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Acriflavin resistant *r*II deletions of bacteriophage T4

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A dispensable region adjacent to the *r*IIB cistron of phage T4 has been revealed by *r*II deletions which extend beyond the border of the B cistron. Physical (Bautz & Bautz, 1967) and genetic (Dove, 1968) mapping of the length of these B terminal deletions indicate that the dispensable segment is about the size of the *r*II region. Although the function(s) controlled by this dispensable segment is (are) not well known, the results of Bautz & Bautz (1967) indicate that it is transcribed and those of Dove (1968) suggest that it may be involved in control of acriflavin resistance. In this note evidence will be presented which indicates that the *ac* locus (Edgar & Epstein, 1961) coincides with the *r*II distal portion of the dispensable region.

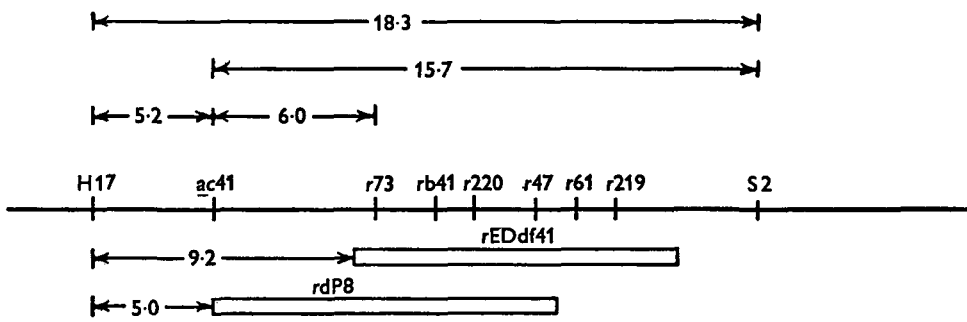

Fig. 1. Genetic map of the gene 52 (*am*H17) to gene 39 (*am*S2) region. The recombination frequency for the *ac*41–*r*73 interval is from Edgar & Epstein (1961) and that for the *ac*41–*am*S2 interval is from Doermann (unpublished data). Interval sizes are not drawn proportional to map length.

The T4D mutation *r*dP8 is a non-reverting, multisite *r*II mutation extending from the interval between *r*47 and *r*61 in the *r*IIA cistron to the *r*IIA-distal border of the B cistron or further (Fig. 1). In crosses homozygous for *r*dP8 the frequency of recombination for the outside markers *am*H17 (gene 52) and *am*S2 (gene 39) is reduced from 18·3 % to 9·5 % (Fig. 1). On the basis of these properties *r*dP8 is considered to be a deletion. Although *r*dP8 was isolated as a spontaneous mutant in an acriflavin-sensitive stock, it showed acriflavin-resistance similar to that of the resistant mutant *ac*41 (Table 1). This result suggested that *r*dP8 was either (1) an *r*II acriflavin-resistant double mutant, or (2) that the deletion extended into the *ac* gene thereby inactivating it. The latter alternative is plausible because *ac*41 is recessive and therefore may be considered to result in a loss of function (Edgar & Epstein, 1961), and because there are no known conditional lethals between *ac*41 and the *r*IIB cistron. To distinguish between the alternatives,

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*r*dP8 was crossed to T4D wild-type and r^+ acriflavin-resistant progeny selected. The frequency of r^+ acriflavin-resistant progeny was $1{\cdot}7 \times 10^{-5}$, which was not statistically different from the mutation index to acriflavin resistance determined in a parallel control. On this basis the second alternative was considered to be correct.

Experiments were then performed to determine if the acriflavin resistance of *r*dP8 was functionally and genetically allelic to *ac*41. If they are functionally allelic, then mixedly infected bacteria should be resistant, while if they are not, the infected bacteria would be sensitive. Cells of *E. coli* CR63 mixedly infected with *ac*41 and *r*dP8 or with either mutant alone, produced infective centres when plated on CR63 supplemented with 2·0 μg of acriflavin per ml of bottom agar (Parma, 1968). However, cells infected with *ac*41 and wild-type or with *r*dP8 and wild-type did not. These results show that *r*dP8 like *ac*41 is recessive and that *ac*41 and *r*dP8 are functionally allelic.

Table 1. *Efficiency of plating of T4D wild-type* ac41 *and* rdP8*

Bacterium ...	S/6			CR63			
Ac. concn.† ...	0	0·25	0·50	0	0·50	1·0	2·0
Strain							
T4D r^+ ac^+	1	$< 1{\cdot}5 \times 10^{-5}$	$< 1{\cdot}5 \times 10^{-5}$	1	$1{\cdot}5 \times 10^{-2}$	4×10^{-5}	$< 1{\cdot}4 \times 10^{-5}$
*ac*41	1	$0{\cdot}82 \pm 0{\cdot}10$	$0{\cdot}26 \pm 0{\cdot}05$	1	$0{\cdot}86 \pm 0{\cdot}09$	$1{\cdot}00 \pm 0{\cdot}10$	$1{\cdot}00 \pm 0{\cdot}10$
*r*dP8	1	$0{\cdot}72 \pm 0{\cdot}06$	$0{\cdot}16 \pm 0{\cdot}03$	1	$0{\cdot}98 \pm 0{\cdot}07$	$1{\cdot}02 \pm 0{\cdot}07$	$0{\cdot}93 \pm 0{\cdot}07$

* For a given host the titre without acriflavin supplement is take as 1 and the titre on acriflavin-supplemented plates is expressed as a fraction of that value. The confidence interval for the titre on Ac.-supplemented plates is twice the square root of the plaque count.

† Acriflavin-neutral concentration in μg/ml added to bottom agar only. Plates were incubated at 37°.

To determine if *r*dP8 and *ac*41 are genetically allelic, *r*dP8 was crossed to *ac*41 and recombinants sought which carry r^+ and are acriflavin sensitive. Among 700 progeny tested no r^+ acriflavin-sensitive (nor *r* acriflavin-sensitive) progeny were found suggesting that *r*dP8 extends nearly to or beyond *ac*41. A similar conclusion is suggested by crosses of *r*dP8 and of *ac*41 to *am*H17. The observed recombination frequencies were 5·0 and 5·2 % for *r*dP8 × *am*H17 and *ac*41 × *am*H17 and were not statistically different. Although this latter comparison is subject to the criticism that *r*dP8 is a deletion and *ac*41 a point mutant, it is worth noting that an acriflavin-sensitive *r*II deletion, *r*EDdf41, gives 9·2 % recombination with *am*H17 (Fig. 1).

The acriflavin sensitivity of three additional T4D B-terminal deletions (*r*EDdf41, *r*db117, and *r*db145) and of six T4B B-terminal deletions (*r*1241, *r*187, *r*A105, *r*1272, *r*NB411, and *r*NB5437) was tested. The amount of the dispensable region deleted by the last four has been estimated by Bautz & Bautz (1967) as 0, 10–15, 10–15 and 97 % respectively. Of the nine, only *r*NB5437 was resistant (Parma, 1968). As in the case of *r*dP8, its acriflavin-resistance did not segregate when crossed to wild-type (L. Boehner, unpublished data).

The results presented here are readily understood in terms of the following proposal: the *ac* locus is situated in the *r*II distal portion of the dispensable region which has been described by Bautz & Bautz (1967) and by Dove (1968); the proximal segment, which is probably large enough to accommodate one or two cistrons, is not concerned with acriflavin resistance as defined here nor is it required for growth on *E. coli* CR63 or B. Very long terminal deletions are acriflavin resistant by virtue of deleting all or part of the *ac* locus. Shorter B-terminal deletions which do not extend into the *ac* locus are acriflavin

sensitive. The size of the dispensable region (Bautz & Bautz, 1967) and Goldberg's (1966) estimate of the physical distance from the *r*II region to *ac*41, 1·2% of the genome, are consistent with the proposed location of the *ac* gene.

Dove (1968) reported that several B-terminal deletions of intermediate length were partially resistant to acriflavin. In the present experiments *r*1241 and *r*1272 did not show a partial resistance. It seems likely that differences in the plating conditions are responsible for this discrepancy (see Table 1, and Dove (1968)).

SUMMARY

The extent and phenotype of acriflavin-resistant *r*II deletions have been examined. The properties of these deletions confirm that acriflavin resistance may result from a loss of function at the *ac* locus and that the *ac* locus coincides with the *r*II distal portion of the dispensable region which is adjacent to the *r*IIB cistron.

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