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Identification and Genetic Characterization of Mutants of Bacteriophage T4 Defective in the Ability to Induce Exonuclease A[1]

HUBER R. WARNER, D. PETER SNUSTAD, JAMES F. KOERNER, AND J. D. CHILDS

Department of Biochemistry and Department of Genetics and Cell Biology, College of Biological Sciences, University of Minnesota, St. Paul, Minnesota 55101; Department of Biochemistry, Medical School, University of Minnesota, Minneapolis, Minnesota 55455; and Biology and Health Physics Division, Atomic Energy of Canada Limited, Chalk River, Ontario, Canada

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A mutant of bacteriophage T4 unable to induce exonuclease A has been isolated. The mutation responsible for this defect maps between genes 39 and 56, in a region of the chromosome devoid of other known markers. Four deletion mutants lacking part of the genome located between genes 39 and 56 also fail to induce exonuclease A. The ability of all of these mutants to replicate suggests that exonuclease A is not essential for replication of phage T4.

We have recently described a method for isolation of bacteriophage T4 mutants defective in the ability to degrade the deoxyribonucleic acid (DNA) of their host, *Escherichia coli* (25). Phage were subjected to heavy mutagenesis with hydroxylamine, and mutants were identified which were unable to replicate in the presence of hydroxyurea, an inhibitor of ribonucleoside diphosphate reductase of phage-infected cells (23). One mutant (*nd28*) obtained in this manner lacked the ability to induce T4 endonuclease II (19) and did not induce the normal pattern of degradation of host DNA (25). A second mutant, designated *nd100*, also failed to replicate in the presence of hydroxyurea, but this mutant was found to induce normal levels of T4 endonuclease II (P. Sadowski, *personal communication*), suggesting that another defect is responsible for its hydroxyurea sensitivity.

Experiments described in this report demonstrate that *nd100* is a double mutant with defects for induction of deoxycytidylate hydroxymethylase and T4 exonuclease A. A temperature-sensitive deoxycytidylate hydroxymethylase is responsible for the hydroxyurea sensitivity of this phage. The mutation affecting exonuclease A induction is located in a gene not previously characterized. The mapping of this mutation and some of its properties are described in this paper.

[1] This publication is Scientific Paper no. 7771 of the Minnesota Agricultural Experiment Station. A preliminary account of this work has been presented (J. F. Koerner and H. R. Warner, Fed. Proc. 30:1263, 1971).

MATERIALS AND METHODS

Growth of bacteria and phages. The phage *amber* mutants (mutants which replicate in *E. coli* CR63 but not in *E. coli* B) were the generous gift of R. S. Edgar and have been described earlier (7, 9). In this paper, the gene mutated is indicated in parentheses after the number of the mutant. The phage deletion mutant *del*(39-56)1, isolated as an *rII* diploid (*r1589/r1236*), was the generous gift of J. Weil and T. Homyk. This mutant is one of several which were shown to contain a deletion lying between genes 39 and 56 (26). The deleted region of *del*(39-56)1 has been physically mapped by Bujard et al. (3), who designated the deleted segment of this mutant Dj. *Amber* mutants were propagated in *E. coli* CR63; other phages were propagated in *E. coli* B. Methods for growing and titering cells and phage have been described (25).

Purification and recombination. A hydroxyurea-sensitive mutant, *nd100*, was isolated as previously described (25). The original isolate was backcrossed once with wild-type T4. The progeny from this cross were assayed for their ability to induce deoxycytidylate hydroxymethylase and T4 exonuclease A as described below. One of the exonuclease A-deficient lines from this cross, designated *nd100'*, was then crossed with various phage bearing single *am* mutations, and the *am* progeny were assayed for capacity to induce exonuclease to determine the per cent recombination of the exonuclease A mutation with the *am* mutations. Crosses were performed by using the procedure described by Edgar (6). This procedure involved infecting permissive host cells with a multiplicity of infection (MOI) of approximately 7.5 of each parental phage and determining the frequency of recombinants among the progeny. KCN was used to prevent superinfection exclusion; T4 antiserum was used to inactivate unad-

sorbed phage. All adsorption tubes and growth tubes were aerated by slow bubbling while incubated at 30 C in a water bath. The *nd*100′ mutant was also crossed with the multiple *am* mutant, *amS2*(39)-*amE51*(56)-*amN81*(41)-*amN82*(44)-*amN130*(46), and the progeny were screened as described by Doermann and Boehner (5). Only progeny resulting from one obvious crossover were selected and assayed for exonuclease to determine which crossovers affected the linkage of the *nd*100′ mutation with its neighboring *am*$^+$ genes.

Preparation of extracts. Ten milliliters of a culture of *E. coli* B on a rotary shaker was infected at an MOI of about 4. After incubation for 15 min at 37 C, the cells were harvested by centrifugation (5 min at 10,000 × *g*). The pellet was suspended in 1 ml of 10 mM tris(hydroxymethyl)aminomethane (Tris)-chloride buffer, *p*H 8.5. Then 0.25 ml of a solution containing 0.2 mg of lysozyme and 0.7 mg of ethylenediaminetetraacetic acid (EDTA) in 125 mM Tris-chloride buffer, *p*H 8.1, was added. The suspension was incubated for 20 min at 0 C, 0.25 ml of 100 mM $MgCl_2$ was added, and the mixture was twice-frozen with dry ice-acetone and thawed. The lysed culture was centrifuged (5 min at 10,000 × *g*), and the supernatant solution was stored frozen.

Enzyme assays. All extracts were assayed for total protein (13) and deoxycytidylate hydroxymethylase (28) to confirm that infection had occurred and that a suitable extract had been obtained. In some cases, the extracts were also assayed for deoxycytidine triphosphatase (17) and dihydrofolate reductase (24).

Exonuclease activity was measured by an automatic system using components of a Technicon autoanalyzer. The system is described in detail in Fig. 1 and is designed to measure the rate of enzymatic formation of dialyzable material from a relatively nondialyzable substrate (2). For these assays, partially degraded DNA, the acid-insoluble oligonucleotide mixture prepared from a partial pancreatic deoxyribonuclease digest of salmon sperm DNA, was used for substrate (15). The exonuclease activity of samples measured by this instrument and converted to standard units, as described in the legend to Fig. 1, was found to agree closely with the values obtained for the same samples by the old manual procedure based on release of acid-soluble material (15, 20, 21).

Chromatographic separation of nucleases. Cultures were centrifuged, and the cells were disrupted by sonic treatment. The extracts were incubated with pancreatic deoxyribonuclease and ribonuclease, and the protein was precipitated by the addition of ammonium sulfate to attain 70% saturation (12). A solution of the 70% ammonium sulfate precipitate was freed of ammonium sulfate by passage through a column of Sephadex G-25 which had been equilibrated with the starting buffer for the subsequent diethylaminoethyl (DEAE) cellulose chromatography. A sample of protein of conductivity approaching that of the starting buffer and containing 100 mg of protein was applied to a DEAE cellulose column (Whatman DE52) of 1-cm^2 area and 24-cm length which had been equilibrated with a solution of 10 mM NaCl-2.5 mM K_2HPO_4-1 mM KH_2PO_4-1 mM trisodium EDTA. The protein was eluted with a concave gradient generated by six identical vessels connected in series (16). The total gradient volume was 324 ml. All vessels were initially filled with the starting buffer and allowed to reach hydrostatic equilibrium. Then the passages between chambers were closed. Solid NaCl was dissolved

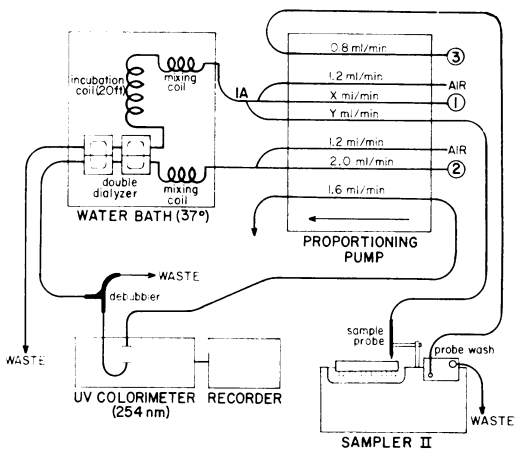

FIG. 1. *Automated system for assay of exonuclease activity. The modules and fittings are autoanalyzer components (Technicon Corporation) except the ultraviolet colorimeter (LKB model 4701 A) and recorder (Sargent model SRLG). The colorimeter was modified so the mercury arc (254 nm) is filtered by a 1-cm layer of 1.75 M $NiSO_4$-0.5 M $CoSO_4$-0.25 mM p-nitrophenol-0.1 M HCl (1), and the effluent from the dialyzer is passed through a flow cuvette of 1-cm light path. Substrate solution (Solution 1) and sample are proportioned to dilute suitably the sample and give a flow rate (X + Y) of 1.7 ml per min. Sample dilutions of 3.5-, 10-, and 40-fold are used routinely. The substrate solution (Solution 1) is made up so the mixture of sample plus substrate solution (Solution 1A) contains enzyme; partially degraded DNA (15), 1 mg/ml; magnesium acetate, 10 mM; Tris-acetate buffer, pH 9.0, 25 mM; EDTA, 0.5 mM; and Brij 58 [polyoxyethylene(20)cetyl ether; Atlas Chemical Industries], 0.05%. The dialysis solution (Solution 2) contains 500 mM NaCl in a solution of the same buffer, salts, and detergent as the incubation mixture. The probe wash solution is 10 mM Tris-acetate buffer, pH 9.0. The combined sample and substrate incubate at 37 C for 9 min in the coils before entering the dialyzer. A fraction of the nucleotides released by action of exonucleases passes into the dialysis solution. The absorbance of this solution is monitored by the ultraviolet colorimeter and automatically recorded. Samples are assayed at a rate of 30 per hr; each sample is pumped for 80 sec, and the probe is then washed for 40 sec. The efficiency of dialysis of nucleotides (approximately 20%) is determined by sampling a solution of dCMP of known absorbance at 254 nm. The colorimeter readings are used to calculate enzyme activity which is expressed in the units previously defined (15). This calculation must take into account the dilution factor used for the assay, the efficiency of dialysis, and the incubation time.*

in vessels 4 and 6 to give final concentrations of 350 mM and 1,000 mM, respectively. (Before NaCl was added to these vessels, a sufficient volume of buffer was removed from them to maintain hydrostatic equilibrium despite the increased density and volume to be engendered by adding the salt.) The passages between the vessels were opened, elution was conducted at a flow rate of 15 ml/hr, and 4-ml fractions were collected. The absorbance of each fraction at 280 nm, its conductivity, and its exonuclease activity were measured.

RESULTS

Identification of an exonuclease-deficient mutant. Of 100 isolates from heavily mutagenized phage stock, four lacked ability to replicate in the presence of hydroxyurea but could replicate in its absence (25). One of these isolates, *nd100*, failed to induce the usual increase in exonuclease activity after infection. As shown in Table 1, experiment 1, extracts of cells infected with *nd100* contain more exonuclease activity than extracts of uninfected cells but much less activity than extracts of cells infected with T4. Assays for other phage-induced enzymes showed that *nd100* induces normal levels of deoxycytidine triphosphatase and dihydrofolate reductase but does not induce deoxycytidylate hydroxymethylase. Because of the heavy mutagenesis used, multiple mutants are likely to be found, but finding a viable mutant defective in deoxycytidylate hydroxymethylase, an essential enzyme (28), is surprising. One possibility is that this mutant induces an altered enzyme which is inactivated under the conditions for in vitro assay but is at least partially active in vivo. This possibility was supported by finding that the *nd100* enzyme was 20% as active as the wild-type enzyme when assayed at 15 C or less.

TABLE 1. *Relative specific activities of enzymes in extracts of Escherichia coli B infected with mutants of phage T4*

Extract	Exonuclease	Deoxycytidylate hydroxymethylase	Deoxycytidine triphosphatase	Dihydrofolate reductase
Expt 1				
T4	1.00	1.00	1.00	1.00
nd100	0.37	0.00	1.10	1.00
Uninfected	0.28	0.00	0.00	0.14
Expt 2				
T4	1.00	1.00		
nd100'	0.28	0.92		
Uninfected	0.23	0.00		
Expt 3				
T4	1.00	1.00		
del(39-56)1	0.41	1.15		
Uninfected	0.18	0.00		

TABLE 2. *Hydroxyurea sensitivity of progeny isolated from a cross of nd100 with wild-type phage T4*

Enzyme activity		Isolates screened[a]		Hydroxyurea sensitivity[b]
Exonuclease	Deoxycytidylate hydroxymethylase	Expt 1	Expt 2	
−	−	7	1	Sensitive
−	+	6	3	Resistant
+	−	0	1	Sensitive
+	+	9	0	Resistant

[a] Twenty-five and twenty progeny were isolated from two different crosses and assayed for enzymatic activity in experiments 1 and 2, respectively, but only the number indicated were screened for hydroxyurea sensitivity.

[b] Resistant phage produce 10 to 15 progeny per infected cell in the presence of 10 mM hydroxyurea at 37 C; sensitive phage fail to replicate (25).

Separation of the exonuclease and deoxycytidylate hydroxymethylase mutations. The *nd100* mutant was crossed with wild-type T4 in an attempt to separate the mutations responsible for exonuclease and deoxycytidylate hydroxymethylase deficiencies. In one experiment, 25 progeny from such a cross included no phage of a phenotype defective for hydroxymethylase but normal for inducing exonuclease; therefore, 20 progeny from a second cross were assayed and one such mutant was found. A total of 27 isolates of different phenotypes were assayed for hydroxyurea sensitivity, and the results are presented in Table 2. In all cases, the phage lacking ability to induce the hydroxymethylase also failed to replicate in the presence of hydroxyurea at 37 C, whereas those inducing this activity all produced 10 to 15 progeny per cell as does wild-type T4 (23). Thus it is clear that the deoxycytidylate hydroxymethylase mutation and not the exonuclease mutation of *nd100* is responsible for its hydroxyurea sensitivity.

One recombinant line deficient for induction of exonuclease was designated *nd100'* and was used for further experiments. As shown in Table 1, experiment 2, this mutant induces a normal level of deoxycytidylate hydroxymethylase. Also shown in Table 1, experiment 3, are data for *del*(39-56)1, a mutant which, as discussed below, was suspected to bear a deletion mapping in the same region of the T4 chromosome as *nd100'*.

Confirmation of T4 exonuclease A deficiency. Since exonuclease A is the major exonuclease activity assayed in extracts of cells infected with T2 or T4 (8), it was presumed that deficiency of

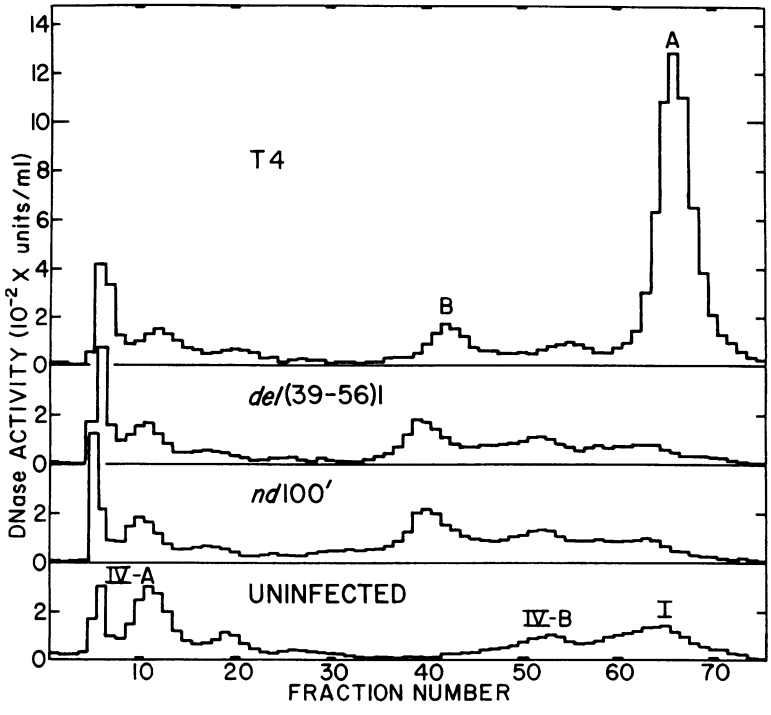

FIG. 2. *DEAE cellulose chromatography of protein fractions from uninfected E. coli B and E. coli B infected with T4, nd100', and del(39-56)1 phage. Peaks IVA, IVB, and I correspond to E. coli exonucleases IVA, IVB, and I, respectively (12). Peaks B and A correspond to phage-induced DNA polymerase and exonuclease A, respectively (20, 21).*

this enzyme accounted for the exonuclease deficiency of cells infected with *nd100'*. This was confirmed by chromatography of extracts on DEAE cellulose under conditions in which the major exonuclease activities are separated. The results of this experiment are shown in Fig. 2. The only significant difference between extracts infected with T4 and *nd100'* is the complete absence of exonuclease A in the latter. The chromatographic profiles of *nd100'* and *del*(39-56)1 were identical. These chromatographic data suggest that most of the small increase of exonuclease activity observed in crude extracts of cells infected with *nd100'* or *del*(39-56)1 can be attributed to the exonuclease accompanying T4-induced DNA polymerase (10, 20).

Mapping of the nd100' mutation. Since no measurable phenotypic difference was found between T4 and *nd100'* except the exonuclease deficiency in extracts infected with the latter phage, mapping of this mutant could be accomplished only by using exonuclease assays. The gene responsible for this phenotype was designated *dexA* (for DNA exonuclease A), in accordance with the suggestion of Hercules et al. (11).

Three procedures were used for locating the *dexA* gene with reasonable accuracy without the necessity for preparing a large number of extracts for enzyme assays. The first experiments used crosses between phage bearing single *amber* mutations and the phage *nd100'*. As shown in Table 3, experiment 1, the results of such crosses with four *amber* mutants suggested that the *dexA* gene is located near gene 56. More extensive analysis of progeny from crosses between *nd100'* and *amber* mutants of genes 39 and 56 showed that *dexA* maps between these two genes, with considerable uncertainty as to a more precise location (Table 3, experiment 2). [Ten experimental crosses of *amE56*(56) with *amN116*(39) (see Table 6), in which a total of 2,543 plaques were scored, gave a recombination frequency of 0.187 ± 0.023. The observed standard deviation can be attributed in part to random sampling error, with a calculated standard deviation of 0.008, and to other experimental variables which are presumed to contribute a standard deviation of 0.022. For the crosses of *nd100'*(*dexA*) with *amE51*(56) and with *amN116*(39) (Table 3), an estimate of their standard deviations can be made by assuming that the standard deviation

TABLE 3. *Mapping of the dexA gene*

Cross	No. of isolates screened	No. of double mutants	Recombination frequency
Expt 1			
amH39 (gene 30) × *nd100'*	24	5	0.21
amA453 (gene 32) × *nd100'*	22	4	0.18
amE51 (gene 56) × *nd100'*	25	0	<0.04
amN82 (gene 44) × *nd100'*	25	3	0.12
Expt 2			
amN116 (gene 39) × *nd100'*	100	5	0.05
amE51 (gene 56) × *nd100'*	99	8	0.08

due to experimental variables is proportional to the recombination frequency observed (0.022/0.187 × recombination frequency) and combining the variance derived therefrom with the variance due to sampling error. (If this assumption is valid, the sampling error for these crosses greatly outweighs the experimental variation.) Then the sum of these frequencies and its standard deviation are 0.115 ± 0.032. This result can be tested statistically for consistency with recombination data for a cross between *amE51* and *amN116*, the recombination frequency of this cross being first augmented by a factor derived from the four-parameter switch model of Stahl et al. (22) to correct for negative interference. The best available estimate for this value and its standard deviation are 0.234 ± 0.029. This was actually derived from the recombination data between *amE56* and *amN116* mentioned above and can be used for this purpose since *amE51* and *amE56* do not recombine (29). Statistical analysis of these results indicates a probability for the observed experimental outcome (Table 3) of only 0.006. This suggests that there may be an experimental circumstance, as yet unidentified, which results in an underestimate of the number of recombinants of *nd100'* with the *amber* mutants. Statistical analysis justifies including both experiments presented in Table 3 for an estimate of the recombination frequency of *amE51* with *nd100'*. The probability of occurrence of the observed experimental result, if both samples were derived from the same parent population, is 0.15.]

The second procedure for mapping involved crossing *nd100'* with the multiple mutant *amS2*(39)-*amE51*(56)-*amN81*(41)-*amN82*(44)-*amN130*(46). [These mutations are listed in their clockwise order on the standard T4 genetic map (7).] As shown in Table 4, progeny with various *amber* genotypes were isolated and assayed for exonuclease activity. The data indicated that crossovers between genes 39 and 56 most drastically affect the distribution of the *dexA* mutation in the progeny isolated. These data are in agreement with the distribution predicted from the results presented in Table 3. The two $dexA^+$ and one $dexA^-$ recombinants found in the $++---$ and $---++$ progeny, respectively, probably originated from double crossovers in the longer interval between genes 39 and 56. The data from this experiment can be used to calculate the location of the *dexA* gene more precisely than is possible using the data presented in Table 3, since a total of 37 recombinants are available for these calculations.

The third procedure for mapping was the use of a deletion mutant characterized by Weil and Terzaghi (26). Their mutant *del*(39-56)1 was obtained from the progeny of a cross between *r1589* and *r1236*. The r^+ diploid parental strain carrying *del*(39-56)1 contains both a duplication of the *rII* region and a deletion of the region between genes 39 and 56; its *r1589* haploid segregant produces a plaque about two-thirds the diameter of the normal *rII* plaque. The existence of a mutant with a deletion between genes 39 and 56 confirms that this region includes nonessential genes, a result previously suspected because of the absence of conditionally lethal *am* or *ts* markers. Assays for exonuclease activity in crude extracts of cells infected with *del*(39-56)1 indicated that this mutant fails to induce T4 exonuclease A (Table 1, experiment 3), and chromatography of these extracts confirmed this result (Fig. 2). The deletion Dj of *del*(39-56)1 has been physically mapped by Bujard et al. (3), whose map thus provides an approximate location for the *dexA* gene derived independently from re-

TABLE 4. *Mapping the dexA gene by characterization of progeny from a cross of nd100' with amS2(39)-amE51(56)-amN81(41)-amN82(44)-amN130(46)*

Progeny *am* genotype	No. screened	Progeny phenotype		% ExoA+	
		ExoA+	ExoA−	Found	Predicted[a]
+−−−−	18	3	15	17	45
−++++	19	12	7	63	55
++−−−	11	2	9	18	0
−−+++	7	7	0	100	100
+++−−	5	0	5	0	0
−−−++	11	10	1	91	100
++++−	5	0	5	0	0
−−−−+	5	5	0	100	100

[a] From data in Table 3, experiment 2.

combination data; this is based on the assumption that the exonuclease A deficiency of these mutants is caused by intrusion of the deletion into the *dexA* gene.

We found that three other deletion mutants [*del*(39-56)2, *del*(39-56)3, and *del*(39-56)4] (furnished by J. Weil and T. Homyk) also fail to induce exonuclease A, and these also map between genes 39 and 56 (Homyk and Weil, *personal communication*).

Effect of dexA mutations on breakdown of host DNA. Both *nd100'* and *del*(39-56)1 induce enzymatic apparatus sufficient to effect breakdown of the host DNA. The fact that cells infected with these mutants produce progeny in the presence of hydroxyurea suggests at least partial activity of this pathway. Additional evidence for breakdown is that phage bearing a double mutation of genes 44 and *dexA* convert labeled host DNA to acid-soluble material (Fig. 3). The gene 44 mutation blocks synthesis of viral DNA from material derived from host DNA, so the integrity of the degradative pathway can be easily measured experimentally in these double mutants (25).

Effect of dexA mutations on recombination. The effect of the presence of the *nd100'* mutation on the frequency of recombination between phage bearing *amber* mutations was investigated. As shown in Table 5, the recombination frequency between mutants of the widely separated genes 32 and 44 was not altered significantly by the presence of this *dexA* mutation. Likewise, this mutation did not influence the recombination frequency between two closely linked *amber* mutants of gene 32.

The effect of the deletion mutant, *del*(39-56)1, on recombination was also investigated, and the results are summarized in Table 6. The results indicate that this deletion has little, if any, effect on recombination between *amber* mutants of genes 56 and 44, two markers located to one side of the deletion on the T4 map. These results make it unlikely that T4 exonuclease A is essential for normal genetic recombination. As would be expected, however, the removal of a definite region of the genome by introduction of this deletion between mutants of genes 39 and 56 reduces their recombination frequency.

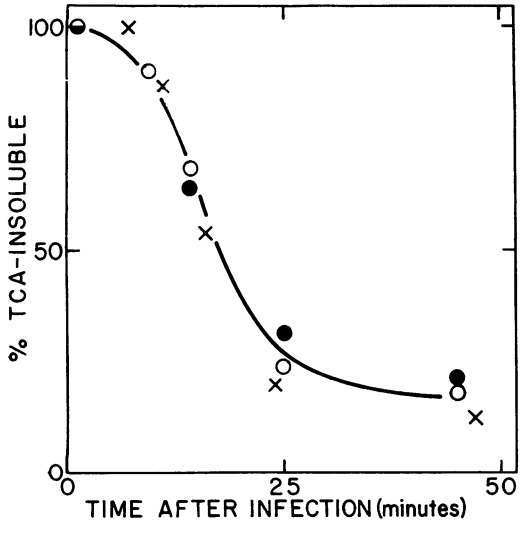

FIG. 3. *Degradation of host DNA to acid-soluble material after phage infection. The E. coli B DNA was labeled with ^{14}C-thymidine before infection. The multiplicity of infection was 5. The percentage of acid-insoluble labeled DNA remaining at various times after infection was measured. Cultures were infected with the T4 mutant amN82(44) (●) and the double mutants amN82(44)-nd100'(dexA) (○) and amN82(44)-del(39-56)1 (×). Results of this assay for other nuclease-deficient mutants have been described (25).*

DISCUSSION

Physiological properties of nd100. The mutant *nd100* originally was detected because of its failure to produce progeny in the presence of hydroxyurea. Hydroxyurea sensitivity should, from consideration of the metabolism of infected cells (25), be useful for identifying phage which induce defective degradation of host DNA, and this expectation has been supported by practical results (11, 19, 25). However, the results of the study reported here suggest a different mechanism

TABLE 5. *Effect of nd100' on recombination*

Cross	Avg. burst size[a] (phage/bacterium)	Recombination frequency[a]
amN82 (gene 44) × *amA453* (gene 32)	558	0.31, 0.29
nd100'-amN82 × *nd100'-amA453*	562	0.31, 0.31
amH18 (gene 32) × *amA453* (gene 32)	756	0.0059, 0.0057 (Expt 1)
	460	0.0057, 0.0070 (Expt 2)
nd100'-amH18 × *nd100'-amA453*	707	0.0047, 0.0046 (Expt 1)
	532	0.0052, 0.0056 (Expt 2)

[a] Each cross was performed twice in each experiment.

TABLE 6. *Effect of del(39-56)1 on recombination*

Cross	Avg. burst size[a] (phage/bacterium)	Recombination frequency[b]
amE56 (gene 56) × *amN82* (gene 44)	237	0.213
del(39-56)1-*amE56* × *del*(39-56)1-*amN82*	205	0.195
amE56 (gene 56) × *amN116* (gene 39)	273	0.187
del(39-56)1-*amE56* × *del*(39-56)1-*amN116*	189	0.095

[a] Since phage development is much slower in crosses homozygous for *del*(39-56)1, comparable burst sizes were obtained by inducing premature lysis with chloroform. In the *am* × *am* crosses, portions were lysed at 1-min intervals at 34 to 38 min after dilution to the growth tube. In the analogous crosses homozygous for *del*(39-56)1, portions were lysed at 5-min intervals at 50 to 70 min after dilution to the growth tube.

[b] Each value is the average recombination frequency obtained from 10 measurements, 5 in each of two experiments.

for the hydroxyurea sensitivity of *nd100*. Of two separable genetic defects identified in this mutant, hydroxyurea sensitivity was found to be associated clearly with recombinants defective for induction of deoxycytidylate hydroxymethylase and not with those defective for induction of T4 exonuclease A. The mechanism for hydroxyurea sensitivity of a deoxycytidylate hydroxymethylase mutant is not apparent. One possibility is that the altered enzyme induced by *nd100* is unstable in vivo in the presence of hydroxyurea due to a low intracellular level of deoxycytidylate; this nucleoside monophosphate has previously been shown to stabilize this enzyme (27).

Physiological properties of dexA mutants. Some physiological data were obtained for two mutants presumed to have defective *dexA* genes, the hydroxylamine-induced mutant *nd100'* and the deletion mutant *del*(39–56)1. Both mutants induce progeny production in the presence of hydroxyurea. There appears to be considerably impaired production by *del*(39–56)1 in the presence of this antimetabolite. It is unclear whether this sensitivity is due to the lack of function of *dexA* or another adjacent unknown gene involved in host DNA breakdown. However, it is evident that an appreciable conversion of host DNA to progeny DNA does occur in cells infected with either of these mutants. In the case of the mutant *nd100'* the possibility of activity in vivo of an altered exonuclease A must be considered; in the case of *del*(39–56)1, this circumstance is improbable. There is also the possibility that exonuclease A normally functions in host DNA degradation but that other enzymes such as *E. coli* exonuclease IV-A or the exonuclease activity of T4-induced DNA polymerase may adequately substitute in its absence (8). Thus the role of T4 exonuclease A in host DNA degradation remains obscure although infected cells defective in this function are now available for study. With regard to other possible unique roles for T4 exonuclease A, only participation in genetic recombination has been examined. Although exonuclease A is clearly not essential for this role, there again remains the possibility that other intracellular enzymes may substitute for its normal function.

Other than exonuclease deficiency, only one distinctive physiological feature is known for these mutants. This is the characteristic plaque morphology, the *minute* phenotype, exhibited by *del*(39–56)1 (26). No evidence is presently available to suggest whether this feature is caused by the lack of function of *dexA* or an unknown adjacent gene of the T4 chromosome.

Mapping of dexA. The available data pertaining to the mapping of *dexA* are summarized in Fig. 4. These data are all consistent with the location of this gene between genes 39 and 56. The possibility that *dexA* lies outside but adjacent to this region would be difficult to support with the data of Table 3, plotted on map C, although these data provide only a rough approximate location within this region. Also supporting a location between genes 39 and 56 are the more reliable data of Table 4, plotted on map D. Consistent with these results is the fact that the deletion mutant *del* (39–56)1 fails to induce T4 exonuclease A. As shown in Fig. 4, the location of the *dexA* gene, as deduced from the data plotted on either map C or map D, can be made congruent with this deletion as plotted on map B.

The data summarized in Fig. 4 also illustrate consistencies and discrepancies pertaining to our knowledge of the location of other genes of this region of the T4 chromosome. A discrepancy between the locations of the *rII* region and gene 60 derived from the recombination map of Edgar and Wood (7) (map A) and the physical mapping of *rII* by Bujard et al. (3) (map B) is evident. Also, the distance between genes 39 and 56 has not been precisely defined; however, here the discrepancies appear to be well within the statistical errors of the various methods. The recombination map of Edgar and Wood (7), plotted as map A, suggests a separation of 14,000 base pairs between these genes. Our recombination data, based on 2,543 recombinants between *amE56*(56) and *amN116*(39), give a separation of 11,400 base pairs (map C). These data are closely comparable

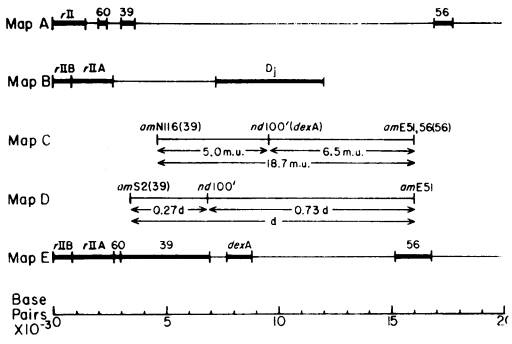

FIG. 4. *Location of dexA on the T4 genetic map. Map A, a portion of the T4 genome as diagrammed by Edgar and Wood (7) from recombination data. Map A was drawn to scale from their map assuming a total T4 genome of 2 × 10⁵ base pairs. Map B, the location of the deletion Dj [del(39-56)1] and the rII region as physically mapped by Bujard et al. (3). Map C, the location of nd100' obtained by recombination data (Table 3). The value of 11,400 base pairs for the distance between amE56(56) and amN116(39) was calculated from the data of Table 6, using the four-parameter switch model of Stahl et al. (22). The gene 56 mutants amE51 and amE56 are known not to recombine (29). Map C was arbitrarily centered over the corresponding region of Map A. Map D, the location of nd100' obtained by recombination frequencies of progeny of a multiple mutant (Table 4). The location of amE51 was copied from Map C. The distance between amS2(39) and amE51(56) was made 12,600 base pairs (4). (The data used for plotting Maps C and D cannot be used to deduce reliably the order of amS2 and amN116 within gene 39). Map E, an idealized diagram of this portion of the phage T4 genome. The rII cistrons were copied from Map B. Gene 60 was assigned a length of 300 base pairs. Gene 39 was arbitrarily assigned a length of 4,000 base pairs and genes 39, 60, and rII were made contiguous. The ends of genes 39 and 56 nearest rII were separated by 12,200 base pairs (14) and the length of gene 56 was made 1,600 base pairs. The center of the gene dexA was plotted 0.3 of the distance between the centers of genes 39 and 56, and the length of dexA was made 1,100 base pairs. (The data used for assigning lengths to the various genes are discussed in the text). It should be emphasized that other genes may occur between rII and gene 39, and dexA may also abut gene 39; the diagram shows one plausible arrangement based on available evidence.*

to Mosig's value of 12,200 base pairs for the separation between the ends of genes 39 and 56 which lie closest to *rII* (14), and Childs' value of 12,600 base pairs between *amS2* in gene 39 and *amE51* in gene 56 (4).

Although the results from these four sources are in close agreement on the distance between genes 39 and 56, the value given by Mosig (14) of 10,400 base pairs for the distance between *r73* (*rIIB*) and that portion of gene 39 nearest *r73* whose removal causes loss of function is higher than the value of 6,200 base pairs found by Childs (4) for the distance between the left-hand end of *rIIB* (Fig. 4) and *amS2* in gene 39. It is also higher than would be expected from the recombination map of Edgar and Wood (7) and the physical map of Bujard et al. (3). According to the latter, the *rII* distal end of gene 39 should not be more than 7,245 base pairs from *r73*. If gene 39 is as large as 4,000 base pairs (see below), then the *rII* proximal end of gene 39 should not be more than 3,245 base pairs from *r73*. Mosig's estimate of this distance (10,400 base pairs) would suggest that *del*(39–56)1 (or Dj) is not located between genes 39 and 56, an assignment in conflict with the conclusion of Weil and Terzaghi (26) and our data of Table 6. It would also render invalid the hypothesis that the exonuclease A deficiency of *del*(39–56)1 is caused by intrusion of this deletion into the *dexA* gene.

On the basis of extensive intragenic recombination data, the size of gene 60 is now estimated to be 300 base pairs, and gene 39 appears to be very large; these two genes probably occupy most of the region between *rII* and the deletion *del*(39-56)1 (S. Mufti and H. Bernstein, *personal communication*). Gene 56 is known to be the structural gene for deoxycytidine triphosphatase (17), an enzyme with a molecular weight of about 59,000 (18). If this protein is composed of a single peptide chain, it requires a structural gene of approximately 1,600 base pairs. An approximate molecular weight of 40,000 for T2 exonuclease A has been reported (21); if the *dexA* gene proves to be the structural gene for an enzyme of comparable size, it will require approximately 1,100 base pairs.

These tentative estimates of gene size and locations are summarized in Fig. 4, map E. It is apparent that much available data can be compiled to give an accordant map of the region between *rII* and gene 56, although some aspects of this map may be considerably modified in the future. Certainly the region between genes 39 and 56, until recently of entirely unknown function, may accommodate nonessential structural genes for as many as 10 additional proteins. The *dexA* marker may be useful for mapping mutants of other adjacent genes when such mutants are found. It should be particularly useful for detecting and locating deletions in this region.

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LITERATURE CITED

1. Backström, H. L. J. 1940 Über die Ultraviolett-Absorption der Kobaltsalze und über einige Lichtfilter für das ultraviolette Strahlengebiet. Arkiv Kemi Mineral. Geol. 13A (No. 24):1-17.
2. Barrera, H., K. S. Chio, and A. L. Tappel. 1969. Automation of enzyme reactions: ribonuclease. Anal. Biochem. 29:515-525.
3. Bujard, H., A. J. Mazaitis, and E. K. F. Bautz. 1970. The size of the *r*II region of bacteriophage T4. Virology 42: 717-723.
4. Childs, J. D. 1971. A map of molecular distances between mutations of bacteriophage T4D'. Genetics 67:455-468.
5. Doermann, A. H., and L. Boehner. 1970. The identification of complex genotypes in bacteriophage T4. I. methods. Genetics 66:417-428.
6. Edgar, R. S. 1963. Some technical considerations concerning experiments on phage recombination, p. 19-36. *In* W. J. Burdette (ed.), Methodology in basic genetics. Holden-Day, Inc., San Francisco.
7. Edgar, R. S., and W. B. Wood. 1966. Morphogenesis of bacteriophage T4 in extracts of mutant-infected cells. Proc. Nat. Acad. Sci. U.S.A. 55:498-505.
8. Elam, J. S., and J. F. Koerner. 1970. Sources of the nucleotide pools in *Escherichia coli* after infection with bacteriophage T2. J. Biol. Chem. 245:1012-1019.
9. Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Susman, G. H. Denhardt, and A. Lielausis. 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. 28:375-392.
10. Goulian, M., Z. J. Lucas, and A. Kornberg. 1968. Enzymatic synthesis of deoxyribonucleic acid. XXV. Purification and properties of deoxyribonucleic acid polymerase induced by infection with phage T4. J. Biol. Chem. 243: 627-638.
11. Hercules, K., J. L. Munro, S. Mendelsohn, and J. S. Wiberg. 1971. Mutants in a nonessential gene of bacteriophage T4 which are defective in the degradation of *Escherichia coli* deoxyribonucleic acid. J. Virol. 7:95-105.
12. Jorgensen, S. E., and J. F. Koerner. 1966. Separation and characterization of deoxyribonucleases of *Escherichia coli* B. I. Chromatographic separation and properties of two deoxyribo-oligonucleotidases. J. Biol. Chem. 241:3090-3096.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
14. Mosig, G. 1968. A map of distances along the DNA molecule of phage T4. Genetics 59:137-151.
15. Oleson, A. E., and J. F. Koerner. 1964. A deoxyribonuclease induced by infection with bacteriophage T2. J. Biol. Chem. 239:2935-2943.
16. Peterson, E. A., and H. A. Sober. 1959. Variable gradient device for chromatography. Anal. Chem. 31:857-862.
17. Price, A. R., and H. R. Warner. 1968. A structural gene for bacteriophage T4-induced deoxycytidine triphosphate-deoxyuridine triphosphate nucleotidohydrolase. Virology 36:523-526.
18. Price, A. R., and H. R. Warner. 1969. Bacteriophage T4-induced deoxycytidine triphosphate-deoxyuridine triphosphate nucleotidohydrolase: its properties and its role during phage infection of *Escherichia coli*. Virology 39: 882-892.
19. Sadowski, P. D., H. R. Warner, K. Hercules, J. L. Munro, S. Mendelsohn, and J. S. Wiberg. 1971. Mutants of bacteriophage T4 defective in the induction of T4 endonuclease II. J. Biol. Chem. 246:3431-3433.
20. Short, E. C., Jr., and J. F. Koerner. 1965. Separation of deoxyribo-oligonucleotidases induced by infection with bacteriophage T2. Proc. Nat. Acad. Sci. U.S.A. 54:595-600.
21. Short, E. C., Jr., and J. F. Koerner. 1969. Separation and characterization of deoxyribonucleases of *Escherichia coli* B. II. Further purification and properties of an exonuclease induced by infection with bacteriophage T2. J. Biol. Chem. 244:1487-1496.
22. Stahl, F. W., R. S. Edgar, and J. Steinberg. 1964. The linkage map of bacteriophage T4. Genetics 50:539-552.
23. Warner, H. R., and M. D. Hobbs. 1969. Effect of hydroxyurea on replication of bacteriophage T4 in *Escherichia coli*. J. Virol. 3:331-336.
24. Warner, H. R., and N. Lewis. 1966. The synthesis of deoxycytidylate deaminase and dihydrofolate reductase and its control in *Escherichia coli* infected with bacteriophage T4 and T4 *amber* mutants. Virology 29:172-175.
25. Warner, H. R., D. P. Snustad, S. E. Jorgensen, and J. F. Koerner. 1970. Isolation of bacteriophage T4 mutants defective in the ability to degrade host deoxyribonucleic acid. J. Virol. 5:700-708.
26. Weil, J., and B. Terzaghi. 1970. The correlated occurrence of duplications and deletions in phage T4. Virology 42: 234-237.
27. Wiberg, J. S., and J. M. Buchanan. 1964. Studies on labile deoxycytidylate hydroxymethylases from *Escherichia coli* B infected with temperature-sensitive mutants of bacteriophage T4. Proc. Nat. Acad. Sci. U.S.A. 51:421-428.
28. Wiberg, J. S., M.-L. Dirksen, R. H. Epstein, S. E. Luria, and J. M. Buchanan. 1962. Early enzyme synthesis and its control in *E. coli* infected with some *amber* mutants of bacteriophage T4. Proc. Nat. Acad. Sci. U.S.A. 48:293-302.
29. Wiberg, J. S. 1967. Amber mutants of bacteriophage T4 defective in deoxycytidine diphosphatase and deoxycytidine triphosphatase. On the role of 5-hydroxymethylcytosine in bacteriophage deoxyribonucleic acid. J. Biol. Chem. 242:5824-5829.