Point Mutants in the D2a Region of Bacteriophage T4 Fail to Induce T4 Endonuclease IV

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We have studied the properties of presumptive point mutants in the D2a region of bacteriophage T4. Dominance tests showed that the D2a mutation was recessive to the wild-type allele. The mutations were shown to map in the D2a region by complementation against *rII* deletions. The D2a mutations were also located between gene 52 and *rIIB* by two- and three-factor crosses. The mutants are located at at least two distinct loci in the D2a region. The point mutants grow normally on all hosts tested and none of the mutants makes T4 endonuclease IV. We propose the name "*denB*" for the D2a locus.

It was suspected for several years that *rII* deletion mutants of bacteriophage T4 might extend past the right-hand end of the *rIIB* cistron into a region which is nonessential for growth (3, 24). Bautz and Bautz (2) defined the right-hand boundaries of several of such deletion mutants by using the techniques of RNA-DNA hybridization, and they named this region the "D region." The extension of several *rII* deletion mutations into the D region was confirmed by Dove by using genetic crosses (9). Kasai and Bautz (13) studied the kinetics of synthesis of mRNA from the D region and were able to distinguish two classes of D message. The message synthesized from the D1 region was the "early" type and had the same kinetics of synthesis as that from the *rII* region. The D2 message was the "pre-early" type and was synthesized maximally at 90 s after infection. Sederoff, Bolle, and Epstein (21) subdivided the D region into three portions called D1, D2a, and D2b by competition hybridization analysis.

The finding that *rII* mutations were able to suppress a normally lethal mutation in the structural gene for T4 polynucleotide ligase led to speculation that the *rII* region might control an endonuclease which introduced single-strand breaks into duplex DNA (4, 11, 12, 15). This prompted Bruner, Souther, and Suggs (6) to investigate whether the deletion of an *rII*-controlled nuclease might permit the accumulation of cytosine-containing phage DNA in a gene 56² (deoxycytidine triphosphatase⁻) mutant. Gene 56 mutants appear to synthesize cytosine-containing phage DNA which is rapidly broken down (16). Bruner et al. (6) did find that certain

rII deletions permitted the accumulation of DNA when coupled to a gene 56 amber mutation. This phenomenon was called "anomalous DNA synthesis" (ADS) and occurred only if the *rII* deletion extended into the adjacent D2a region. These authors postulated that the D2a region controlled a nuclease specific for cytosine-containing DNA. We subsequently showed (20) that if the D2a region of T4 was deleted there was no detectable induction of T4 endonuclease IV. This enzyme had previously been shown to be specific for single-stranded, cytosine-containing DNA (18).

No function has been assigned to the D1 region. It seems that the D2b region controls a function responsible for disruption of the host nucleus after phage infection (22; D. P. Snustad and H. R. Warner, personal communication). DePew and Cozzarelli have discovered the *stp* locus (*stp* = suppressor of three-prime phosphatase mutation) which appears to map to the right of the D2b region and is distinct from *ac* (8). They have postulated that the *stp* region may control an endonuclease which generates 3'-phosphoryl termini in DNA. Finally, the *ac* locus lies to the right of the *stp* locus and controls resistance of T4 to acriflavine hydrochloride. A schematic map of the D region and adjoining loci is presented in Fig. 1.

Bruner, Solomon, and Berger (5) recently reported the isolation of presumptive point mutants in the D2a region of T4. These were isolated after heavy hydroxylamine mutagenesis of a gene 56 amber mutant (either with or without an additional *rII* deletion) and screening survivors for ADS. However, the mutations

FIG. 1. Schematic diagram of the *rII* region and the adjacent *D* region. The distances are not to scale.

were not definitively localized to the D2a region and some of the mutants still contained an *rII* deletion.

We wished to determine if the point mutations in these mutants were actually localized to the D2a region. Furthermore, we wished to demonstrate that the expression of the D2a phenotype (ADS or failure to induce T4 endonuclease IV) did not depend upon an accompanying *rII* mutation. Finally, the availability of point mutants would facilitate studies of the function of the D2a region in the absence of other mutations.

We have carried out genetic, biochemical, and physiological studies of the D2a point mutants isolated by Bruner et al. (5). We have removed extraneous mutations by back-crossing and have localized the mutations to the D2a region by complementation analysis and by genetic crosses. We have demonstrated that none of the point mutants induces detectable T4 endonuclease IV. Finally, we have found that the D2a region seems to be nonessential, since the D2a point mutants grow normally in several different hosts.

MATERIALS AND METHODS

Bacterial and phage strains. *Escherichia coli* B was obtained from J. Wiberg; *E. coli* CR63 was used as the permissive host for amber mutants and was obtained from the laboratory of J. Hurwitz. *E. coli* Y-10 (λ t68), the nonpermissive host for T4rII mutants, was obtained from H. Eisen. *E. coli* 594 (*str^rsup⁻*) was obtained from E. Signer.

Wild-type T4 phage was from the collection of J. Hurwitz. T4amE51x5 and T4amH17, amber mutants in gene 56 and gene 52, respectively, were obtained from J. Wiberg. The designation "x5" indicates that the strain had been purified genetically by back-crossing five times against wild-type phage. These strains will be referred to as 56am and 52am. T4r6 and T4r73 which contain short deletions in the *rIIA* and *rIIB* cistrons, respectively, were obtained from J. Wiberg. We received three presumptive D2a point mutants from Robert Bruner (5): DS37-(D2a-6, 56am, *rII*1241), DS42-(D2a-23, 56am), and DS44-(D2a-2, 56am). The extended *rII* deletions used were *rII*1241 (obtained from R. Bruner) which extends into the D1 region but not into the D2a region and *rII*1272 which extends into the D2a region (from R. DePew and N. Cozzarelli). The *rII*H23 deletion used previously (20) was believed to extend only into the D2a region but we found that it was resistant to acriflavine. Since we have reason to doubt the end point of the H23 deletion (R. DePew and N. Cozzarelli, personal communication), we used the *rII*1272 deletion in this study as a deletion mutant

which is deleted into the D2a region but not beyond. T4ac41 (resistant to acriflavine-hydrochloride) was obtained from H. Bernstein.

Media. GCA medium (19) and GC medium (17) have been described previously. H broth was described by Chase and Doermann (7).

Other materials. [3 H]thymidine (5 Ci/mmol) was obtained from Amersham/Searle. Hydroxyurea was purchased from Calbiochem. Unlabeled thymidine was purchased from Schwarz-Mann. Acriflavine-hydrochloride was purchased from British Drug Houses.

Measurement of ADS. D2a mutants are characterized by their ability to permit the incorporation of [3 H]thymidine into DNA when the D2a mutation is coupled to a mutation in gene 56 (dCTPase). The gene 56 mutant alone is DNA negative. ADS was measured as follows (method A). *E. coli* B was grown at 37°C to a cell concentration of 2×10^8 per ml in GCA medium. L-Tryptophan was added to a concentration of 10 μ g per ml, and 1-ml samples of cells were pipetted into disposable plastic tubes (17 by 100 mm). Phage to be tested were added in 0.1 ml of medium to give a multiplicity of infection of 10 to 20 phage per cell. The tubes were shaken in a reciprocating shaker at 37°C. Fifteen minutes after infection, 10 μ Ci of [3 H]thymidine (in 0.1 ml of water) was added to each tube and shaking was continued for a further 1.5 min. The pulse was terminated by quickly pipetting 5 ml of cold 10% trichloroacetic acid containing 100 μ g of unlabeled thymidine per ml into each tube. The tubes were chilled on ice for 5 min and acid-insoluble material was collected on a Millipore filter. The filter was washed with 15 ml of 1% trichloroacetic acid and dried. Radioactivity was determined in a liquid scintillation counter. In most experiments greater than 99% of the cells were killed as determined by viable cell titer. The relative incorporation of thymidine by infected versus uninfected cells was about 5:1 for T4⁺ phage, about 1:1 for 56am phage, and 5-10:1 for 56am, D2a phage (Table 1). Complementation tests were done by using this method, and each of the two phages to be tested was added at a multiplicity of 10. Positive complementation is indicated by a depression of ADS, whereas negative complementation is accompanied by preservation of ADS.

The maximum number of tubes that could be handled conveniently by the above method was 25. Since we wished to screen several progeny of phage crosses for the D2a phenotype we devised the following modification of the above procedure (method B). Individual plaques were stabbed with a toothpick to a lawn of *E. coli* CR63 and the plates were allowed to incubate at 37°C until the size of the cleared spots was 2 to 3 mm in diameter (6 to 16 h). The entire spot was removed with a Pasteur pipette and placed in 1 ml of GCA medium containing L-tryptophan, 10 μ g per ml. Logarithmically growing *E. coli* cells (10^7) were added in a volume of 50 μ liters with an Eppendorf automatic pipette. The tubes were shaken gently at 30°C for 30 min. A 1- μ Ci amount of [3 H]thymidine in 1 ml of GCA medium was added with a Cornwall continuous pipettor. After 2.5 min at 30°C the infection was terminated with cold 10% trichloroacetic acid as described above. Acid-insoluble material was collected on Whatman GF/C glass filters.

With this assay, the amounts of thymidine incorpo-

rated were about 8,000 counts/min for uninfected cells, 25,000 counts/min for T4⁺-infected cells, 2,000 counts/min for 56*am*-infected cells, and 15,000 to 30,000 counts/min for 56*am*, D2a-infected cells. In reconstruction experiments, 110 phage plaques of known genotype (either 56*am*, or 56*am* D2a) were picked to CR63 and the test was performed (by D. V.) without knowing the genotypes. The correct genotype was determined in 109 of the 110 phage plaques tested, thus vindicating the use of this test for screening phage progeny from genetic crosses. By using this assay, one person could carry out up to 200 tests per day.

Genetic crosses. Crosses for mapping studies were done using the method of Chase and Doermann (7) except that unadsorbed phage were removed by centrifugation and T4 antiserum was not used. Presumptive D2a point mutants were back-crossed against T4 56*am* x 5 phage to remove extraneous mutations. *E. coli* CR63 was grown at 30°C in GCA medium to 2×10^8 cells per ml and was infected with multiplicities of 10 T4 56*am* x 5 and 1 T4 56*am*, D2a phage per cell. The cultures were shaken vigorously at 30°C for 3 to 4 h and lysed with chloroform. The progeny were plated on CR63 and tested for ADS by method B.

Other methods. Phage stocks from a single plaque were obtained by picking the contents of the plaque to 10 ml of cells growing in GCA medium (2×10^8 /ml) at 37°C and shaking vigorously. Lysis occurred within 5 h. Single-step growth curves were done as described by Adams (1). Sensitivity of T4 to hydroxyurea was carried out as described by Hercules et al. (10). Preparation of T4 phage-infected cells for assay of endonuclease IV was done as described previously (17). The methods for the assay of T4 endonuclease IV and for phosphocellulose chromatography have been described (18, 20).

RESULTS

Back-crossing of presumptive D2a point mutants. Each of the three presumptive D2a point mutants was back-crossed against 56*am* phage to remove extraneous mutations. The progenies of each cross were screened for anomalous DNA synthesis (method B), and phage showing the ADS phenotype were complemented against 56*am* (D2a⁺) and 56*am*, rIIH23 (D2a). D2a mutants are complemented by 56*am* but not by 56*am*, rIIH23 (see below). After five such back-crosses an *am*⁺ revertant was isolated by plating the phage on *E. coli* B. Such *am*⁺ revertants still expressed the D2a mutation as shown by the fact that some of the progenies of a cross between the revertant and T4 56*am* phage exhibited ADS.

After the fifth back-cross of T4DS37, the rII mutation (rII1241) was removed by crossing against 56*am* and selecting for an *r*⁺ recombinant. When the progenies of this back-cross were screened for ADS two types of phage were isolated. One gave high values of anomalous DNA synthesis, whereas the other gave inter-

TABLE 1. Dominance relationships of D2a⁺ and D2a genotypes as measured by ability to produce ADS^a

Genotype	Anomalous DNA synthesis	
	Counts/min ($\times 10^{-4}$) ^b	Ratio (counts per min per uninfected counts per min) ^b
1. Uninfected cells	6.9	
2. T4 ⁺	36.4	5.3
3. 56 <i>am</i>	7.5	1.1
4. 56 <i>am</i> , rII1241	7.2	1.0
5. 56 <i>am</i> , rII1272	58.4	8.5
6. 56 <i>am</i> +56 <i>am</i> , rII1272	7.9	1.1
7. 56 <i>am</i> , rII1241+56 <i>am</i> , rII1272	11.7	1.7
8. 56 <i>am</i> , D2a68	63.3	9.2
9. 56 <i>am</i> +56 <i>am</i> , D2a68	9.9	1.4

^a ADS was determined by method A. *E. coli* B cells were infected with the appropriate phage at a multiplicity of infection of 20 for single inputs and 10 for each phage of a mixed infection.

^b Results are expressed as gross acid-insoluble counts per minute and as a ratio of the observed radioactivity to that incorporated by uninfected cells.

mediate values which were still definitely higher than uninfected cells. These two phages were named D2a-68 and D2a-62, respectively. It seemed possible that the original DS37 phage contained two D2a mutations. Complementation analysis (see below, Table 2) showed that both D2a-68 and D2a-62 were located in the D2a region. When crossed against each other, wild-type recombinants were readily detectable, thus confirming that the mutations were at different sites.

Complementation tests of D2a point mutants. The dominance relationships of the D2a mutations were determined by measuring ADS after infection of *E. coli* B with various pairs of phages (Table 1). Phages which were D2a⁺ (56*am*, and 56*am*, rII1241) showed no ADS (lines 3 and 4, Table 1), whereas D2a mutants (56*am*, rII1271 and 56*am*, D2a-68) showed ADS (lines 5 and 8, Table 1). Mixed infection with a D2a⁺ and a D2a phage abolished the ADS, showing that the D2a mutation is recessive. If the ADS is due to the absence of T4 endonuclease IV, this result suggests that endonuclease IV is freely diffusible within the cell.

Complementation analyses of the four D2a point mutants are presented in Table 2. With the exception of T4⁺, all phage examined contained the gene 56 amber mutation. Each phage was tested for complementation with rII1241 (D2a⁺) and rII1272 (D2a) by measuring ADS.

TABLE 2. Complementation analysis of D2a point mutants as measured by ADS^a

Mutant	Anomalous DNA synthesis	
	Counts/min ($\times 10^{-9}$) ^b	Ratio (counts per min per uninfected counts per min) ^b
Uninfected cells	6.9	
T4 ⁺	36.4	5.3
56am	7.5	1.1
56am, rII1241	7.2	1.0
56am, rII1272	58.4	8.5
56am, D2a-68	63.3	9.2
56am, D2a-68+56am, rII1272	57.0	8.3
56am, D2a-68+56am, rII1241	16.6	2.4
56am, D2a-62	29.8	4.3
56am, D2a-62+56am, rII1272	36.5	5.3
56am, D2a-62+56am, rII1241	12.0	1.7
56am, DS42 $\times$ 5	52.8	7.6
56am, DS42 $\times$ 5+56am, rII1272	50.6	7.3
56am, DS42 $\times$ 5+56am, rII1241	16.3	2.4
56am, DS44 $\times$ 5	42.3	6.1
56am, DS44 $\times$ 5+56am, rII1272	45.8	6.6
56am, DS44 $\times$ 5+56am, rII1241	17.2	2.5

^a The complementation tests were performed by infecting *E. coli* B with the appropriate phage(s) at a multiplicity of infection of 20 for single phages and 10 for each phage of a mixed infection. ADS was measured by method A.

^b Results are expressed both as gross acid-insoluble radioactivity and as a ratio of the observed radioactivity to that obtained with uninfected cells.

The results show that none of the presumptive point mutants was complemented by rII1272 (i.e., ADS was maintained) and that all of them were complemented by rII1241 (i.e., ADS was greatly reduced).

The above data indicate that the four D2a point mutants are located in the D2a region. Strong support for this conclusion was obtained from the results of three-factor and two-factor genetic crosses. In the three-factor crosses, an rIIA (r6), D2a phage was crossed against an rIIB (r73), D2a⁺ phage and r⁺ recombinants were selected on a lambda lysogen. These were tested for the D2a phenotype (ADS) by method B (see Fig. 2a). A similar cross was performed except that the D2a mutation was replaced by an amber mutation in gene 52 (H17) (Fig. 2b). All phage contained the 56am mutation. If the D2a mutation is closer to rIIB than is the gene 52 mutation, the percentage of r⁺, D2a⁺ double recombinants would be less than the percentage of r⁺, am⁺ recombinants. This indeed proved to be the case. As can be seen in Table 3, the

number of r⁺, D2a⁺ recombinants was about 5%, whereas the proportion of r⁺, 52am⁺ recombinants was about 20%. Thus the D2a point mutants are localized between rIIB and gene 52 (Fig. 1).

Further confirmation of this location was obtained from two-factor crosses which showed that one D2a point mutant (D2a-68) was closer to rIIB than was the ac41 mutation (3.3% recombination for D2a-68 versus 5.8% recombination for ac41). Two-factor crosses also showed that D2a-68 was closer to rIIB than to rIIA (3.3% recombination with rIIB versus 8.2% recombination with rIIA). These results are consistent with a location of the D2a mutant between rIIB and ac.

The above genetic data would also be compatible with the location of the new mutants within the D2b region or the stp locus. This was excluded because D2a-68 causes nuclear disruption.

FIG. 2. Three-factor crosses to locate D2a point mutants between rII and gene 52. All phage contained the 56am mutation (E51). r⁺ recombinants were selected on a lambda lysogen and screened for the D2a phenotype (ADS) by method B (Fig. 2a) or for the presence of the 52am mutation (H17) by spot testing (Fig. 2b). The percentages show the proportion of double cross-overs obtained as indicated by the dotted line.

TABLE 3. Results of three-factor crosses to localize D2a point mutants^a

Outside marker	Total no. of progenies screened	Double cross-overs (%)
1. D2a-68	582	4.6
2. D2a-62	545	3.9
3. D2a-23	291	6.1
4. D2a-2	291	5.9
5. am52	464	20.0 ^b

^a The crosses are diagrammed in Fig. 2. Methods are described in the text. All phage contained the 56am mutation.

^b The genotype of the progeny (i.e., 56am or 56am, 52am) was determined by complementation spot-testing on a lawn of *E. coli* 594.

tion normally (D. P. Snustad, personal communication) and a D2b point mutant (*ndd98*) (coupled to 56*am*) does not cause ADS and induced T4 endonuclease IV normally (data not shown). The D2a phenotype is not attributable to the *stp* mutation, since the phage *rII1272* is D2a but is *stp*⁺ (R. DePew and N. Cozzarelli, personal communication). This phage also fails to induce T4 endonuclease IV. Finally, all D2a point mutants are sensitive to acriflavine, excluding the possibility that the mutations lie in the *ac* locus.

It was of interest to know if the various point mutants were at different sites in the D2a region. A cross of D2a-68 against D2a-62 yielded 3.5% wild-type recombinants and a cross of D2a-68 against DS44x5 yielded 1% wild-type recombinants, suggesting that D2a-68 is at a separate site from D2a-62 and DS44x5. DS44x5 crossed against Ds42x5 yielded no wild-type recombinants in 200 progenies screened, suggesting that these two mutations may be at the same site, or are very closely linked.

Fine structural mapping within the D2a region was not attempted because of the laborious assay needed to screen for the D2a phenotype. We were unable to find a host which failed to plate D2a point mutants among strains deficient in endonuclease I, DNA polymerase I, DNA ligase, *recA* function, or the *recBC* nuclease. Screening over 10,000 clones from a mutagenized stock of *E. coli* B failed to uncover a strain which was unable to plate D2a mutants. We also failed to find a host restrictive for D2a mutants among 27 of the Cal Tech Hospital Strains which are sensitive to T4 phage.

Physiological studies of D2a point mutants. The finding of a host which failed to plate D2a point mutants would have been useful not only for fine structural mapping studies but also for studying the role of the D2a region in phage replication. As noted above, we did not find a host which was unable to plate D2a mutants. The back-crossed, *am*⁺ D2a point mutants plated normally on all hosts tested and the morphology of the plaques was identical with T4⁺ phage plaques on all hosts. The D2a mutants plated normally on a lambda lysogen, indicating that they do not carry an *rII* mutation. Single step growth curves of each of the D2a point mutants showed that the kinetics of release of phage were similar to T4⁺. The burst sizes obtained were also similar to T4⁺ (Table 4). A D2a point mutant had a slight effect on the recombination frequency between the *rIIA* and *rIIB* markers. In four experiments the recombination between the *rIIA* and *rIIB* markers averaged 2.9% for D2a⁺ phage and 1.8%

TABLE 4. Burst sizes of T4⁺ and D2a phages^a

Phage	Burst size
T4 ⁺	180
D2a-62	195
D2a-68	185
D2a-2	160
D2a-23	230

^a Burst sizes of the phages grown on *E. coli* B were determined by the method described by Adams (1).

when the phage contained the D2a mutation. The biological significance of this result is uncertain especially since T4 endonuclease IV does not act on T4 DNA (11). The D2a point mutants were no more sensitive to hydroxyurea than wild-type T4, confirming previous studies of Souther, Bruner, and Elliott (23) with D2a deletions. This means that the D2a point mutants, like the D2a deletions studied by these authors, are probably not grossly deficient in the destruction of cellular DNA.

Enzymologic studies of D2a point mutants. Our previous studies had shown that a mutant which deleted the D2a region displayed ADS and induced no detectable T4 endonuclease IV. It was therefore of interest to know whether the D2a point mutants would also fail to induce this enzyme. When the back-crossed (*am*⁺) D2a point mutants were used to infect *E. coli* B, no T4 endonuclease IV activity was detectable after phosphocellulose chromatography, whereas the peak of T4 endonuclease III activity was readily detectable (Fig. 3). Identical results were obtained with all four of the D2a point mutants. These results provide strong evidence that the D2a region controls T4 endonuclease IV activity. They also show that the *rII* region does not control T4 endonuclease IV.

DISCUSSION

We have investigated the properties of the D2a point mutants isolated by Bruner, Solomon, and Berger (5). All the mutants were shown genetically to map in the D2a region and to be located at two or perhaps three separate loci within the D2a region. Because the original mutants were isolated after heavy mutagenesis, we purified them by back-crossing against T4 56*am* to remove extraneous mutations. The properties of causing anomalous DNA synthesis and the inability to induce T4 endonuclease IV were preserved throughout these crosses. Hence it is likely that ADS is caused by an absence of T4 endonuclease IV.

It has been pointed out previously (6, 20) that the D2a phenotype (ADS and failure to induce

FIG. 3. Detection of T4 endonuclease III and endonuclease IV by phosphocellulose chromatography. Methods have been described previously (18, 20). (a) T4⁺ infected *E. coli* B cells; (b) T4 D2a-68x6 infected *E. coli* B cells.

T4 endonuclease IV) might require the presence of an *rII* mutation in addition to the D2a mutation. This possibility is now definitely excluded since the D2a point mutants preserve the D2a phenotype and yet are *r*⁺.

We are uncertain whether the D2a region is the structural gene for T4 endonuclease IV. None of the D2a point mutants showed temperature sensitivity of the D2a phenotype (ADS). Thus we did not look for a temperature-sensitive T4 endonuclease IV. Because of the laborious screening procedures for the D2a phenotype (ADS or measurement of T4 endonuclease IV), we have not pursued the search for a D2a temperature-sensitive mutant or fine structural

mapping studies within the D2a region. Such studies would be greatly aided by the availability of a host which cannot plate D2a mutants. A search for such a strain has been unsuccessful.

The availability of such a strain might also facilitate studies of the function of T4 endonuclease IV. The studies of Souther, Bruner, and Elliott (16) suggest that the enzyme plays at best a minor role in degradation of host DNA. The enzyme may play a role in preventing the synthesis of cytosine-containing DNA (either host or phage), although such a function does not seem to be essential in the hosts tested.

It is of interest that the D2a region which controls T4 endonuclease IV activity has two other loci adjacent to it that control functions which may also involve nuclease action. The *ndd* locus lies in the D2b region and is responsible for disruption of the host nucleus. Although this still occurs in the absence of destruction of host DNA (22), it is still possible that nuclear disruption is mediated by a nuclease. The *stp* locus which appears to lie between D2b and *ac*, may also control a nuclease (8). The significance of this apparent grouping of functions is unclear but it might suggest that these regions are transcribed in a coordinate manner. The D2 message is thought to be the "pre-early" type (13) and nuclear disruption (controlled by the *ndd* or D2b region) occurs within 2 to 3 min after infection (22). This grouping might be advantageous if the three gene products act spatially within the cell at a similar site (e.g., the host chromosome) or if they must form a complex before acting. In the latter case the proteins would have a small distance to diffuse before aggregating into the complex. Similar arguments have been considered in explaining the clustering within morphogenetic regions of phage T4 (14).

Finally, we wish to propose that the D2a region be called the *denB* locus (*den* = DNA endonuclease). The *denA* locus controls the synthesis of T4 endonuclease II (10).

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