Presumptive D2a Point Mutants of Bacteriophage T4

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Mutants of bacteriophage T4 have been isolated that show phenotypes seen previously with those *rII* deletions that extend into the nearby D2a region. Analysis indicates that *rII* mutations are not necessary in order to get the D2a phenotypes.

We have recently reported a number of phenotypes that are associated with the presence of certain extended *rII* deletions in bacteriophage T4. These phenotypes deal with (i) the stability of phage cytosine-containing DNA (C-DNA), made by gene 56 mutants (1), (ii) the degradation of the host chromosome (3), and (iii) effects on DNA synthesis in an *in vitro* system (J. Elliott, M.A. thesis, Rice University; J. Elliott, C. Richter, A. Souther, and R. Bruner, submitted for publication). The *rII* deletions that result in these phenotypes are those that extend through the adjacent D1 region into the D2a region (see Fig. 1 of ref. 1). That is, mutations such as *rIIH23*, which extend into D2a, result in these phenotypes, whereas deletion mutations such as *rII1241*, which do not extend into D2a, do not result in these phenotypes, and thus in this regard are like *rII*⁺. Thus, we have inferred that the phenotypes under consideration are due to D2a mutations; we refer to the extended *rII* deletions such as *rIIH23* as "D2a mutations", and to the phenotypes as "D2a phenotypes". However, as we have pointed out in each of our preceding papers, we have not been able to exclude the possibility that *rII* or D1 mutations, or both, are necessary, in addition to D2a mutations, in order to produce the D2a phenotypes. This is because the only D2a mutations available to us were those extended *rII* deletions.

As the number of D2a phenotypes increased, it seemed worthwhile to attempt to isolate new D2a mutations, hopefully point mutations, so that the genetics of the D2a phenotypes could be resolved. Additionally, we hoped to isolate mutant phages that are temperature-sensitive

for the D2a phenotypes; such phages would be useful for a number of experiments.

We report here the isolation of three presumptive D2a point mutants and their preliminary characterization. The results so far suggest that D2a mutations alone are necessary for the D2a phenotypes. We are reporting these preliminary observations now since none of us will be continuing to work on this project. We will be happy to make available to others additional details of our work, as well as any of our phage strains, including the partially characterized new mutants described below. Inquiries should be directed to R. Bruner at Cetus Scientific.

Since no method of selecting for D2a mutants is known, survivors of heavy mutagenesis were individually screened for a D2a phenotype. The D2a phenotype best adapted to the screening is the stabilization of phage C-DNA (1). In order to increase the chance of getting a D2a mutation if, in fact, other mutations are also required to produce the D2a phenotype, two different starting phage strains were subjected to the mutagenesis and screening program. One simply carried a gene 56 mutation: *amE51*, referred to as *am56*. The other carried, in addition, an *rII* deletion mutation that deletes *rII* and D1, but not D2a, and that does not lead to D2a phenotypes: *am56-rII1241* (see Fig. 1 of ref. 1).

Phage were treated with hydroxylamine, according to the procedure of Tessman (4), for 93 h, resulting in about $7 \times 10^{-4}\%$ survivors. Survivors were plated on *Escherichia coli* CR63. Plaques were picked, resuspended in broth, and replated on CR63. Stocks were grown from single plaques. After overnight growth, chloroform and DNase (5 $\mu\text{g/ml}$) were added to the stocks. For the quick, initial screening, 0.1 ml of such stocks was used to infect 1 ml of bacteria. DNA synthesis (at 37 C) was measured as described by Bruner et al. (1). Stocks that

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appeared to be potential D2a mutants, i.e., that showed more DNA synthesis than expected from the number of surviving bacteria, were then tested more carefully. By this method two presumptive D2a mutants were obtained from *am56-rII1241*, and one presumptive D2a mutant was obtained from *am56* (out of 12 survivors tested in each case). Except as noted below, these strains have not been backcrossed to nonmutagenized stocks to free them of extraneous mutations. We briefly note the following about these mutants. All showed the dependence of DNA synthesis on the multiplicity of infection that we find characteristic of *am56*-D2a phage (Table 1; cf. Table 3 of ref. 1). All showed the D2a phenotype at 30, 37, and 42 C. We have no reason to doubt that more extensive searches may turn up temperature-sensitive or cold-sensitive D2a mutants, particularly if the temperature of initial screening is different. All retained the *am56* mutation. Judging by the frequency of *am*⁺ revertants in the stocks (about 10⁻⁴%), they did not contain additional amber mutations in essential genes.

Two of the presumptive D2a mutants have been tested for stimulation of DNA synthesis in vitro (J. Elliott et al., submitted for publication). One (D2a6) clearly showed the stimula-

tion expected for D2a mutations. The other (D2a2) showed a smaller stimulation.

Although the genetic characterization of these strains is quite preliminary, the following arguments suggest that D2a mutations alone are responsible for the D2a phenotypes. (i) Hydroxylamine probably causes only point mutations (2). (ii) D2a mutants were obtained readily from the *am56* phage, as well as from *am56-rII1241*. (iii) The D2a mutant obtained from *am56* plates normally on lambda lysogens; it thus appears to be *rII*⁺. We cannot test for D1 mutations. (iv) One of the D2a mutants isolated from *am56-rII1241* was crossed with *rII*⁺ phage. Among the progeny could be found *am56-rII*⁺ phage which showed the D2a phenotype (see below).

Are our presumptive D2a mutations actually in the D2a gene, or in some other (new?) gene? Our first crosses suggest that our mutations are more tightly linked to *rII* than to gene 38, as expected for D2a mutations. In these crosses, the *am56-rII1241*-D2a strains were crossed with an *am38* strain (*amB262*) at equal input. *am56(rII*⁺ - 38⁺) phage were isolated by usual T4 genetic procedures, and tested individually for the D2a phenotype of stabilizing phage C-DNA (1). In one case (D2a2), two of nine such *am56* phages tested showed the D2a phenotype, and in the other case (D2a6) none of eight *am56* phages tested showed the D2a phenotype; these results indicate the tighter linkage of D2a to *rII* than to 38.

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TABLE 1. DNA synthesis after infection with presumptive D2a mutants of phage T4

Phage strain	MOI ^a	Surviving bacteria ^b	DNA synthesis ^{b, c}
<i>am56-rII1241</i> -D2a2	1.2	0.30	0.75
	1.9	0.15	0.99
	2.8	0.06	1.33
<i>am56-rII1241</i> -D2a6	1.3	0.28	0.89
	2.4	0.09	1.15
	3.5	0.03	1.49
<i>am56</i> -D2a23	1.2	0.31	0.54
	2.2	0.12	0.60
	4.0	0.02	0.96

^a Multiplicity of infection; calculated from surviving bacteria by using Poisson distribution.

^b As fraction of uninfected control. Typical values for uninfected control are 3 × 10⁸ bacteria/ml and 1,500 ³H-thymidine counts/min incorporated.

^c Measured 15 min after infection at 37 C.