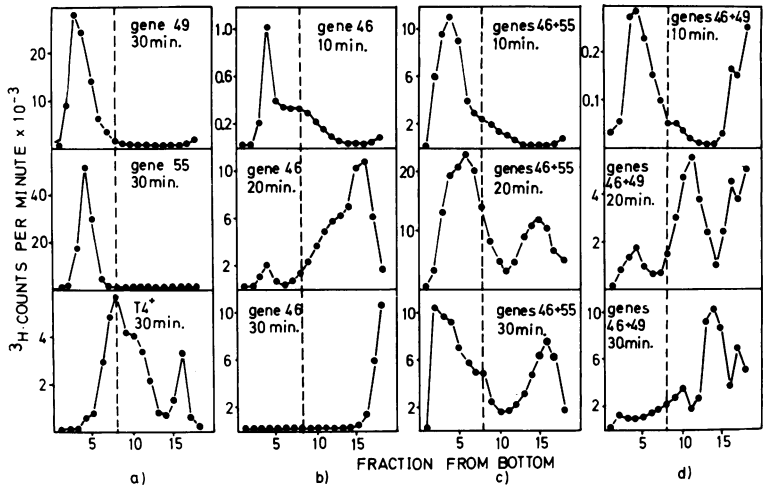

FIG. 2. *Analysis of newly synthesized DNA in T4-infected cells. E. coli B23 was infected with various T4 strains as described in the legend of Fig. 1. At the time indicated in each graph, a 1-ml sample was removed from the culture and placed in an Erlenmeyer flask on ice. Two minutes later, 1 ml of lysis mix (2 mg of lysozyme per ml, 5 x 10^{-2} M EDTA, pH 8, 0.1 M KCN in 10^{-2} M Tris, pH 8) was added, and the incubation continued for 15 min on ice. After this time, Brij 58 was added to give a final concentration of 1%. Five minutes later, when the lysate had cleared up, 0.2 or 0.5 ml was put on top of a 4.5 or 4.8 5 to 20% sucrose gradient (sucrose dissolved in 0.05 M Tris, pH 8.0, 0.05 M EDTA, and 0.05 M NaCl) underlaid with 0.2 ml of CsCl solution (1.8 g/ml). Centrifugations were performed in a Spinco ultracentrifuge at 20 C for 20 min at 18,000 rpm in an SW50.1 rotor as described by Shah and Berger (14). Gradient fractions (0.2 ml) were collected into ice-cold 5% trichloroacetic acid, and the trichloroacetic acid-insoluble material was determined as described in the legend of Fig. 1. The recovery of radioactivity was between 80 and 95%. The dashed line indicates the position of whole phage (1,000S) which were run as a control for each experiment in a parallel tube. The mutants are indicated by the appropriate gene number in each panel. For their further specification, see the legend of Fig. 1.*

suppressed by the elimination of another nuclease, exonuclease I (sbcB phenotype [12]).

The work of Frankel et al. (7) on the characterization of the function of gene 49 suggests that this gene controls nucleolytic activity. If that is true, it cannot be a nuclease which is necessarily involved in the release of the DNA from the fast-sedimenting complex, because it was shown in the present communication that a mutation in gene 49 was not able to prevent the DNA degradation that appeared in a gene 46 mutant. In addition, two more T4-induced, late-controlled nucleases have been recently described (1, 11). Unfortunately, no mutants of these nucleases are available at present, and further studies are necessary before their influence on the DA phenotype can be investigated.

The second explanation follows the suggestion of Shalitin and Naot (15), who consider the function(s) of genes 46 and 47 to be essential for DNA synthesis. In suppressor mutants, however, these functions can be carried out by some other still-unknown gene product(s). MD mutants in genes 55 and 33 have been shown to be unable to turn off early genes at the regular time, which results in an overproduction of early gene products. The substituting function(s) could then belong to this class of early genes. If this is true, mutants can be predicted which do not allow a gene 46 mutation to be suppressed by MD mutants. Such mutants are presently being looked for in our laboratory.

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