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The extent of *rII* deletions in phage T 4

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The distribution of end-points of deletion mutations in the *rII* region of phage T4 is non-random. The gross distribution of spontaneous and nitrous acid induced deletions is shown in Table 1.

These data demonstrate that a disproportionately large fraction of the deletions extend to the terminus of the B cistron. The bias is stronger for nitrous acid induced deletions than for spontaneous deletions.

This distribution has been noted by Tessman (1962). In addition Tessman has shown that for nitrous acid induced deletions the left-hand end-points within the A cistron recur at single sites.

The preponderance of B terminal deletions may arise from the fact that a non-essential region is adjacent to the B terminus, but not to the A terminus. If this non-essential region is long, the stronger bias of nitrous acid induced deletions relative to spontaneous deletions would merely reflect the fact that nitrous acid induced deletions are longer than spontaneous ones. This note reports mapping experiments which reveal the presence of a long non-essential region adjacent to the *rII* B cistron. Independent confirmation of this by the detection of m-RNA has been published by Bautz & Bautz (1967). The mapping results and the m-RNA results are compared and the phenotype controlled by this non-essential region is analysed.

The extent of an *rII* terminal deletion, $rII\nabla_i$, has been estimated by a three factor cross-diagrammed in Fig. 1.

In such a cross, rII^+am^+ recombinants were directly selected on KB, and then scored for their *ac* character on *E. coli* B with 0·25 μg/ml acriflavine. Within the sampling error, the extent of the deletion beyond an extreme B point mutant, X 172, can be calculated from the ratio of ac^S to ac^r recombinants in the rII^+am^+ class. This crude analysis omits consideration of multiple exchanges. One known error exists in this mapping procedure: in T4, as in other organisms, recombination is suppressed at the ends of a deletion mutation (Bode, 1963). Thus, the deletion will appear artificially long when mapped by this procedure. The magnitude of this error was estimated by mapping the end-point of the deletion *r*1231, which ends within the B cistron. The error is significant, but does not account for more than 35 % of the extent of the longest deletions studied here.

The results of these crosses are presented in Table 2.

These results establish that *rII* deletions can extend beyond the B terminus almost to the *ac* locus. The extent can be estimated in nucleotide pairs from the mapping function of Stahl, Edgar & Steinberg (1964). Using the mapping data of Benzer (1961) and of Edgar *et al.* (1962) to estimate the true end-point of *r*1231, it can be seen that the error in this mapping method is about 400 nucleotide pairs. The longest deletions appear to extend 1300 nucleotide pairs beyond the B terminus. Although these calculations are

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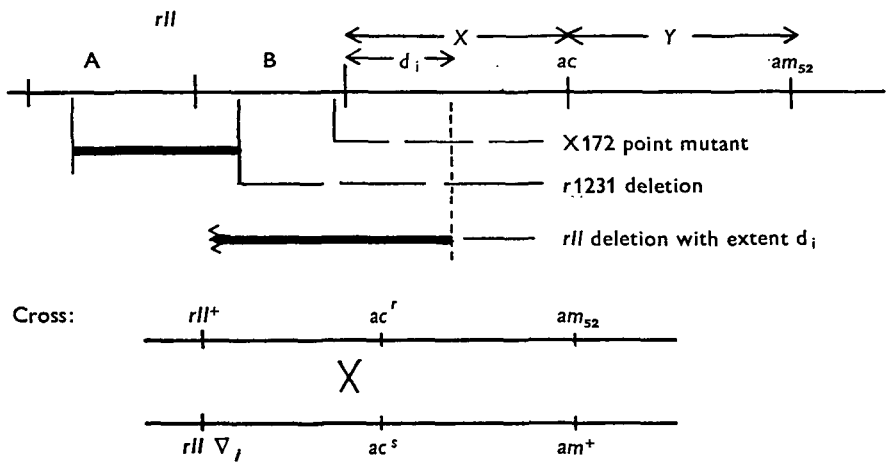
Table 1. *End-points of rII deletions*

	A terminus	Within A cistron	Within B cistron	Within A cistron	Within B cistron	B terminus
		rII				
		A			B	B terminus
		Left-hand end			Right-hand end	
Spontaneous deletions	16	—	—	0	0	16
	—	116	—	←——91—→		25
	—	—	33	—	19	14
Nitrous acid induced deletions	9	—	—	0	0	9
	—	32	—	2	1	29
	—	—	9	—	0	9

Spontaneous deletion end-points are taken from Tessman (1962) and Benzer (1961). Nitrous acid induced deletion end-points are taken from Tessman (1962).



Among recombinants rII^+am^+

$$ac^s/ac^r = \frac{X - d_i}{Y}.$$

For $X172$, $d_1 = 0$.

Thus,
$$\frac{(ac^s/ac^r)_{X112}}{(ac^s/ac^r)\nabla_i} = \frac{X/Y}{(X-d_i)/Y} = \frac{X}{X-d_i},$$

or,
$$d_i = X\,\frac{(ac^s/ac^r)_{X172} - (ac^s/ac^r)\,\nabla_i}{(ac^s/ac^r)_{X112}}.$$

Fig. 1. Mapping of extent of deletions. The *rII* locus is described by Benzer (1955, 1961). The *ac* locus controls sensitivity to low concentrations of acridine dyes, e.g. 0·25 μg/ml acriflavine. The ac^s allele is dominant to the ac^r allele (Edgar & Epstein, 1961; Silver, 1965). The am_{52} site lies in gene 37, controlling the synthesis of tail-fibre antigens (Edgar & Lielausis, 1965). The *rII* and am_{52} mutants were from Dr S. Brenner. The ac^r mutant was induced in T4 Benzer by bromouracil.

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crude, it is clear that deletions can extend beyond the *rII* B terminus almost to the *ac* locus, a distance equal to at least one cistron.

Bautz & Bautz have extended the work reported here by measuring the extent of these and other B-terminal deletions by determining the amount of T4-specific m-RNA which will fail to hybridize with these deletions. Their results are best compared with these by making the assumption that the longest deletion studied extends to the *ac* site. This gives an upper limit to the length measured by their method and expressed in nucleotide pairs. These extents are plotted in Fig. 2 along with the extents calculated from the mapping data reported here.

Comparison of these measurements confirms that the extent of deletions is overestimated by the mapping method by 400 nucleotide pairs. Also, the general agreement between the two sets of measurements provides confirmation of the difficult hybridization technique of Bautz & Bautz. Finally, the agreement confirms the assumption of Bautz & Bautz that the *rII-ac*^{r} region is transcribed at the same rate as is the *rII* region.

Table 2. *Results of mapping of extent of deletions*

Mutant	AC Genotypes AC^S	AC Genotypes AC^R	AC^S/AC^R	d_i/X	d_1 (nucleotide pairs)
X172	37	115	0·322	0	0
*r*1231	41	115	0·357	−0·11	−165 ± 28
A105	37	118	0·314	0·02	30 ± 6
*r*638	30	126	0·238	0·26	390 ± 78
PB28	27	128	0·211	0·34	510 ± 98
*r*1241	23	123	0·187	0·42	630 ± 130
*r*1272	19	135	0·141	0·56	840 ± 200
PT1	19	231	0·082	0·75	1120 ± 270
PB296	21	229	0·092	0·71	1070 ± 810
NB3157	20	448	0·045	0·86	1290 ± 280
PT8	18	468	0·038	0·88	1320 ± 330

The cross diagrammed in Fig. 1 was analysed as described in the text. The extents were converted into nucleotide pairs by using 6 map units as the distance from the B terminus to the *ac* locus, and the mapping function of Stahl *et al.* (1964). The errors are calculated from the Poisson distribution for the numbers of genotypes scored.

A search has been made for phenotypes associated with the region deleted in these long deletions. By extensive mutation with ethyl methanesulphonate or by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, no derivatives of non-lysogenic K12 strains could be found in which the deletion PT *ac*^{r} failed to grow. A number of lysogenic strains were screened. Lysogens for lambda and for two temperate phages, C1021 and C1029 (isolated by G. Bertani), did prevent the growth of PT1 *ac*^{r}. These latter phages, which differ from each other and from lambda in their immune specificity, are similar to lambda in preventing the growth of T4 *rII* mutants. Five mutants of T4 were selected solely for their ability to grow on C1021. They failed also to grow on C1029 and on lambda lysogens, and display an *r* phenotype on *E. coli* B. Thus, the block to growth of PT1 *ac*^{r} is probably due to its *rII* defect, and no host has been found for which the *rII-ac* region is essential for T4 growth.

The acridine sensitivity of a number of these deletions was studied. As is shown in Fig. 2, some deletions display partial resistance to 0·25 μg/ml acriflavine on strain BB at 44 °C. These deletions generally end near the centre of the region under study. Either there are three separate cistrons in this region, with the centre one exerting some control over acridine sensitivity, or else there are two, and deletions ending near the beginning

of the right-hand one generate a polar effect on the ac^s cistron. It will be valuable to distinguish these two possibilities by studying the acridine resistance phenotype of the larger set of deletions whose extent has been mapped by Bautz & Bautz.

The existence of a long non-essential region beyond the *rII* B terminus can account for the preponderance of apparent B-terminal deletions observed in Table 1.

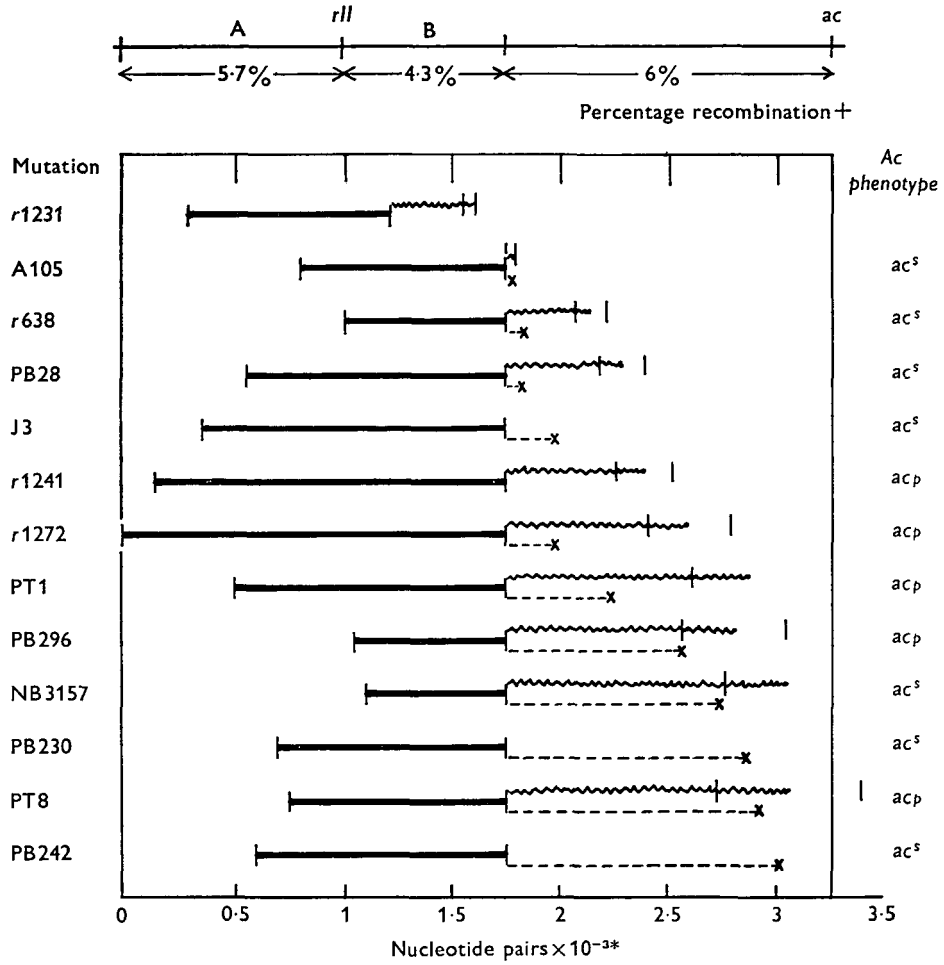

Fig. 2. Extent and phenotype of *rII* deletions. +, from Edgar *et al.* (1962) and Edgar & Epstein (1961). *, from four-parameter switch function of Stahl *et al.* (1964). ——, from deletion mapping, Benzer (1961). ∿∿∿, from crosses reported here. - - - -, from Bautz & Bautz (1967), assuming 'D' extends to *ac* locus. ac^s means an efficiency of plating on BB at 44° with 0·25 μg/ml acriflavine of approximately 01^{-4}. ac^p means an efficiency of plating of approximately 10^{-1}.

Other non-random features of the distribution of end-points might be attributed to a non-random distribution of reactive residues. (Tessman, 1962). Another interesting and testable possibility to be considered both for spontaneous deletions and for those produced by treatment of the packaged phage chromosome is that there may be a non-random distribution of end-points of the phage chromosome in the selection of the phage headful of DNA during T4 maturation (Sechaud *et al.* 1965).

SUMMARY

The extent of certain *rII* deletions in phage T4 has been mapped by genetic means beyond the terminus of the B cistron. They extend to varying degrees, nearly to the ac^s locus. The phenotypes of these mutants indicate that there are two or three cistrons between *rII* B and ac^s, and that the functions coded for by these cistrons are not essential for growth in any known *E. coli* K12 strain or *E. coli* lysogen.

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