**das Mutation in Bacteriophage T4D Does Not Suppress an Amber Mutation in T4 Gene 59**

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Mutations termed *das* were isolated originally (Hercules and Wiberg, 1971) as partial suppressors of mutants in phage T4 genes 46 and 47. Since mutants in genes 46, 47, and 59 exhibit both an early arrest of phage DNA synthesis and the loss of this arrest in the presence of chloramphenicol or of mutations in T4 genes 33 and 55, we asked whether a *das* mutation can also suppress a gene 59 mutant. We find that it cannot—either at the level of phage production or DNA synthesis.

T4D mutants in genes 46 and/or 47 exhibit early arrest of DNA synthesis, an inability to convert host DNA to acid-soluble products, a defect in recombination, and sufficiently low phage production (10 phage per cell) that they produce no plaques (1, 2, 4, 6, 12, 15). Boile et al. (3) and Hosoda et al. (10) found that amber mutations in genes 33 and 55 largely reverse the DNA arrest of gene 46 and 47 mutants; chloramphenicol shows a similar effect when added at the proper time. Hercules and Wiberg (9) described spontaneous mutations in T4D that partially suppress nearly all the defective phenotypes exhibited by gene 46 and 47 mutants (4, 9, 13), including the DNA arrest; these mutations were named *das*, for DNA arrest suppressor. Thus, the *das* mutations (nonlethal) and the 33 and 55 mutations (lethal, by blocking the transcription of essentially all maturation genes) can all suppress the DNA arrest of gene 46 and 47 mutants.

Warner and Hobbs (14) showed that T4 mutants in gene 59 also exhibit DNA arrest; however, gene 59 mutants degrade host DNA normally (15, 16). Wu et al. (16) found that a mutation in gene 33 or 55, or the properly timed addition of chloramphenicol, suppresses the DNA arrest in gene 59 mutant infections. This similarity in suppressibility between gene 46, 47, and 59 mutants prompted us to ask whether a *das* mutation would also suppress a gene 59 mutation.

As before (9), for rapid gene recognition, specific phage mutants are referred to by the gene number or symbol enclosed in brackets: *amB14X5* = [46]; *amC5X5* = [59]; *das13* = [das]. The designation "X3" or "X5" indicates that these mutants have been purified genetically in this laboratory (15). All T4 amber mutants were obtained from R. Edgar. *Escherichia coli* CR63 was used as the amber (*am*)-permissive host, and *E. coli* B was used as the *am*-nonpermissive host. Other procedures, including plating techniques and preparation of phage and bacterial stocks, have been described previously (8, 9, 15).

The *das* mutation only weakly suppresses [46] and [47]: [46] and [47] give no plaques on *E. coli* B. [das] gives a large (normal) plaque, and [das, 46] and [das, 47] give tiny plaques. Thus, we first sought [das, 59] as a plaque intermediate in size between [das] and [59] among the progeny of a cross between [das] and [59]. Since none was found, we concluded that the *das* mutation either suppresses [59] very well or not at all. Wu and Yeh found that [59] itself gives tiny plaques (17). We have confirmed this and found a plating efficiency of about 70% on *E. coli* B; however, under our plating conditions (8), we found that no plaques are formed above 40 C. To test whether the leakiness of [59] might be due to ribosomal ambiguity (11), growth of [59] and another mutant (*amH628X3*) that maps at a different site in gene 59 was tested on *E. coli* BB str; this is a streptomycin-resistant strain exhibiting low ribosomal ambiguity and was obtained from J. D. Karam and P. V. O'Donnell. We have confirmed its low ribosomal ambiguity with a number of T4 *am* mutants. We found that both *am* mutants in gene 59 give a tiny plaque (below

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40°C) at high efficiency on *E. coli* BB str*, which argues that their leakiness on *E. coli* B is not due to ribosomal ambiguity.

We then deliberately constructed [das, 59] as follows. *E. coli* B at 5 × 10⁹ cells per ml was infected in glycerol-Casamino Acids medium (7) at 37°C with a phage mixture having a multiplicity of infection of 10 [das] and 1 [59]. After 60 min the cells were lysed with CHCl₃, and the progeny were grown at 30°C on plates of *E. coli* CR63 (permissive for all the progeny). Progeny plaques were stabbed and printed onto both *E. coli* CR63 at 30°C (the replica plate) and *E. coli* B at 42°C (the test plate on which [59] does not grow). We made the working assumption that [das, 59] would not grow any better than [59]. Lysates of seven of the [59]-containing progeny were grown on *E. coli* CR63 at 30°C. To test these lysates for the presence of das, we crossed each with [46] at multiplicity of infection of 5 for each phage and plated the progeny on *E. coli* B at 41°C; the only progeny that should give tiny plaques at 41°C are [das, 46] recombinants. Three of the seven candidates produced a high frequency of tiny plaques from such a cross, indicating that these three were [das, 59]. To confirm that the tiny plaques were [das, 46], more progeny of the cross of the presumed [das, 59] with [46] were stabbed from the replica plate (CR63) and tested on spot plates. Several gave a pattern unique to [das, 46]: (i) a hazy spot (weak growth) on *E. coli* B at 41°C, (ii) no complementation with helper [46], and (iii) strong complementation with helper [59]. We also recovered [46, 59] and [das, 46, 59] from the same cross of [46] with [das, 59] and confirmed their identities by similar techniques.

The effect of the das mutation on phage production by [59] was then tested in liquid culture. It can be seen in Table 1 that there is no effect (E, F); the typical suppression of [46] by the das mutation is also shown (C, D). [59] is quite "leaky" (E), as was already indicated by its tiny plaque on *E. coli* B and as seen by others (6, 17). The double mutant [46, 59] produces fewer phage than either [46] or [59], yet introduction of the das mutation into this double mutant (H) increases phage production several-fold. Thus, the das mutation can still show some suppression of [46] even when gene 59 is defective.

It seemed quite possible that, despite the failure of the das mutation to suppress [59]'s defect in phage production, the das mutation might suppress the DNA arrest phenotype of [59]. Thus, the experiment shown in Fig. 1 was performed. In Fig. 1 the incorporation of [H]dThd shows that [das, 59] and [59] are indistinguishable with respect to DNA synthesis: both make very little DNA, and both have stopped by 14 min. Thus, the das mutation does not suppress [59] at the level of DNA synthesis. DNA synthesis in various control experiments is also shown in Fig. 1: (i) [das] alone exhibits normal DNA synthesis; (ii) [das, 46] exhibits typically greater synthesis than does [46]; (iii) [46] appears to make two to three times as much DNA as does [59]; (iv) [46, 59] appears to make DNA at a level intermediate between the levels seen with [46] and [59], and addition of the das mutation to [46, 59] has no significant effect. That a small effect of the das mutation on [46, 59] occurred in phage production (Table 1) might be due to a difference in structure of the vegetative DNAs between [46, 59] and [das, 46, 59] infections that could affect their maturability into phage particles; this has not been tested. DNA synthesis by all of these phages has also been examined chemically by using an indole reagent (5); the results (not shown) support all of our interpretations of Fig. 1.

Thus we find that, although a das mutation partially suppresses mutants in genes 46 and 47, it does not suppress a gene 59 mutant, even though mutants in all three genes—46, 47 and

### Table 1. Effect of das mutation on phage production by [46] and [59]*

<table>
<thead>
<tr>
<th>Phage</th>
<th>Phage per cell</th>
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<tbody>
<tr>
<td></td>
<td>Expt 1*</td>
</tr>
<tr>
<td>A. T4D-wild</td>
<td>231 (100)</td>
</tr>
<tr>
<td>B. [das]</td>
<td>(104)</td>
</tr>
<tr>
<td>C. [46]</td>
<td>9.6 (4.2)</td>
</tr>
<tr>
<td>D. [das, 46]</td>
<td>84 (37)</td>
</tr>
<tr>
<td>E. [59]</td>
<td>27 (12)</td>
</tr>
<tr>
<td>F. [das, 59]</td>
<td>24 (10)</td>
</tr>
<tr>
<td>G. [46, 59]</td>
<td>5.3 (2.3)</td>
</tr>
<tr>
<td>H. [das, 46, 59]</td>
<td>17 (7.5)</td>
</tr>
</tbody>
</table>

*E. coli* B was grown to 4.6 × 10⁴ and 5.0 × 10⁴ colony-forming cells per ml (in experiments 1 and 2, respectively) in glycerol-Casamino Acids medium at 37°C from a 1:100 dilution of an overnight culture. Portions of 1 ml were infected at 37°C with 0.1 ml of phage (multiplicity of infection, 10) on a Gyrotory shaker (New Brunswick Scientific Co.). At 50 min after infection, a sample was diluted through CHCl₃-saturated broth and assayed for plaque-forming ability on *E. coli* CR63 at 30°C.

*Values in parentheses are percentages (compared with the wild-type value as 100%).

c [das] was tested in a separate experiment in which the corresponding phage production by wild type was 223 and *E. coli* B was at a concentration of 3.7 × 10⁹/ml.

[H]dThd shows that [das, 59] and [59] are indistinguishable with respect to DNA synthesis: both make very little DNA, and both have stopped by 14 min. Thus, the das mutation does not suppress [59] at the level of DNA synthesis. DNA synthesis in various control experiments is also shown in Fig. 1: (i) [das] alone exhibits normal DNA synthesis; (ii) [das, 46] exhibits typically greater synthesis than does [46]; (iii) [46] appears to make two to three times as much DNA as does [59]; (iv) [46, 59] appears to make DNA at a level intermediate between the levels seen with [46] and [59], and addition of the das mutation to [46, 59] has no significant effect. That a small effect of the das mutation on [46, 59] occurred in phage production (Table 1) might be due to a difference in structure of the vegetative DNAs between [46, 59] and [das, 46, 59] infections that could affect their maturability into phage particles; this has not been tested. DNA synthesis by all of these phages has also been examined chemically by using an indole reagent (5); the results (not shown) support all of our interpretations of Fig. 1.
59—exhibit a DNA arrest phenotype that is suppressible by chloramphenicol or mutations in genes 55 and 33. Wu and Yeh (17) have recently isolated mutations, termed dar (for DNA-arrested restoration), that fully and specifically suppress the defective phenotype of gene 59 mutants with respect to both DNA synthesis and phage production, but which do not suppress mutants in genes 46 and 47. The dar mutations map between genes 24 and 25 and are in a late gene (17), whereas das mutations map between mutants in genes 33 and 34 and are in an early gene, as judged by their ability to cause some degradation of host DNA in an infection by [das, 42, 46] (9); here the 42-mutation prevents the appearance of late proteins.

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LITERATURE CITED


