FURTHER STUDIES ON INDUCED REVERSE MUTATIONS OF PHAGE T4 rII MUTANTS*

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The experimentally observed bipartition of rII mutants into transition and non-transition mutants (Freese 1959), as well as into highly and little (or not) reversion inducible mutants (Freese et al. 1961), can be interpreted without regard to the existence of suppressor mutations. But if one wishes to propose that transition mutants, highly reversion inducible by Hydroxylamine (HA), have a G-C pair at their mutant site, or that non-transition mutants, reversion inducible by low pH or Ethylethane sulfonate (EES) arose by a transversion (E. B. Freese 1961), it is necessary to examine the nature of the induced revertants.

Although results of such examinations have been mentioned in earlier publications (Freese 1959, Freese et al. 1961) it seems worthwhile to publish more detailed results, especially since suppressor mutations are getting more and more attention (i.e. Brenner et al. 1961).

It should be realized that for the above-mentioned interpretations, it is irrelevant whether an r mutant yields some or even mostly "false revertants" which are due to suppressor mutations. The only important question is whether for a given mutant some revertants are induced which, by functional as well as recombinational tests, behave like the standard type phages.

We have tested spontaneous and induced revertants of the following rII mutants: (1) "transition-mutants" induced by 2 Aminopurine, AP 12, 28, 41, 61, 70, 72, 114, 275; by Bromouracil, N 7, 12, 19, 24, 29, 31; by Hydroxylamine, HA 45; and (2) "non-transition mutants" induced by Ethylethanesulfonate,

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Abbreviations: HA = Hydroxylamine; EES = Ethylethanesulfonate.

EES 4, 64, 66, 116, 122. As mentioned previously, AP 12, 28 (located at the same genetic site), and AP 41 produce tiny plaques on Bacteria E. coli K12 (λ) (=K), in addition to the revertants of standard phenotype (Freese 1959). Since for measuring the rates of reversion induction these tiny plaques were as carefully excluded as possible, they have not been tested further. Otherwise we have isolated for each of the AP, HA and N mutants 20 wildtype revertants from bact. K, before and after HA treatment, and for mutants AP 70, 114 and 275, also after EES treatment. Again for the EES induced mutants, 20 wildtype revertants were picked before and after treatment with EES and pH 4.2. These isolated revertants were tested in the following way:

Test of functional properties

Since on bacteria K only revertants can grow, while on E. coli B (=B), r and standard type plaques can be distinguished (Benzer 1955), all isolated revertants were replated on both bacteria E. coli B and K.

From these experiments we can classify the mutants tested into 3 groups:

(a) Mutants whose revertants gave only standard plaques on bacteria B and K or showed only $<30\%$ revertants that looked different from standard wild on Bacteria B: AP 72, AP 41 (tinies excluded), AP 61, AP 275; N 7, N 12, N 24, N 29, N 31; HA 45; EES 4, EES 64, EES 66, EES 116, EES 122

(b) Mutants whose wildtype revertants on B were only about 30% while the rest looked different from wildtype: AP 114, N 19

(c) Mutants whose revertants were exclusively r or big wild on B: AP 12, 28 (tinies excluded) and AP 70.

These results show that for many of the rII mutants tested partial revertants exist; they were not analyzed further. However, for most of the above mutants, the majority of revertants tested seem to have the standard phenotype on both B and K.

Wildtype revertants of mutants EES 66, 116, AP 41, 72, 114, 275 were also tested in parallel experiments on Tryptone-agar (T) plates and Hershey-agar (H) plates (Adams 1959) and plating on bacteria B and K. Plaques on H

plates are slightly larger and clearer than those on T plates. To test this further, we mixed in equal number standard type phages and revertants previously scored on T plates as phages of standard phenotype; these were plated together on the same plates. The plaques look uniform; we could not detect any difference in plaque size or shape between standard and revertant phages on the same plate. The morphological difference between standard plaques on T and H plates can therefore be attributed to the difference in the media.

It should be mentioned here that it is important for testing the plaque morphology always to use plates with media of the same age, the same amount of agar in the media, approximately the same amount of medium per plate, to store the plates under the same conditions, and to evaluate the plaques after about 16 hours of incubation at 37°C.

Genetic Crosses

Revertants of standard phenotype (on K and B) were backcrossed to standard type phages in order to determine whether suppressor mutations might be detectable. The backcrosses were done in the following way:

Bacteria E. coli B were grown in T-broth to 4×10^8/ml. KCN was added to give 1/200 M to prevent exclusion of the superinfecting phages. After 5 minutes in 37°C a phage mixture containing 2×10^9/ml each of the revertant stock and standard type phage was added. Phages were allowed to absorb for 10 minutes, followed by a 10^4 fold dilution in T-broth, and aeration at 37°C. After 90 minutes, chloroform was added. The lysate was diluted and plated on Bacteria B on 20 T plates to give about 500 plaques per plate.

For crosses with revertants of EES mutants, a standard type stock was used whose frequency of r mutants was 6.4×10^{-4} (11 r's among 17 106 standard type plaques), while for crosses with revertants of AP mutants, another standard type stock was used whose frequency of r mutants was 5.4×10^{-4} (5 r's among 8 981 plaques tested).

The results of these crosses are given in Table I. None of the lysates of a cross showed any significant increase in the frequency of r-type plaques

above the values expected from the background in the stocks. Thus in these crosses we have not found evidence for suppressor mutations.

TABLE I - Revertants Backcrossed to Standard Type Phage
(Bacteria B Infected with Multiplicity 5)

Mutant	Revertant No.	Revertant Induced by	Spontaneous Backgrounds in Revertants w	r	r in 10^4	After Cross w	r	r in 10^4
EES 66	1	EES	11 430	6	5.2	8 914	3	3.4
	2	Induction	7 990	4	5.0	8 406	3	3.6
	3	Factor:	11 474	4	3.5	9 414	3	3.9
	4	2.4	13 218	3	2.2	9 526	4	4.2
	5		12 420	15	12.1	10 500	10	9.5
	6		3 784	8	21.1	1 800	3	16.6
EES 116	1	EES	11 025	12	10.9	8 160	5	6.2
	3	Induction Factor: 2.9	10 782	18	34.3	9 450	20	21.2
AP 41	1	HA	3 350	2	5.9	5 000	4	8.0
	2	Induction	6 250	4	6.4	10 433	6	5.7
	3	Factor:	10 652	2	2.0	10 393	3	2.9
	4	139	14 710	5	3.4	9 613	5	5.2
	5		5 420	1	1.8	11 173	4	3.6
AP 72	1	HA	1 747	1	5.7	6 453	6	9.3
	2	Induction	5 243	1	1.9	10 153	2	2.0
	3	Factor:	4 473	2	4.5	7 567	8	3.9
	4	123.7	5 487	3	5.5	6 827	5	7.3
	5		8 793	3	3.4	2 571	1	3.9
AP 114	2	HA	8 625	3	3.5	9 960	6	6.0
	5	Induction	4 800	1	2.0	10 440	3	2.9
	7	Factor:	6 517	1	1.5	11 280	3	2.6
	9	53.9	5 037	5	9.9	9 640	4	4.1
	11		5 243	2	3.8	11 280	4	3.5
AP 275	1	HA	7 370	3	4.1	12 340	4	3.2
	3	Induction	7 827	3	3.8	13 240	8	6.0
	4	Factor:	8 523	3	3.5	11 607	9	7.8
	5	100.2	8 478	3	3.5	10 633	9	8.5
	6		5 515	3	5.4	5 410	4	7.4
	7		6 040	4	6.6	3 890	4	10.3

Abbreviations: w = wild=standard-type plaques
r = r-type plaques

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