JOURNAL OF VIROLOGY, Jan. 1972, p. 184-186
Vol. 9, No. 1

Genetic Location of a Mutant of Bacteriophage T4 Deficient in the Ability to Induce Endonuclease II

PURNIMA RAY, NAVIN K. SINHA, HUBER R. WARNER, AND D. PETER SNUSTAD

Department of Genetics and Cell Biology and Department of Biochemistry, College of Biological Sciences, University of Minnesota, St. Paul, Minnesota 55101

Received for publication 30 September 1971

Reciprocal three-factor crosses and the use of a partial revertant of a putative ribonucleotide reductase mutant of *Escherichia coli* B/5 as indicator have made it possible to map *denA* (deficient in endonuclease II) between *nrd-11* (ribonucleotide reductase gene B) and *amM69* (gene 63) on the bacteriophage T4 chromosome.

Preliminary studies (4) indicated that the nonessential gene *denA* (2), which has been identified by several mutations resulting in the inability to induce endonuclease II (3), maps between gene 32 and gene 63 on the bacteriophage T4 chromosome. Since this region is known to contain at least four other nonessential genes, *frd* (dihydrofolate reductase), *td* (thymidylate synthetase), and *nrdA* and *nrdB* (two ribonucleotide reductase cistrons; reference 5 and Tessman, *personal communication*), it was of interest to determine the location of *denA* relative to these other nonessential genes, particularly since the latter all control functionally related products.

It became possible to map *denA* relative to ribonucleotide reductase mutants *nrd-10* and *nrd-11* with the isolation of a temperature-sensitive hydroxyurea-resistant mutant of *Escherichia coli* B/5 which permitted us to distinguish wild-type T4 plaques from *nrd-10* and *nrd-11* plaques. This *E. coli* mutant, designated NS214, was isolated as a putative ribonucleotide reductase mutant and is presently being characterized in this respect (N. K. Sinha, *unpublished data*). It is *amber*-restrictive like B/5. When a partial revertant (less temperature-sensitive) of this putative ribonucleotide reductase mutant is used as plating indicator and the plates are incubated at 40 C, wild-type plaques are easily distinguished from *nrd-10* and *nrd-11* plaques (Fig. 1) and *nd-28* plaques (4); *nrd-18* plaques are indistinguishable from wild-type plaques under these conditions.

Our preliminary studies (4) indicated that *nd-28* (gene *denA*) was closely linked to *amM69* (gene 63). Yeh et al. (5) and Tessman (*personal communication*) have shown that *nrd-11* is also closely linked to *amM69*. We have therefore performed reciprocal three-factor crosses to determine the order of *nrd-11*, *nd-28*, and *amM69* relative to *amA453* (gene 32) and *amN54* (gene 31). The results of these three-factor crosses (Table 1) indicate the order: *amA453–nrd-11–nd-28–amM69–amN54*. Extensive two-factor cross

TABLE 1. *Reciprocal three-factor cross data*

Crosses	Per cent recombination[a]	Order indicated
amA453 – amM69 × nrd-11	2.6	*amA453 – nrd-11 – amM69*
amA453 – nrd-11 × amM69	4.2	
amA453 – amM69 × nd-28	2.5	*amA453 – nd-28 – amM69*
amA453 – nd-28 × amM69	3.5	
amA453 – nd-28 × nrd-11	2.1	*amA453 – nrd-11 – nd-28*
amA453 – nrd-11 × nd-28	2.9	
amN54 – amM69 × nrd-11	3.5	*nrd-11 – amM69 – amN54*
amN54 – nrd-11 × amM69	1.9	
amN54 – amM69 × nd-28	2.7	*nd-28 – amM69 – amN54*
amN54 – nd-28 × amM69	1.7	
amN54 – nd-28 × nrd-11	2.9	*nrd-11 – nd-28 – amN54*
amN54 – nrd-11 × nd-28	1.9	

[a] Wild-type recombinants were determined on *Escherichia coli* strain NS214 and total progeny were determined on CR63. Each value is the average of two crosses. All crosses were performed in log-phase CR63 cells by the procedures of Edgar (1).

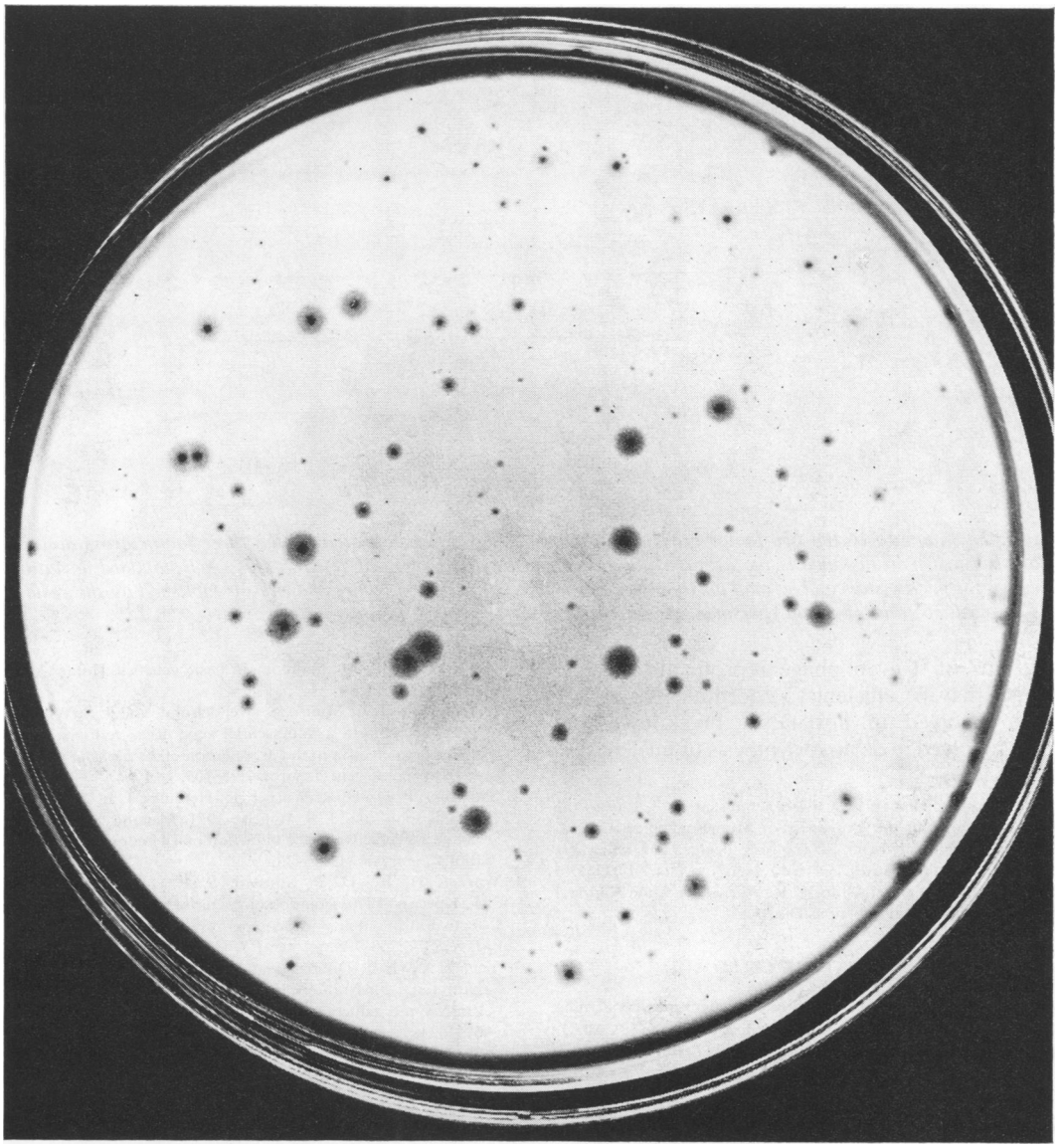

FIG. 1. *Comparative plaque size of T4 nrd+, nrd-10, and nrd-11 on E. coli strain B/5 isolate NS214 after incubation at 40 C. The wild-type plaques are much larger than the nrd-11 plaques which in turn are larger than the nrd-10 plaques. In reconstruction experiments, approximately 4% of the small plaques scored as nrd were actually nrd+; thus, map distances derived from these data are slightly underestimated.*

data, summarized in Fig. 2, are also consistent with the above order. These data along with the results of Yeh et al. (5) and Tessman (*personal communication*) establish the following sequence of markers between genes 31 and 32 on the T4 chromosome: gene 32–*frd-1–td-8–nrd-18–nrd-10–nrd-11–nd-28–amM69–cd-9*–gene 31.

A question of particular interest is whether genes controlling functionally related products are contiguous to facilitate coordinate regulation. If this is the case, it might seem unusual to have a gene controlling a catabolic function, such as endonuclease II, map in a cluster of genes controlling anabolic functions. As far as phage T4 is concerned, however, endonuclease II may be just another enzyme involved in the production of precursors for deoxyribonucleic acid (DNA) synthesis, since nucleotides produced by breakdown of host DNA are incorporated into progeny DNA just as efficiently as are those synthesized de novo.

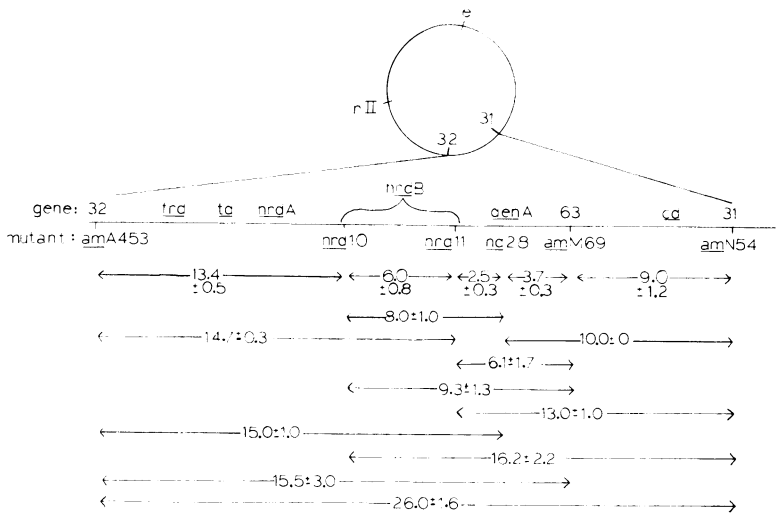

FIG. 2. *Summary of the two-factor cross data, assuming the order indicated by the three-factor cross data of Table 1. Each map distance is the average value obtained in either three or four crosses; ±1 standard deviation is shown for each distance. The map positions of genes frd, td, nrdA, and cd are shown as established by the results of Yeh et al. (5) and Tessman (personal communication).*

In terms of T-even phage reproduction, therefore, it may be efficient to coordinately regulate genes involved in host-DNA breakdown and genes involved in de novo synthesis of nucleotides.

These results are part of a thesis submitted by P. Ray to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the M.S. degree. This work was supported by Public Health Service research grants AI-07946 to D. P. Snustad and AI-07898 to H. R. Warner from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. 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.
2. 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.
3. 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.
4. 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.
5. Yeh, Y. C., E. J. Dubovi, and I. Tessman. 1969. Control of pyrimidine biosynthesis by phage T4: mutants unable to catalyze the reduction of cytidine diphosphate. Virology 37: 615–623.