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Effect of a Gene-Specific Suppressor Mutation (*das*) on DNA Synthesis of Gene 46-47 Mutants of Bacteriophage T4D[1]

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Mutants in genes 46 and 47 of bacteriophage T4 exhibit early cessation of DNA synthesis, inability to form a normal rapidly sedimenting DNA intermediate (200*S*), reduced genetic recombination, and reduced viable phage production. A gene-specific suppressor mutation called *das* partially restores many of the pleiotropic effects of gene 46-47 mutants (13). Our results indicate that this partial suppression by *das* is associated with (i) the synthesis of a small fraction of DNA containing long single chains not detectable in 46-47 infection and (ii) a decrease in an "early" function which participates in the degradation of DNA synthesized in the absence of 46-47 functions. However, *das* does not restore the formation of a normal rapidly sedimenting (200*S*) DNA intermediate.

During the growth of bacteriophage T4, the phage DNA associates with host cell components, presumably membrane, and forms an intracellular complex (2, 8, 9, 15). The DNA in this complex appears to undergo a sequence of changes resulting in the formation of one or more DNA intermediates, characterized by their sedimentation behavior on neutral sucrose gradients (2, 10). Although the precise organization of DNA in these intermediates is still unclear, a portion of the DNA can be shown to consist of single strands longer than those of mature phage DNA (12). Normal function of genes 46 and 47 (DNA arrest) is required for the formation of these intermediates (11, 14, 17, 18). Comparative studies of the sedimentation properties of intracellular DNA from wild-type and gene 46-47 mutant infections show that (i) the 46-47 DNA dissociates prematurely from the host membrane complex at a time which coincides with the arrest of DNA synthesis; (ii) the rapidly sedimenting intracellular DNA is seen only transiently after 46-47 infection; and (iii) single chains longer than those in mature phage are not seen in 46-47 infection.

A new mutation, *das* (DNA-arrest suppressor), has recently been described which partially suppresses many of the pleiotropic effects of gene 46-47 mutants, namely, arrested DNA synthesis, inviability, failure to degrade host DNA, and reduced genetic recombination (13). In this paper we examine the replicative intermediates produced after 46-47 *das* infection. Our results indicate that 46-47 *das* does not produce the complement of replicative intermediates seen after wild-type infection. However, the *das* mutation does allow the production of at least some long single chains, characteristic of wild-type infection.

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MATERIALS AND METHODS

Phage and bacterial strains. The amber mutants of T4D—*am*N130 (gene 46) and *am*A456 (gene 47)—were supplied by R. S. Edgar. The triple mutant *am*B14-*am*A456-*das*13 (genes 46, 47, *das*) was kindly supplied by J. S. Wiberg. Stocks of multiple mutants were constructed by recombination.

Escherichia coli strains B and CR63 were used as nonpermissive and permissive hosts. Phage and bacteria were assayed on Hershey agar as described by Adams (1).

Media and infection procedures. An overnight peptone broth culture was diluted 1:50 into Tris-Casamino Acids-glucose medium of Thomas and Abelson (19) supplemented with 40 μg of L-tryptophan/ml. The cells were aerated at 37 C to a density of 3×10^8 cells/ml. After centrifugation in the cold, the cells were resuspended in fresh medium at 30 C to a density of 10^9 cells/ml. After 5 min of aeration at 30 C, the cells were infected at a multiplicity of infection of 5 to 7, and 4 min later the culture was diluted to a final density of 3×10^8 cells/ml. Addition of phage marks the start of the experiment at 30 C.

Pulse labeling and lysis procedures. Methods for pulse labeling with ^{3}H-thymidine and subsequent isolation of the DNA by lysozyme-Sarkosyl or by the Brij-58 gentle lysis procedure are given in lysis methods I to III of Shah and Berger (17).

Density gradients. Procedures for analytical gradients have been previously described (17). When single strands of DNA were to be resedimented, they were first centrifuged in a 25-ml alkaline sucrose gradient for 12 h at 13,000 rpm in an SW25.1 rotor. Approximately 1.0-ml fractions were collected through a hole in the bottom of the polyallomer tubes. Samples (0.1 ml) were analyzed for trichloroacetic acid-insoluble radioactivity, and the indicated fractions were dialyzed for 4 h against 100 volumes of 0.01 M EDTA and 1.0 M NaCl adjusted to pH 12.1. Portions of the dialyzed samples were mixed with a similarly processed marker phage DNA peak fraction and recentrifuged in 4.6-ml alkaline sucrose gradients.

Other methods. The procedure for the preparation of ^{32}P-labeled phage, sample application, fraction collection, and radioactivity measurements were as described in Shah and Berger (17).

RESULTS

Analysis of DNA by zone sedimentation in sucrose gradients. The sedimentation characteristics of 46-47 *das* intracellular DNA were investigated by pulse labeling with ^{3}H-thymidine for 2 min at 5 min after infection.

A rapidly sedimenting DNA intermediate (200*S*) is detectable in neutral sucrose gradients at 9 min after 46-47 *das* infection (Fig. 1a). Unlike wild-type infection, where a large fraction of the early pulse label is still present in a rapidly sedimenting DNA intermediate at 20 min after infection (Fig. 1f), it is present only transiently in 46-47 *das* infection (Fig. 1b, c). By 20 min (Fig. 1c), most of the labeled DNA sediments as a biphasic band, a fraction cosediments with marker phage DNA, and the rest sediments at a slower rate. These sedimentation profiles of DNA produced up to 20 min after 46-47 *das* infection are similar to 46-47 DNA studied previously (17). At late times,

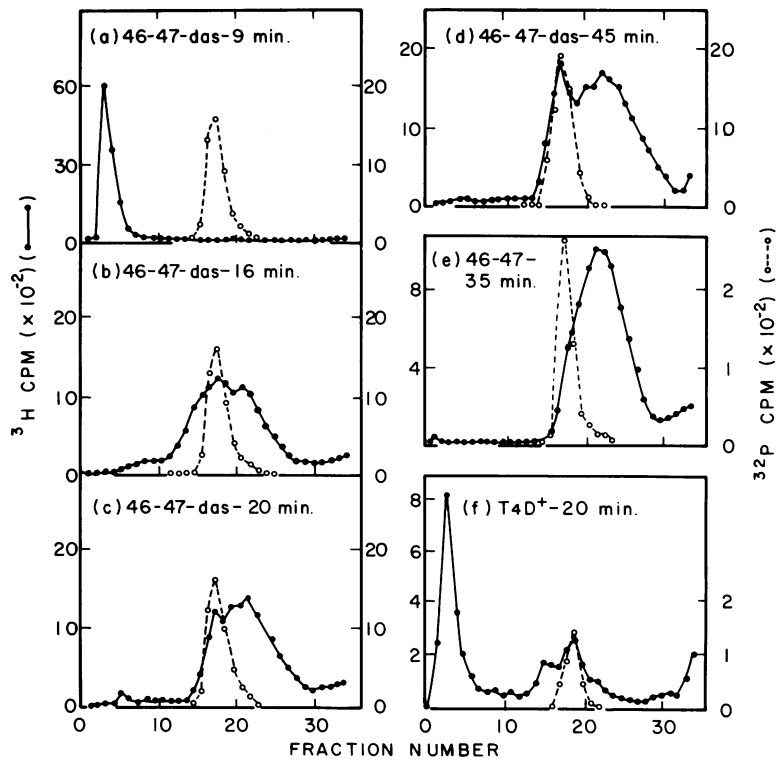

FIG. 1. *Fate of pulse-labeled DNA after infection: neutral sucrose gradient. E. coli B cells were infected with 46-47 das, 46-47, or T4D$^+$ phage at a multiplicity of infection of 5. [^{3}H]thymidine (10 μCi/ml) was added at 5 min after infection. The pulse was terminated 2 min later in 46-47 das infection and 1.5 min later in 46-47 or T4D infection by the addition of unlabeled thymidine. Samples of the cultures were lysed in the presence of ^{32}P-labeled phage by the lysozyme-Sarkosyl high-temperature method (17) at the indicated times. Samples were centrifuged for 100 min at 30,000 rpm at 20 C through 5 to 20% neutral sucrose gradients. The gradients had a cushion of dense material at the bottom to insure recovery. Sedimentation is from right to left in this and subsequent figures. Symbols: -●-●-, ^{3}H-labeled DNA; -O-O-, ^{32}P-labeled marker DNA.*

however, there is accumulation of mature-size DNA after 46-47 *das* infection (Fig.1d). Such molecules are not present after 46-47 infection (Fig. 1e, and reference 17).

Analyses at 13, 20, 30, and 45 min (Fig. 2) of the pulse-chased DNA on alkaline sucrose gradients indicate that (i) the early DNA (Fig. 2a) contains a heterogeneous population of single chains, some cosedimenting with mature phage single chains (73*S*); (ii) by 20 min (Fig. 2b), a portion of the labeled single chains are degraded, and at 30 min (Fig. 2c) they are in fragments with average sedimentation values of 60*S* and 45*S*; (iii) at 20 min, approximately 10% of the total label sediments at a rate higher than marker single chains; and (iv) late in infection mature-size single chains accumulate (Fig. 2d).

The major difference between these results and our previous findings with the 46-47 mutant (17) is the presence of long single chains. However, the amount of pulse label sedimenting at a rate higher than marker single chains between 16 and 20 min after 46-47 *das* infection is about 25% of that seen after wild-type infection (17) at similar times.

Since only a small percentage of the total label sediments faster than marker in alkaline gradients, we confirmed that this DNA is longer than mature molecules by resedimentation. Samples taken at 18 min after 46-47 *das* or 46-47 infection were denatured and sedimented through 25-ml preparative alkaline sucrose gradients (Fig. 3A, C). The fractions indicated by the arrows were mixed with a peak fraction of ^{32}P marker DNA which had also been denatured and sedimented in an alkaline gradient. The sedimentation profiles after recentrifugation show that 46-47 *das* produces single chains longer than marker single chains (Fig. 3B). Only mature-size single chains are detectable after 46-47 infection (Fig. 3D; also see Fig. 2b in reference 17).

When chloramphenicol is added at 11.5 min to 46-47-infected cells, DNA synthesis does not arrest, but continues at near wild-type rates, although phage yields remain low (6, 17). Under these conditions, early pulse-labeled mature-size single chains are further degraded (Fig. 4B), even in the presence of chloramphenicol. This occurs presumably by a nuclease(s) present prior to the addition of chloramphenicol. After removal of chloramphenicol and on further incubation for 20 min, some additional nicking is evident (Fig. 4D). However, in the case of 46-47 *das* infection a sample treated with chloramphenicol and sampled at 30 min after infection does not show degradation of early pulse-labeled DNA (compare Fig. 2a and 4A). In addition, removal of chloramphenicol and further incubation result in no loss of and perhaps a slight increase in mature size chains (Fig. 4C).

The arrest of DNA synthesis after 46-47 infection is coincident with premature dissociation of the parental and newly synthesized DNA

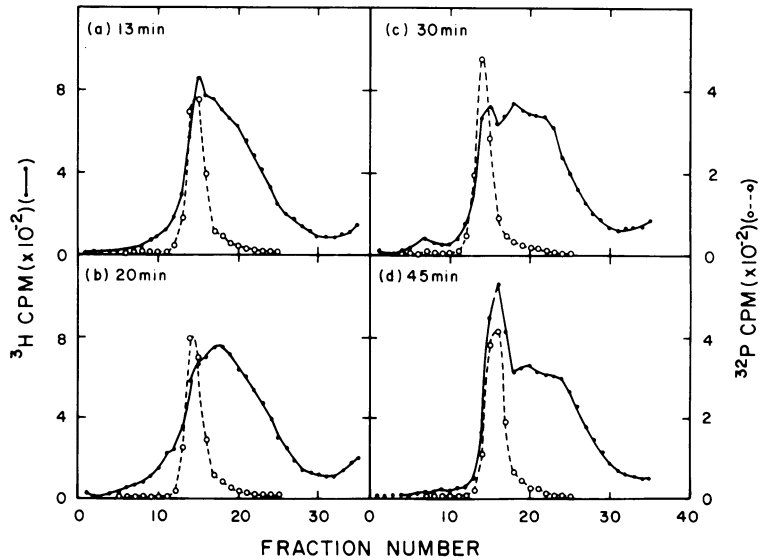

FIG. 2. *Fate of pulse-labeled DNA after 46-47 das infection: alkaline sucrose gradients. Samples of the infected cells described in the legend to Fig. 1 were lysed by the lysozyme-Sarkosyl low-temperature method (17) at the indicated times. Sedimentation was through 5 to 20% alkaline sucrose gradients for 100 min at 30,000 rpm at 20 C. Symbols: ●-●-●, ^3H-labeled DNA; O-O-O, ^{32}P-labeled marker single chains.*

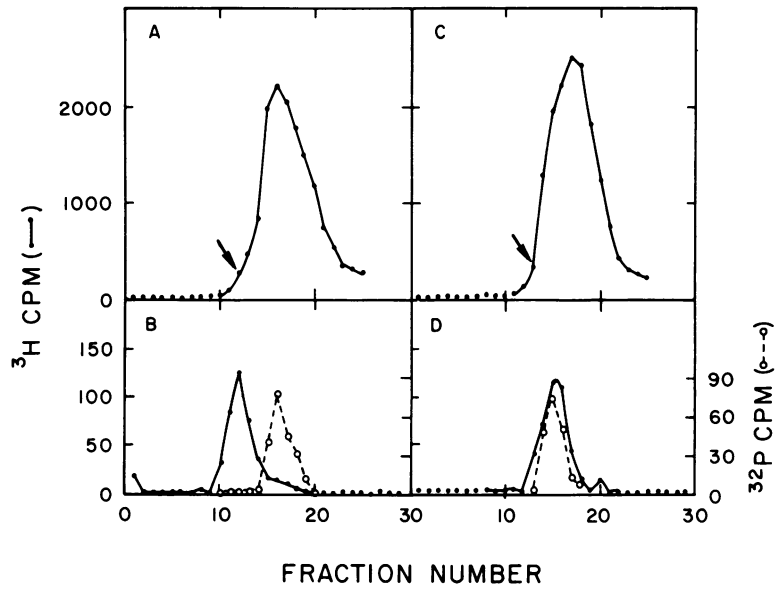

FIG. 3. *Recentrifugation of newly synthesized DNA single chains after 46-47 das or 46-47 infection: alkaline sucrose gradients. At 18 min after infection, samples were lysed by the lysozyme-Sarkosyl low-temperature method (17) and sedimented in 25-ml alkaline sucrose gradients as described in Materials and Methods. (A) 46-47 das DNA; (C) 46-47 DNA. Fractions indicated by the arrow were treated as described in Materials and Methods, mixed with ^{32}P-labeled marker DNA peak fraction, and resedimented in analytical alkaline sucrose gradients. (B) 46-47 das DNA; (D) 46-47 DNA. Symbols: -●-●-, ^{3}H-labeled DNA; -O-O-, ^{32}P-labeled marker DNA.*

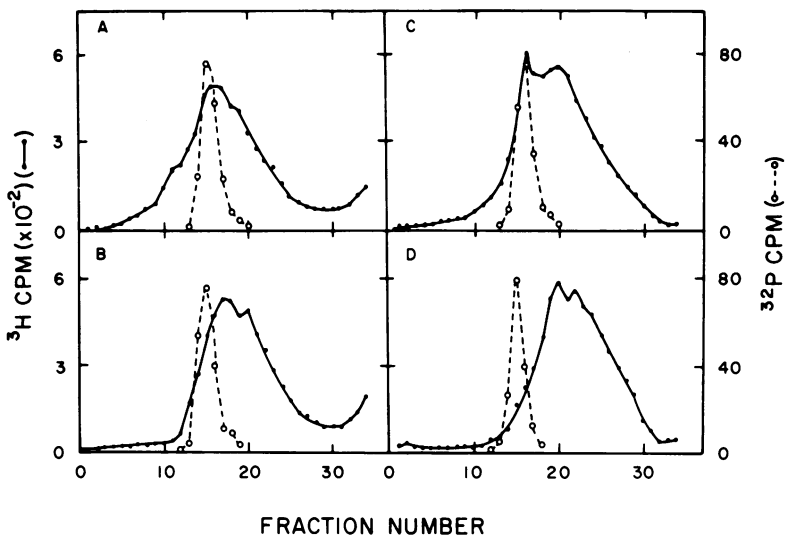

FIG. 4. *Effect of chloramphenicol on the fate of newly synthesized 46-47 das and 46-47 DNA: alkaline sucrose gradients. E. coli B cells infected with 46-47 das or 46-47 phage were pulsed with ^{3}H-thymidine (10 μCi/ml) at 5 min after infection and chased 2 min later with cold thymidine. Chloramphenicol (100 μg/ml) was added at 11.5 min, and samples were withdrawn and lysed at 30 min after infection. After removal of chloramphenicol at 30 min, the cultures were incubated for 20 additional min and lysed. (A) 46-47 das and (B) 46-47 DNA lysed at 30 min, before removal of chloramphenicol; (C) 46-47 das and (D) 46-47 DNA after removal of chloramphenicol. Lysis procedure and sedimentation as in legend to Fig. 2. Symbols: -●-●-●, ^{3}H-labeled DNA; -O-O-, ^{32}P-labeled marker DNA.*

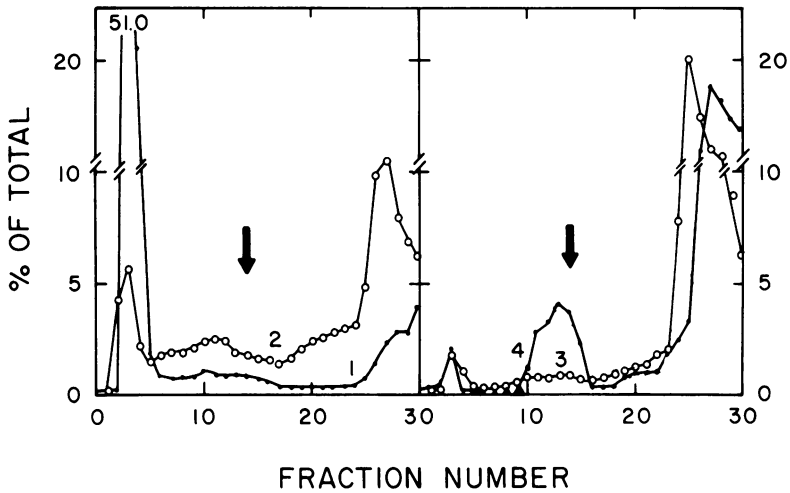

FIG. 5. *Neutral sucrose gradient sedimentation of ^{32}P-labeled 46-47 das-infected cells after gentle lysis by the Brij technique. E. coli B cells were infected with ^{32}P-labeled phage at a multiplicity of infection of 5. The culture was divided into 2 parts. At 9 (curve 1), 15 (curve 2), 20 (curve 3), and 45 (curve 4) min after infection, samples were withdrawn and lysed by the Brij technique (17). Sedimentation was through 5 to 20% neutral sucrose gradients for 15 min at 18,000 rpm at 20 C. The arrow indicates the position of whole phage (800S) in a parallel tube.*

from a rapidly sedimenting complex, presumably associated with the host cell membrane (17). The sedimentation patterns of ^{32}P-labeled parental DNA isolated after gentle lysis of 46-47 *das*-infected cells with the nonionic detergent Brij-58 shows that early in infection (Fig. 5, curve 1, 9 min) the phage DNA is associated with the rapidly sedimenting membrane complex. Whereas wild-type phage DNA remains associated with this complex up to 20 min after infection (17), the 46-47 *das* DNA begins to dissociate from the membrane by 15 min (Fig. 5, curve 2), and at 20 min (Fig. 5, curve 3) approximately 85% of the label is found at the top of the gradient. Late in infection about 15 to 20% of the label appears in mature phage (Fig. 5, curve 4). Since the *das* mutation causes a low-level suppression of the DNA arrest phenotype of gene 46 and 47 mutants, it was possible to administer 30-s pulses of ^{3}H-thymidine between 25 and 40 min after infection and examine the incorporated label for localization in a membrane complex. We found a variable amount (10 to 30%) of a late pulse after 46-47 *das* infection and 65 to 75% of a late pulse after wild-type infection in the rapidly sedimenting membrane complex (data not shown). It is possible that the late-time replication of 46-47 *das* results from the association of a small amount of DNA with the membrane complex.

DISCUSSION

Our comparative studies on intracellular 46-47 (17) and 46-47 *das* DNA indicate that partial suppression by *das* of gene 46-47 mutants is associated with (i) the synthesis of a small fraction of DNA containing long single chains, and (ii) a decrease in the degradation of DNA synthesized in the absence of 46-47 functions.

The defect in 46-47 mutants is as yet unidentified, although indirect experiments suggest these genes are responsible for the appearance of phage-specific nuclease(s) capable of acting upon host (20) and phage DNA (16, 20). The numerous consequences of 46-47 infection can be explained on this assumption, although a positive statement must await isolation and identification of the purported nuclease(s).

Our results do not permit us to precisely define the function controlled by *das*. As previously suggested, *das* could act by overproduction or change in specificity of another T4 nuclease to compensate for the lack of 46-47 function. This nuclease could act to partially restore recombination by providing single-stranded regions believed to be necessary for synapsis. The generation of long single strands observed after 46-47 *das* infection may also require this nuclease action. Suppressor mutants which restore recombination to *rec*$^-$ bacterial mutants have recently been described (3, 7). In the case of *sbc* A suppressors of *E. coli*, the suppression has been linked to the overproduction of exonuclease I which compensates for the *rec* B, C exonuclease defect. It is possible that *das* partially suppresses the 46-47 phenotype in an analogous fashion.

Our observation that *das* reduces degradation

of DNA synthesized in the absence of 46-47 function is possibly explained by the idea that DNA synthesized in the absence of 46-47 function is abnormal DNA (e.g., it does not contain long single strands) and is thus susceptible to attack by degradative nucleases present at early times after infection. In the presence of 46-47 function or, to a small extent, in the presence of the *das* mutation without 46-47 function, DNA is produced which is resistant to degradation. Presumably such degradation-resistant DNA is "competent" for continued DNA replication and production of viable progeny phage.

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LITERATURE CITED

1. Adams, M. 1959. Bacteriophages. Interscience, New York.
2. Altman, S., and L. S. Lerman. 1970. Kinetics and intermediates in the intracellular synthesis of bacteriophage T4 deoxyribonucleic acid. J. Mol. Biol. **50**:235-261.
3. Barbour, S. D., H. Nagaishi, A. Templin, and A. J. Clark. 1970. Biochemical and genetic studies of recombination proficiency in *E. coli*. II. rec$^+$ revertants caused by indirect suppression of rec$^-$ mutations. Proc. Nat. Acad. Sci. U.S.A. **67**:128-135.
4. Berger, H., A. J. Warren, and K. E. Fry. 1969. Variations in genetic recombination due to amber mutations in T4D bacteriophage. J. Virol. **33**:171-175.
5. Bernstein, H. 1968. Repair and recombination in phage T4. I. Genes affecting recombination. Cold Spring Harbor Symp. Quant. Biol. **33**:325-331.
6. Bolle, A., R. H. Epstein, W. Salser, and E. P. Geiduschek. 1968. Transcription during bacteriophage T4 development: requirements for late messenger synthesis. J. Mol. Biol. **33**:339-362.
7. Clark, A. J. 1971. Toward a metabolic interpretation of genetic recombination of *E. coli* and its phages. Annu. Rev. Microbiol. **25**:437-464.
8. Earhart, C. F., G. Y. Tremblay, M. J. Daniels, and M. Schaechter. 1968. DNA replication studies by a new method for the isolation of cell membrane-DNA complexes. Cold Spring Harbor Symp. Quant. Biol. **33**:707-714.
9. Frankel, F. R. 1966. The absence of mature phage DNA molecules from the replicating pool of T-even-infected *Escherichia coli*. J. Mol. Biol. **18**:109-126.
10. Frankel, F. R. 1966. Studies on the nature of replicating DNA in T4-infected *Escherichia coli*. J. Mol. Biol. **18**:127-143.
11. Frankel, F. R. 1966. Studies on the nature of the replicating DNA in *Escherichia coli* infected with certain amber mutants of phage T4. J. Mol. Biol. **18**:144-155.
12. Frankel, F. R. 1968. Evidence for long strands in the replicating pool after T4 infection. Proc. Nat. Acad. Sci. U.S.A. **59**:131-138.
13. Hercules, H., and J. S. Wiberg, 1971. Specific suppression of mutations in genes 46 and 47 by *das*, a new class of mutations in bacteriophage T4D. J. Virol. **8**:603-612.
14. Hosada, J., E. Mathews, and B. Jansen. 1971. Role of genes 46-47 in bacteriophage T4 reproduction. I. In vivo deoxyribonucleic acid replication. J. Virol. **8**:372-387.
15. Kozinski, A. W., and T. H. Lin. 1965. Early intracellular events in the replication of T4 phage DNA. I. Complex formation of replicative DNA. Proc. Nat. Acad. Sci. U.S.A. **54**:273-278.
16. Kutter, E. M., and J. S. Wiberg. 1968. Degradation of cytosine-containing bacterial and bacteriophage DNA after infection of *Escherichia coli* B with bacteriophage T4D wild type and with mutants defective in genes 46, 47, and 56. J. Mol. Biol. **38**:395-411.
17. Shah, D. B., and H. Berger. 1971. Replication of gene 46-47 amber mutants of bacteriophage T4D. J. Mol. Biol. **57**:17-34.
18. Shalatin, C., and Y. Naot. 1971. Role of gene 46 in bacteriophage T4 deoxyribonucleic acid synthesis. J. Virol. **8**:142-153.
19. Thomas C. A., and J. Abelson. 1966. The isolation and characterization of DNA from bacteriophage, p. 533-561. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acid research. Harper and Row, New York.
20. Wiberg, J. S. 1966. Mutants of bacteriophage T4 unable to cause breakdown of host DNA. Proc. Nat. Acad. Sci. U.S.A. **55**:614-621.