UNSTABLE rII MUTANTS OF BACTERIOPHAGE T4.

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Genetic analyses of the phage T4 rII region have been generally confined to stable mutants possessing reversion frequencies from 0 to 1 x 10^{-6} (1,2,3). At least ten percent of ultraviolet or spontaneous rII mutants are U type (unstable), reverting to r^+ phenotype with extraordinarily high frequencies of 10 x 10^{-6} to 5 x 10^{-1} (1,3). UrII mutants are not particularly amenable to normal mapping or mutagen testing procedures by virtue of their identifying characteristic of instability; as a class they do not appear to fit clearly any model for mutation. Hence studies on UrII mutants were begun with the object of delineating their genetic nature. This report summarizes studies on the properties of the r^+ revertants produced by UrII mutants. To anticipate results, r^+ phage obtained from UrII mutants can specifically back mutate to the original UrII type with regard to genetic site and reversion index.

Media and general techniques have been described in a previous article (1). For this study, 3 UF rII mutants of UV origin (1) have been selected from our total group of 65. The range of reversion frequencies of the entire group was 5 x 10^{-1} to 5 x 10^{-5} in a continuous distribution, while those of the 3 discussed here are listed in Table 1.

Table 1 : Reversion Frequencies of UrII mutants

stock no.	Cistron-segment location[a]	r^+/min. reversion frequency[b]
UF-137	A-2	5.4×10^{-5}/o
UF-152	B-8	3.2×10^{-4}/o
UF-102	B-7	2.2×10^{-4}/o

a) Each UrII mutant was spot tested to the six overlapping deletions of Benzer and Freese (4).
b) Reversion frequencies were determined from the averages of r^+ phage frequencies of at least 3 stocks (as per ref. 4). Since some of our UrII mutants yield r^+ and minute phenotypes on K or B bacteria, the frequencies of each are presented as above.

UrII reversion could occur by recombination, nucleotide base pair alteration, or some other mechanism. To determine which mechanism was operating, over 100 single r^+ bursts from UrII-*E. coli* B complexes (moi $\lesssim$ 0.1) plated on *E. coli* K-12(λ) were examined for each of the 3 UrII mutants. When the log accumulated frequency of r^+ bursts is graphed against clone size (5) a slope of -0.35 to -0.61 is observed. Single burst r^+ recombinants between closely linked T4 rII markers yield, on the same graph, a slope of -3.2 (data of Streisinger reported by Steinberg and Stahl, 1,6), while single burst forward mutation for r and w mutants of T_2 yield a slope of -1 (5). Slopes of -1 indicate a clonal origin of mutants, random in time. More negative slopes, as observed for recombinants, are explained by the relatively late origin of r^+ recombinants. Our less negative slopes of -0.35 to -0.61 suggests that UrII r^+ revertants arise *early* in development by a mechanism other than mutation or recombination.

To ascertain the genetic nature of UrII r^+ revertants, efforts were directed toward an analysis of UF-137 r^+ phenotypes. A set of 8 r^+ plaques on K were picked, replated on B, and single isolated r^+ plaques were employed to prepare phage stocks on the non-selective host bacteria E. coli Bb. Each of the 8 r^+ stocks were then scanned on E. coli B for its content and type of rII phage. A total of 110 r mutants were isolated, 108 were rII, and the overall mutation frequency from r^+ to rII was 5.56×10^{-4}, 8.3 fold higher than spontaneous rII mutation frequencies of 6.7×10^{-5} (1). Four of the 8 r^+ stocks from which 89 of the 108 rII mutants were obtained yielded 7 rII mutants which mapped at identical sites to UF-137 in segment 2, with reversion frequencies (r^+/minute x 10^{-6}) of 6.8/o, 25/250, 0.3/o, 18/75, 24/805, 0/o, and 25.6/o. The balance of the rII mutants were similar to spontaneous mutants in distribution and type as far as can be determined with this small sample of mutants. UF-137 was the only representative of its site from a sample of 262 rII mutants representing 53 UV and 43 spontaneous sites maintained in these laboratories (1). Thus, the UF-137 site does not appear to be even a mild "hot spot" of spontaneous mutation and its reappearance from UF-137 r^+ revertants seems to be a property of these revertants.

Similar results regarding site specificity were observed in smaller samples of UF-102 and UF-152 r^+ revertants. From 27 rII mutants obtained from 5 UF-102 r^+ revertants, one site was identical to UF-102. From 6 rII mutants of one UF-152 r^+ revertant, 2 sites were identical to UF-152. Further investigation of the spectrum of UrII $\rightarrow r^+ \rightarrow$ rII mutants is currently underway.

When "r^+" UF-137 revertants are backcrossed to $T4Br^+$ no detectable increase in rII mutants is observed. Internal suppression (2,7) does not appear to explain that preferential direction of rII site specific mutation.

No evidence for episomal (10) or mutator control of UrII mutability was evident from the following experiments. UF-137 was crossed to $T4Br^+$ and 123 of the rII progeny were assayed for reversion frequencies, all of which were well within the UF-137 frequency of 5.4×10^{-5}. Growth of UF-137 in phenylethanol-treated E. coli B (8) yielded progeny phage only of input type regarding reversion indices.

Our results are best explained by employing the "loop" model evoked by Benzinger and Hartman (9) to explain aberrant recombination behavior of the his-24 allele in Salmonella. For our case, we hypothesize UrII mutants contain a short DNA loop of intrastrand complementarity, while r^+ revertants represent that fraction of phage genomes possessing interstrand base pairing. This model generates two alternate states of the UrII site; the "off" state as the loop form and the "on" state as the straight form. Such a model provides for a continuous spectrum of UrII reversion rates, and explains the early reversion of UrII mutants observed in single burst studies. Furthermore, if the loop and straight forms are alternate states, site specific mutation as observed here can be explained. The loop model for unstable genes can also explain the data of Barnett and De Serres (11) for similar mutants in Neurospora.

The possibility that glucosylation of hydroxymethyl cytosine might be aberrant in UrII mutants and related to loop formation is currently being

investigated due to the similar properties of UrII mutants and host induced modification (12).

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